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The identification of differentially expressed cell cycle - related genes in breast and colon cancer cell lines in response to chemotherapeutic drugs

Rupnarain, Charleen

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CHAPTER ONE: INTRODUCTION

By 2020, global cancer rates are estimated to increase by 50% to 15 million new cases. Cancer accounts for over 25% of deaths in many countries, with incidence rates in developing countries having increased to those in industrialised countries. Worldwide, high breast cancer incidence correlates with a high incidence of colon cancer (WHO Cancer Report, April 2003).

For the malignant phenotype to be created, approximately five - ten specific genetic aberrations must occur. Genetic instability in tumours enhances these aberrations, giving cancer cells the opportunity to overthrow the balance between cell growth and cell death and enforce unrestrained proliferation (Renan, 1993; Evan and Vousden, 2001). Cancer is regarded as a cell cycle disease as the majority of tumours have aberrations that overcome cell cycle control (Malumbres and Carnero, 2003). This stated, it is imperative to increase our understanding of the genetic pathways targeted by cancer cells, and to identify new and improved targets to increase the efficiency and specificity of chemotherapeutic agents. Spurred by the Human Genome Project from the 1980s, genomics is the critical factor in drug development. This study aimed to identify cell cycle-related genes that are influenced by the novel chemotherapeutic drugs (curcumin, SAHA, lycopene and thalidomide) with regards to gene expression levels in breast and colon cancer cell lines, with the intention of isolating potential genetic targets to increase the characterisation of these cancers and the molecular pathways affected by the drugs. These genes provide potential targets for manipulation in future studies with the objective of improving on the efficiency of these drugs, in response to the need to streamline

chemotherapeutic treatment through specific genetic targets. 84 cell cycle-related genes are being studied. The cell cycle-related genes in the SuperArray PCR Array are selected by the manufacturers following an extensive PubMed literature search. The final selection in the Cell Cycle pathway array is approved by a scientific advisory board.

While these drugs have been previously studied, this particular study further identifies influenced genes that have not been documented in prior research. With the emphasis for increasing the effectiveness and decreasing the side effects of chemotherapeutic treatments in patients, it is vital to ultimately characterise all of the genes that are influenced by these agents. In achieving this, the full spectrum of the respective drug action is understood, and the drug can be administered with full knowledge of the expected outcome.

AIMS OF STUDY

- Identify the influence of the chemotherapeutic drugs curcumin, SAHA, lycopene and thalidomide on the cell cycle in HT-29, MCF-7, MDA-MB-231 and 184A1 cell lines using flow cytometry.
- Identify genes whose expression is influenced by curcumin, SAHA, lycopene and thalidomide in HT-29, MCF-7, MDA-MB-231 and 184A1 cell lines using the Genefishing system.
- Identify cell cycle-related genes whose expression is influenced by curcumin, SAHA, lycopene and thalidomide in HT-29, MCF-7, MDA-MB-231 and 184A1 cell lines using the SuperArray PCR Array.
- Draw conclusions as to how the drugs influence the oncogenic state of the cancer cell lines HT-29, MCF-7 and MDA-MB-231, and if these drugs influence the normal cancer cell line, 184A1.

1.1 Breast cancer

1.1.1 Statistics

One million new breast cancer cases are diagnosed worldwide every year. Breast cancer accounted for 1.6% of all female deaths in 2000, approximately 400 000 deaths (McNeill *et al.*, 2007). In developed countries, breast cancer accounted for 2% of all female deaths and 0.5% in developing countries in 2000. Breast cancer mortality rates are decreasing in North America, Western Europe and Australia because of early detection and improved treatment, with the five-year survival rate in most developed countries greater than 75% (WHO Cancer Report, April 2003). In Africa, breast cancer incidence is second to

cervical cancer in women (Ferlay *et al*., 2000). By 1999, breast cancer was the most common cancer in South Africa, and the leading cause of cancer-related death in women. It is the most prominent cancer in Caucasian, Coloured and Asian women (Mqoqi *et al*., 2004).

1.1.2 Physiology

The normal breast consists of ductal epithelium surrounded by stroma, which provides nutrition and structural support. The normal non-lactating breast responds to oestrogen and progesterone during the ovulation cycles. These hormones increase the proliferation of epithelial cells and their withdrawal at the start of a new cycle induces apoptosis to maintain epithelium balance. Successive cycles can promote the formation of transformed cells through the accumulation of genetic aberrations, thereby initiating malignancy (King, 1993; Pike *et al*., 1993). Consequently, breast cancer risk rises with increasing reproductive period and increasing ovulatory cycles (Hankinson and Hunter, 2002). While most cancers originate from epithelial cells, they can also develop in the stroma (Bissell and Radisky, 2001; McCaulley and Matrisian, 2001; Wiseman and Werb, 2002).

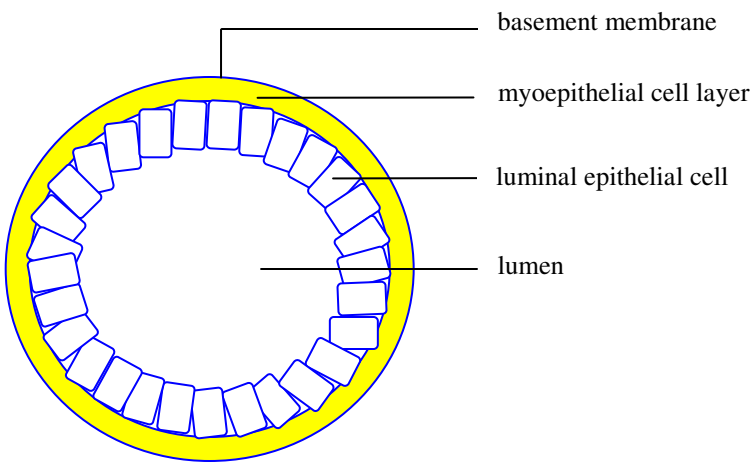


Figure 1.1: Diagrammatic representation of cross-section of normal breast (www.breast-cancer-research.com)

1.1.3 Epidemiology

Breast cancer is induced by reproductive history, genes, radiation, a high calorie diet, obesity, excessive alcohol intake and a sedentary lifestyle (WHO Cancer Report, April 2003; Magnusson *et al.*, 1998; Hamajima *et al.*, 2002). Incidence rates increase swiftly before menopause (ages 40-50) and decrease after menopause, in correlation with low oestrogen levels (Henderson *et al.*, 1988). Oral contraceptives and hormone replacement therapy increase the risk of breast cancer, with cancers usually diagnosed at a clinically unadvanced stage (Collaborative Group on Hormonal Factors in Breast Cancer, 1996; Beral, 2003). However, HRT has been shown to not increase the risk of breast cancer. The increase in breast cancer occurrence is partially due to environmental chemicals e.g.

endogenous oestrogens such as estradiol, and environmental oestrogens such as DDT (Verma *et al*., 1998; Falck *et al.*, 1992; Dewailly *et al*., 1994).

Cancer is initiated and progresses as a result of chromosomal aberrations, chromosomal instability brought about by abnormalities in chromosomal segregation; and structural changes as a result of irregularities at cell cycle checkpoints, in the damage response or telomere defects (Lingle *et al*., 2002; Maser and DePinho, 2003; Feldser *et al*., 2003). Most breast carcinomas develop gradually from defined precursor lesions, with the majority of breast cancers displaying luminal epithelial characteristics (Berardo *et al*., 1998; Abd El-Rehim *et al*., 2005; Nielsen *et al*., 2004). Approximately 5-10% of breast cancers are caused by germline mutations in a set of high-penetrance genes (Prokopcova *et al*., 2007). The molecular template of breast cancer is present from the early stages i.e. from atypical ductal hyperplasia. The genes that confer this hyperplastic state progressively alter expression levels with progression to ductal carcinoma *in situ* (DCIS) and invasive ductal carcinoma (Ma *et al*., 2003). The most common breast malignancy is invasive ductal breast carcinoma, accounting for approximately 80% of all invasive breast cancers (Greenlee *et al*., 2000). Of the differentially expressed genes in DCIS, 15% are involved in the cell cycle (Abba *et al*., 2004). Advanced breast cancer frequently results in metastasis to the bone, inducing incapacitating skeletal complications (Coleman, 2006).

1.1.4 Markers

Oestrogen receptor (ER), progesterone receptor (PR) and HER-2/neu are most commonly used as markers for breast cancer. Breast cancer tumours are steroid hormone responsive. Oestrogen is important in breast and reproductive morphogenesis, functioning through the activation of oestrogen receptors ERα and ERβ, and promotes breast tumour cell growth (Matthews and Gustafson, 2003; Ando *et al*., 2002). Oestrogens and anti-oestrogens control proliferation by regulating the expression of cyclin D1, cyclin A, cell division cycle 25A homologue (CDC25A), p21 and p27, amongst others (Foster *et al*., 2001; Cariou *et al*., 2000). Oestrogen binds to ER, forming an active complex that dimerises and binds to oestrogen responsive elements, promoting tumour growth by stimulating genes that promote cell cycle progression and inhibiting genes that suppress the cell cycle. More than 1% of genes are targeted by oestrogen and approximately 50% of these are inhibited (Frasor *et al*., 2003). ER also functions in a non-genomic or epigenetic pathway, activating a kinase pathway that promotes cell cycle progression (Giancotti, 2006). Inhibition of the ER/oestrogen complex is instrumental in suppressing breast tumour cell proliferation. This can be achieved by preventing oestrogen from interacting with ER e.g. the drug tamoxifen binds to ER, blocking oestrogen association and inhibiting cell cycle progression with arrest at G_1 (Freedman *et al.*, 2005).

60-80% of breast cancers are associated with breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) (Nathanson *et al*., 2001). While BRCA1 and BRCA2 mutations are associated with hereditary breast cancer, these mutations are rarely seen in sporadic breast cancer (Futreal *et al*., 1994). The majority of breast tumours are treated with a

combination of surgery, adjuvant systemic therapy (e.g. chemotherapy, biological therapy and hormonal treatment) and radiation therapy. While a large percentage of patients with advanced breast cancer respond well to hormonal therapy, recurrence causes the development of aggressive hormone-unresponsive tumours that have increased invasiveness (Stendahl *et al*., 2004). Breast cancer assessment can be made more efficient by the use of molecular markers. It is imperative to be able to differentiate between patients that respond to certain treatments and those that do not.

1.1.5 Cell lines

Although cell lines derived from human tumours have differences compared to normal and cancerous tissues, they are used as experimental modes for cancer because of the inaccessibility of human tissues (Ross *et al.*, 2000). The Serial Analysis of Gene Expression (SAGE) Database identifies genes that induce differences between the response of cell lines and solid tumours to chemotherapeutic drugs, highlighting the cell lines most suitable for cancer and drug research i.e. those that most closely depict the *in vivo* state (Stein *et al*., 2004). Only 34 of the sixty cell lines studied are similar to the tissues from which they were derived. Genes functioning in cell cycle progression, protein processing and turnover, and metabolic pathways are upregulated in cell lines compared to tissues. These include genes such as CCNB1, CDC20, CDKN3, CKS2, MCM3, MCM4 and PCNA, and are upregulated in all of the six cell lines studied: breast, central nervous system, colon, ovary, prostate and renal (Ertel *et al*., 2006). Genes that are downregulated in cell lines are involved in cell adhesion and membrane signalling (Sandberg and Ernberg, 2005). In breast cancer cell lines, 66% of genes are upregulated

compared to tumours, with significant alterations in the cell cycle pathway (Ertel *et al*., 2006). This may be a consequence of cultured cells grown in medium that is rich in metabolites, growth factors, cytokines and other factors that *in vivo* cells have to normally compete for (Kufe *et al*., 2003).

Established cell lines, such as MCF-7, MDA-MB-231, 184A1 and HT-29, accumulate mutations during passages and immortalisation. During continuous culture, cell lines are susceptible to genotypic and phenotypic aberrations and the development of sub-populations, as seen in MCF-7 (Osborne *et al.*, 1987). Differences observed between MCF-7 cell lines include different growth rates and changes in hormone receptor content, karotype and clonogenicity (Osborne *et al*., 1987; Bahia *et al*., 2002). On average long-established cell lines have 25 times more alterations than tumours, but the most prominent alterations are common to the tumour and the cell line (Forozan *et al*., 2000).

MCF-7 and MDA-MB-231 have a high level of genetic instability due to variations in selective pressure from different culture conditions (Butler *et al*., 1986; Nugoli *et al*., 2003). MCF-7 has high levels of aneuploidy and varies from hypertriploid to hypertetraploid, with chromosome number ranging from 60 to 140 (Butler *et al*., 1986). While MCF-7 and MDA-MB-231 display aneuploidy, this is also common in tumour cells. This aberrant chromosome number is mutual to cells of the same tumour, implying that mitotic and cytokinesis aberrations are common throughout the tumour (Rajagopalan and Lengauer, 2004).

Most breast cancer cell lines are derived from tumour metastases i.e. more aggressive, usually metastatic tumours, and not primary breast tumours. These are unrepresentative of breast tumour diversity, such as with MDA-MB-231 which is an aneuploid, metastatic breast cancer cell line. As most drug therapies are directed against primary tumours, these cell lines are therefore not clinically relevant (Burdall *et al*., 2002). Most breast cancers develop from luminal cell lineage. While the majority of breast cancer cell lines originate in basal cell lineage, MCF-7 has luminal origin. It is therefore more representative of *in vivo* breast cancer, and is the most widely studied model of human breast cancer (Neve *et al*., 2006; Levenson and Jordan, 1997).

1.2 Colorectal cancer

Colorectal cancer refers to cancers of the colon and rectum, whilst colon cancer specifically refers to cancer of the colon. HT-29, which is used in this study, is a colon cancer cell line.

1.2.1 Statistics

Worldwide, 940 000 new colorectal cancer cases are diagnosed annually (WHO Cancer Report, April 2003). While colorectal cancer is rare in developing countries, it is the second most common cancer in industrialised nations, responsible for 500 000 deaths annually. In developed countries, colorectal cancer is diagnosed at a yearly rate of 580 000. In South Africa, Asian men have the highest incidence of colorectal cancer compared to all other cancers; in Caucasian women, it is the second most prevalent and the third most prevalent cancer in Caucasian men (based on statistics from 1993-1995;

Sitas *et al.*, 1998). Colorectal cancer accounted for 9.4% of all cancers diagnosed in 2002, ranking fourth highest in men and third in women. After breast cancer, it is the second most prevalent cancer, with around 2.8 million people affected (Parkin *et al.*, 2005).

1.2.2 Physiology

The colon and rectum make up the last section of the digestive canal, beginning at the ileocaecal valve at the end of the small intestine and ending at the anus. The colon is lined with a highly proliferative epithelium, subdivided into crypts containing 1000-4000 cells that are renewed every five days, and 1-10 stem cells at the crypt base (Yatabe *et al.*, 2001; Bach *et al.*, 2000). This epithelial lining is continuously and rapidly renewed; this facilitates the study of molecular mechanisms of development and proliferation (Karam, 1999). As the stem cells divide, the daughter cells move up the crypt, dividing until they reach halfway up the crypt, where they begin differentiating. At the top of the crypt, the cells undergo apoptosis (Michor *et al.*, 2005). Colorectal cancer is initiated in colonic epithelial cells lining the lumen, forming single crypt lesions and progressing to small benign tumours or adenomatous polyps. These neoplastic colon adenomas extend into the lumen, developing into disordered and dysplastic villous structures to malignant carcinomas (Markowitz *et al.*, 2002; Vogelstein and Kinzler, 2001). The cancer is visible when invasive cells reach the epithelial basement membrane (Markowitz *et al.*, 2002). Colon cancer is an ideal model for research as its initiation and progression have been well characterised.

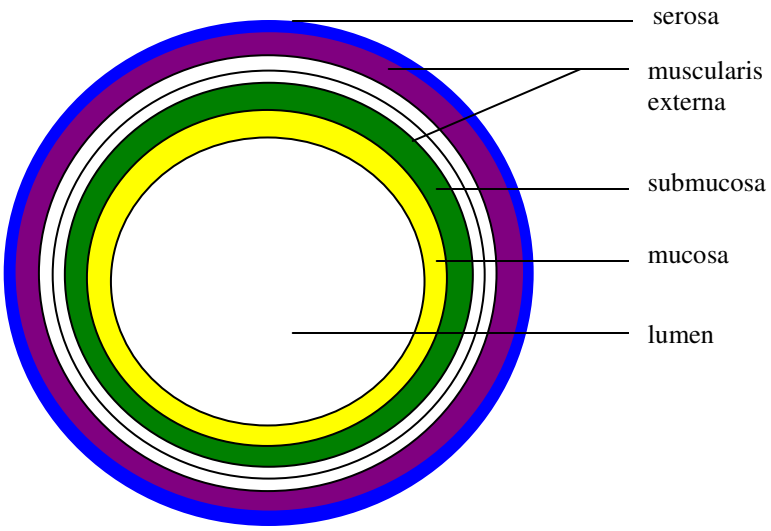


Figure 1.2: Diagrammatic representation of cross-section of normal colon (www.coloncancer.about.com)

1.2.3 Epidemiology

The risk of colon cancer increases with a diet high in fat, refined carbohydrates, red meat, low folate and an inactive lifestyle (Willett, 2001). Less than 5% of cases are due to a genetic cause (WHO Cancer Report, April 2003). Environmental influence is strong as migrant populations acquire the increased risk of the adopted country. Colonoscopy and improved treatment has increased the five year survival rate to 50% (WHO Cancer Report, April 2003). While the most applied treatment is surgical resection, metastasis often hinders its success. Within five years of diagnosis, 20-50% of colorectal cancer patients die. The majority of these deaths are due to extensive metastasis (Ballantyne and Quin, 1993). Upon diagnosis, 20% of patients display metastases in the liver (Weiss *et al.*, 1986).

Colon neoplasia is a multistep pathway culminating in colon cancer over several years. Colorectal carcinogenesis involves the chromosomal instability pathway (CIN) and the microsatellite instability (MIN) pathway (Lengauer *et al*., 1997). In CIN, parts of, or entire chromosomes are lost or gained. This pathway features mutations and loss of heterozygosity (LOH), occurring in 85% of all sporadic colorectal cancers (Lengauer *et al.*, 1997; Arribas *et al*., 1999). This pathway increases the inactivation of tumour suppressor genes, including p53 and K-ras (Michor *et al*., 2005). In the MIN pathway, the DNA mismatch repair system (MMR), important in DNA replication, is deregulated (Modrich, 1991). MIN confers a two - three times greater rate of nucleotide mutation in cancer cells than seen in normal cells (Lengauer *et al.*, 1997). This type of genetic instability is seen in 15% of sporadic colorectal cancers. In hereditary non-polyposis colon cancer, autosomal germline mutations in the MMR complex cause an 80% chance of developing colon cancer (Markowitz, 2000). MMR inactivation causes spontaneous mutation rates to increase by almost a thousand fold and induces MIN (Eshelman *et al.*, 1995; Yamamoto *et al*., 2002).

Familial hereditary colon cancer that is caused by germline mutations, accounts for 3-7% of colon cancer cases yearly (Kinzler and Vogelstein, 1996). Adenomatous polyposis coli germline mutations give rise to the familial adenomatous polyposis syndrome where the lifetime risk of developing colon cancer is nearly 100% (Goss and Goden, 2000). The adenomatous polyposis coli and p53 pathways are altered in 95% of colorectal tumours and are the earliest genetic disruptions in colorectal tumourigenesis (Powell *et al*., 1992;

Fearon and Vogelstein, 1990). The inactivation of adenomatous polyposis coli increases the transcription of growth-promoting genes (Su *et al*., 1993).

1.2.4 Cell line

The HT-29 cell line is isolated from a primary colon adenocarcinoma (grade II) in a 44-year-old Caucasian female. Under standard growth conditions, it has an undifferentiated phenotype. The majority of genes that are downregulated in colorectal tumours, compared to normal colon, are involved in differentiation. The majority of upregulated genes in colorectal tumours function in growth. Many of the differences in gene expression between normal colon and colorectal tumour cells *in vivo* are evident *in vitro*. However, only 47 of 228 genes that are upregulated in colorectal cancer cell lines compared to normal colon cell lines are upregulated in colorectal cancer compared to normal colon (Zhang *et al.*, 1997).

1.3 Chemotherapeutic drugs

1.3.1 Curcumin

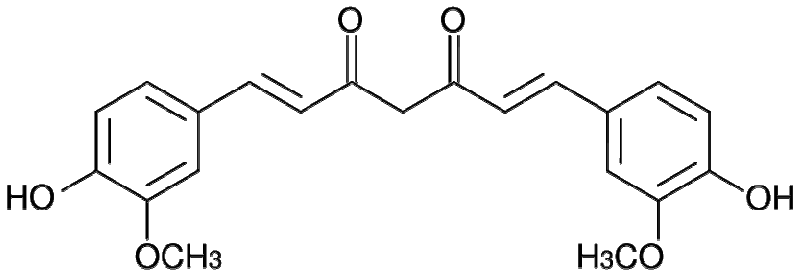


Figure 1.3: Chemical structure of curcumin

The polyphenolic pigments, curcuminoids, are derived from *Curcuma longa* Linn. (turmeric) and includes curcumin (Ammon and Wahl, 1991). *Curcuma longa* belongs to the *Zingiberaceae* (ginger) family. Turmeric is used extensively as a spice, a medicinal agent and in religious ceremonies. The active compounds, curcuminoids, are obtained by ethanol extraction from turmeric. Curcumin has anti-inflammatory, anti-oxidant and anti-carcinogenic traits (Lin and Lin-Shiau, 2001; Joe *et al*., 2004). It induces apoptosis in cancers of the colon, liver, breast and leukaemia by targeting mitochondria, which affects p53-related signalling or inhibits the activation of NF-κB (Leu and Maa, 2002; Chen *et al*., 1999; Jiang *et al*., 1996; Simon *et al*., 1998; Kuo *et al*., 1996). It also shows beneficial potential in lymphoma, ovarian cancer, lung cancer, melanoma, neurological cancers, multiple sclerosis and Alzheimer's, amongst others (Anand *et al*., 2008; Limtrakul, 2007; Zhang *et al*., 2006). Curcumin does display some degree of non-specificity in that it inhibits proliferation in both normal and malignant cells. It does, however, induce apoptosis to a greater extent in cancerous cells (Ramachandran and You, 1999). Tumour cells display a preferential uptake of curcumin in comparison to normal cells (Kunwar *et al*., 2008).

Curcumin is involved in reversing multi-drug resistance in cancer cells (Limtrakul, 2007). It is administered to patients in the early stages of cancer, or to sensitise ovarian cancer cells to the drug cisplatin (Cheng *et al*., 2001; Chan *et al*., 2003). Curcumin enhances the cytotoxicity of other chemotherapeutic drugs including doxorubicin and tamoxifen, and also enhances radiation treatment (Harbottle *et al*., 2001; Verma *et al*., 1998; Bharti *et al*., 2003; Chendil *et al*., 2004; Khafif *et al*., 2005). Phase I clinical studies show that the

continuous administration of curcumin for up to four months is safe for humans (Garcea *et al*., 2005). The molecular and cellular characteristics of curcumin action include DNA laddering, condensation of chromatin, membrane budding, cell shrinkage and cleavage of ribosomal RNA (Jiang *et al*., 1996; Hail, 2008).

The tyrosine kinases, EGFR and HER-2/neu, are overexpressed in the majority of cancers (Lengyel *et al*., 2007). Curcumin decreases proliferation by inhibiting EGFR activity and degrading HER-2/neu protein (Korutla *et al*., 1995; Hong *et al*., 1999). It also selectively inhibits histone acetyltransferases, inhibits metastasis and induces apoptosis by inhibiting the ubiquitin-proteasome pathway (Marcu *et al.* 2006; Banerji *et al*., 2004; Hong *et al*., 2006; Qui *et al*., 2000; Drexler *et al*., 2000; Jana *et al*., 2004). Curcumin, however, has some carcinogenic activity as it inhibits p53 activity and the transactivation of p53-mediated genes (Moos *et al*., 2004; National Toxicology Program, 2001; Sharma *et al*., 2001). It also induces carcinogenesis by promoting hyperplasia in rats and mice, increasing the incidence of hepatocellular adenoma in mice, and causing chromosomal aberrations in CHO cells (National Toxicology Program, 2001). Furthermore, curcumin interferes with chemotherapy treatment in breast cancer patients (Somasundaram *et al*., 2002). The carcinogenic effect of curcumin reflects other instances where chemoprevention agents have anti-carcinogenic action in one organ system, but are carcinogenic in another e.g. tamoxifen prevents breast cancer but aggravates uterine cancer (Fisher *et al*., 1998).

1.3.1.1 Curcumin and breast cancer

Curcumin inhibits Ki-67, PCNA, p53 mRNA, COX-1 and COX-2, and upregulates p21 and p27 in breast cancer cells (Aggarwal *et al*., 2007; Aggarwal *et al*., 2007b; Goel *et al*., 2008). It induces apoptosis in HT-29 cells and breast cancer cells by activating p53 to inhibit anti-apoptotic BCL2 and upregulate pro-apoptotic BAX (Song *et al*., 2005; Choudhuri *et al*., 2002). It disrupts spindle structures and inhibits telomerase reverse transcriptase in MCF-7 cells to promote apoptosis and inhibit cell division (Holy, 2002; Ramachandran *et al*., 2002). Curcumin is unable to induce G_0 cell cycle arrest in cells with a high cyclin D1 expression and instead induces cell cycle arrest and apoptosis at G_2/M (Choudhuri *et al*., 2005). This is significant as cells arrested at G_2/M are more responsive to the effects of radiation (Aggarwal *et al*., 2003).

Unregulated checkpoints and DNA damage induce aberrant mitosis, which causes mitotic catastrophe through compromised spindle structure, malfunctioning at anaphase and production of multinucleated cells, consequently inducing cell death (Castedo *et al*., 2004). Curcumin disrupts the mitotic spindle structure in MCF-7 cells to induce mitotic catastrophe (Holy, 2002). It is more effective at inhibiting the growth of ER- cells than ER+ cells.

1.3.1.2 Curcumin and colon cancer

Curcumin induces cell shrinkage, chromatin condensation and DNA fragmentation in HT-29 cells. It induces apoptosis in colon cancer cells by inducing BID cleavage, promoting caspase-3 and caspase-8, and inhibiting BCL2, BCL-xL, STAT3 and STAT5

(Aggarwal *et al*., 2007; Aggarwal *et al*., 2003; Bharti *et al*., 2003). Curcumin is, however, unable to induce apoptosis in chemoresistant colon adenocarcinomas cells that lack BAX or BAK (Zhang *et al*., 2000). It upregulates the GADD genes and downregulates c-*myc* and EGR-1 (survivor genes); IAP; and mutant p53 (Radhakrishna Pillai *et al*., 2004). Curcumin activates p53 and downregulates MDM2 to promote p53 activity (Li *et al*., 2007; Song *et al*., 2005). It downregulates COX-2, MMP-9, adhesion molecules, cyclin D1, cyclin E and NF-κB to inhibit proliferation and cancer progression (Cho *et al*., 2007; Mukhopadhyay *et al*., 2002; Shishodia *et al*., 2005).

1.3.2 Lycopene

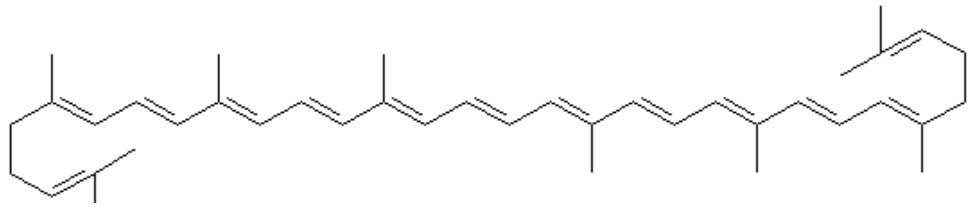


Figure 1.4: Chemical structure of lycopene

A tomato-rich diet lowers the risk of prostate cancer and cardiovascular disease (Mills *et al*., 1989; Giovannucci, 2002; Bhuvaneswari and Nagini, 2005). This diet has, however, not shown any effect in colorectal cancer, oral premalignant lesions and bladder cancer (Mannisto *et al*., 2007; Maserejian *et al*., 2007; Michaud *et al*., 1999). The carotenoid lycopene is accredited for the anti-carcinogenic action of tomato and tomato-based products with regards to prostate cancer (Fleshner and Klotz, 1998). It is an isomer of β-carotene, and is twice as potent as β-carotene (Di Mascio *et al*., 1989). Lycopene accumulates in androgen-sensitive tissues such as the prostate (Erdman, 2005). It

protects DNA to prevent carcinogenesis, decreasing the occurrence of DNA lesions and chromosomal aberrations (Di Mascio *et al*., 1989; Scolastici *et al*., 2007).

Oxidation-induced DNA damage is a significant contributor of cancer (Slupphaug *et al*., 2003). Lycopene is ten times more potent than α-tocopherol as an antioxidant (Di Mascio *et al*., 1989). In clinical trials using tomato products, lycopene protected plasma lipoproteins, lymphocyte DNA and serum proteins from oxidative damage. It decreased the levels of prostate-specific antigen and inhibited prostate tumour aggressiveness (Basu and Imrhan, 2007). It suppresses carcinogenesis by scavenging ROS, promoting detoxification systems, apoptosis and differentiation, and inhibiting proliferation, cell cycle progression and DNA damage (Astorg *et al*., 1997; Muller *et al*., 2002; Kotake-Nara *et al*., 2002; Pastori *et al*., 1998; Bhuvaneswari and Nagini, 2005; Astley *et al*., 2004). Lycopene inhibits the onset of chronic diseases, including osteoporosis, coronary heart disease and type II diabetes (Das *et al*., 2005; Rao *et al*., 2006).

Lycopene decreases the risk of prostate cancer and hepatoma through its antioxidant role (Clinton, 1998; Kotake-Nara *et al*., 2001; Okajima *et al*., 1998). It reduces the growth rates of prostate cancer cell lines and induces G_0/G_1 and S-phase arrest in hepatoma cell lines (Tang *et al*., 2005; Park *et al*., 2005). It also inhibits the phosphorylation of p53 and RB to induce G_0/G_1 arrest in mouse hepatocytes (Matsushima-Nishiwaki *et al*., 1995). MMP-9 is overexpressed in cancers and is involved in metastasis and angiogenesis (Chandler *et al*., 1997; Liabakk *et al*., 1996). Lycopene inhibits MMP-2 and MMP-9 secretion, restricting migration and invasion of cancer cells (Hwang and Lee, 2006).

Lycopene upregulates CDK6 and decreases the activity of CDK4, CDK2, cyclin D1, cyclin D3 and p21 (Harbour *et al*., 1999; Nahum *et al*., 2001). It does not affect the proliferation of normal oral epithelial cells but has an anti-proliferative effect on oral cancer cells (Livny *et al*., 2003). It does, however, inhibit the proliferation and growth of the normal prostate epithelial cell line, PrEC, in a dose-dependent manner (Obermuller-Jevic *et al*., 2003).

1.3.2.1 Lycopene and breast cancer

Lycopene inhibits proliferation in a dose-dependent manner in breast, prostate, oral tumour and hepatoma cells (Chalabi *et al*., 2004; Fornelli *et al*., 2007; Hwang and Bowen, 2004; Kim *et al*., 2002; Levy *et al*., 1995; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005b; Pastori *et al*., 1998). There are very few studies where no effect of lycopene on proliferation is observed (Hantz *et al*., 2005). MCF-7 and MDA-MB-231 cells treated with 10μM lycopene for 48 hours present differential expression in 391 genes. These genes are involved in apoptosis, cell communication, MAPK, cell cycle, fatty acid biosynthesis and gap junctional intercellular communication. Lycopene promotes gap junction communication to inhibit oncogenic transformation (Bertram, 1993). Genes that are upregulated include CDK6, CCND1, CCNB3 and CCNE1. However, it downregulates cyclin D1 to induce G_0/G_1 arrest in MCF-7 and ECC1 endometrial cancer cells. Genes that are differentially expressed between MCF-7 and MDA-MB-231 include ATM, BUB3, CCNB2, CCNB3, CCND1, CCNE1, CDK6, HDAC8, MAD1L1 and MCM4 (Nahum *et al*., 2006). Genes that are differentially

expressed between cancerous and non-cancerous (MCF10A) cells include CDKN1B, GSK3B and PTTG1 (Chalabi *et al*., 2007).

Lycopene increases BRCA1 and BRCA2 mRNA expression in ER+ MCF-7 and decreases expression in ER- MDA-MB-231 (Chalabi *et al*., 2004). This suggests that lycopene interacts with oestrogen receptors. In ER+ cells, lycopene associates with RARα to increase BRCA1 and BRCA2 expression, while in ER- cells it interacts with RARβ to decrease BRCA1 and BRCA2 mRNA expression because of the low expression of RARs (Chalabi *et al*., 2004). It decreases RB phosphorylation and inhibits G_1/S progression in MCF-7 by downregulating CCND, which inhibits CDK4 activity (Nahum *et al*., 2001). Lycopene suppresses the activity of thymidylate synthetase in mammary tumours; this enzyme is required for DNA synthesis. It also inhibits prolactin, the hormone that stimulates cell division in breast cancer development (Levy *et al*., 1995).

1.3.3 Suberoylanilide hydroxamic acid (SAHA)

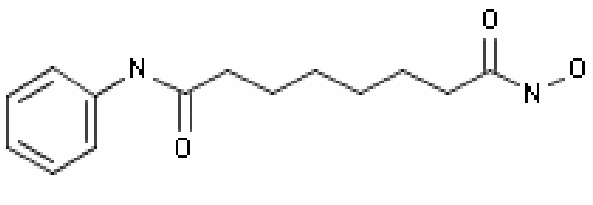


Figure 1.5: Chemical structure of SAHA

SAHA, also known as Vorinostat, is a histone deacetylases inhibitor (HDACi) that instigates growth arrest, differentiation and apoptosis in a range of cancer cells, such as bladder transitional cell carcinoma and breast adenocarcinoma (O'Connor, 2006). It

induces growth arrest by promoting the accumulation of acetylated proteins, such as core nucleosomal histones e.g. p53. SAHA has been tested in phase I and II clinical trials and has been shown to restrain HDACs in peripheral mononuclear cells and in tumours at tolerable doses. Administered orally, it has very good bioavailability, inducing growth arrest/differentiation in a range of human cancer cells including breast, colon, prostate, ovarian and bladder transitional cell cancer (Garcia-Manero and Issa, 2005; Chobanian *et al*., 2004; Butler *et al*., 2000; Huang and Pardee, 2000). SAHA enhances the radiosensitivity of tumour cell lines (Munshi *et al*., 2006). The Federal Drug Administration (FDA) has permitted the marketing of SAHA (as Zolinza) (Merck & Co.) in the USA for the therapy of persistent skin lesions resulting from cutaneous T-cell lymphoma.

HDAC enzymes induce the deletion of acetyl from lysine residues in proteins and an overexpression of these enzymes is seen in cancers (Richon *et al*., 2006). HDAC overexpression causes hypo-acetylation of core nucleosomal histones, correlated with condensed chromatins and transcriptional inhibition. When HDAC activity is inhibited, acetyl groups on the histone lysine residues can accumulate, promoting an open chromatin structure and the activation of transcription (Munshi *et al*., 2006). HDACi induce the hyperacetylation of nucleosomal histones to express genes that are aberrantly repressed in cancers, inciting growth arrest, differentiation and apoptosis in cancer cells (Glaser, 2007; Wagner and Roemer, 2005). HDACi inhibit angiogenesis by inhibiting VEGF and HIF1α (Ocker and Schneider-Stock, 2007). HDACi induce aberrant histone

acetylation in heterochromatin and centromere domains, causing mitotic defect and resulting in apoptosis, mitotic death or mitotic catastrophe (Cimini *et al*., 2003).

SAHA targets most of the human Class I and Class 2 HDACs where it blocks the catalytic site of HDAC enzymes and induces the accumulation of acetylated histones (Marks *et al*., 2001; Richon *et al*., 1996). However, it does not target the Class 3 HDACs. HDAC1 is overexpressed in gastric, colon and prostate cancer, and promotes proliferation in breast cancer (Ocker and Schneider-Stock, 2007; Kawai *et al*., 2003). HDAC2 is overexpressed in cervical, colorectal and gastric cancer (Zhu *et al*., 2004; Ocker and Schneider-Stock, 2007). HDACs 1-3 are overexpressed in breast cancer cell lines (Feng *et al*., 2007).

By inhibiting histone deacetylases, SAHA induces growth arrest, differentiation and apoptosis in a range of cancers (Kelly *et al*., 2003; Butler *et al*., 2002). Cell cycle arrest is induced at G_1 and G_2/M with the onset of apoptosis by upregulating pro-apoptotic BAK and BIM and inhibiting HER-2, BIRC5, BCL2 and BCL-xL (Bali *et al*., 2005; Huang and Pardee, 2000). SAHA induces the expression of specific genes only (Peart *et al*., 2005). Furthermore, it induces polyploidy to promote senescence in transformed cells (Xu *et al*., 2005). SAHA induces G_2/M arrest, downregulates RAD51, cyclin B1 and cyclin D1, and promotes p21 in various cancer cells (Chinnaiyan *et al*., 2005; Heider *et al*., 2006). While SAHA does not affect cyclin D1 mRNA levels, it is involved in inhibiting the translation of the protein (Kawamata *et al*., 2007). The increase in p21

correlates with increased histone acetylation around the p21 promoter (Kumagai *et al*., 2007).

1.3.3.1 SAHA and breast cancer

SAHA induces apoptosis via caspase-3 activation in breast cancer cell lines, including MCF7 and MDA-MB-231 (Shao *et al*., 2004). It induces the accumulation of MCF-7 cells at G_1 and at higher concentrations at G_2/M. SAHA induces the expression of milk fat globule protein, milk fat membrane globule protein and neutral lipid droplets in breast cancer cells. Morphological changes include flattening and enlargement of the cytoplasm, a decrease in the ratio of nucleus: cytoplasm, and a decrease in the nucleoli and mitotic figures, indicative of differentiation (Munster *et al*., 2001). SAHA, and other HDAC inhibitors, suppress Erα protein, with Erα mRNA inhibited and Erα degraded in MCF-7 cells (Marguerron *et al*., 2004; Yi *et al*., 2008). This degradation downregulates Erα transcriptional activity to inhibit cell growth. SAHA also reduces CDK4 and BIRC5 (Yi *et al*., 2008).

While tamoxifen has been the endocrine treatment of choice for breast cancer, tamoxifen resistance can develop with prolonged use (Ali and Coombes, 2002). SAHA has the potential to treat Erα-dependent breast cancer. It also inhibits mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/AKT signalling activity to inhibit MCF-7 growth (Yi *et al*., 2008). Activated EGFR and HER-2 receptors promote MAPK and PI3K/AKT pathways. These protein kinases can phosphorylate and activate Erα, promoting tamoxifen resistance (Levin, 2003). While the RB protein is usually

necessary for differentiation, SAHA can induce differentiation in MCF-7 and other breast cancer cell lines in both the presence and absence of RB (Munster *et al*., 2001).

1.3.4 Thalidomide

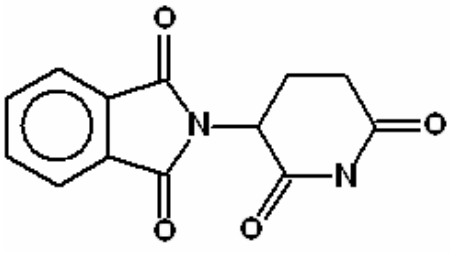


Figure 1.6: Chemical structure of thalidomide

The sedative/hypnotic drug thalidomide was removed from the market because of its extreme teratogenicity (Hashimoto, 2002). It has now been effectively used to treat lepromatous leprosy (approved for use in the USA), and several other diseases including myeloma, AIDS and metastatic thyroid carcinoma (Teo *et al*., 2005; Hashimoto, 2002; Calabrese and Fleisher, 2000; Ain *et al*., 2007). Currently in phase III of American clinical trials for multiple myeloma, it is further applied in the clinical study of various cancers including colon and breast where the multi-target angiogenic and pro-apoptotic drug inhibits TNFα (Noguchi *et al*., 2002; Makonka-Wekeyoon *et al*., 1993; Kaplan, 1994). Preclinical models show that thalidomide acts in synergy with cytotoxic agents (Ching *et al*., 1998).

Thalidomide inhibits COX-1 and COX-2 and is an anti-inflammatory (Noguchi *et al*., 2002). It inhibits tumour growth in a concentration-dependent manner in MCF-7 and

HL-60 cells, which both express COX-2. In cell lines that do not express COX-2, thalidomide does not have an effect on tumour growth. Thalidomide exerts little or no inhibition of DNA synthesis in multiple myeloma cells (Hideshima *et al*., 2000). It reduces angiogenesis by inhibiting VEGF, TNFα, IL-6 and COX-2 (Sampaio *et al*., 1991; Kedar *et al*., 2004; Fujita *et al*., 2001). It inhibits NF-κB activity to decrease the regulation of the transcription of cytokines (e.g. TNF-α), apoptotic proteins (e.g. the BCL2 family) and angiogenic factors (e.g. VEGF, TNF-α) (Keifer *et al*., 2001; Kucharczak *et al*., 2003).

Possible mechanisms for thalidomide action against cancer cells include anti-angiogenic activity, induction of apoptosis, G_1 arrest, inhibition of growth factor production and reducing tumour immunity (Hattori and Iguchi, 2004). It promotes the intrinsic apoptosis pathway by inhibiting anti-apoptotic BCL2 members (Marriott *et al*., 2003). Thalidomide promotes the extrinsic apoptosis pathway by downregulating inhibitors of FAS and TRAIL (Mitsiades *et al*., 2002). It can promote p53-induced inhibition of p21 to induce apoptosis, or induce p21 for G_1 arrest in multiple myeloma cells (Hideshima *et al*., 2000).

1.4 The cell cycle

From the experiments conducted in this study, numerous cell cycle-related genes are differentially expressed in controls and drug-treated cells. These genes function primarily in proliferation, the DNA damage responses, promotion and inhibition of the oncogenic state, in the ubiquitin-proteasome pathway, differentiation and senescence.

These sub-divisions of the cell cycle are consequently the areas of focus in this section. Following this, is a comprehensive characterisation of these significant genes as identified through these experiments.

Via a series of controlled events, the cell cycle functions to replicate all the components of a growing cell to produce two daughter cells with the exact cellular make-up of the parent cell (Ghosh and Bose, 2006). It is vital in regulating the transition from the quiescent state (G_0) to proliferation, and its checkpoints are essential in maintaining genetic integrity. The cell cycle consists of the stages of interphase, which is divided into G_0, G_1, S and G_2 phases, and the M phase (mitosis phase), which is divided into karyokinesis and cytokinesis. During G_0, the cells are still active, with cellular functions and growth occurring under strict regulation. The restriction point controls cell cycle entry. Beyond this point, cell cycle progression is immune to external stimuli such as nutrients and mitogens (Pardee, 1974). While normal cells only enter the cell cycle in response to mitogenic stimulation, cancer cells enter and remain in the cell cycle indefinitely, inducing unrestrained tumour growth.

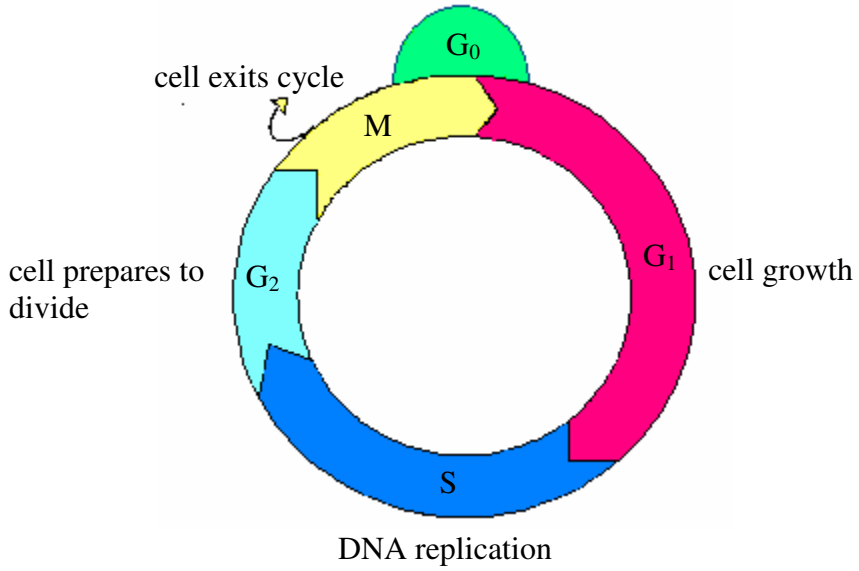


Figure 1.7: Diagrammatic representation of the cell cycle

During G_1 and G_2, the cellular entities, excluding DNA, are duplicated and cell growth occurs. G_1 is a long phase where nutrients and growth factors act upon the cells, establishing the cell's progression through the cell cycle or its exit to G_0. The cell cycle elements are commonly targeted by dysregulation control G_1 progression and transition from G_1 to S-phase. These molecules regulate the restriction point at mid-to-late G_1 when cell cycle progression is independent of growth factors *in vitro*. These molecules are specifically targeted instead of elements at S, G_2 and M because of the significant role that G_1/S events play in obligating the cell to division (Malumbres and Carnero, 2003). If the preceding stages are not satisfactorily completed or DNA damage occurs, cell cycle arrest ensues at the checkpoints to repair the damaged DNA (Ghosh and Bose, 2006). Cancer cells acquire the ability to bypass DNA and spindle checkpoints, increasing genetic instability.

Cell cycle progression is induced by the cyclin dependent kinases (CDKs), which form complexes with the cyclins and are inhibited by the cyclin dependent kinase inhibitors (CKIs) (Nigg, 2001). The expression levels of cyclins fluctuate in a controlled manner during the cell cycle (Johnson and Walker, 1999). In cancer, checkpoint integrity is lost as a consequence of CKI inactivation or cyclin overexpression. Chemotherapeutic agents target the cell cycle, particularly the CDKs, because of the high occurrence of cell cycle irregularities in carcinogenesis. The inhibition of CDKs induces cell cycle arrest and apoptosis (Shah and Schwartz, 2006).

The INK4 family ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, $p19^{Ink4d}$) inhibits G_1/S progression, apoptosis and proliferation (Carnero and Hannon, 1998; Ivanchuk *et al*., 2001; Sherr, 2001). The Cip/Kip family ($p21^{Cip1/Waf1}$, $p27^{Kip1}$, $p57^{Kip2}$) inhibits G_1/S progression, consists of pro- and anti-apoptotic members and suppresses proliferation (Hengst and Reed, 1998; Gartel, 2005). The majority of CKI agents inhibit CDK2 and CDC2 and to a lesser extent CDK4 and CDK6. When cell cycle regulatory proteins are aberrantly expressed, cell cycle progression can occur despite conditions that would normally incite cell cycle arrest and apoptosis (Evan *et al.*, 1996).

1.4.1 Mitosis

At G_2/M, the cell has committed to dividing and this transition is strictly controlled for correct division of replicated chromosomes to each end of the spindle (Pines and Rieder, 2001). The spindle allows for alignment of the replicated sets of chromosomes at metaphase, facilitating the equal distribution to two cells (Gadde and Heald, 2004). The

initial stage of mitosis is prophase where the nuclear envelope is disassembled. This is followed by prometaphase, during which the kinetochores attach to the spindle. In metaphase, the chromosomes arrange themselves along the metaphase plate. At the spindle assembly checkpoint, chromosomes that are not correctly aligned at the metaphase plate incite a cell cycle delay. This checkpoint is regulated by the inhibition of anaphase (Gutierrez and Ronai, 2006). In anaphase the sister chromatids separate and migrate to the opposite poles of the spindle. Telophase occurs with decondensation of the chromatids and reformation of the nuclear envelope and spindle disassembly. Cytokinesis commences from anaphase as the cell starts to divide (Pines, 2006).

1.4.2 DNA damage checkpoints

The series of cell cycle checkpoints are aimed at maintaining genomic integrity by regulating DNA replication. This prevents damaged or incorrectly replicated genomes from entering mitosis (Elledge, 1996). These damage response pathways include DNA repair, cell cycle arrest and apoptosis. These pathways are downregulated in carcinogenesis as the cells aim for unrestricted proliferation and growth, without differentiation, creating a system of chaos and promoting transformation (Cahill *et al*., 1998). The pathways constituting the cell cycle checkpoints have five components. The first component of checkpoint sensors include the RAD9-HUS1-RAD1 (9-1-1), the RAD17-replication factor C (RFC) clamp loading complex and the MRE11A-RAD50-NBS1 (MRN) complex (Melo and Toczyski, 2002; Petrini and Stracker, 2003). The second component includes the checkpoint mediators such as BRCA1 (Petrini and Stracker, 2003; Bartek and Lukas, 2003). The third component is made up of the apical

signal transducing kinases from the PI3K-like family that includes ATM and ATR kinases. In the fourth component are the distal serine/threonine signal transducing kinases (the effector kinases) comprised of CHEK1/CHK1 and CHEK2/CHK2. The fifth component is made up of the checkpoint effector proteins which include cell cycle regulators such as CDC25 phosphatases, DNA repair proteins, chromatin components and transcription factors, including p53 (Bartek and Lukas, 2003; Donzelli and Draetta, 2003).

The majority of double strand breaks (DSBs), and the consequent chromosomal rearrangement, occur in the S-phase from replication using a damaged DNA template (Michel *et al.*, 2004). During DNA replication, DNA dissociates into single strand DNA (ssDNA) templates. If a lesion occurs in this template, the DNA polymerase complex halts, causing the replication fork to stall. When the stalled fork is processed, it forms a DSB. If replication recommences without the template lesion being repaired, a daughter strand gap (DSG) forms, which cannot be filled by DNA polymerase (Nagaraju and Scully, 2007). DSBs and DSGs are repaired by sister chromatid recombination where the intact sister chromatid acts as a repair template to the damaged chromatid by homologous recombination (Michel *et al.*, 2004). Tumour suppressor genes involved in homologous recombination include BRCA1, BRCA2, NBS1, MRE11, ATM, ATR and CHK2 (Venkitaraman, 2002; Stewart *et al.*, 1999; Shiloh, 2003; O'Driscoll *et al.*, 2003).

Checkpoints for DNA damage occur at the G_1/S and G_2/M boundaries, at the S-phase and during mitosis (Lukas *et al.*, 2004). As cells pass through G_1, ATM/ATR and

CHK1/CHK2 are activated. Upon activation by ATM and ATR, CHK1 and CHK2 phosphorylate proteins such as p53, MDM2, BRCA1 and CDC25A, inducing S-phase arrest and DNA repair mechanisms (Sorensen *et al.*, 2003; Shiloh, 2003; Maya *et al.*, 2001). With very high levels of DNA damage, apoptosis is induced (Gasser and Raulet, 2006; Xu *et al*., 2001; Zhou *et al*., 2002). DNA damage response markers do not occur in epithelial cells that are proliferating normally or in inflammatory lesions. The DNA damage response system is activated in pre-cancerous and early cancerous lesions of the breast and colon (Bartkova *et al.*, 2005; Gorgoulis *et al.*, 2005).

1.4.3 Senescence

Telomeres are DNA and protein caps that stabilise chromosomal ends to impede their degradation or fusion. Telomere base pairs are lost with each cell cycle, causing progressive shortening of telomeres. Telomerase adds replacement base pairs to the telomere caps but most cells do not express telomerase (Greider, 1988). When telomeres have reached the dysfunctional state, proliferation is halted and replicative/cellular senescence ensues, with cells at G_1 (Kim *et al*., 2002b; Beausejour *et al*., 2003). Senescence is also induced by DNA damage, aberrant chromatin arrangement and specific oncogenes (Campisi *et al.*, 2001). p53 and RB proteins are essential for senescence and maintain growth arrest to inhibit cancer development (Beausejour *et al.*, 2003; Campisi *et al*., 2001).

1.4.4 Cyclin dependent kinases (CDKs)

The CDKs are protein kinases with enzymatic activity regulated by protein-protein interactions and phosphorylation. Nine CDKs (CDC2-CDK9) and twenty cyclins (cyclin A – cyclin T) have been identified. The CDKs are activated by cyclin association and phosphorylation by cyclin activating kinase (CAK), a complex comprised of CDK7, cyclin H and MAT1. While cyclin levels vary with cell cycle progression, CDK protein levels are stable (Vermeulen *et al.*, 2003).

Cell division cycle 2 (CDC2)

CDC2 promotes proliferation through G_1/S and G_2/M, and is pro-apoptotic (Wolowiec *et al.*, 1995). Cyclin B/CDC2 comprises the M-phase promoting factor (MPF), which is essential for mitotic entry. The checkpoint kinase, CHK, phosphorylates CDC25 to prevent it from activating CDC2 (Kumagai and Dunphy, 1999). Mitosis is also inhibited by GADD45, which is induced by p53 to prevent cyclin B1/CDC2 formation (Zhan *et al*., 1999). The inactivation of MPF by APC2 is essential for mitotic exit (Peters *et al*., 1998; Itzhaki *et al*., 1997). CDC2 phosphorylates and destabilises RNA polymerase II (pol II) to inhibit transcription (Cisek and Corden, 1989; Gebara *et al*., 1997; Zawel *et al*., 1993). p53 and p21 inhibit CDC2 transcription (Taylor *et al*., 1999).

CDK2

Pro-apoptotic CDK2 promotes G_1/S and proliferation (Morgan, 1997; Golsteyn, 2005; Berthet *et al*., 2003). It is inhibited by p27. C-*myc* activates cyclin D1 and cyclin D2

gene expression to alleviate this inhibition and promote cell cycle progression (Perez-Roger *et al*., 1999).

CDK4

CDK4 is a ubiquitin ligase that mediates cyclin E degradation (Strohmaier *et al.*, 2001). It is amplified and overexpressed in various cancers: including breast cancer, gliomas and sarcomas. In addition, this is associated with MDM2 amplification and p53 degradation (Deshpande *et al*., 2005; Ortega *et al*., 2002; Sherr, 1996; Hall and Peters, 1996). The gene encoding CDK4 is mutated in breast cancer cell lines (Strohmaier *et al*., 2001).

CDK5

CDK5 is important in neuronal differentiation, migration, axon outgrowth and the formation of synapses (Lee and Kim, 2004; Smith *et al*., 2001). Its activity is regulated by the catalytic subunits p39, p35 and p25 (the truncated product of p35) (Lew *et al*., 1994). While the highest levels of mRNA and protein occur in neurons, inactivated CDK5 is expressed in numerous tissues and cell lines, including testes, developing muscle and differentiated monocytes (Lin *et al*., 2006; Sahlgren *et al*., 2003; Chen *et al*., 2000). CDK5-mediated phosphorylation of RB and p53 regulates apoptotic events (Lee *et al*., 1997; Zhang *et al*., 2002). CDK5 promotes p53 nuclear accumulation, increases p53 trascriptional activity and promotes apoptosis (Lee and Kim, 2007; Zhang *et al*., 2002b). Activated CDK5/p35 expression occurs in colon cancer cells, with an upregulation compared to normal colonic mucosa where levels are very low.

CDK6

CDK4 and CDK6 form active complexes with the D-type cyclins to phosphorylate RB (Kwon *et al.*, 1995; Takaki *et al*., 2005; Ericson *et al*., 2003). CDK6 regulates RB in differentiation and CDK4 may compensate for CDK6 loss (Ogasawara *et al*., 2004; Malumbres *et al*., 2004). CDK4 and CDK6 upregulation has a tumourigenic effect (Liu, 2006). CDK6 is amplified in cancers, including squamous cell carcinoma, gliomas, neuroblastomas and lymphoid tumours (Ortega *et al*., 2002).

CDK7

CDK7 forms part of the CAK complex that phosphorylates and activates CDKs, and is part of the transcription factor TFIIH (Harper and Elledge, 1998). CDK7 can be activated *in vitro* by its targets, CDC2 and CDK2 (Garrett *et al*., 2001).

CDK8

Cyclin C/CDK8, cyclin H/CDK7 and cyclin T/CDK9 regulate pol II transcription through phosphorylation (Kornberg, 2005; Price, 2000). Cyclin C/CDK8 is transcriptionally activated by p21 and CDK8 enhances p53-regulated transcription (Donner *et al*., 2007). Cyclin C/CDK8 also phosphorylates cyclin H to inhibit CDK7 activity and repress transcription (Akoulitchev *et al*., 2000).

1.4.5 Cyclins

There are two classifications of cyclins: those functioning at G_1/S (the D-type cyclins and cyclin E) and the mitotic cyclins (A-type and B-type cyclins), which induce transition

from G_2 to M (Roberts, 1999). The mitotic cyclins are synthesised and degraded at mitosis. Cyclin A is activated at the S-phase and activates CDC2 and CDK2. The B-type cyclins and CDC2 are associated with G_2 exit and mitosis (Coqueret, 2003). Cyclin C binds to CDK8. Cyclins D1, D2, D3 and C are activated from G_0 to the S-phase and bind to CDK4 and CDK6. Cyclin E activates CDK4 during G_1/S transition. The G- and T-type cyclins associate with CDK5 and CDK9 (Johnson and Walker, 1999). Cyclin availability is greatly influenced by the ubiquitin-proteasome system and levels increase after CDC25-mediated dephosphorylation.

Cyclin B (CCNB)

Cyclin B1 and cyclin B2 regulate G_2/M, and promote apoptosis, proliferation and survival (Pines and Hunter, 1989; Borgne *et al*., 2006; Coqueret, 2003). At the end of G_2, CDC25 dephosphorylates CDC2 to activate cyclin B/CDC2 (the MPF complex) to initiate mitosis (Morgan, 1995; Glotzer *et al*., 1991). The spindle checkpoint prevents APC^{CDC20} from ubiquitinating cyclin B1/CDC2 until the paired sister chromatids are aligned on the mitotic spindle (Pines, 2006).

Cyclin B1 protein levels increase at S and G_2 to increase cyclin B1/CDC2 complexes (Clute and Pines, 1999). Cyclin B1 and B2 are inhibited by p53 (Krause *et al*., 2000). They are overexpressed in colorectal and lung cancer, amongst others, where they alter the spindle checkpoint and promote chromosomal instability (Wang *et al*., 1997; Soria *et al*., 2000). Cytoplasmic cyclin B1 expression in breast cancer cells correlates with histological grade, mitosis, Ki-67, p53 and c-*myc*. The nuclear expression of cyclin B1 in

breast cancer correlates with tumour size, lymph node metastasis, histological grade, mitosis, Ki-67, PLK1 and poor prognosis. The translocation of overexpressed cyclin B1 from the cytoplasm to the nucleus may therefore confer its oncogenic role (Suzuki *et al*., 2007). Elevated cyclin B1 levels occur frequently and as an early event in colorectal carcinoma, with expression increasing with tumour progression (Wang *et al*., 1997). High levels of cyclin B1 are associated with high levels of p53 in adenomas and high levels of Ki-67 in adenomas and primary carcinomas (Li *et al.*, 2003). Cyclin B2 expression correlates with colorectal adenocarcinomas (Park *et al.*, 2007).

Cyclin C (CCNC)

CCNC encodes cyclin C. It is expressed at its highest at G_0 exit (Liu *et al*., 1998). At G_0, the cell's growth is physiologically arrested and the majority of transcription is turned off. With the availability of growth factors and nutrients, the cell re-enters the cell cycle. Cells also enter G_0 after mitotic exit to differentiate or to enter senescence. E2F-based complexes with HDAC activity repress the transcription of many genes at G_0 (Takahashi *et al*., 2000). Cyclin C/CDK3 phosphorylates the RB family at G_0 for cell cycle entry (Ren and Rollins, 2004).

Cyclin C/CDK8 phosphorylates cyclin H, which inhibits TFIIH and its transcriptional influence through CDK7 activity inhibition (Akoulitchev *et al*., 2000). Cyclin C also induces CDC2 to promote mitotic entry and further inhibit transcription by phosphorylating RNA pol II (Liu *et al*., 1998; Cisek and Cordon, 1989; Gebara *et al*.,

1997). Cyclin C is also important for entry through the restriction point at G_1/S (Liu *et al.*, 1998).

Cyclin D1 (CCND1)

CCND1 encodes cyclin D1. The D-type cyclins link the extracellular environment to the cell cycle machinery by picking up and transmitting external stimuli. Cyclin D1 promotes G_1 entry and cell survival (Schwartz and Shah, 2005; Hinds *et al.*, 1994). It is degraded by SKP2-mediated proteasomal degradation from G_1/S. This is essential for S-phase entry and the initiation of DNA replication (Yu *et al.*, 1998). Its degradation is enhanced by phosphorylation by CDK4 (Fukami-Kobayashi and Mitsui, 1999). Cyclin D1 degradation is also induced by DNA damage and extracellular stress, inciting G_1 arrest (Agami and Bernards, 2000).

CAK phosphorylates and activates both cyclin D1/CDK4 and cyclin D1/CDK6, localising them to the nucleus (Matsuoka *et al.*, 1994). These complexes inactivate the inhibitors of S-phase gene expression, including RB and the CKIs, to promote cell cycle progression (Lundberg and Weinberg, 1998). Cyclin D1/CDK4 translocates the tumour suppressors p27 and p21 out of the nucleus to activate cyclin E/CDK2, inactivate RB and promote G_1 progression (Perez-Roger *et al.*, 1999). Furthermore, cyclin D1 represses DNA synthesis by binding to PCNA and CDK2 (Fukami-Kobayashi and Mitsui, 1999).

Cyclin D1 is important in the regulation of breast epithelial growth and differentiation (Sicinski *et al.*, 1995; Fantl *et al.*, 1995; Ormandy *et al.*, 2003; Musgrove *et al.*, 1994;

Sicinski and Weinberg, 1997). It is overexpressed in 50% of breast cancers at the initial stages of breast carcinogenesis and this is sustained throughout breast cancer progression (Ormandy *et al.*, 2003; Bartkova *et al.*, 1994). It is also overexpressed early in tumourigenesis in multiple myelomas, uterine, cervical, stomach, oesophageal, lung and gastric cancers (Sakamaki *et al*., 2006; Hoechtler-Vollmar *et al*., 2000; Cheung *et al.*, 2001; Dobashi, 2005).

Cyclin D2 (CCND2)

Cyclin D2 function is often redundant to cyclin D1 and is expressed by all proliferating cells. Its overexpression in cancer cells occurs less frequently than cyclin D1 overexpression (Malumbres and Barbacid, 2001). While cyclin D1 is frequently overexpressed in breast cancer and its overexpression may contribute to breast cancer development and progression, cyclin D2 expression is very low or absent in the majority of breast cancer cell lines due to promoter methylation (Buckley *et al*., 1993; Bartkova *et al*., 1994; Hui *et al*., 1996; Tam *et al*., 1994; Lukas *et al*., 1995; Evron *et al*., 2001). Cyclin D2 mRNA and protein are present at high levels in some normal breast epithelial cells (Lukas *et al*., 1995). Its accumulation relieves the inhibition induced by p27 to induce cell cycle entry (Bouchard *et al.*, 1999). Cyclin D2 expression is altered in ovarian granulosa cell tumours, testicular germ cell tumour cell lines, gastric, prostate and breast cancer (Sicinski *et al*., 1996; Takano *et al*., 2000; Padar *et al*., 2003; Evron *et al*., 2001). Its overexpression in gastric cancer correlates with progression and poor prognosis (Takano *et al*., 2000).

Cyclin E (CCNE)

The E-type cyclins are not essential for cell cycle progression but are vital for re-entry into the cell cycle (Geng *et al.*, 2003; Parisi *et al*., 2003). Cyclin E functions at late G_1 and early S-phase where it promotes G_1 progression, apoptosis, proliferation, survival and oncogenic transformation (Koff *et al.*, 1991; Mazumder *et al.*, 2004; Dobashi, 2005; Akama *et al*., 1995). Cyclin D/CDK4 and cyclin D/CDK6 phosphorylate RB to release E2F. The E2F transcription factors stimulate cyclin E synthesis and activation just before S-phase onset (Geng *et al.*, 1996). Cyclin E/CDK2 also phosphorylates RB, p130 and p107 to increase E2F levels and further promote cyclin E transcription (Le Cam *et al*., 1999).

Cyclin E/CDK2 is activated by CAK (Dulic *et al.*, 1992). The complex phosphorylates and inactivates p27, inducing p27 SKP1-cullin1-F-box (SCF) ^{SKP2}-mediated ubiquitination for proteasomal degradation (Elledge and Harper, 1998; Bloom and Pagano, 2003). This relieves p27 inhibitory effects and cyclin E/CDK2 therefore activates itself (Sheaff *et al.*, 1997). Cyclin E/CDK2 induces replication origin firing to stimulate S-phase entry (Cook *et al*., 2002; Coverley *et al.*, 2002). Cyclin E levels must decrease late in S-phase and at G_2. The phosphorylation of CDK2-bound cyclin E induces ubiquitination and degradation of cyclin E at early S-phase by SCF^{Fbw7} E3 ligase (Singer *et al.*, 1999; Welcker *et al*., 2003). This resets the cell cycle to mitogen stimulation for G_1 entry (Singer *et al.*, 1999).

Cyclin E decreases the length of G_1, prompting faster progression to the S-phase, increasing cyclin E activity and decreasing genomic stability (Spruck *et al.*, 1999; Akli and Keyomarsi, 2004). It influences the checkpoints at G_1, S, G_2 and M (Keyomarsi *et al*., 1995). Tumours overexpressing cyclin E with inactivated RB have increased proliferation (Sakamaki *et al*., 2006). Its expression is important in the prognosis of sarcomas, non-small-cell lung cancer, leukaemias, lymphomas and cervical cancer (Wolowiec *et al*., 1995; Fukuse *et al*., 2000; Molendini *et al*., 1998; Keating *et al*., 2001). Amplified in breast cancer, cyclin E mRNA can be expressed throughout the cell cycle in these cells and correlates with a poor prognosis in patients (Keyomarsi and Pardee, 1993; Keyomarsi *et al.*, 1995; Keyomarsi *et al*., 2002). Cyclin E and D1 overexpression in breast carcinoma both cause inactivation of the RB pathway (Loden *et al*., 2002). Levels of cyclin E are the best independent predictive factor of survival in breast cancer stages I-III (Keyomarsi *et al*., 2002). Aberrant expression of cyclin E is also seen in ovarian and gastrointestinal cancers (Dobashi, 2005).

Cyclin F (CCNF)

CCNF encodes cyclin F. It has yet to be determined which CDK cyclin F binds to. It may bind to cyclin B1 at G_2/M to direct it into the nucleus (Kong *et al*., 2000). Cyclin F destruction is important for G_2/M progression. Its overexpression accumulates cells at G_2/M (Bai *et al.*, 1994). Cyclin F function at S/G_2 positively influences G_0/G_1 and G_1/S transitions in the next cell cycle. Cyclin F levels also increase slightly in response to DNA damage (Fung *et al*., 2002). It binds SKP1 protein, an essential element of SCF ubiquitin ligase and is therefore involved in proteolysis (Tezlaff *et al*., 2004).

Cyclin G1 (CCNG1)

The G cyclins inhibit the cell cycle and assist in maintaining quiescence in differentiated cells (Bennin *et al*., 2002). Encoded by CCNG1, cyclin G1 is pro-apoptotic and promotes cell growth (Okamoto and Prives, 1999; Smith *et al.*, 1997). It is expressed throughout the cell cycle and does not have an identified CDK partner (Horne *et al*., 1997). Cyclin G1 transcription is induced by p53, p63 and p73 (Okamoto and Prives, 1999; Irwin and Kaelin, 2001). It induces growth suppression in association with p53 stabilisation and activation, and in association with RB (Zhao *et al*., 2003). Cyclin G1 is upregulated following p53 activation in response to DNA damage and participates at G_2/M arrest (Reimer *et al*., 1999; Kimura and Nojima, 2002).

Cyclin G1 overexpression in certain cancer cell lines promotes growth (Smith *et al*., 1997). However, in other studies cyclin G1 levels are high in differentiated tissues and in G_2-arrested hepatocytes, respond to DNA damage, and are associated with growth arrest (Okamoto and Prives, 1999; Home *et al*., 1996; Shimizu *et al*., 1998). While some propose cyclin G1 as a tumour suppressor, others suggest an oncogenic role where it associates with PCNA and increases cancer cell growth rate (Baek *et al*., 2003; Smith *et al*., 1998). This discrepancy may be because the growth suppressive functions of cyclin G1 depend on the level of its expression. Low levels may not inhibit growth or may promote growth, while high levels inhibit growth (Zhao *et al*., 2003). Conflicting work shows cyclin G1 to both suppress and activate p53 (Jensen *et al*., 2003; Zhao *et al*., 2003). Cyclin G1 is overexpressed in osteosarcomas, breast and prostate cancer, amongst others (Reimer *et al*., 1999).

Cyclin G2 (CCNG2)

Cyclin G2 inhibits cell cycle progression by inhibiting CDK2 and inducing G_1/S arrest, and is upregulated in response to DNA damage (Bennin *et al*., 2002; Bates *et al*., 1996). It is a centrosomal and nucleoplasmic shuttling protein, regulating microtubule stability and influencing p53-dependent inhibition of the cell cycle (Arachchige Don *et al*., 2006). Its levels increase in apoptotic cells and decrease in proliferating cells. Cyclin G2 overexpression induces cell cycle arrest and abnormal DNA structures through unregulated mitosis (Bennin *et al*., 2002).

Cyclin H (CCNH)

CDK7 associates with cyclin H and MAT1 to form the CAK complex that is part of the TFIIH complex, which is essential in transcription and DNA repair (Svejstrup *et al*., 1996; Schaeffer *et al*., 1993). CK2 phosphorylates cyclin H to activate the CAK complex (Schneider *et al*., 2002). Enhanced by MAT1, cyclin H/CDK7 phosphorylates CDK2 and RNA pol II to induce elongation following transcription (Andersen *et al*., 1997; Akoulitchev *et al*., 1995).

Cyclin T1 (CCNT1)

CCNT1 encodes cyclin T1. Cyclin T1 is implicated in cell proliferation, activation of peripheral blood lymphocytes, differentiation and HIV-1 gene expression (Garriga *et al*., 1998; Herrmann *et al*., 1998). It is upregulated as part of the innate immune response (Liou *et al*., 2006). Both cyclin T1 and cyclin T2 associate with CDK9 to form the positive transcription elongation factor b (P-TEFb) (Peng *et al*., 1998; Fu *et al*., 1999).

This complex hyperphosphorylates RNA pol II to induce transcription elongation and phosphorylates negative factors to prevent inhibition of polymerase elongation (Palancade and Bensaude, 2003; Fujinaga *et al*., 2004). It is essential for the transcription of numerous genes (Chao and Price, 2001). Cyclin T1 regulates P-TEFb-mediated transcription (Kiernan *et al*., 2001). It is recruited by ERα to ERα target genes in breast cancer (Wittmann *et al*., 2005). Cyclin T1/CDK9 is overexpressed in malignant lymphomas (Bellan *et al*., 2004).

Cyclin T2 (CCNT2)

Cyclin T2 has a higher transcriptional activation potential than cyclin T1 (Napolitano *et al*., 2000). Cyclin T2/CDK9 is essential for differentiation (Simone *et al*., 2002; Bagella *et al*., 2000). Cyclin T2 and CDK9 forms P-TEFb, this activates RNA pol II elongation and is essential for the transcription of many genes (Price, 2000).

1.4.6 Other cell cycle-related genes

Abelson murine leukaemia viral oncogene homologue 1 (ABL1)

c-ABL is the cellular homologue of the v-ABL oncogene from the Abelson murine leukaemia virus and encodes a kinase occurring in proliferating cells. c-ABL functions in the activation of DNA damage-induced apoptosis in proliferating cells (Wang, 2000). RB binds to c-ABL at G_0/G_1 to inhibit its activity. The phosphorylation of RB at G_1/S relieves this inhibition and c-ABL moves into the nucleus at the S-phase (Welch and Wang, 1995). The activation of c-ABL in response to DNA damage therefore only

occurs at the S-phase when RB is inactivated (Wang, 2000). c-ABL shuttles between the nucleus and cytoplasm, transmitting the DNA damage signal (Taagepera *et al*., 1998).

c-ABL inhibits MDM2-mediated degradation of p53 to promote p53 accumulation and p21 expression, and also stabilises p73 following DNA damage (Sionov *et al*., 1999; Goga *et al*., 1995; Gong *et al*., 1999; Agami *et al*., 1999). The overexpression of c-ABL does not independently induce oncogenic transformation (Wang, 2000).

Anaphase-promoting complex subunit 2 (ANAPC2)

The anaphase-promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase that is important in mitosis and G_1, and ubiquitinates cell cycle regulators at the metaphase/anaphase transition and M/G_1 transition. APC^{CDC20} ubiquitinates the B-type cyclins in metaphase and anaphase to inactivate CDC2 and activate CDH1. The degradation of the B-type cyclins is essential for mitotic exit. CDC2 inactivation promotes separase activity and the separation of sister chromatids (Stemmann *et al*., 2001). The degradation of cyclin B is delayed by the spindle assembly checkpoint until the chromosomes are properly attached to the spindle (Amon, 1999). The checkpoint proteins MAD2 and BUBR1 inhibit APC^{CDC20} (Peters, 2002).

The catalytic subcomplex of APC consists of APC11 and the cullin domain in APC2, encoded by ANAPC2. APC11/APC2 bind to E2 ubiquitin ligase enzymes and catalyse the ubiquitination of APC substrates (Leverson *et al*., 2000; Tang *et al*., 2001). It occurs at chromosome 19p13.3, a region frequently lost in sporadic cancers, such as breast and

particularly ovarian cancer (Bignell *et al*., 1998). APC2 is a potential tumour suppressor gene (Jarrett *et al*., 2001).

Anaphase promoting complex subunit 4 (ANAPC4)

APC4 is one of the subunits of the ubiquitin ligase APC that regulates the G_1 checkpoint and spindle assembly checkpoint (Peters, 2002). APC1/APC4/APC5 make up the structural complex of APC/C. (Thornton and Toczyski, 2006). The APC2/APC11/DOC1 complex binds to APC1 of the structural complex and recruits E2. The TPR complex recruits the CDH1 and CDC20 adaptors, which recruit the substrate. This complex binds to APC1 via APC4 and APC5 (Thornton and Toczyski, 2006). APC4 and CDC16 are mutated in colon cancer cell lines (Wang *et al*., 2003).

Ras homologue member I (ARHI/DIRAS3)

The ARHI tumour suppressor gene inhibits motility, growth and invasion. Only a single functional allele needs to be inactivated through LOH for an imprinted tumour suppressor gene to lose function (Peng *et al*., 2000). ARHI induces the tumour suppressor p21, inhibits cyclin D1 transcription, interrupts EGFR-mediated signalling and induces apoptosis (Bao *et al*., 2002). Mapped on chromosome 1p31, this site displays LOH in 40% of breast and ovarian cancers with the remaining allele methylated in 10-15% of cases. It is downregulated in 60% of breast and ovarian cancers. This loss of expression correlates with increased breast tumourigenesis and poor prognosis in ovarian cancer (Wang *et al*., 2003b).

Ataxia telangiectasia mutated (ATM)

The ATM gene encodes a protein that normally occurs as an inactive dimer/multimer complex. ATM autophosphorylates in response to DNA damage to dissociate the inactive complex. It activates the G_1/S checkpoint and its substrates include p53, MDM2, c-ABL, RAD51, BRCA1, CHK1 and CHK2 (Falck *et al*., 2002; Bakkenist and Kastan, 2003). ATM first phosphorylates histone H2AX at DNA damage sites. Phosphorylated H2AX recruits the DNA repair enzyme RAD51, MRN complex and BRCA1 to these sites (Burma *et al*., 2001). The phosphorylation of p53 by ATM increases its stability and activity, allowing it to transcriptionally activate the p21 gene, amongst others (Banin *et al*., 1998). MDM2 is phosphorylated by ATM, which prevents association with p53 to increase p53 stabilisation and inhibit its degradation (Khosravi *et al*., 1999).

The MRN complex responds to DSBs and induces the ATM-dependent response (Costanzo *et al*., 2000). ATM and ATR phosphorylate BRCA1 in response to recombinogenic DNA lesions (Tibbetts *et al*., 2000). ATM phosphorylates the NF-κB inhibitor, IκB, causing its dissociation from NF-κB. Activated NF-κB is then able to regulate the transcription of anti-apoptotic genes (Piret *et al*., 1999). ATM inhibits origin firing at G_1/S and downregulates CDK2 to inhibit cell cycle progression (Costanzo *et al*., 2000). It predominately phosphorylates CHK2 to prevent mitotic entry (Abraham, 2001). The ATM/CHK2 pathway prevents CDC45 loading onto chromatin to inhibit DNA unwinding by MCM at replication origins (Falck *et al*., 2001). ATM also detects and responds to severely shortened telomeres (Verdun and Karlseder, 2006). ATM and ATR

promote the re-initiation of collapsed replication forks to prevent DSB accumulation (Trenz *et al*., 2006).

Hereditary mutations in the ATM gene cause the rare autosomal recessive genomic instability syndrome, *ataxia telangiectasia* (AT) which is associated with an elevated cancer risk (Savitsky *et al*., 1995). Methylation of the ATM promoter is associated with decreased mRNA levels in locally advanced breast cancers (Vo *et al*., 2004). DNA methylation is the main genomic modification influencing gene expression and is closely linked to carcinogenesis (Curradi *et al*., 2002). There are over 400 ATM mutations that cause diseases, including leukaemia (Schaffner *et al*., 2000).

Ataxia telangiectasia and Rad3 related (ATR)

Ataxia telangiectasia and Rad3 related (ATR) is indispensable for chromosomal integrity and cell viability (Brown and Baltimore, 2000; de Klein *et al*., 2000). ATR is recruited to stalled replication forks induced by DNA damage to phosphorylate CHK1, RAD17 and BRCA1 and activate the checkpoints (Dart *et al*., 2004; Andreassen *et al*., 2004). This involves the RAD9-1-1 complex and the RAD17-RFC2-5 complex. It induces cell cycle arrest, stabilises stalled replication forks and promotes the revival of collapsed forks (Sancar *et al*., 2004). The ATR pathway stabilises the replisome at stalled forks, inhibits fork cleavage, prevents the formation of reversed forks and represses ssDNA accumulation (Cobb *et al*., 2005). It requires ATM in its response to DSBs at the S-phase (Jazayeri *et al*., 2006).

Re-replication may cause amplification, chromosomal translocation and chromosomal loss, weakening the chromosomal integrity of the cell. With the initiation of re-replication, ATR induces S-phase checkpoint activation before DSBs are formed. The re-licensing of origins prompts unregulated DNA unwinding by MCM proteins and accumulates ssDNA. ATR phosphorylates MCM2 and MCM7 to prevent this from occurring (Cortez *et al*., 2004). ATR is further assisted by ATM in activating the G_2/M checkpoint following re-replication (Liu *et al*., 2007).

BCL2-associated X protein (BAX)

BAX, BAD, BID and BAK inhibit cell cycle progression, are pro-apoptotic and inhibit cell survival (Zinkel *et al*., 2006; Reed, 1997; Antonsson and Martinou, 2000). Apoptotic stimuli increase the activity of BID, which induces conformational change of the tumour suppressor BAX, prompting its translocation from the cytosol to the mitochondria. BID activates BAX and BAK to form oligomers and influence the permeability of the mitochondrial outer membrane by forming pores for the release of pro-apoptotic proteins, such as cytochrome *c* and SMAC/Diablo, during apoptosis (Korsmeyer *et al*., 2000). BAX overexpression sensitises cells to apoptosis and decreases anti-apoptotic BCL2 protein levels (Raisova *et al*., 2001; Otter *et al*., 1998; Yin *et al*., 1997).

p53 activates BAX transcription (Miyashita and Reed, 1995; Attardi *et al*., 2000). Lack of p53 or PUMA decreases BAX activation and decreases cytochrome *c* release, thereby suppressing the induction of apoptosis (Ding *et al*., 2007). BAX and BCL-xL expression is also influenced by hypoxia-inducible factor (HIF-1α) in colorectal cancer (Wincemicz

et al., 2007). The downregulation of BAX in metastatic breast cancer correlates with poor prognosis: reduced period of survival, accelerated progression and chemotherapeutic resistance (Krajewski *et al*., 1999).

BRCA2 and CDKN1A interacting protein (BCCIP)

BCCIP exists as two isoforms, BCCIPα and BCCIPβ. BCCIP promotes BRCA2 activity to enhance RAD51 in homologous recombination (Lu *et al*., 2007; Yang *et al*., 2002; Meng *et al*., 2004). Its downregulation consequently reduces BRCA2 and RAD51 nuclear focus formation and critically decreases homologous recombination in response to DSBs (Lu *et al*., 2005). Decreased levels also impair the activation of the G_1/S checkpoint in response to DNA damage (Meng *et al*., 2004b). BCCIP functions as a tumour suppressor by increasing p21 stability and p53-regulated expression of p21 (Meng *et al*., 2004; Meng *et al*., 2006).

It is located on chromosome 10q26.1, which is associated with LOH in several cancers, including squamous cell carcinoma, gliomas, prostate cancer and endometrial cancer (Petersen *et al*., 1998; Rasheed *et al*., 1999; Ittmann, 1998; Peiffer *et al*., 1995). Both isoforms are downregulated in various cancer tissues. BCCIP is expressed in various cancers including colon, breast, stomach and prostate (Meng *et al*., 2003). Its overexpression inhibits cell growth by enhancing p21 inhibition of CDK2, as seen in the inhibition of breast cancer and brain cancer cell growth (Liu *et al*., 2001b; Ono *et al*., 2000).

B-cell lymphoma 2 (BCL2)

BCL2 inhibits cell cycle progression at G_1/S, inhibits apoptosis and has antioxidant properties (Mazel *et al*., 1996; Hockenberry *et al*., 1993). It functions as both an anti-apoptotic molecule and as a proto-oncogene, and promotes tumourigenesis by enhancing DNA damage (Deng *et al*., 2006). Its activity is inhibited by p53 (Chipuk *et al*., 2004; Mihara *et al*., 2003). Apoptotic stimuli induce BCL2 conformational change (Kim *et al*., 2004). BCL2 is then able to suppress BAX oligomerisation to inhibit p53-dependent and p53-independent activation of apoptosis (Dlugosz *et al*., 2006; Deng *et al*., 2006). BCL2 overexpression inhibits nucleotide excision repair, DNA replication and homologous recombination repair to promote mutagenesis (Liu *et al*., 1997; Saintigny *et al*., 2001). It can also inhibit cell cycle entry and this may enhance the cell's response to stress as cells tolerate stress more capably at G_0/G_1 than during DNA replication and mitosis (Mazel *et al*., 1996; Gazitt *et al*., 1998).

In the early stages of breast carcinogenesis, p16 inactivation promotes COX-2 induction and causes genomic instability (Crawford *et al*., 2004; Singh *et al*., 2007). COX-2 induces BCL2 expression (Liu *et al*., 2001). BCL2 downregulation, however, correlates with a poor prognosis in metastatic breast carcinoma (Thomadaki *et al*., 2007). It is overexpressed in various cancers, including prostate, colon and breast (Tsuji *et al*., 1998; Flohil *et al*., 1996; Binder *et al*., 1995).

Baculoviral IAP repeat-containing protein (BIRC5/survivin)

BIRC5/survivin inhibits apoptosis and regulates mitosis (Altieri, 2003). It promotes S-phase progression in association with p21 and CDK4 (Fukuda *et al*., 2004; Sui *et al*., 2002). Mitochondrial survivin inhibits apoptosis in cancer cells and promotes anchorage-independent tumour cell growth (Dohi *et al*., 2004). Its downregulation promotes spindle checkpoint abnormalities, p53/p21-mediated G_2/M arrest, irregular cytokinesis and multinucleated cells to induce mitotic catastrophe and cell death (Yang *et al*., 2004; Beltrami *et al*., 2004).

BIRC5 is uniformly overexpressed in cancers, correlating with increased proliferation, decreased apoptosis, chemotherapy resistance, increased rate of recurrence, increased aggressiveness and poor prognosis (Fields *et al*., 2004; Tanaka *et al*., 2000; Zaffaroni *et al*., 2002; Swana *et al*., 1999; Velculescu *et al*., 1999). It is expressed early in breast tumourigenesis (Barnes *et al*., 2006). Its upregulation in breast cancer can, however, be associated with a poor or good prognosis (Tanaka *et al*., 2000; Kennedy *et al*., 2003). Its overexpression is a positive prognostic factor in osteosarcoma, gastric and non-small-cell lung cancer (Zhang *et al*., 2001; Trieb *et al*., 2003; Okada *et al*., 2001; Vischioni *et al*., 2004).

Breast cancer 1 and 2 (BRCA1, BRCA2)

BRCA1 and BRCA2 encode large proteins that occur in the nuclei of dividing cells in the majority of tissues (Scully and Livingston, 2000; Welcsh *et al*., 2000). BRCA1 and BRCA2 gene products are involved in DNA damage repair and in maintaining genomic

integrity (Venkitaraman, 2002). They associate with RAD51 and function in DNA DSB repair (Haber, 1999; Davies *et al*., 2001). In the mammary gland, BRCA1 and BRCA2 expression fluctuates with development (Rajan *et al*., 1996). In familial breast cancer, the allele of BRCA1 or BRCA2 is lost due to a germline mutation and the other allele may be inactivated (Staff *et al*., 2000). Although sporadic breast cancer does not involve BRCA gene mutations, loss of a BRCA allele and loss of BRCA protein and mRNA expression do frequently occur (Wilson *et al*., 1999; Lambie *et al*., 2003). Loss of BRCA1 or BRCA2 causes spontaneous chromosome aberrations (Tirkonnen *et al*., 1997).

BRCA1

BRCA1 localises to DNA damage sites, particularly at DSBs (Celeste *et al*., 2002). It directs spindle assembly and chromosomal separation at the G_2/M checkpoint (Welcsh *et al*., 2000). It also induces G_1/S arrest and inhibits telomerase activity (Aprelikova *et al*., 1999; Wang *et al*., 1998). BRCA1 and DNA repair proteins form the BRCA1-associated genome surveillance complex (BASC), consisting of mismatch repair proteins, DNA DSB repair proteins, DNA replication proteins, and proteins involved in recombination (Wang *et al*., 2000). Depending on the type of DNA damage, BRCA1 may be hyperphosphorylated by ATM, ATR or CHK2, inducing its translocation to a DNA replication complex (Scully *et al*., 1997). DNA repair is then induced by RAD51, BRCA2 and the MRN complex (Yoshida and Miki, 2004). BRCA1 also occurs within the RNA pol II complex and promotes transcriptional activity (Anderson *et al*., 1997; Cabart *et al*., 2004). It stabilises p53 and stimulates numerous p53-regulated genes that

function in DNA repair and the cell cycle, but does not induce genes involved in apoptosis (Ongusaha *et al*., 2003).

The BRCA1 gene can be inactivated in sporadic breast cancer by promoter hypermethylation (Eyfjord and Bodvarsdottir, 2005). This is associated with high histologic grade and metastasis to the lymph nodes, indicative of increased aggressiveness (Lambie *et al*., 2003). It is also implicated in the development of familial breast and ovarian cancers (Fraser *et al*., 2003). BRCA1 mutation correlates with a high risk of various cancers, including breast, ovarian, pancreatic, uterine, cervical and prostate (Gayther *et al*., 1995; Thompson and Easton, 2002; Struewing *et al*., 1997).

BRCA2

BRCA2 enhances the assembly and disassembly of RAD51 filaments in homologous recombination, and responds to ssDNA (Galkin *et al*., 2005; Yang *et al*., 2002; Liu *et al*., 2001). It is associated with genomic stability, particularly by stabilising DNA at stalled replication forks (Lomonosov *et al*., 2003). The absence of BRCA2 results in a degeneration of replication intermediates and a build-up of DSBs (Lomonosov *et al*., 2003). Sporadic breast tumours with BRCA2 mRNA overexpression are associated with higher histologic grade and a poor prognosis (Egawa *et al*., 2002).

Cell cycle division 16 (CDC16)

CDC16 is part of the TPR arm of APC that connects the adaptor proteins, CDC20 and CDH1, to APC for substrate recruitment (Thornton and Toczyski, 2006). CDC2

phosphorylates APC to fully activate APC^{CDC20}. CDC16 is one of the APC subunits that has CDC2 consensus sites and contributes to APC^{CDC20} activity (Rudner and Murray, 2000). CDC16 localises to the spindle/centrosome during mitosis where it is phosphorylated and activated (Tugendreich *et al*., 1995; Peters *et al*., 1996). The three main APC components CDC16, APC8/CDC23 and APC3/CDC27 are mutated in cancer cell lines. CDC16 in particular is mutated in colon cancer cell lines (Wang *et al*., 2003). Inactivation of these components induces premature mitotic cyclin activity and aberrant replication (Zhao *et al*., 1998).

Cell division cycle 20 (CDC20)

CDC20 activates APC, which binds to CDH1 to degrade cyclins for mitotic exit and interphase entry (Pfleger *et al*., 2001). As an adaptor protein, CDC20 activates ubiquitination and enhances binding to APC (Vodermaier *et al*., 2003). Inadequate CDC20 delays the initiation of anaphase while excess CDC20 induces the premature onset of anaphase (Hwang *et al*., 1998).

The inhibitory mitotic checkpoint complex is formed with CDC20, MAD2, MAD3/BUBR1 and BUB3 (Sudakin *et al*., 2001). If kinetochores are unattached, CDC20 is bound in this complex and inhibited by MAD2 and BUBR1, and cannot activate APC. Anaphase is consequently delayed (Tang *et al*., 2001b; Fang, 2002). CDC2 phosphorylates APC in mitosis, increasing the affinity of CDC20 to bind to APC (Lahav-Baratz *et al*., 1995; Fang *et al*., 1998). CDC2 is inactivated at mitotic exit as the mitotic cyclins are degraded. APC dephosphorylation induces the dissociation of the

APC^{CDC20} complex and CDC20 is degraded. From late anaphase to early G_1, CDC20 is ubiquitinated and degraded by APC^{CDH1} (Prinz *et al*., 1998; Huang *et al*., 2001). It is upregulated in breast cancer cell lines where it enhances defective checkpoints and genomic instability (Yuan *et al*., 2006).

Cell cycle division 34 (CDC34)

CDC34 is an E2 conjugating enzyme that enhances SCF E3 ligase function to regulate cell cycle regulator levels (Wu *et al*., 2002). SCF induces ubiquitin transfer from CDC34 to the substrate (Petroski and Deshaies, 2005). CDC34 targets p27 for ubiquitination at G_1, S and G_2, promoting G_1/S progression and is associated with G_2/M transition and spindle function (Butz *et al*., 2005; Kaiser *et al*., 2000; Reymond *et al*., 2000). CK2 phosphorylates CDC34 to enhance its ubiquitin activity (Sadowski *et al*., 2007; Block *et al*., 2001). It is upregulated in paediatric acute lymphoblastic leukaemia, hepatocellular carcinoma, multiple myeloma and other cancers (Eliseeva *et al*., 2001; De Vos *et al*., 2002; Chauhan *et al*., 2004). Overexpression inhibits chromosome alignment at metaphase (Liu *et al*., 2006).

Cyclin-dependent kinase 5 regulatory subunit 1 (CDK5R1)

CDK5R1 encodes for p35 (Gupta and Tsai, 2003). CDK5 does not have enzymatic activity on its own but must be activated by p35 binding. p35 is selectively localised in the central nervous system, which is where CDK5 is mainly active (Dhavan and Tsai, 2001). CDK5/p35 kinase is important in neurite outgrowth, synaptic transmission, neuronal migration and differentiation (Nikolic *et al*., 1996; Halachmi and Lev, 1996;

Tsai *et al*., 1993). It also regulates exocytosis, transcription, tissue regeneration, senescence, apoptosis and hormone-regulation (Rosales and Lee, 2006; Moncini *et al*., 2007). CDK5RAP1, CK2, importin-β, importin-5 and importin-7 compete with CDK5 for binding with p35, thereby inhibiting CDK5 activation (Lim *et al*., 2004; Fu *et al*., 2006).

CDK5 regulatory subunit associated protein 1 (CDK5RAP1)

CDK5 exists in three states: one of these is CDK5RAP1, a form associated with p35 that is expressed in the brain and placenta (Lee *et al*., 1996; Zou *et al*., 2004). It inhibits CDK5 by binding to p35 and preventing CDK5 activation (Ching *et al*., 2002). CDK5RAP1 is associated with RNA pol II elongation and has histone acetyltransferase activity (Winkler *et al*., 2001; Wittschieben *et al*., 1999). It is involved in regulating tRNA modification and translation, post-translational modification and anaerobic oxidation (Anantharaman *et al*., 2001; Sofia *et al*., 2001).

Cyclin-dependent kinase inhibitor 1A (CDKN1A)

CDKN1A encodes $p21^{Cip1/Waf1}$ (CDK-interacting protein 1/wild-type p53-activated fragment 1). p21 inhibits cyclin A/CDK2 and cyclin E/CDK2 to induce p53-dependent G_1 cell cycle arrest (Maddika *et al*., 2007). It is an essential effector of p53 in response to DNA damage; functions as an adaptor protein for cyclin D/CDK4; restricts DNA replication by binding to PCNA; and associates with caspase-3 and caspase-8 to inhibit apoptosis (el-Deiry *et al*., 1993; Ocker and Schneider-Stock, 2007). It is transactivated by p53 and p73 (Lohrum and Vousden, 2000). The loss of p53/p21 expression in cells

prevents sustained cell cycle arrest, causing uncontrolled nuclear division and cell death (Vogelstein and Kinzler, 2001).

p21 is commonly hypermethylated and inactivated in tumours (Fang and Lu, 2002). Normal breast epithelium displays low p21 activity (Diab *et al*., 1997). High levels of nuclear p21 promote cell cycle arrest and high levels of cytoplasmic p21 inhibit apoptosis (Asada *et al*., 1999; Zhou *et al*., 2001). High levels of p21 can, however, correlate with normal functioning of p53, as commonly seen in colon carcinomas (El-Deiry *et al*., 1995).

Cyclin-dependent kinase inhibitor 1B (CDKN1B)

CDKN1B encodes $p27^{Kip1}$ (CDK-interacting protein 1). This growth inhibitory protein inhibits cyclin E/CDK2 and cyclin A/CDK2 to inhibit G_1/S progression (Munoz-Alonso *et al*., 2005). It also promotes the formation of cyclin D/CDK4 and cyclin D/CDK6 complexes (Sherr and Roberts, 2004). It promotes apoptosis and regulates differentiation (Kayatose *et al*., 1997; Zabludoff *et al*., 1998; Robker and Richards, 1998). The D-type cyclins, in particular cyclin D2, are important inhibitors of p27 at G_0/G_1 (Solvason *et al*., 2000). Cyclin E/CDK2 phosphorylates p27 for recognition by SCF^{SKP2} to allow cyclin E/CDK2 levels to accumulate for G_1/S and G_2/M progression (Nakayama *et al*., 2001; Bloom and Pagano, 2003; Montagnoli *et al*., 1999). The downregulation of CDC34, which is involved in ubiquitination, increases p27 protein levels and inhibits proliferation (Butz *et al*., 2005).

p27 mutations are rare in cancers. Its downregulation in tumours is a result of high protein turnover. Decreased p27 expression correlates with increased SKP2 expression as seen in squamous cell carcinoma, colorectal adenocarcinomas, prostate and pituitary cancer, and lymphomas (Kudo *et al*., 2001; Hershko *et al*., 2001; Ben-Izhak *et al*., 2003; Musat *et al*., 2002; Chiarle *et al*., 2002). Decreased p27 expression is associated with increased tumour grade and poor prognosis (Slingerland and Pagano, 2000; Sherr, 1996). p27 cytoplasmic localisation in colon cancer and other cancers correlates with a poor prognosis (Min *et al*., 2004; Baldassare *et al*., 1999; Sgamboto *et al*., 1999). Loss of nuclear p27 in stage III colorectal cancer is associated with recurrence and poor survival and with poor differentiation at stage II (Manne *et al*., 2004).

Cyclin-dependent kinase inhibitor 2A (CDKN2A)

Over 80% of cancers have mutations or epigenetic changes influencing INK4-mediated cell cycle arrest (Ortega *et al*., 2002). CDKN2A encodes the tumour suppressor $p16^{Ink4a}$, which suppresses cyclin C/CDK4 and cyclin C/CDK6 binding by forming complexes with CDK4 and CDK6. This prevents the phosphorylation and inactivation of RB, inhibits E2F-mediated transcription and inhibits entry into the S-phase (Serrano *et al*., 1993). It inhibits angiogenesis and metastasis, and regulates DNA hypermethylation in homeobox genes (Gibson *et al*., 2005; Wang *et al*., 2006; Reynolds *et al*., 2006). The RB/p16 pathway of tumour suppression is inactivated in cancers due to RB or p16 inactivation or aberration, and the overexpression of cyclin D1, CDK4 or CDK6 (Nobori *et al*., 1994). The complete CDKN2A-CDKN2B locus is frequently deleted in cancers as

both p16 and p15 must be inactivated for effective malignant transformation (Krimpenport *et al*., 2007).

p16 overexpression induces RB-dependent G_1/S arrest (Lukas *et al*., 1995). Decreased p16 expression promotes colorectal tumour growth and metastasis, negatively influencing the prognosis (Zhao *et al*., 2006). Altered expression of p16 protein, but not gene alteration, correlates with increased breast cancer cell proliferation (Emig *et al*., 1998). Expression is lost through methylation or mutations in breast cancers and colorectal cell lines (Brenner *et al*., 1998; Zheng *et al*., 2000).

Cyclin-dependent kinase inhibitor 2B (CDKN2B)

CDKN2B encodes $p15^{Ink4b}$. p15 inhibits the phosphorylation of RB by cyclin D/CDK4 and cyclin D/CDK6 to induce G_1 cell cycle arrest (Reynisdottir *et al*., 1995). This pathway is frequently affected in numerous cancers including melanoma, prostate and colorectal tumours (Maddika *et al*., 2007; Herman *et al*., 1996; Ishiguro *et al*., 2006). Overexpression induces the dissociation of cyclin D/CDK complexes, promotes p27 activity, causes senescence and represses telomerase activity (Swarbrick *et al*., 2000; Fuxe *et al*., 2000). The inactivation of p15 promotes escape from senescence and enhances malignant transformation (Hitomi *et al*., 2007).

CDKN2A and CDKN2B occur on chromosome 9p21 which experiences frequent LOH through deletions, associated with various cancers. The inactivation of CDKN2A and CDKN2B promotes unrestrained proliferation(Herman *et al*., 1996). Some histone

deacetylases inhibitors (HDACi's) may increase p21 and p15 mRNA and protein to cause G_1 arrest in tumour cells (Hitomi *et al*., 2003).

Cyclin dependent kinase inhibitor 3 (CDKN3)

The CDKN3 oncogene is located at 14q22, the site at which numerous chromosomal aberrations associated with cancers are located (Demetrick *et al*., 1995; Gilladoga *et al*., 1992; Roulston *et al*., 1998). CDKN3 dephosphorylates and inactivates CDK2, CDC2 and CDK3 to inhibit cell cycle progression (Hannon *et al*., 1994; Gyuris *et al*., 1994). CDKN3 recruits numerous complexes for gene silencing to regulate transcription e.g. it binds to MDM2 to inhibit p53 transcriptional activity and p21 induction (Schultz *et al*., 2001; Schultz *et al*., 2002; Okamoto *et al*., 2006). ATM inactivates CDKN3 following DNA damage (Ziv *et al*., 2006). CDKN3 is overexpressed in breast and prostate cancer and may promote tumourigenesis (Yeh *et al*., 2003; Lee *et al*., 2000).

CHK1 checkpoint homologue 1 (CHEK1)

CHK1 regulates DNA replication, cell cycle progression, apoptosis and chromatin restructuring (Bartek and Lukas, 2003). It is needed for replication fork progression in the S-phase and prevents the accumulation of DNA breaks (Petermann *et al*., 2006; Durkin *et al*., 2006). CHK1 must be phosphorylated for the checkpoint-dependent release of CHK1 from chromatin, which is essential for proper functioning (Smits *et al*., 2006). When localised at centrosomes, CHK1 is restricted from prematurely activating cyclin B/CDC2 (Kramer *et al*., 2004). CHK1 inhibition promotes the premature activation of cyclin B/CDC2, causing chromosomal misalignment at metaphase,

chromosome lagging at anaphase and kinetochore defects (Tang *et al*., 2006). This inhibition enhances tetraploid cell death during or after mitosis (Vitale *et al*., 2007).

ATM phosphorylates CHK1 and CHK2 following ionising radiation to prevent mitotic entry. CHK1 and CHK2 both participate in the G_2/M checkpoint where they phosphorylate CDC25 to prevent CDC2 dephosphorylation and activation of MPF (Gatei *et al*., 2003). Consequently, mitotic entry at the DNA damage checkpoint is suppressed. They also phosphorylate p53 and BRCA1 to induce G_1/S and G_2/M cell cycle arrest, DNA repair and apoptosis (Pommier *et al*., 2005). RAD1, ATR, RAD9, RAD17 and HUS1 are essential at the G_1/S and G_2/M checkpoints for CHK1 and CHK2 to be phosphorylated (Walworth and Bernards, 1996; Lindsay *et al*., 1998). p53 induces p21 and RB to inhibit CHK1 transcription in colorectal cancer (Gottifredi *et al*., 2001).

CHK2 checkpoint homologue 2 (CHEK2)

CHK2 functions at the S-phase checkpoint (Falck *et al*. 2002). Present as a monomer in normal cells, CHK2 forms a dimer in response to DNA damage and is activated by multiple phosphorylations (Ahn *et al*., 2002). CHK2 phosphorylates and stabilises E2F-1, regulating its transcriptional activity in response to DNA damage (Stevens *et al*., 2003). It phosphorylates and destabilises CDC25A to increase its susceptibility to $SCF^{\beta\text{-TRCP}}$-mediated degradation (Busino *et al*., 2003). This inhibits cyclin E/CDK2 and induces G_1 and S checkpoints (Bartek *et al*., 2001). CHK2 phosphorylates CDC25C, resulting in its nuclear export to prevent CDC25C from activating CDC2, which promotes G_2 checkpoint activation and inhibits G_2/M progression (Chehab *et al*., 2000;

Hirao *et al*., 2000). ATM activates CHK2, which phosphorylates and stabilises p53 to promote p53 transcriptional activation, regulate G_1 arrest and induce apoptosis following DNA damage (Chehab *et al*., 2000; Takai *et al*., 2002). In the absence of p53, the CHK-CDC25-CDC2 pathway is activated to induce G_2 arrest (Nurse, 1997; Poon *et al*., 1997). p53 downregulates CHK2 in several cancer cell lines (Shigeishi *et al*., 2002). Its mutation is associated with an increased susceptibility to familial breast cancer.

CDC28 protein kinase regulatory subunit 1B (CKS1B)

CKS1B regulates CDKs (Egan and Solomon, 1998). It is a key accessory protein for SCF^{SKP2} ubiquitin ligase (Cardozo and Pagano, 2004). It mediates SKP2-ubiquitinylation of p27 and interacts with CDKs, such as CDC2 (Ganoth *et al*., 2001; Bourne *et al*., 1996). CKS1B degradation is mediated by APC/C^{CDH1} (Bashir *et al*., 2004). Its protein levels correlate with SKP2 protein levels and inversely correlate with p27 in colorectal and breast cancer (Shapira *et al*., 2004; Slotky *et al*., 2005). CKS1B increases the affinity of SKP2 for phosphorylated p27, enhancing p27 ubiquitinylation and its rapid degradation. Its overexpression in aggressive cancers decreases p27 levels (Slotky *et al*., 2005). CKS1B hyperactivation promotes cell survival through tumour cell proliferation and possible drug resistance (Bloom and Pagano, 2003; Zhan *et al*., 2007).

CKS1B upregulation correlates with a poor prognosis in colorectal, breast, oral and gastric carcinoma (Shapira *et al*., 2005; Slotky *et al*., 2005; Kitajima *et al*., 2004; Masuda *et al*., 2003). Its expression increases in poorly differentiated colorectal tumours (Shapira *et al*., 2005). In breast cancer, high CKS1 levels are associated with poor tumour

differentiation, ER- status, PR- status, shorter disease-free survival, increased recurrence and relapse. It is upregulated in established immortalised breast cancer cell lines, in comparison to non-immortalised breast cancer cell lines derived from breast cancer patients (Fernandez-Cobo *et al*., 2006).

CDC28 protein kinase regulatory subunit 2 (CKS2)

CKS2 induces CDK activity by binding to the catalytic subunit. p53 downregulates CKS2 to inhibit progression from metaphase to anaphase. This downregulation is at the transcriptional and protein level (Rother *et al*., 2007).

Cullin 1 (CUL1)

Proteolysis is essential for protein turnover and degrading aberrantly folded and damaged proteins. Protein degradation mediated by ubiquitination is vital in signalling cascades. Cullin-based ubiquitin ligases consist of a cullin (CUL1-5) and a RING domain protein ROC1/RBX1. The cullin-based ubiquitin ligases have a strong association with cancer. The cullin subunit mediates binding to substrate recognition subunits. It also acts as an E2 ubiquitin-conjugating enzyme providing a docking site for the E2 ubiquitin ligase enzyme (Deshaies, 1999). CUL1 and SKP1 bind F-box-containing substrate recognition subunits such as SKP2 to form SCF E3 ubiquitin ligases (Chew *et al*., 2007). CUL1-based complexes regulate the protein levels of numerous cell cycle regulators, tumour suppressors and oncogenes (Deshaies, 1999).

Cullin 2 (CUL2)

CUL2 and its adaptor protein Elongin B bind von Hippel-Lindau (VHL)-containing substrate recognition subunits. Ubiquitin-related Elongin B forms a heterodimer with SKP1-related Elongin C (Conaway and Conaway, 2002). RBX1 binds to CUL2 to promote the recruitment of the E2 ubiquitin ligase enzyme to the complex (Kawakami *et al*., 2001). CUL2, elongin B/C, RBX1 and VHL-1 form the SCF-like E3 ubiquitin ligase complex ECV. ECV targets HIFα subunits for proteasomal degradation (Cockman *et al*., 2000; Tarimoto *et al*., 2000). HIF1α inhibits pro-apoptotic BAX and BCL-xL, therefore ECV induces apoptosis (Wincemicz *et al*., 2007).

Cullin 3 (CUL3)

In CUL3-based ubiquitin ligases, CUL3 proteins bind to substrate recognition subunits independently of an adaptor protein. CUL3 can form homodimers to enhance cullin-based ubiquitin ligase activity (Chew *et al*., 2007). It is important for chromosomal alignment at metaphase, spindle assembly and cytokinesis (Sumara *et al*., 2007). CUL3-based ubiquitin ligases regulate cyclin E levels (McEvoy *et al*., 2007).

DEAD/H box polypeptide 11 (DDX11)

DDX11 encodes the DNA helicase, CHL-1 like helicase (ChlR1), that is only expressed in proliferating cells and binds to ssDNA and dsDNA (Hirota and Lahti, 2000). DNA helicases are essential in all cells and are vital for DNA metabolic pathways such as DNA replication, repair, transcription and recombination (Tuteja and Tuteja, 2004).

Aberrations in helicase function confer genomic instability and elevated mutation rates, resulting in an increased predisposition to cancer (van Brabant *et al*., 2000).

ChlR1 binds cohesin and is essential for the cohesion of sister chromatids (Parish *et al.*, 2006). Cohesin holds sister chromatids together until the initiation of anaphase when chromatid separation is triggered by separase (Hoque and Ishikawa, 2001; Uhlmann *et al*., 2000). Inadequate levels of ChlR1 delay prometaphase through abnormal sister chromatid cohesion. ChlR1 promotes the correct assembly of cohesin onto DNA during the S-phase. ChlR1 depletion causes abnormal cohesion of sister chromatids, mitotic aberrations and aneuploidy, a common feature in cancers (Parish *et al*., 2006).

Dynamin-2 (DNM2)

The DNM2 signalling GTPase functions in endocytosis, regulation of the actin network, cytokinesis, centrosome cohesion, MAPK signalling pathway and signalling at the immunological synapse (De Camilli *et al*., 1995; Orth and McNiven, 2003; Konopka *et al*., 2006; Thompson *et al*., 2004; Kranenburg *et al*., 1999; Gomez *et al*., 2005). It activates p53 and induces apoptosis (Fish *et al*., 2000). Mutations result in autosomal dominant centronuclear myopathy, a group of rare neuromuscular disorders (Bitoun *et al*., 2005). Loss of dynamin function suppresses neurite outgrowth and axon formation (Yoo *et al*., 2002). DNM2 overexpression activates caspase-3, inducing p53-dependent apoptosis (Fish *et al*., 2000). The GTPase domain of DNM2 is required for apoptosis induction by dynamin (Soulet *et al*., 2006).

E2F transcription factor 4 (E2F4)

The E2F transcription factors are important cell cycle regulators that form homodimers and bind to DNA. E2F4 is the predominant form of E2Fs (Moberg *et al*., 1996). When occurring as heterodimers with a member of the DRTF-1 polypeptide (DP) family (DP1 or DP2), the DNA binding activity is elevated (de Bruin *et al*., 2003; Lam and La Thangue, 1994). E2F4 promotes exit from the cell cycle into terminal differentiation (Fajas *et al*., 2002; Humbert *et al*., 2000). E2F transcriptional activity is inhibited by the pocket proteins RB, p107 and p130, causing cell cycle arrest (O'Connor *et al*., 1995). By phosphorylating RB, CDKs release E2F from its association with RB, thereby promoting G_1/S transition. p53 transactivates p21 to inhibit CDK2 and CDK4, thereby maintaining the E2F/RB association (Xiong *et al*., 1993). E2F4 binds to 500-700 genes, including CCNB1, CCNB2, CCNG2, CDC23, CDC6, CDK2, MAD2L1, RBL1, PCNA, BRCA1, RAD51 and CHK2 (Leone *et al*., 2000; Conboy *et al*., 2007).

E2F4 promotes G_1/S transition in colon epithelial crypt cells where it is important in maintaining the intestinal crypt compartment (Rempel *et al*., 2000). High levels of E2F4 here correlate with Ki-67 expression (Garneau *et al*., 2007). E2F4 is localised at 16q22, a site associated with frequent allelic imbalance in various cancers such as breast, prostate and hepatocellular carcinoma (Ginsberg *et al*., 1994). It functions in cancer progression and is an indicator of poor prognosis (Rakha *et al*., 2004). It is also commonly overexpressed in colon cancer; however, it appears to promote apoptosis in colon cancer and various cell lines (Mady *et al*., 2002; Chang *et al*., 2000).

Growth arrest and DNA-damage-inducible alpha (GADD45A)

GADD45A regulates the cell cycle, genomic integrity, differentiation, survival and the cytoskeleton (Yamauchi *et al*., 2007; Takekawa and Saito, 1998). It is induced by stress responses such as radiation, and promotes intracellular signalling pathways (Fornace *et al*., 1988; Juriloff and Harris, 2000). GADD45A induces G_2/M arrest by inhibiting cyclin B1/CDC2 (Jin *et al*., 2002). It associates with the core histones around damaged DNA and may be involved in regulating the availability of repair nuclear proteins to DNA (Carrier *et al*., 1999). It is regulated by p53, but can also stabilise the p53 protein after DNA damage (Jin *et al*., 2003; Bulavin *et al*., 2003). Defects in the GADD45A gene confer genomic instability, as seen in cancers. Overexpression inhibits cell growth in tumour cell lines (Zhan *et al*., 1994).

General transcription factor IIH (GTF2H1)

GTF2H1 encodes TFIIH, which is involved in transcription, DNA damage repair and cell cycle control. TFIIH consists of the TFIIH core complex and CAK complex. The core complex allows for nucleotide excision repair and is essential for cell cycle progression (Matsuno *et al*., 2007). The CDK-containing complex is responsible for phosphorylating and activating RNA pol II and various nuclear receptors (Araujo *et al*., 2000; Keriel *et al*., 2002). TFIIH is important for nuclear receptor activation, E2F degradation and RNA polymerase phosphorylation (Keriel *et al*., 2002; Vandel and Kouzarides, 1999; Makela *et al*., 1995). TFIIH also has E3 ubiquitin ligase activity (Takagi *et al*., 2005).

G_2 and S-phase expressed (GTSE-1)

The GTSE1 protein is localised at microtubules and is induced by p53 in cells with a tetraploid DNA content (Utrera *et al*., 1998). It shuttles from the cytoplasm to the nucleus in response to nuclear export signals. Following DNA damage, it is stabilised in a p53-independent manner, promoting its nuclear accumulation (Ko and Prives, 1996). GTSE-1 protein inhibits long-term p53 activation at S and G_2 to control DNA damage-induced apoptosis (Monte *et al*., 2003). p53 localisation in the cytoplasm is highest at S and G_2 when GTSE-1 expression is at its peak (David-Pfeuty *et al*., 1996). GTSE-1 overexpression delays G_2/M progression in response to DNA damage and protects cells arrested at the G_2/M checkpoint from p53-dependent apoptosis (Monte *et al*., 2003).

Hect domain and RLD 5 (HERC5)

HERC5 functions as an E3 ligase for ISGylation (the conjugation of interferon-stimulated genes [ISGs] to specific cell proteins) (Dastur *et al*., 2006). ISGs are induced in the cell antiviral response and ISG15 may function in a ubiquitin/SUMO-like manner (Kaivakolanu, 2003; Ritchie and Zhang, 2004). HERC5 expression is therefore upregulated following pro-inflammatory stimuli and may be important in the immune response (Dastur *et al*., 2006; Wong *et al*., 2006). It is a late inflammatory response gene in endothelial cells via the NF-κB pathway (Kroismayr *et al*., 2004). HERC5 is highly expressed in the testis, where the ubiquitin system is specifically needed for protein breakdown in spermatogenesis (Wong *et al*., 2002; Baarends *et al*., 2000). mRNA levels

are very low in other tissues, and as yet, it is not associated with any disease (Kroismayr *et al*., 2004; Scheffner and Staub, 2007).

HUS1 checkpoint homologue

HUS1 interacts with ATR and CHK1 in response to DNA damage induced by UV and DNA replication blocks. It enhances the phosphorylation of CHK1 and RAD17, and functions in checkpoint activation (Weiss *et al*., 2002; Zou *et al*., 2002; Bermudez *et al*., 2003). It forms part of the 9-1-1 complex, which is essential in the DNA damage response. PCNA subunits form a clamp around DNA to anchor DNA-metabolising enzymes at the DNA replication site (Caspari *et al*., 2000). The 9-1-1 complex forms a PCNA-like structure at DNA where damage is detected, anchored by the RAD17-RFC complex (checkpoint protein RAD17 and four RFC subunits) (Shiomi *et al*., 2002). RAD9-1-1 then promotes phosphorylation and activation of CHK1 by ATR to regulate S-phase, G_2/M arrest and stabilisation of replication forks (Parrilla-Castellar *et al*., 2004). Inactivation or decreased levels of RAD1, HUS1 or RAD9 promote spontaneous chromosomal aberrations, morphology change and carcinogenesis (Bao *et al*., 2004). As HUS1 is unstable, RAD1 protects it from ubiquitination and degradation (Hirai *et al*., 2004).

HUS1 deletion causes critical shortening at telomeres (Francia *et al*., 2006). This may result in a poorly regulated telomerase complex producing aberrant DNA that consequently may cause mass loss of telomeric repeats following recombination (Wang *et al*., 2004). HUS1 and RAD9 are involved in homologous recombination repair;

therefore, aberrations in this pathway promote greater telomere instability (Wang *et al*., 2006b).

Karyopherin α 2 (KPNA2)

KPNA2 is important in signal-transduction pathways regulating epidermal proliferation and differentiation (Umegaki *et al*., 2007). KPNA proteins are essential in nucleocytoplasmic transport by binding cargo-proteins to the karyopherin β transport protein (importin β) (Dankof *et al*., 2007). Nuclear transport mechanisms, however, promote oncogenic transformation (Poon and Jans, 2005). KPNA2 instigates the transport of molecules such as BRCA1, p53, and the MRN complex from the cytoplasm through the nuclear core and into the nucleus (Thakur *et al*., 1997; Kim *et al*., 2000; Tseng *et al*., 2005). It is particularly important for NBS1 nuclear localisation. KPNA2-NBS1 association is essential for the DNA damage cell cycle checkpoint (Tseng *et al*., 2005). Overexpressed KPNA2 is a molecular marker for breast cancer with nuclear protein expression correlated with a poor prognosis (Dahl *et al*., 2006). Its upregulation is an early event in breast carcinogenesis while its downregulation inhibits proliferation (Dankof *et al*., 2007; Umegaki *et al*., 2007).

Mitotic arrest deficient-like 1 (MAD2L1)

The MAD2L1 gene encodes the MAD2 checkpoint protein that interacts with the kinetochores of unattached chromosomes and inhibits APC activation by CDC20 to induce cell cycle arrest at metaphase (Chen *et al*., 1996b; Fang *et al*., 1998). APC inhibition regulates kinetochore attachment to the spindle for controlled induction of

anaphase by stipulating correct chromatid separation. MAD2 binds to MAD1, which directs MAD2 to unattached kinetochores and enhances MAD2 binding to CDC20 (Sironi *et al*., 2001). When chromosomes are correctly aligned at metaphase, MAD2 is not present (Chen *et al*., 1996b). It is upregulated in familial adenomatous polyposis and sporadic colorectal cancer and this is associated with APC mutations (Abal *et al*., 2007). MAD2L1 is also upregulated in ductal breast carcinoma, basal-like breast cancer and breast cancer cell lines, where it promotes defective checkpoints and genomic instability (Scintu *et al*., 2007; Yuan *et al*., 2006; Rouzier *et al*., 2005). It is, however, downregulated in some breast and ovarian cancers (Wang *et al*., 2002).

Mitotic arrest deficient-like 2 (MAD2L2)

Like MAD2L1, MAD2L2 encodes essential components of the spindle checkpoint. The inactivation of these checkpoint components induces aneuploidy (Burds *et al*., 2005). MAD2L2 interacts with enzymes that are associated with DNA-damage repair (Ying and Wold, 2003). It also associates with the APC co-activators CDC20 and CDH1 to inhibit APC activation at late mitosis (Pfleger *et al*., 2001). MAD2 haploinsufficiency induces premature entry into anaphase and CIN (Michel *et al*., 2001). MAD2L1 and MAD2L2 mutations are rare in aneuploid solid tumours but have been identified in cancers of the digestive tract, lungs, breast and hepatocellular carcinoma (Imai *et al*., 1999; Percy *et al*., 2000; Hernando *et al*., 2001). MAD2L2 is upregulated at different stages of colorectal cancer with expression correlating with decreased survival in patients (Rimkus *et al*., 2006). It is upregulated in breast cancer cell lines where it promotes defective checkpoints and genomic instability (Yuan *et al*., 2006).

Minichromosome maintenance deficient 2 (MCM2)

During DNA replication, double-stranded DNA must be unwound by an ATP-dependent helicase into single strands, as DNA polymerases need a ssDNA template on which to work (Kawasaki and Sugino, 2001). The minichromosome maintenance (MCM) proteins function as the replicative helicase (Bell and Dutta, 2002). In the initial steps of DNA replication, the origin recognition complex enhances MCM complex loading onto the DNA. The MCM complex permits binding of proteins, such as CDC45, to chromatin. This is essential for replication origin firing, which allows replication protein A (RPA) and DNA polymerases to access unwound DNA (Yoo *et al*., 2004). Re-replication is prevented by a licensing factor that binds to origins of replication during mitosis. Once the origins have fired in the S-phase, the licensing factor is released. The MCM complex forms part of the licensing factor, limiting DNA synthesis to once per cell cycle (Blow and Laskey, 1988; Yan *et al*., 1991). MCM proteins vacate chromatin at S-phase and bind to them again at the end of mitosis (Liang and Stillman, 1997; Coue *et al*., 1996). MCM proteins also control DNA elongation (Labib *et al*., 2000).

The MCM2-7 proteins are essential for DNA replication, forming a ring-shaped structure around DNA (Ying and Gautier, 2005). Decreased MCM2-7 levels do not suppress normal DNA synthesis (Cortez *et al*., 2004; Tsao *et al*., 2004). Besides DNA synthesis, MCM2-7 may also function in DNA pumping, checkpoint activation, transcription and chromatin remodelling (Laskey and Madine, 2003; Cortez *et al*., 2004; Tsao *et al*., 2004; Fitch *et al*., 2003; Dziak *et al*., 2003). ATR, ATM, CDC2, CDK2 and CK2

phosphorylate MCM2 at the S-phase checkpoint (Cortez *et al*., 2004; Jiang *et al*., 1999; Montagnoli *et al*., 2006).

While it is expressed in normal and tumour tissues, expression is higher in tumours (Todorov *et al*., 1998). MCM2 mRNA and protein levels vary little with cell cycle progression in cell lines, but levels drop sharply in slowly proliferating cells (Todorov *et al*., 1991). Increased MCM2 expression is associated with increased carcinoma grade and poor survival rates (Dudderidge *et al*., 2005; Hashimoto *et al*., 2004). Cell proliferation is an important prognostic factor in breast carcinomas (Keshgegian and Cnaan, 1995). However, Ki-67 and PCNA immunostaining is not a good prognostic factor in breast cancer (Hall and Woods, 1990). MCM2/BM28 is a cell proliferation marker that correlates with proliferation and might be a prognostic factor alternative to PCNA and Ki-67 (Todorov *et al*., 1998).

MCM3

MCM3 is the most abundant MCM protein (Lei *et al*., 1996). It inhibits the initiation of DNA replication but not elongation (Takei *et al*., 2002). The MCM3 nuclear protein has a nuclear localisation signal, allowing MCM3 to direct the nuclear localisation of the protein bound to it (Takei and Tsujimoto, 1998). With entry into the quiescent state, MCM3 protein shows a delayed downregulation as p27 protein expression increases (Endl *et al*., 2001). Its expression is absent in differentiated cells (Musahl *et al*., 1998). Unlike Ki-67, MCM3 is expressed in non-proliferating cells and is therefore not a reliable proliferation marker (Endl *et al*., 2001).

MCM4

The MCM subcomplex of MCM 4, 6 and 7 has intrinsic ATP-dependent DNA helicase activity (Davey *et al*., 2003). Helicase activity is inhibited by the CDKs at S and G_2/M to enable cells to sustain arrested replication forks (Ishimi and Komamura-Kohno, 2001; Sogo *et al*., 2002). MCM4 participates in the DNA replication checkpoint (Ishimi *et al*., 2004). ATR-CHK1 and CDK2 phosphorylate MCM4 in response to DNA damage, inhibiting MCM helicase activity (Ishimi *et al.*, 2004; Zhu *et al*., 2005). When this phosphorylation is repressed, replication is re-initiated and genomic instability ensues. Cyclin B/CDC2 and cyclin A/CDK2 phosphorylate chromatin-detached MCM4 at G_2/M, inhibiting re-association with chromatin (Fujita *et al*., 1998; Findeisen *et al*., 1999). As MCM2 and MCM3/5 inhibit this activity, they may regulate MCM4/6/7 helicase function (Sato *et al*., 2000). MCM4 is therefore the critical member of the MCM complex that prevents re-replication (Ishimi *et al*., 2000). Increased MCM4 mRNA levels are seen in various tumours such as uterine cervical and oesophageal (Ishimi *et al*., 2003; Huang *et al*., 2005).

MCM5

The MCM5/MCM3 subcomplex activates STAT1 transcription in response to cytokines (Snyder *et al*., 2005). The STAT family of transcription factors regulate numerous cytokine-regulated gene transcriptions (Levy and Darnell, 2002). MCM5 expression increases in dysplastic cells (Murphy *et al*., 2005). Normally MCM5 is only expressed in proliferating cells and not in quiescent or differentiated cells (Stoeber *et al*., 2002). E2F regulates MCM5 promoter activity, thereby regulating its expression (Ohtani *et al*., 1999).

Antigen identified by monoclonal antibody Ki-67 (MKI67)

The nuclear non-histone protein Ki-67 functions in DNA organisation and maintenance, and ribosomal synthesis during mitosis (MacCallum and Hall, 2000). It is an important cell proliferative biomarker with phosphorylation sites for numerous kinases (Endl and Gerdes, 2000). Besides G_0, it is expressed at all stages of the cell cycle and is used as a measurement of tumour proliferation (Scagliotti *et al*., 1993). It is not expressed in differentiated cells but is expressed in cells that have been arrested, as they still have the potential to proliferate once arrest has been relieved (Nagata *et al*., 1998; Scholzen and Gerdes, 2000). Its expression correlates with apoptosis, with an inverse correlation with BCL2 (Lipponen, 1999; Bottini *et al*., 2001).

Expression is very low in normal breast tissue (Harper-Wynne *et al*., 2002; Clarke *et al*., 1997; de Lima *et al*., 2003). It is not expressed in ER+ normal breast tissues but is expressed in ER+ breast cancer cells (Clarke *et al*., 1997; Shoker *et al*., 1999). Breast cancers with mutated or overexpressed p53 have higher Ki-67 levels due to high proliferation (Bottini *et al*., 2001; Liu *et al*., 2001b; Moriki *et al*., 1996; Pietilianen *et al*., 1996). Overexpression in breast cancer correlates with increased histology grade, poor metastasis-free survival and death (Spyratos *et al*., 2002; Trihia *et al*., 2003; Colozza *et al*., 2005). The prognostic value of Ki-67 has also been validated in cancers of the lung, cervix, prostate and brain and in multiple myeloma, amongst others, correlating with increased aggressiveness and invasiveness (Shiba *et al*., 2000; Miguel-Garcia *et al*., 1995; Cummings *et al*., 2000; Ziotta and Schulman, 2000; Indinnimeo *et al*., 2000).

Mènage-á-trois (MNAT1/MAT1)

MAT1 is an essential component of the CAK complex, that is also comprised of CDK7 and cyclin H. It stabilises cyclin H and CDK7 association, and confers substrate specificity to CAK (Tassan *et al*., 1995; Rossignol *et al*., 1997; Busso *et al*., 2000). CAK is a component of the TFIIH complex, functioning in CDK activation and pol II-mediated transcription (Tassan *et al*., 1995). As a component of CAK and TFIIH, MAT1 enhances the phosphorylation of p53, RB and ERα (Ko *et al*., 1997; Wu *et al*., 2001; Chen *et al*., 2000b). It is also essential for the interaction of CAK with MCM7 (Wang *et al*., 2000b). MAT1 is frequently overexpressed in breast tumours (Talukder *et al*., 2003).

Meiotic recombination 11 homologue A (MRE11A)

MRE11A is an essential component of the MRE11/RAD50/NBS1 (MRN) complex. It is vital for proliferation and the repair of DSB ends and hairpins (Yamaguchi-Iwai *et al*., 1999; Paull and Gellert, 1998). It is phosphorylated in an NBS1-dependent manner following DNA damage (Dong *et al*., 1999). The MRN complex responds to the early phase of DNA damage response, associating with DSBs in an ATM-dependent manner, where it acts as a sensor for breaks (Mirzoeva and Petrini, 2001). It maintains genome stability by initiating homologous recombination, checkpoint activation and apoptosis (Lee and Paull, 2005). MRE11 has DNA unwinding function and unwinds DNA ends to induce ATM activity (Zhou *et al*., 2006; Lee and Paull, 2005). MRE11 aberrations are associated with sporadic tumours (Fukuda *et al*., 2001).

Nijmegen breakage syndrome 1 (NBS1)

NBS1 forms part of the MRN complex that detects and processes DSBs and enlists ATM to DNA damage sites (Stracker *et al*., 2004). ATM phosphorylates NBS1 to regulate MRN activity at the S and G_2/M checkpoints (Carson *et al*., 2003). Phosphorylated NBS1 in turn phosphorylates CHK2 to activate ATM (Lee and Paull, 2005). The MRN complex also maintains telomeres, preventing telomeric fusion and aberrant telomere recombination (Ranganathan *et al*., 2001). NBS1 is important in transporting MRE11 and RAD50 into the nucleus in conjunction with KPNA2. MRE11 and RAD50 are directly involved in DNA repair (Hopfner *et al*., 2001). NBS1 associates with BRCA1 at DNA damage sites and localises at stalled replication forks (Komatsu *et al*., 2007). NBS1 also associates with RPA, which interacts with ATR at damage sites (Shiloh, 2001; Stiff *et al*., 2005). It enhances ATR localisation at DNA damage sites to increase the ATR signal (Stiff *et al*., 2005).

Proliferating cell nuclear antigen (PCNA)

PCNA acts as a central loading platform in DNA replication and repair, coordinating the activity of multiple proteins (Maga and Hubscher, 2003). It forms a ring around DNA to mediate protein interaction with DNA, and is essential for nucleotide excision repair, base excision repair, mismatch repair and DSB repair (Shivji *et al*., 1992; Levin *et al*., 2000; Jiricny, 2006; Dorazi *et al*., 2006). The RFC complex loads PCNA onto DNA during DNA replication. PCNA, however, accumulates independently of the complex at DNA repair sites (Hashiguchi *et al*., 2007). p21 and RB bind to PCNA to inhibit DNA replication (Maki and Howley, 1997; Sever-Chroneos *et al*., 2001). PCNA has a high

expression in proliferating cells (Kawakita *et al*., 1992). It is a proliferation marker but may not be as reliable as Ki-67 as it functions in DNA repair as well as DNA replication (Prosperi, 1997).

RAD1

RAD1 forms part of the 9-1-1 DNA damage response complex and functions in checkpoint activation (Bermudez *et al*., 2003). The 9-1-1 complex identifies DNA lesions as part of the checkpoint response and functions in checkpoint signalling pathways (Burtelow *et al*., 2000).

RAD17

RAD17 is important in genomic stability and essential in ATR-dependent checkpoint signal induction (Wang *et al*., 2003c). RAD17 protein associates with chromatin and is required for recruiting the 9-1-1 complex to chromatin in an ATR-dependent or – independent manner. It is also required for activating CHK1 in response to DNA damage. ATM phosphorylates RAD17 in response to DSBs and ATR maintains this phosphorylation (Zou *et al*., 2002). This phosphorylation is required for the S-phase checkpoint but not for the G_2/M checkpoint (Wang *et al*., 2006c). Cells devoid of RAD17 accumulate DSBs to induce genomic instability (Wang *et al*., 2003c).

RAD17 mutant cells initiate a second round of DNA synthesis before mitosis. RAD17, ATR and cyclin/CDK associate with fired origins to prevent re-initiation. Alternately, the ATR/RAD17 pathway produces an ongoing replication signal that blocks licensing of

origins (Wang *et al*., 2003c). ATR-dependent signalling is repressed in the absence of RAD17 or RAD1 (Bao *et al*., 2004).

RAD51

The RAD51 recombinase binds to ssDNA to promote homologous pairing between two DNA duplexes in homology-directed repair (Sung and Klein, 2006). In homologous recombination, broken DSB ends are repaired using homology to intact DNA sequences. RAD51, BRCA1 and BRCA2 form foci at DNA damage sites (Chen *et al*., 1999b). p53 inhibits the formation of RAD51 nuclear foci in response to DSBs. RAD51 interacts with p53 and consequently is associated with cell cycle regulation (Marmorstein *et al*., 1998).

BCL2 and BCL-xL overexpression inhibits RAD51-mediated homologous recombination (Saintigny *et al*., 2001). Elevated RAD51 protein in tumour cell lines correlates with increased rates of recombination, promoting chromosomal rearrangement and aneuploidy (Vispe *et al*., 1998; Richardson *et al*., 2004). Overexpression is also associated with p21 upregulation, transcriptional downregulation of MMR genes and increased p53 levels (Raderschall *et al*., 2002; Orre *et al*., 2006). p21 induces cell cycle arrest in cells overexpressing RAD51 (Henning and Sturzbecher, 2003). As cells are arrested at G_2 when homologous recombination is most active, this arrest may increase homologous recombination and increase genomic instability since improper recombinations have a greater probability of occurring e.g. translocations (Klein, 2008). The inactivation of both alleles of RAD51 allows chromosome aberrations to accumulate, causing cell death (Sonoda *et al*., 1998).

RAD51 nuclear foci increase in colon cancer cell lines (Raderschall *et al*., 2002). Expression is decreased in breast tumours (Soderlund *et al*., 2007). LOH of the RAD51 gene region is associated with ER- status, PR- status, increased histologic grade and increased tumour stage (Gonzalez *et al*., 1999). Different studies reveal both high and low RAD51 protein expression in breast cancer (Maacke *et al*., 2000; Yoshikawa *et al*., 2000).

RAD9A

RAD9 is an essential component of the 9-1-1 complex that responds to DNA damage and enhances cell viability (Brandt *et al*., 2006). RAD9 maintains genomic integrity by regulating the DNA damage response, controlling the checkpoints, DNA repair, apoptosis, regulating transcription and synthesising ribonucleotides (Ishikawa *et al*., 2006). RPA associates with ssDNA to stimulate RAD17-RFC clamp loader recruitment. RAD9 shuttles 9-1-1 into the nucleus and directs it to RPA-associated sites, and the RAD17-RFC clamp loads 9-1-1 around DNA (Hirai and Wang, 2002; Wu *et al*., 2005; Zou *et al*., 2003; Bermudez *et al*., 2003). Its localisation at DSBs is independent of ATM but it is phosphorylated by ATM for G_1/S checkpoint activation (Greer *et al*., 2003).

Chromatin-bound 9-1-1 promotes ATR-dependent CHK1 phosphorylation and activation at the G_1/S and G_2/M checkpoints following DNA damage (Bao *et al*., 2004; Roos-Mattjus *et al*., 2003). Furthermore, it activates p21 transcription to contribute to the G_1/S checkpoint (Yin *et al*., 2004). 9-1-1 is also associated with enzymes functioning in base

excision repair (Brandt *et al*., 2006). RAD9 interacts with anti-apoptotic BCL2 and BCL-xL to induce apoptosis (Lindsey-Boltz *et al*., 2004). It is located at 11q13.1-13.2. This region is frequently amplified in cancers and is also the location of the CCND1 gene (Lieberman *et al*., 1996; Schwab, 1998). Amplification of this region correlates with poor prognosis in breast cancer (Borg *et al*., 1991; Cheng *et al*., 2005).

Retinoblastoma susceptibility gene (RB)

RB encodes a tumour suppressor protein, with mutations in a number of cancers (Cavanee *et al*., 1983; Friend *et al*., 1987; Weinberg, 1992). It is an essential factor in tumour suppression, maintaining stem cells, tissue regeneration, development and differentiation (Maddika *et al*., 2007). Phosphorylation of the RB protein by CDKs, particularly cyclin D/CDK4, inactivates its tumour suppressor properties. These complexes are activated by p16 inactivation or p27 degradation (Miwa *et al*., 2006). Hypo-phosphorylated activated RB arrests cells at G_1, S and G_2 in response to DNA damage and protects them from apoptosis (Harrington *et al*., 1998; Knudsen *et al*. 2000). Caspases cleave the RB protein, prompting its degradation during apoptosis (Tan and Wang, 1998). Increased MDM2 expression incites the degradation of RB and p53 through the ubiquitin-proteasome pathway (Uchida *et al*., 2005).

Hypo-phosphorylated RB antagonises E2F and its heterodimer partner DP (Jackson and Pereira-Smith, 2006). The RB/E2F complex regulates apoptosis and maintains genomic and chromosomal integrity (Tell *et al*., 2006). At G_1/S, CDKs are hyper-phosphorylated and active. RB is then unable to interact with E2F (Blais and Dynlacht, 2004; Dimova

and Dyson, 2005). This leaves E2F free to activate genes involved in promoting DNA synthesis and cell cycle progression (Harbour and Dean, 2000). Mutations also affect the ability of RB to repress E2F. In neoplasias, 80% have mutations in genes encoding the proteins involved in the cyclin D/CDK-INK4-RB-E2F cascade or mutations in their upstream regulators (Ortega *et al*., 2002).

Of the genetic abnormalities occurring in the RB pathway, more than 80% are implicated in cancer (Miwa *et al*., 2006). RB is frequently inactivated but only in selected cancers such as retinoblastoma, bladder carcinoma and osteosarcoma (Sherr and McCormick, 2002; Deshpande *et al*., 2005; Weinberg, 1995). It is rarely inactivated in colon carcinoma while RB function is lost in 17-26% of breast tumours (Ali *et al*., 1999; Dublin *et al*., 1998).

Retinoblastoma binding protein 8 (RBBP8)

RBBP8 is a co-factor for CtBP, which represses the transcriptional activity of several DNA-binding transcriptional factors (Meloni *et al*., 1999). RBBP8 also associates with RB, BRCA1 and ATM (Meloni *et al*., 1999; Yu and Chen, 2004; Li *et al*., 2000). Following DNA damage, ATM-dependent phosphorylation of RBBP8 relieves transcriptional repression. p21 and GADD45 are subsequently upregulated to inhibit CDC2 at the G_2/M checkpoint and suppress mitosis (Li *et al*., 1999; Li *et al*., 2000). RBBP8 is also phosphorylated by CDK activity. RBBP8 binds to its promoters and CCND1 to activate the transcription of RBBP8 and cyclin D1 at G_1/S transition (Liu and Lee, 2006). After the removal of RB from its promoter, RBBP8 promotes E2F

transcriptional activation to induce S-phase entry. BRCA1 is able to bind to phosphorylated RBBP8 to activate CHK1 in an ATM/ATR-dependent manner to induce G_2/M checkpoint activation, and repress specific genes during proliferation and differentiation (Wu and Lee, 2006). RBBP8 levels peak at G_2/M in association with the G_2/M checkpoint (Yu *et al*., 2006). It is also present at the G_1/S transition (Liu and Lee, 2006).

The RBBP8/BRCA1/ZBRK1 complex suppresses specific genes in mammary gland differentiation. When this repression is lifted, mammary epithelial outgrowth is promoted and differentiation is lost, as seen in breast cancers with mutated BRCA1 (Wu and Lee, 2006). RBBP8 gene aberrations occur in some cancer cells including colon cancer (Wong *et al*., 1998; Vilkki *et al*., 2002).

Retinoblastoma like 1 (RBL1)

RBL1 encodes the pocket protein p107, which inhibits the transcriptional activity of E2F4 and E2F5 (Lipinski and Jacks, 1999). Binding of the pocket proteins to E2F represses the transcription of genes in DNA synthesis, cell cycle progression, differentiation and DNA damage checkpoints (Cam and Dynlacht, 2003; Stevaux and Dyson, 2002). This regulation is essential to limit S-phase entry e.g. entry is inhibited during DNA damage.

p107 inhibits cyclin E/CDK2 and cyclin A/CDK2 activity to impede cell cycle progression (Woo *et al.* 1997). It also promotes the transcription of differentiation-

related genes (Vanderluit *et al*., 2007). p107 inhibits SKP2 expression and promotes the proteasomal degradation of SKP2 to increase p27 accumulation. This inhibits CDK2 and G_1 progression (Rodier *et al*., 2005). In association with RB, p107 inhibits various carcinomas and sarcomas (Dannenberg *et al*., 2004).

Retinoblastoma-like 2 (RBL2)

RBL2 encodes the p130 pocket protein. It is located at 16q12.2, a region with frequent alterations in cancers such as breast (Yeung *et al*., 1993). p130 maintains G_0 and regulates G_1/S transition (Grana *et al*., 1998). It inhibits E2F4 and E2F5 transcriptional activity to inhibit numerous cell cycle genes (Lipinski and Jacks, 1999; Ren *et al*., 2002). p130 accumulates at G_0 and protein levels decrease with cell cycle entry through proteasomal degradation (Tedesco *et al*., 2002; Grana *et al*., 1998). Cyclin D/CDK4, cyclin D/CDK6, cyclin A/CDK2 and cyclin E/CDK2 phosphorylate and inactivate p130 in arrested and terminally differentiated cells (Canhoto *et al*., 2000; Garriga *et al*., 1998b; Hansen *et al*., 2001). RB prevents p130 and p107 from inducing E2F4 and E2F5 transcriptional activity at G_0 and early G_1 (Vairo *et al*., 1995; Beijersbergen *et al*., 1994). p130 also inhibits cyclin E/CDK2 and cyclin A/CDK2 activity to induce G_1 arrest (Woo *et al*., 1997; Hansen *et al*., 2001). At late G_1, phosphorylation of the pocket proteins by cyclin E/CDK2 dissociates them from E2F. E2Fs are now able to induce transcription of genes for S-phase entry (Sun *et al*., 2007).

Replication protein A3 (RPA3)

RPA forms part of the origin recognition complex and functions in stabilising ssDNA during DNA replication (Bochkarev *et al*., 1997). It also participates in DNA recombination and repair, apoptosis and gene expression (He *et al*., 1995; Iftode *et al*., 1999). ATM phosphorylates a range of proteins to regulate the S-phase checkpoint, including RPA. This inhibits the progression of DNA replication. The RPA/ssDNA complex promotes the recruitment of RAD17/RFC complex, which induces the phosphorylation and activation of CHK1 (Foray *et al*., 2003). The RPA protein has three subunits: RPA1, RPA2 and RPA3 (Wold, 1997). RPA1 and RPA2 proteins are widely expressed in colon carcinoma, implying an involvement in cancer growth and progression (Givalos *et al*., 2007). RPA3 is important for RPA trimerisation (Bochkareva *et al*., 2000).

SERTA domain-containing 1 (SERTAD1)

SERTAD1 is important for transcriptional regulation (Aasland *et al*., 1995; Marmorstein *et al*., 2001). It promotes E2F1/DP1 transcriptional activity to promote G_1/S progression. RB inhibits E2F1/DP1/SERTAD1 (Hsu *et al*., 2001). SERTAD1 also promotes cell growth (Sim *et al*., 2006). It binds to CDK4 to inhibit p16, alleviating p16 inhibition of cyclin D/CDK4 (Sugimoto *et al*., 1999). Protein overexpression promotes anchorage-independent growth and tumourigenesis (Tang *et al*., 2005b; Tang *et al*., 2002). It is overexpressed in ovarian carcinomas, ovarian cancer cell lines and head and neck carcinomas, where it enhances aberrant cell growth by increasing resistance to p16 (Tang *et al*., 2002; Li *et al*., 2005; Tang *et al*., 2005b).

S-phase kinase-associated protein 2 (SKP2)

SCF^{SKP2} is a member of the SCF E3 ligases and targets proteins involved in cell cycle progression for ubiquitin-mediated proteolysis including p21, p27 and cyclin D1 (Kim *et al*., 2003; Bornstein *et al*., 2003; Nakayama *et al*., 2000; Ganiatsas *et al*., 2001). It induces degradation of the FOXO tumour suppressor, releasing cyclin D from its inhibition (Huang *et al*., 2005b; Schmidt *et al*., 2002). SKP2 promotes proliferation and oncogenesis, and enhances adhesion-independent tumour cell growth (Signoretti *et al*., 2002). Expression at late G_1 induces SCF^{SKP2} translocation to the nucleus to promote ubiquitination (Nakayama *et al*., 2000).

SKP2 loss causes CKI accumulation, hypophosphorylates active RB and inhibits G_1/S progression (Hara *et al*., 2001; Nakayama *et al*., 2004). Its overexpression induces S-phase entry (Gstaiger *et al*., 2001; Chiarle *et al*., 2002). It is overexpressed in approximately 50% of breast cancers and is associated with a poor prognosis (Traub *et al*., 2006; Signoretti *et al.*, 2002). High levels of SKP2 are inversely correlated with p27 expression in breast cancer due to increased degradation (Signoretti *et al*., 2002). High levels also correlate with increased cyclin A, cyclin B1, cyclin E and CDK2 in primary colorectal carcinomas, and with increased Ki-67 in colorectal adenomas and primary carcinomas. This overexpression promotes colorectal carcinogenesis and metastasis, and is also associated with a poor prognosis. Expression increases with progression from normal mucosa to adenoma to primary carcinoma, from mild to severe dysplasia in adenomas, and from primary carcinoma to lymph node metastasis (Li *et al*., 2004).

Small ubiquitin-related modifier (SUMO1)

SUMO1 protein is homologous to ubiquitin (Muller *et al.*, 2001). It binds to and modifies ubiquitin-like proteins post-translationally and regulates nucleocytoplasmic transport, transcription, intra-nuclear targeting, chromosome segregation, DNA repair, DNA recombination and genome stability (Bossis and Melchior, 2006; Muller *et al.*, 2001; Seeler *et al.*, 2007). SUMO1 inhibits BRCA1 transcriptional activity by stimulating HDAC activity at GADD45A, p27 and p21 promoters, which are regulated by BRCA1. This causes histone deacetylation and transcriptional repression (Park *et al.*, 2008). SUMO1 modifies MDM2 to protect it from self-degradation and increase p53 degradation (Buschmann *et al.*, 2000).

Transcriptional factor DP-1 (TFDP1)

TFDP1 encodes transcriptional factor DP-1, which forms heterodimers with the E2F transcription factors (Bandara *et al.*, 1993). DP1 and DP2 cannot bind to DNA without E2F association (Zhang *et al.*, 1995). E2F/DP1 binds to E2F sites to activate the transcription of proteins involved in G_0/G_1, G_1/S, apoptosis, DNA synthesis and oncogenic transformation (Schulze *et al.*, 1995; Shan *et al.*, 1996). It promotes apoptosis by promoting BAD, BAK1 and BID expression and p53 function, and inhibiting NF-κB (Stanelle *et al.*, 2002; Hitchens and Robbins, 2003). p53 competes with E2F1 for binding to DP1 and decreases E2F/DP complexes (Sorensen *et al.*, 1996).

TFDP1 expression levels correlate with CCNE1 levels, which promote G_1/S progression (Yasui *et al.*, 2002). Cyclin A phosphorylates E2F/DP1 to promote S-phase progression

(Krek *et al*., 1995). This phosphorylation also increases E2F/DP/RB association where RB inhibits E2F/DP transcriptional activity (Peeper *et al*., 1995). DP1 upregulation is associated with p21 and inhibits entry into the S-phase (Magae *et al*., 1999). It is overexpressed in tumours with levels upregulated in various cell lines and tissues (Yasui *et al*., 2002; Gopalkrishnan *et al*., 1996; Wu *et al*., 1995; Yasui *et al*., 2003).

Transcriptional factor DP-2 (TFDP2)

DP2 binds to E2F proteins to enhance the DNA binding and transcriptional activity of E2F (Zhang and Chellappan, 1995). Like DP1, DP2 affects the phosphorylation, localisation and expression level of E2F/DP (Hitchens and Robbins. 2003). E2F/DP is important in regulating the cell cycle, DNA replication, RB function and apoptosis (Dyson, 1998).

Tumour protein p53 (TP53)

p53 deletions or mutations occur in 50% of cancers. The other 50% usually exhibit inactivated wild-type p53 through genetic and epigenetic alterations (Olivier *et al*., 2002; Vousden and Lu, 2002). p53 can induce either cell cycle arrest or apoptosis. Low stress levels or DNA damage induces the transcription of growth arrest genes. G_1 arrest allows for DNA damage to be repaired, preventing potentially harmful mutations from being replicated. With very high cellular stress, if the damage is irreparable, p53 induces apoptosis to maintain genomic integrity (Maddika *et al*., 2007). Stress stimuli such as DNA damage, oncogene activation and hypoxia increase p53 stability. The resulting

protein accumulation induces cell cycle arrest, DNA repair, senescence, differentiation, apoptosis and inhibits angiogenesis (Finlan and Hupp, 2005; Giono and Manfredi, 2006).

p53 upregulates GADD45, p21 and p48 to promote DNA repair and BAX, p53-AIP and FAS/APO1/CD95 to induce apoptosis (Maddika *et al*., 2007; El-Diery *et al*., 1994; Miyashita and Reed, 1995). CHK1/CHK2 and ATM/ATR phosphorylate p53 at the G_1 checkpoint in response to DNA damage to produce a sustained cell cycle arrest. MDM2 is the E3 ubiquitin ligase of p53. ATM/ATR target MDM2 following DNA damage, leading to p53 accumulation and an increase in p53 transcriptional activity. p53 identifies dysfunctional telomeres as DNA damage, consequently increasing p21 expression to prevent phosphorylation and inhibition of RB (Sherr and Roberts, 1999). Inactivated p53 or RB allows cells to proliferate in spite of dysfunctional telomeres (Hara *et al*., 1991).

Ubiquitin-activating enzyme E1 (UBE1)

UBE1 is an E1 activating enzyme that activates ubiquitin in an ATP-dependent manner. It induces the initial steps of the ubiquitin-conjugating systems and is therefore important for regulation of this system (Varshavsky, 1997). UBE1L is located on chromosome 3p21 which is frequently deleted in pre-neoplastic and neoplastic epithelial tissues (Zabarovsky *et al*., 2002). As an activating enzyme, UBE1L is essential in ubiquitin conjugation (Pickart, 2001). Besides proteolysis, it also functions in DNA repair and cell cycle progression (Jentsch *et al*., 1990; Zacksenhaus and Sheinin, 1990; Handley-Gearhart *et al*., 1994). High levels of UBE1L expression correlate with low levels of

cyclin D1 expression, therefore it may inhibit cyclin D1 (Lonardo *et al*., 1999; Pitha-Rowe *et al*., 2004). UBE1L expression is inhibited in cancer cell lines (Pitha-Rowe *et al*., 2004b). It is a tumour suppressor in lung cancer (Kok *et al*., 1993).

CHAPTER TWO: METHODS AND MATERIALS

For details on the reagents and solutions used in this study, please consult Appendix I.

2.1 Materials

Higher degree approval was obtained from the Postgraduate Committee at the University of the Witwatersrand on 7 July 2006. Ethics approval for use of the human cell lines was obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, protocol number M060410, on 19 June 2006.

2.1.1 Cell lines

The human colon epithelial HT-29 cell line was purchased from European Collection of Cell Cultures (ECACC). The human breast epithelial-like MCF-7 cell line was purchased from ECACC. The human breast epithelial cell line MDA-MB-231 was accessed from frozen stock in the Division of Medical Oncology. The normal breast epithelial cell line 184A1 was purchased from American Type Culture Collection (ATCC). Following drug treatment, the cell lines were used for flow cytometry for analysis of the cell cycle and for RNA extractions for purposes of gene expression studies.

2.1.2 Drugs

These cells were exposed to the chemotherapeutic drugs, curcumin (Sigma, Steinheim, Germany), SAHA (courtesy of Merck Research Laboratories, New Jersey, USA), lycopene (Sigma, Steinheim, Germany) and thalidomide (Sigma, Steinheim, Germany). As controls, cells were also treated with the solvents, DMSO (Sigma, Missouri, USA) and THF/BHT (Sigma, Steinheim, Germany). These drugs were selected because of their

potential use in South African cancer patients and for the interesting results that have been obtained with them in research studies (**chapter one**). These treated cells were used for flow cytometry and consequently, RNA extractions.

2.2 Methods

2.2.1 Cell culture

2.2.1.1 Medium

HT-29

HT-29 colon cancer cells are cultured in a DMEM/F-12 1:1 mixture (Lonza, Maryland, USA) containing L-glutamine, 15mM HEPES buffer and 3.151g/l glucose, and supplemented with 2% foetal bovine serum (FBS) (Invitrogen Corporation, New York, USA), and penicillin/streptomycin (Lonza, Maryland, USA) (**appendix 1**). Cells are cultured in 75cm^2 cell culture flasks.

MCF-7

MCF-7 breast cancer cells are cultured in MEM Eagle with Earle's BSS (EMEM) medium (Lonza, Maryland, USA) containing L-glutamine. This is supplemented with 10% FBS and penicillin/ streptomycin (**appendix 1**). Cells are cultured in 25cm^2 cell culture flasks.

MDA-MB-231

MDA-MB-231 breast cancer cells are cultured in DMEM/F-12 with L-glutamine, 15mM HEPES buffer, 3.151g/l glucose, and supplemented with 10% FBS and penicillin/streptomycin (**appendix 1**). Cells are cultured in 25cm^2 cell culture flasks.

184A1

184A1 normal breast cells are cultured in MEGM medium (Lonza, Maryland, USA) supplemented with 0.005mg/ml transferrin (Sigma, Missouri, USA) and 1ηg/ml cholera toxin (Sigma, Steinheim, Germany) (**appendix 1**). Transferrin provides iron to the cells, and cholera toxin stimulates human epithelial cell growth. Cells are cultured in 25cm^2 cell culture flasks.

2.2.1.2 Subculturing cells

1. Cells are grown at 37ºC in 5% CO_2 in an incubator. Medium is changed every two-four days depending on the change in pH, indicated by the colour of the medium.
2. Cells reach 100% confluency after approximately 2-4 days. The cells are washed with 2-5ml of phosphate buffered saline (PBS) (Sigma, Steinheim, Germany).
3. 2ml of trypsin/versene (Highveld Biological, Lyndhurst, South Africa) is added to detach the cells from the flask's surface.
4. 5ml of 10% PBS/FBS is added to deactivate the trypsin/versene and the cells are centrifuged for 4 minutes at low speed. The solution is poured off.
5. The pellet is suspended in 4-10ml of FBS-containing medium and divided between two flasks.

6. Alternatively, the pellet is suspended in 1ml of FBS-containing medium and 1ml of cryoprotective medium (Cambrex, Maryland, USA) at 4ºC. This is divided between two 1ml cryovials and frozen at -70˚C as stock.

2.2.1.3 Drug treatment

Cells at 80% confluency are serum-starved overnight (i.e. kept in FBS-free medium) to synchronise cells at G_0/G_1. Thus, with the introduction of the drug, all cells are influenced from the same point in the cell cycle. The cells were at near confluence when the serum-free treatments were added to test the survival of the cells in relation to the treatments.

Curcumin

1. A 5mM curcumin stock solution in DMSO is made up (**appendix 1**).
2. The medium is removed from the cells and they are starved overnight in serum-free medium.
3. The cells are exposed to different concentrations of curcumin (25µM, 50µM, 75µM and 100µM) for different periods (12, 24, 48 and 72 hours). This does not contain serum.
4. The control cells are treated with 50µl DMSO in 10ml curcumin-free medium for the respective periods.

SAHA

1. A 5mM stock solution of SAHA/Zolinza is made up with DMSO and stored at -20˚C (**appendix 1**).
2. Dilutions of 1µM, 2µM, 5µM and 7.5µM are made up fresh with medium lacking serum and added to serum-starved cells at 80% confluence for 12, 24, 48 and 72 hours (**appendix 1**).
3. 10ml SAHA-free medium containing 50µl DMSO is added to control cells for the respective times.

Lycopene

1. A 2mM stock solution of lycopene is made up with tetrahydrofuran with 250ppm butylated hydroxytoluene (THF/BHT) (**appendix 1**).
2. Fresh dilutions of 2µM, 5µM, 10µM and 20µM are made up in serum-free medium and added to 80% confluent, serum-starved cells at concentrations for 12, 24, 48 and 72 hours (**appendix 1**).
3. Control cells are treated with 5µl THF/BHT in 5ml lycopene-free medium for the respective times.

Thalidomide

1. A 10mM stock solution of thalidomide is made up with DMSO (**appendix 1**).
2. Fresh dilutions are made up in serum-free medium and added to 80% confluent, serum-starved cells at 10µM, 30µM, 100µM and 300µM for 12, 24, 48 and 72 hours (**appendix 1**).

3. Control cells are treated with 6µl DMSO in 5ml thalidomide-free medium for the respective times.

2.2.2 Flow Cytometry

With flow cytometry, various cellular parameters can be quantified. For the purposes of this study, the BD FACSCalibur (BD Biosciences) system was used to quantify the DNA present at each of the three interphase stages of the cell cycle. Single cells with propidium iodide-stained DNA pass in front of a laser beam in the flow cytometer. Each cell absorbs light and emits fluorescence that is proportional to the DNA content of the cell. This measured fluorescence is digitally converted to an electric pulse and the data is visualised as DNA histograms (Dey, 2004).

2.2.2.1 Isolating and staining cells

1. Drug-treated and control cells are harvested by collecting the medium in a centrifuge tube and spinning for 4 minutes at low speed.
2. The remaining cells that are still attached to the flask surface are released by adding 2ml of trypsin/versene.
3. Upon detachment, 5ml of 10% PBS/FBS is added to deactivated the trypsin/versene and centrifuged for 4 minutes at low speed.
4. The solution is poured off and the pellet re-suspended in the remaining solution. This is added to the pellet collected above.
5. The cells are washed with 5ml of PBS. This is aspirated into a single cell state by pipetting.

6. 1-2ml of cold 70% ethanol (Merck, Gauteng, South Africa) is added drop-wise to the centrifuge tube during vortexing.
7. The fixed cells are held at -20˚C overnight.
8. The cells are washed twice in 5ml of PBS. 1-2ml of PBS is added to the final re-suspended pellet.
9. 20µl of 0.1mg/ml RNase A (Sigma, Steinheim, Germany) is added to eliminate RNA from the solution.
10. 70µl of 0.5mg/ml propidium iodide (Boehringer Mannheim, Mannheim, Germany) is added to stain the DNA.
11. The cells are held at 4˚C overnight and are run on the BD FACSCalibur in the morning.
12. The results generated are exported into ModFit LT^{TM} (Verity Software House) for analysis.

2.2.3 RNA extraction

RNA of high integrity, concentration and purity is necessary for the downstream applications i.e. SuperArray PCR array and Genefishing Differential Display. The Qiagen RNeasy Mini Kit (Hilden, Germany) is used for the purification of total RNA from the control and treated cells, using a silica-based membrane and centrifugation protocol. All centrifugations are at 10 000rpm.

1. 2ml of trypsin/versene is added to cells. Upon detachment, 5ml of 10% PBS/FBS is added and the solution is centrifuged at low speed for 4 minutes. The solution is poured off and the pellet is re-suspended in the remaining solution.

2. 350µl of Buffer RLT is added to the pellet obtained from the breast cells (25cm^2 culture flasks), and 600µl of Buffer RLT is added for HT-29 cells (75cm^2 culture flasks).
3. The lysate is pipetted into a QIAshredder spin column in a 2ml collection tube and centrifuged for 2 minutes.
4. An equal volume of 70% ethanol is added to the lysate and mixed by pipetting.
5. The sample is transferred to an RNeasy spin column in a 2ml collection tube, and centrifuged for 15 seconds. The flow-through is discarded.
6. 700µl of Buffer RWI is added. This is centrifuged for 15 seconds and the flow-through discarded.
7. 500µl of Buffer RPE is added to the spin column and centrifuged for 15 seconds. The flow-through is discarded.
8. 500µl of Buffer RPE is added to the spin column and centrifuged for 2 minutes.
9. The spin column is placed in a new 2ml collection tube and centrifuged for 1 minute.
10. The spin column is placed in a 1.5ml collection tube and 30-50µl of RNase-free water is added to the membrane. This is centrifuged for 1 minute to elute the RNA.
11. 1µl of sample is run on the Nanodrop 1000 (Thermo Scientific) spectrophotometer to calculate the concentration and purity of RNA by taking readings at 280ηm, 260ηm and 230ηm.
12. RNA is run on a 1% denaturing agarose (Bio-Rad, California, USA) gel stained with ethidium bromide. This is visualised by UV light to ascertain the integrity of the rRNA bands.

2.2.4 Genefishing

The Seegene (Seoul, Korea) Genefishing^{TM} DEG101 and 102 Premix Kits identify differentially expressed genes using arbitrary primers and a two-stage PCR. It utilises a unique tri-partite primer design, with the 3' and 5' ends separated by a regulator. It claims to increase the sensitivity of PCR amplification and to be less expensive than other differential display methods. However, this was not found to be an effective experiment in terms of the amount of results generated. The PCR products are visualised on an agarose gel, cloned and sequenced.

2.2.4.1 Reverse transcription

This produces cDNA with the universal sequence of the primer (dT-ACP1) at the 5' end. The sequence of dT-ACP1 as provided by Seegene is:

dT-ACP1: 5'-CTGTGAATGCTGCGACTACGATXXXXX$(T)_{18}$-3'

All centrifugations are at 10 000rpm.

1. The reverse transcription cocktail is made up as follows:

   3µg total RNA

   2µl 10µM dT-ACP1

   RNase-free water to make up to 9.5µl

2. This is incubated in the Hybaid thermal reactor at 80˚C for 3 minutes and then on ice for 2 minutes.
3. The tube is centrifuged for 15 seconds and the following reagents added to it:

   4µl 5x RT buffer (Promega, Wisconsin, USA)

   5µl 2mM dNTP (Promega, Wisconsin, USA)

0.5µl 40u/µl RNase inhibitor (Promega, Wisconsin, USA)

1µl 200u/µl M-MLV reverse transcriptase (Promega, Wisconsin, USA)

4. The tube is incubated at 42˚C for 90 minutes and heated at 94˚C for 2 minutes.
5. It is kept on ice for 2 minutes and centrifuged for 15 seconds.
6. 80µl of DNase-free water is added to dilute the cDNA and this is stored at -20˚C.

2.2.4.2 PCR

The first-strand cDNA is used to amplify DNA complementary to the arbitrary primer (ACP1-20) used. It is used in conjunction with the dT-ACP2 primer. In the first stage, the 3' end of the arbitrary primer anneals to the first-strand cDNA. The second strand cDNA produced has the complementary sequence of the universal sequence of dT-ACP1 at its 3' end and the universal sequence of the arbitrary primer at its 5' end. In the second stage, dT-ACP2 binds to the 5' end of the second strand cDNA and the arbitrary primer binds to the 3' end. The cDNA is then amplified.

The sequence of dT-ACP2 as provided by Seegene is:

dT-ACP2: 5'-CTGTGAATGCTGCGACTACGATXXXXX$(T)_{15}$-3'

The primer sequences of the twenty arbitrary primers as provided by Seegene are:

ACP1: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGCCATCGACC-3'

ACP2: 5'-GTCTACCAGGCATTCGCTTCATXXXXXAGGCGATGCC-3'

ACP3: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCCGGAGGATG-3'

ACP4: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGCTGCTCGCG-3'

ACP5: 5'-GTCTACCAGGCATTCGCTTCATXXXXXAGTGCGCTCG-3'

ACP6: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGGCCACATCG-3'

ACP7: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCTGCGGATCG-3'

ACP8: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGGTCACGGAG-3'

ACP9: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGATGCCGCTG-3'

ACP10: 5'-GTCTACCAGGCATTCGCTTCATXXXXXTGGTCGTGCC-3'

ACP11: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCTGCAGGACC-3'

ACP12: 5'-GTCTACCAGGCATTCGCTTCATXXXXXACCGTGGACG-3'

ACP13: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGCTTCACCGC-3'

ACP14: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGCAAGTCGGC-3'

ACP15: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCCACCGTGTG-3'

ACP16: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGTCGACGGTG-3'

ACP17: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCAAGCCCACG-3'

ACP18: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCGGAGCATCC-3'

ACP19: 5'-GTCTACCAGGCATTCGCTTCATXXXXXCTCTGCGAGC-3'

ACP20: 5'-GTCTACCAGGCATTCGCTTCATXXXXXGACGTTGGCG-3'

1. These reagents are added to a PCR tube and kept on ice:

   3-7µl diluted first-strand cDNA (~50ηg)

   2µl 5µM arbitrary ACP

   1µl 10µM dT-ACP2

   10µl 2x SeeAmp ACP master mix

   Distilled water to make up to 20µl

2. The tube is placed in the BioRad MJ Mini thermal cycler preheated to 94˚C.
3. PCR is run as follows:

    1 cycle: 94˚C for 5 minutes

    1 cycle: 50˚C for 3 minutes

    1 cycle: 72˚C for 1 minute

    40 cycles: 94˚C for 40 seconds

    65˚C for 40 seconds

    72˚C for 40 seconds

    1 cycle: 72˚C for 5 minutes
4. The PCR products are run on a 2% agarose gel stained with ethidium bromide and viewed under UV light.
5. The differentially expressed bands are extracted from the gel.

2.2.4.3 Gel extraction

The QIAquick Gel Extraction Kit (Qiagen) is used to clean up DNA from bands excised from agarose gels. The downstream applications are then to clone the DNA for sequencing. It utilises a silica membrane spin-column technology. Centrifugations are at 13 000rpm.

1. The gel slice is weighed. 3 volumes of Buffer QG are added to 1 volume of gel.
2. The tube is incubated at 50ºC for 10 minutes until the gel has dissolved, with vortexing every 2-3 minutes.
3. 1 volume of isopropanol (Merck, Gauteng, South Africa) is added.

4. The sample is added to a QIAquick spin column in a 2ml collection tube. This is centrifuged for 2-5 minutes and the flow-through is discarded.
5. 0.5ml of Buffer QG is added to the column and centrifuged for 1 minute. The flow-through is discarded.
6. 0.75ml of Buffer PE is added to the column. This stands for 2-5 minutes and is centrifuged for 1 minute. The flow-through is discarded.
7. The column is centrifuged for 1 minute.
8. 30µl of distilled water at 55ºC is added to the column, and centrifuged for 2-5 minutes.
9. The DNA is stored at -20ºC for cloning.

2.2.4.4 Cloning

The Fermentas CloneJET^{TM} PCR Cloning Kit (Maryland, USA) is used to ligate the DNA isolated above into a pJET1.2/blunt cloning vector that only propagates with the ligation of a DNA insert. This is used to transform *E.cloni* cells to produce multiple clones for sequencing.

1. The blunting reaction is set up as follows:

    10µl 2x reaction buffer

    2µl DNA

    5µl nuclease-free water

    1µl DNA blunting enzyme

2. The mixture is vortexed and centrifuged at 10 000rpm for 5 seconds.
3. It is incubated at 70ºC for 5 minutes, and chilled on ice.
4. The following reagents are added to the blunting reaction mixture:

1µl 50ηg/µl pJET 1.2/blunt cloning vector

1µl 5u/µl T4 DNA ligase

5. This is vortexed and centrifuged at 10 000rpm for 5 seconds.
6. The ligation mixture is incubated at 22ºC for 5 minutes.

2.2.4.5 Transformation

The *E. cloni* Chemically Competent Cells (Lucigen® Corporation, Wisconsin, USA) were transformed with the ligated vectors prepared above to propagate numerous clones of the DNA insert for sequencing of the differentially expressed band.

1. Agar plates are prepared using YT agar powder (Lucigen® Corporation, Wisconsin, USA) and 50mg ampicillin (Roche, Mannheim, Germany) (**appendix 1**).
2. *E. cloni* cells are thawed on wet ice.
3. 40µl of *E. cloni* cells are added to a chilled culture tube.
4. 4µl of ligation reaction is added to the tube and mixed.
5. The mixture is incubated on ice for 30 minutes.
6. The tube is heated in a 42ºC water bath for 45 seconds.
7. The tube is chilled on ice for 2 minutes.
8. 960µl of Recovery Medium at room temperature is added.
9. The tubes are shaken at 250rpm for 1 hour at 37ºC.
10. 100µl of transformed cells are plated onto agar plates.
11. The plates are incubated at 37ºC overnight.
12. The colonies are sequenced at Inqaba Biotechnology Industries (Pty) Ltd (Johannesburg, South Africa).

2.2.5 PCR Array

The SuperArray (Maryland, USA) RT^2 Profiler PCR Array System is a real-time PCR-based profiling application to study pathway-focused (cell cycle) gene expression. Each array contains 96 primer sets for 84 cell cycle-related genes, five housekeeping genes, one genomic DNA control, three reverse transcription controls and three positive PCR controls. With this technique, the influence of each drug on the cell cycle-related genes was quantified in the respective cell lines.

2.2.5.1 First strand synthesis

The SuperArray RT^2 PCR Array First Strand Kit is used to convert RNA into first strand cDNA.

1. The annealing mixture is prepared as follows:

   1µg total RNA

   1µl primer and external control mix

   RNase-free water to make up to 10µl

   This is mixed by pipetting and centrifuged at 10 000rpm for 15 seconds.
2. This is incubated in the Hybaid thermal reactor for 3 minutes at 70˚C.
3. It is put on ice for 1 minute.
4. The RT cocktail is made up as follows:

   4µl 5x RT Buffer

   4µl RNase-free water

   1µl RNase inhibitor

1µl RT enzyme mix II

5. 10µl of RT cocktail is added to each 10µl of annealing mixture.
6. It is mixed by pipetting and incubated for 60 minutes at 37˚C in the Hybaid thermal reactor, and 5 minutes at 95ºC.
7. 91µl of double distilled water is added to each 20µl cDNA synthesis reaction. This first strand cDNA synthesis reaction is kept on ice until the next step.

2.2.5.2 Real-time PCR

This step employs SYBR Green I as the principle fluorescent dye and ROX as the reference dye. Using the cDNA produced from the step above, the 96 primer sets amplified the DNA in real-time. The amount of fluorescence emitted is proportional to the amount of PCR product.

1. The PCR cocktail is made up as follows in a 5ml tube:

   1275µl 2x SuperArray PCR master mix

   102µl diluted first strand cDNA synthesis reaction

   1173µl double distilled water

2. 25µl of this cocktail is pipetted into each well of the PCR array.
3. The array is sealed with optical thin-wall 8-cap strips and put on ice.
4. The program is set up on the ABI 7500 as follows:

   1 cycle for 10 minutes at 95˚C

   40 cycles for 15 seconds at 95˚C

   1 minute at 60˚C

5. The threshold cycle (C_t) for each well is calculated using the instrument's software.
6. The baseline values are set from cycle two to two cycle values before the earliest amplification, but not greater than fifteen.
7. The threshold values are set above the background signal in the linear phase of the amplification plot.
8. The threshold cycle values are exported to Excel.
9. The results are analysed using a SuperArray web-based analysis programme.

CHAPTER THREE: RESULTS

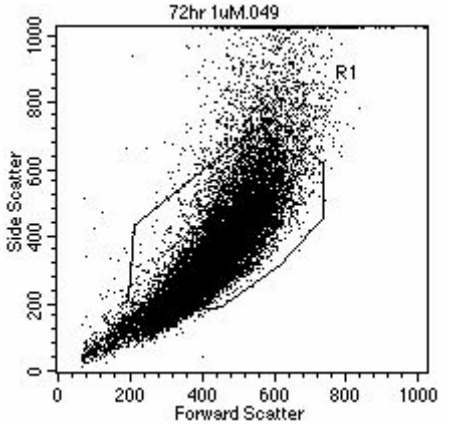
3.1 Flow Cytometry

Flow cytometry measures the relative DNA content of fluorescently stained cells. Cells are treated with RNase to digest RNA, ensuring that only DNA is stained. Cells must first be fixed, as propidium iodide is only able to cross the damaged plasma membranes of dead cells. Propidium iodide intercalates into the DNA helix of fixed, permeabilised cells. As a cell absorbs light from the laser beam in the flow cytometer, it emits fluorescence that is proportional to the DNA content of the cell. This fluorescence is read by various detectors (Dey, 2004). Cells at different stages of the cell cycle have a defined amount of DNA and different physical sizes. This allows for discrimination into the three interphase stages. Cells at G_0/G_1 have a diploid chromosomal DNA content; at S-phase cells have diploid-tetraploid DNA levels; at G_2 and M phase, cells have a diploid content and are bigger than cells at G_0/G_1.

3.1.1 HT-29 cell line

Time and dose trials are performed to establish the optimal length of drug exposure and the optimal drug concentration in each cell line. This is subject to variables such as variation from the control samples, high cell number, low debris and low level of aggregates. 10 000 – 15 000 gated events are read. The data is first collected in 1D scatter plots on the BD FACSCalibur software. A negative control is initially run to set the detectors. Side scatter (SSC) correlates with the complexity of the cell e.g. nucleus shape, granularity, membrane roughness, and forward scatter (FSC) correlates with cell volume. The results for HT-29 cells treated with 1µM SAHA for 72 hours are used as an

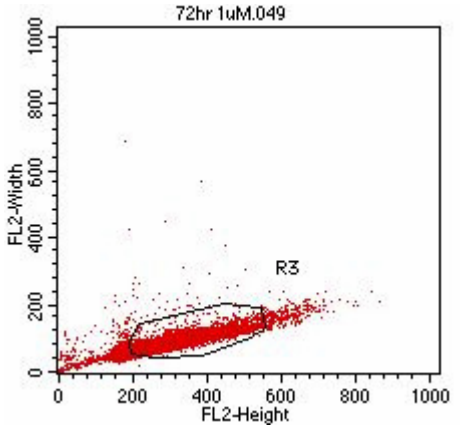
example to illustrate this set-up. The protocol is uniform for all the cell lines and treatments. The scatter plot in **figure 3.1** represents the data generated from 20 103 events. The gated area represents 11 149 events.



R1 = gate 1

Figure 3.1: Side scatter versus forward scatter dot plot for HT-29 cells treated with 1µM SAHA for 72 hours.

The maximum number of cells with the minimum amount of debris and apoptotic matter is selected by gating, to generate a second plot of height versus width (**figure 3.2**) Propidium iodide is detected at the FL2 channel of the flow cytometer. A second gate is set to further remove debris and aggregates from the selected population of cells.



R3 = gate 2

Figure 3.2: FL2-Width versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours.

The dot plot of side scatter versus FL2-Height provides a crude estimate of DNA distribution among the cell cycle stages (**figure 3.3**).

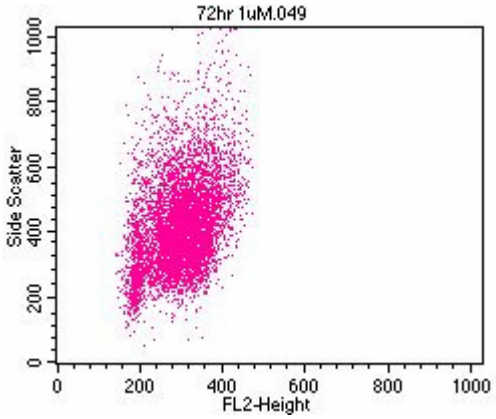


Figure 3.3: Side scatter versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours.

The population gated in **figure 3.2** is then represented in a histogram (**figure 3.4**), with the first peak correlating to G_0/G_1, the second peak corresponding to the S-phase and the area thereafter correlating to G_2/M. The ModFit-generated histogram defines these areas with higher precision (**figure 3.7**). Events occurring before 200 on the FL2-height axis represent debris, and events occurring after 600 represent aggregates.

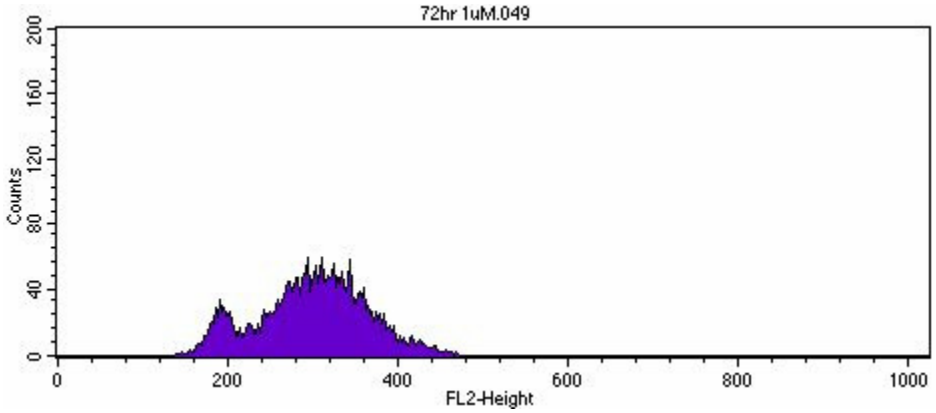
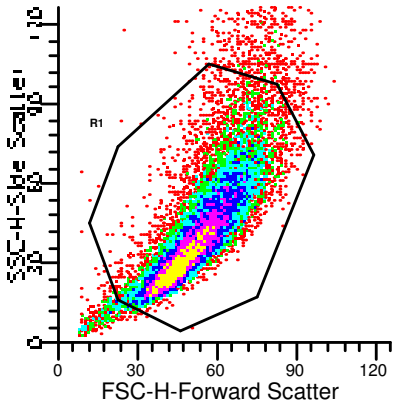


Figure 3.4: Counts versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours.

This data is exported into the ModFit LT^{TM} analysis software programme. This programme applies algorithms to model the three cell cycle stages, and to differentiate the overlap existing between G_0/G_1 and G_2/M. It eliminates debris and aggregates from the analysis for a more accurate calculation of the percentage of cells at each stage of the cell cycle. As with the data in the FACSCalibur analysis, a side scatter versus forward scatter dot plot is first generated, and the population representing the maximum number of cells with minimum debris and aggregates is gated, illustrated in **figure 3.5**. This analyses 16 340 events, with the gated region segregating 16 211 events. Therefore, the

number of events that are analysed by ModFit is significantly greater than that analysed by the FACSCalibur software. This allows for a greater representation of the cells used in the treatment.



R1 = gate 1

Figure 3.5: ModFit-generated side scatter versus forward scatter for HT-29 cells treated with 1μM SAHA for 72 hours.

The gated population is presented in a FL2-width versus FL2-height dot plot (**figure 3.6**). The population representing the maximum number of cells with minimum debris and aggregates is gated.

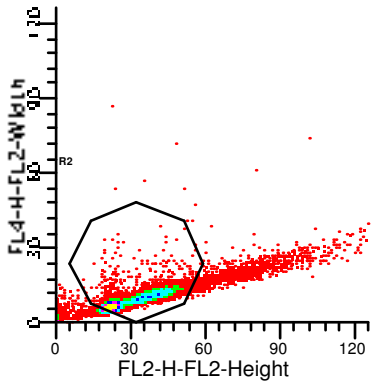


Figure 3.6: ModFit-generated FL2-Width versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours.

The population represented by this second gate is illustrated in a histogram (**figure 3.7**).

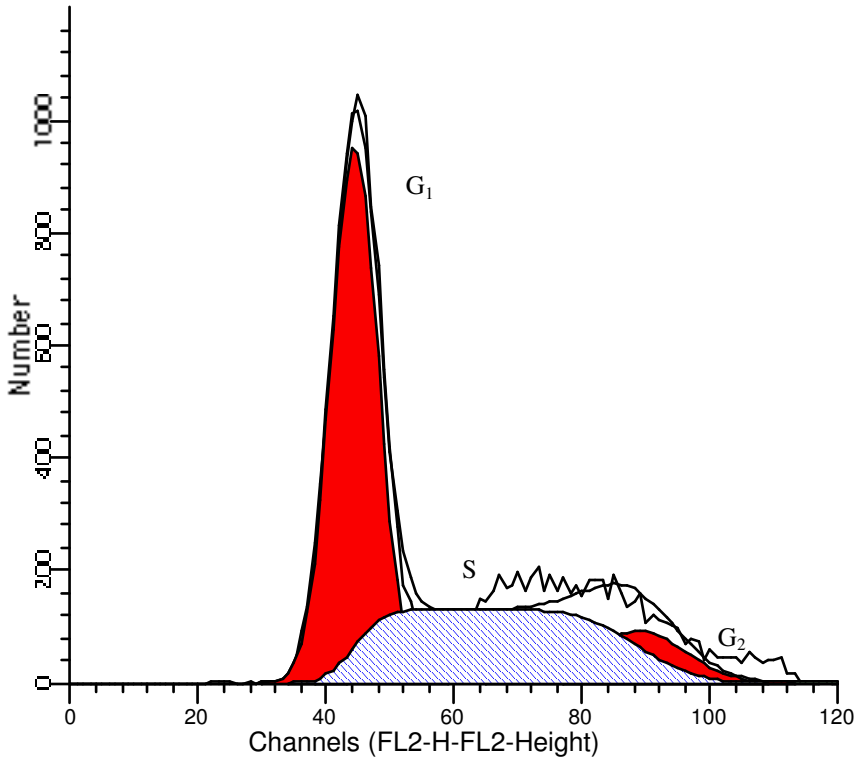


Figure 3.7: ModFit-generated DNA histogram of HT-29 cells treated with 1µM SAHA for 72 hours.

The percentages of DNA at each cell cycle stage are calculated from this histogram. With this programme, the differentiation of the three cell cycle phases is clearly defined. In addition, ModFit calculates the percentage of debris and aggregates included in the analysis, allowing one to minimise these values. While the majority of cells are diploid, aneuploidy is common in cancer cell lines and primary tumours. The optimal concentration and period of exposure is selected by identifying a substantial difference from the control treatment, an arrest of cells at G_1 and a high number of cells to indicate a low toxicity. For instance, **table 3.1** illustrates the percentages of DNA obtained by ModFit from the flow cytometry analysis using HT-29 cells treated with SAHA.

Table 3.1: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of SAHA to HT-29 cells.

The highlighted treatment is the optimal treatment used in this study.

	12 hours	**24 hours**	**48 hours**	**72 hours**
Control				
G_1	70.94	61.52	44.08	33.29
S	29.06	35.23	55.92	66.71
G_2	0	3.25	0	0
1µM				
G_1	51.81	58.42	43.86	53.87
S	48.19	40.18	56.14	35.77
G_2	0	1.40	0	10.96
2.5µM				

G_1	56.86	67.70	45.22	62.91
S	43.14	29.02	54.78	31.20
G_2	0	3.28	0	5.89
5µM				
G_1	62.64	58.47	66.73	36.48
S	37.36	36.71	33.27	63.52
G_2	0	4.83	0	0
7.5µM				
G_1	64.50	63.34	75.57	53.87
S	35.50	30.83	24.43	46.13
G_2	0	5.84	0	0

Confluent cells are serum starved overnight before the drug is introduced into the medium. This induces the synchronisation of cells at G_1. Predictably, at 12 hours there is little variation in cell cycle distribution from the control. SAHA inhibits tumourigenesis by arresting cells at G_1 and G_2/M. As the percentage of cells at G_2 is very low here, SAHA induces G_1 arrest in HT-29. While the highest arrest at G_1 is in cells treated with 7.5µM SAHA for 48 hours, there are considerably less cells in this treatment as the higher concentration induces greater cell death. This is therefore not a viable option, as a high cell number is needed for purposes of RNA extraction. The optimal treatment is 5µM for 48 hours as it has the second highest percentage of cells arrested at G_1, a high cell number, and the largest difference compared to the control. Similarly, Munster *et al.* (2001) observed complete growth arrest of MCF-7 cells treated with 5µM SAHA. The

analysis of this treatment is denoted in **figure 3.8**. Here, 66.73% of the DNA is at the G_1 phase, 33.27% is at the S-phase and 0% at the G_2 phase.

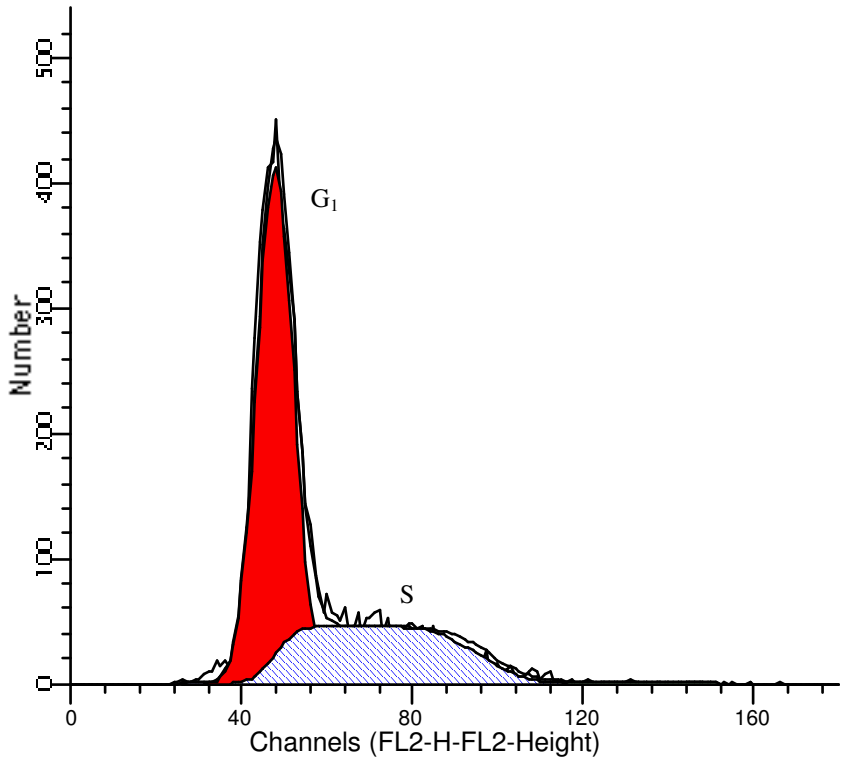


Figure 3.8: ModFit-generated DNA histogram of HT-29 cells treated with 5µM SAHA for 48 hours.

The optimal treatment for HT-29 cells treated with curcumin is 50µM curcumin for 24 hours. Curcumin induces G_2/M arrest in HT-29 (Hanif *et al.*, 1997). While high percentages at G_2 were not observed in these cells, the difference compared to the control cells is high. The control cells are analysed in **figure 3.9** and the treated cells in **figure 3.10**.

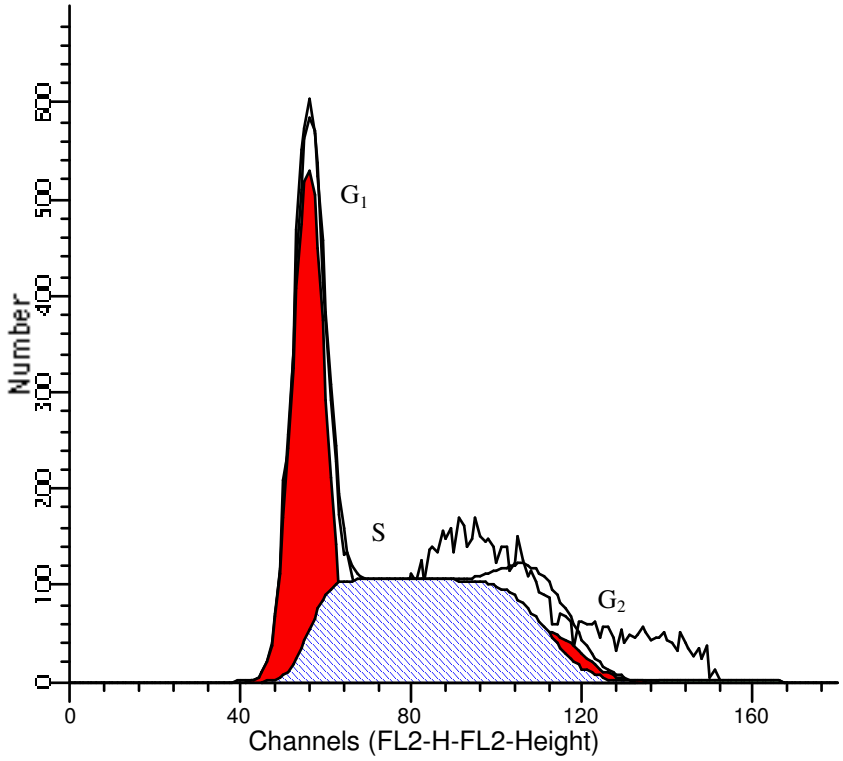


Figure 3.9: ModFit-generated DNA histogram of HT-29 cells treated with DMSO for 24 hours.

In this figure, 42.49% of the DNA is at the G_1 phase, 49.17% is at the S-phase and 8.34% is at the G_2 phase. As cells are synchronised at G1 before the addition of control solvents and drugs, this suggests that DMSO does not have a significant influence in 24 hours in terms of cell cycle progression since the majority of DNA is at G_1 and S-phase.

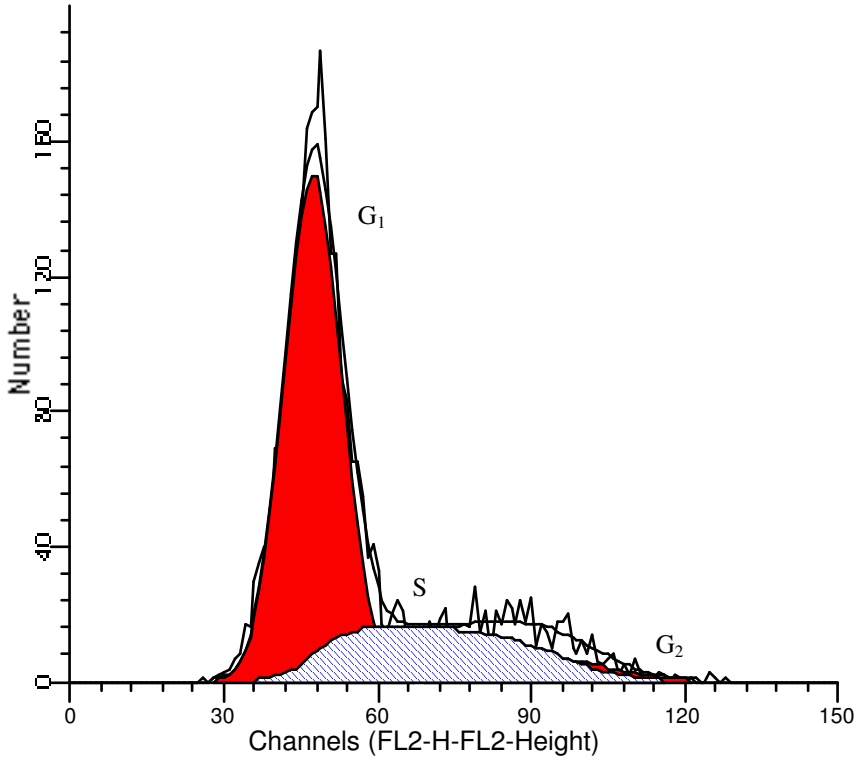


Figure 3.10: ModFit-generated DNA histogram of HT-29 cells treated with 50µM curcumin for 24 hours.

Here, 68.95% of the DNA is at the G_1 phase, 24.97% is at the S-phase and 6.08% is at the G_2 phase. In comparison to the control cells in **figure 3.9**, curcumin influences the cell cycle of HT-29 by promoting accumulation at G_1, thereby inhibiting DNA synthesis at the S-phase, and growth and mitosis at G_2/M.

3.1.2 MCF-7 cell line

Table 3.2: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of thalidomide to MCF-7 cells.

The highlighted treatment is the optimal treatment used in this study.

	12 hours	24 hours	48 hours	72 hours
Control				
G_1	87.26	87.83	87.14	86.58
S	11.0	0.78	6.52	8.11
G_2	1.74	11.40	6.34	5.31
10µM				
G_1	81.54	86.93	86.73	78.18
S	11.80	0.32	6.71	18.88
G_2	6.66	12.75	6.57	2.94
30µM				
G_1	78.93	85.80	90.33	78.09
S	16.87	1.70	4.97	17.50
G_2	4.20	12.49	4.71	4.41
100µM				
G_1	80.79	89.86	93.21	70.56
S	12.66	1.97	2.36	21.49
G_2	6.55	8.17	4.42	7.75
300µM				
G_1	85.51	78.60	81.68	81.32

S	10.90	7.78	14.15	18.68
G_2	3.58	13.62	4.16	0

The optimal treatment for MCF-7/thalidomide is 100µM for 48 hours, as deduced from **table 3.2**. The largest difference in cell cycle distribution compared to the controls is 100µM for 72 hours. However, at this period of exposure, very few cells are left, limiting the RNA available for extraction. The difference in cell number is illustrated in **figure 3.11** and **figure 3.12**, validating the choice of cells treated with 100µM thalidomide for 48 hours. This analysis is represented in **figure 3.13**.

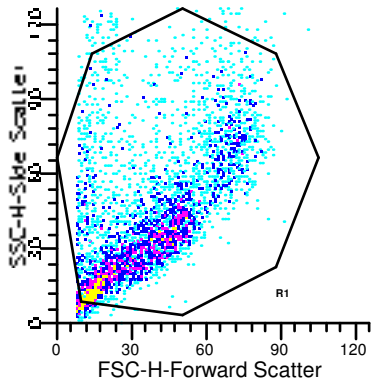


Figure 3.11: ModFit-generated scatter plot of side scatter versus forward scatter for MCF-7 cells treated with 100µM thalidomide for 48 hours.

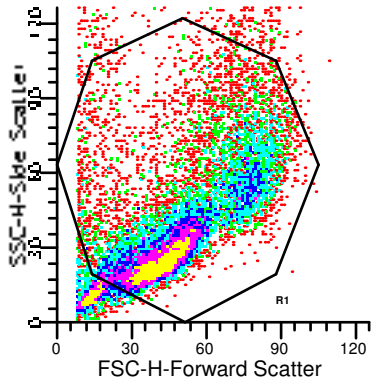


Figure 3.12: ModFit-generated scatter plot of side scatter versus forward scatter for MCF-7 cells treated with 100µM thalidomide for 48 hours.

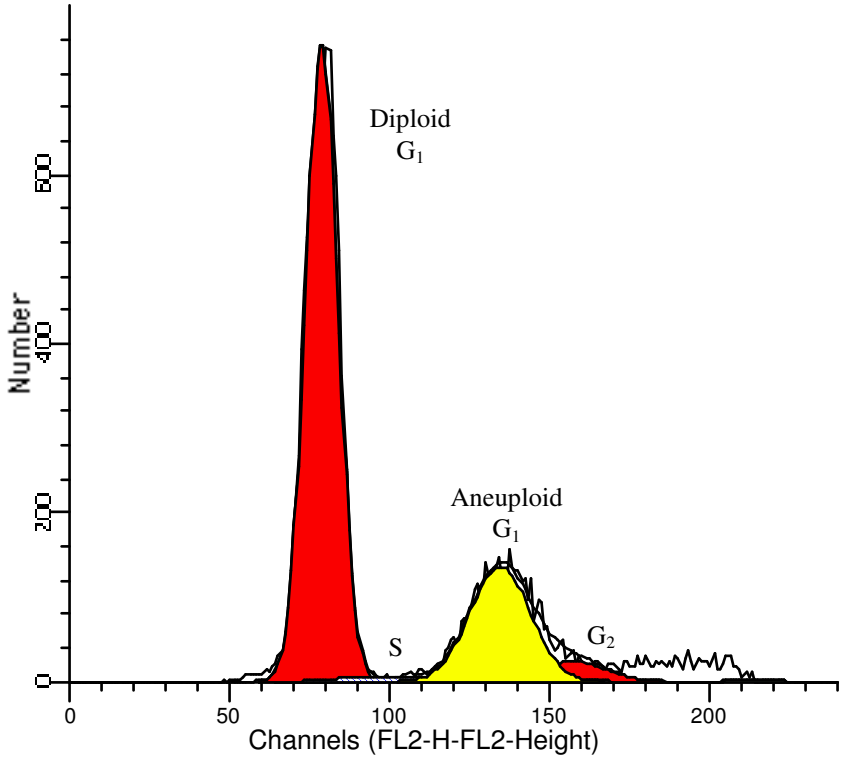


Figure 3.13: ModFit-generated DNA histogram of MCF-7 cells treated with 100µM thalidomide for 48 hours.

Aneuploid cells, as seen in **figure 3.13**, are common in cancer cell lines and are caused by aberrant spindle checkpoints due to gene mutations or deletions (Dey, 2004). ModFit distinguishes between the diploid and aneuploid state, and calculates the percentage of each relative to the total number of gated events.

For the lycopene treatment, the optimal treatment is 10µM lycopene for 24 hours. Lycopene delays G_1/S progression. At this treatment, the highest difference is seen in comparison to the control cells (**figure 3.14**), with a large number of viable cells for RNA extraction. This treatment has a high percentage of cells arrested at G_1, with zero cells entering G_2 (**figure 3.15**).

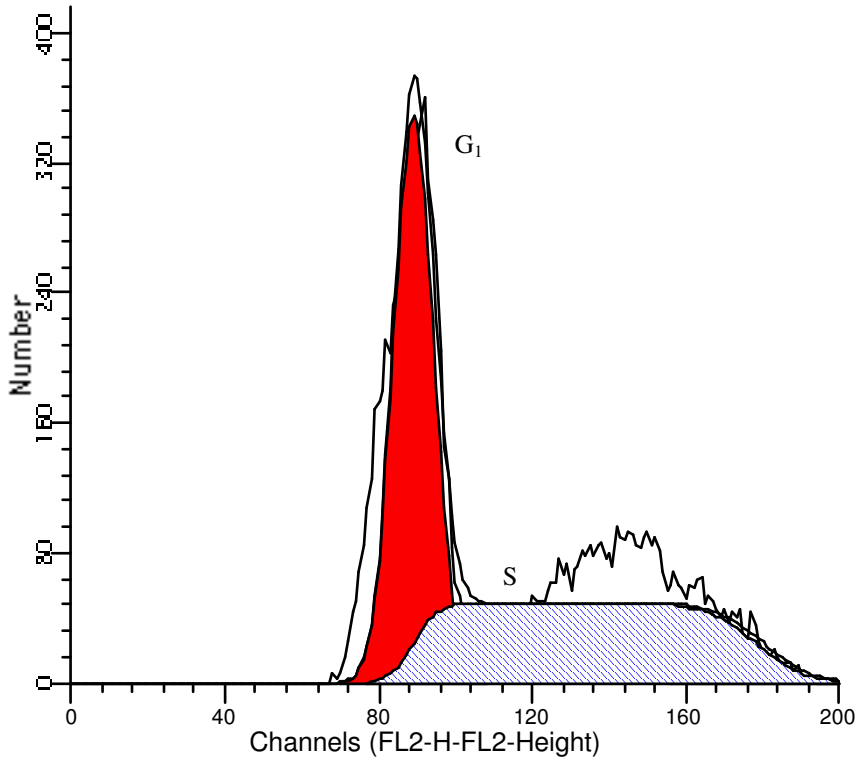


Figure 3.14: ModFit-generated DNA histogram of MCF-7 cells treated with THF for 24 hours.

Here, 50.52% of the DNA is at the G_1 phase, 48.27% is at the S-phase and 1.21% is at the G_2 phase.

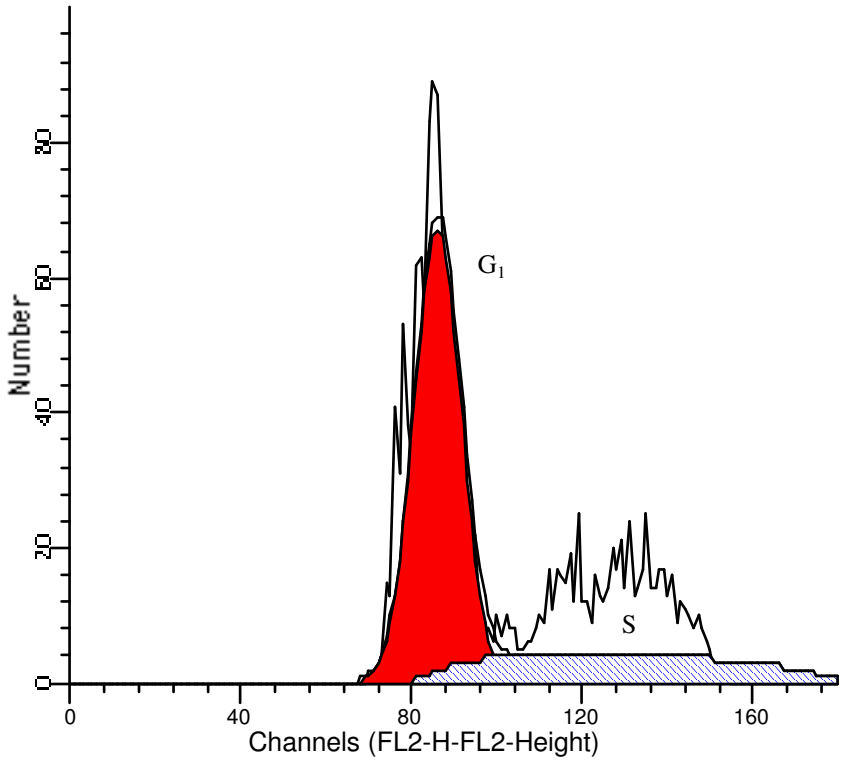


Figure 3.15: ModFit-generated DNA histogram of MCF-7 cells treated with 10µM lycopene for 24 hours.

In **figure 3.15**, 75.48% of the DNA is at the G_1 phase, 25.42% is at the S-phase and 0% is at the G_2 phase. In comparison to the control cells in **figure 3.14**, it is evident that lycopene induces G_1/S arrest. As the majority of cells are at G_1, lycopene inhibits both DNA synthesis and mitosis.

3.1.3 MDA-MB-231 cell line

Table 3.3: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of thalidomide to MDA-MB-231 cells.

The highlighted treatment is the optimal treatment used in this study.

	12 hours	**24 hours**	**48 hours**	**72 hours**
Control				
G_1	55.30	65.35	56.31	73.10
S	40.86	31.50	23.82	20.99
G_2	3.85	3.15	19.87	5.91
10µM				
G_1	60.07	65.95	73.36	64.62
S	37.19	28.96	19.18	15.51
G_2	2.74	5.09	7.46	19.88
30µM				
G_1	61.72	72.86	66.50	71.79
S	35.75	23.87	32.17	24.43
G_2	2.53	3.25	0.93	3.78
100µM				
G_1	56.04	75.47	71.29	69.34
S	37.27	18.38	22.52	21.39
G_2	6.69	6.16	6.19	9.27
300µM				
G_1	70.20	83.75	73.47	72.83

S	29.73	11.65	20.28	19.09
G_2	0.07	4.60	6.28	8.08

The optimal treatment for MDA-MB-231/thalidomide as per the results in **table 3.3** is 300µM for 24 hours (**figure 3.17**). This treatment demonstrates the greatest difference in cell cycle distribution compared to the control (**figure 3.16**). There are a large percentage of cells arrested at G_1, with very few entering into G_2. Although 300µM is the maximum concentration, it has been shown to be a non-toxic concentration (Kim and Choi, 2005). Thalidomide typically has a weak anti-proliferative activity against MDA-MB-231.

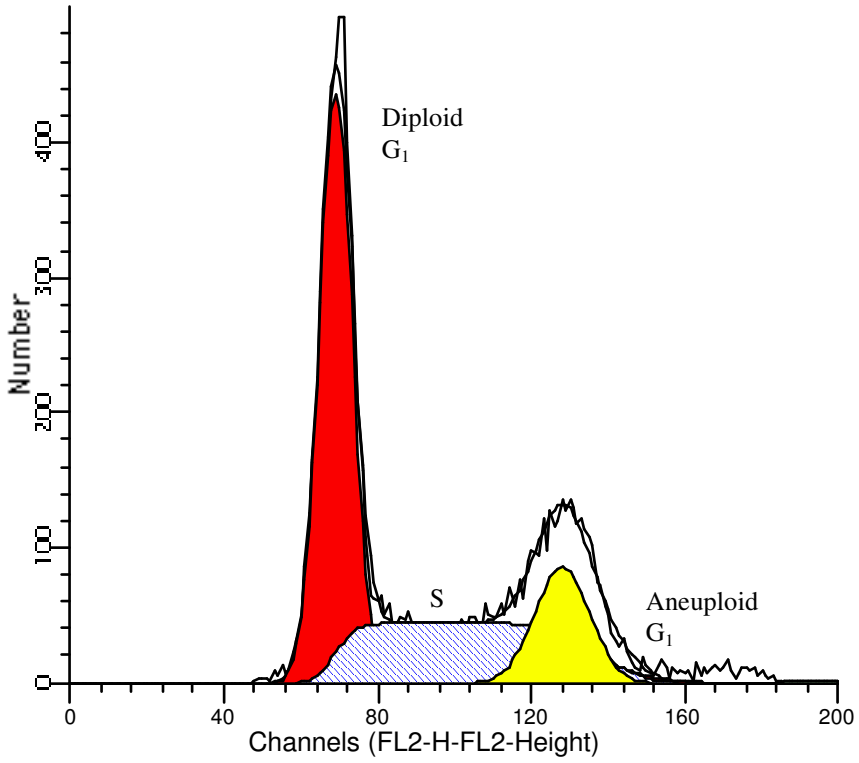


Figure 3.16: ModFit-generated DNA histogram of MDA-MB-231 cells treated with DMSO for 24 hours.

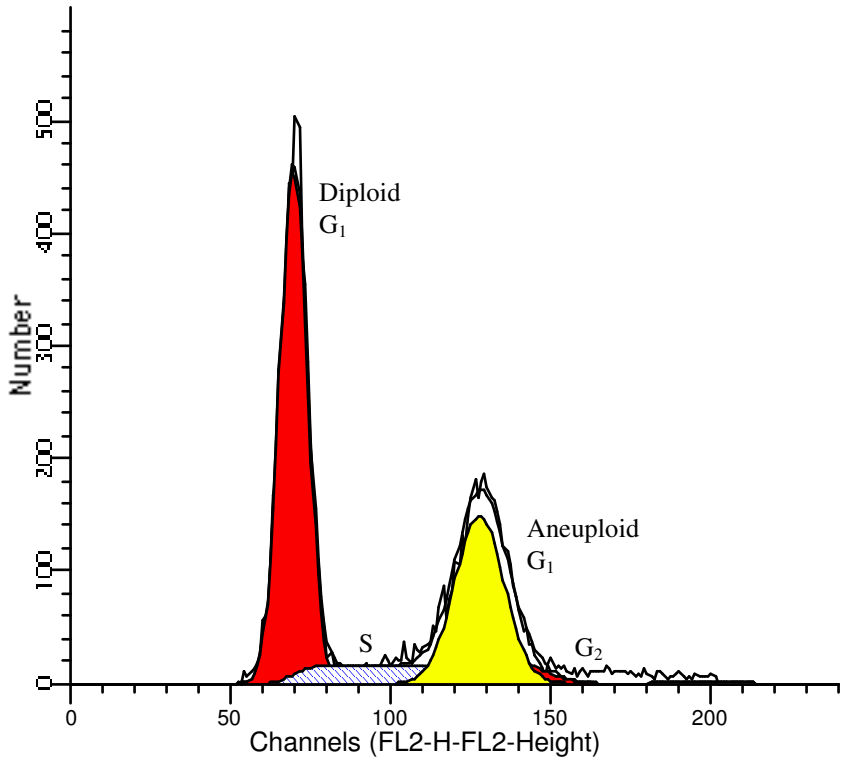


Figure 3.17: ModFit-generated DNA histogram of MDA-MB-231 cells treated with 300µM thalidomide for 24 hours.

In **figure 3.16**, 65.35% of the DNA is at the G_1 phase, 31.50% is at the S-phase and 3.15% is at the G_2 phase. In **figure 3.17**, 83.75% of the DNA is at the G_1 phase, 11.65% is at the S-phase and 4.60% is at the G_2 phase. Thalidomide therefore accumulates cells at G_1 to reduce DNA synthesis (S-phase), growth (G_2 phase) and mitosis (M-phase).

The optimal treatment for lycopene-treated MDA-MB-231 cells is 5µM for 72 hours (**figure 3.19**). Lycopene induces G_1 arrest, and at this treatment the highest percentage of cells at G_1 is observed. There are very few cells occurring at G_2, indicating that

progression from the S-phase is restricted by lycopene. This treatment also has the greatest difference in cell cycle distribution compared to the control (**figure 3.18**).

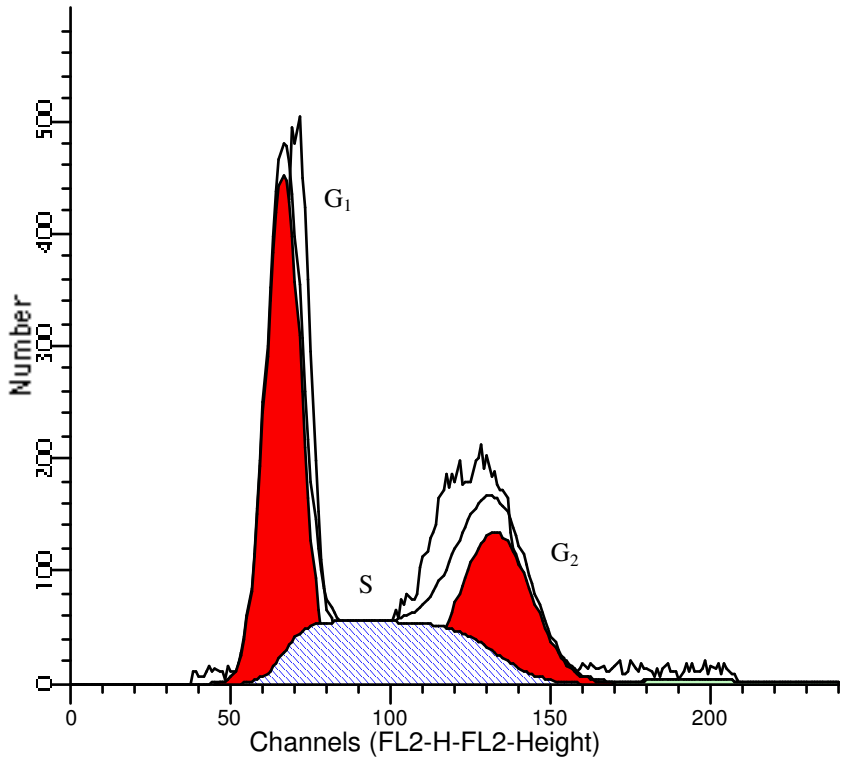


Figure 3.18: ModFit-generated DNA histogram of MDA-MB-231 cells treated with THF for 72 hours.

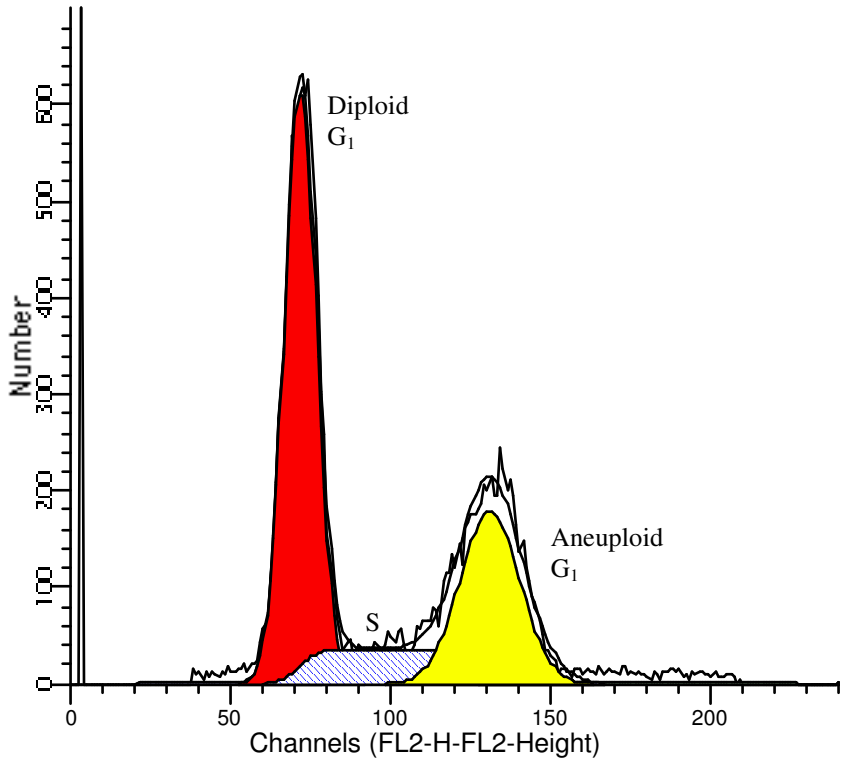


Figure 3.19: ModFit-generated DNA histogram of MDA-MB-231 cells treated with 5µM lycopene for 72 hours.

In the control cells in **figure 3.18**, 46.05% of the DNA is at the G_1 phase, 26.64% is at the S-phase and 27.31% is at the G_2 phase. In the treated cells in **figure 3.19**, 81.23% of the DNA is at the G_1 phase, 17.74% is at the S-phase and 1.03% is at the G_2 phase. This demonstrates that lycopene induces G_1 arrest in MDA-MB-231 as it inhibits entry into the S-phase and G_2 phase.

3.1.4 184A1 cell line

Table 3.4: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of lycopene to 184A1 cells.

The highlighted treatment is the optimal treatment used in this study.

	12 hours	24 hours	48 hours	72 hours
Control				
G_1	86.99	83.46	91.07	84.72
S	7.06	9.55	8.85	8.98
G_2	5.95	6.99	0.08	6.30
2µM				
G_1	90.05	39.70	79.58	85.08
S	8.59	60.29	16.30	8.30
G_2	1.37	0.01	4.13	6.62
5µM				
G_1	70.25	82.99	82.53	85.84
S	14.71	9.01	17.25	8.03
G_2	15.04	8.00	0.26	6.13
10µM				
G_1	91.75	79.64	92.01	95.18
S	5.58	12.36	4.01	4.82
G_2	2.67	8.00	3.98	0
20µM				
G_1	80.97	85.90	91.11	92.46

S	14.93	6.10	8.87	7.03
G_2	4.11	8.00	0.03	0.52

The optimal treatment for lycopene-treated 184A1 cells is 10µM for 48 hours (**table 3.4**). Lycopene induces G_1 arrest. At 72 hours, there are significantly less viable cells; therefore, this period of exposure is not appropriate for obtaining sufficient concentrations of RNA. With the exception of these treatments, the highest percentage of cells are arrested at G_1 at 10µM after 48 hours, with few cells at the S-phase and G_2 (**figure 3.20**). The differences induced by lycopene are not significantly large compared to the controls. This suggests that lycopene inhibits cell cycle progression through G_1/S as it this was not the case, the cells would not remain accumulated at G_1 as they are in the synchronised controls.

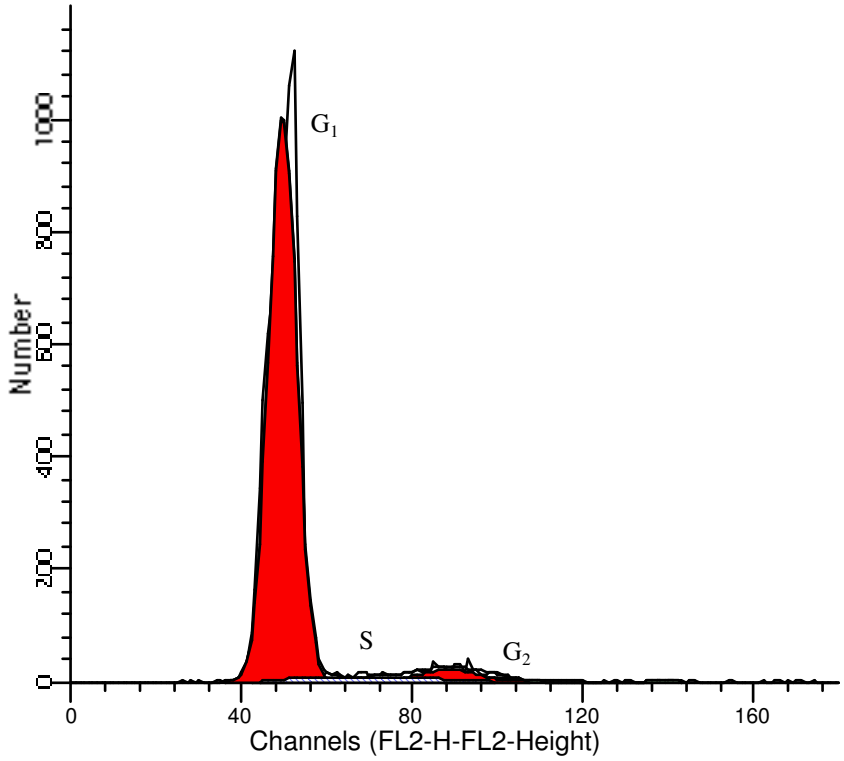


Figure 3.20: ModFit-generated DNA histogram of 184A1 cells treated with 10µM lycopene for 48 hours.

Here, 92.01% of the DNA is at the G_1 phase, 4.01% is at the S-phase and 3.98% of the DNA is at G_2 phase.

The optimal treatment selected for thalidomide-treated 184A1 cells is 100µM thalidomide for 48 hours (**figure 3.21**).

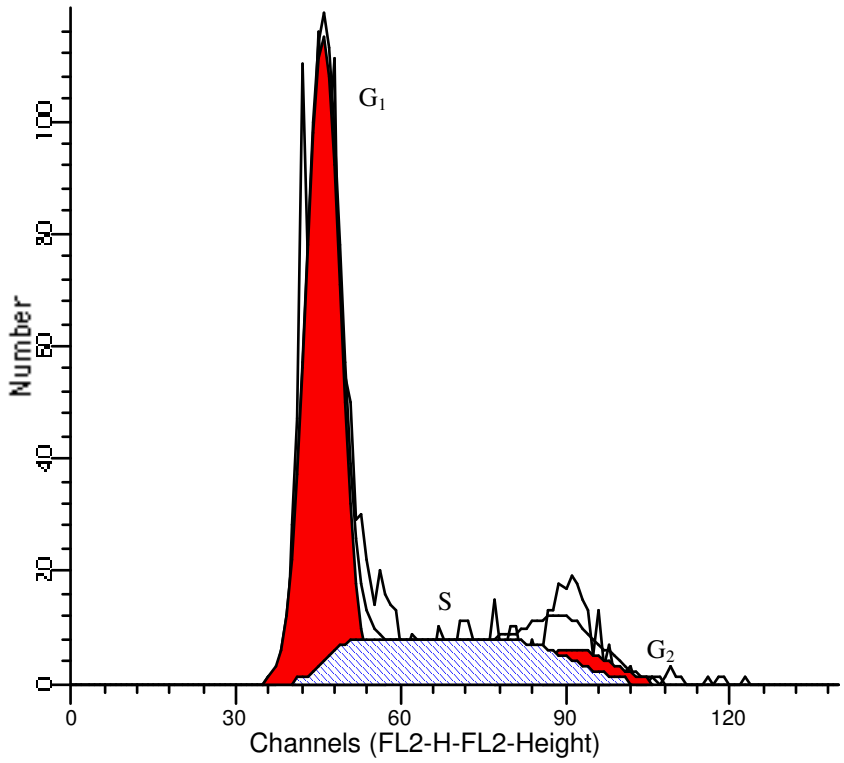


Figure 3.21: ModFit-generated DNA histogram of 184A1 cells treated with 100µM thalidomide for 48 hours.

In **figure 3.21**, 66.38% of the DNA is at the G_1 phase, 26.40% is at the S-phase and 7.22% is at the G_2 phase. In the control cells, 88.12% of the DNA is at the G_1 phase, 11.53% is at the S-phase and 0.35% is at the G_2 phase. This suggests that thalidomide may not exert a significant influence on cell cycle distribution as if it did, the cells would not have continued to progress through the cell cycle as suggested by these results.

3.1.5 Summary

Flow cytometry and ModFit analysis allows for the quantification of DNA in the three interphase stages. By comparing the results of the drug-treated cells with the controls and with previous research, the optimal treatments for each drug in the respective cell line are determined. This assures that the treatment administered to the cell line, with regards to concentration and time, does produce a result attributed to the actions of the drug. This technique is essential in establishing the parameters for the downstream applications to follow. **Appendix II** contains a full reference of the flow cytometry and ModFit analysis results.

Table 3.5: Summary of treatments selected.

Cell line/Treatment	Concentration (µM)	Time (hours)
HT-29/SAHA	5	48
HT-29/Curcumin	50	24
MCF-7/Thalidomide	100	48
MCF-7/Lycopene	10	24
MDA-MB-231/Thalidomide	300	24
MDA-MB-231/Lycopene	5	72
184A1/Lycopene	10	48
184A1/Thalidomide	100	48

3.2 RNA

For RNA to be successfully reverse transcribed and used in the Genefishing and SuperArray PCR array systems, it has to meet certain criteria: the A_{260}/A_{280} ratio must be greater than 2.0, and A_{260}/A_{230} ratio must be greater than 1.7. This ensures a pure concentration of RNA. A_{260} measures the nucleic acid concentration, A_{280} measures the protein concentration and A_{230} measures contaminants e.g. carbohydrates, guanidine thiocyanate, nucleotides, EDTA and phenol. **Table 3.5** illustrates the purity and concentration of RNA for untreated and treated cells.

Table 3.6: RNA purity and concentration ratios for untreated and treated cells

	A_{260}/A_{280}	**A_{260}/A_{230}**	**ηg/µl**
HT-29 untreated	2.08	1.86	813.4
HT-29/DMSO	2.09	2.16	1033.7
HT-29/curcumin	2.13	2.24	763.9
HT-29/SAHA	2.10	1.77	1562.1
MCF-7 untreated	2.17	2.18	797.6
MCF-7/DMSO	2.18	2.12	1099.4
MCF-7/THF	2.17	1.88	797.4
MCF-7/thalidomide	2.11	1.82	561.8
MCF-7/lycopene	2.12	1.79	964.6
MDA-MB-231 untreated	2.17	2.11	810.3
MDA-MB-231/DMSO	2.17	2.28	1491.2
MDA-MB-231/THF	2.13	2.24	885.2

MDA-MB-231/thalidomide	2.15	2.11	813.3
MDA-MB-231/lycopene	2.12	2.07	623.5
184A1 untreated	2.04	1.78	231.7
184A1/DMSO	2.06	1.72	241.5
184A1/THF	2.04	1.80	288.1
184A1/thalidomide	2.05	1.71	333.8
184A1/lycopene	2.04	1.77	307.4

Figure 3.22 demonstrates the 28S and 18S ribosomal RNA (rRNA) subunits in lanes 1, 2 and 3. This validates that these RNA samples are intact and of high integrity. However, in **figure 3.23**, lane 1 shows an RNA sample that only has the 28S rRNA subunit, and lanes 2 and 3 do not have crisp bands. The smearing here indicates degraded RNA that is not suitable for use in downstream experiments.

1 2 3



Figure 3.22: RNA gel of good integrity RNA.

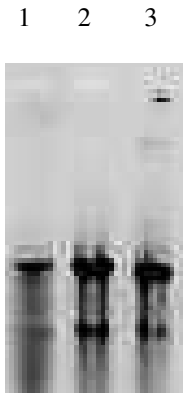


Figure 3.23: RNA gel showing unsuitable RNA samples as indicated by smearing

3.2.1 Summary

It is essential to acquire high quality RNA for gene expression analysis i.e. PCR array and differential display. For the PCR arrays, 1.0µg of total RNA is consistently used for the first strand synthesis. For the differential display, 3µg of total RNA is used as a standard amount for the reverse transcription reactions.

3.3 Genefishing

The Seegene Genefishing^{TM} DEG101 and 102 Premix Kits are used to identify differentially expressed genes between HT-29, MCF-7, MDA-MB-231 and 184A1 cells using controls and drug treatments. This involves a reverse transcription and a two-stage PCR using twenty arbitrary primers. Arbitrary refers to a general primer that anneals to a wide range of genetic sequences. In PCR the use of one arbitrary primer with a common secondary primer generates multiple DNA products. This system is therefore applied to differential gene expression studies.

3.3.1 Positive control

Before running the reverse transcription and PCR of the test samples, a positive control experiment is conducted to ascertain the correct PCR conditions. The manufacturers specify the band detail that must be obtained by running the PCR products on a 2% agarose gel. As per **figure 3.24**, using control kidney cDNA and control liver cDNA, the experiment conditions are optimal as the bands obtained in this gel correspond to this specification.

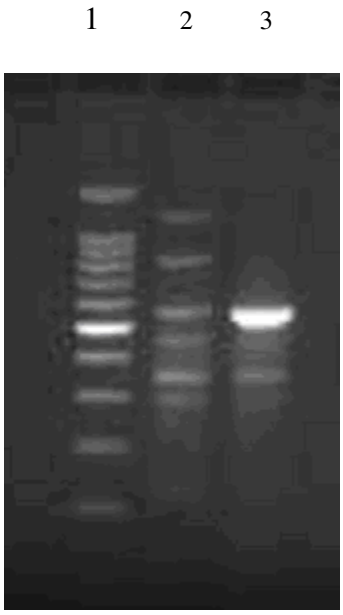
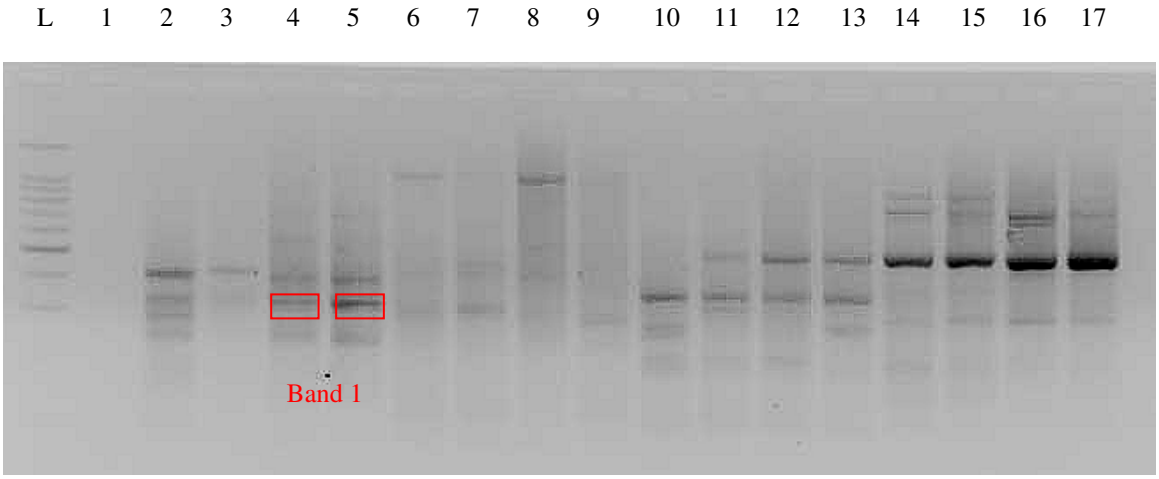


Figure 3.24: Positive control gel with the 100bp DNA ladder in lane 1, control kidney DNA in lane 2, and control liver DNA in lane 3.

3.3.2 HT-29

Using the twenty arbitrary primers, nine bands are differentially expressed in the untreated cells compared to the treated samples. Nine bands are differentially expressed in the DMSO-treated cells. Ten bands are differentially expressed in the SAHA-treated cells, and eleven bands are differentially expressed in the curcumin-treated cells. These results are represented by figures 3.25, 3.27 and 3.28. Four of these bands are successfully sequenced, and the others are too low in molecular weight for sequencing by Inqaba Biotec. The sequencing results are represented by figures 3.26, 3.28, 3.29 and 3.30.



Lane L: 100bp DNA ladder

Lane 1: negative PCR control

Lane 2: HT-29 untreated ACP6

Lane 3: HT-29/DMSO ACP6

Lane 4: HT-29/SAHA ACP6

Lane 5: HT-29/curcumin ACP6

Lane 6: HT-29 untreated ACP7

Lane 7: HT-29/DMSO ACP7

Lane 8: HT-29/SAHA ACP7

Lane 9: HT-29/curcumin ACP7

Lane 10: HT-29 untreated ACP8

Lane 11: HT-29/DMSO ACP8

Lane 12: HT-29/SAHA ACP8

Lane 13: HT-29/curcumin ACP9

Lane 14: HT-29 untreated ACP9

Lane 15: HT-29/DMSO ACP9

Lane 16: HT-29/SAHA ACP9

Lane 17: HT-29/curcumin ACP9

Figure 3.25: Gel image of HT-29 ACP6 – ACP10 PCR reactions.

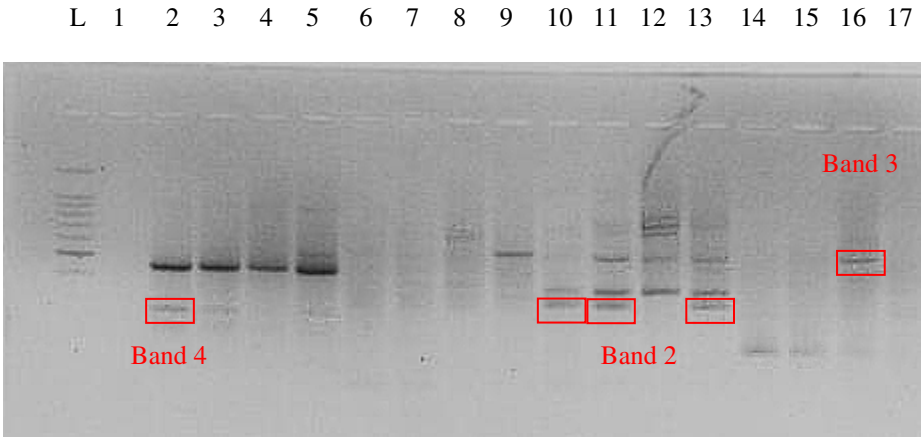
Band 1 is present in HT-29/SAHA and HT-29/curcumin, evident in **figure 3.25**. Basic local alignment search tool (BLAST) localises this band at chromosome 22, at base pairs 42723549 – 42723738. **Figure 3.26** illustrates its sequence.

GAAGCGGAGGCGGCACCTTCTGAGAGGCGCATGCTCAGTGAGTCGTGAAGAT
GGCAGGGCTGGCGGAGCGGCCGCCGCATCTGATCTCTCCCCTTTTTTTAGGAA
TGTGATGGCGTCCAGTTTGGAGCTGGGATAAGGTTCCTGTAGCCGACACCCCT
ACAGGAGAAGCTCTGGGACTGGGGCAGCAGCAAGGCGCCCATGCCACACAC
CGTCTCTCGAGGAAACGCGGTTCAGCGATTCTTTGACTGCGGACCCTGTGGG
AAACCCCGTCAATAAATGTTAAAGACACACTCCGAGGCAGCGTGGATGTGGT
TTGCTTTCACTCAACAGTATGGCCCGCGTGGCCTTTCCCCGGGACCACCCGTG
TTCTCACACACCCGCCCAGTGTC

Figure 3.26: Sequence of band 1, with the matching bases in blue. The light blue base represents a splice site (BLAST).

Ensembl identifies this sequence as corresponding to the Sorting and Assembling Machinery Component 50 Homologue (SAMM50) gene located at base pairs 42673949 - 42723744. It is present in the mitochondrial outer membrane in eukaryotes. It is an essential element of the protein insertion machinery, where it is involved in transporting mitochondrial pre-proteins into the mitochondrion (Endo *et al*., 2003; Truscott *et al*., 2003). Defects in mitochondrial function are implicated in cancer development and progression (Carew and Huang, 2002). By inducing the expression of SAMM50, SAHA and curcumin are promoting mitochondrial function i.e. respiration and apoptosis (Talarico *et al*., 2001; Olivero *et al*., 1997). Curcumin protects mitochondria from reactive oxygen species (ROS) and the lipid peroxidation of the mitochondrial membranes (Morin *et al*., 2001).

Curcumin also increases the permeability of the mitochondrial membrane to decrease membrane potential and induce apoptosis (Morin *et al*., 2001). Similarly, curcumin induces apoptosis in HT-29, as seen in SuperArray PCR arrays, by inducing the upregulation of CDK5R1, CUL2, RAD9A and RPA3, and the downregulation of BCL2, BIRC5, CDC16, CDK5RAP1, CKS1B, CUL3 and GTF2H1. Curcumin also promotes apoptosis in other colon cancer cells, in breast cancer, liver cancer and leukaemia (Aggarwal *et al*., 2007; Leu and Maa, 2002; Chen *et al*., 1999; Jiang *et al*., 1996; Simon *et al*., 1998; Kuo *et al*., 1996). SAHA induces cytochrome *c* release and ROS production to promote mitochondrial-mediated apoptosis (Ruefli *et al*., 2001). SAHA promotes apoptosis in HT-29 by inducing the upregulation of ARHI, CCNE1, CCNG2, CUL2, RAD9A, RB1 and RPA3, and the downregulation of CCNF and CDK4 (**chapter 3.4**). Furthermore, SAHA induces apoptosis in bladder transitional cell carcinoma, breast cancer, pancreatic cancer, endometrial cancer and lymphoma (O'Connor, 2006; Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005).



Lane L: 100bp DNA ladder

Lane 1: negative PCR control

Lane 2: HT-29 untreated ACP10

Lane 3: HT-29/DMSO ACP10

Lane 4: HT-29/SAHA ACP10

Lane 5: HT-29/curcumin ACP10

Lane 6: HT-29 untreated ACP11

Lane 7: HT-29/DMSO ACP11

Lane 8: HT-29/SAHA ACP11

Lane 9: HT-29/curcumin ACP11

Lane 10: HT-29 untreated ACP12

Lane 11: HT-29/DMSO ACP12

Lane 12: HT-29/SAHA ACP12

Lane 13: HT-29/curcumin ACP12

Lane 14: HT-29 untreated ACP13

Lane 15: HT-29/DMSO ACP13

Lane 16: HT-29/SAHA ACP13

Lane 17: HT-29/curcumin ACP13

Figure 3.27: Gel image of HT-29 ACP10 – ACP13 PCR reactions.

Band 2 is present in HT-29/untreated, HT-29/DMSO and HT-29/curcumin, but not in HT-29/SAHA. This is illustrated in the gel image in **figure 3.27**. Sequencing identifies its location at chromosome 7, from base pairs 23281019 – 23281252 (**figure 3.28**).

GGTGGGAGTATTTTGGTGACAACCTACTTTGCTTGGCTGAGTGAAGGAATGA
TATTCATATATTCATTTATTCCATGGACATTTAGTTAGTGCTTTTTATATACCA
GGCATGATGCTGAGTGACACTCTTGTGTATATTTCCAAATTTTTGTACAGTCG
CTGCACATATTTGAAATCATATATTAAGACTTTCCAAAGATGAGGTCCCTGGT
TTTTCATGGCAACTTGATCAGTAAGGATTTCACCTCTGTTTGTAACTAAAACC
ATCTACTATATGTTAGACATGACATTCTTTTTCTCTCCTTCCTGAAAAATAAA
GTGTGGGAAGAGACAAGACCTTGGTATTGTAATTATTTTTCTGATTCATCAT
TTGCTTTTCAATTTCACTTACAGTTGTTTCTTTTTTTTAGATGAGGGTCTCCCT
ATATTGTCCA

Figure 3.28: Sequence of band 2, with matching bases in blue (BLAST).

This sequence corresponds with the glycoprotein non-metastatic melanoma protein B (GPNMB) gene, localised at base pairs 23252841 - 23281248 (Ensembl). While this gene correlates with low metastasis in cell lines, it is also expressed in aggressive melanoma, glioma and breast cancers, where it corresponds with metastasis (Weterman *et al.*, 1995). This suggests that SAHA inhibits metastasis in HT-29. Similarly, SAHA induces the upregulation of CDKN2A in HT-29 in the SuperArray PCR array study to inhibit metastasis (**chapter 3.4**) (Wang *et al.*, 2006). Angiogenesis is essential for successful metastasis. SAHA inhibits angiogenesis by inducing the upregulation of CUL2 to inhibit HIF-1α in this PCR array study (**chapter 3.4**) (Ocker and Schneider-Stock, 2007).

Band 3 is identified in HT-29/SAHA (**figure 3.27**). It is localised at chromosome 2, at base pairs 131995178 – 131995616 (**figure 3.29**) (BLAST).

CCTCCCAGAGCCCAGGTGCTTCACCGCAGACCGCAAGCCATTCACCCAAAGC
AGTGGCCAACATCGCGGACCCCTGTGCCTTGTCACAGATGGGTGCTGGTCCC
AGGCGTTGGGGACACTGCTGGGTCGATGGGGTCGGATTCTGCCAGTTTCTGCT
CTGCAGCCAAAGATGGTCAGAAGCATTGTCACTTCAGTAACATCAAGTGCTC
AAAGACATGGCAACCGTTCAGTGGTACTTAAGTATTCAAAATATACAACTAC
AGATTCTCTGACAGAAACCAGCACGGGGTCTTCACCTTCATTCACCCCACAGC
GACATGCGAGGGAGAACAGCATCTCAGTGGTGATTTCCAAACCAAGCCTTTG
TTTTCGGTGTGGGGTTTTGGGGGTTTGCTTTAATGTTTTTGAAATTGTAAATGT
TGGGCTTTGTATTTTGATGTAAACTGAGAATAATGGCATTTTAGGGCCTGTGA
CCAAAAATGAAGCTTGTAACGACCATGGATCTGAATAAACATGTCCTTGCTT
CTGAGTCTTCTGGCACCTGGGCTCAGTCTCTAGGAGGTTCACCTATACAGAGT
CCCTGAGCACACGGGGGCTGCAGAAACAACTGTTGGGCACCC TGTCGGCCC

Figure 3.29: Sequencing of band 3, with matching bases highlighted.

This corresponds to the Q6ZWB7 gene, localised at base pairs 131989755 - 131995584. The function of this gene is not well characterised.

Band 4 is present in HT-29/untreated and not in any of the DNA from the treatments (**figure 3.27**). It is localised at chromosome 17, base pairs 17227376 – 17227487 (**figure 3.30**).

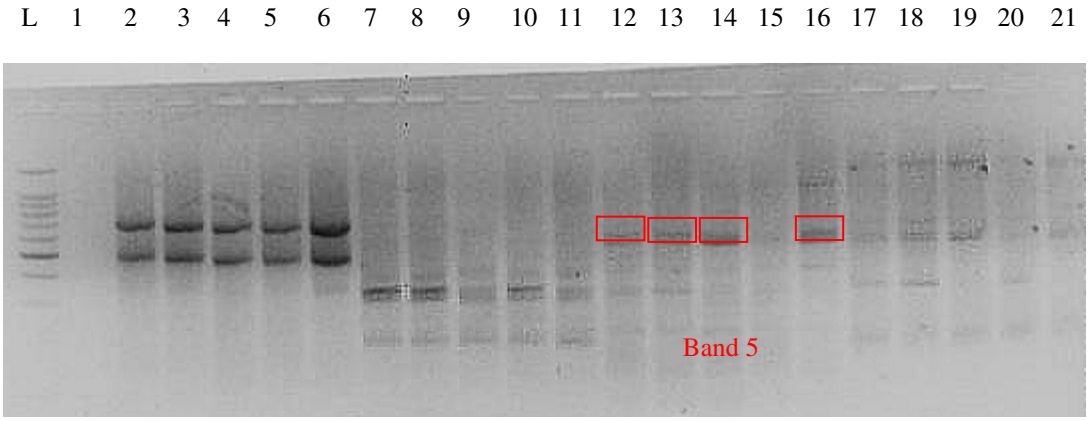
GCCCATCCGGAATGTCTATAAGAAGGAGAAAGCTCGAGTGATCACTGAGGAA
GAGAAGAATTTCAAAGCCTTCGCTAGTCTCCGTATGGCCCGTGCCAACGCCC
GGCTCTTCGGCATACGGGCAAAAAGAGCCAAGGAAGCCGCAGAACAGGATG
TTGAAAAGAAAAAATAAAGCCCTCCTGGGGACTTGGAATAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAGGAGGTTACATACAGCTTTCCATCTGGCCCAACAA
CCCTACTTCTGGGTACATGCCCAGAAGAATTAAAGACAGGTATTCAAACCAA
AAC

Figure 3.30: Sequencing of band 4, with matching bases in blue.

There are no mapped genes at this region.

3.3.3 MCF-7

From the DNA resulting from PCR using the twenty primers, seven differentially expressed bands are produced in the untreated cells, seven in the THF-treated cells, five in the DMSO-treated cells, four in the lycopene-treated cells and five in the thalidomide-treated cells. The bands are represented in figures 3.31, 3.33 and 3.36. Four of these bands are successfully sequenced by Inqaba Biotec. The sequences are represented in figures 3.32, 3.34, 3.35 and 3.37.



Lane L: 100bp DNA ladder

Lane 1: negative PCR control

Lane 2: MCF-7 untreated ACP1

Lane 3: MCF-7/THF ACP1

Lane 4: MCF-7/DMSO ACP1

Lane 5: MCF-7/lycopene ACP1

Lane 6: MCF-7/thalidomide ACP1

Lane 7: MCF-7 untreated ACP2

Lane 8: MCF-7/THF ACP2

Lane 9: MCF-7/DMSO ACP2

Lane 10: MCF-7/lycopene ACP2

Lane 11: MCF-7/thalidomide ACP2

Lane 12: MCF-7 untreated ACP3

Lane 13: MCF-7/THF ACP3

Lane 14: MCF-7/DMSO ACP3

Lane 15: MCF-7/lycopene ACP3

Lane 16: MCF-7/thalidomide ACP3

Lane 17: MCF-7 untreated ACP4

Lane 18: MCF-7/THF ACP4

Lane 19: MCF-7/DMSO ACP4

Lane 20: MCF-7/lycopene ACP4

Lane 21: MCF-7/thalidomide ACP4

Figure 3.31: Gel image of MCF-7 ACP1 – ACP4 PCR reactions.

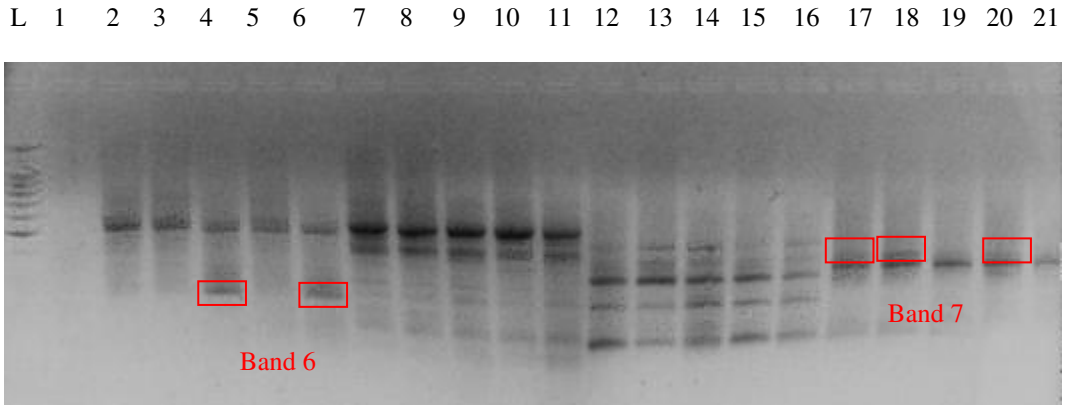
Band 5 is present in MCF-7 untreated, MCF-7/THF, MCF-7/DMSO and MCF-7/thalidomide, but not in MCF-7 lycopene (**figure 3.31**). It is localised at chromosome 17 at base pairs 7422381 – 7422630 (**figure 3.32**).

AGAATGGACCAAAAGGAACGAGACGTGATTATGAGGGAGTTTCGTTCTGGCT
CTAGCARGTTTTGATTACCACTGACCTGCTGGCCAGAGGCATTGATGTGCAGC
AGGTTTCTTTAGTATCAACTATGACCTTCCCACCAACAGGGAAAACTATATCC
ACAGAATCGGTCGAGGTGGCGGTTTGGCCGTAAAGGTGTGGCTATTAACATG
GTGACAGAAGAAGACAAGAGGACTCTCGAGACATTGAGACCTTCTACAACAC
CTCCATTGAGGAAATGCCCCTCAATGTTGCTGACTCATCTGAGGGGCTGTCCT
GCCACCCAGCCCCAGCCAGGGCTCAATCTCTGGGGGCTGGGAGCAGCAGGAG
GGGGGAGGGAAGGGAGCCAAGGGATGGACATCTTGTCATTTTTTTT

Figure 3.32: Sequencing of band 5, with matching bases in blue.

The eukaryotic protein synthesis initiation factor 4A (E1F4A1) gene is mapped at this location, occurring at base pairs 7403807 - 7426066. This RNA helicase functions in proliferation, where it is essential in initialising protein synthesis by unwinding mRNA for ribosome binding (Cordin *et al*., 2005). It is overexpressed in hepatocellular carcinoma, in melanoma cells where it is associated with tumourigenesis, and is associated with metastasis in early stage non-small cell lung cancer (Shuda *et al*., 2000; Eberle *et al*., 1997; Eberle *et al*., 2002; Wang *et al*., 2002b). As it is not expressed in MCF-7/lycopene, this suggests that lycopene induces the downregulation of E1F4A1 to

inhibit proliferation and metastasis. Similarly, lycopene inhibits proliferation in MCF-7 in the SuperArray PCR array study by inducing the upregulation of ATR, BCL2, CCNG2, CDKN1B, CDKN2B and RBL2, and the downregulation of ANAPC2, CCNB2, CDC2, DDX11 and MKI67 (**chapter 3.4**). This concurs with other studies where lycopene inhibits proliferation in MCF-7 and in hepatoma, prostate, endometrial, breast and lung cancer cells (Fornelli *et al*., 2007; Nahum *et al*., 2001; Park *et al*., 2005b; Hwang and Bowen, 2004; Levy *et al*., 1995). Lycopene also inhibits metastasis in multiple sclerosis, colorectal cancer and hepatoma (Chandler *et al*., 1997; Liabakk *et al*., 1996; Huang *et al*., 2007).



Lane L: 100bp DNA ladder

Lane 1: negative PCR control

Lane 2: MCF-7 untreated ACP13

Lane 3: MCF-7/THF ACP13

Lane 4: MCF-7/DMSO ACP13

Lane 5: MCF-7/lycopene ACP13

Lane 6: MCF-7/thalidomide ACP13

Lane 7: MCF-7 untreated ACP14

Lane 8: MCF-7/THF ACP14

Lane 9: MCF-7/DMSO ACP14

Lane 10: MCF-7/lycopene ACP14

Lane 11: MCF-7/thalidomide ACP14

Lane 12: MCF-7 untreated ACP15

Lane 13: MCF-7/THF ACP15

Lane 14: MCF-7/DMSO ACP15

Lane 15: MCF-7/lycopene ACP15

Lane 16: MCF-7/thalidomide ACP15

Lane 17: MCF-7 untreated ACP16

Lane 18: MCF-7/THF ACP16

Lane 19: MCF-7/DMSO ACP16

Lane 20: MCF-7/lycopene ACP16

Lane 21: MCF-7/thalidomide ACP16

Figure 3.33: Gel image of MCF-7 ACP13-ACP16 PCR reactions.

Band 6 is present in MCF-7/DMSO and MCF-7/thalidomide (**figure 3.33**). Upon sequencing, it is identified at chromosome MT (mitochondrial), from base pairs 8207 – 8295 (**figure 3.34**). Abnormalities in MTDNA are associated with cancer.

ACAGTTTYCTKGCCCWTCGTCCTARAATTAATTCCCCTAAAAATCTTTGAAAT
AGGGCCCGTATTTACCCTATAGCACCCCCTCTACCCCCTCTAGAGCCAAAAAA
AAAAAAAAAAAMC

Figure 3.34: Sequence of band 6, with matching bases highlighted.

This corresponds to the cytochrome *c* oxidase subunit 2 (COII) gene, which is localised at base pairs 7587 – 8295. This is a catalytic subunit of cytochrome *c* oxidase (complex IV) of the respiratory chain in the inner membrane of mitochondria. This complex transfers electrons from cytochrome *c* to oxygen, and provides a binding site for substrates. Although it is elevated in breast tumours, COII is not present in the untreated or control treatments (Sharp *et al*., 1992). This suggests that MCF-7 is not truly representative of the *in vivo* state. As its overexpression is associated with breast tumourigenesis, this implies that DMSO and thalidomide are promoting the oncogenic state of MCF-7 by inducing the expression of COII. This can be accredited to the influence of DMSO, which has been shown via the SuperArray PCR array study to have a negative influence on the function of thalidomide i.e. DMSO induces the downregulation of ANAPC2, and the upregulation of BCL2 and CCND2 to promote tumourigenesis in MCF-7 cells treated with thalidomide (**chapter 3.4**). On the other hand, the expression of COII induced by

thalidomide and DMSO may increase the sensitivity of MCF-7 to thalidomide. Cancer cell lines that lack MTDNA display resistance to chemotherapeutic drugs and photodynamic therapy (Singh *et al*., 1999). To determine the exact significance of COII expression as induced by thalidomide, further work on this subject is necessary.

Band 7 is identified in MCF-7/untreated, MCF-7/THF and MCF-7/lycopene (**figure 3.33**). It occurs on chromosome MT, at base pairs 9014 – 9206 (**figure 3.35**).

AACATTACTGYWGGCCACCTACTCATGCACCTAATTGGAAGCGCCACCCTAG
CAATATCAACCATTAACCTTCCCTCTACACTTATCATCTTCACAATTCTAATTC
TACTGACTATCCTARAAATCGCTGTCGCCTTAATCCAAGCCTACGTTTTCACA
CTTCTAGTAAGCCTCTACCTGCACGACAACACATAAAAAAAAAAAAAAAAA
MMCC

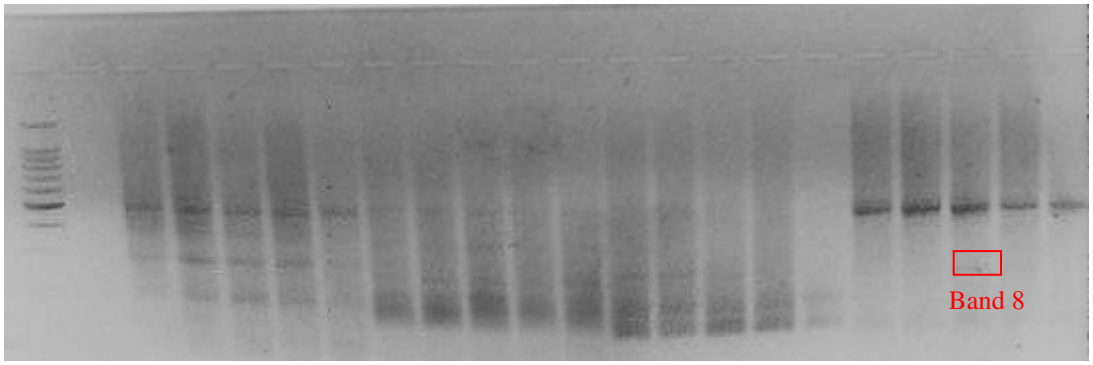
Figure 3.35: Sequence of band 7, with matching bases highlighted.

This sequence and position corresponds to two genes. The first is the cytochrome *c* oxidase subunit III (COIII) gene, at base pairs 9035 – 9991, one of the catalytic subunits of cytochrome *c* oxidase (Mather *et al*., 1993). It is involved in mitochondrial electron transport, converting cytochrome *c* to oxygen. As it is essential for the biosynthesis of functional cytochrome *c* oxidase, deletion of this gene induces deficiency of the enzyme complex (Haltia *et al*., 1991; Keightley *et al*., 1996). The levels of COIII are reduced in colon adenomas and carcinomas in comparison to normal mucosa (Heerdt *et al*., 1990).

While the expression of this gene has not been characterised in breast cancer, it implies that lycopene induces the expression of COIII to promote mitochondrial-induced apoptosis, and inhibit the oncogenic state of MCF-7. Similarly, lycopene induces apoptosis in MCF-7 in this SuperArray PCR array study by promoting the upregulation of ABL1, CDKN1B and RPA3, and the downregulation of ANAPC2 and CDC2 (**chapter 3.4**). In other studies, lycopene promotes apoptosis in colon cancer, Burkitt's lymphoma and T lymphoblast cells (Ivanov *et al*., 2007; Salman *et al*., 2007; Muller *et al*., 2002b).

The second gene to which the sequence of band 7 corresponds to is the mitochondrially encoded ATP synthase 6 (MT-ATP6) gene, at base pairs 8528 – 9208. It is one of the subunits of ATP synthase (complex V) of the respiratory chain/oxidative phosphorylation system in the mitochondrion. This complex is essential in converting ADP to ATP by oxidative phosphorylation. Mitochondria are vital for providing cells with energy and inducing apoptosis (Ferri and Kroemer, 2001; Wang, 2001). Mitochondrial dysfunction is associated with cancer development and progression. ATP synthase is downregulated in cancers of the liver, kidney, colon, lung, breast and in gastric cancer. This downregulation inhibits electron flux to the respiratory chain, promoting superoxide radicals, DNA damage and cell death (Shin *et al*., 2005). The decrease in oxidative phosphorylation capability reduces apoptosis (Dey and Moraes, 2000). As lycopene induces the expression of this gene, it promotes mitochondrial function to limit radical-induced DNA damage and promote apoptosis, as is seen with the PCR array study where lycopene induces apoptosis in MCF-7 (**chapter 3.4**).

L 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21



Lane L: 100bp DNA ladder

Lane 1: negative PCR control

Lane 2: MCF-7 untreated ACP17

Lane 3: MCF-7/THF ACP17

Lane 4: MCF-7/DMSO ACP17

Lane 5: MCF-7/lycopene ACP17

Lane 6: MCF-7/thalidomide ACP17

Lane 7: MCF-7 untreated ACP18

Lane 8: MCF-7/THF ACP18

Lane 9: MCF-7/DMSO ACP18

Lane 10: MCF-7/lycopene ACP18

Lane 11: MCF-7/thalidomide ACP18

Lane 12: MCF-7 untreated ACP19

Lane 13: MCF-7/THF ACP19

Lane 14: MCF-7/DMSO ACP19

Lane 15: MCF-7/lycopene ACP19

Lane 16: MCF-7/thalidomide ACP19

Lane 17: MCF-7 untreated ACP20

Lane 18: MCF-7/THF ACP20

Lane 19: MCF-7/DMSO ACP20

Lane 20: MCF-7/lycopene ACP20

Lane 21: MCF-7/thalidomide ACP20

Figure 3.36: Gel image of MCF-7 ACP17-ACP20 PCR reactions.

Band 8 is present in MCF-7/DMSO as seen in **figure 3.36**, and is localised at chromosome 19, from base pairs 57159421 – 57159525 (**figure 3.37**).

ARACCAGRGCCAGTGTGGAGGATTAATTGAAAACCAGGACCATAAACTTTCA

RAACTTTGTATTATGCARAGGGATCTGTGAAACTAGTCAATTTGTTTCATTAA

ACAAATATTTTAAAATTAAAAAAAAAAAAAAAAAMMCCCC

Figure 3.37: Sequence of band 8, with matching bases highlighted.

This corresponds to the zinc finger protein 350 (ZNF350) gene that occurs at base pairs 57159406 – 57181891. This is a potential breast cancer susceptibility gene, with its mutation correlating with inherited breast cancer (Rutter *et al*. 2003). ZNF350 inhibits GADD45A transcription (Yun and Lee, 2003). GADD45A induces cell cycle arrest and DNA repair (Jin *et al*., 2002; Carrier *et al*., 1999). Although GADD45A is upregulated by DMSO in MCF-7, but not with a significant fold change, DMSO does induce the downregulation of ATM and RAD9A, and the upregulation of BCL2 to decrease DNA damage repair (**chapter 3.4**) (Liu *et al*., 2007; Toueille *et al*., 2004; Saintigny *et al*., 2001). DMSO is therefore implicated in promoting genomic instability by inhibiting the DNA damage responses, consequently increasing the oncogenic potential of MCF-7. As this gene is not expressed in lycopene-treated and thalidomide-treated cells, lycopene and thalidomide are implicated in inhibiting its expression to promote GADD45A function and inhibit breast tumourigenesis. Accordingly, lycopene induces the upregulation of GADD45A in MCF-7 in the PCR array study (**chapter 3.4**).

3.3.4 MDA-MB-231

The PCR reactions generated four differentially expressed bands in the untreated cells, five differentially expressed bands in the THF-treated cells, seven in the DMSO-treated cells, six in the lycopene-treated cells, and seven in the thalidomide-treated cells. These bands were of very low molecular weight and could not be successfully sequenced.

3.3.5 184A1

From the twenty arbitrary primers used in the PCR reactions, five differentially expressed bands were identified in the untreated cells, two in the THF-treated cells, seven in the DMSO-treated cells, ten in the thalidomide-treated cells and seven in the lycopene-treated cells. However, these differentially expressed bands were too small in size to be successfully sequenced.

3.3.6 Summary

Overall, the Genefishing experiment did not prove be a successful technique. Although numerous differentially expressed bands were obtained, due to the majority being of low molecular weight and concentration, only eight bands were successfully sequenced. The manufacturers recommend that the PCR reaction be repeated two – three times to improve the band intensity for cloning. Another recommendation is to use two – three times the concentration of primer combination during the PCR reaction. These are not practical solutions as the kit is equipped with only enough reagents to run twelve PCR reactions using each of the twenty primers.

It is significant to highlight that four mitochondrial-associated genes are differentially expressed with drug treatment. SAHA and curcumin induce the expression of the SAMM50 gene in HT-29 to promote metabolism and mitochondrial-induced apoptosis. Lycopene also promotes mitochondrial-induced apoptosis in MCF-7 and inhibits the oncogenic state by inducing the expression of COIII. Lycopene inhibits DNA damage and promotes apoptosis in MCF-7 by inducing the expression of MT-ATP6. Thalidomide is implicated in increasing the sensitivity of MCF-7 to the drug by inducing the expression of COII, but this expression may also suggest that DMSO is promoting tumourigenesis. SAHA inhibits metastasis in HT-29 by inducing the downregulation of GPNMB, and lycopene, likewise, inhibits proliferation and metastasis in MCF-7 by inducing the downregulation of E1F4A1. DMSO promotes tumourigenesis in MCF-7 by inducing the expression of ZNF350. Alternatively, lycopene and thalidomide inhibit tumourigenesis in MCF-7 by inducing the downregulation of ZNF350. The majority of these results correlate with the results obtained from the SuperArray PCR Array experiments (**chapter 3.4**).

3.4.SuperArray RT^2 Profiler PCR Array

The PCR array data analysis web portal normalises data to the housekeeping genes to generate fold changes (changes in expression levels of the target genes in comparison to the selected control). Since the values for the housekeeping genes are taken as an average, there is always a fold change value. Changes in fold regulation between -two and two are not significant for target genes and housekeeping genes. If the fold regulation for the housekeeping genes is significant (less than -two and greater than two) it is an indication that the treatment is affecting the expression of these genes. Under these circumstances, these housekeeping genes are removed from the normalisation. For the target genes, only significant upregulations and downregulations are analysed.

3.4.1 HT-29

The data for the HT-29 treatments, in comparison to untreated cells, are normalised against the RPL13A and ACTB housekeeping genes as the expression of these genes is not significantly influenced. For comparison to DMSO-treated cells, the data is normalised against the HPRT1, RPL13A, GAPDH and ACTB housekeeping genes, which have insignificant fold regulations.

3.4.1.1 Distribution of C_t Values

The results for the gene expression in HT-29 untreated cells are used as an example (**table 3.7**).

Table 3.7: Distribution of C_t values of HT-29 untreated cells

Percent distribution of C_t values	
C_t Range	
<25	50.0%
25-30	32.29%
30-35	6.25%
Absent calls	11.46%

3.4.1.2 Reverse transcription control

The reverse transcription control determines the efficiency of the RT^2 PCR Strand Kit using an RNA control. The positive PCR control determines the efficiency of the PCR using a DNA control. These controls are in replicates to ascertain the consistency of the PCR array plate.

HT-29 untreated

ΔCt = average reverse transcription control (RTC) – average positive PCR control (PPC)

$\Delta Ct = 22.86 – 20.69$

$= 2.17$

Reverse transcription (RT) efficiency: pass.

HT-29 DMSO

$\Delta Ct = 21.19 – 20.58$

$= 0.61$

RT efficiency: pass.

HT-29 curcumin

$\Delta Ct = 22.29 – 20.36$

$= 1.93$

RT efficiency: pass.

HT-29 SAHA

$\Delta Ct = 21.09 – 22.18$

$= -1.09$

RT efficiency: pass.

3.4.1.3 Genomic DNA contamination

The Genomic DNA Control (GDC) tests for non-transcribed genomic DNA contamination. For the sample to pass this test, the Ct (GDC) value as calculated by the software must be 35.

HT-29 untreated

C_t (GDC) = 35

Result: pass

HT-29 DMSO

C_t (GDC) = 35

Result: pass

HT-29 curcumin

C_t (GDC) = 35

Result: pass

HT-29 SAHA

C_t (GDC) = 35

Result: pass

Table 3.8: Fold regulation of genes, in comparison to untreated HT-29 cells, due to various treatments with significant fold changes highlighted.

	DMSO	Curcumin	SAHA
ANAPC4	3.0695	1.3933	3.191
ARHI	-4.2723	-3.7179	1.5562
ATM	1.8213	2.4889	2.7837
ATR	1.7544	-1.6172	2.6427
BCCIP	1.9602	-1.1971	2.1992
BCL2	1.0519	-3.0053	2.1947
BIRC5	-1.295	-5.5194	-1.2746
BRCA2	2.1705	-2.1354	2.0951
CCNB1	-1.2117	-3.4882	-1.2269
CCNB2	-2.6666	-5.2616	-1.4113
CCNC	1.5042	-1.5643	2.6281
CCND1	4.9212	2.7236	7.7328
CCNE1	1.4621	-1.4923	2.7568
CCNF	-2.4301	-7.4928	-2.0139
CCNH	-1.1181	1.7882	2.8167
CCNT1	1.2449	1.3627	2.5883
CCNT2	1.2772	-1.2946	2.2689
CDC16	1.2431	-2.0216	1.46
CDC2	-2.4589	-3.7934	-1.795
CDK2	-1.6403	-2.1969	1.0056

CDK4	-1.3996	-3.4738	-2.0097
CDK5R1	-1.1196	3.9875	1.5497
CDK5RAP1	-1.0007	-2.1192	1.0175
CDK6	1.4054	-1.6661	2.7721
CDK7	1.4152	1.482	2.1067
CDK8	1.1983	-1.0031	3.0001
CDKN1A	1.1826	5.1676	1.7691
CDKN1B	-2.082	-2.857	-1.3324
CDKN2A	-1.1181	1.7882	2.8167
CDKN2B	-1.1181	1.7882	2.8167
CDKN3	-2.0691	-6.2355	1.0504
CKS1B	-1.232	-2.7349	1.2719
CUL2	-1.1181	N/A	2.8167
CUL3	2.0335	-1.7213	2.5315
DDX11	1.4917	-1.2803	7.5266
GTF2H1	1.7231	-1.6696	2.2784
HERC5	-1.1181	1.7882	2.8167
HUS1	1.9132	1.9609	3.1427
KNTC1	1.3031	-1.9272	2.0936
MAD2L2	-1.389	-1.3694	2.5527
MCM2	1.5241	-5.9155	1.8583
MCM3	-1.2906	-7.6661	1.4054
MCM5	-1.1688	-5.6353	-1.1088

MKI67	1.1672	-7.6185	1.4876
MNAT1	1.2083	-17.8952	2.1795
NBS1	1.7496	1.1341	2.5865
PCNA	-1.1181	1.7882	2.8167
RAD1	1.3177	-1.5817	2.3359
RAD17	1.5021	1.1046	2.9363
RAD9A	-1.1181	1.7882	2.8167
RB1	2.172	2.4717	2.1901
RBBP8	1.6211	-3.0115	1.8125
RBL1	1.1975	-2.3303	1.5519
RBL2	-1.0807	-2.2245	1.8764
RPA3	-1.1181	1.7882	2.8167
SERTAD1	1.2843	2.0272	1.0324
SKP2	1.057	-4.3939	1.1188
TFDP1	-1.226	-3.7051	1.4959
TP53	2.5973	-1.0073	1.0161
UBE1	-1.9145	-2.1939	20.53

Table 3.9: Fold regulation of genes, in comparison to DMSO-treated HT-29 cells, due to various treatments with significant fold changes highlighted.

	Curcumin	SAHA
ARHI	1.6344	6.4097
BCL2	-2.2226	2.0115
BIRC5	-2.9964	-1.0208
BRCA2	-3.2586	-1.0746
CCNB1	-2.0241	-1.0503
CCNF	-2.1678	1.1633
CCNG2	1.2014	2.2568
CCNH	2.8437	3.0362
CCNT1	1.557	2.0045
CDK5R1	6.35	1.6728
CDK8	1.1832	2.4137
CDKN1A	6.215	1.4422
CDKN2A	2.8437	3.0362
CDKN2B	2.8437	3.0362
CDKN3	-2.1188	2.0954
CUL2	2.8437	3.0362
CUL3	-2.461	1.2002
DDX11	-1.3428	4.8644
GADD45A	3.7031	2.0227
GTF2H1	-2.0227	1.2748

HERC5	2.8437	3.0362
MAD2L2	1.4427	3.4182
MCM2	-6.339	1.1755
MCM3	-4.1764	1.7487
MCM5	-3.3899	1.0162
MKI67	-6.2517	1.2288
MNAT1	-15.2026	1.739
PCNA	2.8437	3.0362
RAD9A	2.8437	3.0362
RBBP8	-3.4325	1.0779
RPA3	2.8437	3.0362
SERTAD1	2.2451	-1.2903
SKP2	-3.2654	1.0205
TFDP1	-2.1247	1.7682
TP53	-1.8394	-2.6514
UBE1	1.2412	37.9033

3.4.1.4 HT-29/treatments

The treated cells are first compared to untreated HT-29 cells as the control, and treated cells are then compared to DMSO-treated cells as the control. This isolates the effect of the drug, without the influence of the solvent, on the genes. This is a similar approach to the methods of Moos *et al*. (2004) and Xia *et al*. (2007).

ANAPC4

APC4 is upregulated in response to DMSO treatment in comparison to untreated HT-29 cells. SAHA also upregulates APC4 in relation to untreated cells. However, as it is not upregulated by SAHA in relation to DMSO-treated control cells, but is also upregulated by DMSO treatment, this upregulation is due to the influence of DMSO. It is a structural subunit of the APC/C ubiquitin ligase (Thornton and Toczyski, 2006). It therefore functions in regulating the G_1 checkpoint and spindle assembly checkpoint; degrading mitotic cyclins for mitotic exit and initiation of a new cycle (Peters, 2002; Wasch and Cross, 2002). APC4 is mutated in colon cancer cell lines and other cancer cell lines (Wang *et al*., 2003). This mutation negatively influences cyclin B1 ubiquitination and induces premature mitotic cyclin activity to impair replication and genomic integrity. Although the APC/C subunit is upregulated by DMSO, the mutation promotes aberrant replication. This decreases the genomic integrity and stability of HT-29 to promote tumourigenesis. Consequently, DMSO is not an appropriate solvent for SAHA.

ARHI

DMSO treatment downregulates expression of the tumour suppressor ARHI in relation to untreated cells. Curcumin downregulates ARHI in relation to untreated cells and in DMSO-treated cells. ARHI downregulation decreases activity of the tumour suppressor p21, promotes cyclin D1, increases EGFR-mediated signalling and reduces apoptosis (Bao *et al*., 2002). It is a result of E2F1 and E2F4 binding, in complexes with HDAC, to ARHI promoters (Lu *et al*., 2006). ARHI expression may decrease through histone acetylation (Fujii *et al*., 2003). However, DMSO promotes histone hypoacetylation in

leukaemia cells (Terada *et al*., 1978; Nudel *et al*., 1977; Scher *et al*., 1977). DMSO promotes p21 in hybridoma 7TD1 cells and inhibits cyclin D1 in HL-60 and N1E-115 cells (Ponzio *et al*., 1998; Burger *et al*., 1994; Jiang *et al*., 1994). This downregulation by curcumin promotes HT-29 proliferation and decreases genomic stability. As it is not downregulated by curcumin in relation to DMSO-treated control cells, it is induced by DMSO. DMSO is therefore not suitable as a solvent for curcumin.

SAHA upregulates ARHI, in comparison to DMSO-treated control cells, and this is due to curcumin without the influence of DMSO. This increases p21 activity, inhibits cell cycle progression and induces apoptosis (Bao *et al*., 2002). SAHA activates p21 expression to induce growth arrest; promotes p21 activity in pancreatic cancer cells, endometrial cancer, lymphoma, bladder carcinoma and leukaemia cells; and induces apoptosis in numerous cell types (Zhao *et al*., 2006; Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005; Gui *et al*., 2004; Vrana *et al*., 1999; O'Connor, 2006; Zhang *et al*., 2005). SAHA decreases HT-29 proliferation and increases genomic stability to inhibit the oncogenic state.

ATM

Curcumin upregulates ATM in comparison to untreated cells. It is not upregulated in comparison to DMSO-treated control cells, and is therefore induced by the combination of curcumin with DMSO. SAHA also upregulates ATM in comparison to untreated cells. This is due to the combination of SAHA with DMSO. The upregulation of this tumour suppressor gene inhibits cell cycle progression and promotes checkpoint activation, by

promoting p53 and p21, and inhibits DNA replication and apoptosis (Lee and Paull, 2005; Banin *et al*., 1998; Brown and Baltimore, 2003; Greer *et al*., 2003; Falck *et al*., 2002; Liu *et al*., 2007; Ocker and Schneider-Stock, 2007; Burma *et al*., 2001). Curcumin upregulates p21 in HT-29. This inhibits proliferation and promotes the genomic stability of HT-29. Similarly, curcumin activates p53 and cell cycle arrest in HT-29 cells, and upregulates p21 and p53 in prostate cancer cells and HepG2 cells (Song *et al*., 2005; Hour *et al*., 2002; Lv *et al*., 2007).

SAHA also promotes p53 and p21 activity, and induces cell cycle arrest in numerous cancer cell types, including colon cancer (Munshi *et al*., 2006; Zhao *et al*., 2006; Kumagai *et al*., 2007; Garcia-Manero and Issa, 2005; Butler *et al*., 2000; Chobanian *et al*., 2004). ATM inhibits DNA replication following DNA damage (Costanzo *et al*., 2000). SAHA reduces HT-29 proliferation and promotes genomic stability to inhibit tumourigenesis.

ATR

SAHA upregulates ATR in comparison to untreated cells. As it is not upregulated in comparison to DMSO-treated control cells, it is induced by the combination of SAHA with the solvent DMSO. ATR is essential at the S and G_2 checkpoints (Walworth and Bernards, 1996; Lindsay *et al*., 1998). It localises at stalled replication forks to phosphorylate CHK1 and induce checkpoint activation and cell cycle arrest (Dart *et al*., 2004). This stabilises the stalled forks, promotes their re-initiation, prevents ssDNA accumulation and promotes DNA repair (Cobb *et al*., 2005). It also reacts to S-phase

DSBs in an ATM-dependent manner (Jazayeri *et al*., 2006). ATR phosphorylates BRCA1 at these sites for DNA repair and cell cycle arrest (Andreassen *et al*., 2004). It inhibits DNA replication and promotes DNA repair by phosphorylating MCM2 and MCM7 (Cortez *et al*., 2004). ATR induces activation of the S-phase and G_2/M checkpoint in response to re-replication (Liu *et al*., 2007). It also localises at telomeres at late S-phase and may function in telomere replication (Verdun and Karlseder, 2006). SAHA induces G_1/S and G_2/M arrest in previous studies, including in MCF-7 cells (Bali *et al*., 2005; Huang and Pardee, 2000; Kumagai *et al*., 2007; Munster *et al*., 2001). G_2/M arrest sensitises tumour cells to radiation treatment (Aggarwal *et al*., 2003; Munshi *et al*., 2006). SAHA decreases proliferation and increases genomic integrity and stability to reduce the oncogenic state of HT-29.

BCCIP

SAHA upregulates the tumour suppressor BCCIP, in comparison to untreated cells. This is due to SAHA with the influence of DMSO. BCCIP inhibits proliferation by enhancing p21 stability and expression. This increases the inhibition of CDK2 to reduce cycle cell progression (Liu *et al*., 2001; Ono *et al*., 2000). It promotes BRCA2, which is upregulated by SAHA, and RAD51 nuclear focus formation during homologous recombination (Lu *et al*., 2005). BCCIP prevents ssDNA and DSB accumulation (Lu *et al*., 2007). SAHA also inhibits proliferation in mantle cell lymphoma cell lines (Heider *et al*., 2006). SAHA decreases HT-29 proliferation and increases DNA integrity to inhibit tumourigenesis.

BCL2

Anti-apoptotic BCL2 is downregulated by curcumin in comparison to both untreated and DMSO-treated control cells. This is induced by curcumin with and without DMSO influence. This promotes BAX and induces apoptosis and G_1/S progression (Kim *et al*., 2004; Dlugosz *et al*., 2006; Mazel *et al*., 1996; Hockenberry *et al*., 1993). It promotes E2F transcriptional activity to increase DNA mismatch repair (Youn *et al*., 2005). BCL2 is inhibited by the tumour suppressor p53 (Chipuk *et al*., 2004; Mihara *et al*., 2003). This downregulation increases proliferation and genomic integrity and stability. Likewise, curcumin inhibits BCL2 in HT-29, lung cancer, bladder cancer and prostate cancer cells (Song *et al*., 2005; Radhakrishna Pillai *et al*., 2004; Tian *et al*., 2008; Chendil *et al*., 2004).

SAHA upregulates BCL2, in relation to untreated and DMSO-treated control cells. This is induced by SAHA with and without DMSO influence. This inhibits BAX, apoptosis and cell cycle progression (Kim *et al*., 2004; Dlugosz *et al*., 2006; Mazel *et al*., 1996; Hockenberry *et al*., 1993). Its overexpression inhibits DNA repair and DNA replication and induces genomic instability (Saintigny *et al*., 2001; Youn *et al*., 2005; Liu *et al*., 1997). This contradicts studies where HDACi inhibit BCL2 and anti-apoptotic BCL-xL to promote cell cycle arrest and apoptosis (Bali *et al*., 2005; Huang and Pardee, 2000). This upregulation decreases cell cycle progression but reduces the genomic integrity of HT-29.

BIRC5

Anti-apoptotic survivin is downregulated by curcumin in relation to untreated and DMSO-treated control cells. This is induced by curcumin both with and without DMSO influence. This is associated with decreased proliferation to inhibit tumour cell growth (Altieri, 2003; Zaffaroni *et al*., 2002; Dohi *et al*., 2004). It decreases microtubule assembly and spindle checkpoint regulation (Giodini *et al*., 2002; Lens and Medema, 2003). The inhibition of p21 is reduced to inhibit S-phase progression (Fukuda *et al*., 2004). Curcumin upregulates p21 in HT-29. The nuclear translocation of survivin is stimulated by CDK4 to induce cell cycle entry (Sui *et al*., 2002). As CDK4 is also downregulated by curcumin, this is repressed. Caspase-9 activation increases to promote apoptosis, inhibiting anchorage-independent growth (Sarela *et al*., 2000). Curcumin also decreases survivin expression in bladder cancer cells, apoptosis-resistant BCR-ABL-expressing cells and T-cell leukaemia cells to induce G_2/M arrest, mitotic catastrophe and cell death (Tian *et al*., 2008; Wolanin *et al*., 2006; Tomita *et al*., 2006). G_2/M arrest sensitises cells to radiation (Aggarwal *et al*., 2003). Curcumin also promotes apoptosis and inhibits proliferation in various cell types (Leu and Maa, 2002; Jiang *et al*., 1996; Simon *et al*., 1998; Kuo *et al*., 1996; Aggarwal *et al*., 2003). This downregulation reduces proliferation and increases genomic stability to decrease the oncogenic state of HT-29.

BRCA2

DMSO upregulates BRCA2. This upregulation promotes the assembly and disassembly of RAD51 filaments during homologous recombination in DSB repair (Davies *et al*.,

2001; Galkin *et al*., 2005). It stabilises DNA at stalled replication forks and is a component of the DNA replication complex with BRCA1, PCNA, and RAD51 (Lomonosov *et al*., 2003; Scully *et al*., 1997). BRCA2 binds to ssDNA and BCCIP (Yang *et al*., 2002; Meng *et al*., 2004). It also regulates replication and centrosome migration (Nakanishi *et al*., 2007). DMSO promotes the genomic integrity and replication of stabilised DNA.

Curcumin downregulates BRCA2 in relation to untreated and DMSO-treated control cells. This is due to curcumin both with and without DMSO influence. This decreases DNA damage repair and DNA replication. However, BRCA2 overexpression in breast cancer correlates with poor prognosis, suggesting it promotes oncogenic transformation (Egawa *et al*., 2002). Curcumin may be downregulating overexpressed BRCA2 found in untreated HT-29 cells to a positive level for DNA damage response functioning to increase genomic stability. Curcumin promotes genomic integrity and proliferation in HT-29.

SAHA upregulates BRCA2, in relation to untreated cells, to increase the DNA damage responses and DNA replication (Galkin *et al*., 2005; Lomonosov *et al*., 2003). As it is also upregulated in DMSO-treated cells, but not by SAHA in relation to DMSO-treated cells, this upregulation is due to DMSO influence. SAHA increases the proliferation of stable HT-29 DNA. However, SAHA inhibits proliferation in mantle cell lymphoma cell lines (Heider *et al*., 2006). This upregulation demonstrates the unsuitability of DMSO as a solvent for SAHA.

CCNB1

Cyclin B1 is downregulated by curcumin treatment in comparison to untreated and DMSO-treated control cells. This is induced by curcumin with and without DMSO influence. Cyclin B1 downregulation inhibits apoptosis and inhibits proliferation and cell survival (Pines and Hunter, 1989; Coqueret, 2003). The other component of the M-phase-promoting factor (MPF) complex, CDC2, is also downregulated by curcumin to decrease mitosis (Morgan, 1995). Nuclear cyclin B1/CDC2 promotes chromosomal condensation and degradation of the nuclear envelope during apoptosis (Hagting *et al*., 1999). Cyclin B1 is inhibited by p53 (Krause *et al*., 2000). Curcumin also downregulates cyclin B1 in human umbilical vein endothelial cells (Park *et al*., 2002). This decreases proliferation of HT-29.

CCNB2

Cyclin B2 is downregulated by DMSO in comparison to untreated cells. It forms part of the MPF complex that induces mitosis (Morgan, 1995). It is inhibited by the tumour suppressor p53 (Krause *et al*., 2000). The other component of the MPF complex, CDC2, is also downregulated here, therefore MPF activity is inhibited by DMSO treatment and mitotic entry is suppressed. This is in conflict with work with HL-60 and N1E-115 cells where DMSO promotes cyclin B overexpression (Burger *et al*., 1994; Jiang *et al*., 1994). This downregulation decreases proliferation of HT-29 and reduces tumourigenesis.

Curcumin downregulates cyclin B2, in relation to untreated cells, to inhibit mitotic entry and apoptosis (Morgan, 1995; Pines and Hunter, 1989). This downregulation is due to

DMSO influence. CDC2 is also downregulated by curcumin. The overexpression of cyclin B2 induces chromosomal instability in colorectal cancer (Wang *et al*., 1997). Curcumin inhibits HT-29 proliferation and decreases tumourigenesis.

CCNC

SAHA upregulates cyclin C, in relation to untreated cells. It is not upregulated in comparison to DMSO-treated cells, and hence is due to the combination of SAHA and DMSO. Cyclin C mediates pRB phosphorylation and inactivation at G_0 to promote cell cycle entry. It induces CDC2 for G_1/S and G_2/M progression (Liu *et al*., 1998). Cyclin C/CDK8 inhibits transcription by phosphorylating cyclin H to inhibit CDK7 and TFIIH (Akoulitchev *et al*., 2000). It also inhibits transcription by phosphorylating pol II (Hengartner *et al*., 1998). Cyclin C upregulation promotes cell cycle progression but inhibits transcription. DMSO is not an appropriate solvent for SAHA.

CCND1

Cyclin D1 is upregulated by DMSO in relation to untreated cells. It promotes G_1 entry, cell survival and regulates transcription factor activity (Schwartz and Shah, 2005; Hinds *et al*., 1994; Coqueret, 2002). Cyclin D/CDK4 and cyclin D/CDK6 inactivate the CKIs to promote cell cycle progression (Lundberg and Weinberg, 1998). Cyclin D1/CDK4 translocates p27 and p21 to the nucleus to activate cyclin E/CDK2, inactivate RB and promote cell cycle progression (Perez-Roger *et al*., 1999). Cyclin D1 represses premature DNA synthesis by inhibiting PCNA and CDK2 (Fukami-Kobayashi and Mitsui, 1999). Accumulation at G_2 promotes entry into the next cycle of cell division

(Yang *et al*., 2006). By upregulating cyclin D1, DMSO is promoting proliferation and cell survival in HT-29 cells. However, DMSO inhibits cyclin D1 expression in HL-60 and N1E-115 cells (Burger *et al*., 1994; Jiang *et al*., 1994).

Curcumin upregulates cyclin D1 in relation to untreated cells. As it is not upregulated in relation to DMSO-treated control cells, but is upregulated in DMSO-treated cells, this upregulation is induced by DMSO. Curcumin downregulates cyclin D1 in leukaemia cells, LnCap prostate cells and various breast cancer cell lines, including MCF-7 (Tomita *et al.*, 2006; Mukhopadhyay *et al*., 2002). This upregulation promotes HT-29 proliferation and survival, consequently increasing the oncogenic state. DMSO is not an appropriate solvent for curcumin.

Cyclin D1 is also upregulated by SAHA in comparison to untreated cells. This is due to SAHA with DMSO influence. SAHA, however, inhibits cyclin D1 protein translation in mantle cell lymphoma cell lines to induce growth arrest (Glaser, 2007; Heider *et al*., 2006; Kawamata *et al*., 2007). UBE1 is upregulated by SAHA as well, and inhibits cyclin D1 to reduce cell cycle progression (Lonardo *et al*., 1999; Pitha-Rowe *et al*., 2004). The increase in HT-29 proliferation by cyclin D1 may be reduced by the upregulation of UBE1. The upregulation of cyclin D1 increases proliferation and genomic integrity in HT-29. This is accredited to DMSO influence and consequently this affirms that DMSO is not a suitable solvent for SAHA.

CCND2

Cyclin D2 is downregulated by DMSO. It promotes G_1 entry and cell survival (Schwartz and Shah, 2005; Hinds *et al*., 1994). It has redundant roles to cyclin D1 with overexpression in cancer occurring less frequently (Malumbres and Barbacid, 2001). If accumulated, cyclin D2 would relieve p27 inhibition of CDK2. Similarly, DMSO inhibits cyclin D2/CDK4 and inactivates CDK2 in hybridoma 7TD1 cells (Ponzio *et al*., 1998). DMSO is inhibiting HT-29 proliferation.

CCNE1

SAHA upregulates cyclin E1, in relation to untreated cells. It is not upregulated in comparison to DMSO-treated control cells, and is due to the combination of SAHA with DMSO. Cyclin E1 promotes G_1 exit, S-phase progression and apoptosis (Koff *et al*., 1991; Mazumder *et al*., 2004; Dobashi, 2005). It inactivates the tumour suppressor p27 to promote CDK2 activity and cell cycle progression (Elledge and Harper, 1998). Cyclin E/CDK2 regulates DNA synthesis with CDC6 and PCNA and regulates the delivery of MCM2-7 and CDC45 to replication origins to promote assembly of the pre-replication complex. Cyclin E/CDK2 then induces replication origin firing to initiate S-phase entry (Cook *et al*., 2002; Coverley *et al*., 2002). Cyclin E promotes faster G_1/S progression, decreases genomic stability and promotes oncogenic transformation (Spruck *et al*., 1999; Akli and Keyomarsi, 2004; Akama *et al*., 1995). Cyclin E upregulation increases proliferation and tumourigenesis of HT-29 and therefore DMSO is not an appropriate solvent for SAHA.

CCNF

Cyclin F is downregulated by DMSO in comparison to untreated cells. This decreases binding to cyclin B1 at G_2/M and reduces mitosis (Kong *et al*., 2000). Its influence on G_0/G_1 and G_1/S transitions in the next cell cycle is decreased. RB-E2F-cyclin E function decreases to reduce cyclin E transcription and CDK2 activation (Tezlaff *et al*., 2004). E2F inhibition increases to reduce S-phase entry and DNA synthesis (Lavia and Jansen-Durr, 1999). Cyclin F has a role in proteolysis as it binds Skp1 protein, an essential element of SCF ubiquitin ligase (Tezlaff *et al*., 2004). Its downregulation therefore induces apoptosis (Jana *et al*., 2004). DMSO inhibits cell cycle progression of HT-29 cells and reduces tumourigenesis.

Cyclin F is downregulated by curcumin in comparison to both untreated and DMSO-treated control cells. This is induced by curcumin with and without DMSO influence. Through this downregulation, curcumin suppresses HT-29 proliferation to decrease tumourigenesis. Other studies verify that curcumin inhibits the proliferation of colon cancer cells (Aggarwal *et al*., 2003).

SAHA also downregulates cyclin F in relation to untreated cells and this is due to the combination of SAHA with DMSO. SAHA inhibits the cell cycle and induces apoptosis in various cancer cell types, including colon cancer (O'Connor, 2006; Garcia-Manero and Issa, 2005; Chobanian *et al*., 2004; Butler *et al*., 2000; Yi *et al*., 2008; Kumagai *et al*., 2007; Bali *et al*., 2005; Huang and Pardee, 2000). SAHA decreases HT-29 proliferation and increases genomic stability to inhibit tumourigenesis.

CCNG2

SAHA induces an upregulation in cyclin G2 in comparison to DMSO-treated control cells. This is accredited to SAHA without the influence of DMSO. Cyclin G2 inhibits the cell cycle, maintains quiescence in differentiated cells and regulates microtubule stability (Bennin *et al*., 2002; Arachchige Don *et al*., 2006). It inhibits CDK2 to induce G_1/S arrest (Bennin *et al*., 2002). Cyclin G2 levels, dependent on p53, are low in proliferating cells and increase in apoptotic cells (Horne *et al*., 1997). DMSO inhibits proliferation, increases genomic stability and reduces the oncogenic state of HT-29. The increased cyclin G2 levels in SAHA-treated cells indicate that apoptosis is occurring at a higher rate than in untreated cells.

CCNH

Curcumin upregulates cyclin H in comparison to DMSO-treated control cells. This upregulation is induced by curcumin without DMSO influence. Cyclin H forms the TFIIH complex with CDK7 and MAT1 to promote transcription and DNA repair (Svejstrup *et al*., 1996; Schaeffer *et al*., 1993). TFIIH phosphorylates CDK2 to inhibit cell cycle progression (Andersen *et al*., 1997). It also phosphorylates RNA pol II to induce elongation following transcription (Akoulitchev *et al*., 1995). SAHA also upregulates cyclin H, in comparison to untreated and DMSO-treated control cells. This is therefore due to SAHA with and without DMSO influence. The upregulation of cyclin H promotes transcription and genomic integrity of HT-29.

CCNT1

Cyclin T1 is upregulated by SAHA, in relation to untreated and DMSO-treated control cells. This upregulation is induced by SAHA with and without DMSO influence. Cyclin T forms part of the positive transcription elongation factor b (P-TEFb) that phosphorylates RNA pol II and activates transcriptional elongation (Palancade and Bensaude, 2003). P-TEFb is essential for the transcription of numerous genes (Garber *et al*., 1998; Chao and Price, 2001). Cyclin T1/CDK9 is required for cell differentiation and its expression is higher in terminally differentiated tissues (De Falco and Giordano, 2002; Simone *et al*., 2002). Cyclin T1 is upregulated as part of the innate immune response (Liou *et al*., 2006). SAHA induces differentiation in bladder carcinoma, breast adenocarcinoma, colon cancer, prostate cancer, ovarian cancer and pancreatic cancer cells (O'Connor, 2006; Garcia-Manero and Issa, 2005; Chobanian *et al*., 2004; Butler *et al*., 2000; Kumagai *et al*., 2007). SAHA promotes transcription and differentiation of HT-29 cells to increase its genomic stability and inhibit tumourigenesis.

CCNT2

SAHA upregulates cyclin T2, in relation to untreated cells. This is induced by SAHA in combination with DMSO. Cyclin T2 forms part of P-TEFb to phosphorylate RNA pol II and induce the transcription of many genes (Palancade and Bensaude, 2003). It has a higher transcriptional activation potential than cyclin T1 (Napolitano *et al*., 2000). Cyclin T2/CDK9 is required for differentiation and its expression is higher in terminally differentiated tissues (Simone *et al*., 2002). SAHA does promote differentiation in various other cancer cell types (O'Connor, 2006; Garcia-Manero and Issa, 2005;

Chobanian *et al*., 2004; Butler *et al*., 2000; Kumagai *et al*., 2007). SAHA induces HT-29 differentiation to decrease its transformed state.

CDC16

CDC16 is downregulated by curcumin in relation to untreated cells. As it is not upregulated in relation to DMSO-treated control cells, it is induced by the combination of curcumin with DMSO. CDC16 is one of the main subunits of the APC/C E3 ubiquitin ligase complex, forming part of the TPR arm for adaptor binding, and is essential for complex activation through CDK1 phosphorylation (Rudner and Murray, 2000). APC/C activity is essential for metaphase/anaphase and M/G_1 transition. CDC16 is mutated in colon cancer cell lines and other cancer cell lines (Wang *et al*., 2003). Its downregulation decreases cell cycle progression and induces apoptosis (Jana *et al*., 2004). Similarly, curcumin inhibits proliferation in colon cancer cells and induces apoptosis in various cells, including HT-29, MCF-7 and prostate cancer cells (Aggarwal *et al*., 2003; Song *et al*., 2005; Xia *et al*., 2007; Shankar and Srivastava, 2007). CDC16 downregulation reduces proliferation and increases genomic integrity of HT-29 to decrease tumourigenesis.

CDC2

DMSO treatment downregulates CDC2 in relation to untreated cells. This inhibits mitotic entry (Peters *et al*., 1998). The phosphorylation of RNA pol II, and subsequent inhibition of mRNA production during mitosis, decreases (Kobor and Greenblatt, 2002). The tumour suppressors p53 and p21 repress CDC2 transcription. p53 induces GADD45

inhibition of cyclin B1/CDC2 formation. CDC2 downregulation decreases the phosphorylation and activation of APC^{CDC20}, and reduces anaphase progression (Rudner and Murray, 2000). Low CDC2 levels are sufficient for mitotic entry but higher levels are required for mitotic progression and the onset of anaphase (Lindqvist *et al*., 2007). DMSO decreases HT-29 proliferation to decrease the oncogenic state.

CDC2 is downregulated by curcumin treatment in comparison to untreated cells and this is due to curcumin in combination with DMSO. Through this downregulation, curcumin decreases HT-29 proliferation to reduce tumourigenesis. This concurs with studies where curcumin downregulates CDC2 in human umbilical vein endothelial cells (Park *et al*., 2002).

CDK2

CDK2 is downregulated by curcumin in relation to untreated cells. This is induced by the combination of curcumin with DMSO. This decreases replication origin firing and transcription (Yoo *et al*., 2004). p27 activity is promoted through decreased degradation induced by SKP2, and consequently G_1/S and G_2/M progression is inhibited (Fischer *et al*., 2004). The inactivation of CDC16 at S-phase by cyclin A/CDK2 decreases and this may increase re-replication (Biermann *et al*., 2002; Petersen *et al*., 2000). The downregulation of CDK2 inhibits proliferation in HT-29, but may promote genomic instability.

CDK4

CDK4 is downregulated by curcumin in relation to untreated cells. As it is not upregulated in relation to DMSO-treated control cells, this is induced by the combination of curcumin with DMSO. Cyclin E degradation and cell cycle progression are reduced (Strohmaier *et al*., 2001). Likewise, curcumin inhibits cyclin E in HaCaT cells and in breast cancer (Cho *et al*., 2007; Aggarwal *et al*., 2007; Goel *et al*., 2008). Cyclin D/CDK4 promotes the cell cycle at early G_1 (Pines, 1995). Cyclin D is upregulated by curcumin but as CDK4 is downregulated, G_1 progression is not promoted. The phosphorylation and inactivation of the tumour suppressor RB decreases. As elevated levels of CDK4 exert a strong tumourigenic effect, this downregulation reduces tumourigenesis in HT-29 by inhibiting proliferation (Liu, 2006). Curcumin also inhibits CDK4 in other studies and suppresses cell cycle progression in colon cancer cell lines and breast cancer (Choudhuri *et al*., 2005; Mukhopadhyay *et al*., 2002; Aggarwal *et al*., 2003; Aggarwal *et al*., 2007b).

SAHA also downregulates CDK4 in comparison to untreated cells as a result of SAHA activity in combination with DMSO. SAHA also inhibits CDK4 in MCF-7 cells (Yi *et al*., 2008). Its downregulation decreases MDM2 levels to increase p53 tumour suppressor activity (Ortega *et al*., 2002; Hall and Peters, 1996). It has ubiquitin ligase activity and its inhibition induces apoptosis (Strohmaier *et al*., 2001; Jana *et al*., 2004). CDK4 is overexpressed in colorectal carcinomas and SAHA is inducing its downregulation compared to untreated HT-29 cells (Zhao *et al*., 2006). SAHA decreases HT-29 proliferation and increases its genomic stability to reduce tumourigenesis.

CDK5RAP1

CDK5RAP1 is downregulated by curcumin in comparison to untreated cells. This is due to the combination of curcumin and DMSO. This downregulation promotes CDK5 activation and increases apoptosis, differentiation, transcription and senescence (Ching *et al*., 2002; Zhang *et al*., 2002; Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007). Curcumin also induces apoptosis in several cell types, including HT-29, other colon cancer cells and MCF-7 (Song *et al*., 2005; Aggarwal *et al*., 2003; Xia *et al*., 2007). CDK5RAP1 histone acetyltransferase activity is inhibited to decrease transcription (Anantharaman *et al*., 2001). Curcumin inhibits histone acetyltransferases by promoting p300 proteasomal degradation (Marcu *et al*., 2006). This downregulation increases genomic stability and decreases the oncogenic state of HT-29.

CDK5R1

CDK5R1 is upregulated by curcumin, in comparison to both untreated and DMSO-treated control cells, to promote apoptosis, differentiation, transcription and senescence by activating CDK5 (Zhang *et al*., 2002; Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007). This upregulation is due to curcumin with and without DMSO influence. CDK5/p35 is upregulated in colon cancer cells in comparison to normal colonic mucosa (Lee *et al*., 1997; Zhang *et al*., 2002). Similarly, curcumin induces apoptosis in colon cancer, breast cancer, liver cancer, leukaemia, neuroblastoma cells and bladder cancer cells (Aggarwal *et al*., 2007; Xia *et al*., 2007; Jiang *et al*., 1996; Simon *et al*., 1998; Kuo *et al*., 1996; Liontas and Yeger, 2004; Park *et al*., 2006). Curcumin promotes genomic stability and inhibits the oncogenic state of HT-29.

CDK6

SAHA upregulates CDK6 in relation to untreated cells and this is induced by SAHA in combination with DMSO. Cyclin D/CDK6 phosphorylates and inhibits pRB to relieve the inhibition of E2F, and promote cyclin E (Geng *et al*., 1996). This promotes S-phase progression (Kwon *et al*., 1995). CDK6 overexpression stimulates HER2/neu upregulation to accelerate entry into the S-phase and promote cyclin D1 transcription (Timms *et al*., 2002; Lenferink *et al*., 2001). p16, which is upregulated by SAHA, inhibits CDK6 (Parry *et al*., 1999). Its overexpression in cancers strongly promotes tumourigenesis (Ortega *et al*., 2002; Liu, 2006). This increases the oncogenic potential of HT-29 cells (Timms *et al*., 2002; Liu, 2006). This is accredited to DMSO as it is not upregulated by SAHA in comparison to DMSO-treated control cells. This supports the evidence of DMSO not being a suitable solvent for SAHA.

CDK7

SAHA upregulates CDK7, in comparison to untreated cells. This is due to the combination of SAHA and DMSO, as CDK7 is not upregulated by SAHA in relation to DMSO-treated control cells. CDK7 is a component of the CAK complex that activates the CDKs, and forms part of the TFIIH transcription factor (Harper and Elledge, 1998). TFIIH initiates transcription and DNA repair. CAK phosphorylates TFIIH to induce elongation following transcription (Akoulitchev *et al*., 1995). CDK7 phosphorylates RNA pol II for transcription (Shiekhattar *et al*., 1995). This upregulation promotes HT-29 proliferation; consequently, DMSO is not an appropriate solvent for SAHA.

Cyclin C and CDK8 are also upregulated by SAHA and therefore inhibit cyclin H/CDK7 activity (Akoulitchev *et al*., 2000).

CDK8

SAHA upregulates CDK8 in comparison to both untreated and DMSO-treated control cells. This upregulation is due to SAHA with and without DMSO influence. CDK8 is a subunit in the Mediator complex, inhibiting transcription by phosphorylating and destabilising transcription factors (Hengartner *et al*., 1998; Chi *et al*., 2001). Cyclin C/CDK8 phosphorylates and inhibits cyclin H/CDK7 to inhibit CAK and TFIIH activity (Akoulitchev *et al*., 2000). Cyclin C/CDK8 is also transcriptionally activated by p21 and enhances p53-regulated transcription (Donner *et al*., 2007). SAHA inhibits proliferation and increases genomic integrity and stability to reduce the oncogenic state of HT-29.

CDKN1A

Curcumin upregulates the tumour suppressor p21 in relation to both untreated and DMSO-treated control cells. This upregulation is induced by curcumin, with and without the influence of the DMSO solvent. p21 promotes p130/E2F4 binding to the CDC2 promoter to inhibit mitotic entry (Taylor *et al*., 2001). It inhibits cyclin A/CDK2 and cyclin E/CDK2 to induce p53-dependent G_1 arrest (Maddika *et al*., 2007). It is stimulated by p53 in response to DNA damage where it prevents pRB phosphorylation and inhibition (Sherr and Roberts, 1999; Lohrum and Vousden, 2000). p21 inhibits PCNA and suppresses DNA replication. It inhibits caspase-3 and caspase-8 to inhibit apoptosis (el-Deiry *et al*., 1993; Ocker and Schneider-Stock, 2007). Similarly, curcumin

upregulates p21 in breast cancer cells, prostate cancer cells and HepG2 cells (Aggarwal *et al*., 2007; Goel *et al*., 2008; Choudhuri *et al*., 2005; Hour *et al*., 2002; Lv *et al*., 2007). Curcumin reduces HT-29 proliferation and promotes genomic stability.

CDKN1B

DMSO treatment and curcumin treatment downregulate p27 in comparison to untreated cells. This promotes G_1/S progression by increasing cyclin E/CDK2 and cyclin A/CDK2, and inhibits apoptosis and differentiation (Kayatose *et al*., 1997; Zabludoff *et al*., 1998; Robker and Richards, 1998). Cyclin E/CDK2 phosphorylates p27 for ubiquitination at G_1, S and G_2 mediated by CDC34 (Malek *et al*., 2001). SKP2 levels are inversely correlated with p27 protein levels in colorectal adenocarcinomas (Hershko *et al*., 2001). Cyclin D2 is another important inhibitor of p27 at G_0/G_1 (Solvason *et al*., 2000). This downregulation increases proliferation and the oncogenic state of HT-29 to promote tumourigenesis. In contrast, DMSO induces differentiation in HL-60 and N1E-115 cells and increases p27 levels in CHO cells (Burger *et al*., 1994; Jiang *et al*., 1994; Fiore and Degrassi, 1999). DMSO also promotes p27 inhibition of cyclin/CDK2 in hybridoma 7TD1 cells (Ponzio *et al*., 1998).

The downregulation by curcumin is due to DMSO as it is also upregulated in DMSO-treated cells, but not by curcumin in comparison to DMSO-treated control cells. Curcumin upregulates p27 in prostate cancer cells and in breast cancer (Hour *et al*., 2002; Aggarwal *et al*., 2007; Goel *et al*., 2008). As the upregulation is induced by DMSO, DMSO is not an appropriate solvent for curcumin.

CDKN2A

The tumour suppressor p16 is upregulated by curcumin treatment, in relation to DMSO-treated control cells. As it is not upregulated in DMSO-treated cells or by curcumin in comparison to untreated control cells, this upregulation is induced by curcumin without the influence of DMSO. SAHA also upregulates p16 in relation to untreated cells. This is due to the effect of SAHA in combination with DMSO. p16 induces G_1/S arrest by activating RB to suppress E2F-mediated transcription (Brenner *et al*., 1998). p16 expression increases with stress, including oncogenic stress (Parry *et al*., 1999). It also inhibits metastasis and angiogenesis (Wang *et al*., 2006; Gibson *et al*., 2005). Curcumin inhibits metastasis in breast cancer (Aggarwal *et al*., 2007; Goel *et al*., 2008). As well as inhibiting proliferation and inducing arrest in numerous cancers, SAHA also inhibits angiogenesis (O'Connor, 2006; Garcia-Manero and Issa, 2005; Butler *et al*., 2000; Chobanian *et al*., 2004; Bali *et al*., 2005; Ocker and Schneider-Stock, 2007). This upregulation inhibits proliferation and the oncogenic state and promotes genomic stability.

CDKN2B

Curcumin upregulates p15 in relation to DMSO-treated control cells. This is induced by curcumin without DMSO influence. SAHA also upregulates p15 in comparison to both untreated and DMSO-treated control cells. This upregulation is induced by SAHA with and without the influence of DMSO. This tumour suppressor induces G_1 arrest by inhibiting RB phosphorylation and increasing p27 activity (Reynisdottir *et al*., 1995; Massague, 2004). Its upregulation induces senescence and inhibits telomerase activity

(Swarbrick *et al*., 2000; Fuxe *et al*., 2000). The activation of telomerase increases the telomere stability of cancer cells and increases their ability to proliferate and escape senescence (Buys, 2000; Hastie *et al*., 1999). Curcumin also decreases telomerase activity in MCF-7 cells, and induces G_1 arrest in MCF-7 and umbilical vein endothelial cells (Ramachandran *et al*., 2002; Xia *et al*., 2007; Park *et al*., 2002). SAHA induces cell cycle arrest in various cancers, including breast, colon, prostate and ovarian cancer, and promotes senescence in transformed cells (Garcia-Manero and Issa, 2005; Butler *et al*., 2000; Chobanian *et al*., 2004; Xu *et al*., 2005). Curcumin and SAHA inhibit proliferation and the malignant nature of HT-29.

CDKN3

DMSO downregulates CDKN3 in relation to untreated cells. The dephosphorylation and inactivation of CDK2 decreases to promote the cell cycle (Hannon *et al*., 1994). Decreased levels promote p53 and p21, and p21 is upregulated by DMSO in HT-29 (Okamoto *et al*., 2006). CDKN3 is phosphorylated and inactivated by ATM following DNA damage (Ziv *et al*., 2006). As it is inactivated following DNA damage, this implies that there is a higher rate of DNA damage in the DMSO-treated cells compared to the untreated cells. This downregulation increases HT-29 proliferation.

Curcumin also downregulates CDKN3 in comparison to untreated and DMSO-treated control cells. This is due to curcumin both with and without DMSO influence. CDKN3 overexpression promotes tumourigenesis (Lee *et al*., 2000). Curcumin upregulates p21 in HT-29. This downregulation increases proliferation but also increases genomic integrity

and stability by upregulating the tumour suppressors. Similarly, curcumin promotes p53 and p21 in breast cancer, prostate cancer, umbilical vein endothelial cells, neuroblastoma and HT-29, amongst others (Choudhuri *et al*., 2005; Hour *et al*., 2002; Park *et al*., 2002; Liontas and Yeger, 2004; Song *et al*., 2005).

CDKN3 is upregulated by SAHA in relation to DMSO-treated control cells. This upregulation is due to SAHA without the influence of DMSO. This inhibits p53 transcriptional activity and represses the cell cycle (Schultz *et al*., 2001; Schultz *et al*., 2002; Chinami *et al*., 2005). ATM, which inactivates CDKN3 following DNA damage, is upregulated here by SAHA in HT-29. This upregulation inhibits proliferation; however, genomic integrity and stability may decrease.

CKS1B

Curcumin downregulates CKS1B in relation to untreated cells. This is due to the combination of curcumin with DMSO. This decreases binding to CDC2, which is also downregulated by curcumin, and decreases CDK regulation (Egan and Solomon, 1998). As it is an accessory protein for SCF^{SKP2} ubiquitin ligase, its downregulation induces apoptosis (Cardozo and Pagano, 2004; Jana *et al*., 2004). SKP2-mediated degradation of p27 decreases to reduce proliferation and cell survival (Bloom and Pagano, 2003). Likewise, curcumin inhibits proliferation and induces apoptosis in various cells, including HT-29 (Song *et al*., 2005; Aggarwal *et al*., 2003). CKS1B correlate with SKP2 levels and inversely correlate with p27 levels in colorectal and breast cancer (Shapira *et al*., 2004; Slotky *et al*., 2005). In accordance, SKP2 is downregulated by curcumin in

HT-29. Curcumin decreases HT-29 proliferation and increases its genomic stability to reduce the oncogenic state.

CUL2

CUL2 is upregulated by curcumin, in comparison to DMSO-treated control cells. This is due to curcumin action, without the influence of DMSO. SAHA upregulates CUL2 in comparison to untreated and DMSO-treated control cells. This is due to SAHA with and without DMSO influence. This upregulation promotes ECV ubiquitin ligase activity in the ubiquitination of hypoxia-inducible factor (HIFα) subunits (Cockman *et al*., 2000; Tarimoto *et al*., 2000). This induces apoptosis by promoting pro-apoptotic BAX and BCL-xL expression, and inhibits angiogenesis (Wincemicz *et al*., 2007; Ocker and Schneider-Stock, 2007). Curcumin and SAHA decrease proliferation and inhibit the oncogenic state of HT-29. This concurs with previous studies where curcumin upregulates BAX and inhibits anti-apoptotic BCL2 to induce apoptosis in HT-29, MCF-7 and bladder cancer cells (Song *et al*., 2005; Holy, 2002; Ramachandran *et al*., 2002; Tian *et al*., 2008). In other studies, SAHA increases apoptosis in cancer cells and inhibits angiogenesis by inhibiting HIF1α (O'Connor, 2006; Bali *et al*., 2005; Huang and Pardee, 2000; Kumagai *et al*., 2007; Ocker and Schneider-Stock, 2007).

CUL3

CUL3 is upregulated by DMSO in relation to untreated cells. CUL3-BTB ubiquitin ligases are important for chromosomal alignment at metaphase, spindle assembly and cytokinesis (Sumara *et al*., 2007). CUL3-based ubiquitin ligases have a tumour

suppressive function with RhoBTB2 (Wilkins *et al*., 2004). DMSO promotes the genomic integrity and stability of HT-29.

Curcumin downregulates CUL3 in relation to DMSO-treated cells and this is due to curcumin without the influence of DMSO. Metaphase and anaphase are consequently inhibited (Sumara *et al*., 2007). Chromosomal instability increases during anaphase through this downregulation, but the inhibition of ubiquitin-mediated degradation induces apoptosis in these cells (Jana *et al*., 2004). By disrupting the spindle structure through CUL3 downregulation, curcumin induces mitotic catastrophe and cell death, as seen in MCF-7 cells (Holy, 2002). Curcumin decreases HT-29 proliferation and increases genomic stability. Curcumin also inhibits proliferation in other colon cancer cell lines (Aggarwal *et al*., 2003).

SAHA upregulates CUL3 in relation to untreated cells. As CUL3 is upregulated in DMSO-treated cells, but not by SAHA in relation to DMSO-treated cells, this upregulation is induced by DMSO. This upregulation increases HT-29 genomic stability.

DDX11

SAHA upregulates DDX11, in relation to untreated and DMSO-treated control cells. This is induced by SAHA with and without the influence of DMSO. It encodes the DNA helicase ChlR1 that is essential in replication, repair, transcription and recombination (Tuteja and Tuteja, 2004). ChlR1 promotes genome stability. It accumulates at the spindle poles from the onset of mitosis through telophase. ChlR1 binds cohesin and is

essential for the cohesion of sister chromatids until anaphase initiation (Parish *et al*., 2006; Hoque and Ishikawa, 2001; Uhlmann *et al*., 2000). DDX11 is only expressed in proliferating cells and its expression increases with rapid proliferation (Amann *et al*., 1997). SAHA promotes proliferation, genomic integrity and stability.

GADD45A

GADD45A is upregulated by curcumin in comparison to DMSO-treated control cells. This upregulation is induced by curcumin without the influence of the DMSO solvent. SAHA also upregulates GADD45A, in relation to DMSO-treated control cells. This upregulation is induced by SAHA without the influence of DMSO. GADD45A upregulation inhibits cyclin B/CDC2 to induce G_2/M arrest and promotes DNA repair in response to stress, such as DNA damage and oncogenic stress (Jin *et al*., 2002; Carrier *et al*., 1999). Its upregulation inhibits growth in cancer cell lines (Zhan *et al*., 1994). Previous work confirms that curcumin upregulates the GADD genes (Radhakrishna Pillai *et al*., 2004). G_2/M arrest enhances the cell's response to radiation treatment (Aggarwal *et al*., 2003). Curcumin and SAHA inhibit HT-29 proliferation and increase its genetic stability. Similarly, SAHA induces G_2/M arrest in breast cancer cells, including MCF-7, pancreatic cancer, endometrial cancer and lymphoma (Bali *et al*., 2005; Huang and Pardee, 2000; Munster *et al*., 2001; Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005).

GTF2H1

Curcumin downregulates GTF2H1 in relation to DMSO-treated control cells. This is induced by curcumin, independently of DMSO influence. GTF2H1 encodes TFIIH, which functions in nucleotide damage repair, transcription, nuclear receptor transactivation, E2F degradation and cell cycle control. TFIIH has E3 ubiquitin ligase activity (Takagi *et al*., 2005). Its downregulation decreases cell cycle progression and induces apoptosis (Matsuno *et al*., 2007; Jana *et al*., 2004). Curcumin reduces HT-29 proliferation and although damage repair decreases, with the consequent increase in apoptosis, genomic integrity may be maintained. Similarly, curcumin inhibits proliferation in colon cancer cell lines (Aggarwal *et al*., 2003).

SAHA upregulates GTF2H1 in comparison to untreated cells, and this is induced by the combination of SAHA with DMSO. SAHA increases HT-29 proliferation and its genomic integrity and stability. As proliferation is increased, DMSO is not an appropriate solvent for SAHA. SAHA, on the other hand, inhibits proliferation in mantle cell lymphoma cell lines (Heider *et al*., 2006).

HERC5

Curcumin upregulates HERC5, a ubiquitin ligase that functions in the immune response (Dastur *et al*., 2006; Kaivakolanu, 2003). This upregulation is only observed in comparison to DMSO-treated control cells and is induced by curcumin without DMSO influence. HERC5 is also upregulated by SAHA, in comparison to untreated and DMSO-treated control cells. This upregulation is due to SAHA with and without the

influence of DMSO. Curcumin and SAHA upregulate HERC5 as part of the immune response to promote HT-29 stability and cell survival.

HUS1

SAHA upregulates HUS1 in relation to untreated cells, and this is induced by the combination of SAHA with DMSO. HUS1 is essential at the G_1/S and G_2/M checkpoints and must be present for CHK1 and CHK2 phosphorylation to inhibit mitotic entry (Walworth and Bernards, 1996; Lindsay *et al*., 1998). It forms part of the 9-1-1 complex that is essential in the DNA damage response and regulates telomeres (Francia *et al*., 2006). It interacts with ATR and CHK1 in response to DNA damage to regulate the S-phase, G_2/M arrest and stabilisation of the replication forks (Parrilla-Castellar *et al*., 2004). HUS1 functions in homologous recombination repair (Wang *et al*., 2006). SAHA induces cell cycle arrest in various types of cancer cells, including bladder transitional cell carcinoma, breast adenocarcinoma, pancreatic cancer, endometrial cancer and lymphoma (O'Connor, 2006; Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005). SAHA decreases cell cycle progression and increases the genomic integrity of HT-29 to deviate from its oncogenic state. Similarly, SAHA inhibits proliferation in mantle cell lymphoma cells (Heider *et al*., 2006).

KNTC1

SAHA upregulates KNTC1, in comparison to untreated cells. This is due to the combination of SAHA with DMSO. KNTC1 is involved in maintaining correct

chromosome segregation at mitosis. This promotes genomic integrity but may also correlate with increased proliferation.

MAD2L2

SAHA upregulates MAD2L2, in relation to both untreated and DMSO-treated control cells. This is induced by SAHA with and without DMSO influence. MAD2L2 encodes essential components of the spindle checkpoint (Burds *et al*., 2005). It interacts with DNA damage repair enzymes (Ying and Wold, 2003; Nelson *et al*., 1999). It also associates with CDC20 and CDH1 to inhibit APC activation at late mitosis and inhibit mitotic exit (Nasmyth, 2005). MAD2L2 prevents anaphase initiation until all chromosomes are properly aligned on the spindle plate. This upregulation promotes genomic integrity and stability of HT-29.

MCM2

MCM2 is downregulated by curcumin in relation to untreated and DMSO-treated control cells. This is induced by curcumin with and without DMSO influence. Its downregulation decreases DNA replication and decreases the inhibition of re-replication (Ying and Gautier, 2005; Labib *et al*., 2000). As its expression correlates with proliferation, this denotes decreased proliferation of HT-29 (Todorov *et al*., 1998). MCM2-7 proteins form a ring-shaped structure around DNA during replication and are an essential part of the pre-replication complex. As part of this structure, MCM2 is involved in checkpoint activation and transcription (Cortez *et al*., 2004; Dziak *et al*., 2003). MCM2 expression correlates with the proliferation marker Ki-67, which is also

downregulated by curcumin (Gonzalez *et al*., 2003). The downregulation of MCM2 reduces HT-29 proliferation, but also suggests a decrease in genomic stability.

MCM3

Curcumin downregulates MCM3 in comparison to untreated and DMSO-treated control cells, and this is induced by curcumin with and without the influence of DMSO. As part of the MCM ring structure, MCM3 prevents DNA re-replication, and is involved in checkpoint activation and transcription (Labib *et al*., 2000; Cortez *et al*., 2004; Dziak *et al*., 2003). It is the most abundant MCM protein and is expressed in non-proliferating and proliferating cells, but not in differentiated cells (Lei *et al*., 1996; Endl *et al*., 2001; Musahl *et al*., 1998). It is essential in DNA replication, where it inhibits the initiation of DNA replication but not elongation (Ying and Gautier, 2005; Takei *et al*., 2002). Its downregulation decreases proliferation and the genomic integrity of HT-29.

MCM5

MCM5 is downregulated by curcumin in comparison to untreated and DMSO-treated control cells. This is due to curcumin with and without the influence of DMSO. MCM5 is involved in cell cycle regulation, checkpoint activation and transcription (Labib *et al*., 2000; Cortez *et al*., 2004; Snyder *et al*., 2005). As part of the MCM ring structure around DNA, it prevents DNA re-replication (Labib *et al*., 2000). It is only expressed in proliferating cells and is therefore a proliferation marker (Stoeber *et al*., 2002; Williams *et al*., 1998). Its expression increases in dysplastic cells and is regulated by E2F (Murphy *et al*., 2005; Ohtani *et al*., 1999). This downregulation decreases HT-29 proliferation in

comparison to untreated cells and this may be of greater relevance in suppressing tumourigenesis than the accompanying decrease in genomic integrity. Similarly, curcumin inhibits proliferation in colon cancer cell lines (Aggarwal *et al*., 2003).

MKI67

Curcumin downregulates the proliferation marker Ki-67 in relation to untreated and DMSO-treated cells. This downregulation is a result of curcumin both with and without DMSO influence. It decreases DNA organisation, DNA maintenance and ribosomal synthesis during mitosis (MacCallum and Hall, 2000). Ki-67 is an important proliferative biomarker, but not for breast cancer (Endl and Gerdes, 2000; Hall and Woods, 1990). Likewise, curcumin downregulates Ki-67 in breast cancer (Ramachandran and You, 1999; Aggarwal *et al*., 2007b). This reduces HT-29 proliferation to decrease the oncogenic state.

MNAT1

Curcumin downregulates MAT1 in comparison to untreated and DMSO-treated control cells, and is due to curcumin with and without the influence of DMSO. MAT1 is an essential component of the CAK that activates CDKs. CAK is also part of the TFIIH complex that induces transcription (Tassan *et al.*, 1995). Its downregulation decreases cell cycle progression and transcription. p53, RB and ERα activity is promoted (Ko *et al*., 1997; Wu *et al*., 2001; Chen *et al*., 2000b). This reduces proliferation and increases the genomic stability of HT-29. Curcumin inhibits proliferation in other colon cancer cell lines as well (Aggarwal *et al*., 2003).

MAT1 is upregulated by SAHA, in relation to untreated cells, and is due to the combination of SAHA with DMSO. This upregulation promotes proliferation of HT-29 and decreases genomic stability. DMSO is therefore not a suitable solvent for SAHA.

NBS1

SAHA upregulates NBS1 in comparison to untreated cells and this is accredited to the combination of SAHA with DMSO. NBS1 is essential in the MRN complex that detects and processes DSBs (Stracker *et al*., 2004). The MRN complex maintains telomeres, preventing telomeric fusion and aberrant telomere recombination (Ranganathan *et al*., 2001). NBS1 and KPNA2 transport MRE11 and RAD50 into the nucleus (Carney *et al*., 1998). NBS1 binds to ATM and is then responsible for guiding MRE11/RAD50 and ATM to DNA damage sites (Tseng *et al*., 2005). ATM phosphorylates and activates NBS1 at the G_1/S and G_2/M checkpoints. NBS1 then activates ATM to phosphorylate CHK2 and activate the S-phase checkpoint (Lee and Paull, 2005). NBS1 also associates with BRCA1 at damage sites (Komatsu *et al*., 2007). SAHA increases HT-29 DNA integrity and genomic stability. Similarly, SAHA induces G_1/S and G_2/M arrest in breast cancer, pancreatic cancer, endometrial cancer and lymphoma cells (Bali *et al*., 2005; Huang and Pardee, 2000; Munster *et al*., 2001; Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005).

PCNA

Curcumin upregulates PCNA, in comparison to DMSO-treated control cells, and is due to curcumin without the influence of DMSO. SAHA also upregulates PCNA in relation to

untreated and DMSO-treated control cells. This upregulation is due to SAHA acting in combination with and without DMSO. PCNA acts as a loading platform in DNA replication and repair (Maga and Hubscher, 2003). It induces DNA repair proteins and mediates protein interaction with DNA (Shiomi *et al*., 2002). It is essential for nucleotide and base excision repair, mismatch repair and DSB repair (Shivji *et al*., 1992; Levin *et al*., 2000; Jiricny, 2006; Dorazi *et al*., 2006). PCNA is important in chromatin assembly (Moggs *et al*., 2000). p21 and RB bind to PCNA to inhibit DNA replication (Maki and Howley, 1997; Sever-Chroneos *et al*., 2001). Although it is upregulated, its inhibitors p21 and RB are also upregulated by curcumin and reduce its function in replication. RB is also upregulated by SAHA and inhibits DNA replication. Previous studies confirm that curcumin inhibits PCNA in breast cancer cells (Aggarwal *et al*., 2007b; Ramachandran and You, 1999). Curcumin and SAHA therefore promote HT-29 genomic integrity and stability, but not proliferation.

RAD1

SAHA upregulates RAD1 in comparison to untreated cells, and this is due to SAHA with DMSO influence. RAD1 is an essential component of the 9-1-1 complex that induces cell cycle arrest in response to DNA damage or aberrant DNA replication. 9-1-1 promotes ATR-dependent CHK1 phosphorylation and activation at the G_1/S and G_2/M checkpoints, and activates p21 transcription to contribute to the G_1/S checkpoint (Bao *et al*., 2004; Roos-Mattjus *et al*., 2003; Yin *et al*., 2004). It also promotes base excision repair (Brandt *et al*., 2006). Similarly, SAHA induces cell cycle arrest in various cancers to inhibit cell cycle progression (O'Connor, 2006; Garcia-Manero and Issa, 2005;

Chobanian *et al*., 2004; Butler *et al*., 2000; Kumagai *et al*., 2007). SAHA also increases p21 activity in pancreatic cancer, endometrial cancer, lymphoma, bladder carcinoma cells and myelomonocytic leukaemia cells (Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005; Gui *et al*., 2004; Vrana *et al*., 1999). SAHA increases HT-29 genetic integrity and stability to decrease its oncogenic state.

RAD17

SAHA upregulates RAD17, in relation to untreated cells. As RAD17 is not upregulated by SAHA in relation to DMSO-treated control cells, this upregulation is due to the combination of SAHA with DMSO. RAD17 is essential in ATR-dependent checkpoint signal induction and in genomic stability (Wang *et al*., 2003c). It must be present for CHK1 and CHK2 phosphorylation at the G_1/S and G_2/M checkpoints (Walworth and Bernards, 1996; Lindsay *et al*., 1998). It is phosphorylated by ATM in response to DSBs and this phosphorylation is maintained by ATR (Zou *et al*., 2002). RAD17 loads the 9-1-1 complex onto ssDNA chromatin to promote checkpoint activation (Zou *et al*., 2003; Bermudez *et al*., 2003). It prevents the re-initiation of DNA synthesis (Wang *et al*., 2003c). Likewise, SAHA induces cell cycle arrest in several cancers (O'Connor, 2006; Garcia-Manero and Issa, 2005; Chobanian *et al*., 2004; Butler *et al*., 2000; Kumagai *et al*., 2007). SAHA increases HT-29 genomic integrity and stability.

RAD9A

Curcumin upregulates RAD9A, in relation to DMSO-treated control cells. As RAD9A is not upregulated by curcumin in comparison to untreated cells, this upregulation is

induced by curcumin, without DMSO influence. SAHA also upregulates RAD9A in comparison to untreated and DMSO-treated control cells. This upregulation is induced by SAHA with and without the influence of DMSO. RAD9A is essential in detecting DNA damage for cell cycle arrest, DNA repair and apoptosis (Toueille *et al*., 2004). It is part of the 9-1-1 complex that responds to DNA damage by inducing the checkpoint-signalling cascade. It shuttles the complex into the nucleus and directs it to RPA-associated damage sites (Hirai and Wang, 2002; Wu *et al*., 2005). 9-1-1 then induces DNA repair proteins and associates with enzymes involved in base excision repair (Shiomi *et al*., 2002; Brandt *et al*., 2006). ATM, which is upregulated by both curcumin and SAHA, phosphorylates RAD9A to activate CHK1 and CHK2 at G_1/S and G_2/M checkpoints (Greer *et al*., 2003; Roos-Mattjus *et al*., 2003). 9-1-1 activates p21, which is also upregulated by curcumin, to contribute to the G_1/S checkpoint (Yin *et al*., 2004).

Curcumin induces apoptosis in many cancer cell types, including HT-29 (Aggarwal *et al*., 2007; Chen *et al*., 1999b; Jiang *et al*., 1996; Simon *et al*., 1998; Kuo *et al*., 1996; Song *et al*., 2005; Xia *et al*., 2007; Liontas and Yeger, 2004; Park *et al*., 2006). SAHA induces cell cycle arrest and apoptosis in bladder transitional cell carcinoma, breast adenocarcinoma and pancreatic cancer, amongst others (O'Connor, 2006; Kumagai *et al*., 2007). It promotes p21 activity in pancreatic cancer, endometrial cancer, lymphoma, bladder carcinoma and myelomonocytic leukaemia cells (Kumagai *et al*., 2007; Takai *et al*., 2004; Sakajiri *et al*., 2005; Gui *et al*., 2004; Vrana *et al*., 1999). Curcumin and SAHA therefore increase the genomic integrity and stability of HT-29.

RB1

DMSO treatment and curcumin treatment upregulate RB1 in comparison to untreated cells. The upregulation by curcumin is due to DMSO influence. SAHA also upregulates RB, in relation to untreated cells, and this is due to the influence of DMSO on SAHA. This tumour suppressor protein is essential in development, differentiation, maintaining stem cells, tissue regeneration and senescence (Maddika *et al*., 2007). It induces cell cycle arrest from G_1 to the S phase (Hinds *et al*., 1992). It arrests cells in response to DNA damage (Knudsen *et al*., 2000). RB regulates gene expression by inhibiting E2F-mediated transcription (Jackson and Pereira-Smith, 2006). pRB/E2F regulates apoptosis and maintains genomic and chromosomal integrity (Tell *et al*., 2006). RB is rarely inactivated in colon carcinoma (Ali *et al*., 1999).

Similarly, DMSO induces cell cycle arrest in various cells, including HL-60, Burkitt's lymphoma and hybridoma 7TD1 cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998; Fiore and Degrassi, 1999; Teraoka *et al*., 1996). SAHA induces differentiation in numerous cancers, including colon cancer and breast cancer, and promotes senescence in transformed cells (Garcia-Manero and Issa, 2005; Munster *et al*., 2001; Xu *et al*., 2005). SAHA also hypophosphorylates RB to induce growth arrest (Wagner and Roemer, 2005). RB upregulation increases HT-29 genomic stability, inhibits proliferation and decreases its oncogenic state.

RBBP8

RBBP8 is downregulated by curcumin in relation to untreated and DMSO-treated control cells. This is due to curcumin with and without DMSO influence. RBBP8 downregulation alleviates the inhibition of the transcriptional activity of several DNA-binding transcriptional factors, including p21 and GADD45 (Meloni *et al*., 1999; Li *et al*., 1999; Li *et al*., 2000). Cyclin D1 transcription decreases to inhibit G_1/S progression (Liu and Lee, 2006; Wu and Lee, 2006). Similarly, curcumin promotes the GADD genes in lung cancer, and promotes p21 in breast cancer, prostate cancer and HepG2 cells (Radhakrishna Pillai *et al*., 2004; Choudhuri *et al*., 2005; Hour *et al*., 2002; Lv *et al*., 2007). It downregulates cyclin D1 in leukaemia, breast cancer and prostate cancer cells (Tomita *et al*., 2006; Aggarwal *et al*., 2007). This decreases HT-29 proliferation and increases its genomic stability to reduce tumourigenesis.

RBL1

Curcumin downregulates the tumour suppressor p107 in comparison to untreated cells. As it is not downregulated in comparison to DMSO-treated control cells, it is due to the combination of curcumin with DMSO. This promotes E2F4 and E2F5 transcription of genes involved in DNA synthesis, cell cycle progression and DNA damage checkpoints (Cam and Dynlacht, 2003; Stevaux and Dyson, 2002). It inhibits the transcription of differentiation-related genes (Lipinski and Jacks, 1999; Vanderluit *et al*., 2007). It promotes cyclin E/CDK2 and cyclin A/CDK2 to increase cell cycle progression (Woo *et al*., 1997). SKP2 proteasomal degradation is reduced and p27 ubiquitination increases to promote CDK2 and G_1 progression (Rodier *et al*., 2005). p107 downregulation increases

HT-29 proliferation and promotes genomic instability and the oncogenic state. This affirms that DMSO is not a suitable solvent for curcumin.

RBL2

The tumour suppressor p130 is downregulated by curcumin in relation to untreated cells, and this is due to the combination of curcumin with DMSO. This inhibits quiescence and promotes G_1/S transition (Grana *et al*., 1998). Cyclin E/CDK2 and cyclin A/CDK2 are promoted and cell cycle arrest decreases (Woo *et al*., 1997; Hansen *et al*., 2001). It promotes CDC2 transcription to increase mitotic entry (Taylor *et al*., 2001). The transcription of S-phase entry genes is promoted, even under unfavourable conditions (Iavarone and Massague, 1999). This downregulation increases proliferation and genomic instability to promote tumourigenesis. It is induced by DMSO and DMSO is therefore not an appropriate solvent for curcumin.

RPA3

Curcumin upregulates RPA3, in comparison to DMSO-treated control cells. This is induced by curcumin without DMSO influence. RPA3 is also upregulated by SAHA in comparison to untreated and DMSO-treated control cells. This upregulation is induced by SAHA with and without the influence of DMSO. RPA3 is a subunit of the RPA protein that forms part of the origin recognition complex to stabilise ssDNA during DNA replication (Bochkarev *et al*., 1997). RPA functions in DNA recombination and repair, apoptosis and gene expression (He *et al*., 1995; Iftode *et al*., 1999). RPA/ssDNA promotes the recruitment of the RAD17/RFC complex to induce phosphorylation and

activation of CHK1 (Foray *et al*., 2003). CHK1 promotes replication fork progression, cell cycle arrest, DNA repair and apoptosis (Petermann *et al*., 2006; Durkin *et al*., 2006; Gatei *et al*., 2003; Pommier *et al*., 2005). ATM, which is upregulated by both curcumin and SAHA, phosphorylates RPA at the S-phase checkpoint to inhibit DNA replication.

Similarly, curcumin induces cell cycle arrest and apoptosis in HT-29, other colon cancer cell lines, breast cancer, neuroblastoma and bladder cancer cells (Song *et al*., 2005; Aggarwal *et al*., 2003; Choudhuri *et al*., 2005; Xia *et al*., 2007; Liontas and Yeger, 2004; Sindhwani *et al*., 2001; Park *et al*., 2006). SAHA induces cell cycle arrest and apoptosis in numerous cancer cells (O'Connor, 2006; Bali *et al*., 2005; Huang and Pardee, 2000; Kumagai *et al*., 2007). RPA3 upregulation promotes DNA integrity and genomic stability, and DNA replication is inhibited. This reduces tumourigenesis in HT-29.

SERTAD1

SERTAD1 is upregulated by curcumin in relation to both untreated and DMSO-treated control cells. This upregulation is due to curcumin with and without DMSO influence. SERTAD1 promotes E2F1/DP1 transcriptional activity to promote G_1/S progression. It binds to CDK4 to inhibit p16 and alleviate the inhibition of cyclin D/CDK4 to promote cell cycle progression (Sugimoto *et al*., 1999). It promotes cell growth and its overexpression correlates with tumourigenesis (Sim *et al*., 2006; Gladden and Diehl, 2005; Tang *et al*., 2002). RB, which is upregulated by curcumin, inhibits E2F1/DP1/SERTAD1 (Hsu *et al*., 2001). As the inhibitor of SERTAD1, RB, is upregulated by curcumin, proliferation may not increase in HT-29. Curcumin inhibits

cell cycle progression in colon cancer cell lines and breast cancer (Aggarwal *et al*., 2003; Aggarwal *et al*., 2007b).

SKP2

SKP2 is downregulated by curcumin in comparison to untreated and DMSO-treated control cells. This is induced by curcumin with and without the influence of DMSO. SKP2 is a subunit of SCF^{SKP2} ubiquitin ligase that targets proteins involved in cell cycle progression for ubiquitin-mediated proteolysis. These targets include E2F1, c-*myc*, p21, p27 and cyclin D1 (Marti *et al*., 1999; Kim *et al*., 2003; Bornstein *et al*., 2003; Nakayama *et al*., 2000; Ganiatsas *et al*., 2001). This downregulation inhibits E2F1/DP transcriptional activity to reduce G_1/S (Hsu *et al*., 2001). It decreases adhesion-independent cell growth (Signoretti *et al*., 2002). Its levels correlate with cyclin A, cyclin B1, cyclin E, CDK2 and Ki-67 in colorectal cancer (Li *et al*., 2004). Correspondingly, cyclin B1 and Ki-67 are downregulated by curcumin in HT-29. SKP2 downregulation reduces HT-29 proliferation and oncogenesis (Gstaiger *et al*., 2001). Curcumin inhibits proliferation in other colon cancer cells as well (Aggarwal *et al*., 2003).

TFDP1

DP1 is downregulated by curcumin in relation to both untreated and DMSO-treated control cells. This is due to curcumin with and without DMSO influence. E2F/DP1 heterodimer formation decreases to reduce the transcriptional activity for proteins involved at the G_0/G_1 and G_1/S transition, apoptosis, DNA synthesis and oncogenic transformation (Schulze *et al*., 1995; Shan *et al*., 1996). TFDP1 expression correlates

with CCNE1 levels (Yasui *et al*., 2002). Curcumin inhibits cyclin E in HaCaT cells and in breast cancer (Cho *et al*., 2007; Aggarwal *et al*., 2007; Goel *et al*., 2008). DP1 levels are elevated in various cell lines (Gopalkrishnan *et al*., 1996; Wu *et al*., 1995; Yasui *et al*., 2003). This downregulation inhibits HT-29 proliferation and tumourigenesis. Similarly, curcumin decreases proliferation in colon cancer cell lines (Aggarwal *et al*., 2003).

TP53

DMSO upregulates p53 in relation to untreated cells. This tumour suppressor induces cell cycle arrest, DNA repair, senescence, differentiation and apoptosis. Its stability increases in response to DNA damage, oncogene activation and hypoxia (Finlan and Hupp, 2005; Giono and Manfredi, 2006). p53 is essential for G_0/G_1 transition. It increases p21 expression to prevent pRB inactivation (Sherr and Roberts, 1999). DMSO upregulates p21 in HT-29. p53 upregulates GADD45A to promote DNA repair and BAX to induce apoptosis (Maddika *et al*., 2007; Miyashita and Reed, 1995). It inhibits anti-apoptotic BCL2 (Chipuk *et al*., 2004; Mihara *et al*., 2003). CHK1/CHK2 and ATM/ATR phosphorylate p53 at the G_1/S checkpoint in response to DNA damage to sustain cell cycle arrest. DMSO decreases HT-29 proliferation and promotes genomic stability to inhibit the oncogenic state. DMSO also induces differentiation in HL-60 and N1E-115 cells (Burger *et al*., 1994; Jiang *et al*., 1994).

SAHA conflictingly downregulates p53 in relation to DMSO-treated control cells, and this is due to SAHA without DMSO influence. This downregulation decreases cell cycle arrest, DNA repair, senescence and differentiation and promotes angiogenesis (Finlan and

Hupp, 2005; Giono and Manfredi, 2006). SAHA, however, induces apoptosis and differentiation in various cancers and inhibits angiogenesis (Ocker and Schneider-Stock, 2007). p53 is frequently mutated in colon adenocarcinoma and this may account for this unexpected finding (Fearon and Vogelstein, 1990). Loss of p53 function promotes tetraploidy and SAHA may then promote senescence (Castedo *et al*., 2006; Xu *et al*., 2005).

UBE1

Curcumin downregulates UBE1 in relation to untreated cells and this is induced by curcumin with the influence of DMSO. UBE1 functions in proteolysis, DNA repair and cell cycle progression (Jentsch *et al*., 1990; Zacksenhaus and Sheinin, 1990; Handley-Gearhart *et al*., 1994). Its downregulation decreases ubiquitin activation and ubiquitin conjugation (Pelzer *et al*., 2007; Pickart, 2001). It decreases the activation of the ubiquitin-like ISG15 protein in response to stress (Kim and Zhang, 2003). It promotes cyclin D1 and G_1 cell cycle progression (Lonardo *et al*., 1999; Pitha-Rowe *et al*., 2004). This downregulation increases proliferation and decreases the stress responses. Curcumin, however, inhibits proliferation in colon cancer cell lines (Aggarwal *et al*., 2003). As this is due to DMSO influence, DMSO is not a suitable solvent for curcumin.

SAHA upregulates UBE1 in comparison to untreated and DMSO-treated control cells. This is due to SAHA with and without the influence of DMSO. UBE1 expression in inhibited in cancer cell lines (Pitha-Rowe *et al*., 2004b). SAHA increases UBE1 levels to

inhibit cyclin D1 and cell cycle progression. SAHA inhibits HT-29 proliferation and increases genomic integrity and stability to inhibit the oncogenic state.

3.4.2 MCF-7

The data compared to untreated control cells, is normalised against the HPRT1, RPL13A, GAPDH and ACTB housekeeping genes, which have insignificant fold regulations. Similarly, the thalidomide-treated cells, compared to DMSO-treated cells, are normalised against HPRT1, RPL13A, GAPDH and ACTB. The lycopene-treated cells, compared to THF-treated cells, are normalised against B2M, HPRT1, GAPDH and ACTB.

3.4.2.1 Distribution of C_t Values

The results for the gene expression in MCF-7 untreated cells are used as an example.

Table 3.10: Distribution of C_t values of MCF-7 untreated cells

Percent distribution of C_t values	
C_t Range	
<25	34.38%
25-30	44.79%
30-35	14.58%
Absent calls	6.25%

3.4.2.2 Reverse transcription control

MCF-7 untreated

$\Delta Ct = 23.31 – 19.98$

$= 3.33$

Reverse transcription (RT) efficiency: pass.

MCF-7 DMSO

$\Delta Ct = 22.39 – 25.02$

$= -2.63$

RT efficiency: pass.

MCF-7 THF

$\Delta Ct = 23.07 – 20.18$

$= 2.89$

RT efficiency: pass

MCF-7 lycopene

$\Delta Ct = 22 – 19.66$

$= 2.34$

RT efficiency: pass.

MCF-7 thalidomide

$\Delta Ct = 21.44 – 18.79$

$= 2.65$

RT efficiency: pass.

3.4.2.3 Genomic DNA contamination

MCF-7 untreated

C_t (GDC) = 35

Result: pass

MCF-7 DMSO

C_t (GDC) = 35

Result: pass

MCF-7 THF

C_t (GDC) = 35

Result: pass

MCF-7 lycopene

C_t (GDC) = 35

Result: pass

MCF-7 thalidomide

C_t (GDC) = 35

Result: pass

Table 3.11: Fold regulation of genes in comparison to untreated MCF-7 cells due to treatments, with significant fold changes highlighted

	DMSO	THF	Lycopene	Thalidomide
ABL1	2.0014	-2.0994	2.175	1.0231
ANAPC2	-3.2288	-1.2772	-2.5758	-2.5421
ANAPC4	-3.2966	-1.3947	-1.4152	1.0056
ATM	-3.7659	1.0231	1.5444	1.6911
ATR	1.1096	1.7715	2.7625	3.0844
BCCIP	-2.6281	1.0105	1.7195	1.9917
BCL2	4.8736	2.1199	2.3834	2.0562
BIRC5	1.1471	1.3928	2.4777	1.6313
CCNB1	-2.0907	1.1859	-1.3472	-3.0801
CCNB2	-2.16	1.8519	-1.0666	-2.3702
CCNC	1.5157	1.2675	2.307	1.9917
CCND1	1.9876	1.8213	2.1082	1.2816
CCND2	4.8601	-1.1925	1.5486	2.7492
CCNF	-3.5826	1.805	1.5988	-2.169
CCNG1	2.1629	1.1663	1.6947	1.3918
CCNG2	2.4116	1.1917	2.6519	2.0965

CCNT2	-1.2781	1.2117	-1.4459	2.2548
CDC2	-1.9972	1.7593	-1.136	-13.5761
CDK4	2.0548	-1.1088	1.6947	-1.5594
CDK5RAP1	-8.8214	1.0607	1.1575	-9.6532
CDK6	-1.1142	-2.9938	1.4651	1.0035
CDK7	-1.0063	-1.19	2.0562	2.291
CDK8	1.2719	1.2008	1.4172	2.2284
CDKN1B	1.9588	2.7076	4.541	1.168
CDKN2A	-3.4271	-1.1925	-1.5486	1.3059
CDKN2B	9.2535	2.1361	4.8906	4.952
CDKN3	-2.5053	1.2329	1.0882	1.325
CUL3	6.2767	3.7192	12.8616	3.779
DDX11	-9.2856	-2.3054	-5.4907	-16.4498
E2F4	1.6506	1.9917	2.4777	1.9931
GADD45A	1.752	1.7411	5.4717	1.4054
GTF2H1	-1.1235	1.2159	2.1376	2.0083
GTSE1	-2.6299	1.3832	1.1567	-3.283
HERC5	-1.0345	-1.2269	2.0806	1.3679
KPNA2	-1.36	-8.51	-1.47	1.24
MCM4	2.5793	1.9507	1.8455	1.3472
MKI67	-2.3198	1.35	-1.7703	-1.2897
NBS1	1.5147	1.4743	1.9119	2.3343
RAD17	-1.0432	-1.0331	2.3735	2.4794

RAD51	-2.8759	-2.2191	-4.305	-5.0912
RAD9A	-3.4271	-1.1925	N/A	1.3059
RBL2	1.2075	-1.1027	2.5509	1.601
RPA3	-1.9225	1.3041	2.5298	1.5
SERTAD1	-1.3851	-1.6223	-1.0133	-1.5263

Table 3.12: Fold regulation of genes from lycopene treatments in comparison to THF-treated control MCF-7 cells, and thalidomide treatments in comparison to DMSO-treated control MCF-7 cells, with significant fold changes highlighted.

	Lycopene	Thalidomide
ABL1	4.0553	-1.9561
ANAPC2	-2.2708	1.2702
ANAPC4	-1.1425	3.315
ARHI	-1.4582	2.1332
ATM	1.3405	6.3687
ATR	1.3849	2.7798
BCCIP	1.5113	5.2343
BCL2	-1.0015	-2.3702
CCNB2	-2.2241	-1.0973
CCNH	2.0574	-1.036
CCNT2	-1.9727	2.8819
CDC2	-2.2504	-6.7975
CDK4	1.6688	-3.2043

CDK6	3.8955	1.1181
CDK7	2.1732	2.3054
CDKN2A	1.6401	4.4753
CDKN2B	2.0333	-1.8687
CDKN3	-1.2756	3.3196
CHEK2	1.6688	2.3587
CUL3	3.0712	-1.6609
DDX11	-2.6818	-1.7715
GADD45A	2.791	-1.2466
GTF2H1	1.5614	2.4183
HERC5	2.267	1.4152
HUS1	-1.0419	2.7207
KPNA2	5.158	1.6818
MKI67	-2.6911	1.7988
MRE11A	1.2132	2.181
RAD17	2.1777	2.5865
RAD51	-2.1843	-1.7703
RAD9A	N/A	4.4753
RBL2	2.498	1.3259
RPA3	1.7229	2.8839

3.4.2.4 MCF-7/treatments

ABL1

The ABL1 oncogene is upregulated by DMSO in comparison to untreated cells. It is also upregulated by lycopene in comparison to both untreated and THF-treated control cells. This upregulation is induced by lycopene with and without the influence of THF. ABL1 functions in response to DNA damage by activating RAD51 to induce G_1 arrest and promote apoptosis in proliferating cells (Chen *et al*., 1999; Wang, 2000). ABL1 transmits the DNA damage signal by shuttling between the nucleus and cytoplasm (Taagepera *et al*., 1998). It phosphorylates and stabilises p73, which activates p53-regulated genes and induces apoptosis in numerous cell lines (Gong *et al*., 1999; Agami *et al*., 1999; Levrero *et al*., 1999; Jost *et al*., 1997). By inhibiting MDM2-mediated degradation of p53, ABL1 also promotes p53 accumulation and consequently, p21 expression (Sionov *et al*., 1999; Goga *et al*., 1995). Likewise, DMSO increases p21 mRNA and protein in hybridoma 7TD1 cells (Ponzio *et al*., 1998). DMSO also induces G_1 arrest in various cell lines including HL-60, lymphoid, CHO, hybridoma 7TD1, leukaemic cells, and B cell lines (Nishizawa *et al*., 1998; Teraoka *et al*., 1996; Fiore and Degrassi, 1999; Ponzio *et al*., 1998).

Similarly, lycopene induces cell cycle arrest in MCF-7, MDA-MB-231, Hep3B, ECC1 and mouse hepatocyte cells (Chalabi *et al*., 2004; Park *et al*., 2005; Nahum *et al*., 2006; Matsushima-Nishiwaki *et al*., 1995). Lycopene also induces apoptosis in LNCaP, HuCC, Raji cells and T lymphoblast cells; decreases DNA damage in lymphocytes, H568 and CHO cells; and promotes p21 in hybridoma 7TD1 cells (Ivanov *et al*., 2007; Salman *et*

al., 2007; Muller *et al*., 2002; Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007; Ponzio *et al*., 1998). ABL1 upregulation promotes MCF-7 genomic integrity and stability.

THF downregulates ABL1. As p73 stability is reduced, the transcription of p53-regulated genes decreases, including p21 (Gong *et al*., 1999; Agami *et al*., 1999; Levrero *et al*., 1999; Jost *et al*., 1997; Sionov *et al*., 1999; Goga *et al*., 1995). THF inhibits MCF-7 proliferation but at the cost of incurring DNA damage.

ANAPC2

APC2 is downregulated by DMSO to reduce cell cycle progression. Lycopene also downregulates APC2 in relation to untreated and THF-treated control cells. This is due to lycopene with and without THF influence. APC2 is downregulated by thalidomide in comparison to untreated cells and this is due to DMSO influence. The downregulation of the ubiquitin-proteasome pathway induces apoptosis (Jana *et al*., 2004). DMSO inhibits proliferation in HL-60, hybridoma 7TD1 and N1E-115 cells, and induces apoptosis in CHO cells (Watson *et al*., 1997; Ponzio *et al*., 1998; Kranenburg *et al*., 1995; Fiore and Degrassi, 1999). Likewise, lycopene inhibits cell cycle progression and induces apoptosis in various cancer cells (Levy *et al*., 1995; Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005; Ivanov *et al*., 2007; Salman *et al*., 2007; Muller *et al*., 2002). This reduces MCF-7 proliferation and inhibits its oncogenic potential.

ANAPC4

APC4 is downregulated by DMSO to decrease ubiquitin ligase activity, inhibit cell cycle progression, induce apoptosis and decrease checkpoint function (Peters, 2002; Jana *et al*., 2004; Wasch and Cross, 2002). DMSO inhibits MCF-7 proliferation and increases genomic stability to inhibit tumourigenesis. Similarly, DMSO induces apoptosis in CHO cells (Fiore and Degrassi, 1999).

Thalidomide upregulates APC4, in relation to DMSO-treated control cells. This is induced by thalidomide, without the influence of DMSO. APC4 promotes the G_1/S and spindle checkpoints, and cell cycle progression (Peters, 2002; Wasch and Cross, 2002). Thalidomide does promote the G_1/S checkpoint in other studies (Hattori and Iguchi, 2004). This upregulation increases MCF-7 proliferation, but also genomic stability.

ARHI

The tumour suppressor ARHI is upregulated by thalidomide in comparison to DMSO-treated control cells. This is due to thalidomide without DMSO influence. This promotes p21 and inhibits cyclin D1 to inhibit cell cycle progression, inhibiting cyclin D1 and induce apoptosis (Bao *et al*., 2002). Thalidomide also induces p21 in multiple myeloma cells to promote cell cycle arrest, and induces apoptosis in MCF-7, HL-60 and multiple myeloma cells (Hideshima *et al*., 2000; Du *et al*., 2005; Hattori and Iguchi, 2004). The upregulation of ARHI decreases proliferation and increases genomic integrity to inhibit tumourigenesis in MCF-7.

ATM

ATM is downregulated by DMSO. This decreases cell cycle arrest; the re-initiation of collapsed forks; DNA damage repair and the inhibition of re-replication; and promotes apoptosis and cell cycle progression (Lee and Paull, 2005; Liu *et al*., 2007; Trenz *et al*., 2006; Ocker and Schneider-Stock, 2007; Costanzo *et al*., 2000). It decreases p53 and p21, and increases CDK2 (Banin *et al*., 1998; Khosravi *et al*., 1999; Costanzo *et al*., 2000). DMSO promotes MCF-7 proliferation and reduces genomic stability to increase oncogenic transformation. Although DMSO does induce apoptosis in CHO cells, it induces cell cycle arrest in HL-60, Burkitt's lymphoma and CHO cells, amongst others, and promotes p21 in hybridoma 7TD1 cells (Fiore and Degrassi, 1999; Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998).

Thalidomide upregulates ATM, in relation to DMSO-treated control cells. This is due to thalidomide independently of DMSO influence. ATM increases the DNA damage responses, including p21-induced G_1 arrest and DNA replication inhibition (Lee and Paull, 2005; Banin *et al*., 1998; Ocker and Schneider-Stock, 2007). Thalidomide promotes p21 activity to induce G_1 arrest in multiple myeloma cells (Hideshima *et al*., 2000). ATM is often downregulated in breast cancer therefore, thalidomide reduces MCF-7 proliferation and promotes genomic stability (Ding *et al*., 2004).

ATR

Lycopene upregulates the tumour suppressor ATR, in comparison to untreated cells. As it is not upregulated by lycopene in comparison to THF-treated control cells, this

upregulation is due to the combination of lycopene with THF. ATR is also upregulated by thalidomide in relation to both untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. ATR promotes checkpoint function, cell cycle arrest, the re-initiation of stalled forks and DNA repair, and inhibits DNA replication (Walworth and Bernards, 1996; Lindsay *et al*., 1998; Dart *et al*., 2004; Cobb *et al*., 2005; Andreassen *et al*., 2004; Cortez *et al*., 2004). It increases the levels of phosphorylated BRCA1 and in accordance, lycopene increases phosphorylated BRCA1 in MCF-7 cells (Andreassen *et al*., 2004; Chalabi *et al*., 2004).

Lycopene also promotes the DNA damage responses in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Previous studies confirm that thalidomide inhibits tumour growth in MCF-7, HL-60 and myeloma (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993). Thalidomide also induces cell cycle arrest in multiple myeloma cells (Hideshima *et al*., 2000). ATR upregulation inhibits MCF-7 proliferation and increases genomic integrity to inhibit tumourigenesis.

BCCIP

BCCIP is downregulated by DMSO to promote p21 and inhibit CDK2 to inhibit proliferation (Liu *et al*., 2001; Ono *et al*., 2000). DNA repair is promoted (Lu *et al*., 2005). BCCIP is overexpressed in breast cancer (Meng *et al*., 2003; Liu *et al*., 2001). DMSO downregulates BCCIP in comparison to untreated cells to promote the inhibition of proliferation. Similarly, DMSO inactivates CDK2 and promotes p21 activity in 7TD1

cells (Ponzio *et al*., 1998). This reduces MCF-7 proliferation and promotes genomic integrity to decrease the oncogenic state.

BCCIP is upregulated by thalidomide, in comparison to DMSO-treated control cells. This promotes p21, BRCA2 and RAD51 and inhibits CDK2 to decrease cell cycle progression and promote DNA repair (Meng *et al*., 2003; Liu *et al*., 2001; Ono *et al*., 2000; Lu *et al*., 2005). Thalidomide promotes p21 activity in multiple myeloma cells to induce G_1 arrest (Hideshima *et al*., 2000). This reduces proliferation and increases genomic integrity to inhibit the oncogenic state of MCF-7.

BCL2

DMSO and THF upregulate anti-apoptotic BCL2. Lycopene also upregulates anti-apoptotic BCL2 in comparison to untreated cells. As this upregulation occurs in THF-treated cells but is not upregulated by lycopene, in comparison to THF-treated control cells, it is induced by THF. This upregulation promotes cell survival, and inhibits cell cycle progression, DNA repair and DNA replication (Mazel *et al*., 1996; Dlugosz *et al*., 2006; Youn *et al*., 2005; Saintigny *et al*., 2001; Liu *et al*., 1997). BCL2 inhibits pro-apoptotic BAX, but the upregulation of CUL2 by DMSO promotes BAX expression (Kim *et al*., 2004; Wincemicz *et al*., 2007). BCL2 overexpression in breast cancer inhibits RAD51-mediated DNA repair, promotes mutagenesis, and confers resistance to chemotherapy and irradiation (Saintigny *et al*., 2001; Liu *et al*., 1997; Binder *et al*., 1995; Ramsay *et al*., 1995; Flohil *et al*., 1996; Tsuji *et al*., 1998; Laudanski *et al*., 1992; Campos *et al*., 1993).

Similarly, DMSO upregulates BCL2 in CHO cells to inhibit apoptosis (Fiore and Degrassi, 1999). This decreases proliferation and genomic integrity, but the corresponding upregulation of BAX may maintain genomic stability of MCF-7. THF is therefore not a suitable solvent for lycopene.

Thalidomide also upregulates the anti-apoptotic BCL2 in relation to untreated cells. However, thalidomide downregulates BCL2 in relation to DMSO-treated control cells and this is due to thalidomide without the influence of DMSO. Likewise, thalidomide inhibits NF-κB activity to inhibit BCL2 transcription and promote apoptosis (Kucharczak *et al*., 2003; Keifer *et al*., 2001; Marriott *et al*., 2003). Similarly, thalidomide induces apoptosis in MCF-7, HL-60 and multiple myeloma cells (Du *et al*., 2005; Hideshima *et al*., 2000). It also induces G_1 arrest in multiple myeloma cells to inhibit cell cycle progression (Hideshima *et al*., 2000).

BIRC5

Lycopene upregulates survivin, in relation to untreated cells, to promote proliferation, and inhibit checkpoint function, cell cycle arrest and apoptosis (Dohi *et al*., 2004; Giodini *et al*., 2002; Lens and Medema, 2003; Beltrami *et al*., 2004; Sarela *et al*., 2000). Lycopene, however, inhibits proliferation and promotes cell cycle arrest and apoptosis in several cancer cell lines (Chalabi *et al*., 2004; Nahum *et al*., 2001; Levy *et al*., 1995; Ivanov *et al*., 2007). This upregulation promotes the oncogenic state of MCF-7. It is induced by the combination of lycopene with THF, as it is not upregulated by lycopene in comparison to THF-treated control cells. THF is not an appropriate solvent for lycopene.

CCNB1

Cyclin B1 is downregulated by DMSO. This inhibits cell cycle progression and apoptosis (Borgne *et al*., 2006; Pines and Hunter, 1989). Genomic instability is promoted as the control of prematurely activated mitosis is decreased (Amon, 1999). As it is essential for cell survival, its downregulation denotes decreased proliferation (Morgan, 1995). Cyclin B1 expression in breast cancer correlates with increased proliferation and Ki-67 (Suzuki *et al*., 2007). Accordingly, Ki-67 is also downregulated by DMSO. Its downregulation suggests decreased rates of mitosis (Megha *et al*., 1999). DMSO promotes cyclin B overexpression in HL-60 and N1E-115 cells (Burger *et al*., 1994; Jiang *et al*., 1994). DMSO decreases MCF-7 proliferation but may promote chromosomal instability.

Thalidomide also downregulates cyclin B1 in comparison to untreated cells and this is induced by DMSO influence. Cyclin B1 forms the MPF complex with CDC2. CDC2 is also downregulated by thalidomide (Morgan, 1995). This decreases MCF-7 proliferation but may reduce genomic integrity.

CCNB2

DMSO downregulates cyclin B2. Lycopene also downregulates cyclin B2 in relation to THF-treated control cells and this is induced by lycopene independently of THF influence. This decreases mitosis and apoptosis (Morgan, 1995; Pines and Hunter, 1989). Chromosomal integrity decreases at anaphase (Wang *et al*., 1997). DMSO promotes

cyclin B upregulation in HL-60 and N1E-115 cells (Burger *et al*., 1994; Jiang *et al*., 1994). DMSO inhibits MCF-7 proliferation, but genomic stability may decrease.

Cyclin B2 is inhibited by p53 (Krause *et al*., 2000). The other component of the MPF complex, CDC2, is also downregulated by lycopene. Lycopene decreases cell cycle progression in several cancer cells (Watson *et al*., 1997; Ponzio *et al*., 1998; Kranenburg *et al*., 1995). This reduces MCF-7 proliferation to decrease tumourigenesis.

Cyclin B2 is also downregulated by thalidomide, in relation to untreated cells. CDC2 is also downregulated by thalidomide (Morgan, 1995). This is due to DMSO influence as it is not downregulated by thalidomide in relation to DMSO-treated control cells. Thalidomide reduces MCF-7 proliferation but may also decrease genomic integrity.

CCNC

Cyclin C is upregulated by lycopene in comparison to untreated cells. This upregulation promotes cell cycle entry and progression, but inhibits transcription (Ren and Rollins, 2004; Liu *et al*., 1998; Akoulitchev *et al*., 2000). Lycopene promotes MCF-7 proliferation but previous studies contest this (Nahum *et al*., 2001; Levy *et al*., 1995; Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005). As cyclin C is not upregulated by lycopene in comparison to THF-treated control cells, this upregulation is due to THF action. This affirms that THF is not an appropriate solvent for lycopene.

CCND1

Lycopene upregulates cyclin D1, in comparison to untreated cells, to promote cell cycle progression, survival, transcription (Schwartz and Shah, 2005; Hinds *et al*., 1994; Coqueret, 2002). This upregulation is due to the combination of lycopene with THF. In contrast, lycopene decreases cyclin D1 to induce G_0/G_1 arrest in MCF-7, ECC1 endometrial cancer cells, and the prostate cancer cell lines LNCaP and PC3 (Nahum *et al*., 2001; Nahum *et al*., 2006; Ivanov *et al*., 2007). Another study, however, confirms this finding where lycopene upregulates cyclin D1 in MCF-7 and MDA-MB-231 cells (Chalabi *et al*., 2007). MCF-7 proliferation and oncogenic transformation are promoted. This confirms the unsuitability of THF as a solvent for lycopene.

CCND2

DMSO upregulates cyclin D2 to promote cell cycle progression and survival, and decrease growth arrest (Schwartz and Shah, 2005; Hinds *et al*., 1994; Glaser, 2007). However, DMSO inhibits cyclin D2 and cyclin D2/CDK4 in 7TD1 cells to induce G_1 arrest (Ponzio *et al*., 1998). DMSO promotes MCF-7 proliferation and the oncogenic state.

Thalidomide also upregulates cyclin D2, in relation to untreated cells. It is not upregulated by thalidomide in relation to DMSO-treated control cells, but as it is also upregulated in DMSO-treated cells, this upregulation is due to DMSO. This increases MCF-7 cell cycle progression (Schwartz and Shah, 2005). However, thalidomide inhibits cell cycle progression in various cells (Du *et al*., 2005; Hattori and Iguchi, 2004;

Hideshima *et al*., 2000). As this upregulation promotes MCF-7 proliferation and tumourigenesis, it reiterates that DMSO is not an appropriate solvent for thalidomide.

CCNF

Cyclin F is downregulated by DMSO to decrease cell cycle progression, cyclin E transcription and SCF ubiquitin ligase activity (Kong *et al*., 2000; Tezlaff *et al*., 2004). Thalidomide also downregulates cyclin F in comparison to untreated cells and this is induced by DMSO influence. This induces apoptosis (Jana *et al*., 2004). DMSO reduces MCF-7 proliferation and increases genomic stability to reduce tumourigenesis. DMSO also induces apoptosis in CHO cells (Fiore and Degrassi, 1999). Similarly, thalidomide inhibits tumourigenesis in MCF-7, HL-60 and myeloma, and induces apoptosis in MCF-7 and HL-60, amongst other cells (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

CCNG1

Cyclin G1 is upregulated by DMSO, in comparison to untreated cells, to inhibit cell cycle progression, induce G_2/M arrest, and promote differentiation and apoptosis (Bennin *et al*., 2002; Okamoto and Prives, 1999; Reimer *et al*., 1999). It is upregulated in response to DNA damage (Bates *et al*., 1996). DMSO induces differentiation in HL-60 and N1E-115 cells, and induces growth arrest in HL-60, Burkitt's lymphoma, CHO and 7TD1 cells (Watson *et al*., 1997; Jiang *et al*., 1994; Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998). This inhibits MCF-7 proliferation and promotes genomic integrity to reduce tumourigenesis.

CCNG2

DMSO upregulates cyclin G2. It is also upregulated by lycopene, in comparison to untreated cells. As it is not upregulated by lycopene, in comparison to THF-treated control cells, this upregulation is due to the combination of lycopene with THF. Thalidomide also upregulates cyclin G2, in relation to untreated cells, to inhibit cell cycle progression, increase cell cycle arrest and promote differentiation (Bennin *et al*., 2002; Arachchige Don *et al*., 2006). This is due to DMSO.

Cyclin G2 upregulation inhibits cell cycle progression, and promotes cell cycle arrest, differentiation and microtubule stability (Bennin *et al*., 2002; Arachchige Don *et al*., 2006). It is upregulated following DNA damage (Bates *et al*., 1996). Likewise, DMSO promotes cell cycle arrest and differentiation in various cancer cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998; Watson *et al*., 1997; Jiang *et al*., 1994). Lycopene also induces differentiation in prostate cancer cells (Kotake-Nara *et al*., 2002). This upregulation reduces MCF-7 proliferation and increases genomic stability to decrease the oncogenic state.

CCNH

Lycopene upregulates cyclin H, in relation to THF-treated control cells, to promote cell cycle progression, transcription and DNA repair (Matsuoka *et al*., 1994; Svejstrup *et al*., 1996; Schaeffer *et al*., 1993). This is induced by lycopene without the influence of THF. Similarly, lycopene decreases DNA damage in lymphocytes, H568 cells and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Lycopene does, however,

decrease cell cycle progression in various cells (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005). Cyclin H promotes the proliferation of high integrity DNA to promote genomic integrity.

CCNT2

Cyclin T2 is upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, to promote transcription and differentiation (Palancade and Bensaude, 2003; Simone *et al*., 2002). This is due to thalidomide with and without the influence of DMSO. Cyclin T2 promotes MCF-7 genomic stability and inhibits its oncogenic state. Likewise, thalidomide inhibits tumourigenesis in MCF-7, HL-60 and myeloma (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

CDC2

Lycopene downregulates CDC2 in relation to THF-treated control cells and this is induced by lycopene without THF influence. CDC2 is also downregulated by thalidomide in relation to both untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. This downregulation decreases APC^{CDC20} activation, consequently inhibiting cell cycle progression and transcription, and promotes apoptosis (Peters *et al*., 1998; Kobor and Greenblatt, 2002; Rudner and Murray, 2000; Jana *et al*., 2004). Lycopene also decreases DNA damage in lymphocytes, H568 and CHO cells (Astlely *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). CDC2 is inhibited by GADD45A, which is upregulated by lycopene in MCF-7 (Rudner and Murray, 2000). CDC2 is upregulated in breast cancer (Megha *et al*., 1999).

CDC2 downregulation decreases MCF-7 proliferation and increases genomic integrity to reduce tumourigenesis.

Similarly, lycopene inhibits proliferation in various cancer cells, including MCF-7, and induces apoptosis in LnCaP, HuCC and Raji cells (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005; Ivanov *et al*., 2007; Salman *et al*., 2007). Thalidomide also inhibits cell cycle progression in other studies, including in MCF-7 (Du *et al*., 2005; Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CDK4

CDK4 is upregulated by DMSO to promote cell cycle progression and inhibit p53 and RB tumour suppressor activity (Strohmaier *et al*., 2001; Ortega *et al*., 2002; Reynisdottir *et al*., 1995). CDK4 is upregulated in breast cancer where it promotes p53 degradation (Ortega *et al*., 2002; Hall and Peters, 1996). However, in previous studies DMSO inhibits proliferation in cancer cells (Watson *et al*., 1997; Ponzio *et al*., 1998; Kranenburg *et al*., 1995). DMSO increases MCF-7 proliferation and decreases genomic stability to increase tumourigenesis.

CDK4 is downregulated by thalidomide in comparison to DMSO-treated control cells and this is due to thalidomide independently of DMSO influence. This inhibits cell cycle progression and tumourigenesis, and induces apoptosis (Ortega *et al*., 2002; Pines, 1995; Liu, 2006; Strohmaier *et al*., 2001; Jana *et al*., 2004). MCF-7 proliferation is reduced to

promote genomic stability. Thalidomide also induces apoptosis and inhibits tumourigenesis in MCF-7, HL-60 and multiple myeloma cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

CDK5RAP1

DMSO downregulates CDK5RAP1 to increase CDK5 activation and promote apoptosis, differentiation, transcription and senescence (Zhang *et al*., 2002b; Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007). CDK5RAP1 is also downregulated by thalidomide in comparison to untreated cells, and this is due to DMSO influence on thalidomide. Cell cycle progression is inhibited (Kim *et al*., 2006).

DMSO also induces differentiation in HL-60, murine myeloid and murine neuroblastoma cells, and promotes apoptosis in CHO cells (Watson *et al*., 1997; Marthyn *et al*., 1998; Fiore and Degrassi, 1999). Similarly, thalidomide induces apoptosis and inhibits cell cycle progression in MCF-7, HL-60 and multiple myeloma cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Hideshima *et al*., 2000). This downregulation decreases proliferation, increases genomic stability and reduces tumourigenesis in MCF-7. Thalidomide inhibits tumourigenesis in other studies, including in MCF-7 (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

CDK6

CDK6 is downregulated by THF to decrease cyclin E activation, cyclin D1 transcription and cell cycle progression, and promote RB tumour suppressor function (Geng *et al*., 1996; Timms *et al*., 2002; Lenferink *et al*., 2001; Kwon *et al*., 1995). It is inhibited by p16 and its overexpression induces tumourigenesis (Parry *et al*., 1999; Ortega *et al*., 2002; Liu, 2006). THF decreases MCF-7 proliferation and inhibits its oncogenic potential.

CDK6 is upregulated by lycopene, in comparison to THF-treated control cells, to increase cell cycle progression and tumourigenesis (Kwon *et al*., 1995; Liu, 2006). This promotes cyclin D1 transcription, which is upregulated by lycopene (Lenferink *et al*., 2001). This concurs with previous work where lycopene upregulates CDK6 in MCF-7 and MDA-MB-231 cells (Chalabi *et al*., 2007). Lycopene promotes MCF-7 proliferation and decreases genomic stability.

CDK7

Lycopene upregulates CDK7, in comparison to both untreated and THF-treated control cells, to promote cell cycle progression, transcription and DNA repair (Harper and Elledge, 1998). This is due to lycopene with and without THF influence. Thalidomide also upregulates CDK7, in relation to untreated and DMSO-treated control cells. This is due to thalidomide both with and without DMSO. The proliferation of high integrity, stabilised DNA is promoted. Lycopene has been shown to decrease DNA damage in various cells where it inhibits proliferation (Astley *et al*., 2004; Yeh and Hu, 2000;

Scolastici *et al*., 2007; Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005).

CDK8

Thalidomide upregulates CDK8, in comparison to untreated cells, to increase p53-regulated transcription and inhibit cell cycle progression (Donner *et al*., 2007; Akoulitchev *et al*., 2000). It is activated by the tumour suppressor p21 (Donner *et al*., 2007). This inhibits proliferation and promotes DNA integrity to reduce the oncogenic state. It is induced by the combination of thalidomide with DMSO. Similarly, thalidomide decreases cell cycle progression by inducing cell cycle arrest in various cells (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CDKN1B

The tumour suppressor p27 is upregulated by THF in comparison to untreated cells. It is also upregulated by lycopene, in comparison to untreated cells. This is induced by THF influence. This upregulation inhibits cell cycle progression, and promotes apoptosis and differentiation (Munoz-Alonso *et al*., 2005; Kayatose *et al*., 1997; Robker and Richards, 1998). This reduces proliferation and the oncogenic state of MCF-7, and increases genomic stability.

Likewise, lycopene increases p27 levels in A549 cells to induce G_1/S arrest (Lian *et al*., 2007). Lycopene inhibits proliferation, and induces apoptosis and differentiation in

various cancer cells (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Ivanov *et al*., 2007; Salman *et al*., 2007; Kotake-Nara *et al*., 2002).

CDKN2A

p16 is downregulated by DMSO to increase cell cycle progression, angiogenesis and metastasis and increase pRB phosphorylation and inactivation (Serrano *et al*., 1993; Gibson *et al*., 2005; Wang *et al*., 2006; Brenner *et al*., 1998). However, DMSO inhibits pRB phosphorylation in 7TD1 cells and inhibits proliferation in various cancer cells (Ponzio *et al*., 1998). p16 hypermethylation and inactivation occurs frequently in breast cancers (Nielsen *et al*., 2001). DMSO promotes MCF-7 proliferation and tumourigenesis.

Thalidomide upregulates the tumour suppressor p16 in relation to DMSO-treated control cells. As p16 is not upregulated in relation to untreated cells, this upregulation is induced by thalidomide without the influence of DMSO. This upregulation inhibits cell cycle progression, angiogenesis and metastasis, and promotes RB-mediated G_1 arrest (Serrano *et al*., 1993; Gibson *et al*., 2005; Wang *et al*., 2006; Brenner *et al*., 1998). Similarly, thalidomide inhibits cell cycle progression, angiogenesis, metastasis, and induces G_1 arrest in other studies (Hattori and Iguchi, 2004; Geitz *et al*., 1996; Hideshima *et al*., 2000; D'Amato *et al*., 1994; Kenyon *et al*., 1997; Adeoti *et al*., 1998; Kucharczak *et al*., 2003; Keifer *et al*., 2001; Kedar *et al*., 2004; Fujita *et al*., 2001). p16 decreases MCF-7 proliferation to increase genomic stability and decrease tumourigenesis.

CDKN2B

DMSO and THF upregulate the tumour suppressor p15. It is also upregulated by lycopene, in relation to both untreated and THF-treated control cells. This is induced by lycopene with and without THF influence. Furthermore, it is upregulated by thalidomide in comparison to untreated cells, and this is attributed to DMSO influence. This upregulation induces cell cycle arrest, senescence and p27, and inhibits cell cycle progression and telomerase activity (Reynisdottir *et al*., 1995; Fuxe *et al*., 2000; Massague, 2004; Swarbrick *et al*., 2000).

DMSO induces cell cycle arrest in various cancer cells, including HL-60, Burkitt's lymphoma and hybridoma 7TD1 cells, and promotes p27 in CHO, N1E-115 and 7TD1 cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998; Fiore and Degrassi, 1999; Kranenburg *et al*., 1995). Lycopene inhibits cell cycle progression and induces cell cycle arrest in various cells, including MCF-7 and MDA-MB-231 (Chalabi *et al*., 2004; Park *et al*., 2005; Limpens *et al*., 2006). p15 upregulation therefore reduces the oncogenic state of MCF-7. Likewise, thalidomide induces cell cycle arrest in multiple myeloma cells (Hideshima *et al*., 2000; Hattori and Iguchi, 2004).

CDKN3

DMSO downregulates CDKN3 to increase cell cycle progression and promote tumour suppressor p53 and p21 transcription (Chinami *et al*. 2005; Schultz *et al.* 2002; Okamoto *et al*. 2006). It is overexpressed in breast cancer where it promotes tumourigenesis (Yeh *et al.* 2003; Lee *et al.* 2000). DMSO is decreasing CDKN3 overexpressed levels found in

untreated cells to inhibit MCF-7 proliferation by increasing tumour suppressor transcription. Similarly, DMSO promotes p21 in hybridoma 7TD1 cells (Ponzio *et al.* 1998).

Thalidomide upregulates CDKN3, in comparison to DMSO-treated control cells. It is due to thalidomide without DMSO influence. CDKN3 inhibits CDK2, cell cycle progression and transcription to promote the genomic stability of MCF-7 (Chinami *et al*., 2005; Gyuris *et al*., 1994; Okamoto *et al*., 2006). It inhibits p53-regulated transcription (Schultz *et al*., 2001; Schultz *et al*., 2002). Likewise, thalidomide induces cell cycle arrest in various cells to inhibit cell cycle progression (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CHEK2

CHK2 is upregulated by thalidomide in relation to DMSO-treated control cells and this is induced by thalidomide independently of DMSO. CHK2 participates at the G_1/S and G_2/M checkpoints where it inhibits activation of the mitosis promoting factor to control mitotic entry (Gatei *et al*., 2003). It functions in response to DNA damage, and inhibits cyclin E/CDK2 and cell cycle progression (Ahn and Prives, 2002; Bartek *et al*., 2001). By phosphorylating and stabilising p53 and BRCA1, CHK2 induces cell cycle arrest, DNA repair and apoptosis (Pommier *et al*., 2005). It also phosphorylates and stabilises E2F-1 to regulate its transcriptional activity following DNA damage (Stevens *et al*., 2003). This upregulation increases genomic integrity and stability, and inhibits

proliferation. CHK2 decreases tumourigenesis in MCF-7. Similarly, thalidomide induces G_1 arrest in multiple myeloma cells (Hideshima *et al*., 2000).

CUL3

CUL3 is upregulated by DMSO, and by THF. Lycopene also upregulates CUL3, in comparison to untreated and THF-treated control cells. This is due to lycopene with and without the influence of THF. It is also upregulated by thalidomide, in comparison to untreated cells. This is due to DMSO influence as CUL3 is upregulated in DMSO-treated cells. This upregulation increases cell cycle progression, spindle assembly and chromosomal integrity (Sumara *et al.* 2007; Stewart and Fang, 2005). CUL3-based ubiquitin ligases also have a tumour suppressive function (Wilkins *et al*., 2004). This upregulation increases MCF-7 genomic stability.

DDX11

DMSO and THF downregulate DDX11. It is also downregulated by lycopene in comparison to untreated and THF-treated control cells, and this is due to lycopene both with and without THF influence. Thalidomide also downregulates DDX11 in relation to untreated cells. This is due to thalidomide in combination with DMSO. This downregulation decreases replication, DNA repair and transcription, and increases mitotic aberrations (Amann *et al*., 1997; Tuteja and Tuteja, 2004; Parish *et al*., 2006; Hoque and Ishikawa, 2001; Uhlmann *et al*., 2000). MCF-7 proliferation is decreased but is accompanied by a decrease in genomic stability. Similarly, thalidomide reduces

proliferation in MCF-7, HL-60 and myeloma (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

E2F4

E2F4 is upregulated by lycopene, in relation to untreated cells, to promote cell cycle progression, cell cycle exit and differentiation (Garneau *et al*., 2007; Humbert *et al*., 2000; Fajas *et al*., 2002). While lycopene increases MCF-7 proliferation, differentiation also increases to inhibit tumourigenesis. Similarly, lycopene induces differentiation in prostate cancer cells (Kotake-Nara *et al*., 2002). This is due to THF influence as E2F4 is not upregulated by lycopene in relation to THF-treated control cells.

GADD45A

Lycopene upregulates GADD45A, in comparison to untreated and THF-treated control cells, to induce cell cycle arrest, DNA repair and inhibit growth (Jin *et al*., 2002; Carrier *et al*., 1999; Zhan *et al*., 1994). This is due to lycopene, both with and without THF influence. Lycopene induces cell cycle arrest in various cancer cells, including MCF-7, and decreases DNA damage (Chalabi *et al*., 2004; Park *et al*., 2005; Ivanov *et al*., 2007; Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). GADD45A upregulation inhibits MCF-7 proliferation and increases genomic integrity to inhibit tumourigenesis.

GTF2H1

GTF2H1 is upregulated by lycopene, in comparison to untreated cells and is induced by the combination of lycopene and THF. Thalidomide also upregulates GTF2H1, in

relation to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO. GTF2H1 promotes DNA repair, transcription, cell cycle progression and cell cycle control (Araujo *et al*., 2000; Matsuno *et al*., 2007). Similarly, lycopene reduces DNA damage in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). This increases proliferation and genomic integrity in MCF-7.

GTSE1

GTSE1 is downregulated by DMSO. It is also downregulated by thalidomide in comparison to untreated cells, and this is due to DMSO influence. This microtubule-associated protein is stabilised following DNA damage and accumulates in the nucleus (Ko and Prives, 1996). GTSE1 promotes G_2/M delay following DNA damage and protects these cells at the G_2 checkpoint from p53-dependent apoptosis (Monte *et al*., 2003). It promotes the translocation of p53 to the cytoplasm and its consequent degradation by MDM2 (Monte *et al*., 2004; O'Keefe *et al*., 2003). Its downregulation promotes genomic integrity and p53 tumour suppressor function. This inhibits MCF-7 proliferation and increases genomic stability to inhibit tumourigenesis. Similarly, thalidomide induces apoptosis in MCF-7, HL-60 and multiple myeloma cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Mitsiades *et al*., 2002; Hideshima *et al*., 2000).

HERC5

HERC5 is upregulated by lycopene, in relation to untreated and THF-treated control cells, and is due to lycopene both with and without THF influence. This promotes proteolysis

in conjunction with the immune response (Dastur *et al*., 2006). MCF-7 stability is promoted.

HUS1

HUS1 is upregulated by thalidomide, in comparison to DMSO-treated control cells. As HUS1 is not upregulated in comparison to untreated cells, this upregulation is induced by thalidomide without the influence of DMSO. As part of 9-1-1, it promotes checkpoint function and DNA repair (Francia *et al*., 2006; Parrilla-Castellar *et al*., 2004; Walworth and Bernards, 1996; Wang *et al*., 2006). It promotes CHK1 and CHK2 activity (Walworth and Bernards, 1996; Lindsay *et al*., 1998). Thalidomide increases the genomic integrity and stability of MCF-7 to inhibit its oncogenic state. Thalidomide induces checkpoint function in multiple myeloma cells as well (Hideshima *et al*., 2000).

KPNA2

THF downregulates KPNA2 to inhibit checkpoint function and oncogenic transformation (Tseng *et al*., 2005; Dankof *et al*., 2007; Poon and Jans, 2005). KPNA2 is overexpressed in breast cancer and correlates with a poor prognosis (Dahl *et al*., 2006). This downregulation therefore inhibits tumourigenesis of MCF-7.

Lycopene upregulates KPNA2 in relation to THF-treated control cells and this is due to the lycopene without THF influence. This increases checkpoint function, BRCA1, p53 and MRN function, and differentiation, but may also promote oncogenic transformation (Tseng *et al*., 2005; Thakur *et al*., 1997; Kim *et al*., 2000; Dankof *et al*., 2007; Poon and

Jans, 2005). Lycopene promotes genomic integrity and stability, and decreases the oncogenic potential of MCF-7.

MCM4

MCM4 is upregulated by DMSO. It prevents DNA re-replication, controls DNA elongation and is involved in checkpoint activation and transcription (Labib *et al*., 2000; Cortez *et al*., 2004; Dziak *et al*., 2003). The MCM4/6/7 complex has DNA-helicase activity for DNA replication (Davey *et al*., 2003). It is involved in the DNA replication checkpoint, inducing arrest at the DNA fork following DNA damage (Ishimi *et al*., 2004). DMSO promotes genomic stability and proliferation of MCF-7.

MKI67

Ki-67 is downregulated by DMSO. It is also downregulated by lycopene in comparison to THF-treated control cells. This is induced by lycopene independently of THF influence. This decreases DNA organisation, DNA maintenance and ribosomal synthesis, and correlates with a decrease in proliferation (MacCallum and Hall, 2000; Endl and Gerdes, 2000). The tumourigenic potential of MCF-7 is reduced. Similarly, DMSO induces apoptosis in CHO cells (Fiore and Degrassi, 1999).

MRE11A

MRE11A is upregulated by thalidomide in comparison to DMSO-treated control cells, and this is due to thalidomide without the influence of DMSO. As part of the MRN complex, MRE11A responds to DSBs to induce checkpoint activation, DNA repair and

apoptosis (Lee and Paull, 2005). It is phosphorylated in an NBS1-dependent manner following DNA damage (Dong *et al*., 1999). MRE11A unwinds DNA to induce ATM activity (Lee and Paull, 2005). NBS1 and ATM are upregulated by thalidomide. It is involved in homologous recombination at DSB ends and hairpins, telomere length maintenance and is essential for proliferation (Yamaguchi-Iwai *et al*., 1999; Paull and Gellert, 1998). The upregulation of MRE11A promotes proliferation and genomic integrity in MCF-7. Thalidomide also induces cell cycle arrest and apoptosis in other studies, including in MCF-7 (Hideshima *et al*., 2000; Du *et al*., 2005; Hattori and Iguchi, 2004).

NBS1

Thalidomide upregulates NBS1 in relation to untreated cells and this is due to the combination of thalidomide with DMSO. NBS1 is downregulated in breast cancer and this upregulation promotes checkpoint activation (Lee and Paull, 2005). It activates and is activated by ATM, which is upregulated by thalidomide (Stracker *et al*., 2004; Lee and Paull, 2005). It is part of the MRN complex, and promotes MRE11A and RAD51 activity (Carney *et al*., 1998; Tseng *et al*., 2005). This increases MCF-7 genomic integrity and stability.

RAD17

RAD17 is upregulated by lycopene in relation to both untreated and THF-treated control cells. This is due to lycopene with and without THF influence. It is also upregulated by thalidomide in relation to untreated cells. This is due to the combination of thalidomide

with DMSO. RAD17 promotes ATR and 9-1-1 function, increases checkpoint function and prevents re-replication (Wang *et al*., 2003; Zou *et al*., 2003; Bermudez *et al*., 2003; Walworth and Bernards, 1996; Lindsay *et al*., 1998). ATR is upregulated by lycopene and also activates RAD17 in response to DNA damage (Zou *et al*., 2002). Lycopene decreases DNA damage in various cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Thalidomide promotes checkpoint function in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000). This upregulation promotes genomic integrity and reduces the oncogenic state of MCF-7.

RAD51

DMSO and THF downregulate RAD51. Lycopene also downregulates RAD51, in comparison to untreated and THF-treated control cells, and this is induced by lycopene with and without THF influence. It is also downregulated by thalidomide in comparison to untreated cells. This is induced by thalidomide with DMSO influence. RAD51 is overexpressed in breast cancer and cancer cell lines where it promotes chromosomal rearrangement and aneuploidy by increasing homologous recombination (Maacke *et al*., 2000; Yoshikawa *et al*., 2001; Vispe *et al*., 1998; Richardson *et al*., 2004). By decreasing elevated levels of RAD51, DNA damage repair is promoted (Maacke *et al*., 2000; Raderschall *et al*., 2002; Richardson *et al*., 2004; Gupta *et al*., 1997). This downregulation increases the genomic integrity of MCF-7.

Similarly, lycopene promotes DNA repair in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007).

RAD9A

RAD9A is downregulated by DMSO to decrease checkpoint signalling, cell cycle arrest, DNA repair and apoptosis following DNA damage (Toueille *et al*., 2004). A decrease in RAD9 levels induces chromosomal aberrations, morphology change and carcinogenesis (Bao *et al*., 2004). However, RAD9 is overexpressed in breast cancer (Cheng *et al*., 2005). DMSO is therefore not decreasing RAD9 to promote the DNA damage responses. This increases the genomic integrity of MCF-7.

Thalidomide upregulates RAD9A in relation to DMSO-treated control cells. This is induced by thalidomide without DMSO influence. This promotes checkpoint function, cell cycle arrest, DNA repair and apoptosis (Toueille *et al*., 2004). The 9-1-1 complex, of which RAD9A forms part, is activated by ATM, which is upregulated by thalidomide, and activates the p21 tumour suppressor (Roos-Mattjus *et al*., 2003; Yin *et al*., 2004). This concurs with previous studies where thalidomide induces p21-mediated cell cycle arrest and induces apoptosis (Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Du *et al*., 2005; Marriott *et al*., 2003). Thalidomide increases MCF-7 genomic stability and reduces its oncogenic state.

RBL2

The tumour suppressor p130 is upregulated by lycopene, in relation to untreated and THF-treated control cells. This is induced by lycopene both with and without THF influence. p130 maintains quiescence, induces G_1 arrest and suppresses growth (Grana *et al*., 1998; Woo *et al*., 1997; Hansen *et al*., 2001; Beijersbergen *et al*., 1995). p130/E2F4

and p130/E2F5 inhibit the transcription of cell cycle-related genes (Ren *et al*., 2002). p130/E2F4 inhibits CDC2 transcription to suppress mitosis (Taylor *et al*., 2001). Lycopene downregulates CDC2 in MCF-7. Similarly, lycopene induces G_1/S arrest in MCF-7, MDA-MB-231, ECC1 endometrial cancer cells and Hep3B hepatoma cells, amongst others (Chalabi *et al*., 2004; Nahum *et al*., 2006; Park *et al*., 2005; Lian *et al*., 2007; Bhuvaneswari and Nagini, 2005). p130 reduces MCF-7 proliferation and increases genomic stability to inhibit tumourigenesis.

RPA3

Lycopene upregulates RPA3, in comparison to untreated cells. This is induced by the combination of lycopene and THF. Thalidomide also upregulates RPA3, in comparison to DMSO-treated control cells. This is induced by thalidomide without the influence of DMSO. RPA3 increases genomic integrity during replication by promoting DNA repair and apoptosis (Bochkarev *et al*., 1997; He *et al*., 1995; Iftode *et al*., 1999). Lycopene inhibits the accumulation of DNA damage in other studies (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). RPA3 upregulation promotes proliferation and genomic integrity in MCF-7.

This concurs with previous studies where thalidomide induces apoptosis in various cells (Hattori and Iguchi, 2004; Du *et al*., 2005; Marriott *et al*., 2003; Mitsiades *et al*., 2002; Hideshima *et al*., 2000).

3.4.3 MDA-MB-231

The data for the MDA-MB-231 treatments, in comparison to untreated cells, are normalised against the HPRT1, RPL13A, GAPDH and ACTB housekeeping genes. For comparison of thalidomide-treated cells to DMSO-treated cells, and lycopene-treated cells to THF-treated cells, the data is normalised against the B2M, HPRT1, RPL13A, GAPDH and ACTB housekeeping genes.

3.4.3.1 Distribution of C_t Values

The results for the gene expression in MDA-MB-231 untreated cells are used as an example.

Table 3.13: Distribution of C_t values of MDA-MB-231 untreated cells

Percent distribution of C_t values	
C_t Range	
<25	58.13%
25-30	34.38%
30-35	4.17%
Absent calls	8.33%

3.4.3.2 Reverse transcription control

MDA-MB-231 untreated

ΔCt = average reverse transcription control (RTC) – average positive PCR control (PPC)

ΔCt = 23.44 – 20.42

= 3.02

Reverse transcription (RT) efficiency = pass.

MDA-MB-231 DMSO

ΔCt = 23.22 – 21.07

= 2.15

RT efficiency = pass

MDA-MB-231 THF

ΔCt = 24.28 – 19.93

= 4.35

RT efficiency = pass

MDA-MB-231 lycopene

ΔCt = 22.18 – 17.53

= 4.65

RT efficiency = pass

MDA-MB-231 thalidomide

$\Delta Ct = 22.83 – 19.4$

$= 3.43$

RT efficiency = pass

3.4.3.3 Genomic DNA contamination

MDA-MB-231 untreated

C_t (GDC) = 35

Result: pass

MDA-MB-231 DMSO

C_t (GDC) = 35

Result: pass

MDA-MB-231 THF

C_t (GDC) = 35

Result: pass

MDA-MB-231 lycopene

C_t (GDC) = 34.8

Result: fail

The genomic DNA contamination control has failed here. This is due to DNA contamination introduced genomically, or generally through reagents, pipette tips, tubes etc. Fold changes in gene expression can still be obtained from this array, as it does not represent severe contamination. The Ct value should be 35, and as this value of 34.8 is not a large difference from this, it is nominated to use these results. It is difficult to repeat the first strand synthesis and PCR for this system due to the limited and expensive reagents supplied with the kit.

MDA-MB-231 thalidomide

C_t (GDC) = 35

Result: pass

Table 3.14: Fold regulation of genes, in comparison to untreated MDA-MB-231 cells, due to various treatments with significant fold changes highlighted

	DMSO	THF	Lycopene	Thalidomide
ABL1	1.7857	-1.2527	1.9056	-2.3194
ANAPC4	-1.4176	-1.6234	-1.0244	3.3294
ARHI	3.0346	2.3311	8.2549	51.8492
ATM	1.6906	1.2483	3.9075	5.5146
ATR	1.2618	1.2816	2.0623	3.3432
BCL2	-1.1278	-3.3058	-3.7679	-3.1118
BIRC5	-3.1766	-3.5578	-1.9369	-4.2273
BRCA2	1.8487	1.6679	N/A	-7.9931
CCNC	-1.7082	-2.0577	-1.5081	-1.4023
CCND1	1.7189	1.3296	3.2294	-2.6606
CCND2	1.8334	2.0069	1.0877	1.635
CCNE1	-1.5806	-2.5403	-1.901	-1.0089
CCNF	1.4484	1.2897	N/A	-1.5452
CCNG1	-2.7943	-2.3198	-1.4159	-3.37
CCNG2	2.2	1.1917	3.0935	4.7117
CCNH	1.3486	1.6853	2.6968	9.1976
CCNT1	-1.2046	-1.279	1.3207	2.4322
CCNT2	-1.3875	4.1153	16.339	2.2149
CDC16	-1.4585	-3.5628	-6.5602	-1.1629
CDC2	-1.2299	-1.3957	-2.9542	-1.7312

CDC20	1.2324	3.3242	-2.0066	-3.1816
CDC34	3.1438	1.6702	1.762	1.5234
CDK2	1.1146	1.69	-1.7312	-2.2234
CDK4	-1.6976	1.3031	-1.2064	-7.4475
CDK5R1	-1.2522	-2.9732	1.8833	2.0031
CDK5RAP1	-2.5695	-5.8442	-13.4427	-7.154
CDK6	1.1499	-2.6945	-1.4925	1.1237
CDK7	-1.4525	-2.6629	-2.3698	1.0085
CDK8	-1.8119	-1.7863	-2.3583	-1.73
CDKN1A	-1.3458	2.2284	2.4919	2.5302
CDKN1B	-1.0378	1.5168	1.1633	-11.6863
CDKN2A	-1.0178	-1.1127	1.4889	2.8944
CDKN2B	-1.0178	-1.4379	1.4889	2.8944
CUL1	-1.4167	-2.8859	-1.9142	1.047
CUL2	-1.7094	-1.3861	-2.3929	1.3326
CUL3	-1.0178	3.3753	82.7249	27.3458
DDX11	2.2199	5138.0314	2.5407	3.4564
DNM2	1.0242	4.7502	2.578	-1.0495
E2F4	-1.2715	-3.5851	-5.648	-1.3868
GADD45A	-3.4259	-1.7617	-1.6152	-1.3414
GTF2H1	-2.609	-2.0491	-1.135	-2.6477
GTSE1	1.3328	1.8391	1.7294	1.3989
HERC5	3.9109	7.0567	1.7966	5.3712

HUS1	1.3402	1.6166	1.9362	5.1488
MAD2L1	-1.9786	-2.0279	-2.7146	-2.0234
MCM2	3.804	1853.4532	N/A	3.6131
MCM3	2.6473	2.8779	2.6031	2.9819
MCM4	-1.4245	-1.5508	1.3043	-2.086
MCM5	-3.4907	-1.43	2.1027	-1.6063
MKI67	2.0427	1.1408	1.8179	2.2027
MNAT1	-1.1262	-1.325	-1.4081	1.3016
MRE11A	2.4683	1.4661	1.9215	3.507
NBS1	1.291	1.205	1.0326	2.3122
PCNA	1.4985	2.2958	1.0412	3.2406
RAD1	1.7672	-1.3095	2.6782	1.1682
RAD17	-1.9033	-1.0468	-1.0096	2.6987
RAD51	-4.2916	-5.0315	-3.5671	-4.3886
RAD9A	-1.0178	363.2956	197.7111	2.8944
RB1	-1.4933	-1.295	-1.7003	3.5609
RPA3	-1.0178	2.1361	3.4064	11.7717
SERTAD1	-2.7387	-1.2995	-1.2309	-3.0626
SKP2	-2.5571	-10.4759	-13.7347	-27.1099
SUMO1	-1.9745	-1.883	-2.8496	-1.1653
TFDP2	-2.752	-2.0028	-2.073	-4.1432
TP53	1.868	2.3801	3.5733	N/A
UBE1	-1.2453	23.5067	36.6883	1.3806

Table 3.15: Fold regulation of genes, from lycopene-treated MDA-MB-231 cells in comparison to THF-treated control MDA-MB-231 cells, and thalidomide-treated MDA-MB-231 cells in comparison to DMSO-treated control MDA-MB-231 cells, with significant fold changes highlighted

	Lycopene	Thalidomide
ABL1	2.291	-4.8326
ANAPC4	1.521	4.0452
ARHI	3.3987	14.6436
ATM	3.0042	2.7957
ATR	1.5444	2.2708
BAX	1.238	2.5371
BCL2	-1.1876	-3.2194
BRCA2	N/A	-17.2413
CCNB1	-2.0014	-1.9329
CCND1	2.3311	-5.3362
CCNF	N/A	-2.6114
CCNG2	2.4915	1.8355
CCNH	1.5358	5.845
CCNT1	1.6211	2.5109
CCNT2	3.8106	2.6339
CDC2	-2.2053	-1.6424
CDC20	-6.9499	-4.5751
CDC34	1.0126	-2.4079

CDK2	-3.0483	-2.8915
CDK4	-1.6381	-5.1188
CDK5R1	5.374	2.1498
CDK5RAP1	-2.3966	-3.2486
CDKN1A	1.0733	2.9184
CDKN1B	-1.3585	-13.1391
CDKN2A	1.59	2.5249
CDKN2B	2.0548	2.5249
CDKN3	-2.0505	1.0484
CHEK2	2.0056	-1.3093
CUL3	23.523	23.8547
DDX11	-2107.0371	1.3344
GADD45A	1.0468	2.1889
HERC5	-4.0925	1.1771
HUS1	1.1495	3.2925
KPNA2	-2.5847	1.6202
MCM5	2.8859	1.8624
PCNA	-2.2974	1.8534
RAD1	3.3659	-1.7652
RAD17	-1.0049	4.4021
RAD9A	-1.9145	2.5249
RB1	-1.3679	4.5574
RBL2	2.514	1.2485

RPA3	1.5305	10.2688
SKP2	-1.366	-12.3702

3.4.3.4 MDA-MB-231/Treatments

ABL1

ABL1 is upregulated by lycopene, in comparison to THF-treated control cells, to promote the DNA damage responses, including cell cycle arrest and apoptosis (Chen *et al*., 1999; Wang, 2000). Lycopene induces cell cycle arrest and apoptosis in various cells, including MCF-7 and MDA-MB-231 (Chalabi *et al*., 2004). It decreases DNA damage in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Lycopene increases MDA-MB-231 genomic stability and decreases its oncogenic state. This upregulation is not observed in comparison to untreated cells, and is due to lycopene without the influence of THF.

Thalidomide downregulates ABL1 in comparison to untreated and DMSO-treated control cells. This decreases RAD51 activation, which is downregulated by thalidomide (Chen *et al*., 1999; Wang, 2000). RAD51 is overexpressed in tumour cell lines and breast cancer where it promotes genomic instability through elevated levels of homologous recombination (Vispe *et al*., 1998; Richardson *et al*., 2004; Maacke *et al*., 2000; Yoshikawa *et al*., 2001). G_1 arrest and apoptosis are also decreased (Chen *et al*., 1999; Wang, 2000). However, in previous studies thalidomide promotes G_1 arrest and apoptosis (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003). Thalidomide

promotes genomic stability by inhibiting RAD51 activation but may also decrease integrity.

ANAPC4

Thalidomide upregulates APC4, in relation to untreated and DMSO-treated cells, and this is due to lycopene with and without the influence of DMSO. APC4 promotes the checkpoints and cell cycle progression (Peters, 2002; Wasch and Cross, 2002). This increases MDA-MB-231 proliferation and genomic stability. Similarly, thalidomide promotes checkpoint function by inducing G_1 arrest in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

ARHI

The tumour suppressor ARHI is upregulated by DMSO and by THF, in comparison to untreated cells. It is also upregulated by lycopene, in relation to both untreated and THF-treated control cells, to inhibit cell cycle progression and induce apoptosis (Bao *et al*., 2002). This is due to lycopene with and without THF influence. ARHI is also upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, and this is induced by thalidomide with and without DMSO influence. ARHI upregulation promotes activity of the tumour suppressor p21 (Bao *et al*., 2002). p21 is upregulated by THF. Its inhibitor E2F4 is downregulated by THF (Lu *et al*., 2006). This upregulation decreases MDA-MB-231 proliferation and promotes genomic stability.

Similarly, DMSO induces cell cycle arrest in HL-60, Burkitt's lymphoma cells, CHO, and hybridoma 7TD1 cells, amongst others (Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998). Lycopene inhibits cell cycle progression and promotes apoptosis in various cancers (Levy *et al*., 1995; Chalabi *et al*., 2004; Hwang and Bowen, 2004; Ivanov *et al*., 2007; Salman *et al*., 2007; Muller *et al*., 2002). p21 is upregulated by thalidomide. Thalidomide also induces p21 in multiple myeloma cells to induce cell cycle arrest, and induces apoptosis in various cells (Hideshima *et al*., 2000; Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003).

ATM

Lycopene upregulates the tumour suppressor ATM, in comparison to untreated and THF-treated control cells. This is due to lycopene with and without THF influence. Thalidomide also upregulates ATM in relation to untreated and DMSO-treated control cells; and this is due to thalidomide with and without DMSO influence. This increases the DNA damage responses, including cell cycle arrest, and inhibits cell cycle progression and DNA replication (Lee and Paull, 2005; Liu *et al*., 2007; Costanzo *et al*., 2000). ATM is often downregulated in breast cancer therefore, this upregulation reinstates its functions (Ding *et al*., 2004). MDA-MB-231 proliferation decreases and genomic stability increases.

Lycopene reduces DNA damage in lymphocytes, H568 and CHO cells, and induces cell cycle arrest in several cancer cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007; Chalabi *et al*., 2004; Park *et al*., 2005). Thalidomide promotes p21 activity to

induce G_1 arrest in multiple myeloma cells, and p21 is upregulated by thalidomide in this study (Hideshima *et al*., 2000).

ATR

Lycopene upregulates the tumour suppressor ATR, in comparison to untreated cells. This is due to the combination of lycopene with THF. Thalidomide also upregulates ATR in comparison to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. ATR promotes checkpoint activation following DNA damage, promotes DNA repair, inhibits DNA replication and inhibits re-replication (Dart *et al*., 2004; Andreasson *et al*., 2004; Cortez *et al*., 2004; Liu *et al*., 2007). ATR upregulation decreases MDA-MB-231 proliferation and promotes genomic stability.

Lycopene induces cell cycle arrest and inhibits proliferation in many cancer cells, including MDA-MB-231 (Chalabi *et al*., 2004; Nahum *et al*., 2001; Hwang and Bowen, 2004; Park *et al*., 2005). Thalidomide promotes checkpoint function in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

BAX

Thalidomide upregulates pro-apoptotic BAX in relation to DMSO-treated control cells. This is due to thalidomide without the influence of DMSO. Tumour suppressor p53 activates BAX transcription (Miyashita and Reed, 1995). BAX induces cytochrome *c* release during apoptosis and is essential for activation of the effector caspases

(Korsmeyer *et al*., 2000; Kepp *et al*., 2007). It inhibits cell cycle progression, cell survival and decreases anti-apoptotic BCL2 protein levels (Zinkel *et al*., 2006; Reed, 1997; Antonsson and Martinou, 2000; Otter *et al*., 1998). BCL2 is downregulated by thalidomide. Thalidomide inhibits BCL2 in other studies as well (Du *et al*., 2005; Kucharczak *et al*., 2003; Keifer *et al*., 2001; Marriott *et al*., 2003). Its overexpression sensitises cells to apoptosis and it also functions in homologous recombination (Raisova *et al*., 2001; Dumay *et al*., 2006). This upregulation promotes genomic integrity and stability and reduces proliferation. MDA-MB-231 oncogenic state is inhibited.

BCL2

The anti-apoptotic BCL2 is downregulated by THF to promote apoptosis and cell cycle progression, and promote DNA repair (Dlugosz *et al*., 2006; Mazel *et al*., 1996; Youn *et al*., 2005). It is also downregulated by lycopene in relation to untreated cells. As it is downregulated in THF-treated cells as well, this downregulation is due to THF influence. BCL2 is also downregulated by thalidomide in comparison to untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. Its inhibitor, p53, is upregulated by THF (Chipuk *et al*., 2004; Mihara *et al*., 2003). BCL2 is overexpressed in breast cancer therefore its downregulation promotes DNA integrity and reduces the oncogenic state of MDA-MB-231 (Binder *et al*., 1995; Ramsay *et al*., 1995).

Lycopene promotes apoptosis and decreases DNA damage in other studies (Ivanov *et al*., 2007; Salman *et al*., 2007; Astley *et al*., 2004; Scolastici *et al*., 2007). Thalidomide

inhibits NF-κB activity to decrease BCL2 transcription and promote apoptosis following DNA damage and cell cycle defects (Kucharczak *et al*., 2003; Keifer *et al*., 2001; Marriott *et al*., 2003).

BIRC5

Survivin is downregulated by DMSO and by THF to inhibit cell cycle progression, and promote cell cycle arrest and apoptosis (Dohi *et al*., 2004; Beltrami *et al*., 2004; Sarela *et al.* 2000). Thalidomide also downregulates survivin in relation to untreated cells. This is induced by the combination of thalidomide with DMSO. Its downregulation alleviates the inhibition of the tumour suppressor p21 that is upregulated by both THF and thalidomide, to inhibit cell cycle progression (Fukuda *et al*., 2004). MDA-MB-231 proliferation decreases and genomic integrity increases.

DMSO inhibits proliferation and induces cell cycle arrest in various cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998; Teraoka *et al*., 1996). Thalidomide promotes cell cycle arrest, including p21-mediated arrest, and apoptosis in other cells (Hideshima *et al*., 2000; Hattori and Iguchi, 2004; Du *et al*., 2005; Marriott *et al*., 2003).

BRCA2

Thalidomide downregulates BRCA2 in comparison to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. BRCA2 is overexpressed in breast cancer and promotes oncogenic transformation

(Egawa *et al*., 2002). Its downregulation decreases DNA replication and inhibits elevated homologous recombination by RAD51 (Lomonosov *et al*., 2003; Galkin *et al*., 2005). This decreases proliferation and promotes genomic stability in MDA-MB-231 to reduce tumourigenesis.

CCNB1

Cyclin B1 is downregulated by lycopene, in relation to THF-treated control cells. As it is not upregulated in relation to untreated cells, this upregulation is due to lycopene without the influence of THF. Cyclin B1 downregulation decreases apoptosis and inhibits proliferation (Borgne *et al*., 2006; Pines and Hunter, 1989). Oncogenic transformation and metastasis are inhibited (Suzuki *et al*., 2007; Megha *et al*., 1999). It interacts with CDC2 to promote mitosis (Morgan, 1995). CDC2 is also downregulated by lycopene. Lycopene inhibits proliferation in MCF-7, endometrial cancer, lung cancer, prostate cells, amongst others and inhibits metastasis (Nahum *et al*., 2001; Levy *et al*., 1995; Hwang and Bowen, 2004; Chandler *et al*., 1997). Lycopene decreases MDA-MB-231 proliferation and reduces its oncogenic state.

CCNC

THF downregulates cyclin C to inhibit cell cycle entry and progression, and promote transcription (Ren and Rollins, 2004; Liu *et al*., 1998; Akoulitchev *et al.*, 2000). It can be assumed that to correlate with reduced cell cycle progression, cyclin C promotes the transcription of tumour suppressors. THF increases MDA-MB-231 genomic stability and decreases its proliferation. This decreases tumourigenesis.

CCND1

Lycopene upregulates cyclin D1, in comparison to untreated and THF-treated control cells. This is due to lycopene with and without THF influence. Cyclin D1 increases cell cycle progression, survival and transcription (Schwartz and Shah, 2005; Hinds *et al*., 1994; Coqueret, 2002). In contrast, lycopene downregulates cyclin D1 to induce cell cycle arrest in various cancer cells including MCF-7 and endometrial cancer cells (Nahum *et al*., 2001; Nahum *et al*., 2006). A previous study does, however, confirm the upregulation of cyclin D1 in MDA-MB-231 and MCF-7 cells (Chalabi *et al*., 2007). This upregulation increases MDA-MB-231 proliferation and promotes oncogenic transformation.

Thalidomide downregulates cyclin D1, in relation to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. Cyclin D1 downregulation increases p21 and p27 activity, and inhibits cell cycle entry and cell cycle progression (Perez-Roger *et al*., 1999; Schwartz and Shah, 2005). p21 is upregulated by thalidomide. Thalidomide induces p21 to promote cell cycle arrest in multiple myeloma cells (Hideshima *et al*., 2000). Cyclin D1 is overexpressed in breast cancer (Ormandy *et al*., 2003; Bartkova *et al*., 1994). MDA-MB-231 proliferation decreases to promote genomic stability and reduce tumourigenesis.

CCND2

THF upregulates cyclin D2 to promote cell cycle progression and increase survival (Schwartz and Shah, 2005; Hinds *et al*., 1994). It alleviates the inhibition of CDK2 by the tumour suppressor p27. This upregulation promotes proliferation and tumourigenesis.

CCNE1

Cyclin E1 is downregulated by THF to inhibit cell cycle progression and apoptosis (Mazumder *et al*., 2004). As cyclin E1 promotes oncogenic transformation and is upregulated in breast cancer, this downregulation promotes MDA-MB-231 genomic stability by decreasing proliferation (Akli and Keyomarsi, 2004; Keyomarsi *et al*., 1995).

CCNF

Cyclin F is downregulated by thalidomide in relation to DMSO-treated control cells and this is induced by thalidomide without the influence of DMSO. This decreases cyclin E transcription and cell cycle progression, and promotes apoptosis (Tezlaff *et al*., 2004; Kong *et al*., 2000; Jana *et al*., 2004). Thalidomide induces apoptosis in previous studies (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000). This reduces MDA-MB-231 proliferation to increase genomic stability.

CCNG1

DMSO and THF downregulate cyclin G1. Thalidomide also downregulates cyclin G1 in comparison to untreated cells and this is induced by the combination of thalidomide with DMSO. Cyclin G1 is overexpressed in breast cancer and its downregulation therefore

inhibits the oncogenic state (Reimer *et al*., 1999). Its downregulation inhibits the cell cycle, induces G_2/M arrest in response to DNA damage, maintains quiescence in differentiated cells and promotes apoptosis (Bennin *et al*., 2002; Okamoto and Prives, 1999; Reimer *et al*., 1999). This downregulation promotes genomic stability in MDA-MB-231 and reduces the oncogenic state.

This concurs with other studies where DMSO reduces cell cycle progression (Watson *et al*., 1997; Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998). Thalidomide induces cell cycle arrest and apoptosis in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Du *et al*., 2005; Marriott *et al*. 2003).

CCNG2

DMSO upregulates cyclin G2. It is also upregulated by lycopene, in relation to untreated and THF-treated control cells. This is due to the effect of lycopene with and without the influence of the THF solvent. Cyclin G2 is also upregulated by thalidomide in comparison to untreated cells. As it is not upregulated by thalidomide, in comparison to DMSO-treated control cells, but is upregulated in DMSO-treated cells, this upregulation is due to DMSO influence. The effect is to inhibit cell cycle progression and promote quiescence and apoptosis (Bennin *et al*., 2002; Arachchige Don *et al*., 2006; Home *et al*., 1996). It inhibits CDK2 to induce G_1/S arrest (Bennin *et al*., 2002). DMSO inactivates CDK2 in 7TD1 cells and induces cell cycle arrest in various cancer cells (Ponzio *et al*., 1998; Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999). Cyclin G2 upregulation inhibits MDA-MB-231 proliferation and tumourigenesis.

Similarly, lycopene induces differentiation in prostate cancer cells (Kotake-Nara *et al*., 2002). Lycopene also inhibits cell cycle progression and induces cell cycle arrest in various cells, including MDA-MB-231 (Chalabi *et al*., 2004; Nahum *et al*., 2001; Hwang and Bowen, 2004; Park *et al*., 2005). Thalidomide induces cell cycle arrest in other studies, and upregulates cyclin G2 in MCF-7 cells (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CCNH

Lycopene upregulates cyclin H by lycopene, in comparison to untreated cells. This upregulation is due to the combination of lycopene with THF as it is not upregulated by lycopene in comparison to THF-treated control cells. Thalidomide also upregulates cyclin H, in comparison to untreated and DMSO-treated control cells, and this is induced by thalidomide with and without DMSO influence. This upregulation promotes cell cycle progression; transcription and DNA repair (Matsuoka *et al*., 1994; Svejstrup *et al*., 1996; Schaeffer *et al*., 1993). Although proliferation is increased, genomic integrity also increases.

CCNT1

The upregulation of cyclin T1 by thalidomide, in relation to untreated and DMSO-treated control cells, increases transcription and differentiation (Palancade and Bensaude, 2003; De Falco and Giordano, 2002). It is induced by thalidomide with and without DMSO influence. It is implied that the transcription of differentiation-related genes is promoted. Cyclin T1 is upregulated as part of the immune response, and similarly thalidomide

promotes the immune response to increase tumour cell degradation (Liou *et al*., 2006; Mitsiades *et al*., 2002). Thalidomide inhibits the oncogenic state of MDA-MB-231.

CCNT2

THF upregulates cyclin T2. It is also upregulated by lycopene, in relation to untreated and THF-treated control cells. This is due to lycopene with and without THF influence. Cyclin T2 is also upregulated by thalidomide in comparison to untreated and DMSO-treated control cells. This upregulation is induced by thalidomide with and without DMSO influence. This upregulation promotes transcription and differentiation (Palancade and Bensaude, 2003; Simone *et al*., 2002). It decreases the oncogenic state of MDA-MB-231 and increases its genomic stability. Similarly, lycopene induces differentiation in prostate cancer cells (Kotake-Nara *et al*., 2002).

CDC16

CDC16 is downregulated by lycopene, in comparison to untreated cells, to reduce cell cycle progression and promote apoptosis (Zachariae and Nasmyth, 1996; Rudner and Murray, 2000; Jana *et al*., 2004). As it is not upregulated by lycopene in comparison to THF-treated cells, this upregulation is induced by the combination of lycopene with THF. Lycopene inhibits cell cycle progression and induces apoptosis in various cancer cells (Chalabi *et al*., 2004; Nahum *et al*., 2001; Ivanov *et al*., 2007; Salman *et al*., 2007). Lycopene inhibits MDA-MB-231 proliferation and reduces its oncogenic state.

CDC2

CDC2 is downregulated by lycopene, in comparison to THF-treated control cells and this is induced by lycopene without the influence of THF. CDC2 is overexpressed in breast cancer (Megha *et al*., 1999). Its downregulation inhibits cell cycle progression and transcription, and promotes apoptosis (Peters *et al*., 1998; Kobor and Greenblatt, 2002; Rudner and Murray, 2000; Jana *et al*., 2004). Similarly, lycopene decreases cell cycle progression and induces apoptosis in a range of cancer cells (Chalabi *et al*., 2004; Nahum *et al*., 2001; Ivanov *et al*., 2007; Muller *et al*., 2002). Lycopene decreases MDA-MB-231 proliferation and increases genomic integrity.

CDC20

CDC20 is upregulated by THF to promote MDA-MB-231 cell cycle progression (Pfleger *et al*., 2001). Overexpressed CDC20 promotes the premature onset of anaphase to induce genetic instability (Hwang *et al*., 1998). This increases the oncogenic state of MDA-MB-231.

Lycopene downregulates CDC20, in relation to both untreated and THF-treated control cells. This is due to lycopene with and without the influence of THF. Thalidomide also downregulates CDC20 in comparison to untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. CDC20 is upregulated in breast cancer cell lines and this downregulation inhibits cell cycle progression and promotes apoptosis (Yuan *et al*., 2006; Pfleger *et al*., 2001; Jana *et al*., 2004). Similarly, lycopene reduces cell cycle progression and induces apoptosis in other studies (Chalabi *et al*., 2004;

Nahum *et al*., 2001; Ivanov *et al*., 2007; Salman *et al*., 2007). Thalidomide inhibits proliferation and induces apoptosis in other cells (Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Du *et al*., 2005; Marriott *et al*., 2003). This downregulation decreases MDA-MB-231 proliferation and promotes genomic stability.

CDC34

DMSO upregulates CDC34 in comparison to untreated cells. It is an E2 conjugating enzyme that enhances SCF E3 ligase activity (Wu *et al*., 2002). SCF induces ubiquitin transfer from CDC34 to the substrate to be targeted for proteasomal degradation (Petroski and Deshaies, 2005). CDC34 promotes SCF^{SKP2} ubiquitination of p27 to induce G_1/S progression, G_2/M transition and spindle function (Butz *et al*., 2005; Kaiser *et al*., 2000; Reymond *et al*., 2000). Its overexpression inhibits chromosome alignment at metaphase and is associated with cancers (Liu *et al*., 2006; Eliseeva *et al*., 2001; De Vos *et al*., 2002; Chauhan *et al*., 2004). CDC34 upregulation promotes cell cycle progression and genomic instability to promote tumourigenesis. In contrast, DMSO increases p27 activity in CHO and N1E-115 cells (Fiore and Degrassi, 1999; Kranenburg *et al*., 1995).

CDC34 is downregulated by thalidomide in relation to DMSO-treated control cells and this is due to thalidomide without the influence of DMSO. This promotes p27, inhibits cell cycle progression and induces apoptosis (Butz *et al*., 2005; Kaiser *et al*., 2000; Petroski and Deshaies, 2005; Jana *et al*., 2004). CDC34 downregulation promotes genomic stability and decreases MDA-MB-231 proliferation to reduce the oncogenic

state. Similarly, thalidomide promotes apoptosis in other studies (Du *et al*., 2005; Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Marriott *et al*., 2003).

CDK2

Lycopene downregulates CDK2 in comparison to THF-treated control cells. It is not upregulated in comparison to untreated cells, and is therefore due to lycopene without the influence of THF. Thalidomide also downregulates CDK2 in comparison to untreated and DMSO-treated control cells. This downregulation is induced by thalidomide with and without DMSO influence. CDK2 downregulation inhibits cell cycle progression, DNA replication and cell division (Fischer *et al*., 2004; Yoo *et al*., 2004; Blagden and Glover, 2003). This downregulation promotes p27 and RB function (Fischer *et al*., 2004). Thalidomide upregulates RB in this study. Lycopene inhibits breast tumourigenesis by decreasing cell division, and inhibits proliferation in various cancer cells (Levy *et al*., 1995; Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006). MDA-MB-231 proliferation decreases to reduce tumourigenesis.

CDK4

CDK4 is downregulated by thalidomide in relation to untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. CDK4 is overexpressed in breast cancer (Ortega *et al*., 2002). Its downregulation reduces cell cycle progression and tumourigenesis, and promotes apoptosis (Pines, 1995; Liu, 2006; Strohmaier *et al*., 2001; Jana *et al*., 2004). Similarly, thalidomide promotes apoptosis in other cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Mitsiades *et*

al., 2002; Hideshima *et al*., 2000). CDK4 downregulation decreases proliferation, increases genomic stability and inhibits the oncogenic state.

CDK5R1

CDK5R1 is downregulated by THF and reduces differentiation, transcription and senescence to increase MDA-MB-231 tumourigenesis (Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007).

Lycopene upregulates CDK5R1, in comparison to THF-treated control cells. This is due to the combination of lycopene with THF. Thalidomide also upregulates CDK5R1 in relation to both untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. This increases differentiation, transcription, senescence and tumour suppression (Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007; Lee *et al*., 2007). Its inhibitor, CDK5RAP1, is downregulated by lycopene (Ching *et al*., 2002). Similarly, lycopene induces differentiation in prostate cancer cells (Kotake-Nara *et al*., 2002). CDK5R1 upregulation inhibits tumourigenesis and promotes genomic stability.

CDK5RAP1

DMSO and THF downregulate CDK5RAP1. It is also downregulated by lycopene in relation to untreated and THF-treated control cells. This is due to lycopene with and without the influence of THF. Thalidomide also downregulates CDK5RAP1 in comparison to untreated and DMSO-treated control cells. This is due to thalidomide both

with and without the influence of DMSO. This downregulation promotes CDK5, which is upregulated by thalidomide, to inhibit proliferation, and promote apoptosis, differentiation, transcription and senescence (Ching *et al*., 2002; Kim *et al*., 2006; Zhang *et al*., 2002; Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007; Lee *et al*., 2007). CDK5RAP1 downregulation inhibits transcription (Winkler *et al*., 2001; Wittschieben *et al*., 1999; Anantharaman *et al*., 2001). This decreases MDA-MB-231 proliferation and tumourigenesis, and increases genomic stability.

Similarly, DMSO induces differentiation in HL-60, murine myeloid cells and murine neuroblastoma cells (Watson *et al*., 1997; Marthyn *et al*., 1998). Lycopene promotes differentiation and apoptosis in various cancer cells (Kotake-Nara *et al*., 2002; Ivanov *et al*., 2007; Salman *et al*., 2007). Thalidomide promotes apoptosis and inhibits tumour growth in other cell types (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

CDK6

THF downregulates CDK6 to inhibit proliferation and promote genomic stability (Timms *et al*., 2002; Lenferink *et al*., 2001). Cyclin E synthesis and activation decreases, and in accordance, THF does downregulate cyclin E (Geng *et al*., 1996). This decreases cell cycle progression (Kwon *et al*., 1995). CDK6 downregulation reduces the oncogenic state of MDA-MB-231.

CDK7

CDK7 is downregulated by THF. It is also downregulated by lycopene in relation to untreated cells. As it is downregulated in THF-treated cells but not by lycopene in relation to THF-treated cells, this downregulation is due to THF. This decreases cell cycle progression, transcription and DNA repair (Harper and Elledge, 1998; Akoulitchev *et al*., 1995). CDK7 downregulation reduces MDA-MB-231 proliferation but also genomic integrity. Lycopene inhibits cell cycle progression, but decreases DNA damage in other studies (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Astley *et al*., 2004; Scolastici *et al*., 2007).

CDK8

Lycopene downregulates CDK8 to reduce transcription and cell cycle progression (Chi *et al*., 2001; Akoulitchev *et al*., 2000). It is downregulated in comparison to untreated cells and in THF-treated cells; therefore it is induced by THF. CDK8 downregulation inhibits MDA-MB-231 proliferation to reduce the oncogenic state.

CDKN1A

The tumour suppressor p21 is upregulated by THF. Lycopene also upregulates p21, in comparison to untreated cells. This is due to the combination of lycopene with THF. p21 is also upregulated by thalidomide, in relation to untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. This inhibits cell cycle progression, DNA replication and apoptosis, and promotes cell cycle arrest (Hengst and Reed, 1998; Ocker and Schneider-Stock, 2007; Maddika *et al*., 2007). It inhibits

cyclin E, which is downregulated by THF (Maddika *et al*., 2007). This upregulation reduces proliferation and increases the genomic stability of MDA-MB-231 to decrease tumourigenesis.

Lycopene also increases p21 levels to induce cell cycle arrest in A549 cells (Lian *et al*., 2007). Thalidomide inhibits cell cycle progression and induces p21-mediated G_1 arrest in previous studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CDKN1B

Thalidomide downregulates the tumour suppressor p27 in comparison to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. p27 downregulation increases cell cycle progression and reduces apoptosis and differentiation (Munoz-Alonso *et al*., 2005; Kayatose *et al*., 1997; Robker and Richards, 1998). In contrast, thalidomide inhibits cell cycle progression and induces apoptosis in various cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Hideshima *et al*., 2000). This downregulation promotes MDA-MB-231 proliferation and reduces genomic stability to increase the oncogenic state.

CDKN2A

The tumour suppressor p16 is upregulated by thalidomide in relation to untreated and DMSO-treated control cells. This upregulation is induced by thalidomide with and without the influence of DMSO. This inhibits cell cycle progression, angiogenesis and metastasis, and promotes RB-mediated G_1 arrest (Serrano *et al*., 1993; Gibson *et al*., 2005;

Wang *et al*., 2006; Brenner *et al*., 1998). This concurs with other studies where thalidomide inhibits cell cycle progression, angiogenesis, metastasis, and induces G_1 arrest (Hattori and Iguchi, 2004; Geitz *et al*., 1996; Hideshima *et al*., 2000; D'Amato *et al*., 1994; Kenyon *et al*., 1997; Adeoti *et al*., 1998; Kucharczak *et al*., 2003; Keifer *et al*., 2001; Kedar *et al*., 2004; Fujita *et al*., 2001). Thalidomide reduces MDA-MB-231 proliferation and tumourigenesis.

CDKN2B

Lycopene upregulates the tumour suppressor p15, in relation to untreated and THF-treated control cells. This is due to lycopene with and without THF influence. Thalidomide also upregulates p15, in comparison to untreated and DMSO-treated control cells, and this is due to thalidomide with and without the influence of DMSO. p15 inhibits cell cycle progression, induces cell cycle arrest and promotes senescence (Reynisdottir *et al*., 1995; Fuxe *et al*., 2000). MDA-MB-231 proliferation increases and oncogenic transformation decreases.

Similarly, lycopene inhibits proliferation and induces cell cycle arrest in several cancer cells, including MDA-MB-231 (Chalabi *et al*., 2004; Nahum *et al*., 2001; Levy *et al*., 1995; Park *et al*., 2005). Thalidomide induces G_1 arrest in other cell types, including multiple myeloma cells (Hattori and Iguchi, 2004; Hideshima *et al*., 2000).

CDKN3

Lycopene downregulates CDKN3 in comparison to THF-treated control cells. This is induced by lycopene without the influence of THF. CDKN3 downregulation promotes cell cycle progression and transcription, including that of the tumour suppressors p53 and p21 (Chinami *et al*., 2005; Schultz *et al*., 2002; Okamoto *et al*., 2006). p53 and p21 are upregulated by lycopene. Lycopene promotes proliferation and genomic stability in MDA-MB-231.

CHEK2

Lycopene upregulates CHK2, in comparison to THF-treated control cells, to promote checkpoint function, cell cycle arrest, DNA repair and apoptosis (Gatei *et al*., 2003; Pommier *et al*., 2005). This is induced by lycopene without the influence of the THF solvent. Lycopene induces cell cycle arrest, apoptosis and DNA damage repair in various cancer cells (Chalabi *et al*., 2004; Nahum *et al*., 2001; Ivanov *et al*., 2007; Salman *et al*., 2007; Scolastici *et al*., 2007; Astley *et al*., 2004). CHK2 upregulation increases the genomic integrity and stability of MDA-MB-231.

CUL1

THF downregulates CUL1 to decrease ubiquitin ligase activity and cell cycle progression, and induce apoptosis (Deshaies, 1999; Jana *et al*., 2004). This downregulation promotes the activity of the tumour suppressor p27 (Deshaies, 1999). MDA-MB-231 proliferation decreases and genomic stability increases.

CUL3

THF upregulates CUL3. Lycopene also upregulates CUL3 in relation to both untreated and THF-treated control cells. This is induced by lycopene with and without THF influence. CUL3 is also upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, and this is due to thalidomide with and without DMSO influence. This promotes genomic integrity during replication, promotes anaphase and enhances tumour suppression (Sumara *et al*., 2007; Stewart and Fang, 2005; Wilkins *et al*., 2004). MDA-MB-231 proliferation and genomic stability are increased.

DDX11

DMSO and THF upregulate DDX11 and ChlR1 to increase integrity during mitosis (Parish *et al*., 2006). DNA replication, DNA repair and transcription are also promoted (Tuteja and Tuteja, 2004). This promotes MDA-MB-231 proliferation and increases genomic integrity. DMSO also inhibits proliferation in various cells (Fiore and Degrassi, 1999; Ponzio *et al*., 1998; Kranenburg *et al*., 1995).

Lycopene upregulates DDX11, in comparison to untreated cells. However, it is downregulated by lycopene in comparison to THF-treated control cells. Therefore THF promotes its upregulation but lycopene, without the influence of THF, downregulates it to inhibit proliferation (Amann *et al*., 1997). Similarly, lycopene inhibits proliferation in breast cells, prostate cancer cells, oral tumour cells and hepatoma cells, amongst others (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Limpens *et al*., 2006; Livny *et al*., 2002; Park *et al*., 2005).

Thalidomide also upregulates DDX11 in relation to untreated cells. As it is upregulated in DMSO-treated cells but not in relation to DMSO-treated cells, it is induced by DMSO. DMSO is not an appropriate solvent for thalidomide.

DNM2

THF upregulates DNM2. DNM2 is also upregulated by lycopene, in comparison to untreated cells, and is induced by THF. This promotes tumour suppressor p53 function and induces apoptosis (De Camilli *et al*., 1995; Fish *et al*., 2000). p53 is upregulated by THF. DNM2 increases MDA-MB-231 genomic integrity and stability to reduce its oncogenic state.

E2F4

E2F4 is downregulated by THF. Lycopene also downregulates E2F4, in relation to untreated cells, and this is due to THF influence. This decreases cell cycle progression and differentiation (Humbert *et al*., 2000; Fajas *et al*., 2002). MDA-MB-231 proliferation is reduced but oncogenic transformation is promoted. However, its overexpression promotes tumourigenesis; therefore, this downregulation increases MDA-MB-231 genomic stability (Rakha *et al*., 2004). THF is not an appropriate solvent for lycopene.

GADD45A

GADD45A is downregulated by DMSO to decrease cell cycle arrest and DNA repair, and promote cell cycle progression, differentiation and cell survival (Jin *et al*., 2002; Carrier

et al., 1999; Takekawa and Saito, 1998). DMSO promotes MDA-MB-231 proliferation but oncogenic transformation is decreased through increased differentiation. Genomic integrity is decreased through decreased DNA repair.

GADD45A is upregulated by thalidomide in comparison to DMSO-treated control cells, and this is due to thalidomide without DMSO influence. This inhibits cyclin B1/CDC2 to induce G_2/M arrest, and promotes DNA repair (Jin *et al*., 2002; Carrier *et al*., 1999). GADD45A overexpression inhibits growth in tumour cell lines (Zhan *et al*., 1994). This inhibits MDA-MB-231 proliferation and increases genomic integrity and stability. The oncogenic state is consequently inhibited.

GTF2H1

DMSO and THF downregulate GTF2H1. Thalidomide also downregulates GTF2H1 in comparison to untreated cells. This is due to DMSO influence as it is also downregulated in DMSO-treated cells. This downregulation decreases transcription, DNA repair and cell cycle progression (Araujo *et al*., 2000; Matsuno *et al*., 2007). Apoptosis is promoted (Takagi *et al*., 2005; Jana *et al*., 2004). MDA-MB-231 proliferation decreases. While DNA repair decreases, apoptosis is induced so genomic integrity may not be reduced. DMSO inhibits cell cycle progression in several studies (Fiore and Degrassi, 1999; Ponzio *et al*., 1998; Kranenburg *et al*., 1995). Thalidomide also induces apoptosis in other studies (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

HERC5

DMSO and THF upregulate HERC5. It is also upregulated by thalidomide in relation to untreated cells. It is not upregulated by thalidomide in comparison to DMSO-treated cells, but is upregulated in DMSO-treated cells, and is therefore due to DMSO influence. This promotes the immune response and increases MDA-MB-231 survival (Dastur *et al*., 2006).

Lycopene downregulates HERC5, in comparison to THF-treated control cells, to decrease proteolysis as part of the immune response (Dastur *et al*., 2006). This is due to lycopene without the influence of THF.

HUS1

Thalidomide upregulates HUS1 in comparison to untreated and DMSO-treated control cells. It is induced by thalidomide with and without DMSO influence. HUS1 increases checkpoint function and DNA repair following DNA damage (Francia *et al*., 2006; Walworth and Bernards, 1996; Wang *et al*., 2006). Similarly, thalidomide promotes checkpoint function by inducing cell cycle arrest (Hattori and Iguchi, 2004; Hideshima *et al*., 2000). MDA-MB-231 genomic integrity increases to inhibit its oncogenic state.

KPNA2

KPNA2 is downregulated by lycopene in relation to THF-treated control cells. This is induced by lycopene without THF influence. As it is overexpressed in breast cancer, this downregulation decreases the elevated levels in untreated cells to promote the DNA

damage responses (Dahl *et al*., 2006; Tseng *et al*., 2005). Similarly, lycopene decreases DNA damage in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Lycopene increases MDA-MB-231 genomic integrity and inhibits the oncogenic state.

MAD2L1

THF downregulates MAD2L1. Lycopene also downregulates MAD2L1 in comparison to untreated cells. This is due to the combination of lycopene with THF. MAD2L1 is also downregulated by thalidomide in relation to untreated cells. This is induced by the combination of thalidomide with DMSO. MAD2L1 is overexpressed in breast cancer cell lines; this downregulation promotes anaphase control and cell cycle arrest (Yuan *et al*., 2006; Amon, 1999; Fang *et al*., 1998). As it is only present when chromosomes are incorrectly aligned, its upregulation increases genomic integrity in comparison to untreated cells. This increases MDA-MB-231 genomic stability. Lycopene also induces cell cycle arrest in various cancer cells (Chalabi *et al*., 2004; Nahum *et al*., 2006).

MCM2

MCM2 is upregulated by DMSO and by THF. Thalidomide also upregulates MCM2 in comparison to untreated cells and this is due to the combination of thalidomide with DMSO. to promote proliferation and genomic integrity during DNA replication (Todorov *et al*., 1998; Ying and Gautier, 2005; Labib *et al*., 2000). This increases the proliferation of stabilised MDA-MB-231 DNA.

MCM3

DMSO and THF upregulate MCM3. It is also upregulated by lycopene, in comparison to untreated cells, and this is due to THF. Thalidomide also upregulates MCM3 in relation to untreated cells. This is due to the combination of thalidomide with DMSO. MCM3 upregulation to promotes genomic integrity during replication (Labib *et al*., 2000; Cortez *et al*., 2004; Dziak *et al*., 2003; Ying and Gautier, 2005). This promotes the proliferation of MDA-MB-231 and increases genomic integrity.

MCM4

MCM4 is downregulated by thalidomide in comparison to untreated cells to decrease transcription, checkpoint activation and the inhibition of re-replication (Dziak *et al*., 2003; Cortez *et al*., 2004; Labib *et al*., 2000). Although proliferation is reduced, genomic integrity decreases to contribute to the oncogenic state. This is induced by the combination of thalidomide with DMSO.

MCM5

DMSO downregulates MCM5. This correlates with a decrease in proliferation and increased DNA re-replication (Stoeber *et al*., 2002; Labib *et al*., 2000). MDA-MB-231 proliferation and genomic integrity decreases to promote genomic instability.

Lycopene upregulates MCM5, in comparison to untreated and THF-treated control cells, to promote proliferation, checkpoint activation and transcription, and inhibit

re-replication (Labib *et al*., 2000; Cortez *et al*., 2004; Snyder *et al*., 2005). Lycopene promotes the proliferation of high integrity DNA to increase genomic stability.

MKI67

Ki-67 is upregulated by DMSO. It is also upregulated by thalidomide and this is due to DMSO influence. This correlates with increased proliferation and apoptosis (Endl and Gerdes, 2000; Lipponen, 1999). Ki-67 upregulation increases MDA-MB-231 proliferation and promotes genomic integrity. However, DMSO inhibits proliferation and apoptosis in various cell types (Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998; Fiore and Degrassi, 1999). Thalidomide inhibits tumourigenesis in myeloma and other cell types (Kumar and Rajkumar, 2006; Moreira *et al*., 1993; Du *et al*., 2005; Goswami *et al*., 1998). DMSO is therefore not a suitable solvent for thalidomide.

MRE11A

MRE11A is upregulated by DMSO. Thalidomide also upregulates MRE11A in relation to untreated cells. This is due to DMSO influence as it is also upregulated in DMSO-treated cells, but not in relation to DMSO-treated control cells. This promotes the DNA damage responses, including apoptosis, and promotes proliferation (Lee and Paull, 2005; Paull and Gellert, 1998). It is downregulated in breast cancer and its upregulation increases its levels to optimise its functions (Ding *et al*., 2004). MRE11A upregulation promotes the proliferation of stabilised DNA in MDA-MB-231. Thalidomide promotes checkpoint function and induces apoptosis in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Du *et al*., 2005; Marriott *et al*., 2003).

NBS1

Thalidomide upregulates NBS1 in comparison to untreated cells to promote MRN function in response to DNA damage, including checkpoint activation (Stracker *et al*., 2004; Lee and Paull, 2005). This upregulation is due to the combination of thalidomide and DMSO. NBS1 is downregulated in breast cancer (Ding *et al*., 2004). This increases MDA-MB-231 genomic integrity and stability.

PCNA

THF upregulates PCNA. It is also upregulated by thalidomide in relation to untreated cells and this is induced by thalidomide in combination with DMSO. PCNA upregulation promotes DNA replication and DNA damage repair (Maga and Hubscher, 2003). p21, which is upregulated by THF, inhibits DNA replication induced by PCNA; therefore, PCNA does not promote proliferation (Maki and Howley, 1997). It does increase genomic integrity in MDA-MB-231.

PCNA is downregulated by lycopene in relation to THF-treated control cells. This downregulation is induced by lycopene without THF influence. PCNA downregulation reduces DNA replication and DNA repair (Maga and Hubscher, 2003). Although lycopene inhibits proliferation in various cells, it also decreases DNA damage (Chalabi *et al*., 2004; Park *et al*., 2005; Astley *et al*., 2004; Scolastici *et al*., 2007). Lycopene decreases proliferation and genomic integrity in MDA-MB-231.

RAD1

Lycopene upregulates RAD1, in relation to untreated and THF-treated control cells, to promote checkpoint activation and DNA repair (Bermudez *et al*., 2003; Brandt *et al*., 2006). This is due to lycopene with and without THF influence. Lycopene decreases DNA damage in lymphocytes, H568 and CHO cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007). Lycopene increases MDA-MB-231 genomic integrity to inhibit the oncogenic state.

RAD17

RAD17 is upregulated by thalidomide in comparison to both untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. This promotes checkpoint function and 9-1-1 complex activity, including DNA repair, and inhibits re-replication (Wang *et al*., 2003; Bermudez *et al*., 2003). It is activated by ATM and ATR, which are upregulated by thalidomide (Zou *et al*., 2002). Thalidomide induces cell cycle arrest in previous studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000). This increases genomic integrity and stability in MDA-MB-231 to inhibit its oncogenic state.

RAD9A

THF upregulates RAD9A. It is also upregulated by lycopene, in comparison to untreated cells, and this upregulation is due to THF. RAD9A is upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, and this is induced by thalidomide with and without DMSO influence. RAD9A upregulation promotes

checkpoint function, DNA repair and apoptosis following DNA damage (Toueille *et al*., 2004). It is activated by ATM that is upregulated by thalidomide (Roos-Mattjus *et al*., 2003). RAD 9-1-1 activates p21, which is upregulated by THF and thalidomide, to induce cell cycle arrest (Yin *et al*., 2004). RAD9A increases MDA-MB-231 genomic integrity and reduces its oncogenic potential. Lycopene also induces cell cycle arrest, DNA repair and apoptosis in various cancer cells (Chalabi *et al*., 2004; Park *et al*., 2005; Astley *et al*., 2004; Scolastici *et al*., 2007; Ivanov *et al*., 2007; Salman *et al*., 2007). Thalidomide induces cell cycle arrest, including p21-mediated arrest, and promotes apoptosis in previous studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000; Du *et al*., 2005; Marriott *et al*., 2003).

RAD51

DMSO and THF downregulate RAD51. Lycopene also downregulates RAD51, in relation to untreated cells. This is induced by THF. Thalidomide downregulates RAD51 in relation to untreated cells and this is due to DMSO influence. As it is overexpressed in tumour cell lines, this decrease may return it to a functional level to promote DNA repair through homologous recombination (Vispe *et al*., 1998; Richardson *et al*., 2004; Gupta *et al*., 1997). This promotes genomic stability and integrity in MDA-MB-231.

RB1

Thalidomide upregulates the tumour suppressor RB in relation to untreated and DMSO-treated control cells, to promote differentiation, senescence, cell cycle arrest and genomic and chromosomal integrity (Maddika *et al*., 2007; Hinds *et al*., 1992; Tell *et al*., 2006).

This upregulation is induced by thalidomide with and without DMSO influence. Thalidomide induces cell cycle arrest in other studies (Hattori and Iguchi, 2004; Hideshima *et al*., 2000). Thalidomide promotes genomic stability and inhibits tumourigenesis in MDA-MB-231.

RBL2

Lycopene upregulates the tumour suppressor p130, in comparison to THF-treated control cells. This is induced by lycopene without the influence of THF. p130 promotes quiescence, inhibits cell cycle progression and promotes cell cycle arrest (Grana *et al*., 1998; Hansen *et al*., 2001). In accordance with previous studies on lycopene, lycopene reduces proliferation and increases genomic stability (Chalabi *et al*., 2004; Nahum *et al*., 2006).

RPA3

RPA3 is upregulated by THF. It is also upregulated by lycopene, in relation to untreated cells, and this is due to THF influence. THF is therefore not an appropriate solvent for lycopene. Thalidomide upregulates RPA3, in comparison to untreated and DMSO-treated control cells. It is induced by thalidomide with and without DMSO influence. RPA3 upregulation promotes integrity during DNA replication and promotes DNA repair (Bochkarev *et al*., 1997; He *et al*., 1995). This increases proliferation and genomic stability in MDA-MB-231. Similarly, lycopene decreases DNA damage and induces apoptosis in various cancer cells (Astley *et al*., 2004; Yeh and Hu, 2000; Muller

et al., 2002; Ivanov *et al*., 2007). Thalidomide promotes apoptosis in other studies (Hattori and Iguchi, 2004; Du *et al*., 2005; Marriott *et al*., 2003).

SERTAD1

SERTAD1 is downregulated by DMSO to reduce cell cycle progression (Hsu *et al*., 2001). Similarly, DMSO inhibits cell cycle progression in a range of cancer cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998). SERTAD1 is also downregulated by thalidomide in relation to untreated cells and this is due to DMSO influence. This downregulation decreases the tumourigenic state of MDA-MB-231. Similarly, thalidomide inhibits tumour growth in MCF-7, HL-60 and myeloma (Du *et al*., 2005; Kumar and Rajkumar, 2006; Moreira *et al*., 1993).

SKP2

DMSO and THF downregulate SKP2. It is also downregulated by lycopene, in relation to untreated cells, due to THF influence. SKP2 is downregulated by thalidomide in relation to untreated and DMSO-treated control cells. This is due to thalidomide with and without DMSO influence. This downregulation inhibits cell cycle progression and induces apoptosis (Gstaiger *et al*., 2001; Jana *et al*., 2004). SKP2 is overexpressed in MDA-MB-231 cells and its downregulation reduces proliferation and promotes genomic integrity (Traub *et al*., 2006). Adhesion-independent cell growth is inhibited to decrease tumourigenesis (Signoretti *et al*., 2002).

DMSO also inhibits cell cycle progression in HL-60, Burkitt's lymphoma, and hybridoma 7TD1 cells, amongst others (Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998). Lycopene inhibits proliferation and induces apoptosis in various cancer cells (Chalabi *et al*., 2004; Hwang and Bowen, 2004; Ivanov *et al*., 2007; Salman *et al*., 2007). Thalidomide inhibits tumourigenesis and induces apoptosis in other cells (Kumar and Rajkumar, 2006; Goswami *et al*., 1998; Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

SUMO1

Lycopene downregulates SUMO1, in relation to untreated cells. This is due to the combination of lycopene with THF. SUMO1 downregulation inhibits cell cycle progression and transcription (Park *et al*., 2008; Yang and Sharrocks, 2004). This concurs with other studies where lycopene inhibits proliferation in MCF-7, MDA-MB-231, endometrial cancer and lung cancer cells, amongst others (Chalabi *et al*., 2004; Levy *et al*., 1995). This downregulation promotes GADD45A, p21 and p27 transcription (Park *et al*., 2008). GADD45A and p21 are upregulated by lycopene, similar to observations in A549 cells (Lian *et al*., 2007). Lycopene decreases MDA-MB-231 proliferation and promotes genomic stability to reduce the oncogenic state.

TFDP2

DP2 is downregulated by DMSO and by THF. Lycopene also downregulates TFDP2 in relation to untreated cells and this is due to THF influence. DP2 is downregulated by thalidomide in comparison to untreated cells. This is due to the combination of

thalidomide with DMSO. This reduces cell cycle progression, DNA replication and apoptosis (Dyson, 1998; Stanelle *et al*., 2002). It decreases MDA-MB-231 proliferation and lowers the genomic integrity.

TP53

The tumour suppressor p53 is upregulated by THF. It is also upregulated by lycopene, in comparison to untreated cells, due to THF influence. This increases cell cycle arrest, DNA repair, apoptosis, differentiation and senescence (Finlan and Hupp, 2005; Maddika *et al*., 2007; Miyashita and Reed, 1995). It promotes p21 activity, which is upregulated by THF (Sherr and Roberts, 1999). It inhibits anti-apoptotic BCL2, which is downregulated by THF (Mihara *et al*., 2003). TP53 upregulation inhibits MDA-MB-231 proliferation and promotes genomic stability to decrease the oncogenic state.

Lycopene induces cell cycle arrest and apoptosis in several types of cancer cells, including MDA-MB-231 (Chalabi *et al*., 2004; Ivanov *et al*., 2007; Salman *et al*., 2007). It promotes DNA repair in lymphocytes, H568 and CHO cells, and induces differentiation in prostate cancer cells (Astley *et al*., 2004; Yeh and Hu, 2000; Scolastici *et al*., 2007; Kotake-Nara *et al*., 2002). Lycopene promotes p53 activity in mouse hepatocytes (Matsushima-Nishiwaki *et al*., 1995).

UBE1

THF upregulates UBE1. Lycopene also upregulates UBE1 in comparison to untreated cells, and this is induced by THF influence. This upregulation increases cell cycle

progression, DNA repair and the response to stress (Zacksenhaus and Sheinin, 1990; Handley-Gearhart *et al*., 1994; Kim and Zhang, 2003). UBE1 is downregulated in tumour cell lines (Pitha-Rowe *et al*., 2004; Jentsch *et al*., 1990). This upregulation therefore increases the genomic integrity of MDA-MB-231.

3.4.4 184A1

The data for the 184A1 treatments, in comparison to untreated cells, are normalised against the RPL13A, GAPDH and ACTB housekeeping genes. For comparison of lycopene-treated cells to THF-treated cells, and thalidomide-treated cells to DMSO-treated cells, the data is normalised against the B2M, HPRT1, RPL13A, GAPDH and ACTB housekeeping genes. For comparison of MCF-7 and MDA-MB-231 controls and treatments to 184A1 controls and treatments, the data is normalised against B2M, RPL13A, GAPDH and ACTB housekeeping genes.

3.4.4.1 Distribution of C_t Values

The results for the gene expression in 184A1 untreated cells are used as an example.

Table 3.16: Distribution of C_t values of 184A1 untreated cells

Percent distribution of C_t values	
C_t Range	
<25	12.50%
25-30	47.92%
30-35	31.25%
Absent calls	8.33%

3.4.4.2 Reverse transcription control

184A1 untreated

ΔCt = average reverse transcription control (RTC) – average positive PCR control (PPC)

ΔCt = 25.94 – 22.15

= 3.79

Reverse transcription (RT) efficiency: pass.

184A1 DMSO

ΔCt = 25.59 – 21.95

= 3.64

RT efficiency: pass

184A1 THF

ΔCt = 26.35 – 22.07

= 4.28

RT efficiency: pass

184A1 thalidomide

ΔCt = 23.47 – 21.98

= 1.49

RT efficiency: pass

184A1 lycopene

$\Delta Ct = 25.21 - 22.11$

$= 3.1$

RT efficiency: pass

3.4.4.3 Genomic DNA contamination

184A1 untreated

C_t (GDC) = 35

Result: pass

184A1 DMSO

C_t (GDC) = 35

Result: pass

184A1 THF

C_t (GDC) = 35

Result: pass

184A1 thalidomide

C_t (GDC) = 35

Result: pass

184A1 lycopene

C_t (GDC) = 35

Result: pass

Table 3.17: Fold regulation of genes, in comparison to untreated 184A1 cells, due to various treatments with significant fold changes highlighted.

	DMSO	THF	Lycopene	Thalidomide
ANAPC2	-2.5204	1.5312	-8.9012	-4.2585
ANAPC4	-2.4686	-1.1114	-5.191	-1.9485
ARHI	-6.9967	1.361	-10.9056	-3.4208
ATM	2.7498	4.5054	2.9322	3.188
BAX	-1.5451	1.2173	-1.3041	-2.2852
BCL2	-2.152	1.7773	-4.4691	-2.7176
BIRC5	-1.9101	2.0401	-4.5884	-1.1747
BRCA2	661.8375	14.1494	11.7371	16.6987
CCNB1	-1.4698	-1.6159	-5.5136	-2.2135
CCNB2	-1.1413	1.321	-4.2251	-1.2417
CCNC	219.8435	84.0203	123.8972	189.7119
CCND1	27.9221	59.2879	75.2177	23.0336
CCND2	2.9966	3.2035	3.3753	3.0265
CCNE1	-2.1972	-1.1562	-2.0293	-1.6193
CCNG2	-2.3193	-3.3319	-1.7826	-1.939
CCNH	-1.9802	2.538	-1.428	1.0597

CCNT1	-1.9009	-1.3062	-2.521	-1.6148
CCNT2	31.2619	2.9053	1.9521	24.8241
CDC16	2.0524	4.1516	2.7019	2.9073
CDC2	3.6259	2.7428	1.0303	2.3032
CDC20	2.9656	3.2959	1.0035	1.2938
CDK4	4.0483	1.4426	2.299	1.831
CDK5R1	-2.3064	2.1165	1.3398	-1.6845
CDK5RAP1	-1.7467	-1.0121	-3.9041	-2.0883
CDK7	5.2939	2.5574	4.9485	2.9622
CDK8	25.4278	10.9485	23.1831	21.0196
CDKN1A	11.9202	17.5289	13.1046	7.839
CDKN1B	7.8263	6.2709	12.1341	12.8053
CDKN2A	-5.0164	1.4158	2.3038	-3.4758
CDKN2B	-10.3007	-1.2944	1.2906	1.1217
CDKN3	1.2998	1.2515	-4.2901	-1.5706
CHEK2	1.7343	1.477	1.8289	2.8082
CKS1B	3.2385	3.5768	1.857	2.6311
CKS2	2.7901	1.1248	1.5052	2.6075
CUL2	-11.1014	1.4456	-6.5979	-6.014
CUL3	-2.6568	1.4456	-1.752	-1.5652
DNM2	-1.8695	1.2743	-1.1753	-2.013
E2F4	-1.4217	-1.0976	-2.6592	-1.7391
GTF2H1	-1.9114	-1.1261	-3.934	-2.4172

GTSE1	-1.4158	2.1004	-4.541	-2.144
HERC5	2.5799	7.5092	6.3731	4.2359
KNTC1	-3.2595	1.1588	-8.0389	-4.4702
MAD2L1	1.8042	1.219	2.1962	1.1508
MCM2	68.1827	14.3967	11.1967	44.4671
MCM4	-1.957	1.3019	1.5856	2.2202
MCM5	3.5024	14.3867	10.578	2.4278
MKI67	1.3607	1.1009	1.676	4.3944
MRE11A	4.6407	5.1396	5.1587	10.2366
PCNA	-2.259	-2.4022	-1.9752	-1.5706
RAD1	1.6998	2.5152	1.6392	2.4161
RAD17	3.0681	4.6193	3.0272	3.0837
RAD51	-11.1014	1.4456	2.1886	-4.3873
RAD9A	N/A	1.4456	-1.0852	-6.014
RB1	-2.8063	1.4456	-1.0483	-1.259
RBL1	-1.1824	5.4629	4.0139	3.8602
RBL2	-1.0336	1.3582	2.4453	4.6676
RPA3	-4.674	1.3819	1.0585	-2.8686
SERTAD1	-1.7049	-3.2814	-1.4489	1.3047
SKP2	-2.4044	-4.1911	-3.8397	-2.4138
TFDP1	-2.5877	-1.6962	-2.805	-1.9866
TFDP2	-2.0917	-1.8965	-2.4083	-1.0072
UBE1	1.6625	-3.2543	1.1519	1.3028

Table 3.18: Fold regulation of genes from lycopene-treated 184A1 cells in comparison to THF-treated 184A1 cells, and thalidomide-treated 184A1 cells in comparison to DMSO-treated 184A1 cells, with significant fold changes highlighted.

	Lycopene	Thalidomide
ANAPC2	-16.9663	-2.0743
ANAPC4	-5.8143	1.032
ARHI	-18.4762	1.666
BCL2	-9.8875	-1.5504
BIRC5	-11.6528	1.3245
BRCA2	-1.5007	-48.6578
CCNB1	-4.2475	-1.8488
CCNB2	-6.948	-1.3357
CCNE1	-2.1849	1.1053
CCNF	-3.1158	-1.7048
CCNH	-4.5115	1.7093
CCNT1	-2.4026	-1.0429
CDC2	-3.3141	-1.9327
CDC20	-4.0886	-2.814
CDK4	1.2802	-2.7143
CDK5RAP1	-4.8019	-1.4677
CDK7	1.5544	-2.1941
CDKN2B	1.342	9.4113
CDKN3	-6.6835	-2.5064

CKS1B	-2.3976	-1.5111
CUL2	-11.8729	1.5036
CUL3	-3.1527	1.3826
DDX11	-9.2831	N/A
E2F4	-3.0159	-1.5017
GTF2H1	-4.3487	-1.5525
GTSE1	-11.8729	-1.8591
KNTC1	-11.5964	-1.6837
MCM4	-1.0221	3.5391
MKI67	1.223	2.6306
MNAT1	-2.2825	-1.0804
RAD51	1.2162	2.0611
RBBP8	-3.1637	-1.6241
RBL1	-1.6942	3.7177
RBL2	1.4463	3.9296
TFDP1	-2.0585	1.061
UBE1	3.0113	-1.5666

3.4.4.4 184A1/Treatments

ANAPC2

APC2 is downregulated by DMSO. It is also downregulated by lycopene, as a result of lycopene with and without THF influence. Thalidomide also downregulates APC2 in comparison to untreated and DMSO-treated control 184A1 cells. This is induced by

thalidomide both with and without DMSO influence. This reduces cell cycle progression and induces apoptosis (Pickart, 2001; Jana *et al*., 2004). APC2 decreases proliferation and increases genomic stability to inhibit oncogenic transformation in 184A1. Similarly, lycopene inhibits proliferation in the normal prostate epithelial cell line, PrEC (Obermuller-Jevic *et al*., 2003). Thalidomide induces apoptosis in cancer cells (Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

ANAPC4

APC4 is downregulated by DMSO. It is also downregulated by lycopene, in relation to untreated and THF-treated control cells, and this is induced by lycopene both with and without THF-influence. This downregulation decreases cell cycle progression, induce apoptosis and reduce checkpoint function (Peters, 2002; Jana *et al*., 2004; Wasch and Cross, 2002). This reduces proliferation and the genomic integrity of 184A1, and therefore promotes transformation. DMSO, however, induces cell cycle arrest in CHO cells and various cancer cells (Fiore and Degrassi, 1999; Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998).

ARHI

DMSO downregulates the tumour suppressor ARHI. Lycopene also downregulates ARHI, in comparison to untreated and THF-treated control cells, and this is due to lycopene with and without THF influence. It is also downregulated by thalidomide in relation to untreated cells. As it is also downregulated in DMSO-treated cells, but not by thalidomide in relation to DMSO-treated control cells, this downregulation is due to

DMSO. ARHI downregulation promotes cell cycle progression and reduce apoptosis. This decreases tumour suppressor p21 function and promotes cyclin D1 ((Bao *et al*., 2002). Cyclin D1 is upregulated here by DMSO. This downregulation increases 184A1 proliferation and decreases its genomic integrity, thereby promoting oncogenic transformation. Similarly, DMSO inhibits apoptosis in CHO cells (Fiore and Degrassi, 1999). DMSO is therefore not a suitable solvent for thalidomide.

ATM

ATM is upregulated by DMSO, and by THF. It is also upregulated by lycopene in relation to untreated cells, and this is due to THF influence. Thalidomide also upregulates ATM in relation to untreated cells. This promotes RAD51 and BRCA1 function at DNA damage sites, promotes the re-initiation of collapsed replication forks and inhibits DNA damage accumulation (Burma *et al*., 2001; Trenz *et al*., 2006). It activates RAD9A and promotes ATR, p53 and p21 activity to induce checkpoint function, and inhibit cell cycle progression, apoptosis and re-replication (Greer *et al*., 2003; Falck *et al*., 2002; Costanzo *et al*., 2000; Banin *et al*., 1998; Ocker and Schneider-Stock, 2007; Liu *et al*., 2007). DMSO, THF, lycopene and thalidomide upregulate p21 in 184A1 in this study. Thalidomide also promotes p21 function in multiple myeloma cells (Hideshima *et al*., 2000). ATM upregulation in thalidomide-treated cells is induced by DMSO.

Similarly, DMSO induces cell cycle arrest in other cell types (Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998; Teraoka *et al*., 1996). ATM

upregulation decreases 184A1 proliferation and promotes genomic integrity to inhibit oncogenic transformation.

BAX

Thalidomide downregulates pro-apoptotic BAX, in comparison to untreated cells, to promote cell cycle progression, and inhibit apoptosis and DNA repair (Reed, 1997; Zinkel *et al*., 2006; Dumay *et al*., 2006). This downregulation is due to the combination of thalidomide with DMSO. BAX expression levels are still higher than BCL2, however, as seen in **figure 3.38**. Therefore, apoptosis and DNA repair are still promoted, and cell cycle progression is inhibited. Thalidomide promotes apoptosis in cancer cells in other studies (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

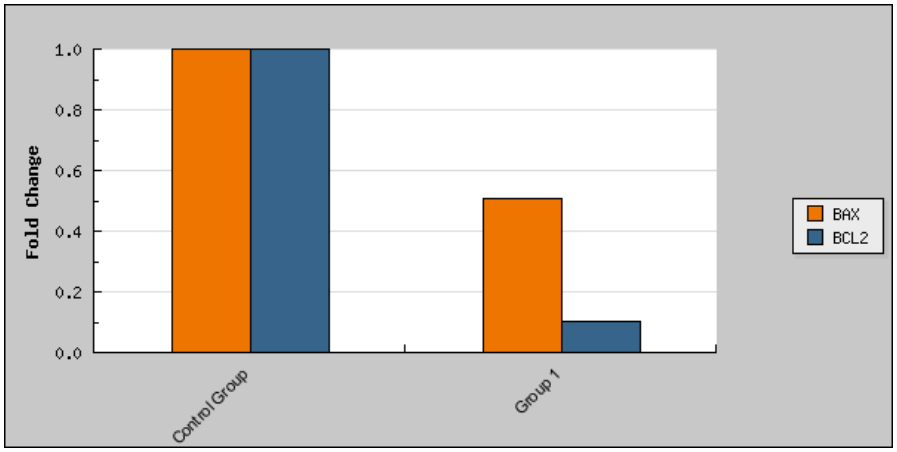


Figure 3.38: Multi-group plot comparing BAX and BCL2 fold changes in control group (THF-treated 184A1 cells) and group 1 (lycopene-treated 184A1 cells).

BCL2

Anti-apoptotic BCL2 is downregulated by DMSO. Lycopene also downregulates BCL2 in comparison to both untreated and THF-treated control cells. It is induced by lycopene with and without THF influence. BCL2 is also downregulated by thalidomide in relation to untreated and DMSO-treated control cells. This is induced by thalidomide with and without DMSO influence. BCL2 downregulation promotes apoptosis, cell cycle progression, DNA repair and replication (Dlugosz *et al*., 2006; Mazel *et al*., 1996; Youn *et al*., 2005; Liu *et al*., 1997). It is inhibited by the tumour suppressor p53. This promotes proliferation and genomic stability of 184A1. Likewise, thalidomide inhibits NF-κB to inhibit BCL2 transcription and induce apoptosis in cancer cells (Du *et al*., 2005; Marriott *et al*., 2003; Kucharczak *et al*., 2003; Keifer *et al*., 2001).

BIRC5

THF upregulates survivin to promote proliferation, checkpoint function and anchorage-independent growth, and decrease apoptosis (Dohi *et al*., 2004; Lens and Medema, 2003; Sarela *et al*., 2000). This decreases genomic stability and promotes oncogenic transformation.

Survivin is downregulated by lycopene, in relation to untreated and THF-treated control cells, to inhibit cell cycle progression, and promote cell cycle arrest and apoptosis (Dohi *et al*., 2004; Beltrami *et al*., 2004; Sarela *et al*., 2000). This is due to lycopene with and without THF influence. The downregulation decreases proliferation and promotes the

genomic stability of 184A1 to inhibit transformation. Lycopene also inhibits proliferation in normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003).

BRCA2

DMSO and THF upregulate BRCA2. It is also upregulated by lycopene, in comparison to untreated cells, and is induced by THF. BRCA2 is also upregulated by thalidomide in comparison to untreated cells and this is attributed to DMSO influence. This promotes DNA replication and repair (Scully *et al*., 1997; Galkin *et al*., 2005). It forms part of the replication complex with BRCA1, RAD51 and PCNA (Lomonosov *et al*., 2003; Scully *et al*., 1997). RAD51 and PCNA are downregulated by DMSO in 184A1. THF downregulates PCNA. Therefore, replication is not significantly promoted by BRCA2 upregulation. This upregulation promotes genomic integrity and stability of 184A1.

CCNB1

Lycopene downregulates cyclin B1 in relation to untreated and THF-treated control cells. It is induced by lycopene with and without THF influence. Thalidomide also downregulates cyclin B1 in comparison to untreated cells. It is not downregulated by thalidomide in comparison to DMSO-treated cells, or in DMSO-treated cells, and is therefore due to the combination of thalidomide with DMSO. This inhibits proliferation and apoptosis (Borgne *et al*., 2006; Pines and Hunter, 1989). High levels of cyclin B1 promote oncogenic transformation (Suzuki *et al*., 2007). This downregulation therefore increases genomic stability and decreases proliferation to inhibit transformation.

While thalidomide induces apoptosis in cancer cells, it can also decrease apoptosis and instead induce cell cycle arrest in multiple myeloma cells (Hideshima *et al*., 2000).

CCNB2

Lycopene downregulates cyclin B2, in comparison to untreated and THF-treated control cells, to decrease cell cycle progression and apoptosis (Morgan, 1995; Borgne *et al*., 2006). This downregulation is due to lycopene with and without THF influence. This decreases proliferation and also the genomic integrity of 184A1.

CCNC

Cyclin C is upregulated by DMSO and by THF. It is also upregulated by lycopene, in relation to untreated cells. This upregulation is induced by THF, as it is also upregulated in THF-treated cells, but not by lycopene in comparison to THF-treated cells. Thalidomide also upregulates cyclin C in comparison to untreated cells. Cyclin C upregulation promotes cell cycle progression and inhibits tumour suppressor transcription (Liu *et al*., 1998; Hengartner *et al*., 1998). It promotes CDC2, which is upregulated here by DMSO, THF, and thalidomide to increase G_2/M progression and further inhibit transcription (Liu *et al*., 1998; Zawel *et al*., 1993). This increases 184A1 proliferation. This upregulation in thalidomide-treated cells is due to DMSO as it is also upregulated in DMSO-treated cells. DMSO is not an appropriate solvent for thalidomide.

CCND1

DMSO and THF upregulate cyclin D1. Lycopene also upregulates cyclin D1 in relation to untreated cells. Cyclin D1 is also upregulated by thalidomide in relation to untreated cells. As it is also upregulated in DMSO-treated cells, but not by thalidomide in relation to DMSO-treated control cells, cyclin D1 upregulation in thalidomide-treated cells is induced by DMSO. This promotes cell cycle progression, survival and replication (Schwartz and Shah, 2005; Hinds *et al*., 1994; Fukami-Kobayashi and Mitsui, 1999). Cyclin D1 upregulation inhibits the tumour suppressors RB, p107, p130, p21 and p27 (Lundberg and Weinberg, 1998; Perez-Roger *et al*., 1999). DMSO downregulates RB in 184A1. It promotes integrity during replication by inhibiting PCNA, which is downregulated by both DMSO and THF, and CDK2 from inducing premature DNA synthesis (Fukami-Kobayashi and Mitsui, 1999). This increases 184A1 proliferation and genomic integrity. As this is due to DMSO in thalidomide-treated cells, DMSO is not an appropriate solvent for thalidomide.

However, lycopene downregulates cyclin D1 in normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003). The upregulation in lycopene-treated cells is induced by THF, as it is not upregulated by lycopene without THF influence, but is upregulated in THF-treated cells.

CCND2

Cyclin D2 is upregulated by DMSO and by THF. It is also upregulated by lycopene, in relation to untreated cells and this is induced by THF influence. Thalidomide also

upregulates cyclin D2 in comparison to untreated cells and in DMSO-treated cells. This upregulation is therefore due to DMSO. Cyclin D2 upregulation increases cell cycle progression and survival, and decreases cell cycle arrest (Schwartz and Shah, 2005; Hinds *et al*., 1994; Glaser, 2007). Proliferation and genomic instability are promoted. This promotes oncogenic transformation. This affirms that DMSO is not suitable as a solvent for thalidomide. In other studies, DMSO inhibits cyclin D2 in cancer cell lines to inhibit growth (Ponzio *et al*., 1998).

CCNE1

DMSO downregulates cyclin E1. It is also downregulated by lycopene in comparison to untreated and THF-treated control cells. It is induced by lycopene with and without THF influence. This inhibits cell cycle progression, apoptosis and replication (Koff *et al*., 1991; Cook *et al*., 2002). Its downregulation relieves inhibition of the tumour suppressor p27, which is upregulated here by DMSO and lycopene (Elledge and Harper, 1998). It regulates DNA synthesis with PCNA, which is downregulated by DMSO (Cook *et al*., 2002; Coverley *et al*., 2002). Cyclin E decreases 184A1 proliferation and increases genomic stability (Akli and Keyomarsi, 2004). This inhibits oncogenic transformation. However, in other studies lycopene does not affect cyclin E expression in normal prostate epithelial cells (Obermuller-Jevic *et al.*, 2003).

CCNF

Lycopene downregulates cyclin F, in relation to THF-treated control cells, to decrease cell cycle progression (Kong *et al*., 2000; Tezlaff *et al*., 2004; Lavia and Jansen-Durr,

1999). This is due to lycopene without the influence of THF. This decreases cyclin B1, CDC2 and cyclin E activity, which are downregulated by lycopene (Kong *et al*., 2000; Tezlaff *et al*., 2004). Similarly, lycopene inhibits proliferation of normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003).

CCNG2

DMSO and THF downregulate cyclin G2 and promotes cell cycle progression (Bennin *et al*., 2002). Cell cycle arrest and differentiation decrease (Arachchige Don *et al*., 2006). This increases 184A1 proliferation and promotes oncogenic transformation. DMSO also promotes differentiation in leukaemia, murine neuroblastoma N1E-115 and murine myeloid cells (Burger *et al*., 1994; Watson *et al*., 1997; Jiang *et al*., 1994; Marthyn *et al*., 1998).

CCNH

THF upregulates cyclin H. This increases transcription and DNA repair, and decreases cell cycle progression (Svejstrup *et al*., 1996; Schaeffer *et al*., 1993; Andersen *et al*., 1997). This increases the genomic integrity of 184A1. Cyclin H is downregulated by lycopene in comparison to THF-treated control cells and this is induced by lycopene without THF influence. This inhibits transcription, DNA repair and cell cycle progression to decrease proliferation and the genomic stability of 184A1 (Svejstrup *et al*., 1996; Schaeffer *et al*., 1993; Andersen *et al*., 1997).

CCNT1

Lycopene downregulates cyclin T1, in relation to both untreated and THF-treated control cells, to decrease transcription and differentiation (Palancade and Bensaude, 2003; De Falco and Giordano, 2002). This promotes oncogenic transformation of 184A1. This downregulation is induced by lycopene with and without THF-influence.

CCNT2

DMSO and THF upregulate cyclin T2 to promote transcription and differentiation (Palancade and Bensaude, 2003; Napolitano *et al*., 2000; Simone *et al*., 2002). Cyclin T2 is also upregulated by thalidomide, in relation to untreated cells. This is induced by thalidomide with DMSO influence. Cyclin T2 upregulation increases the genomic stability of 184A1 to inhibit transformation. Similarly, DMSO induces differentiation in leukaemia cells (Watson *et al*., 1997; Burger *et al*., 1994; Jiang *et al*., 1994).

CDC16

CDC16 is upregulated by DMSO and by THF. It is also upregulated by lycopene, in comparison to untreated cells. Thalidomide also upregulates CDC16, in comparison to untreated cells. As CDC16 is also upregulated in DMSO-treated cells, but not by thalidomide in comparison to DMSO-treated control cells, this pro-tumourigenic upregulation is accredited to DMSO. CDC16 upregulation activates CDC2, which is upregulated by both DMSO and THF, and promotes cell cycle progression and proliferation in 184A1 (Zachariae and Nasmyth, 1996; Rudner and Murray, 2000).

While this upregulation increases 184A1 proliferation, lycopene inhibits proliferation in the normal prostate epithelial cell line, PrEC (Obermuller-Jevic *et al*., 2003). This upregulation in lycopene-treated cells is induced by THF, as it is not upregulated by lycopene in comparison to THF-treated cells, but is upregulated in THF-treated cells. THF is not an appropriate solvent for lycopene.

CDC2

DMSO and THF upregulate CDC2 to promote cell cycle progression and transcription (Peters *et al*., 1998; Kobor and Greenblatt, 2002). Its transcription is inhibited by p21, which is upregulated by DMSO and THF, and p53. CDC2 upregulation increases CDC20 activation (Rudner and Murray, 2000). CDC20 is upregulated by DMSO and THF. This increases 184A1 proliferation. It is also upregulated by thalidomide in comparison to untreated cells and in DMSO-treated cells. This upregulation is induced by DMSO, confirming that it is not an appropriate solvent for thalidomide.

Lycopene downregulates CDC2 in comparison to THF-treated control cells. This is due to lycopene without the influence of THF. It decreases proliferation and promotes apoptosis in 184A1 (Peters *et al*., 1998; Kobor and Greenblatt, 2002; Rudner and Murray, 2000; Jana *et al*., 2004). This downregulation decreases cyclin B1 activity (Peters *et al*. 1998; Kobor and Greenblatt, 2002). Cyclin B1 is downregulated by lycopene in 184A1. The CDC2 inhibitor, p21, is upregulated by lycopene. Lycopene increases genomic stability. This concurs with previous studies where lycopene inhibits proliferation of normal cells (Obermuller-Jevic *et al*., 2003).

CDC20

CDC20 is upregulated by DMSO and THF to promote cell cycle progression and increase proliferation (Pfleger *et al*., 2001). It is induced by CDC2, which is upregulated by DMSO (Peters, 2002). Overexpressed CDC20 promotes genomic instability by prematurely activating anaphase (Hwang *et al*., 1998). This upregulation promotes proliferation and oncogenic transformation of 184A1.

The downregulation of CDC20 by lycopene, in relation to THF-treated control cells, decreases cell cycle progression and induces apoptosis to promote genomic integrity (Pfleger *et al*., 2001; Jana *et al*., 2004). This is induced by lycopene without THF influence. CDC20 activation decreases as CDC2 is downregulated by lycopene as well (Peters, 2002). Thalidomide also downregulates CDC20 in relation to DMSO-treated control cells. It is induced by thalidomide independently of DMSO.

CDK4

CDK4 is upregulated by DMSO. Lycopene also upregulates CDK4, in relation to untreated cells, and this is due to the combination of lycopene with THF. This increases cell cycle progression and decreases genomic integrity (Strohmaier *et al*., 2001; Ortega *et al*., 2002; Reynisdottir *et al*., 1995). Its activity is promoted by cyclin D1, which is upregulated by DMSO, and high levels promote tumourigenesis (Pines, 1995; Liu, 2006). CDK4 upregulation promotes the oncogenic transformation of 184A1. In previous studies, DMSO inhibits CDK4 expression in various cancer cells (Ponzio *et al*., 1998).

CDK4 is downregulated by thalidomide in comparison to DMSO-treated control cells and this is due to thalidomide without DMSO influence. This decreases G_1 progression and promotes RB and p53 tumour suppressor function (Pines, 1995; Reynisdottir *et al*., 1995; Ortega *et al*., 2002). This downregulation inhibits 184A1 proliferation and promotes genomic stability.

CDK5R1

DMSO downregulates CDK5R1 to increase proliferation and decrease apoptosis, differentiation and senescence (Kim *et al*., 2006; Zhang *et al*., 2002; Tsai *et al*., 1993; Moncini *et al*., 2007). The phosphorylation and activation of the tumour suppressors RB and p53 decreases (Lee *et al*., 1997; Zhang *et al*., 2002). This promotes the oncogenic transformation of 184A1.

CDK5R1 is upregulated by THF to inhibit proliferation, and increase apoptosis, differentiation and senescence (Kim *et al*., 2006; Zhang *et al*., 2002; Tsai *et al*., 1993; Moncini *et al*., 2007). Genomic stability is promoted and carcinogenesis is inhibited.

CDK5RAP1

Lycopene downregulates CDK5RAP1 in comparison to both untreated and DMSO-treated control cells. This is induced by lycopene with and without THF influence. Thalidomide also downregulates CDK5RAP1 in relation to untreated cells. This downregulation is induced by thalidomide in combination with DMSO. This decreases transcription, and promotes differentiation, senescence and apoptosis (Winkler

et al., 2001; Ching *et al*., 2002; Tsai *et al*., 1993; Moncini *et al*., 2007; Rosales and Lee, 2006). CDK5RAP1 downregulation increases the genomic stability of 184A1 and inhibits oncogenic transformation.

CDK7

DMSO and THF upregulate CDK7. It is also upregulated by lycopene, in comparison to untreated cells, and this is induced by THF. Thalidomide also upregulates CDK7, in comparison to untreated and DMSO-treated control cells, and this is induced by thalidomide with and without DMSO influence. This upregulation promotes cell cycle progression, transcription and DNA repair (Harper and Elledge, 1998). It is activated by CDC2 and CDK2 (Garrett *et al*., 2001). CDC2 is upregulated here by DMSO and THF. This increases the proliferation of high integrity DNA in 184A1.

CDK8

CDK8 is upregulated by DMSO and by THF. Lycopene also upregulates CDK8 in relation to untreated cells, and this is induced by THF. CDK8 is also upregulated by thalidomide in comparison to untreated cells. As it is also upregulated in DMSO-treated cells, but not by thalidomide in comparison to DMSO-treated control cells, it is induced by DMSO. This decreases cell cycle progression and transcription (Akoulitchev *et al*., 2000; Chi *et al*., 2001; Hengartner *et al*., 1998). It is activated by p21, which is upregulated here by DMSO, THF, and lycopene, and promotes p53-regulated transcription (Donner *et al*., 2007). It increases 184A1 genomic stability and decreases proliferation to inhibit oncogenic transformation.

CDKN1A

DMSO and THF upregulate the tumour suppressor p21. Lycopene also upregulates p21, in comparison to untreated cells. THF induces the upregulation in lycopene-treated cells as it is not upregulated by lycopene in comparison to THF-treated control cells. p21 is also upregulated by thalidomide in relation to untreated cells, due to DMSO influence. This inhibits cell cycle progression and apoptosis, and promotes cell cycle arrest (Hengst and Reed, 1998; el-Deiry *et al*., 1993; Ocker and Schneider-Stock, 2007; Maddika *et al*., 2007). It inhibits PCNA, which is downregulated here by DMSO and THF, to decrease DNA replication, and promotes RB function (Sherr and Roberts, 1999). This increases 184A1 genomic stability and inhibits proliferation and oncogenic transformation. Likewise, DMSO promotes p21 activity in hybridoma 7TD1 cells to inhibit growth (Ponzio *et al*., 1998). Thalidomide promotes p21 in multiple myeloma cells to induce cell cycle arrest (Hideshima *et al*., 2000).

CDKN1B

The tumour suppressor p27 is upregulated by DMSO and by THF. It is also upregulated by lycopene in relation to untreated cells and this is due to THF. Thalidomide also upregulates p27, in comparison to untreated cells, due to DMSO influence. This upregulation inhibits cell cycle progression, and promotes apoptosis and differentiation (Kayatose *et al*., 1997; Robker and Richards, 1998). It inhibits cyclin E, which is downregulated by lycopene. Its levels decrease by SKP2-mediated degradation (Hershko *et al*., 2001). However, SKP2 is downregulated by DMSO and by lycopene. p27 upregulation reduces 184A1 proliferation and increases its genomic stability to inhibit

oncogenic transformation. DMSO also inhibits cell cycle progression and induces apoptosis in cancer cells (Watson *et al*., 1997; Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999). Thalidomide induces apoptosis in cancer cells as well (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

CDKN2A

DMSO downregulates the tumour suppressor p16 to promote cell cycle progression and decrease cell cycle arrest (Serrano *et al*., 1993; Brenner *et al*., 1998). It is also downregulated by thalidomide, in relation to untreated cells and in DMSO-treated cells. This promotes cyclin C activity, which is upregulated by DMSO and by thalidomide, and decreases RB function, which is downregulated by DMSO. (Serrano *et al*., 1993). p16 downregulation increases genomic instability and proliferation, thereby promoting the oncogenic transformation of 184A1. This downregulation is induced by DMSO in thalidomide-treated cells, and consequently, DMSO is not an appropriate solvent for thalidomide.

Lycopene upregulates p16, in comparison to untreated cells, to inhibit cell cycle progression and promote cell cycle arrest (Serrano *et al*., 1993; Brenner *et al*., 1998). This is due to lycopene with THF influence. Similarly, lycopene inhibits proliferation and induces cell cycle arrest in normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003). This upregulation inhibits cyclin C, which is upregulated by lycopene, and increases RB activity (Serrano *et al*., 1993). Lycopene reduces 184A1 proliferation and increases genomic stability to inhibit oncogenic transformation.

CDKN2B

The tumour suppressor p15 is downregulated by DMSO to increase cell cycle progression and reduce cell cycle arrest and senescence (Reynisdottir *et al*., 1995; Fuxe *et al*., 2000). This promotes cyclin D activity and decreases RB function (Reynisdottir *et al*., 1995). Accordingly, cyclin D1 and cyclin D2 are upregulated by DMSO, and RB is downregulated. This promotes the oncogenic transformation of 184A1 by promoting genomic instability.

Thalidomide upregulates p15, in relation to DMSO-treated control cells, to decrease cell cycle progression, and increase cell cycle arrest and senescence (Reynisdottir *et al*., 1995; Fuxe *et al*., 2000). This promotes genomic stability and inhibits oncogenic transformation. As it is not upregulated in relation to untreated cells, p15 upregulation is induced by thalidomide without the influence of DMSO.

CDKN3

Lycopene downregulates CDKN3 in relation to untreated and THF-treated control cells. It is induced by lycopene with and without THF influence. It is also downregulated by thalidomide in comparison to DMSO-treated control cells due to thalidomide without DMSO influence. This promotes p21, which is upregulated by both lycopene and thalidomide, and p53 (Schultz *et al*., 2001; Schultz *et al*., 2002). Similarly, thalidomide promotes p21 in multiple myeloma cells (Hideshima *et al*., 2000). CDKN3 is inactivated by ATM, which is upregulated by lycopene, in response to DNA damage (Ziv *et al*.,

2006). This downregulation promotes 184A1 genomic stability to inhibit oncogenic transformation.

CHEK2

Thalidomide upregulates CHK2, in comparison to untreated cells, to promote checkpoint function, cell cycle arrest, DNA repair and apoptosis in response to DNA damage (Gatei *et al*., 2003; Pommier *et al*., 2005). This increases the genomic stability of 184A1. Thalidomide also induces cell cycle arrest and apoptosis in cancer cells (Hattori and Iguchi, 2004; Hideshima *et al*., 2000). This upregulation is due to the combination of thalidomide with DMSO.

CKS1B

DMSO and THF upregulate CKS1B to increase cell cycle progression. It is also upregulated by thalidomide in comparison to untreated cells and in DMSO-treated cells. This upregulation is not observed by thalidomide in comparison to DMSO-treated control cells, and is induced by DMSO. CKS1B upregulation promotes CDC2 and SKP2 activity (Egan and Solomon, 1998; Cardozo and Pagano, 2004). CDC2 is upregulated here by DMSO, by THF and by thalidomide, but SKP2 is downregulated by all three treatments. CKS1B promotes 184A1 proliferation. DMSO is therefore not an appropriate solvent for thalidomide.

Lycopene downregulates CKS1B, in comparison to THF-treated control cells, to inhibit proliferation and induce apoptosis (Egan and Solomon, 1998; Cardozo and Pagano, 2004;

Jana *et al*., 2004). This downregulation is due to lycopene, without the influence of THF, and increases the genomic stability of 184A1.

CKS2

The upregulation of CKS2 by DMSO promotes cell cycle progression and proliferation of 184A1 (Rother *et al*., 2007). It is also upregulated by thalidomide in comparison to untreated cells. As it is not upregulated by thalidomide, in comparison to DMSO-treated control cells, but is upregulated in DMSO-treated cells, CKS2 upregulation is due to DMSO. This affirms that DMSO is not a suitable solvent for thalidomide.

CUL2

DMSO downregulates CUL2. Lycopene also downregulates CUL2, in comparison to untreated and THF-treated control cells, and this is induced by lycopene with and without THF influence. Thalidomide downregulates in relation to untreated cells. This downregulation is induced by DMSO influence. CUL2 downregulation reduces cell cycle progression and promotes apoptosis (Cockman *et al*., 2000; Ocker and Schneider-Stock, 2007). This decreases proliferation and promotes the genomic integrity of 184A1 to inhibit oncogenic transformation. Similarly, thalidomide induces apoptosis in cancer cells (Du *et al*., 2005; Hattori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000).

CUL3

CUL3 is downregulated by DMSO. It is also downregulated by lycopene, in relation to THF-treated control cells, and this is induced by lycopene without the influence of THF. CUL3 downregulation lowers integrity during replication (Stewart and Fang, 2005; Sumara *et al*., 2007). This decrease in genomic stability promotes the oncogenic transformation of 184A1.

DDX11

DDX11 is upregulated by THF to promote DNA replication, DNA repair, transcription and genomic integrity (Tuteja and Tuteja, 2004; Parish *et al*., 2006; Hoque and Ishikawa, 2001; Uhlmann *et al*., 2000). This upregulation increases proliferation and genomic stability.

Lycopene downregulates DDX11 in comparison to THF-treated control cells and this is induced by lycopene without THF influence. This correlates with decreased proliferation and possibly with decreased genomic integrity during anaphase (Amann *et al*., 1997; Parish *et al*., 2006; Hoque and Ishikawa, 2001; Uhlmann *et al*., 2000). Lycopene reduces 184A1 proliferation and may promote genomic instability.

DNM2

Thalidomide downregulates DNM2, in relation to untreated cells, to decrease p53 function, apoptosis, cytoskeletal integrity and signalling (De Camilli *et al*., 1995; Orth and McNiven, 2003; Konopka *et al*., 2006; Thompson *et al*., 2004; Kranenburg *et al*.,

1999; Gomez *et al*., 2005). This reduces the genomic integrity and stability of 184A1. Oncogenic transformation is consequently promoted. DNM2 downregulation is due to thalidomide with DMSO influence, therefore DMSO is not a suitable solvent for thalidomide.

E2F4

The downregulation of E2F4 by lycopene, in relation to untreated and THF-treated control cells, inhibits cell cycle progression and differentiation, and promotes cell cycle arrest (Garneau *et al*., 2007; Humbert *et al*., 2000; Fajas *et al*., 2002; Maehara *et al*., 2005). It is induced by lycopene with and without THF influence. Lycopene promotes the oncogenic transformation of 184A1.

GTF2H1

GTF2H1 is downregulated by lycopene in comparison to untreated and THF-treated control cells. This downregulation is due to lycopene with and without the influence of THF. GTF2H1 is also downregulated by thalidomide in comparison to untreated cells. It is due to the combination of thalidomide with DMSO. It decreases cell cycle progression, transcription, cell cycle control and DNA repair (Matsuno *et al*., 2007; Araujo *et al*., 2000). This downregulation reduces proliferation, genomic integrity and stability of 184A1. DMSO is not appropriate as a solvent for thalidomide.

GTSE1

The upregulation of GTSE1 by THF inhibits the apoptosis of arrested damaged cells to decrease genomic integrity (Monte *et al*., 2003). This promotes the oncogenic transformation of 184A1.

Lycopene downregulates GTSE1, in relation to both untreated and THF-treated control cells, and is due to lycopene with and without THF influence. Thalidomide also downregulates GTSE1 in relation to untreated cells. This is due to the combination of thalidomide with DMSO. This downregulation promotes apoptosis of arrested, damaged cells to increase the genomic integrity of 184A1 (Monte *et al*., 2003; Monte *et al*., 2004).

HERC5

DMSO and THF upregulate HERC5 to promote ubiquitin ligase activity in response to the immune system to increase 184A1 stability (Dastur *et al*., 2006). Lycopene and thalidomide also upregulate HERC5 in comparison to untreated cells. As it is also upregulated in DMSO-treated cells, this upregulation by thalidomide is induced by DMSO.

KNTC1

DMSO downregulates KNTC1. Lycopene also downregulates KNTC1, in relation to untreated and THF-treated control cells, and this is due to lycopene with and without THF influence. Thalidomide downregulates KNTC1 in relation to untreated cells and in DMSO-treated cells. As it is induced by DMSO, it supports the unsuitability of DMSO

as a solvent for thalidomide. This downregulation reduces genomic integrity and promotes aneuploidy. Oncogenic transformation is promoted. This downregulation also suggests a decrease in proliferation.

MAD2L1

The upregulation of MAD2L1 by lycopene, in relation to untreated cells, increases control and integrity during mitosis (Chen *et al*., 1996b; Amon, 1999; Fang *et al*., 1998). It inhibits CDC20 from prematurely activating APC ubiquitin ligase (Chen *et al*., 1996b; Amon, 1999). It induces cell cycle arrest (Fang *et al*., 1998). This promotes the genomic stability of 184A1. This upregulation is induced by the combination of lycopene with THF.

MCM2

MCM2 is upregulated by DMSO and by THF. Lycopene also upregulates MCM2 in comparison to untreated cells. This is due to THF as it is upregulated in THF-treated cells as well, but not by lycopene in comparison to THF-treated control cells. MCM2 is upregulated by thalidomide in relation to untreated cells, and this is due to DMSO influence. It increases proliferation, checkpoint activation and transcription, and inhibits re-replication (Todorov *et al*., 1998; Ying and Gautier, 2005; Cortez *et al*., 2004; Dziak *et al*., 2003; Labib *et al*., 2000). MCM2 expression correlates with proliferation and Ki-67 (Todorov *et al*., 1998; Gonzalez *et al*., 2003). Ki-67 is upregulated by thalidomide. Its proliferative function is inhibited by ATM and ATR at the S-phase checkpoint (Cortez and Elledge, 2004; Cortez *et al*., 2004). ATM is upregulated here by DMSO and by THF.

MCM2 upregulation promotes proliferation and genomic stability in 184A1. Similarly, DMSO induces cell cycle arrest in HL-60, Burkitt's lymphoma, CHO and hybridoma 7TD1 cells (Nishizawa *et al*., 1998; Lin *et al*., 1995; Fiore and Degrassi, 1999; Ponzio *et al*., 1998).

MCM4

Thalidomide upregulates MCM4, in comparison to untreated and DMSO-treated control cells, to promote checkpoint activation, transcription and DNA replication, and inhibit re-replication (Ishimi *et al*., 2004; Dziak *et al*., 2003; Davey *et al*., 2003; Labib *et al*., 2000). This increases proliferation and the genomic integrity of 184A1. This upregulation is due to thalidomide, both with and without DMSO influence.

MCM5

MCM5 is upregulated by DMSO and by THF. It is also upregulated by lycopene, in comparison to untreated cells. This is induced by THF, as it is also upregulated in THF-treated cells but not by lycopene without THF influence. MCM5 is upregulated by thalidomide in relation to untreated cells, due to DMSO influence. This promotes proliferation, checkpoint activation and transcription, and inhibits re-replication (Stoeber *et al*., 2002; Labib *et al*., 2000; Cortez *et al*., 2004; Snyder *et al*., 2005). DMSO promotes checkpoint function in CHO and various cancer cells (Fiore and Degrassi, 1999; Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998). MCM5 upregulation promotes proliferation and genomic stability in 184A1.

MKI67

Ki-67 is upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, to promote proliferation and apoptosis (MacCallum and Hall, 2000; Lipponen, 1999). It is induced by thalidomide with and without the influence of DMSO. This upregulation promotes genomic stability and inhibits the oncogenic transformation of 184A1.

MNAT1

Lycopene downregulates MAT1, in comparison to THF-treated control cells, and is due to lycopene without THF influence. This decreases cyclin H and CDK7, which are downregulated by lycopene, to reduce the proliferation of 184A1 (Tassan *et al*., 1995). This concurs with other studies where lycopene inhibits proliferation in normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003).

MRE11A

DMSO and THF upregulate MRE11A. It is also upregulated by lycopene, in relation to untreated cells, and this is induced by THF. Thalidomide upregulates MRE11A in relation to untreated cells. This promotes checkpoint activation, DNA repair, apoptosis and proliferation (Lee and Paull, 2005; Paull and Gellert, 1998). It induces ATM, which is upregulated here by DMSO, THF, lycopene and thalidomide, in response to DNA damage (Lee and Paull, 2005). This increases proliferation and the genomic integrity and stability of 184A1.

Likewise, DMSO induces cell cycle arrest in CHO and cancer cells (Fiore and Degrassi, 1999; Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al.*, 1998). Thalidomide promotes apoptosis in cancer cells as well (Du *et al*., 2005; Hatttori and Iguchi, 2004; Marriott *et al*., 2003; Hideshima *et al*., 2000). MRE11A upregulation in thalidomide-treated cells is due to DMSO influence.

PCNA

PCNA is downregulated by DMSO and by THF to decrease DNA replication and DNA repair (Shiomi *et al*., 2002; Maga and Hubscher, 2003). It is inhibited by p21 and RB (Maki and Howley, 1997; Sever-Chroneos *et al*., 2001). p21 is upregulated here by DMSO and THF, and RB is downregulated by DMSO. This lowers proliferation and the genomic integrity of 184A1, which promotes transformation.

RAD1

The upregulation of RAD1 by THF increases 9-1-1 function in inducing cell cycle arrest and DNA repair following DNA damage (Bermudez *et al*., 2003; Yin *et al*., 2004; Brandt *et al*., 2006). It is also upregulated by thalidomide in comparison to untreated cells. This is induced by thalidomide in combination with DMSO. RAD1 promotes CHK1 activation and p21 activity (Bao *et al*. 2004; Roos-Mattjus *et al*., 2003; Yin *et al*., 2004). p21 is upregulated here by THF and by thalidomide. Thalidomide also promotes p21 to induce cell cycle arrest in multiple myeloma cells (Hideshima *et al*., 2000). RAD1 upregulation increases the genomic integrity and stability of 184A1 to inhibit oncogenic transformation.

RAD17

RAD17 is upregulated by DMSO and by THF. Lycopene also upregulates RAD17 in comparison to untreated cells. Thalidomide upregulates RAD17, in relation to untreated cells, and is due to DMSO influence. This increases checkpoint function and is essential for CHK1 and CHK2 activation (Wang *et al*., 2003c; Walworth and Bernards, 1996; Lindsay *et al*., 1998). CHK2 is upregulated here by thalidomide in 184A1. RAD17 promotes 9-1-1 activity following DNA damage and prevents re-replication (Zou *et al*., 2003; Bermudez *et al*., 2003; Wang *et al*., 2003c). It is activated by ATM and ATR (Zou *et al*., 2002). ATM is upregulated here by DMSO, THF and thalidomide. RAD17 increases the genomic integrity and stability of 184A1 to inhibit oncogenic transformation. Similarly, DMSO promotes checkpoint function in CHO and cancer cells (Fiore and Degrassi, 1999; Nishizawa *et al*., 1998; Lin *et al*., 1995; Ponzio *et al*., 1998).

RAD51

DMSO downregulates RAD51 to decrease DNA repair and 184A1 genomic integrity (Gupta *et al*., 1997). Thalidomide also downregulates RAD51 in comparison to untreated cells. However, it is upregulated by thalidomide in comparison to DMSO-treated control cells. This upregulation is due to thalidomide without the influence of DMSO. Although its function in promoted by BRCA2, which is upregulated by DMSO, RAD51 is downregulated (Galkin *et al*., 2005). This reduces genomic stability and promotes oncogenic transformation.

RAD51 upregulation by lycopene, in relation to untreated cells, increases DNA repair (Gupta *et al*., 1997). This is induced by lycopene in combination with THF. BRCA2, which is upregulated by lycopene, promotes RAD51 activity (Galkin *et al*., 2005). This increases the genomic integrity and stability of 184A1.

RAD9A

RAD9A is downregulated by thalidomide, in relation to untreated cells, to decrease checkpoint signalling, cell cycle arrest, DNA repair and apoptosis (Toueille *et al*., 2004). This is due to the combination of thalidomide with DMSO. RAD9A downregulation reduces the genomic integrity and stability of 184A1 and promotes oncogenic transformation. DMSO is therefore not an appropriate solvent for thalidomide.

RB1

The tumour suppressor RB is downregulated by DMSO to promote cell cycle progression and inhibit the DNA damage responses, differentiation and senescence (Hinds *et al*., 1992; Tell *et al*., 2006; Maddika *et al*., 2007). It is inhibited by cyclin D1 and cyclin D2, which are upregulated by DMSO (Knudsen *et al*., 2000). This increases proliferation and the oncogenic transformation of 184A1 as genomic integrity and stability are reduced.

RBBP8

Lycopene downregulates RBBP8 in comparison to THF-treated control cells. This is due to lycopene without THF influence. The downregulation of RBBP8 inhibits cyclin D1

transcription and decreases G_1/S progression (Liu and Lee, 2006; Wu and Lee, 2006). This reduces the proliferation of 184A1.

RBL1

Upregulation of the tumour suppressor p107 by THF inhibits cell cycle progression, suppresses growth and promotes differentiation (Woo *et al*., 1997; Beijersbergen *et al*., 1996; Lipinski and Jacks, 1999). It is also upregulated by lycopene in relation to untreated cells, and this is induced by THF. Thalidomide upregulates p107, in relation to untreated and DMSO-treated control cells, and this is with and without DMSO influence. p107 upregulation inhibits cyclin E, cyclin A and SKP2, and promotes p27 (Woo *et al*., 1997; Rodier *et al*., 2005). Thalidomide downregulates cyclin E1 and SKP2 in this study. p27 is upregulated here by THF and by thalidomide. This decreases 184A1 proliferation and inhibits oncogenic transformation.

RBL2

Lycopene upregulates the tumour suppressor p130, in comparison to untreated cells, to inhibit cell cycle progression, and promote cell cycle arrest and quiescence (Grana *et al*., 1998; Hansen *et al*., 2001). This upregulation is due to the combination of lycopene with THF. It is also upregulated by thalidomide, in comparison to untreated and DMSO-treated control cells, and this is induced by thalidomide with and without the influence of DMSO. p130 inhibits cyclin E, which is downregulated by thalidomide (Woo *et al*., 1997). p130 upregulation increases the genomic stability of 184A1.

Similarly, lycopene inhibits proliferation and induces cell cycle arrest in normal prostate epithelial cells (Obermuller-Jevic *et al*., 2003).

RPA3

RPA3 is downregulated by DMSO to decrease DNA repair, apoptosis and the control of replication (He *et al*., 1995; Iftode *et al*., 1999; Bochkarev *et al*., 1997). It is also downregulated by thalidomide in comparison to untreated cells, and this is due to the combination of thalidomide with DMSO. This downregulation reduces genomic integrity and promotes oncogenic transformation.

SERTAD1

SERTAD1 is downregulated by THF to inhibit cell cycle progression and tumourigenesis (Hsu *et al*., 2001; Sim *et al*., 2006; Gladden *et al*., 2005; Tang *et al*., 2002). It is inhibited by the tumour suppressor RB (Hsu *et al*., 2001). This decreases proliferation in 184A1.

SKP2

DMSO and THF downregulate SKP2. It is also downregulated by lycopene in comparison to untreated cells, induced by THF. Thalidomide downregulates SKP2, in relation to untreated cells, due to DMSO influence. SKP2 downregulation inhibits cell cycle progression and oncogenesis, and induces apoptosis (Gstaiger *et al*., 2001; Jana *et al*., 2004). This increases p21, p27 and cyclin D1, which are upregulated here by DMSO, THF, lycopene and thalidomide (Bornstein *et al*., 2003; Nakayama *et al*., 2000; Ganiatsas *et al*., 2001). It inhibits adhesion-independent cell growth (Signoretti *et al*., 2002).

Similarly, DMSO increases the adhesion to the extracellular matrix in CHO (Fiore and Degrassi, 1999). This decreases proliferation and increases the genomic stability of 184A1 to inhibit oncogenic transformation.

TFDP1

DMSO downregulates DP1. It is also downregulated by lycopene in comparison to untreated and THF-treated control cells. This is induced by lycopene with and without THF influence. DP1 downregulation inhibits cell cycle progression, apoptosis, DNA synthesis and oncogenic transformation (Schulze *et al*., 1995; Shan *et al*., 1996). Its expression correlates with cyclin E1, which is also downregulated by DMSO (Yasui *et al*., 2002). This reduces 184A1 proliferation and increases its genomic stability.

TFDP2

DP2 is downregulated by DMSO. It is also downregulated by lycopene in relation to untreated cells, due to the combination of lycopene with THF. This decreases proliferation and apoptosis (Dyson, 1998; Stanelle *et al*., 2002). DP2 downregulation therefore decreases the genomic integrity of 184A1. Lycopene also inhibits proliferation in the normal prostate epithelial cell line, PrEC (Obermuller-Jevic *et al*., 2003).

UBE1

The downregulation of UBE1 by THF promotes cyclin D1 to increase cell cycle progression (Lonardo *et al*., 1999; Pitha-Rowe *et al*., 2004). Cyclin D1 is upregulated by

THF. The response to stress decreases (Kim and Zhang, 2003). This promotes proliferation and genomic instability, to increase oncogenic transformation.

Lycopene upregulates UBE1, in comparison to THF-treated control cells, to promote cyclin D1, which is upregulated by lycopene, and increase cell cycle progression (Lonardo *et al*., 1999; Pitha-Rowe *et al*., 2004). It is induced by lycopene without THF influence. This increases 184A1 proliferation.

3.4.5 Comparison of breast cancer cell lines with the normal breast cell line

The gene expression of the breast cancer cell lines, MCF-7 and MDA-MB-231, is compared to the gene expression of the normal breast cell line, 184A1. This identifies genes that are differentially expressed between the cancerous and normal cell lines, implicating the pathways that are altered with oncogenic transformation.

Table 3.19: Fold regulation of genes from MCF-7 and MDA-MB-231 untreated cells in comparison to 184A1 untreated cells, with significant fold changes highlighted.

	MCF-7	MDA-MB-231
ABL1	-1.3486	3.7334
ANAPC4	3.0074	-1.6627
ARHI	-24.3104	-72.4292
BAX	-1.4933	2.2728
BCCIP	-1.3713	4.2091
BCL2	-12.8661	3.8278

BIRC5	-1.0464	14.3552
BRCA1	3.3554	12.2737
BRCA2	43.2162	19.2595
CCNB1	-2.0569	1.707
CCNB2	-2.8451	2.3694
CCNC	383.6109	2057.248
CCND1	149.448	75.2438
CCND2	-714.8516	-16.5356
CCNF	2.3546	-1.0024
CCNG2	-1.138	-3.6999
CCNH	-1.8836	-3.6667
CCNT2	9.1928	3.107
CDC16	6.4643	21.3106
CDC2	3.1635	26.364
CDC20	-1.5983	2.6991
CDC34	4.4707	-1.898
CDK2	-1.1871	2.0584
CDK4	3.9055	2.6253
CDK5R1	-2.3014	-1.188
CDK7	12.0629	14.2461
CDK8	25.5367	76.5059
CDKN1A	3.6693	19.2462
CDKN1B	100.6009	40.8284

CDKN2A	-57.6201	-167.3239
CDKN2B	-165.1348	-1073.0488
CDKN3	-1.2548	2.8235
CHEK1	1.1262	7.133
CHEK2	98.4623	1.9691
CKS1B	5.7577	10.4868
CKS2	2.2138	2.2634
CUL1	1.4305	2.8255
CUL3	3.1569	-5.9032
DNM2	1.7189	-4.6219
GADD45A	-7.0445	3.569
HERC5	19.8147	82.5102
HUS1	-4.3939	-3.1199
KPNA2	3.2727	3.2299
MAD2L1	6.89	59.8177
MAD2L2	1.1749	3.4165
MCM2	18.4878	-1.0228
MCM3	3.4955	2.9763
MCM4	1.2004	10.6258
MCM5	30.8993	18.7199
MKI67	5.6824	3.7855
MNAT1	2.371	1.2715
MRE11A	4.5237	4.2649

NBS1	5.1497	2.3563
PCNA	-4.4183	-1.8888
RAD1	10.8491	5.2036
RAD17	7.3233	7.2325
RAD51	3.5418	11.3255
RAD9A	-2.0328	-5.9032
RBL1	5.6982	40.1548
RBL2	3.8225	2.0627
RPA3	-1.9883	-98.4623
SKP2	-15.829	1.707
SUMO1	2.5271	5.0055
TP53	1.6083	-4.964

3.4.5.1 MCF-7 untreated

ANAPC4

APC4 is upregulated in comparison to normal breast cells to increase ubiquitin ligase activity, cell cycle progression and checkpoint function (Peters, 2002; Jana *et al*., 2004; Wasch and Cross, 2002). This promotes proliferation of MCF-7 as well as genomic integrity.

ARHI

The tumour suppressor ARHI is downregulated in MCF-7 in comparison to 184A1. This inhibits p21 and promotes cyclin D1 to increase cell cycle progression and inhibit

apoptosis (Bao *et al*., 2002). This downregulation promotes motility, growth and invasion of MCF-7. ARHI is downregulated in breast cancers by LOH and histone acetylation (Wang *et al*., 2003b; Fujii *et al*., 2003). E2F1, E2F4 and HDAC also bind to ARHI promoters to inhibit its transcription (Lu *et al*., 2006). This downregulation promotes proliferation and reduces genomic integrity to promote oncogenic transformation.

BCL2

BCL2 is downregulated in relation to normal breast cells. This promotes BAX, apoptosis, G_1/S progression, DNA repair and DNA replication (Kim *et al*., 2004; Dlugosz *et al*., 2006; Mazel *et al*., 1996; Hockenberry *et al*., 1993; Youn *et al*., 2005; Saintigny *et al*., 2001; Liu *et al*., 1997). It is inhibited by the p53 tumour suppressor (Chipuk *et al*., 2004; Mihara *et al*., 2003). This downregulation increases MCF-7 proliferation and also genomic integrity.

BRCA1

BRCA1 is upregulated in comparison to 184A1 to increase DNA damage repair, checkpoint function, cell cycle arrest, transcription, replication and spindle stability (Venkitaraman, 2002; Eyfjord and Bodvarsdottir, 2005; Scully *et al*., 1997). CHK2, which is upregulated in MCF-7, activates BRCA1 (Yoshida and Miki, 2004; Williamson *et al*., 2002). It associates with RAD51, which is upregulated in MCF-7, to promote DNA repair (Haber, 1999; Davies *et al*., 2001). However, the overexpression of RAD51 seen in tumour cell lines, amplifies homologous recombination to induce genomic

instability and aneuploidy (Vispe *et al*., 1998; Richardson *et al*., 2004). This upregulation promotes proliferation and genomic instability, and this is implicated in transformation from the normal to the malignant state. The increase in the other DNA damage responses, indicates that there is a greater level of DNA damage in MCF-7 as opposed to 184A1.

BRCA2

BRCA2 is upregulated to promote RAD51-mediated DNA damage repair (Galkin *et al.*, 2005). It is overexpressed in breast cancer, as is RAD51, and this elevates levels of homologous recombination to induce chromosomal rearrangements and aneuploidy (Egawa *et al*., 2002; Vispe *et al*., 1998; Richardson *et al*., 2004). It associates with BRCA1, PCNA, and RAD51 to increase DNA replication (Lomonosov *et al*., 2003; Scully *et al*., 1997). Overexpression in sporadic breast tumours is associated with increased progression and a poor prognosis (Egawa *et al*., 2002). BRCA2 upregulation increases MCF-7 proliferation and genomic instability, which is important in promoting oncogenic transformation.

CCNB1

Cyclin B1 is downregulated to decrease apoptosis, proliferation and cell survival (Hagting *et al*., 1999; Pines and Hunter, 1989; Borgne *et al*., 2006; Coqueret, 2003). Cyclin B1 forms part of the MPF complex that induces mitosis (Morgan, 1995). The other component of complex, CDC2, is upregulated in MCF-7. It is inhibited by p53 (Krause *et al*., 2000). However, cyclin B1 is overexpressed in breast cancer and is

associated with increased tumour size, metastasis and a poor prognosis (Suzuki *et al*., 2007). This downregulation reduces proliferation and the genomic integrity of MCF-7.

CCNB2

Cyclin B2 is downregulated in comparison to 184A1. It forms part of the mitosis-inducing MPF complex (Morgan, 1995). It is inhibited by p53 (Krause *et al*., 2000). The other component of the MPF complex, CDC2, is upregulated in MCF-7. Cyclin B2 downregulation decreases proliferation.

CCNC

Cyclin C is upregulated in comparison to normal cells to promote cell cycle entry. By promoting CDC2, which is also upregulated in MCF-7, it increases G_1/S and G_2/M progression (Liu *et al*., 1998). It also inhibits transcription (Akoulitchev *et al*., 2000; Hengartner *et al*., 1998). This increases MCF-7 proliferation.

CCND1

Cyclin D1 is upregulated, in relation to 184A1, to increase cell cycle progression, survival and replication (Schwartz and Shah, 2005; Hinds *et al*., 1994; Fukami-Kobayashi and Mitsui, 1999). It inhibits the tumour suppressors RB, p107, p130, p27 and p21 (Lundberg and Weinberg, 1998; Perez-Roger *et al*., 1999). It also promotes entry into the next cell cycle (Yang *et al*., 2006). Cyclin D1 overexpression in breast cancer promotes cancer development and progression (Buckley *et al*., 1993; Bartkova *et*

al., 1994). It promotes the proliferation of MCF-7 and decreases genomic stability to increase oncogenic transformation.

CCND2

The downregulation of cyclin D2 decreases entry into the cell cycle and reduces cell survival (Schwartz and Shah, 2005; Hinds *et al*., 1994). It promotes p27 and CDK2 inhibition (Bouchard *et al*., 1999). As cyclin D2 is downregulated in most breast cancer cell lines due to promoter methylation, this decreases the oncogenic state of MCF-7 (Tam *et al*., 1994; Lukas *et al*., 1995; Evron *et al*., 2001).

CCNF

Cyclin F upregulation promotes cell cycle progression, cyclin E transcription and SCF ubiquitin ligase activity (Kong *et al*., 2000; Tezlaff *et al*., 2004). Cyclin E accelerates G_1/S progression, increases genomic instability and its overexpression in breast cancer correlates with a poor prognosis (Spruck *et al*., 1999; Akli and Keyomarsi, 2004; Keyomarsi *et al*., 1995; Keyomarsi *et al*., 2002). Cyclin F also promotes progression of the next cell cycle (Tezlaff *et al*., 2004). It promotes cyclin B1 activity to increase mitosis (Kong *et al*., 2000). This upregulation increases proliferation and genomic instability to promote tumourigenesis.

CCNT2

Cyclin T2 is upregulated in comparison to 184A1 to increase transcription and differentiation (Napolitano *et al*., 2000; Simone *et al*., 2002). The increase in

differentiation reduces the oncogenic state of MCF-7 and this is therefore a beneficial upregulation that inhibits transformation.

CDC16

CDC16 is upregulated, in relation to normal breast cells, to increase APC/C ubiquitin ligase activity and cell cycle progression (Rudner and Murray, 2000; Zachariae and Nasmyth, 1996). This promotes MCF-7 proliferation.

CDC2

CDC2 is upregulated to activate CDC20 and increase cell cycle progression (Rudner and Murray, 2000; Peters *et al*., 1998). CDC20 is downregulated in MCF-7. CDC2 is overexpressed in breast cancer where it promotes mitosis and metastasis (Megha *et al*., 1999). It is inhibited by p53, p21 and GADD45A (Rudner and Murray, 2000). GADD45A is downregulated in MCF-7. This upregulation increases MCF-7 proliferation and tumourigenesis.

CDC34

CDC34 is upregulated in comparison to 184A1 to promote SCF ubiquitin ligase activity, increase cell cycle progression and promote spindle function (Wu *et al*., 2002; Butz *et al*., 2005; Reymond *et al*., 2000). It inhibits the tumour suppressor p27 and is overexpressed in cancers where it decreases genomic integrity during metaphase (Liu *et al*., 2006; Eliseeva *et al*., 2001; De Vos *et al*., 2002; Chauhan *et al*., 2004). This upregulation

increases MCF-7 proliferation and genomic instability to promote oncogenic transformation.

CDK4

CDK4 is upregulated, in relation to normal breast cells, to promote cell cycle progression and inhibit p53 and RB tumour suppressor activity (Strohmaier *et al*., 2001; Ortega *et al*., 2002; Reynisdottir *et al*., 1995). It is upregulated in breast cancer where it promotes p53 degradation (Ortega *et al*., 2002; Hall and Peters, 1996). CDK4 promotes survivin function to promote tumourigenesis (Sui *et al*., 2002). This increases proliferation and reduces the genomic integrity of MCF-7 to promote tumourigenesis.

CDK5R1

CDK5R1 is downregulated in comparison to normal breast cells. This decreases CDK5 activation, differentiation, transcription, senescence and apoptosis (Fung *et al*., 2002; Tsai *et al*., 1993; Rosales and Lee, 2006; Moncini *et al*., 2007; Lee *et al*., 1997; Zhang *et al*., 2002). This downregulation promotes oncogenic transformation and tumourigenesis.

CDK7

CDK7 is upregulated in comparison to increase cell cycle progression, transcription and DNA repair (Harper and Elledge, 1998). It is activated by CDK1 and CDK2 (Garrett *et al*., 2001). CDK7 promotes MCF-7 proliferation and also genomic integrity.

CDK8

CDK8 upregulation promotes genomic integrity and p53-mediated transcription (Chi *et al*., 2001; Hengartner *et al*., 1998; Donner *et al*., 2007). It is activated by p21 (Donner *et al*., 2007). MCF-7 genomic stability is increased in comparison to 184A1. This positive upregulation reduces the oncogenic state of MCF-7.

CDKN1A

The tumour suppressor p21 is upregulated, in relation to normal breast cells, to decrease proliferation and apoptosis, and promote cell cycle arrest (Hengst and Reed, 1998; Sherr and Roberts, 1999; el-Deiry *et al*., 1993; Ocker and Schneider-Stock, 2007; Maddika *et al*., 2007). p21 levels are low in normal breast epithelium (Diab *et al*., 1997). It inhibits CDC2, cyclin A/CDK2 and cyclin E/CDK2 (Taylor *et al*., 2001; Maddika *et al*., 2007). CDC2 is downregulated in MCF-7. This beneficial upregulation promotes genomic stability of MCF-7 and inhibits tumourigenesis.

CDKN1B

The tumour suppressor p27 is upregulated to inhibit cell cycle progression, and promote apoptosis and differentiation (Kayatose *et al*., 1997; Robker and Richards, 1998). It inhibits cyclin E/CDK2 and cyclin A/CDK2 (Munoz-Alonso *et al*., 2005). Its ubiquitination is mediated by CDC34, which is upregulated in MCF-7 (Malek *et al*., 2001). This increases genomic stability and reduces the oncogenic state of MCF-7.

CDKN2A

The tumour suppressor p16 is downregulated in comparison to 184A1. This promotes cyclin C/CDK4 and cyclin C/CDK6 to increase S-phase entry, angiogenesis and metastasis (Serrano *et al*., 1993; Gibson *et al*., 2005; Wang *et al*., 2006). RB activation decreases to reduce G_1/S arrest (Brenner *et al*., 1998). p16 downregulation decreases genomic integrity and increases tumourigenesis in MCF-7. The complete CDKN2A-CDKN2B locus is deleted in cancers as effective transformation requires both p16 and p15 inactivation (Krimpenport *et al*., 2007). Accordingly, both p16 and p15 are downregulated in MCF-7 compared to 184A1. Expression in breast cancer is also lost through methylation and mutation (Brenner *et al*., 1998).

CDKN2B

p15 is downregulated to promote cyclin D/CDK4 and cyclin D/CDK6, which decrease RB activity and inhibit G_1 arrest (Reynisdottir *et al*., 1995). This decreases p27 activity and inhibits senescence (Massague, 2004; Massague *et al*., 2000; Swarbrick *et al*., 2000). Telomerase activity increases to prolong the proliferation of MCF-7 (Fuxe *et al*., 2000). This downregulation reduces genomic stability and increases oncogenic transformation of MCF-7.

CHEK2

CHK2 is upregulated in relation to 184A1 to increase checkpoint activity, cell cycle arrest, DNA repair and apoptosis in response to DNA damage (Gatei *et al.,* 2003; Pommier *et al*., 2005). While this upregulation promotes genomic integrity, it reduces

the sensitivity of MCF-7 to genotoxic drugs (Sordet *et al*., 2005). Its mutation promotes the development of familial breast cancer (Zhou and Bartek, 2004). It is upregulated in response to the increased level of DNA damage in MCF-7 compared to 184A1, but may also promote tumourigenesis.

CKS1B

CKS1B is upregulated in comparison to normal breast cells to promote CDC2 activity and cell cycle progression (Egan and Solomon, 1998; Bloom and Pagano, 2003). It induces degradation of p27 (Bloom and Pagano, 2003). CKS1B overexpression in breast cancer increases tumour aggressiveness and is associated with a poor prognosis (Slotky *et al*., 2005). This increases proliferation and reduces genomic stability to promote transformation. It is upregulated in immortalised breast cancer cell lines in relation to non-immortalised breast cancer cell lines derived from patients (Fernandez-Cobo *et al*., 2006).

CKS2

CKS2 is upregulated to promote cell cycle progression in comparison to normal breast cells (Rother *et al*., 2007).

CUL3

CUL3 is upregulated in relation to 184A1 to increase cell cycle progression, spindle assembly and chromosomal integrity (Sumara *et al*., 2007). It also has tumour suppressor properties (Wilkins *et al*., 2004). This promotes proliferation and genomic integrity.

GADD45A

GADD45A is downregulated to promote cyclin B1/CDC2, mitosis and growth, and decrease DNA repair (Jin *et al*., 2002; Zhan *et al*., 1994; Carrier *et al*., 1999). While cyclin B1 is downregulated in MCF-7, CDC2 is upregulated. This downregulation increases MCF-7 proliferation and reduces genomic integrity to increase tumourigenesis.

HERC5

HERC5 is upregulated to promote ubiquitin ligase activity as part of the immune response (Dastur *et al*., 2006). This promotes MCF-7 stability and survival.

HUS1

The downregulation of HUS1 in comparison to 184A1 decreases G_1/S and G_2/M checkpoints and DNA repair, and promotes mitotic entry (Walworth and Bernards, 1996; Wang *et al*., 2006; Lindsay *et al*., 1998). It is part of the 9-1-1 DNA damage response complex (Francia *et al*., 2006). Stabilisation of the replication forks decreases (Parrilla-Castellar *et al*., 2004). HUS1 downregulation reduces genomic integrity and stability and promotes proliferation to increase tumourigenesis.

KPNA2

KPNA2 is upregulated in comparison to 184A1. KPNA2 promotes the DNA damage responses and oncogenic transformation (Tseng *et al*., 2005; Kim *et al*., 2000; Umegaki *et al*., 2007; Dankoff *et al*., 2007; Poon and Jans, 2005). It promotes BRCA1, p53 and the MRN complex function (Thakur *et al*., 1997; Kim *et al*., 2000; Tseng *et al*., 2005).

BRCA1, MRE11A and NBS1 are upregulated in MCF-7. KPNA2 is overexpressed in breast cancer and correlates with poor prognosis (Dahl *et al*., 2006). This upregulation promotes tumourigenesis.

MAD2L1

MAD2L1 upregulation induces cell cycle arrest to promote integrity during anaphase (Fang *et al*., 1998; Chen *et al*., 1996b; Amon, 1999). It is an important prognostic gene in MCF-7 (Teschendorff *et al*., 2007). It inhibits CDC20 activity, which is downregulated in MCF-7 (Chen *et al*., 1996b; Amon, 1999). MAD2L1 is upregulated and mutated in breast cancer and breast cancer cell lines, where it promotes defective checkpoints and genomic instability (Scintu *et al*., 2007; Yuan *et al*., 2006; Percy *et al*., 2000; Rouzier *et al*., 2005). This upregulation decreases genomic integrity and promotes tumourigenesis in MCF-7.

MCM2

MCM2 is upregulated to inhibit re-replication, promote checkpoint activation and transcription, (Ying and Gautier, 2005; Cortez *et al*., 2004; Dziak *et al*., 2003). It correlates with proliferation and Ki-67 (Todorov *et al*., 1998; Gonzalez *et al*., 2003). MCM2 overexpression correlates with increased tumour progression and poor survival rates (Dudderidge *et al*., 2005; Hashimoto *et al*., 2004). MCM2 increases proliferation and the oncogenic state of MCF-7. The increase in the DNA damage response is due to the higher level of DNA damage compared to 184A1.

MCM3

MCM3 upregulation increases cell cycle arrest, transcription and DNA replication, and inhibits re-replication (Cortez *et al*., 2004; Dziak *et al*., 2003; Ying and Gautier, 2005; Labib *et al*., 2000). It is not expressed in differentiated cells (Endl *et al*., 2001; Musahl *et al*., 1998). This promotes proliferation and genomic integrity in MCF-7, and indicates the increase in DNA damage compared to 184A1.

MCM5

MCM5 is upregulated in relation to 184A1 to promote checkpoint function, transcription and proliferation, and inhibit re-replication (Cortez *et al*., 2004; Snyder *et al*., 2005; Stoeber *et al*., 2002; Williams *et al*., 1998; Labib *et al*., 2000). Its expression correlates with dysplasia (Murphy *et al*., 2005). MCM5 upregulation corresponds with the higher level of DNA damage and genomic instability in MCF-7, and increases proliferation.

MKI67

Ki-67 is upregulated in comparison to normal breast cells and correlates with increased proliferation and apoptosis (Endl and Gerdes, 2000; MacCallum and Hall, 2000). Expression is very low in normal breast tissue (Harper-Wynne *et al*., 2002; Clarke *et al*., 1997; de Lima *et al*., 2003). Ki-67 is inversely correlated with anti-apoptotic BCL2, and accordingly BCL2 is downregulated in MCF-7 (Bottini *et al*., 2001). Ki-67 overexpression in breast cancer increases cancer progression and reduces the survival rate (Spyratos *et al*., 2002; Trihia *et al*., 2003; Colozza *et al*., 2005). This therefore promotes tumourigenesis in MCF-7.

MNAT1

MAT1 upregulation, in relation to normal breast cells, promotes cell cycle progression and transcription by inhibiting p53 and RB activity (Tassan *et al*., 1995; Ko *et al*., 1997; Wu *et al*., 2001). It is overexpressed in breast cancers (Talukder *et al*., 2003). This increases MCF-7 proliferation and decreases its genomic stability to promote tumourigenesis.

MRE11A

MRE11A is upregulated in comparison to 184A1 to promote cell cycle arrest, DNA repair, apoptosis and proliferation (Lee and Paull, 2005; Yamaguchi-Iwai *et al*., 1999; Paull and Gellert, 1998). It is activated by NBS1, which is upregulated in MCF-7, following DNA damage, and induces ATM activity (Dong *et al*., 1999; Lee and Paull, 2005). It forms part of the MRN DNA damage response complex, and the other components of the complex, RAD51 and NBS1, are also upregulated in MCF-7. MRE11A increases proliferation and genomic integrity by responding to the increased occurrence of DNA damage compared to 184A1.

NBS1

NBS1 is upregulated in relation to 184A1 to increase MRN activity and induce cell cycle arrest following DNA damage (Stracker *et al*., 2004; Lee and Paull, 2005; Komatsu *et al*., 2007). The other components of the MRN complex, MRE11A and RAD51, are also upregulated in MCF-7. It is activated by ATM and in turn, activates ATM to induce CHK2 (Lee and Paull, 2005). It also associates with BRCA1 in response to DNA

damage (Komatsu *et al*., 2007). This promotes MCF-7 genomic integrity and stability and is a positive upregulation.

PCNA

PCNA is downregulated in comparison to normal breast cells. It acts as a loading platform in DNA replication and repair (Maga and Hubscher, 2003). This decreases DNA repair (Shiomi *et al*., 2002; Shivji *et al*., 1992; Levin *et al*., 2000; Jiricny, 2006; Dorazi *et al*., 2006). p21 and RB bind to PCNA to inhibit DNA replication (Maki and Howley, 1997; Sever-Chroneos *et al*., 2001). The decrease in genomic integrity promotes the oncogenic state of MCF-7.

RAD1

RAD1 is upregulated, in comparison to normal breast cells, to promote 9-1-1-mediated cell cycle arrest and DNA repair (Bermudez *et al*., 2003; Yin *et al*., 2004; Brandt *et al*., 2006). The other components of the 9-1-1 complex, RAD9A and HUS1, are downregulated in MCF-7. 9-1-1 upregulates p21 to enhance cell cycle arrest (Roos-Mattjus *et al*., 2003; Yin *et al*., 2004). This increases the genomic integrity and stability of MCF-7. It is also due to the higher level of DNA damage in MCF-7 compared to 184A1.

RAD17

RAD17 is upregulated to increase functioning of the checkpoints and inhibit re-replication (Zou *et al*., 2003; Wang *et al*., 2003). It loads the 9-1-1 complex onto

ssDNA to induce cell cycle arrest and DNA repair (Zou *et al*., 2003; Bermudez *et al*., 2003). This increases the genomic stability of MCF-7 to inhibit its oncogenic state.

RAD51

RAD51 is upregulated in comparison to normal breast cells. Although it promotes DNA repair, its overexpression in breast cancer cell lines promotes chromosomal rearrangement and aneuploidy by increased homologous recombination (Raderschall *et al*., 2002; Vispe *et al*., 1998; Richardson *et al*., 2004). It shows both upregulation and downregulation in different breast cancers (Maacke *et al*., 2000; Yoshikawa *et al*., 2001). This upregulation promotes genomic instability and tumourigenesis.

RAD9A

RAD9A is downregulated to reduce checkpoint signalling, cell cycle arrest, DNA repair and apoptosis in response to DNA damage (Toueille *et al*., 2004). It is part of the 9-1-1 complex and this downregulation decreases 9-1-1 translocation to the nucleus and damage sites (Hirai and Wang, 2002; Wu *et al*., 2005). This decreases p21 activation (Yin *et al*., 2004). RAD9A downregulation decreases the genomic integrity and stability of MCF-7 to promote oncogenic transformation. RAD9A is, however, overexpressed in breast cancer and is associated with a poor prognosis (Cheng *et al*., 2005; Borg *et al*., 1991).

RBL1

The tumour suppressor p107 is upregulated in relation to 184A1 to decrease cell cycle progression, DNA synthesis, checkpoints and promote differentiation (Cam and Dynlacht, 2003; Stevaux and Dyson, 2002; Lipinski and Jacks, 1999). It promotes SKP2 degradation to increase p27 (Rodier *et al*., 2005). Accordingly, SKP2 is downregulated in MCF-7. This positive upregulation increases genomic stability and reduces the oncogenic state of MCF-7. The DNA damage response is promoted in response to increased DNA damage compared to 184A1.

RBL2

The tumour suppressor p130 is upregulated, in comparison to normal breast cells, to inhibit cell cycle progression, promote cell cycle arrest and increase genomic stability (Grana *et al*., 1998; Hansen *et al*., 2001; Iavarone and Massague, 1999). This inhibits tumourigenesis. However, the region at which p130 is located (16q12.2) is frequently altered in breast cancer (Yeung *et al*., 1993).

SKP2

SKP2 is downregulated and decreases cell proliferation and oncogenesis (Gstaiger *et al*., 2001). This promotes the expression of E2F1, c-myc, p21, p27 and cyclin D1 (Marti *et al*., 1999; Kim *et al*., 2003; Bornstein *et al*., 2003; Nakayama *et al*., 2000; Ganiatsas *et al*., 2001). p21 and cyclin D1 are upregulated in MCF-7. This degradation is promoted by the upregulation of p107 (Rodier *et al*., 2005). p107 is upregulated in response to the

increased DNA damage in MCF-7 compared to 184A1, and consequently SKP2 is downregulated to increase genomic stability and reduce the oncogenic state.

SUMO1

SUMO1 is upregulated to inhibit the transcription of BRCA1-regulated genes, GADD45A, p27 and p21 (Park *et al*., 2008). GADD45A is downregulated in MCF-7. This promotes MCF-7 proliferation and decreases genomic stability to increase the oncogenic state.

3.4.5.2 MDA-MB-231 untreated

ABL1

ABL1 is upregulated to promote cell cycle arrest and apoptosis in response to DNA damage (Wang, 2000; Sionov *et al*., 1999). p53 and p21 expression increase to inhibit cell cycle progression (Wang, 2000; Sionov *et al*., 1999; Goga *et al*., 1995). MDA-MB-231 proliferation is inhibited and genomic integrity is promoted in comparison to normal breast cells. The increase in the DNA damage response is due to the higher level of DNA damage in MDA-MB-231.

ARHI

The tumour suppressor ARHI is downregulated in MDA-MB-231 to promote cell cycle progression and reduce apoptosis. This is achieved by inhibiting p21 and promoting cyclin D1 (Bao *et al*., 2002). It is downregulated by E2F1, E2F4 and histone acetylation (Lu *et al*., 2006; Fujii *et al*., 2003). ARHI is downregulated in breast cancers by LOH

(Wang *et al*., 2003). ARHI downregulation increases proliferation and genomic instability to promote tumourigenesis.

BAX

Pro-apoptotic BAX is upregulated, in relation to 184A1, to inhibit proliferation and cell survival, and promote DNA repair and apoptosis (Zinkel *et al*., 2006; Dumay *et al*., 2006; Reed, 1997). This beneficial upregulation increases genomic integrity and stability to inhibit the oncogenic state of MDA-MB-231. However, BCL2 is upregulated to a greater extent than BAX in MCF-7 and this inhibits apoptosis (**figure 3.39**).

BCCIP

The tumour suppressor BCCIP is upregulated to inhibit proliferation and promote DNA repair by increasing p21, BRCA1 and RAD51, and decreasing CDK2 activity (Liu *et al*., 2001; Ono *et al*., 2000; Lu *et al*., 2005). It is overexpressed in breast cancer where it inhibits proliferation (Meng *et al*., 2003; Liu *et al*., 2001). This upregulation increases the genomic integrity and stability of MDA-MB-231 and is in response to the elevated levels of DNA damage in comparison to 184A1.

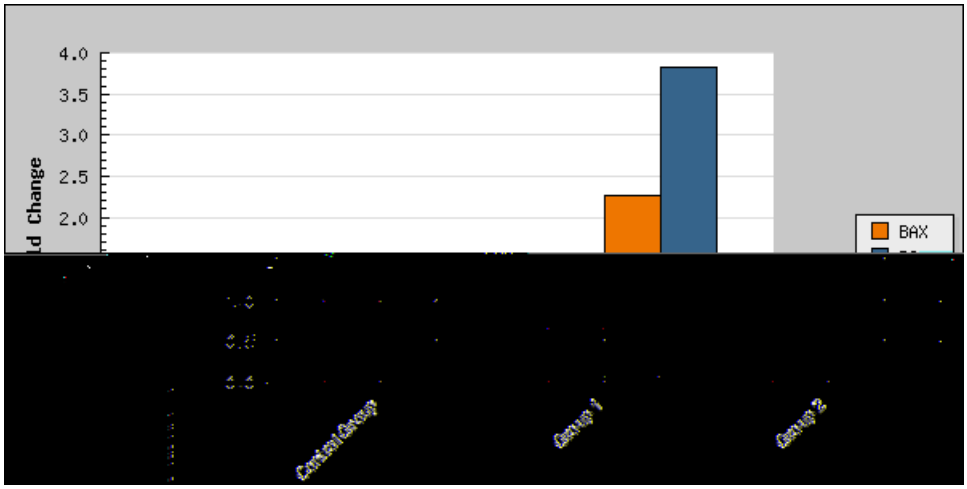


Figure 3.39: Fold change of BAX and BCL2 in MCF-7 untreated (group 1) and MDA-MB-231 untreated (group 2) in comparison to 184A1 untreated (control group).

BCL2

Anti-apoptotic BCL2 is upregulated, in relation to 184A1, to inhibit BAX and reduce apoptosis, cell cycle progression and DNA repair (Dlugosz *et al*., 2006; Mazel *et al*., 1996; Youn *et al*., 2005). It is overexpressed in breast cancer and confers resistance to chemotherapy and irradiation (Binder *et al*., 1995; Ramsay *et al*., 1995; Campos *et al*., 1993). However, BCL2 is downregulated in metastatic breast cancer, from which MDA-MB-231 is derived, and correlates with a poor prognosis (Thomadaki *et al*., 2007). BCL2 promotes tumourigenesis by increasing DNA damage (Deng *et al*., 2006). Although BAX is also upregulated in MDA-MB-231, BCL2 upregulation is greater (**figure 3.39**). This decreases the genomic integrity and stability of MDA-MB-231 and increases the oncogenic state.

BIRC5

Survivin is upregulated, in comparison to 184A1, to increase cell cycle progression, and inhibit cell cycle arrest and apoptosis (Dohi *et al*., 2004; Beltrami *et al*., 2004; Sarela *et al*., 2000). It is promoted by CDK4, which is upregulated in MDA-MB-231 (Sui *et al*., 2002). Survivin is overexpressed in breast cancer cells and correlates with increased aggressiveness and a poor prognosis (Tanaka *et al*., 2000). This increases proliferation and decreases genomic stability to promote the oncogenic state of MDA-MB-231.

BRCA1

BRCA1 is upregulated to promote DNA damage repair, cell cycle arrest, transcription, DNA replication and spindle stability (Venkitaraman, 2002; Eyfjord and Bodvarsdottir, 2005; Scully *et al*., 1997). It interacts with RAD51 to promote DNA repair (Haber, 1999; Davies *et al*., 2001). RAD51 is upregulated in MDA-MB-231. As RAD51 is overexpressed in tumour cell lines and breast cancer and induces aneuploidy, this upregulation promotes genomic instability (Vispe *et al*., 1998; Richardson *et al*., 2004). This increases the oncogenic state of MDA-MB-231. The DNA damage responses increase due to the higher level of damage in comparison to 184A1.

BRCA2

BRCA2 is upregulated, in relation to normal breast cells, to increase RAD51 activity in DNA repair (Galkin *et al*., 2005). BRCA2 and RAD51 are overexpressed in breast cancer and this amplifies homologous recombination to induce genomic instability (Egawa *et al*., 2002; Vispe *et al*., 1998; Richardson *et al*., 2004). BRCA2 also associates

with BRCA1, PCNA, and RAD51 to increase DNA replication (Lomonosov *et al*., 2003; Scully *et al*., 1997). BRCA1 and RAD51 are upregulated in MDA-MB-231. BRCA2 overexpression is associated with increased progression and a poor prognosis in sporadic breast tumours (Egawa *et al*., 2002). This promotes proliferation and decreases genomic integrity to increase tumourigenesis.

CCNB2

Cyclin B2 is upregulated, in relation to 184A1, and promotes cell cycle progression, particularly mitosis, and apoptosis (Morgan, 1995; Borgne *et al*., 2006). It forms the MPF complex with CDC2, which is also upregulated in MDA-MB-231. This increases the proliferation and genomic integrity of MDA-MB-231.

CCNC

Cyclin C is upregulated, in comparison to normal breast cells, to increase cell cycle progression and inhibit transcription (Ren and Rollins, 2004; Liu *et al*., 1998; Akoulitchev *et al*., 2000). It promotes CDC2, which is upregulated in MDA-MB-231 (Liu *et al*., 1998). Since cell cycle progression is promoted, cyclin C may be inhibiting the transcription of tumour suppressors. This promotes tumourigenesis in MDA-MB-231.

CCND1

Cyclin D1 is upregulated in relation to normal breast cells. This increases cell cycle progression, DNA replication and survival (Schwartz and Shah, 2005; Hinds *et al*., 1994; Fukami-Kobayashi and Mitsui, 1999). Premature DNA synthesis is inhibited to promote

genomic integrity (Fukami-Kobayashi and Mitsui, 1999). It inhibits RB, p107, p130, p27 and p21 (Lundberg and Weinberg, 1998; Perez-Roger *et al*., 1999). Cyclin D1 is overexpressed in breast cancer, enhancing the development and progression of breast cancer (Buckley *et al*., 1993; Bartkova *et al*., 1994). This promotes proliferation but decreases the genomic stability of MDA-MB-231 to increase the oncogenic state.

CCND2

Cyclin D2 is downregulated to decrease G_1 entry and cell survival (Schwartz and Shah, 2005; Hinds *et al*., 1994). Unlike cyclin D1, which is overexpressed in breast cancer, cyclin D2 expression is very low or absent in the majority of breast cancer cell lines due to promoter methylation (Buckley *et al*., 1993; Bartkova *et al*., 1994; Hui *et al*., 1996; Tam *et al*., 1994; Lukas *et al*., 1995; Evron *et al*., 2001). This downregulation promotes p27, which is upregulated in MDA-MB-231, and inhibits tumourigenesis (Bouchard *et al*., 1999).

CCNG2

Cyclin G2 is downregulated in MDA-MB-231 to promote the cell cycle, inhibit quiescence and decrease microtubule stability (Bennin *et al*., 2002; Arachchige Don *et al*., 2006). It promotes CDK2 to reduce cell cycle arrest (Bennin *et al*., 2002). Cyclin G2 levels are dependent on p53 and are low in proliferating cells (Horne *et al*., 1997). This correlates with increased proliferation and reduced genomic stability to promote the oncogenic state of MDA-MB-231.

CCNH

Cyclin H is downregulated in comparison to 184A1 to decrease transcription and DNA repair, and promote cell cycle progression (Svejstrup *et al*., 1996; Schaeffer *et al*., 1993; Andersen *et al*., 1997). As cell cycle progression increases, this suggests that the transcription of tumour suppressor genes is inhibited. This reduces genomic integrity and increases proliferation to promote tumourigenesis.

CCNT2

Cyclin T2 upregulation promotes transcription and differentiation (Palancade and Bensaude, 2003; Simone *et al*., 2002). This positive upregulation inhibits the transformation of MDA-MB-231.

CDC16

CDC16 is upregulated to increase APC/C activity and cell cycle progression (Rudner and Murray, 2000; Zachariae and Nasmyth, 1996). This increases MDA-MB-231 proliferation in comparison to 184A1 cells.

CDC2

CDC2 is upregulated in relation to normal breast cells. This activates CDC20 to promote cell cycle progression and transcription (Peters, 2002; Peters *et al*., 1998; Kobor and Greenblatt, 2002; Rudner and Murray, 2000). It is overexpressed in breast cancer and correlates with increased proliferation and metastasis (Megha *et al*., 1999). Its inhibitor

p53 is downregulated in MDA-MB-231 (Rudner and Murray, 2000). CDC2 increases tumourigenesis in MDA-MB-231.

CDC20

CDC20 is upregulated in comparison to 184A1. It is activated by CDC2, which is upregulated in MDA-MB-231 (Peters, 2002). It increases APC/C activation and cell cycle progression (Pfleger *et al*., 2001). Elevated CDC20 prematurely induces anaphase and genetic instability (Hwang *et al*., 1998). Its upregulation in breast cancer cells decreases checkpoint function and induces instability (Yuan *et al*., 2006). This promotes proliferation, genomic instability and tumourigenesis.

CDK2

CDK2 is upregulated in comparison to normal breast cells to increase DNA replication and cell cycle progression, and inhibit re-replication (Yoo *et al*., 2004; Fischer *et al*., 2004; Biermann *et al*., 2002; Peterson *et al*., 2000). It induces p27 degradation and inhibits RB activity (Fischer *et al*., 2004). This upregulation promotes proliferation but also contributes to the genomic integrity of MDA-MB-231.

CDK4

CDK4 is upregulated to increase MDA-MB-231 cell cycle progression (Pines, 1995). It is overexpressed in breast cancer and is associated with p53 degradation (Ortega *et al*., 2002; Hall and Peters, 1996). It inhibits RB activity and promotes survivin, which is upregulated here (Reynisdottir *et al*., 1995; Sui *et al*., 2002). This upregulation increases

proliferation and reduces genomic integrity, consequently promoting oncogenic transformation.

CDK7

CDK7 is upregulated in comparison to normal breast cells and promotes cell cycle progression, transcription and DNA repair (Harper and Elledge, 1998; Akoulitchev *et al*., 1995). This increases MDA-MB-231 proliferation and genomic integrity.

CDK8

CDK8 upregulation promotes genomic integrity through transcriptional control (Donner *et al*., 2007; Hengartner *et al*., 1998). It is induced by p21, which is upregulated in MDA-MB-231, and increases the transcription of p53-mediated genes (Donner *et al*., 2007). This inhibits the oncogenic state of MDA-MB-231.

CDKN1A

The tumour suppressor p21 is upregulated in comparison to 184A1 to inhibit cell cycle progression, DNA replication and apoptosis, and promote cell cycle arrest (Hengst and Reed, 1998; Ocker and Schneider-Stock, 2007; Maddika *et al*., 2007). This increases the genomic integrity and stability of MDA-MB-231 to inhibit the oncogenic state. It occurs at low levels in normal breast cells (Diab *et al*., 1997). The DNA damage responses increase due to the higher levels of genomic instability in MDA-MB-231 compared to 184A1.

CDKN1B

The tumour suppressor p27 is upregulated in relation to normal breast cells. This reduces cell cycle progression, and promotes apoptosis and differentiation (Munoz-Alonso *et al*., 2005; Kayatose *et al*., 1997; Robker and Richards, 1998). This positive upregulation increases genomic stability and reduces oncogenic transformation of MDA-MB-231.

CDKN2A

The downregulation of the tumour suppressor p16 in MDA-MB-231 increases entry into the S-phase, and promotes angiogenesis and metastasis by promoting cyclin C/CDK4 and cyclin C/CDK6 (Serrano *et al*., 1993; Gibson *et al*., 2005; Wang *et al*., 2006). Cyclin C and CDK4 are upregulated in MDA-MB-231. It inhibits RB activation to inhibit cell cycle arrest (Brenner *et al*., 1998). This increases proliferation and decreases genomic integrity to promote tumourigenesis. The complete CDKN2A-CDKN2B locus is deleted in cancers, as oncogenic transformation requires the inactivation of both p16 and p15 (Krimpenport *et al*., 2007). Both p16 and p15 are downregulated in MDA-MB-231 in relation to 184A1. p16 expression is also lost through methylation and mutations in breast cancer (Brenner *et al*., 1998).

CDKN2B

p15 is downregulated in comparison to normal breast cells and reduces cell cycle arrest and senescence. It promotes cyclin D/CDK4 and cyclin D/CDK6, and inhibits p27 (Reynisdottir *et al*., 1995; Massague, 2004; Massague *et al*., 2000; Swarbrick *et al*., 2000). By promoting telomerase activity, it increases the proliferative lifespan of

MDA-MB-231 (Fuxe *et al*., 2000). This decreases genomic stability and increases oncogenic transformation.

CDKN3

CDKN3 upregulation inhibits CDK2 and decreases cell cycle progression and transcription, including that of p53 and p21 (Chinami *et al*., 2005; Schultz *et al*., 2002; Okamoto *et al*., 2006). CDK2 is upregulated in MDA-MB-231. However, CDKN3 is overexpressed in breast cancer where it promotes tumourigenesis (Yeh *et al*., 2003; Lee *et al*., 2000). It is inactivated by ATM following DNA damage (Ziv *et al*., 2006). This upregulation reduces genomic stability and increases tumourigenesis in MDA-MB-231.

CHEK1

CHK1 is upregulated, in relation to normal breast cells, to reduce DNA damage and promote checkpoint function during replication (Petermann *et al*., 2006; Durkin *et al*., 2006; Gatei *et al*., 2003). It phosphorylates p53 and BRCA1 to induce cell cycle arrest, DNA repair and apoptosis (Pommier *et al*., 2005). BRCA1 is upregulated in MDA-MB-231. This increases MDA-MB-231 genomic integrity and stability.

CKS1B

CKS1B upregulation increases CDC2 activity and induces p27 degradation to promote cell cycle progression (Egan and Solomon, 1998; Cardozo and Pagano, 2004; Bloom and Pagano, 2003). It is upregulated in breast cancer where it correlates with increased aggressiveness and a poor prognosis, with levels higher in ER- MDA-MB-231 than in

ER+ MCF-7 (Slotky *et al*., 2005). It is upregulated in immortalised breast cancer cell lines in comparison to non-immortalised cell lines derived from breast cancer patients (Fernandez-Cobo *et al*., 2006). This promotes proliferation and decreases genomic stability to increase tumourigenesis.

CKS2

CKS2 is upregulated, in relation to 184A1, to increase cell cycle progression (Rother *et al*., 2007). This promotes MDA-MB-231 proliferation and tumourigenesis.

CUL1

CUL1 is upregulated to induce p27 degradation and promote G_1/S cell cycle progression (Deshaies, 1999). This increases MDA-MB-231 proliferation and decreases genomic stability to promote tumourigenesis in comparison to normal breast cells.

CUL3

CUL3 is downregulated in comparison to 184A1 and this reduces integrity during mitosis and promotes tumourigenesis (Sumara *et al*., 2007; Wilkins *et al*., 2004). This downregulation increases the oncogenic state of MDA-MB-231.

DNM2

The downregulation of DNM2 reduces p53 activation and apoptosis (De Camilli *et al*., 1995; Fish *et al*., 2000). p53 is downregulated in MDA-MB-231. This increases the oncogenic state of MDA-MB-231 and promotes tumourigenesis.

GADD45A

GADD45A is upregulated to inhibit cyclin B1/CDC2, and increase cell cycle arrest and DNA repair (Jin *et al*., 2002; Carrier *et al*., 1999). It is induced in response to stress, such as oncogenic stress and DNA damage (Fornace *et al*., 1988; Yamauchi *et al*., 2007; Juriloff and Harris, 2000). This upregulation is in response to the increase in DNA damage compared to normal breast cells. It promotes genomic integrity and stability in MDA-MB-231 to inhibit the oncogenic state.

HERC5

HERC5 is upregulated, in comparison to normal breast cells, to promote the immune response and increase MDA-MB-231 stability (Dastur *et al*., 2006).

HUS1

HUS1 is downregulated to reduce checkpoint function, DNA repair and replication fork stability, and increase mitosis (Walworth and Bernards, 1996; Wang *et al*., 2006; Parrilla-Castellar *et al*., 2004; Lindsay *et al*., 1998). It is part of the 9-1-1 DNA damage response complex, and although RAD1 is upregulated, RAD9A is also downregulated (Francia *et al*., 2006). This decreases genomic stability and increases the oncogenic state of MDA-MB-231.

KPNA2

KPNA2 is upregulated in relation to 184A1. It promotes the DNA damage responses but its overexpression in breast cancer correlates with a poor prognosis (Dahl *et al*., 2006;

Tseng *et al*., 2005). KPNA2 therefore increases the oncogenic transformation of MDA-MB-231. It also promotes the activity of BRCA1, p53 and MRN (Thakur *et al*., 1997; Kim *et al*., 2000; Tseng *et al*., 2005). BRCA1, MRE11A, RAD51 and NBS1 are upregulated in MDA-MB-231.

MAD2L1

MAD2L1 is upregulated in comparison to normal breast cells. It promotes checkpoint function and inhibits CDC20 from prematurely activating APC (Amon, 1999; Chen *et al*., 1996b; Fang *et al*., 1998). It is overexpressed in breast cancer and breast cancer cell lines where it inhibits integrity at the checkpoints to induce genomic instability (Scintu *et al*., 2007; Yuan *et al*., 2006; Rouzier *et al*., 2005). This upregulation decreases genomic integrity and induces tumourigenesis in MCF-7.

MAD2L2

MAD2L2 is upregulated in comparison to 184A1. It increases spindle checkpoint function and DNA repair (Yuan *et al*., 2006; Burds *et al*., 2005; Ying and Wold, 2003). It is mutated in breast cancer and its overexpression in breast cancer and breast cancer cell lines promotes defective checkpoints and genomic instability (Percy *et al*., 2000; Yuan *et al*., 2006). MAD2L2 upregulation therefore decreases genomic integrity and induces tumourigenesis in MDA-MB-231.

MCM3

MCM3 upregulation promotes cell cycle arrest, transcription and DNA replication, and prevents re-replication in MDA-MB-231 (Cortez *et al*., 2004; Dziak *et al*., 2003; Ying and Gautier, 2005; Labib *et al*., 2000). As MCM3 is only expressed in undifferentiated cells, this upregulation correlates with an increased oncogenic state (Endl *et al*., 2001; Musahl *et al*., 1998). The increase in genomic integrity is in response to increased DNA damage compared to 184A1.

MCM4

MCM4 is upregulated in relation to 184A1. This inhibits DNA re-replication and promotes cell cycle arrest, transcription and replication (Labib *et al*., 2000; Ishimi *et al*., 2004; Dziak *et al*., 2003; Davey *et al*., 2003). Its replicative activity is inhibited by ATR, CHK1 and CHK2 following DNA damage, however these are not upregulated in MDA-MB-231 (Ishimi *et al*., 2003; Zhu *et al*., 2005). This increases proliferation and the genomic integrity, in response to higher levels of DNA damage.

MCM5

MCM5 is upregulated, in relation to normal breast cells, to inhibit DNA re-replication, and promote checkpoint activation, transcription and proliferation (Labib *et al*., 2000; Cortez *et al*., 2004; Snyder *et al*., 2005; Stoeber *et al*., 2002; Williams *et al*., 1998). This correlates with dysplasia (Murphy *et al*., 2005). MCM5 increases tumourigenesis in MDA-MB-231 and the increase in DNA damage responses is due to the elevated levels of DNA damage in comparison to 184A1.

MKI67

Ki-67 is upregulated in comparison to normal breast cells. It is expressed at very low levels in normal breast tissue (Harper-Wynne *et al*., 2002; Clarke *et al*., 1997; de Lima *et al*., 2003). This upregulation correlates with increased proliferation and apoptosis (Endl and Gerdes, 2000; Lipponen, 1999). Ki-67 upregulation increases breast cancer progression and is associated with a poor prognosis (Spyratos *et al*., 2002; Trihia *et al*., 2003; Colozza *et al*., 2005). Ki-67 promotes oncogenic transformation of MDA-MB-231.

MRE11A

MRE11A is upregulated, in relation to 184A1, and increases cell cycle arrest, DNA repair, apoptosis and proliferation (Lee and Paull, 2005; Yamaguchi-Iwai *et al*., 1999; Paull and Gellert, 1998). It is part of the MRN DNA damage response complex, with NBS1, which is upregulated in MDA-MB-231. NBS1 activates MRE11A following DNA damage (Dong *et al*., 1999). This upregulation promotes proliferation and genomic integrity in response to increased DNA damage.

NBS1

NBS1 upregulation increases checkpoint function in response to DNA damage (Stracker *et al*., 2004). It is part of the MRN complex, of which MRE11A is also upregulated in MDA-MB-231. It associates with BRCA1, which is upregulated here, at DNA damage sites (Komatsu *et al*., 2007). This upregulation increases genomic integrity and stability of MDA-MB-231 in response to the higher level of DNA damage.

RAD1

RAD1 is upregulated in comparison to normal breast cells. It is part of the 9-1-1 DNA damage response complex and promotes checkpoint activation and DNA repair (Bermudez *et al*., 2003; Brandt *et al*., 2006). It promotes p21, which is also upregulated in MDA-MB-231. This increases genomic integrity in response to DNA damage.

RAD17

RAD17 is upregulated to promote checkpoint activation and DNA repair, and inhibit re-replication (Wang *et al*., 2003; Zou *et al*., 2003; Bermudez *et al*., 2003). This increase in the DNA damage response promotes genomic stability following DNA damage.

RAD51

RAD51 is upregulated in MDA-MB-231 in comparison to normal breast cells. It is overexpressed in tumour cell lines where high levels of homologous recombination induce chromosomal rearrangements and instability (Vispe *et al*., 1998; Richardson *et al*., 2004; Gupta *et al*., 1997). Both upregulation and downregulation of RAD51 is observed in breast cancer (Maacke *et al*., 2000; Yoshikawa *et al*., 2000). It is overexpressed in breast cancer cell lines (Raderschall *et al*., 2002). This upregulation decreases genomic integrity and promotes oncogenic transformation.

RAD9A

The downregulation of RAD9A in MDA-MB-231 decreases checkpoint signalling, cell cycle arrest, DNA repair and apoptosis (Toueille *et al*., 2004). It forms the 9-1-1

complex with RAD1 and HUS1. RAD1 is upregulated and HUS1 is downregulated here. The activity of 9-1-1 and p21 decreases and DNA damage accumulates (Hirai and Wang, 2002; Wu *et al*., 2005; Yin *et al*., 2004). This reduces the genomic integrity and stability, and increases the oncogenic state. However, RAD9 is overexpressed in breast cancer, where it correlates with poor prognosis (Cheng *et al*., 2005; Borg *et al*., 1991).

RBL1

The tumour suppressor p107 is upregulated to decrease cell cycle progression, DNA synthesis, checkpoint function and promote differentiation (Cam and Dynlacht, 2003; Stevaux and Dyson, 2002; Vanderluit *et al*., 2007). Its upregulation inhibits MCF-7 growth (Beijersbergen *et al*., 1996). This increases the genomic stability of MDA-MB-231 in response to DNA damage, and inhibits transformation.

RBL2

The tumour suppressor p130 is upregulated in MDA-MB-231 to inhibit cell cycle progression and growth, and promote quiescence and cell cycle arrest (Ren *et al*., 2002; Beijersbergen *et al*., 1995; Grana *et al*., 1998; Woo *et al*., 1997; Hansen *et al*., 2001). It downregulates CDC2 to suppress mitotic entry, and inhibits S-phase entry under unfavourable conditions (Taylor *et al*., 2001; Iavartine and Massague, 1999). This increases genomic stability following DNA damage, and decreases the oncogenic state in comparison to 184A. The p130 region is, however, frequently altered in breast cancer (Yeung *et al*., 1993).

RPA3

RPA3 is downregulated and decreases DNA repair and apoptosis (He *et al*., 1995; Iftode *et al*., 1999). This reduces RAD17 and CHK1 function (Foray *et al*., 2003). The decrease in genomic integrity promotes the oncogenic state of MDA-MB-231.

SUMO1

SUMO1 is upregulated, in relation to normal breast cells, to inhibit GADD45A, p27 and p21 transcription (Park *et al*., 2008). This decreases genomic integrity and promotes the oncogenic transformation of MDA-MB-231.

TP53

The tumour suppressor p53 is downregulated in comparison to 184A1. This decreases cell cycle arrest, DNA repair, senescence, differentiation and apoptosis (Finlan and Hupp, 2005; Giono and Manfredi, 2006). It reduces p21, GADD45A and BAX expression, and RB activation (Sherr and Roberts, 1999; Maddika *et al*., 2007; Miyashita and Reed, 1995). BCL2 is promoted (Chipuk *et al*., 2004; Mihara *et al*., 2003). BCL2 is upregulated in MDA-MB-231. This reduces genomic integrity and stability and increases tumourigenesis.

		Table 3.20: Influence of drugs on gene expression in cell lines	
Gene	**Up/downregulation in cell**	**Result of up/downregulation**	**Influence of drug**
ABL1	MDA-MB-231: Upregulated	Promotes DNA damage response.	Upregulated by lycopene, downregulated by thalidomide.
ANAPC4	MCF-7: Upregulated	Promotes proliferation and transformation.	Upregulated by thalidomide.
ARHI	MCF-7: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis.	Upregulated by thalidomide.
	MDA-MB-231: Downregulated		Upregulated by lycopene and thalidomide.
BAX	MDA-MB-231: Upregulated	Promotes DNA damage response.	Upregulated by thalidomide.
BCCIP	MDA-MB-231: Upregulated	Promotes DNA damage response.	
BCL2	MCF-7: Downregulated	Promotes proliferation and transformation.	Overexpression downregulated by thalidomide.
	MDA-MB-231: Upregulated	Decreases cell cycle arrest and apoptosis.	Downregulated by thalidomide.
BIRC5	MDA-MB-231: Upregulated	Promotes proliferation and transformation.	Downregulated by thalidomide.
BRCA1	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response and oncogenic state.	
	MDA-MB-231: Upregulated		

BRCA2	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response and oncogenic state.	
			Downregulated by thalidomide.
CCNB1	MCF-7: Downregulated	Decreases cell cycle arrest and apoptosis.	
CCNB2	MDA-MB-231: Upregulated	Promotes proliferation, promotes DNA damage response.	
CCNC	MCF-7: Upregulated	Promotes proliferation and transformation.	
	MDA-MB-231: Upregulated		
CCND1	MCF-7: Upregulated	Promotes proliferation and transformation.	Upregulated by lycopene.
	MDA-MB-231: Upregulated		Upregulated by lycopene. Downregulated by thalidomide.
CCNF	MCF-7: Upregulated	Promotes proliferation and transformation.	Downregulated by thalidomide.
CCNG2	MDA-MB-231: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis.	Upregulated by lycopene and thalidomide.
CCNH	MDA-MB-231: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis.	Upregulated by lycopene and thalidomide.
CCNT2	MCF-7: Upregulated	Promotes proliferation, promotes differentiation	Upregulated by thalidomide.

	MDA-MB-231: Upregulated		
CDC16	MCF-7: Upregulated	Promotes proliferation and transformation.	
	MDA-MB-231: Upregulated		Downregulated by lycopene.
CDC2	MCF-7: Upregulated	Promotes proliferation, promotes angiogenesis and metastasis.	
	MDA-MB-231: Upregulated		Downregulated by lycopene.
CDC20	MDA-MB-231: Upregulated	Promotes proliferation and transformation.	Downregulated by lycopene and thalidomide.
CDC34	MCF-7: Upregulated	Promotes proliferation and transformation.	
CDK2	MDA-MB-231: Upregulated	Promotes proliferation and transformation.	Downregulated by lycopene and thalidomide.
CDK4	MCF-7: Upregulated	Promotes proliferation and transformation.	Downregulated by thalidomide.
	MDA-MB-231: Upregulated		Downregulated by thalidomide.
CDK5R1	MCF-7: Downregulated	Inhibits senescence, decreases cell cycle arrest and apoptosis, inhibits differentiation	
CDK7	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response	Upregulated by lycopene and thalidomide.
	MDA-MB-231: Upregulated		Downregulated by thalidomide.
CDKN1A	MCF-7: Upregulated	Promotes DNA damage response.	
	MDA-MB-231: Upregulated		Upregulated by lycopene and thalidomide.

CDKN1B	MCF-7: Upregulated	Promotes DNA damage response, promotes differentiation.	Upregulated by lycopene.
	MDA-MB-231: Upregulated		Downregulated by thalidomide.
CDKN2A	MCF-7: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis, promotes angiogenesis and metastasis	Upregulated by thalidomide.
	MDA-MB-231: Downregulated		
CDKN2B	MCF-7: Downregulated	Promotes proliferation, inhibits senescence, decreases cell cycle arrest and apoptosis	Upregulated by lycopene and thalidomide.
	MDA-MB-231: Downregulated		
CHEK1	MDA-MB-231: Upregulated	Promotes DNA damage response.	
CHEK2	MCF-7: Upregulated	Promotes DNA damage response.	Upregulated by thalidomide.
CKS1B	MCF-7: Upregulated	Promotes proliferation and transformation.	
	MDA-MB-231: Upregulated		
CKS2	MCF-7: Upregulated	Promotes proliferation and transformation.	
	MDA-MB-231: Upregulated		
CUL1	MDA-MB-231: Upregulated	Promotes proliferation and transformation.	
CUL3	MCF-7: Upregulated	Promotes proliferation and transformation.	Upregulated by lycopene.
DNM2	MDA-MB-231: Downregulated	Decreases cell cycle arrest and apoptosis	Upregulated by lycopene.

GADD45A	MCF-7: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis	Upregulated by lycopene.
	MDA-MB-231: Upregulated	Promotes DNA damage response.	
HUS1	MCF-7: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis	Upregulated by thalidomide.
	MDA-MB-231: Downregulated		
KPNA2	MCF-7: Upregulated	Promotes DNA damage response, promotes oncogenic state	Upregulated by lycopene.
	MDA-MB-231: Upregulated		Downregulated by lycopene.
MAD2L1	MCF-7: Upregulated	Promotes DNA damage response, promotes oncogenic state	
	MDA-MB-231: Upregulated		Downregulated by lycopene and thalidomide.
MAD2L2	MDA-MB-231: Upregulated	Promotes DNA damage response, promotes oncogenic state.	
MCM2	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response, promotes oncogenic state	
MCM3	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response.	
	MDA-MB-231: Upregulated		Downregulated by thalidomide.
MCM4	MDA-MB-231: Upregulated	Promotes proliferation, promotes DNA damage	Downregulated by thalidomide.

		response.	
MCM5	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response, promotes oncogenic state	
	MDA-MB-231: Upregulated		Upregulated by lycopene.
MKI67	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response, promotes oncogenic state	
	MDA-MB-231: Upregulated		
MNAT1	MCF-7: Upregulated	Promotes proliferation and transformation	
MRE11A	MCF-7: Upregulated	Promotes proliferation, promotes DNA damage response	Upregulated by thalidomide.
	MDA-MB-231: Upregulated		
NBS1	MCF-7: Upregulated	Promotes DNA damage response.	Upregulated by thalidomide.
	MDA-MB-231: Upregulated		
RAD1	MCF-7: Upregulated	Promotes DNA damage response.	
	MDA-MB-231: Upregulated		Upregulated by lycopene.
RAD17	MCF-7: Upregulated	Promotes DNA damage response.	Upregulated by lycopene and thalidomide.
	MDA-MB-231: Upregulated		Upregulated by thalidomide.
RAD51	MCF-7: Upregulated	Promotes DNA damage response, promotes oncogenic state	Downregulated by lycopene and thalidomide.
	MDA-MB-231: Upregulated		

RAD9A	MCF-7: Downregulated MDA-MB-231: Downregulated	Decreases cell cycle arrest and apoptosis	Upregulated by thalidomide.
RBL1	MCF-7: Upregulated MDA-MB-231: Upregulated	Decreases cell cycle arrest and apoptosis, promotes differentiation	
RBL2	 MDA-MB-231: Upregulated	Promotes DNA damage response.	Upregulated by lycopene.
RPA3	MDA-MB-231: Downregulated	Decreases cell cycle arrest and apoptosis	Upregulated by thalidomide.
SUMO1	MCF-7: Upregulated MDA-MB-231: Upregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis	
TP53	MDA-MB-231: Downregulated	Promotes proliferation, decreases cell cycle arrest and apoptosis, inhibits differentiation.	

3.4.6 Summary

The use of the SuperArray PCR Array system has identified numerous differentially expressed genes, influenced by oncogenic transformation and by the chemotherapeutic drugs. This provides valuable information in deciphering the genetic pathways involved in carcinogenesis. It also increases the understanding of the molecular pathways targeted by these drugs. This information will enable greater control of the drug with regards to its influence on genes. It allows the investigators to screen for genes that may increase the potential of colon and breast cancer after drug administering, as it is unlikely that a perfect drug exists that only has beneficial outcomes.

CHAPTER FOUR: DISCUSSION

4.1 Genefishing

Four mitochondrial-associated genes are differentially expressed and identified between control cells and drug treated-cells, namely SAMM50, COII, COIII and MT-ATP6. This is in accordance with previous studies where chemotherapeutic drugs are observed to localise and accumulate in mitochondria, and induce apoptosis by disrupting the mitochondrial membrane (Talarico *et al*., 2001; Olivero *et al*., 1997; Ruefli *et al*., 2001). By inducing the expression of SAMM50, curcumin and SAHA are promoting mitochondrial-mediated apoptosis in HT-29 cells. Lycopene promotes mitochondrial-induced apoptosis and inhibits tumourigenesis by inducing the expression of COIII and MT-ATP6 in MCF-7 (Heerdt *et al*., 1990; Shin *et al*., 2005). Thalidomide and DMSO may induce the expression of COII to increase the sensitivity of MCF-7 to thalidomide treatment (Singh *et al*., 1999).

Due to the high proliferative rate of cancer cells, mitochondria are not able to supply sufficient ATP for tumour cells in hypoxic conditions; therefore, the glycolytic pathway is upregulated (Gogvadze *et al*., 2008; Wang and Semenza, 1993). While 95% of normal cellular energy is produced by oxidative phosphorylation in mitochondria, the majority of cancer cells have few mitochondria and are highly glycolytic (Singh, 1998; Rempel *et al*., 1996). Tumour cell aggressiveness correlates with glycolytic ATP production (Simonnet *et al*., 2002). Curcumin, SAHA, lycopene and thalidomide induce mitochondrial components to decrease mitochondrial transmembrane potential and increase

cytochrome *c* release. This implies a decrease in tumour cell proliferation and aggressiveness, and an increase in apoptosis.

SAHA and lycopene inhibit proliferation and metastasis in HT-29 and MCF-7 respectively by inducing the downregulation of GPNMB and E1F4A1. Similarly, lycopene and thalidomide are implicated in inhibiting carcinogenesis in MCF-7 by inducing the downregulation of ZNF350. This concurs with results obtained from the SuperArray PCR Array experiments.

The problem arising from using the Genefishing differential display system is that the differential expression cannot be quantified as with the SuperArray system. The investigator must use qualitative assessment to decide as to whether the band intensity is sufficiently bold to qualify as a differentially expressed band. The majority of the differentially expressed bands show weak amplification and/or small size (<300 base pairs). Consequently, these cannot be successfully sequenced. A further problem is that the arbitrary primers are too long for sequencing. It was therefore necessary to synthesise a universal sequencing primer for the PCR reactions leading up to sequencing. The sequencing of the differentially expressed bands generated from the Genefishing system had a low success rate and this system did not provide much information on the effects of the drugs on gene expression in breast and colon cancer.

4.2 SuperArray

Cancer progression is driven by a subpopulation of cells in a tumour, not all the cells, to produce tumours with cellular heterogeneity (Al-Hajj *et al*., 2003). The genes encoding cell cycle-regulatory proteins are frequently altered in cancer. These alterations target the cell cycle components and machinery directly or components of upstream signalling cascades that trigger cell cycle steps. The accumulation of multiple alterations, such as oncogene activation and tumour suppressor inactivation, gives cells a selective advantage to escape internal control and proliferate without restraint, forming tumours.

4.2.1 Are lycopene and thalidomide associated with the inhibition of oncogenic transformation in MCF-7 and MDA- MB-231?

4.2.1.1 MCF-7

In breast cancer cell lines, 61% of genes are upregulated compared to breast tumours; with the cell cycle pathway being significantly altered (Ertel *et al*., 2006). Low-grade breast tumours are ER+ with low levels of genetic aberrations (Simpson *et al*., 2005). MCF-7 is an ER+ breast cancer cell line. Oestrogen promotes tumour growth in ER+ cells by inducing the transcription of cell cycle promoting genes, and inhibiting genes that suppress the cell cycle (Frasor *et al*., 2003). CDKN1A is upregulated in MCF-7 compared to 184A1, and its expression is regulated by oestrogens (Foster *et al*., 2001). It is overexpressed early in breast cancer, in DCIS and invasive breast cancer, and correlates with differentiation, ER+ and a negative p53 status (Wang *et al*., 2003; Mommers *et al*., 2001). While TP53 is upregulated in MCF-7, it is not a significant fold change compared to 184A1.

While there is no evidence that proliferation in normal breast epithelium and benign breast lesions is a marker of breast cancer risk, proliferation and apoptosis do increase with the progression from normal to malignant epithelium (Bai *et al*., 2001). Accordingly, genes involved in proliferation are upregulated in malignant breast cells compared to normal breast cells (Grigoriadis *et al*., 2006). The genes that are upregulated in MCF-7, relative to 184A1, that may promote proliferation are: ANAPC4, BRCA1, BRCA2, CCNC, CCND1, CCNF, CCNT2, CDC16, CDC2, CDC34, CDK4, CDK7, CKS1B, CKS2, CUL3, MCM2, MCM3, MCM5, MKI67, MNAT1, MRE11A and SUMO1. In breast cancers, 60-80% of cases are associated with BRCA1 and BRCA2 (Nathanson *et al*., 2001). BRCA1 is upregulated by chromosomal gain at 17q in breast cancer cell lines compared to tumours (Forozan *et al*., 2000).

CCND1 and MKI67 are involved in the progression of DCIS to invasive carcinoma (Grigoriadis *et al*., 2006; Menter *et al*., 2001; Hisatomi *et al*., 2002). Oestrogens regulate the expression of CCND1, and breast tumours overexpressing cyclin D1 have a very poor prognosis (Foster *et al*., 2001; Ahnstrom *et al*., 2005). CKS1B at chromosome 1q is upregulated by chromosomal gain in breast cancer cell lines compared to tumours (Forozan *et al*., 2000). In accordance with other studies, MCM2, MCM3, MCM5 and MKI67 are overexpressed in MCF-7 cells (Whitfield *et al*., 2006). MCM2, at chromosome 3q, is upregulated in breast cancer cell lines compared to tumours (Forozan *et al*., 2000).

Genes that are downregulated in MCF-7 and that are involved in promoting proliferation are ARHI, BCL2, CDKN2A, CDKN2B, GADD45A and HUS1. ARHI (chromosome 1p), BCL2 (chromosome 18q) and GADD45A (chromosome 1p) are downregulated in MCF-7, and these chromosomes are commonly lost in breast cancer cell lines compared to tumours (Forozan *et al*., 2000). CDKN2A transcription is silenced by promoter hypermethylation in solid tumours (Shahjehan *et al*., 2001; Herman *et al*., 1995). CDKN2B is downregulated here in comparison to 184A1, but has been shown in other studies to be upregulated in MCF-7 by oestrogens (Cariou *et al*., 2000). Although PCNA is frequently overexpressed in cancer cells, it is downregulated in MCF-7, but not with a significant fold change (Whitfield *et al*., 2006; Ertel *et al*., 2006). These specific genes may contribute to oncogenic transformation (the transformation of a normal cell to a cancerous cell) by promoting proliferation.

Senescence is induced by DNA damage and dysfunctional telomeres, and arrests proliferation to inhibit cancer development (Campisi *et al*., 2001; Kim *et al*., 2002; Beausejour *et al*., 2003). During tumourigenesis, cells bypass senescence checkpoints to achieve immortality and full malignancy (Yaswen and Stampfer, 2002). CDK5R1 and CDKN2B are downregulated in MCF-7, in comparison to 184A1, and consequently may inhibit senescence. CDKN2B transcription is silenced by promoter hypermethylation in multiple myeloma, suggesting a similar occurrence here (Ng *et al*., 1997).

The therapeutic mechanisms of cell cycle arrest and apoptosis eradicate damaged cells (Choudhuri *et al*., 2005). Cancer cells bypass DNA and spindle checkpoints to increase

genetic instability (Fischer *et al*., 2004). Checkpoint integrity is lost in cancer cells because of CKI inactivation or cyclin overexpression, consequently promoting transformation (Shahjehan *et al*., 2001; Cahill *et al*., 1998). The upregulation of SUMO1 is implicated in decreasing cell cycle arrest and apoptosis in this study. Genes that are downregulated in MCF-7 and that may decrease cell cycle arrest and apoptosis are ARHI, CCNB1, CDK5R1, CDKN2A, CDKN2B, GADD45A, HUS1 and RAD9A. The downregulation of the ARHI, CDKN2A, CDKN2B and GADD45A tumour suppressor genes has been shown to give the cell a selective advantage to escape internal control and to proliferate clonally (Folkman, 2001). ARHI expression is lost by LOH and the other allele by promoter methylation in breast cancer (Bao *et al*., 2002).

The DNA damage response markers do not occur in cells that are proliferating normally. This response system is activated in pre-cancerous and early cancerous breast lesions (Bartkova *et al*., 2005). Genes that are upregulated in MCF-7 and that may promote the DNA damage responses and DNA repair are ANAPC4, BRCA1, BRCA2, CCNF, CDK7, CDKN1A, CDKN1B, CHEK2, KPNA2, MAD2L1, MCM2, MCM3, MCM5, MKI67, MRE11A, NBS1, RAD1, RAD17, RAD51 and RBL2. However, the overexpression of BRCA1, BRCA2, CCNF, KPNA2, MAD2L1, MCM2, MCM5, MKI67 and RAD51 is demonstrated to promote the oncogenic state (cancer-causing state) and correlates with a poor prognosis in breast cancer patients (Vispe *et al*., 1998; Richardson *et al*., 2004; Keyomarsi *et al*., 2002; Dahl *et al*., 2006; Scintu *et al*., 2007; Yuan *et al*., 2006; Dudderidge *et al*., 2005; Hashimoto *et al*., 2004; Murphy *et al*., 2005; Trihia *et al*., 2003; Colozza *et al*., 2005).

During metastasis, cancer cells migrate from the tissue of origin to new sites via the circulatory system. An essential requirement for growth and metastasis is angiogenesis, where tumour cells induce the formation of blood vessels to supply the tumour with nutrients and oxygen, and remove waste products (Folkman, 2001). The upregulation of CDC2 and the downregulation of CDKN2A in MCF-7 may promote angiogenesis and metastasis. Differentiated tumour cells have similar structure and function as normal cells. They grow and metastasise at a slower rate than undifferentiated tumour cells that proliferate uncontrollably. The upregulation of CCNT2, CDKN1B and RBL1 is implicated in promoting differentiation and inhibiting the oncogenic state of MCF-7. CDK5R1 is downregulated in MCF-7 and may inhibit differentiation and promote tumourigenesis (the production of a new tumour).

Forty genes are differentially expressed in MCF-7 compared to 184A1. By characterising their functions, lycopene is associated with a positive, anti-tumourigenic influence on 27.5% of their activities, and a negative, carcinogenic influence on 10% of their activities. Thalidomide is implicated as having a positive therapeutic influence on 57.5% of their activities, and a negative, carcinogenic influence on 7.5% of their activities. With regards to MCF-7, while lycopene demonstrates a good anti-tumourigenic effect (dissuades the production of a tumour) on the genes, thalidomide clearly has a more profound influence as a chemotherapeutic drug through its association with inhibiting the oncogenic transformation of breast cells.

4.2.1.2 MDA-MB-231

Genes that are upregulated in MDA-MB-231, in comparison to 184A1, and that may promote proliferation are: BIRC5, BRCA1, BRCA2, CCNC, CCND1, CCNT2, CDC16, CDC2, CDC20, CDK2, CDK4, CDK7, CKS1B, CKS2, CUL1, MCM3, MCM4, MCM5, MKI67, MRE11A and SUMO1. BIRC5 (chromosome 17q), CDC20 (chromosome 1p), CKS1B (chromosome 1q), CUL1 (chromosome 7q) and MCM4 (chromosome 8q) are upregulated in breast cancer cell lines compared to tumours by chromosomal gains (Forozan *et al.* 2000). Genes that are downregulated that may promote proliferation are ARHI, CCNG2, CCNH, CDKN2A, CDKN2B, HUS1 and TP53. ARHI is downregulated in breast cancer cell lines compared to tumours due to chromosomal loss at chromosome 1p (Forozan *et al*., 2000). CDKN2B and TP53 are downregulated in MDA-MB-231 and are implicated in inhibiting senescence. Genes that are upregulated in MDA-MB-231 and that may consequently decrease cell cycle arrest and apoptosis are BCL2, BIRC5, RBL1 and SUMO1. Chromosomal gain at chromosome 20q, where RBL1 is located, has been shown to induce upregulation of the gene in breast cancer cell lines (Forozan *et al*., 2000). Genes that are downregulated in MDA-MB-231 and that may decrease cell cycle arrest and apoptosis are ARHI, CCNG2, CCNH, CDKN2A, CDKN2B, DNM2, HUS1, RAD9A, RPA3 and TP53. Previous work illustrates that RAD9A is localised at chromosome 11q, where chromosomal loss occurs in breast cancer cell lines, and this induces the downregulation of this gene (Forozan *et al*., 2000).

Genes that are upregulated in MDA-MB-231 and that are implicated in promoting the DNA damage responses and DNA repair are: ABL1, BAX, BCCIP, BRCA1, BRCA2,

CCNB2, CDK7, CDKN1A, CDKN1B, CHEK1, GADD45A, KPNA2, MAD2L1, MAD2L2, MCM3, MCM4, MCM5, MKI67, MRE11A, NBS1, RAD1, RAD17, RAD51 and RBL2. However, the overexpression of BRCA1, BRCA2, KPNA2, MAD2L1, MAD2L2, MCM5, MKI67 and RAD51 may promote the oncogenic state of MDA-MB-231 and has been shown to correlate with a poor prognosis in breast cancer patients. GADD45A (chromosome 1p), KPNA2 (chromosome 17q), MAD2L2 (chromosome 1p), NBS1 (chromosome 8q) and RAD51 (chromosome 5p) are upregulated due to chromosomal gains in breast cancer cell lines compared to tumours (Forozan *et al*., 2000). The upregulation of CDC2 and the downregulation of CDKN2A in MDA-MB-231 may promote angiogenesis and metastasis. Genes that are upregulated and that are associated with promoting differentiation in MDA-MB-231 are CCNT2, CDKN1B and RBL1. TP53 is downregulated in MDA-MB-231 and this is implicated in inhibiting differentiation.

MDA-MB-231 is an ER- cell line. ER- breast tumours are high-grade with chromosomal gain at the chromosomes at which the upregulated BIRC5 (17q), CCND1 (11q), MCM4 (8q), MRE11A (11q), RBL1 (20q), BRCA1 (17q), CHEK1 (11q), KPNA2 (17q), NBS1 (8q) and RBL2 (16q) genes are located. Prior work has identified the sites of chromosomal losses (Simpson *et al*., 2005). This includes the chromosome at which the downregulated RAD9A (11q) gene is located.

The effect of lycopene and thalidomide on the functions of the forty-eight differentially expressed genes in MDA, relative to 184A1, is analysed. Lycopene exhibits a positive,

anti-carcinogenic influence on 45.8% of these functions, and a negative, carcinogenic effect on 6.3% of the functions. Thalidomide is associated with a beneficial therapeutic influence on 69% of the functions, and a negative influence on 7.5%. As with MCF-7, although lycopene displays a highly significant anti-tumourigenic effect, thalidomide displays a much stronger anti-tumourigenic influence on MDA-MB-231 than lycopene does in association with inhibiting oncogenic transformation.

4.2.2 Is lycopene associated with inhibiting tumourigenesis in MCF-7 and MDA-MB-231 cells?

4.2.2.1 MCF-7

Lycopene is associated with inhibiting proliferation in MCF-7 cells by upregulating CDKN1B, CDKN2B and RBL2, and downregulating ANAPC2, CCNB2, CDC2, DDX11 and MKI67. Lycopene inhibits cell division in breast cancer by reducing prolactin levels, as demonstrated in previous work (Levy *et al*., 1995). However, it may promote proliferation here by upregulating CCNH, CDK6 and CDK7. Lycopene is implicated in increasing cell cycle arrest, apoptosis and DNA repair by upregulating ABL1, CCNH, CDK7, CDKN1B, CDKN2B, GADD45A, KPNA2, RAD17 and RBL2, and downregulating ANAPC2, CDC2 and RAD51. It is associated with the promotion of differentiation by upregulating KPNA2. While the upregulation of CUL3, HERC5 and RAD17, and the downregulation of RAD51, may inhibit the oncogenic state, lycopene may also promote the oncogenic state by upregulating KPNA2 and downregulating DDX11. The upregulation of CDKN2B is associated with promoting senescence.

In MCF-7, twenty-seven genes are differentially expressed in response to lycopene/THF treatment. Nine of these (ATR, BCL2, BIRC5, CCNC, CCND1, CCNG2, E2F4, GTF2H1 and RPA3) are due to the combination of lycopene with THF, but in comparison to THF-treated cells, are not significant fold changes. CDKN1B is due to the combination of lycopene with THF, with a significant fold change. Of the eighteen genes differentially expressed in response to lycopene, and highlighting that the majority of genes are multi-functional, lycopene is associated with an anti-tumourigenic influence on seventeen (94.4%) of the genes, and a negative, pro-tumourigenic influence on five (27.8%) of the genes. If the influence of THF is removed from the analysis, lycopene is associated with a chemotherapeutic influence on sixteen (94.1%) of the seventeen genes, and a tumourigenic influence on five (29.4%). Lycopene induces the upregulation of twelve (66.7%) of the genes, and induces the downregulation of six (33.3%). Without THF influence, it upregulates 64.7% of the seventeen genes, and downregulates 35.3%. From the eighty-four cell cycle-related genes studied, lycopene treatment is suggested to have a therapeutic influence on 20.2% and a negative influence on 6% of the genes. Without THF influence, it is implicated with having a therapeutic influence on 19% of the eighty-four genes, and a carcinogenic influence on 6%. Lycopene may therefore inhibit tumourigenesis in MCF-7, mainly in a pro-active manner by upregulating genes. THF influences lycopene activity and is therefore not a suitable solvent.

4.2.2.2 MDA-MB-231

Lycopene may inhibit proliferation in MDA-MB-231 by inducing the upregulation of ARHI, ATM, CCNG2, CDKN2B, RBL2 and UBE1, and the downregulation of CCNB1,

CDC16, CDC2, CDC20, CDK2, DDX11, E2F4, PCNA and SKP2. However, it may also promote proliferation by upregulating CCND1, CUL3 and MCM5, and downregulating CDKN3. Lycopene is implicated with increasing cell cycle arrest, apoptosis and DNA repair by upregulating ABL1, ARHI, ATM, CCNG2, CDKN2B, CHEK2, MCM5, RAD1, RBL2 and UBE1; and downregulating CDC16, CDC2, CDC20, CDK5RAP1, CDKN3, E2F4, KPNA2 and SKP2. It may decrease cell cycle arrest, apoptosis and DNA repair in MDA-MB-231 by downregulating CCNB1 and PCNA. Lycopene is associated with promoting differentiation by upregulating CCNG2, CCNT2 and CDK5R1, and downregulating CDK5RAP1. The downregulation of E2F4 is implicated in inhibiting differentiation. Lycopene may inhibit the oncogenic state of MDA-MB-231 by downregulating CCNB1 and upregulating CUL3 and MCM5. It may also promote the oncogenic state by downregulating HERC5. Lycopene is associated with inhibiting angiogenesis and metastasis by downregulating CCNB1. Senescence is proposed to be promoted by the upregulation of CDK5R1 and CDKN2B, and the downregulation of CDK5RAP1.

Lycopene treatment induces the differential expression of forty-two genes. Five of these are induced by THF, without lycopene influence. Furthermore, eleven genes are differentially expressed due to the combination of lycopene with THF, but in comparison to THF-treated cells, are not significant fold changes. Of the twenty-six genes that are differentially expressed in MDA-MB-231 in response to lycopene, lycopene is suggested to have an anti-tumourigenic effect on twenty-five (96.2%). As most of the genes are multi-functional, lycopene exhibits a pro-tumourigenic influence on eight (30.8%) of the

genes. Lycopene exerts its influence by upregulating thirteen (50%) of the twenty-six genes, and downregulating thirteen (50%). Of the eighty-four cell cycle-related genes that are studied, lycopene is proposed to have a chemotherapeutic effect on 29.8% of the genes, and a negative carcinogenic effect on 9.5% of the genes.

CDC16, E2F4, SKP2 and UBE1 are differentially expressed due to the combination of lycopene with THF, and not by lycopene or the solvent alone. By removing THF influence from the analysis, lycopene exhibits an anti-tumourigenic effect on twenty-one (95.5%) of the twenty-two genes, and a carcinogenic effect on seven (31.8%) of the genes. In this manner, it induces the upregulation of twelve (54.5%), and downregulates ten (45.5%) of the genes. Without THF influence, lycopene is associated with exerting a positive influence on 25% of the eighty-four analysed genes, and a negative tumourigenic effect on 8.3%. Lycopene therefore displays a therapeutic ability against metastatic ER- breast cancer cells, and this is achieved to a slighter greater effect by pro-active influence on genes i.e. promoting gene expression. The results suggest that lycopene is more influential as a therapeutic drug in MDA-MB-231 than in MCF-7. Lycopene induces the upregulation of ABL1, CDKN2B and RBL2 in both MCF-7 and MDA-MB-231 cells. Similarly, it induces the downregulation of CDC2 and DDX11 in both MCF-7 and MDA-MB-231. However, this analysis illustrates that THF is not an appropriate solvent for lycopene due to the considerable effect it has on the drug's activity.

4.2.3 Is thalidomide associated with inhibiting tumourigenesis in MCF-7 and MDA-MB-231 cells?

4.2.3.1 MCF-7

Thalidomide is associated with inhibiting proliferation in MCF-7 by inducing the upregulation of ARHI, ATM, BCCIP, CDK8, CDKN2A, CDKN3 and CHEK2, and the downregulation of ANAPC2, CDC2, CDK4 and DDX11. However, it may promote proliferation by inducing the upregulation of ANAPC4, CDK7, GTF2H1 and MRE11A, and the downregulation of BCL2. Thalidomide is associated with increasing cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of ANAPC4, ARHI, ATM, ATR, BCCIP, CDK7, CDKN2A, CHEK2, GTF2H1, HUS1, MRE11A, NBS1, RAD17, RAD9A and RPA3, and the downregulation of BCL2 and DDX11. Thalidomide is implicated in promoting differentiation by inducing the upregulation of CCNT2. It induces the upregulation of CDKN2A and this may inhibit angiogenesis and metastasis. Thalidomide induces the downregulation of RAD51 and this is associated with inhibiting the oncogenic state of MCF-7. The differential expression of ANAPC2, CDK8, DDX11, GTF2H1, NBS1 and RAD51 is due to the combination of thalidomide with DMSO.

Although thirty-three genes are differentially expressed in MCF-7 in response to thalidomide, nine of these are due to DMSO without the influence of thalidomide. Although CCND2, CCNF, CCNG2, CDKN2B are upregulated by thalidomide/DMSO treatment, this is due to DMSO influence and not thalidomide, i.e. the up/downregulation is greater in DMSO-treated cells than in thalidomide/DMSO-treated cells. The differential expression of CCNB1, CCNB2, CDK5RAP1 and GTSE1 is due to the

combination of thalidomide/DMSO, but the difference with DMSO-treated cells is not greater than two and is therefore not significant. Of the twenty-four differentially expressed genes, thalidomide is implicated with an anti-tumourigenic effect on all (100%) of the genes. As most of the genes have more than one role in the cell cycle, thalidomide is also associated with a pro-tumourigenic influence on five (20.8%) of these genes. It exerts its influence by inducing the upregulation of eighteen (75%) of the twenty-four genes, and the downregulation of six (25%). Of the eighty-four genes studied, thalidomide is proposed to inhibit cancer progression and the oncogenic state in 28.6% of the genes, and is associated with promoting the oncogenic state in 6% of the genes.

Without the influence of DMSO, thalidomide displays an anti-tumourigenic influence on 100% of the genes and a tumourigenic influence on 22.2 % of the eighteen differentially expressed genes. It induces the upregulation of 83.3% of the genes and the downregulation of 16.7%. Thalidomide is implicated in having a chemotherapeutic effect on 21.4% of the 84 genes and a carcinogenic effect on 3.6% of the genes, without DMSO influence. Thalidomide is therefore associated with an anti-tumourigenic influence on MCF-7 cells and this is mainly exerted by upregulating genes, therefore it has a pro-active approach to inhibiting cancer. This analysis affirms that DMSO is not a suitable solvent for thalidomide as it has a significant influence on the drug.

4.2.3.2 MDA-MB-231

Thalidomide may inhibit the proliferation of MDA-MB-231 by inducing the upregulation of ARHI, ATM, ATR, BAX, CCNG2, CDKN1A, CDKN2A and GADD45A, and the

downregulation of BRCA2, CCND1, CCNF, CDC20, CDC34, CDK2, CDK4, CDK5RAP1 and SKP2. It is associated with promoting proliferation by inducing the upregulation of ANAPC4, CCNH and PCNA, and the downregulation of BCL2 and CDKN1B. Thalidomide is implicated in increasing cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of ANAPC4, ARHI, ATM, ATR, BAX, CCNG2, CCNH, CDK5R1, CDKN1A, CDKN2A, CDKN2B, GADD45A, HUS1, NBS1, PCNA, RAD17, RAD9A, RB1 and RPA3; and the downregulation of BCL2, CCND1, CCNF, CCNG1, CDC20, CDC34, CDK2, CDK4, CDK5RAP1, MAD2L1 and SKP2. It may decrease cell cycle arrest by inducing the downregulation of ABL1 and CDKN1B. Thalidomide is associated with promoting differentiation by inducing the upregulation of CCNT1, CCNT2, CDK5R1 and RB1, and the downregulation of CDK5RAP1. The downregulation of CDKN1B is in involved in inhibiting differentiation. Thalidomide is implicated in inhibiting the oncogenic state of MDA-MB-231 by inducing the upregulation of CUL3 and HERC5, and the downregulation of ABL1, BRCA2 and SKP2. CDKN2A upregulation may inhibit metastasis. Thalidomide is associated with promoting senescence by inducing the upregulation of CDK5R1, CDKN2B and RB1, and the downregulation of CDK5RAP1.

Of the forty-nine genes that are differentially expressed in MDA-MB-231 by thalidomide/DMSO, twelve are due to the influence of DMSO and not thalidomide. Thalidomide influences the expression of thirty-seven genes. It is implicated in having an anti-tumourigenic effect on thirty-six (97.3%) of the genes, and, highlighting the fact that many genes are multi-functional, may have a pro-tumourigenic influence on six

(16.2%) of the genes. Thalidomide exerts its influence on MDA-MB-231 by inducing the upregulation of twenty-three (62.2%) of the thirty-seven genes, and inducing the downregulation of fourteen (37.8%). Of the eighty-four cell cycle-related genes studied, thalidomide is proposed to have a positive, chemotherapeutic effect on 42.9% of the genes, and a negative carcinogenic effect on 7.1% of the genes.

CCNG2, HERC5, MAD2L1, NBS1 and PCNA are differentially expressed due to the combination of thalidomide with DMSO. If the influence of DMSO is removed from this analysis, thalidomide is proposed to have an anti-tumourigenic effect on thirty-one (96.9%) of the thirty-two differentially expressed genes, and a tumourigenic effect on five (15.6%); it also induces the upregulation of nineteen (59.4%) genes and the downregulation of twelve (37.5%). Independently of DMSO influence, thalidomide displays a chemotherapeutic effect on 36.9% of the eighty-four genes, and a carcinogenic effect on 6%. It induces its proposed anti-tumourigenic influence more in a pro-active manner (promoting tumour suppressors) than by inhibiting tumourigenic genes. DMSO is not an appropriate solvent for thalidomide as it has a clear influence on its activity. As observed with lycopene, thalidomide displays a greater beneficial influence in MDA-MB-231 cells than in MCF-7 with regards to its involvement in inhibiting tumourigenesis.

4.2.4 Does lycopene influence normal breast cells?

MCF10A is used as a normal breast control in many studies; however, it contains markers for myoepithelial and luminal phenotypes (Gordon *et al*., 2003). Consequently,

184A1 is used as a normal breast cell line in this study. Lycopene may inhibit proliferation in 184A1 by inducing the downregulation of ANAPC2, ANAPC4, BIRC5, CCNB1, CCNB2, CCNE1, CCNF, CCNH, CDC2, CDC20, CDK5RAP1, CKS1B, CUL2, CUL3, DDX11, E2F4, GTF2H1, KNTC1, MNAT1, TFDP1, and inducing the upregulation of CDKN1B and CDK8. It may also promote proliferation by inducing the upregulation of CCNC, CCND1, CDK7 and UBE1, and the downregulation of ARHI and BCL2. Lycopene is associated with promoting cell cycle arrest, DNA repair and apoptosis by inducing the upregulation of CDKN1B and CDK7, and the downregulation of BCL2, BIRC5, CDC2, CDC20, CDK5RAP1, CDKN3, CKS1B, CUL2, CUL3, E2F4 and GTSE1. However, it may inhibit cell cycle arrest, DNA repair and apoptosis by inducing the upregulation of CDK8, and the downregulation of ANAPC4, ARHI, CCNB1, CCNB2, CCNE1, CCNH, GTF2H1 and TFDP1. Lycopene is implicated in promoting differentiation in 184A1 by inducing the upregulation of CDKN1B, and the downregulation of CDK5RAP1. It may also inhibit differentiation by inducing the downregulation of CCNT1 and E2F4. The downregulation of KNTC1 is implicated in promoting oncogenic transformation, while the upregulation of CCND1 and downregulation of TFDP1 is implicated in inhibiting transformation. Lycopene may promote senescence by inducing the downregulation of CDK5RAP1.

Lycopene treatment influences the expression of fifty genes in 184A1. Twelve of these are due to THF, without lycopene influence. The differential expression of CCND2, CDK4, CDKN2A, MAD2L1, MRE11A and RAD51 is due to the combination of lycopene and THF, but in comparison to THF-treated cells, they do not have a significant

fold change. Therefore, lycopene influences the expression of thirty-two genes in 184A1. Of these, it is associated with a positive, anti-transforming effect on twenty-eight (87.5%) of the genes, but a transforming influence on seventeen (53%) of the genes. It induces the upregulation of six (18.8%) of the thirty-two genes, and induces the downregulation of twenty-six (81.3%) genes. From the eighty-four genes that are included in this study, lycopene is proposed to positively influence the expression of 33.3% and proposed to have a negative carcinogenic influence on 20.2% of the genes.

CCNC, CCND1, CDK7, CDK8 and CDKN1B are upregulated due to the combination of lycopene with THF. Without the influence of THF, lycopene influences the expression of twenty-seven genes. It is associated with an anti-tumourigenic effect on twenty-four (88.9%) of the genes, and a carcinogenic effect on thirteen (48.1%) of the genes. It induces the upregulation of one (4.2%) of the twenty-seven genes, and downregulates twenty-six (96.3%). Lycopene is implicated to have a positive influence on 28.6% of the eighty-four genes studied, and a negative influence on 15.5% of the genes. From this it can be deduced, that lycopene does influence normal breast cells. Although it is associated with inhibiting carcinogenesis in 184A1, it also has an involvement in promoting oncogenic transformation in these cells. This indicates that firstly, it is not a highly selective drug, and secondly, that it is implicated in inducing transformation in normal cells. This has important implications in its administering to patients despite the therapeutic influence it has on breast cancer cells.

4.2.5 Does thalidomide influence normal breast cells?

Thalidomide is associated with inhibiting proliferation in 184A1 by inducing the upregulation of CDKN1B, CDKN2B and RBL1, and the downregulation of ANAPC2, CDC20, CDK4 and GTF2H1. However, thalidomide may also promote proliferation by inducing the upregulation of BRCA2, CDK7, MCM4, MKI67 and MRE11A, and the downregulation of BAX and CDKN3. It is implicated in promoting cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of BRCA2, CDK7, CDKN1B, CDKN2B, CHEK2, MCM4, MKI67, MRE11A, RAD1, RAD51 and RBL2, and the downregulation of ANAPC2, CDK4, CDK5RAP1, CDKN3 and GTSE1. It may inhibit cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of RBL1, and the downregulation of BAX, DNM2, GTF2H1 and RAD9A. Thalidomide is associated with promoting differentiation by inducing the upregulation of CDKN1B and RBL1, and the downregulation of CDK5RAP1. It is implicated in inhibiting oncogenic transformation by inducing the upregulation of MCM4, and the downregulation of CCNB1 and CDKN3. Thalidomide may promote senescence in 184A1 by inducing the upregulation of CDKN2B and the downregulation of CDK5RAP1.

Thalidomide treatment influences the expression of forty-five genes in 184A1. The differential expression of ARHI, CCNC, CCND1, CCNT2, CDC2, CDK8, CDKN1A, CDKN2A, CKS1B, CKS2, CUL2, MCM2, MCM5 and RPA3 is induced by DMSO influence, without thalidomide. Furthermore, ATM, BCL2, CCND1, CDC16, HERC5, KNTC1, RAD17 and SKP2 are differentially expressed due to the combination of thalidomide with DMSO, but these are not significant fold changes when compared to

DMSO-treated cells. Hence, thalidomide influences the expression of twenty-three genes. It is implicated in inhibiting the oncogenic transformation of 184A1 by influencing the expression of twenty (87%) of the genes, but is associated with a carcinogenic effect on eleven (47.8%) of the 23 genes. It induces the upregulation of ten (43.5%) and the downregulation of thirteen (56.5%) of these genes. Thalidomide is associated with a positive, anti-carcinogenic influence on 23.8% of the eighty-four genes that are included in this study. However, it is also associated with promoting oncogenic transformation by influencing the expression of 13.1% of the genes.

BAX, CCNB1, CDK5RAP1, CDKN1B, CHEK2, DNM2, GTF2H1, GTSE1, MRE11A, RAD1 and RAD9A are differentially expressed due to the combination of thalidomide with DMSO. Without the influence of DMSO in the analysis, thalidomide influences the expression of twelve genes. It is associated with a positive, anti-transforming influence on all of the genes (100%), and a negative carcinogenic influence on six (50%) of the genes. It induces the upregulation of six (50%) of the genes and induces the downregulation of six (50%). From the eighty-four genes, thalidomide is associated with inhibiting oncogenic transformation by influencing the expression of 14.3%, and associated with promoting transformation by influencing the expression of 7.1%. While thalidomide does not have a highly significant effect on 184A1 gene expression, it does influence the expression of various important cell cycle-related genes. The fact that it is implicated in promoting oncogenic transformation by inducing the differential expression of specific genes indicates that there are limitations to its treatment of breast cancer.

4.2.6 Is curcumin associated with inhibiting tumourigenesis in HT-29?

Curcumin is associated with inhibiting proliferation in HT-29 by inducing the upregulation of CCNH and CDKN1A, and the downregulation of BIRC5, CCNB1, CCNF, CDC16, CDK4, CUL3, GTF2H1, MCM2, MCM3, MCM5, MKI67, MNAT1, RBBP8, SKP2 and TFDP1. Curcumin typically targets proliferating cells more efficiently than differentiated cells (Aggarwal *et al*., 2003). It is involved in promoting proliferation by inducing the upregulation of RPA3 and SERTAD1, and the downregulation of BCL2, BRCA2, CDKN3 and RBL1. Curcumin is associated with increasing cell cycle arrest, apoptosis and DNA repair in HT-29 by inducing the upregulation of CCNH, CDK5R1, CDKN1A, CDKN2A, CDKN2B, CUL2, GADD45A, PCNA, RAD9A and RPA3, and the downregulation of BCL2, BIRC5, BRCA2, CDC16, CUL3, GTF2H1, RBBP8 and RBL1. It is also associated with decreasing cell cycle arrest, apoptosis and DNA repair by inducing the downregulation of CCNB1, MCM2, MCM3, MCM5 and MKI67.

Curcumin is implicated in promoting differentiation by inducing the upregulation of CDK5R1 and in inhibiting differentiation by inducing the downregulation of RBL1. It is associated with promoting senescence by inducing the upregulation of CDK5R1 and CDKN2A. Curcumin may inhibit angiogenesis and metastasis by inducing the upregulation of CDKN2A and CUL2. It is associated with inhibiting the oncogenic state of HT-29 by inducing the upregulation of HERC5, and the downregulation of BIRC5, BRCA2, CCNB2, CDK4, CDKN3 and SKP2. However, it is also associated with promoting the oncogenic state by inducing the upregulation of SERTAD1 and the

downregulation of MCM2, MCM3 and MCM5. This is in agreement with previous findings where curcumin induces carcinogenesis in rats, mice and in CHO cells (http://ntp-server.niehs.nih.gov/htdocs/LT-studies/tr427.html).

Of the forty-three genes that are differentially expressed in HT-29 following curcumin treatment, ARHI and CCND1 gene expression is influenced by DMSO and not curcumin. Furthermore, the expression of nine genes is influenced by the combination of curcumin with DMSO, but do not have significant fold changes in comparison to DMSO-treated cells i.e. ATM, CDC2, CDK2, CDK5RAP1, CDKN1B, CKS1B, RB1, RBL2 and UBE1. Of the thirty-two genes that are influenced by curcumin, curcumin is associated with having an anti-tumourigenic effect on thirty-one (96.9%) of the genes, and a pro-tumourigenic effect on eleven (34.4%). Curcumin induces the upregulation of twelve (37.5%) of the thirty-two genes, and the downregulation of twenty (62.5%). Of the eighty-four genes analysed, curcumin is proposed to have a chemotherapeutic influence on 36.9%, and a tumourigenic influence on 13.1% of the genes.

CCNB2, CDC16, CDK4 and RBL1 are downregulated due to the combination of curcumin with DMSO. Without the influence of DMSO, curcumin influences the expression of twenty-eight genes. It is associated with an anti-tumourigenic effect on twenty-seven genes (96.4%) and a carcinogenic effect on ten genes (35.7%). Curcumin induces the upregulation of twelve (42.9%) of the genes, and the downregulation of sixteen (57.1%). From the eighty-four genes included in this study, curcumin, without DMSO influence, is implicated to have a chemotherapeutic influence on 32% and a

tumourigenic influence on 11.9%. It is proposed to mainly exert its chemotherapeutic effect by inducing the downregulation of genes i.e. a defensive rather than a pro-active role. While it may have an important effect in inhibiting tumourigenesis in colon cancer cells, it is also associated with some carcinogenic influence therefore, its administering must be carefully evaluated. It is evident that DMSO is not an appropriate solvent for curcumin due to the considerable influence it has on the drug's actions.

4.2.7 Is SAHA associated with inhibiting tumourigenesis in HT-29?

SAHA is implicated in inhibiting the proliferation of HT-29 by inducing the upregulation of ARHI, BCL2, CCNG2, CCNH, CDK8, CDKN2A, CDKN3, HUS1 and MAD2L2, and the downregulation of UBE1. However, it is also implicated in promoting proliferation by inducing the upregulation of CCND1, DDX11, PCNA and RPA3. SAHA is associated with promoting cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of ARHI, CCNG2, CCNH, CDK8, CDKN2A, CDKN2B, CUL2, DDX11, GADD45A, HUS1, MAD2L2, PCNA, RAD9A and RPA3. It may also decrease cell cycle arrest, apoptosis and DNA repair by inducing the upregulation of BCL2 and CDK8. SAHA is associated with promoting differentiation by inducing the upregulation of CCNG2 and CCNT1, and is associated with promoting senescence by inducing the upregulation of CDKN2B and the downregulation of TP53. TP53 is frequently mutated in colon adenocarcinoma and this downregulation induces tetraploidy and senescence. SAHA is implicated in inhibiting angiogenesis and metastasis by inducing the upregulation of CDKN2A and CUL2. SAHA is involved in inhibiting the oncogenic state of HT-29 by inducing the upregulation of CCND1, GADD45A, HERC5 and RPA3.

Of the forty-one genes that are differentially expressed in HT-29 due to SAHA treatment, two of these, BRCA2 and CCNF, are due to DMSO influence. The differential expression of another eighteen is induced by the combination of SAHA and DMSO, but the fold changes in comparison to DMSO-treated cells, are not significant. These are ANAPC4, ATM, ATR, BCCIP, CCNC, CCNE1, CCNT2, CDK4, CDK6, CDK7, CUL3, GTF2H1, KNTC1, MNAT1, NBS1, RAD1, RAD17 and RB1. Of the twenty-one genes that are influenced by SAHA, SAHA is proposed to have an anti-tumourigenic effect on all (100%) of the genes. As many genes are multi-functional, SAHA is also proposed to have a pro-tumourigenic influence on six (28.6%) of the genes. SAHA induces the upregulation of twenty (95.2%) of the twenty-one genes, and the downregulation of one (4.8%). From the eighty-four genes included in this study, SAHA is implicated in having a chemotherapeutic influence on 25% and a tumourigenic influence on 7.1% of the genes.

CCND1 and HUS1 are upregulated due to the combination of SAHA with DMSO. By eliminating DMSO influence from the analysis, SAHA is proposed to have an anti-carcinogenic influence on all (100%) of the nineteen genes, and a carcinogenic influence on five (26.3%) of the genes. It induces the upregulation of eighteen (94.7%) of the genes, and induces the downregulation of one (5.3%). SAHA is associated with a chemotherapeutic effect on 22.6% of the eighty-four genes analysed in this study, and a tumourigenic effect on 6%. Due to the influence of DMSO on gene expression, it is concluded to not be an appropriate solvent for SAHA.

4.2.8 From this study, which genes have not previously been documented in studies with these drugs?

Numerous genes have not previously been identified with regards to the specific interaction with lycopene, thalidomide, curcumin and SAHA in the respective cell lines. Genes that have not been documented in MCF-7 cells treated with lycopene and that are consequently documented in this study are: ANAPC2, ANAPC4, ARHI, BCL2, BIRC5, CCNB1, CCNB2, CCNC, CCNF, CCNG2, CCNT1, CCNT2, CDC16, CDC20, CDK2, CDKN2B, CDK5R1, CDK5RAP1, CDK8, CDKN3, CHEK2, CKS1B, COIII, CUL2, CUL3, DDX11, E1F4A1, E2F4, GTF2H1, GTSE1, HERC5, KNTC1, MCM5, MKI67, MNAT1, MT-ATP6, PCNA, RAD1, SKP2, TFDP1 and UBE1.

Genes that have not been documented in MDA-MB-231 cells treated with lycopene are: ANAPC2, ANAPC4, BCL2, BIRC5, CCNB2, CCNC, CCNF, CCNH, CCNT1, CDK7, CDK8, CDKN2B, CKS1B, CUL2, DDX11, GADD45A, GPNMB, GTF2H1, GTSE1, KNTC1, MKI67, MNAT1, RAD17, RAD51, RBL2 and TFDP1.

Similarly, genes that have not been documented in 184A1 cells treated with lycopene are: ABL1, ANAPC2, ATM, CCNB2, CCNG2, CCNT2, CDC16, CDK2, CDK5R1, CDK6, CDKN1B, CDKN2B, CHEK2, GADD45A, HERC5, KPNA2, MCM5, MKI67, PCNA, RAD1, RAD17, RAD51, RBL2 and SKP2.

The interaction of the following genes in MCF-7 with thalidomide has not been previously documented: ABL1, BAX, BRCA2, CCNB1, CCND1, CCNF, CCNG1, CCNG2, CCNH, CCNT1, CDC20, CDC34, CDK2, CDK5R1, CDK5RAP1, CDKN1A,

CDKN1B, CDKN2B, COII, CUL3, DNM2, E1F4A1, GADD45A, GTSE1, HERC5, MAD2L1, MCM4, MKI67, PCNA, RAD1, RB1, RBL1, RBL2 and SKP2.

Genes that have not been documented in MDA-MB-231 cells treated with thalidomide are: ANAPC2, BCCIP, CCNB1, CDC2, CDK7, CDK8, CDKN3, CHEK2, COII, DDX11, DNM2, GTF2H1, GTSE1, MCM4, MKI67, MRE11A, RAD1, RAD51, RBL1 and RBL2.

The interaction of genes in 184A1 cells with thalidomide that have not been documented are: ABL1, ANAPC4, ARHI, ATM, ATR, BCCIP, BCL2, CCND1, CCNF, CCNG1, CCNG2, CCNH, CCNT1, CCNT2, CDC2, CDC34, CDK2, CDK5R1, CDK8, CDKN1A, CDKN2A, CUL3, DDX11, GADD45A, HERC5, HUS1, MAD2L1, NBS1, PCNA, RAD17, RB1, RPA3 and SKP2.

HT-29 cells treated with SAHA revealed an interaction with the following genes that have not been previously documented: ARHI, BCL2, CCND1, CCNG2, CCNH, CCNT1, CDK8, CDKN2A, CDKN2B, CDKN3, CUL2, DDX11, GADD45A, HERC5, HUS1, MAD2L2, PCNA, RAD9A, RPA3, SAMM50, TP53 and UBE1.

Similarly, HT-29 cells that were treated with curcumin interacted with the following genes, that have not been previously documented: BIRC5, BRCA2, CCNB1, CCNF, CDC16, CDK4, CDK5R1, CDKN1A, CDKN2B, CDKN3, CUL2, CUL3, GADD45A, GPNMB, GTF2H1, MCM2, MCM3, MKI67, MNAT1, RBBP8, RBL1, RPA3, SERTAD1, SKP2 and TFDP1.

CHAPTER FIVE: CONCLUSION

Normal cell development relies on the regulation of gene expression, but in diseases such as cancer, loss of this regulation induces the differential expression of genes and contributes to the malignant state. It is important to increase the understanding of the molecular mechanisms involved in the initiation and progression of cancer to develop new, and improve on existing, therapies. There has been a shift in clinical medicine and research and the focus is currently on developing cancer therapies that target one or more particular genes of interest, thereby acting only on pre-determined pathways. This increases the effectiveness of the drug with less impact on normal cells. Some cancers are unresponsive to treatment or develop resistance to treatment. By using a combination of targeted therapies, two or three pathways that are essential to the survival of the cancer cell can be targeted for maximum impact. The outcome of this work will potentially lead to the improvement of the use of the chemotherapeutic drugs studied by contributing to the characterisation of drug action, increasing their specificity and effectiveness with regards to how they influence cell cycle-related genes.

The objective of this study is to identify genes that are differentially expressed in response to chemotherapeutic drugs and hence generate a framework for genes that can serve as genetic targets to increase the therapeutic activity of these drugs. Lycopene is exhibits a slight effect in potentially inhibiting the oncogenic transformation of MCF-7 relative to 184A1 (normal breast cells) by influencing the expression of CDC2, CDKN2B, GADD45A, MKI67 and RAD51. Likewise, lycopene has a minor association in inhibiting the oncogenic transformation of MDA-MB-231 by inducing the differential

expression of ARHI, CCNG2, CDC16, CDC2, CDC20, CDKN2B and KPNA2. It is important to highlight that numerous genes are differentially expressed in both MCF-7 and MDA-MB-231 to possibly promote the oncogenic transformation from normal breast cell to the cancerous state. These genes are ARHI, BRCA1, BRCA2, CCNC, CCND1, CCNT2, CDC16, CDC2, CDK4, CDK7, CDKN2A, CDKN2B, CKS1B, CKS2, HUS1, KPNA2, MAD2L1, MCM3, MCM5, MKI67, MRE11A, RAD51, RAD9A and SUMO1. These genes can therefore potentially serve as markers for an increased susceptibility to transformation and tumour development.

Lycopene has a strong involvement in exerting an anti-tumourigenic effect on MCF-7, potentially inhibiting the development and progression of the oncogenic state, by influencing the expression of ABL1, ANAPC2, CCNB2, CCNH, CDC2, CDK7, CDKN1B, CDKN2B, COIII, CUL3, DDX11, E1F4A1, GADD45A, HERC5, KPNA2, MKI67, MT-ATP6, RAD17, RAD51, RBL2 and ZNF350. It displays high therapeutic ability in inhibiting the oncogenic state of MDA-MB-231 by influencing the expression of ABL1, ARHI, ATM, CCNB1, CCNG2, CCNT2, CDC16, CDC2, CDC20, CDK2, CDK5R1, CDK5RAP1, CDKN2B, CDKN3, CHEK2, CUL3, DDX11, E2F4, KPNA2, MCM5, PCNA, RAD1, RBL2, SKP2 and UBE1. Lycopene influences the expression of mutual genes in MCF-7 and MDA-MB-231, namely ABL1, CDC2, CDKN2B, CUL3, DDX11 and RBL2, and these are associated with inhibiting tumourigenesis. While there are many genes that are not influenced in common, this relationship above suggests that lycopene functions with consistency to a certain degree, despite the difference between ER- and ER+ cells. In both cell types, the majority of differentially expressed genes are

implicated in the promotion of the DNA damage responses: cell cycle arrest, apoptosis and DNA repair. Therefore, lycopene is involved in limiting DNA damage in the cancerous cell and increasing genomic integrity by restricting the replication of damaged DNA. The primary difference between the effect of lycopene on MCF-7 and the effect on MDA-MB-231 is that lycopene induces the differential expression of significantly more genes in MDA-MB-231 to potentially inhibit the oncogenic state. This suggests that lycopene may be more effective in treating metastatic, ER- breast cancer than ER+ breast cancer.

Lycopene is implicated in having a carcinogenic effect on MCF-7 by influencing the expression of CCNH, CDK6, CDK7, DDX11 and KPNA2. Similarly, it is associated with promoting the oncogenic state of MDA-MB-231 by influencing the expression of CCNB1, CCND1, CDKN3, CUL3, E2F4, HERC5, MCM5 and PCNA. The identification of these genes has the potential to enhance the therapeutic potential of lycopene in breast cancer as necessary measures can be taken to limit the influence of lycopene on the expression of these genes before and during administering. For genes that are upregulated by lycopene and subsequently may promote tumourigenesis (CCND1, CCNH, CDK6, CDK7, CUL3, KPNA2 and MCM5) gene silencing is a possible step to overcome this negative effect. It is interesting to note that while lycopene is associated with promoting the oncogenic state of MCF-7 and MDA-MB-231 by influencing gene expression, none of these genes are common to the two cell lines. This suggests that lycopene may promote tumourigenesis via different pathways, depending on the ER status of the cell.

As with lycopene, thalidomide is implicated in inhibiting the oncogenic transformation of MCF-7, but does not show high potential in doing this. This potential role is suggested from its influence on the expression of CDKN2A, CDC2, CDK4, HUS1, RAD9A and RAD51. Thalidomide has a more pronounced association in inhibiting oncogenic transformation in MDA-MB-231 in comparison to MCF-7. This stems from the finding that thalidomide influences the expression of six genes in MCF-7, and influences the expression of fifteen genes in MDA-MB-231 to reduce transformation. These are ARHI, BCL2, BRCA2, CCND1, CCNG2, CCNH, CDC20, CDK2, CDK4, CDKN2A, CDKN2B, HUS1, MAD2L1, RAD9A and RPA3. Thalidomide influences the expression of CDK4, CDKN2A, HUS1 and RAD9A in both MCF-7 and MDA-MB-231. This indicates that thalidomide functions in pathways common to both types of breast cancer, and that these genes are important targets as they are mutual to both cell lines. These findings are beneficial in elucidating the genetic pathways of breast cancer initiation, consequently identifying potentially new gene targets to hinder oncogenic transformation.

Thalidomide has a stronger involvement in inhibiting tumourigenesis and the oncogenic state of MCF-7, than in potentially inhibiting oncogenic transformation in this cell line. This is achieved by inducing the differential expression of ANAPC2, ANAPC4, ARHI, ATM, ATR, BCCIP, BCL2, CCNT2, CDC2, CDK4, CDK7, CDK8, CDKN2A, CDKN3, CHEK2, DDX11, GTF2H1, HUS1, MRE11A, NBS1, RAD17, RAD51, RAD9A, RPA3 and ZNF350. Thalidomide is implicated in inhibiting the oncogenic state of MDA-MB-231 by stimulating the differential expression of ABL1, ANAPC4, ARHI, ATM, ATR, BAX, BCL2, BRCA2, CCND1, CCNF, CCNG1, CCNG2, CCNH, CCNT1,

CCNT2, CDC20, CDC34, CDK2, CDK4, CDK5R1, CDK5RAP1, CDKN1A, CDKN2A, CDKN2B, CUL3, GADD45A, HERC5, HUS1, MAD2L1, NBS1, PCNA, RAD17, RAD9A, RB1, RPA3 and SKP2; thirty-six genes in comparison to the twenty-four genes influenced by thalidomide in MCF-7. Thalidomide influences the expression of common genes in MCF-7 and MDA-MB-231 and these are associated with the inhibition of tumourigenesis. These genes are ANAPC4, ARHI, ATM, ATR, BCL2, CCNT2, CDK4, CDKN2A, HUS1, NBS1, RAD17, RAD9A and RPA3. These can potentially be targeted to increase the efficiency of thalidomide in breast cancer cells. In MCF-7 and MDA-MB-231, the majority of the genes that are differentially expressed by thalidomide are involved in promoting the DNA damage responses. Thalidomide's primary mode of action in inhibiting the oncogenic state of breast cancer cells is proposed to be the suppression of the replication of damaged DNA and the enforcement of DNA repair to increase genomic integrity.

Thalidomide is, however, implicated in promoting tumourigenesis in MCF-7 by influencing the expression of ANAPC4, BCL2, CDK7, GTF2H1 and MRE11A, and in MDA-MB-231 by influencing the expression of ABL1, ANAPC4, BCL2, CCNH, CDKN1B and PCNA. As the genes that thalidomide influences to potentially promote the oncogenic state have been identified in this study, attention can be paid to the expression of these genes during drug administering to prospectively limit the negative effect of thalidomide. Thalidomide induces the upregulation of ANAPC4, CCNH, CDK7, GTF2H1, MRE11A and PCNA, and this differential expression is associated with the promotion of tumourigenesis. During thalidomide treatment, intervening steps must be

incorporated with the aim of decreasing the expression of these genes, possibly through gene silencing. Thalidomide induces the downregulation of ABL1, BCL2 and CDKN1B. This is implicated in an increase in tumourigenesis, therefore in a parallel vein, the expression of these genes must be promoted during the administering of thalidomide to patients.

It cannot be elucidated as to whether curcumin and SAHA inhibit the oncogenic transformation of HT-29 in comparison to normal colon cells, as the undiseased cell line selected, FHC, proved to be a slow-growing, problematic cell line. As a result, the focus is on the influence of these drugs on tumourigenesis. Curcumin is involved in inhibiting the oncogenic state of HT-29 by influencing the expression of BCL2, BIRC5, BRCA2, CCNB1, CCNB2, CCNF, CCNH, CDC16, CDK4, CDKN1A, CDKN2A, CDKN2B, CDK5R1, CDKN3, CUL2, CUL3, GADD45A, GTF2H1, HERC5, MCM2, MCM3, MCM5, MKI67, MNAT1, PCNA, RAD9A, RBBP8, RBL1, RPA3, SAMM50, SKP2 and TFDP1. It is proposed to inhibit tumourigenesis primarily by suppressing proliferation and promoting the DNA damage responses. While it is associated with a strong anti-tumourigenic effect, curcumin is also implicated in promoting the oncogenic state of HT-29 by influencing the expression of BCL2, BRCA2, CDKN3, MCM2, MCM3, MCM5, RBL1, RPA3 and SERTAD1. This suggests that while it proposes a therapeutic impact on colon cancer cells, it must be administered with caution, particularly with regards to the genes through which it promotes tumourigenesis. This study provides beneficial insight into ultimately improving the efficiency of treatment with curcumin by identifying genes that may hamper its therapeutic function.

SAHA is implicated in inhibiting the oncogenic state of HT-29 by influencing the expression of ARHI, BCL2, CCND1, CCNG2, CCNH, CCNT1, CDK8, CDKN2A, CDKN3, CUL2, DDX11, GADD45A, GPNMB, HERC5, HUS1, MAD2L2, PCNA, RAD9A, RPA3, SAMM50, TP53 and UBE1. It is proposed to inhibit tumourigenesis largely by inducing the differential expression of genes to promote the DNA damage responses, and hence, increase DNA repair and decrease the replication of damaged DNA. SAHA, however, is also implicated in promoting the oncogenic state by influencing the expression of BCL2, CCND1, CDK8, DDX11, PCNA and RPA3. By identifying the genes that SAHA influences to potentially promote tumourigenesis, investigators have greater control over the action of the drug and can take necessary measures to limit this carcinogenic effect.

In summary, curcumin and SAHA display their capabilities as anti-cancer agents in colon cancer although both have the ability to be pro-carcinogenic as well. The same holds true for lycopene and thalidomide in the treatment of breast cancer. Lycopene and thalidomide have also displayed their ability in potentially inhibiting the oncogenic transformation of breast cells. DMSO and THF have both demonstrated their unsuitability to be used as solvents for curcumin, SAHA, lycopene and thalidomide. As a solvent, neither should have an influence on the gene expression levels of the cell lines. Since they both induce the differential expression of various genes, they cannot be used as solvents for unbiased drug treatment. This would apply to both *in vitro* and *in vivo* work. The influence of the drugs is therefore analysed here both with and independently of the solvents.

The output of this research identifies the genetic pathways through which lycopene, thalidomide, curcumin and SAHA function in cancer treatment and proposes several genes that can potentially be manipulated to amplify the efficiency and success of these drugs. In addition, numerous cell cycle-related genes have been identified, with regards to the interaction with these drugs, that have not previously been documented in studies. Studies such as these are very important in streamlining cancer treatment, enabling researchers and clinicians to increase the specificity of treatment for improved responses in patients. It is important not to merely target cells that are rapidly proliferating, but to target and inhibit specific molecules that are promoting carcinogenesis. By individualising treatments based on the specific molecular targets of the tumour, the success rates of treatments can increase and side effects will decrease. The outcome that all clinicians and researchers aim for is to improve on the success rates of cancer therapies, and studies such as these provide the knowledge to achieving this.

Future research

With the identification of these genes that are influenced by lycopene, thalidomide, curcumin and SAHA, successive studies can apply this data to validate the expression of the genes in response to the drugs i.e. by silencing gene expression *in vitro* and in animal models, the influence of the gene in response to the respective drug can be analysed. This would provide verification on the genes that are potential genetic targets and facilitate the process of improving on the effectiveness of these drugs. The effects of these drugs can be analysed in different pathways as SuperArray manufactures PCR arrays focusing on, for example, apoptosis and the cancer pathway. The effects of

lycopene and thalidomide on colon cancer and the effects of curcumin and SAHA on breast cancer can be studied, with little need for purchasing more reagents. In future studies, tumour sections can be probed for the novel genes using *in situ* hybridisation. In this way, their potential as cancer biomarkers can be assessed relative to tumour grade. Furthermore, during drug trials this may provide useful markers to assess the *in vivo* effects of the treatments.

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