

https://dspace.org/

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

Rupnarain, Charleen

2010-01-27T11:15:07Z
http://hdl.handle.net/10539/7479

Downloaded from a DSpace repository. https://dspace.org/

THE IDENTIFICATION OF DIFFERENTIALLY EXPRESSED CELL CYCLE–RELATED GENES IN BREAST AND COLON CANCER CELL LINES IN RESPONSE TO CHEMOTHERAPEUTIC DRUGS

Charleen Rupnarain

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2009

DECLARATION

I (Charleen Rupnarain) declare that this thesis is my own, unaided work. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

_____

(Signature of candidate)

_____ day of _____ 200__

ABSTRACT

With the high prevalence and high mortality rate of cancer in the global community, it is increasingly essential to accelerate our understanding of the disease, to identify new genetic targets for therapy, and to pursue avenues for improving on the therapies in development and in current use. The aim of this study is to identify cell cycle-related genes whose expression is influenced by the chemotherapeutic drugs curcumin, SAHA, lycopene and thalidomide in breast and colon cancer and normal cell lines. These drugs are currently not in clinical use for cancer in South Africa, and while there have been investigative studies of these chemotherapeutic agents, this study aims to identify the specific genes that are influenced by the drugs. The result of this is that several genes that were not previously documented as targets of these drugs are highlighted. The cell cycle pathway is the area of focus as loss of regulation in the cell cycle is one of the important factors involved in promoting cancer initiation and progression.

In the first instance, flow cytometry was used to identify optimal drug concentrations relative to the cell cycle stages. Following this, alterations in gene expression were assessed using a PCR-based differential display after each drug treatment. Subsequently, a more focussed approach was taken in a PCR-array analysis of panels of cell cycle-related genes.

A subset of genes is identified that is implicated in oncogenic transformation in breast cancer. This has the potential to inhibit the genetic pathways involved in breast malignancy by providing targets that perhaps may not be manipulated in current

therapies. The gene expression studies here suggest that lycopene and thalidomide function in inhibiting this transformation, and play significant roles in suppressing the oncogenic state of breast cancer. Curcumin and SAHA also exhibit important functions in inhibiting tumourigenesis in colon cancer. While the results propose that the drugs have clear roles in inhibiting breast and colon cancer, they are also implicated in promoting cancer. This research has defined the genes that must be carefully monitored during drug administering as they may promote these and other cancers. The availability of these results to researchers will aid in selecting the criteria for assessing the success rate of these drugs.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Dr Clement Penny and Prof. Paul Ruff, for academic support and advice during my research project, as well as my labmate Dr Victoria Gibbon.

My thanks to Karolina Kuun (Scientific Group), Christiaan Labuschagne (Inqaba Biotech) and Catherine Scollick (SuperArray Bioscience) for technical assistance.

I would like to thank Dr Pascal Willem for use of the ABI7500.

I would like to acknowledge and thank Merck Research Laboratories, New Jersey, USA for provision of SAHA/Vorinostat for this research study.

I am grateful to the NRF, FRC, the Department of Internal Medicine, Wits Health Consortium and Canon Collins for funding.

I would like to extend my thanks to the University of the Witwatersrand for the Postgraduate Merit Award.

TABLE OF CONTENTS Page

Declaration		ii
Abstract		iii
Acknowledgements		v
List of figures		xiii
List of tables		xvii
Abbreviations		xix
Chapter one – Introduction		1
	Aims of Study	3
1.1	Breast cancer	3
1.1.1	Statistics	3
1.1.2	Physiology	4
1.1.3	Epidemiology	5
1.14	Markers	7
1.1.5	Cell lines	8
1.2	Colorectal cancer	10
1.2.1	Statistics	10
1.2.2	Physiology	11
1.2.3	Epidemiology	12
1.2.4	Cell line	14
1.3	Chemotherapeutic drugs	14
1.3.1	Curcumin	14

1.3.1.1	Curcumin and breast cancer	17
1.3.1.2	Curcumin and colon cancer	17
1.3.2	Lycopene	18
1.3.2.1	Lycopene and breast cancer	20
1.3.3	SAHA	21
1.3.3.1	SAHA and breast cancer	24
1.3.4	Thalidomide	25
1.4	The cell cycle	26
1.4.1	Mitosis	29
1.4.2	DNA damage checkpoints	30
1.4.3	Senescence	32
1.4.4	CDKs	33
1.4.5	Cyclins	35
1.4.6	Other cell cycle-related genes	44

Chapter two – Methods and Materials

2.1	Materials	92
2.1.1	Cell lines	92
2.1.2	Drugs	92
2.2	Methods	93
2.2.1	Cell culture	93
2.2.1.1	Medium	93
2.2.1.2	Subculturing cells	94

2.2.1.3	Drug treatment	95
2.2.2	Flow cytometry	97
2.2.2.1	Isolating and staining cells	97
2.2.3	RNA extraction	98
2.2.4	Genefishing	100
2.2.4.1	Reverse transcription	100
2.2.4.2	PCR	101
2.2.4.3	Gel extraction	103
2.2.4.4	Cloning	104
2.2.4.5	Transformation	105
2.2.5	PCR Array	106
2.2.5.1	First strand synthesis	106
2.2.5.2	Real-time PCR	107

Chapter three – Results

3.1	Flow cytometry	109
3.1.1	HT-29 cell line	109
3.1.2	MCF-7 cell line	120
3.1.3	MDA-MB-231 cell line	125
3.1.4	184A1 cell line	130
3.1.5	Summary	134
3.2	RNA	135
3.2.1	Summary	137

3.3	Genefishing	138
3.3.1	Positive control	138
3.3.2	HT-29	139
3.3.3	MCF-7	146
3.3.4	MDA-MB-231	156
3.3.5	184A1	156
3.3.6	Summary	156
3.4	SuperArray RT^2 Profiler PCR Array	158
3.4.1	HT-29	158
3.4.1.1	Distribution of C_t values	158
3.4.1.2	Reverse transcription control	159
3.4.1.3	Genomic DNA contamination	160
3.4.1.4	HT-29/treatments	166
3.4.2	MCF-7	210
3.4.2.1	Distribution of C_t values	210
3.4.2.2	Reverse transcription control	211
3.4.2.3	Genomic DNA contamination	212
3.4.2.4	MCF-7/treatments	217
3.4.3	MDA-MB-231	246
3.4.3.1	Distribution of C_t values	246
3.4.3.2	Reverse transcription control	247
3.4.3.3	Genomic DNA contamination	248
3.4.3.4	MDA-MB-231/treatments	255

3.4.4 184A1 290

3.4.4.1 Distribution of C_t values 290

3.4.4.2 Reverse transcription control 291

3.4.4.3 Genomic DNA contamination 292

3.4.4.4 184A1/Treatments 297

3.4.5 Comparison of breast cancer cell lines with the normal breast cell line 329

3.4.5.1 MCF-7 untreated 332

3.4.5.2 MDA-MB-231 untreated 349

3.4.6 Summary 375

Chapter four – Discussion

4.1 Genefishing 376

4.2 SuperArray 378

4.2.1 Are lycopene and thalidomide associated with the inhibition of oncogenic transformation in MCF-7 and MDA-MB-231? 378

4.2.1.1 MCF-7 378

4.2.1.2 MDA-MB-231 383

4.2.2 Is lycopene associated with inhibiting tumourigenesis in MCF-7 and MDA-MB-231 cells? 385

4.2.2.1 MCF-7 385

4.2.2.2 MDA-MB-231 386

4.2.3 Is thalidomide associated with inhibiting tumourigenesis in MCF-7 and MDA-MB-231 cells? 389

4.2.31 MCF-7 389

4.2.3.2 MDA-MB-231 390

4.2.4 Does lycopene influence normal breast cells? 392

4.2.5 Does thalidomide influence normal breast cells? 395

4.2.6 Is curcumin associated with inhibiting tumourigenesis in HT-29? 397

4.2.7 Is SAHA associated with inhibiting tumourigenesis in HT-29? 399

4.2.8 From this study, which genes have not previously been documented in studies with these drugs? 401

Chapter five – Conclusion 403

Future research 410

References 412

Appendix I: Reagents and Solutions 624

Appendix II: Results 629

LIST OF FIGURES Page

Figure 1.1: Diagrammatic representation of cross-section of normal breast 5

Figure 1.2: Diagrammatic representation of cross-section of normal colon 12

Figure 1.3: Chemical structure of curcumin 14

Figure 1.4: Chemical structure of lycopene 18

Figure 1.5: Chemical structure of SAHA 21

Figure 1.6: Chemical structure of thalidomide 25

Figure 1.7: Diagrammatic representation of the cell cycle 28

Figure 3.1: Side scatter versus forward scatter dot plot for HT-29 cells treated with 1µM SAHA for 72 hours 110

Figure 3.2: FL2-Width versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours 111

Figure 3.3: Side scatter versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours 111

Figure 3.4: Counts versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours 112

Figure 3.5: ModFit-generated side scatter versus forward scatter for HT-29 cells treated with 1µM SAHA for 72 hours 113

Figure 3.6: ModFit-generated FL2-Width versus FL2-Height for HT-29 cells treated with 1µM SAHA for 72 hours 114

Figure 3.7: ModFit-generated DNA histogram of HT-29 cells treated with 1µM SAHA for 72 hours 114

Figure 3.8: ModFit-generated DNA histogram of HT-29 cells treated with 5µM SAHA for 48 hours 117

Figure 3.9: ModFit-generated DNA histogram of HT-29 cells treated with DMSO for 24 hours 118

Figure 3.10: ModFit-generated DNA histogram of HT-29 cells treated with 50µM curcumin for 24 hours 119

Figure 3.11: ModFit-generated scatter plot of side scatter versus forward scatter for MCF-7 cells treated with 100µM thalidomide for 48 hours 121

Figure 3.12: ModFit-generated scatter plot of side scatter versus forward scatter for MCF-7 cells treated with 100µM thalidomide for 48 hours 122

Figure 3.13: ModFit-generated DNA histogram of MCF-7 cells treated with 100µM thalidomide for 48 hours 122

Figure 3.14: ModFit-generated DNA histogram of MCF-7 cells treated with THF for 24 hours 123

Figure 3.15: ModFit-generated DNA histogram of MCF-7 cells treated with 10µM lycopene for 24 hours 124

Figure 3.16: ModFit-generated DNA histogram of MDA-MB-231 cells treated with DMSO for 24 hours 126

Figure 3.17: ModFit-generated DNA histogram of MDA-MB-231 cells treated with 300µM thalidomide for 24 hours 127

Figure 3.18: ModFit-generated DNA histogram of MDA-MB-231 cells treated with THF for 72 hours 128

Figure 3.19: ModFit-generated DNA histogram of MDA-MB-231 cells treated with 5µM lycopene for 72 hours 129

Figure 3.20: ModFit-generated DNA histogram of 184A1 cells treated with 10µM lycopene for 48 hours 132

Figure 3.21: ModFit-generated DNA histogram of 184A1 cells treated with 100µM thalidomide for 48 hours 133

Figure 3.22: RNA gel of good integrity RNA 136

Figure 3.23: RNA gel showing unsuitable RNA samples 137

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 3 139

Figure 3.25: Gel image of HT-29 ACP6-ACP10 PCR reactions 140

Figure 3.26: Sequence of band 1 141

Figure 3.27: Gel image of HT-29 ACP10-ACP13 PCR reactions 143

Figure 3.28: Sequence of band 2 144

Figure 3.29: Sequencing of band 3 145

Figure 3.30: Sequencing of band 4 146

Figure 3.31: Gel image of MCF-7 ACP1-ACP4 PCR reactions 147

Figure 3.32: Sequencing of band 5 148

Figure 3.33: Gel image of MCF-7 ACP13-ACP16 PCR reactions 150

Figure 3.34: Sequence of band 6 151

Figure 3.35: Sequence of band 7 152

Figure 3.36: Gel image of MCF-7 ACP17-ACP20 PCR reactions 154

Figure 3.37: Sequence of band 8 155

Figure 3.38: Multi-group plot comparing BAX and BCL2 fold changes 300

Figure 3.39: Fold change of BAX and BCL2 351

Appendix II: Figures 1-260 629-775

LIST OF TABLES Page

Table 3.1: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of SAHA to HT-29 cells 115

Table 3.2: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of thalidomide to MCF-7 cells 120

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 125

Table 3.4: Percentages of DNA occurring at cell cycle stages at different concentrations and periods of exposure of lycopene in 184A1 cells 130

Table 3.5: Summary of treatments selected 134

Table 3.6: RNA purity and concentration ratios for untreated and treated cells 135

Table 3.7: Distribution of C_t values of HT-29 untreated cells 159

Table 3.8: Fold regulation of genes, in comparison to untreated cells 162

Table 3.9: Fold regulation of genes, in comparison to DMSO-treated cells 165

Table 3.10: Distribution of C_t values of MCF-7 untreated cells 210

Table 3.11: Fold regulation of genes in comparison to untreated cells 213

Table 3.12: Fold regulation of genes from lycopene treatments in comparison to THF-treated control cells, and thalidomide treatments in comparison to DMSO-treated control cells 215

Table 3.13: Distribution of C_t values of MDA-MB-231 untreated cells 246

Table 3.14: Fold regulation of genes, in comparison to untreated cells 250

Table 3.15: Fold regulation of genes, from lycopene-treated cells in comparison to THF-treated control cells, and thalidomide-treated cells in comparison to DMSO-treated control cells 253

Table 3.16: Distribution of C_t values of 184A1 untreated cells 290

Table 3.17: Fold regulation of genes, in comparison to untreated cells 293

Table 3.18: Fold regulation of genes from lycopene-treated cells in comparison to THF-treated cells, and thalidomide-treated cells in comparison to DMSO-treated cells 296

Table 3.19: Fold regulation of genes from MCF-7 and MDA-MB-231 untreated cells in comparison to 184A1 untreated cells 329

Table 3.20: Influence of drugs on gene expression in cell lines 368

ABBREVIATIONS

9-1-1	RAD9-HUS1-RAD1
A230	absorbance at 230ηm
A260	absorbance at 260ηm
A280	absorbance at 280ηm
ABL1	Abelson murine leukaemia viral oncogene homologue 1
ACTB	actin, beta
AIDS	acquired immune deficiency syndrome
ACP	annealing control primer
ANAPC2	anaphase promoting complex subunit 2
ANAPC4	anaphase promoting complex subunit 4
APC	anaphase promoting complex
ARHI	Ras homologue member I
AT	*Ataxia telangiectasia*
ATCC	American type culture collection
ATM	*Ataxia telangiectasia* mutated
ATP	adenosine triphosphate
ATPase	adenosine triphosphate hydrolase
ATR	*Ataxia telangiectasia* mutated and Rad3 related
B2M	beta-2-microglobulin
BAD	BCL2-associated death promoter
BAK	BCL2 homologous antagonist killer
BARD1	BRCA1 associated RING domain 1

BAX	BCL2-associated X protein
BCCIP	BRCA2 and CDKN1A interacting protein
BCL2	B-cell lymphoma 2
BCL-xL	basal cell lymphoma-extra large
BHT	butylated hydroxytoluene
BID	BH3 interacting domain death antagonist
BIM	BCL2-interacting mediator of cell death
BIRC5	baculoviral IAP repeat-containing 5
BLAST	basic local alignment search tool
bp	base pair
BRCA1	breast cancer 1
BRCA2	breast cancer 2
BTB	Bric-a-brac/Tramtrack/Broad
BUBR1	benzimidazole receptor 1
CAK	cyclin activating kinase 2
CCN	cyclin
CDC	cell division cycle
CDH1	cadherin 1
CDK	cyclin dependent kinase
CDK5R1	cyclin dependent kinase 5, regulatory subunit 1
CDK5RAP1	CDK5 regulatory subunit associated protein 1
CDKN	cyclin dependent kinase inhibitor
cDNA	circular DNA

CHEK1/CHK1	checkpoint homologue 1
CHEK2/CHK2	checkpoint homologue 2
CHO	Chinese hamster ovary
CIN	chromosomal instability
CIP1/KIP1	CDK-interacting protein 1
CK2	casein kinase 2
CKI	cyclin dependent kinase inhibitor
CKS1B	CDC28 protein kinase regulatory subunit 1B
CKS2	CDC28 protein kinase regulatory subunit 2
COII	cytochrome *c* oxidase subunit II
COIII	cytochrome *c* oxidase subunit III
COX-2	cyclooxygenase-2
C_t	threshold cycle
CtBP	C-terminal binding protein
CUL	cullin
DCIS	ductal carcinoma *in situ*
DDT	dichloro-diphenyl-trichloroethane
DDX11	DEAD/H box polypeptide 11
DEG	differentially expressed gene
DMEM	Dulbecco's modified essential medium
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
DNM2	dynamin 2

dNTP	deoxyribonucleic triphosphate
DP	DRTF-1 polypeptide
DSB	double strand break
DSG	daughter strand gap
E1F4A1	eukaryotic protein synthesis initiation factor 4A1
ECACC	European collection of cell culture
ECV	elongins/CUL2/pVHL
EDTA	ethylene diamine-tetra-acetic acid
EGFR	epidermal growth factor receptor 1
EMEM	Eagle's minimal essential medium
ER	oestrogen receptor
ER+	oestrogen receptor positive
ER-	oestrogen receptor negative
FACS	fluorescent-activated cell sorting
FBS	foetal bovine serum
FOXO3A	forkhead box O3A
FSC	forward scatter
G_1	gap phase 1
G_2	gap phase 2
GADD45A	growth arrest and DNA damage inducible alpha
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GDC	genomic DNA control
GPNMB	glycoprotein non-metastatic melanoma protein B

GSK3B	glycogen synthase kinase 3 beta
GTF2H1	general transcription factor IIH
GTPase	guanosine triphosphate hydrolase
GTSE1	G_2 and S-phase expressed 1
HDAC	histone deacetylase
HDACi	histone deacetylase inhibitor
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid
HER-2/neu	human epidermal growth factor receptor 2
HERC5	Hect domain and RLD5
HIF-1α	hypoxia inducible factor 1α
HPRT1	hypoxanthine phosphoribosyltransferase 1
IAP	inhibitor of apoptosis
IκB	inhibitor of NF-κB
IL-6	interleukin-6
INK4	inhibitor of cyclin dependent kinase 4
Inqaba Biotec	Inqaba Biotechnology Industries (Pty) Ltd
ISG	interferon-stimulated gene
KNTC1	kinetochore associated 1
KPNA2	karyopherin alpha 2
LOH	loss of heterozygosity
MAD2L1	mitotic arrest deficient-like 1
MAD2L2	mitotic arrest deficient-like 2
MAPK	mitogen-activated protein kinase

MCM	minichromosome maintenance deficient
MDM2	murine double minute 2
MEGM	mammary epithelium growth medium
MIN	microsatellite instability
MKI67	antigen identified by monoclonal antibody Ki-67
M-MLV	Moloney murine leukaemia virus
MMP	matrix metalloproteinase
MMR	mismatch repair system
MNAT1/MAT1	menage á trois 1
MPF	mitosis-phase promoting factor
MRE11A	meiotic recombination 11 homologue A
MRN	MRE11A-RAD50-NBS1
mRNA	messenger ribonucleic acid
MT	mitochondrial
MT-ATP6	mitochondrially encoded ATP synthase 6
NBS1	Nijmegen breakage syndrome 1
NEDD8	neural precursor cell expressed, developmentally downregulated 8
NF-κB	nuclear factor-κB
NRF2	NF-E2-related factor 2
OCT1	octamer transcription factor
PBS	phosphate buffered saline
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction

PEST	proline, glutamic acid, serine, threonine
PHD	plant homeo domain
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinase C
pol II	polymerase II
PPC	positive PCR control
ppm	parts per million
PR	progesterone receptor
P-TEFb	positive transcription elongation factor b
PTTG1	pituitary tumour-transforming 1
PUMA	p53 upregulated modulator of apoptosis
RB1	retinoblastoma 1
RBBP8	retinoblastoma binding protein 8
RBL1	retinoblastoma-like 1
RBL2	retinoblastoma-like 2
RBX1	RING-BOX 1
RFC	replication factor C
RhoBTB2	Rho-related BTB domain containing 2
RING	really interesting new gene
RNA	ribonucleic acid
rRNA	ribosomal RNA
ROC1	RING-BOX finger protein
ROS	reactive oxygen species

ROX	6-carboxyl-x-rhodamine
RPA3	replication protein A3
RPL13A	ribosomal protein L13A
rpm	revolutions per minute
RT	reverse transcription
RTC	reverse transcription control
SAGE	serial analysis of gene expression
SAHA	suberoylanilide hydroxamic acid
SAMM50	sorting and assembling machinery component 50 homologue
SCF	SKP1-Cullin1-F-box
SERTAD1	SERTA domain containing 1
SKP2	S-phase kinase-associated protein 2
SSC	side scatter
ssDNA	single strand DNA
STAT1	signal transducer and activator of transcription 1
SUMO1	SMT3 suppressor of mif 2 3 homologue 1
SWM1	spore wall maturation protein 1
TBE	Tris borate EDTA
TE	Tris EDTA
TFDP1	transcription factor DP1
TFDP2	transcription factor DP2
TFIIH	transcription factor II H
THF	tetrahydrofuran

TNF	tumour necrosis factor
TP53	tumor protein p53
TPR	tetratricopeptide repeat
TRAIL	TNF-related apoptosis-inducing ligand
tRNA	transfer ribonucleic acid
UBE1	ubiquitin-activating enzyme E1
UV	ultraviolet
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau
WAF1	wild-type p53-activated fragment 1
WHO	World Health Organisation
ZBRK1	zinc finger and BRCA1-interacting protein with a KRAB domain 1
ZNF350	zinc finger protein 350