CHAPTER ONE

GENERAL INTRODUCTION

1.1

REASONS FOR THIS STUDY

Oestrogen is a modulator of cellular growth and differentiation especially in those cells associated with reproduction and mammary gland development (Laidlaw *et al*, 1995). Oestrogens are also involved in the growth and development of uterine and mammary cancers (Miller and O'Neil, 1989). The mechanism by which the effects of oestrogen are mediated in both normal and neoplastic cells is via two specific intracellular receptors ERα and ERβ (Kuiper *et al*, 1996). These ERs are members of the steroid/thyroid/retinoid receptor gene superfamily of ligand-activated nuclear transcription factors and their presence has been an indicator of a favourable prognosis in breast cancer with ERα-positive tumours being more sensitive to endocrine treatment with agents such as tamoxifen. Unfortunately, a large proportion of patients eventually develop resistance towards this treatment (Clarke and McGuire, 1988). A number of ERα and ERβ variants have been identified (Fuqua *et al*, 1991; Wang and Miksicek, 1991; Dotzlaw *et al*, 1992; Fuqua *et al*, 1992; Zhang *et al*, 1996; Chappell *et al*, 2000; Poola and Speirs, 2001; Poola *et al*, 2002a; Taylor *et al*, 2010) and it has been suggested that these ER variants may play a role in the development of drug resistance. These variants have also been found to coexist with wildtype ER and may interfere with its normal

function. This study attempted to examine these ER variants and assess their clinical relevance as well as their significance with respect of each other and the role they each may play in the pathogenesis of breast disease.

In order to examine the progression of disease the T-47D breast cancer cell line was utilized as a model for the effects of various breast cancer treatments on the ERs. The T-47D cell line is an ERα positive cell line that, although it has been found to be sensitive to tamoxifen, an oestrogen antagonist, it seems to also be genetically unstable. This cell line may therefore be in transition from an oestrogen responsive state to an oestrogen resistant state and possibly become ER-negative. The effects of oestrogen, in the form of 17-β-oestradiol, are therefore important when assessing hormone responsiveness and the possible influence of each ERα exon variant or ERβ region.

Tamoxifen is the most widely used hormonal therapy for all stages of breast cancer provided that the cells are ER-positive (Fisher, 1999). Not only has it been used for the treatment of advanced disease in pre- and postmenopausal women but also for the prevention in women at high risk of developing breast cancer (Levenson and Jordan, 1999). In breast tissue, tamoxifen binds to ERα and competitively blocks the action of oestrogen (Jordan and Dowse, 1976). ERβ also binds to tamoxifen but with a higher affinity than ERα does (Kuiper *et al*, 1997). An important drawback to tamoxifen treatment, however, is the development of drug resistance that occurs in many patients. This may also take the form of tamoxifen stimulation of the tumour thus suggesting both oestrogen agonist and antagonist activities (Katzenellenbogen *et al*, 1997; McDonnell *et al*, 2002). The mechanism

of tamoxifen resistance in ER-positive breast cancer is unknown despite extensive studies (Osborne, 1998; Ali and Coombs, 2002). This study examines the potential role of oestrogen receptor variants in the development of oestrogen resistance in T-47D breast cancer cells by observing growth in medium containing tamoxifen.

The majority of breast carcinomas arise after menopause when ovarian production of oestrogen and progesterone decline. Oestrogen synthesis, however, also occurs in muscle, skin and adipose tissue through the conversion of either androstenedione or testosterone to oestrone and oestradiol by the enzyme, aromatase, a cytochrome P-450. Cytochrome P-450 is also involved in the conversion of cholesterol to pregnenolone, the basic precursor of androgens, oestrogens and glucocorticoids. Cholesterol has, therefore, also been implicated in increasing breast cancer risk in postmenopausal women (Furberg *et* al, 2004). Concentrations of oestradiol have been reported to be significantly higher in breast carcinoma than in peripheral tissue in postmenopausal women (van Landegham *et al*, 1985; Szyczak *et al*, 1998; Chetrite *et al*, 2000; Miyoshi *et al*, 2001) suggesting that oestrogen production within the breast and by breast cancers may be critical in stimulating tumour proliferation in these patients. Aromatase mRNA levels have been found to be increased in invasive (Utsumi *et al*, 1996) and non-invasive (Suzuki *et al*, 2007) breast carcinoma compared to normal breast tissue indicating that aromatase is the key enzyme responsible for the increase in intratumoural oestrogen levels in breast cancer. Systemic treatment of breast cancer patients with aromatase inhibitors should block synthesis of oestrogen in all tissue sites and therefore reduce the progression of breast tumours especially in postmenopausal women.

One of the early aromatase inhibitors was aminoglutethimide. In fact it was the prototype for nonsteroidal aromatase inhibitors (Cocconi, 1994). Aminoglutethimide blocks several cytochrome P-450-mediated steroid hydroxylation steps including those required for cholesterol to pregnenolone conversion and for the aromatization of androgens to oestrogens. The T-47D cell line provides a model in order to establish the effects of aromatase action (by observing growth in the presence of cholesterol and androstenedione) and aromatase inhibition (by the addition of aminoglutethimide to the growth medium) on ER variants.

Oestriol, a weak oestrogen and a metabolite of oestradiol and oestrone, has been used successfully as a hormone replacement therapy (HRT) in treating postmenopausal women for the relief of climactic symptoms (Takahashi *et al*, 2000). Menopausal HRT has, however, been associated with increased breast cancer risk (Rosenberg *et al*, 2006) although the presence of oestriol has also indicated some prevention of mammary carcinogenesis (Lakshmanaswamy *et al*, 2004). T-47D breast cancer cells grown in medium containing oestriol would provide information on hormone response and the effect of this oestrogen metabolite on ERs.

1.2

AIM

The purpose of this thesis is to define a pattern of oestrogen receptor α (ERα) and oestrogen receptor β (ERβ) mRNA variants in clinical breast cancer using RT-PCR

and sequence analysis in order to establish their relationships with clinical parameters, prognosis and treatment outcome and thus their role in the alteration of oestrogen action that occurs during tumourigenesis and perhaps in the acquisition of anti-oestrogen resistance. To further examine progression of disease the rate and location of variations in the exons of ERα and ERβ caused by oestrogens, anti-oestrogens, aromatase inhibitors and oestrogen precursors and their implications on hormone responsiveness are examined in an *in vitro* system using the human breast cancer cell line, T-47D, as a model.