In Touch with your Feminine Side: How Oestrogen Metabolism Impacts Prostate Cancer

Rahman, Habib-Ur; Hofland, Johannes; Foster, Paul

DOI:
10.1530/ERC-16-0118

Accepted Preprint first posted on 18 May 2016 as Manuscript ERC-16-0118

In Touch with your Feminine Side: How Oestrogen Metabolism Impacts Prostate Cancer

[1]Habibur P. Rahman, [2]Johannes Hofland, [1,3]Paul A. Foster

[1] Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, B15 2TT, UK.

[2] Department of Internal Medicine, Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands.

[3] Centre for Endocrinology, Diabetes and Metabolism, Birmingham Healthcare Partners, Birmingham, B15 2TH, UK.

Tel: +44 (0)121 414 3776

Fax: +44 (0)121 415 8712

Short title: Oestrogens and Prostate Cancer

Proofs and Correspondence to:

Dr. P.A. Foster

Institute of Metabolism and Systems Research,

College of Medical and Dental School,

University of Birmingham,

Birmingham,

B15 2TT.

UK.

Declaration of Interest

The authors have nothing to declare.

Funding

No specific funding was received for this review.

Abstract

Prostate cancer is the primary male cancer with increasing global incidence rates making this malignancy a significant healthcare burden. Androgens promote normal prostate maturity but also influence the development and progression of prostate cancer. Intriguingly, evidence now suggests endogenous and exogenous oestrogens, in the form of phytoestrogens, may be equally as relevant as androgens in prostate cancer growth. The prostate gland has the molecular mechanisms, catalysed by steroid sulphatase (STS), to unconjugate and utilise circulating oestrogens. Furthermore, prostate tissue also expresses enzymes essential for local oestrogen metabolism, including aromatase (CYP19A1) and 3β- and 17β-hydroxysteroid dehydrogenases. Increased expression of these enzymes in malignant prostate tissue compared to normal prostate indicates oestrogen synthesis is favoured in malignancy and thus may influence tumour progression. In contrast to previous reviews, here we comprehensively explore the epidemiological and scientific evidence on how oestrogens impact prostate cancer, particularly focusing on pre-receptor oestrogen metabolism and subsequent molecular action. We analyse how molecular mechanisms and metabolic pathways involved in androgen and oestrogen synthesis intertwine to alter prostate tissue. Furthermore, we speculate on whether oestrogen receptor status in the prostate affects progression of this malignancy.

Introduction

In the UK prostate cancer is the number one male malignancy accounting for 25% of all new cancer diagnoses in men (Siegel, et al. 2012). In 2011, there were almost 42,000 new cases with an age-standardised incidence rate of 104.7 per 100,000. Prostate cancer is the second leading cancer killer in UK men and 4th most common cause of cancer death in the general population. Similarly, in Europe prostate cancer is the most common cancer in males and third most common cancer overall (Jacob and Henrik 2006) . It is the third most common cause of cancer deaths in men and sixth overall. Currently, prostate cancer is the second most common cancer in males worldwide after lung cancer. However, it is predicted that prostate cancer will become the most common cancer in men globally (Parkin, et al. 2001).

Survival statistics from prostate cancer have improved dramatically over the last four decades which may be attributed to earlier detection and treatment granted by prostate specific antigen (PSA) testing and transurethral resection of the prostate (TURP). The UK 10-year survival has improved from 25% when diagnosed in 1970 to 84% in 2010 (Quaresma, et al. 2015). Prostate cancer primarily affects the elderly with 99.9% of patients diagnosed over the age of 50 and the mean age at diagnosis being 73 (Parkin, et al. 1997). Furthermore, from autopsy studies of non-cancer-related deaths, there is histological evidence of prostate neoplasms in more than 50% of men in their 50s (Sakr, et al. 1993). As average male life expectancy gradually increases, it is foreseeable that men will live longer with the disease and may experience a poorer quality of life.

There are significant geographical variations between prostate cancer incidences around the world with up to a 24-fold difference between the regions with the highest rates (in Australia, North America and Western Europe) and the lowest rates (in India, Japan and China) (Center, et al. 2012). While some of the discrepancies might be explained by disparities in healthcare

access, diagnostic methods, screening programmes and reporting systems; environment and lifestyle remain considerable factors. Studies comparing the incidence of prostate cancer in first and second generation Asian immigrants to USA with age-matched controls in their native countries have found that migrants travelling from low risk countries to high risk countries adopt the higher risk (Cook, et al. 1999). This advocates that environmental risk factors may have a higher precedence than genetic associations in determining risk of prostate cancer. Furthermore, environmental and lifestyle factors, diet in particular, fundamentally alter endogenous hormones including sex steroids (Barazani, et al. 2014). Indeed, factors such as smoking, increased physical exercise and a vegetarian diet increased serum androgen concentrations in British men while obesity, high fat diet and sedentary occupation reduced serum androgen concentrations (Allen et al. 2002). Such hormonal changes have the propensity to subsequently affect tumour initiation and progression (Kolonel, et al. 2004).

Sex Steroids and Prostate Cancer

Both males and females produce sex steroid hormones; the predominant androgens are testosterone and the more biologically active dihydrotestosterone (DHT) and the predominant oestrogens are oestrone (E_1) and the more biologically active oestradiol (E_2). However, the ratio of the two hormones differs between the sexes significantly. In the prostate, androgens are required for normal development and function. However, the role of oestrogens in normal prostate development is ill defined as biochemical mechanisms are still under investigation; the current dogma being that oestrogens are involved in the differentiation of epithelial tissue (Chen, et al. 2012; Francis, et al. 2013) and regulation of prostatic angiogenesis (Montico, et al. 2013).

Androgens have been implicated in prostate carcinogenesis since 1941 when Huggins published his Nobel winning study showing testosterone injections exacerbate prostate cancer in patients with late-stage disease and androgen deprivation alleviated the disease (Huggins and Hodges 1941), this suggested prostate cancer as an androgen-dependent malignancy. The primary source of androgens in males is testosterone secreted by the testicles, however, the adrenal glands secrete 100-500 times greater amounts of dehydroepiandrostrone sulphate (DHEAS), a testosterone precursor which can be converted peripherally in the prostate into testosterone and DHT (Labrie, et al. 2005). Androgen ablation therapy is initially successful in the vast majority of prostate cancers but relapse is common as tumours become castration resistant; they still however continue to express androgen receptors which respond to very low concentrations (as low as 10 pM) of peripherally synthesised testosterone and DHT (Chen, et al. 2004; Mohler, et al. 2004). Using microarray experiments on LNCaP and LAPC4 cell lines, Chen et al. (2004) showed an increase in androgen receptor mRNA and protein expression *in vitro* and *in vivo* in castrated xenograft murine models which correlated with tumour growth. Increased expression of androgen receptors amplified signals from low levels of androgen ligands to confer castration resistance. Mohler et al (2004) demonstrated using immunostaining and radioimmunoassays that activation of androgen receptors occur even in human prostate cancer samples retrieved from chemically castrated patients. This explains why surgical or medical castration is not 100% effective.

Previously, second-line hormonal therapy has proven to improve survival in patients with castration-resistant disease, both before and after docetaxel chemotherapy. Both inhibition of steroidogenic enzyme CYP17A1 using abiraterone and androgen receptor antagonism by enzalutamide have successfully ablated continued androgen receptor activation and prostate cancer growth (Beer , et al. 2014; de Bono , et al. 2011; Ryan , et al. 2013; Scher , et al.

2012). However, as with other androgen ablation therapy, resistance to abiraterone and enzalutamide inevitably develops.

Even though molecular mechanisms were not elucidated, oestrogens were traditionally considered to protect against prostate cancer. Therapeutic use of oestrogens was based on their anti-androgenic effects. Huggins reported exogenous oestrogens had protective properties mediated by a negative feedback effect on the hypothalamic-pituitary-gonadal (HPG) axis which reduced stimulation for androgen secretion from the testes (Huggins and Hodges 1941). Diethylstilbestrol (DES), a synthetic non-metabolised oestrogen is still used in certain clinics as a non-first line therapy to chemically castrate patients with metastatic prostate cancer (Bosset, et al. 2012; Clemons, et al. 2013). DES negatively feedbacks on the pituitary gland to reduce secretion of luteinizing hormone which reduces the stimulus for the testes to synthesise sex hormones In addition to the effects oestrogens have on the HPG axis, demonstrated by quantitative PCR,DES inhibits androgen-stimulated telomerase activity and gene expression and induces apoptosis in LNCaP and PC3 prostate cancer cell lines in both the presence and absence of androgens (Geier, et al. 2010). On the contrary, while DES is still licensed in the UK for treatment of prostate cancer it is infrequently used as secondary treatment due to the accompanied high rates of cardiovascular toxicity (Malkowicz 2001).

Importantly, the interactions of oestrogens on androgen receptors should be considered. For example, E_2 can activate both wildtype and, with greater efficacy, mutated (T877A) androgen receptors in LNCaP cells (Susa et al J Cell Physiolog 2015; Yeh et al. 1998; Veldscholte et al J Steroid Biochem Mol Biol. 1992). Mutations of the androgen receptor are uncommon in the early stages of prostate cancer but are much more frequent in late-stage disease. In one study, out of 99 patients diagnosed with early stage prostate cancer none were found to have

mutations in the androgen receptor. On the contrary, eight tumours out of 38 patients with advanced prostate cancer were found to harbour androgen receptor mutations (Marcelli, et al. 2000; Brooke and Bevan 2009). There is, however, mounting evidence that oestrogens may be involved in the initiation and progression of prostate cancer, although compelling evidence confirming oestrogen binding affinity to AR is lacking.

Impact of Endogenous Oestrogens in Prostate Cancer

Males are exposed to a high oestrogen/androgen (E/T) ratio twice in their lifetime. The first is as a foetus, during the third trimester when the maternal E_2 levels increase and foetal androgen levels decrease. Raised E_2 levels stimulate the developing epithelial cells of the prostate to proliferate but also cause morphological changes. For example, the prostate glands of neonatal rats and mice show abnormal proliferation and cell structure when the pregnant mother is injected with E_2. (Wernert, et al. 1990). This early exposure may imprint intracellular changes by modulating expression pathways of steroid enzymes and receptors as shown in rat models where the response to endogenous androgens and oestrogens becomes abnormal, thus predisposing the animal to prostate cancer after sexual maturation (Rajfer and Coffey 1978). Moreover, studies in mice show that when exposed to high levels of oestrogens in utero, foetal prostate tissue develops abnormalities including intraepithelial neoplasia and predisposition to carcinogenesis in adult life (Prins, et al. 2006). This hypothesis is supported by epidemiological evidence obtained from African-American men having twice as high a risk of developing prostate cancer than comparable Caucasian men which correlates with African-American women having a higher serum oestrogen level during pregnancy compared to Caucasian women (Henderson, et al. 1988).

The second time men are exposed to a high E/T ratio is during old age when serum testosterone decreases, partly due to a dampened HPG axis and partly due to reduced Leydig cell function in the testes. In addition to this, sex hormone-binding globulin (SHBG), which has a higher affinity to testosterone than E_2 (Knochenhauer, et al. 1998), also increases with age which further decreases free serum testosterone relative to free serum E_2 (Samaras, et al. 2012). Furthermore, there is evidence that E_1 and E_2 not only remain at the same level, but in fact increase with age even when accounted for BMI and other metabolic diseases (Jasuja, et al. 2013). While the evidence for an association between serum oestrogen concentration and risk of prostate cancer is unclear and inconsistent, increased serum oestrogen concentrations may stimulate the prostate stroma and epithelia to proliferate and subsequently become neoplastic. Indeed a higher oestrogen:androgen ratio stimulates proliferation of normal prostate stromal (PrSC) and normal epithelial (PrEC) cell lines in vitro (King, et al. 2006).

Another interesting population which is exposed to a high E/T ratio are transsexual male to female individuals. Often in this group of former males, individuals are orchiectomised and then supplemented with anti-androgens to relinquish masculine secondary sex characteristics. They are also supplemented with oestrogens to acquire and enhance feminine characteristics. Their prostates, however, remain unadulterated. A study observing such a cohort of transsexual persons for over 30 years has not identified any increase in risk for prostate cancer (Gooren and Morgentaler 2014). However the study has suggested that when presenting these patients are more likely to be diagnosed with a later stage disease. One limitation admitted by the authors is that the majority of the cohort has not reached the mean age at which prostate cancer is typically diagnosed (Gooren and Morgentaler 2014). Observations made to this cohort over the next two or three decades will be most

enlightening in ascertaining whether oestrogens have any significant effects in the development of prostate cancer.

Oestrogen Metabolism in Adipose and Prostate Cancer

While in pre-menopausal females the primary source of oestrogens are the ovaries, in males there is no central organ which produces substantial quantities of E_2. Instead, peripheral conversion of oestrogen precursors is the main source of oestrogen in men. Local synthesis of E_1 and E_2 is regulated by a plethora of enzymes. DHEA secreted from the zona reticularis of the adrenal glands, and stored in the blood as a reservoir as DHEAS, is the ultimate precursor. Adipose tissue is another notable source of oestrogen synthesis (Cui, et al. 2013). White adipose tissues (the predominant type in obesity) express significant quantities of cytochrome P450 aromatase enzyme (*CYP19A1*) in the abdominal adipose fat of male human samples, which is the final catalyst in the conversion of androgens to oestrogens (Polari, et al. 2015; Wang, et al. 2013). There is also a positive correlation between the amount of visceral adipose tissue and serum E_2 levels as shown in a study of 229 man with a mean age of 53.6 years where visceral fat was measured using magnetic resonance imaging (Gautier, et al. 2013).

There have been conflicting reports as to whether obesity is a risk factor for prostate cancer as some suggest it decreases risk while others have found the opposite. Allott *et al.* have summarised the findings published between 1991 to 2012 in their review and conclude obesity is associated with aggressive prostate cancer (Allott, et al. 2013). There is further

robust evidence that obese patients are more likely to present with aggressive high-grade prostate cancer (De Nunzio, et al. 2013; Vidal, et al. 2014). It is possible that the risk associated with obesity may in fact be due to elevated circulating oestrogen levels secondary to increased adipose deposition. If this is the case, it would parallel the effects of oestrogen that have been observed in colorectal cancer where oestrogen exposure in the form of hormone replacement therapy or oral contraceptives are initially protective against colorectal cancer but when patients present, they present with a later stage disease (Foster 2013). The intra- and extracellular handling and metabolism of oestrogens within the prostate gland may clarify what effects oestrogens have on tumours. However, studies are lacking regarding the exact intra-tumoural metabolism of oestrogens in prostate cancer cells and human prostate cancer tissue.

Impact of Exogenous Oestrogen on Prostate Cancer

Exogenous oestrogen intake and subsequent availability to the prostate should be considered when determining whether oestrogens affect the development and progression of prostate cancer. A Western diet comprising of high meat, saturated fat, and dairy products has been associated with increased risk of prostate cancer as highlighted by numerous epidemiological studies (Grönberg 2003; Howell 1974; Whittemore, et al. 1995). Additionally, it has been observed that such a Western diet is more likely to cause men diagnosed with prostate cancer to die from the disease when compared to a diet rich in fruits, vegetables, and whole grain cereals (Yang, et al. 2014). Supporting this, it has been widely speculated that dietary oestrogenic compounds from plant sources, termed phytoestrogens, are protective against prostate cancer and are the reason behind lower incidence rates in East Asia where per capita consumption of phytoestrogen-rich foods, such as soya beans, are considerably higher than

the Western world (Adlercreutz, et al. 2000; Goetzl, et al. 2007; Strom, et al. 1999). It is possible that phytoestrogens reduce the risk of prostate cancer through multiple mechanisms. In rodent models phytoestrogens can upregulate SHBG synthesis in the liver leading to a higher circulating concentration (Pilšáková, et al. 2010). Increased SHBG is anti-androgenic as it binds to free testosterone with a higher affinity than oestrogens (Knochenhauer et al. 1998) implementing a net reduction of testosterone relative to E_2 (Ronde, et al. 2005). This reduction in androgen is thought to be important in the reduction of risk. In addition to chelation of free testosterone via SHBG, phytoestrogens have a negative feedback effect on the HPG axis directly leading to reduced secretion of luteinising hormone and consequently reduced stimulation of androgen and oestrogen synthesis (Goetzl et al. 2007).

Phytoestrogen compounds are similar enough to endogenous oestrogens to be able to bind to oestrogen receptors (ER) and evoke ligand-specific intracellular responses (Usui 2006). Preference for different types of nuclear ER varies between phytoestrogens (see section on oestrogen receptors). Isoflavones and coumestans are two main categories of phytoestrogens and are structurally similar to E_2 (Figure 1). The prostate cancer cell lines LNCaP and DU145 are more sensitive to apoptotic factors when treated with isoflavones *in vitro*. A dose-response relationship between concentration of biochanin A and apoptosis was observed using cytotoxicity and lactate dehydrogenase release assays, flow cytometry and fluorescence microscopy (Szliszka, et al. 2013). Coumestans are able to induce caspase-dependent apoptosis in LNCaP, DU145 and PC3 cells. When treated with wedelolactone, a plant derived coumestan, there was dose-dependent apoptosis in androgen-sensitive cell lines (LNCaP) and androgen-independent cell lines (DU145 and PC3). However, normal non-cancerous PrEC prostate epithelial cells were not affected as harshly showing 90% cell viability compared to circa 20% in cancerous cell lines at concentrations of 30μM. (Sarveswaran, et al. 2012).

While in vitro evidence argues that phytoestrogens are protective against prostate cancer, clinical trials looking at the relationship between consumption of dietary phytoestrogens and progression of prostate cancer have been inconclusive (Goetzl et al. 2007). One double blind randomised control trial in which 81 healthy men were either given a soy protein drink with high isoflavone concentration (83mg/day) or a drink with low isoflavone concentration (3mg/day) showed no significant difference in PSA over 12 months (Adams, et al. 2004). Another trial offering men with confirmed prostate cancer who had either failed medical/surgical therapy or had chosen active surveillance a high dose (450mg/day) oral isoflavone supplement for 6 months showed only a clinically insignificant improvement in PSA in the active surveillance group with no difference in the failed therapy group (deVere White, et al. 2004). Furthermore, a study following up 3628 men with diagnosed prostate cancer for a median duration of 11.5 years showed an increased risk of advanced prostate cancer (HR: 1.62) but a reduced risk of non-advanced prostate cancer (HR: 0.88) in the higher dietary intake of isoflavones group. Dietary intake of phytoestrogens was measured using a validated food frequency questionnaire and so exact doses of phytoestrogens are subject to variation (Reger et al. 2015). This preliminary evidence could infer that dietary phytoestrogens might protect against initiation of prostate cancer, however may promote the progression of advanced prostate cancer.

Steroid metabolism in the prostate

Androgens

The metabolism of oestrogens and oestrogen precursors is important for availability of biologically active E_2 to prostate cancer cells. Oestrogens are synthesised from androgens which themselves are synthesised from progestogens (Khurana 2008). In addition to circulating androgens secreted from the testes, normal prostate tissues have the potential to produce androgens from circulating C19 steroids DHEA and androstenedione (Figure 2). There have been conflicting reports on the possibility of prostate cancer to synthesize androgens *de novo* through the conversion of progestogens via cytochrome P450 17A1 (17-hydroxylase and 17, 20 lyase enzyme [*CYP17A1*]). In prostate cancer, the expression of cytochrome P450 17A1 was reportedly increased in LNCaP and LuCaP cells and human prostate tissue samples ascertained by PCR and immunoblotting (Locke, et al. 2008; Montgomery, et al. 2008); however not all studies support this (Ellem and Risbridger 2009; Hofland, et al. 2010). Although DHT formation from cholesterol was detected using mass spectrometry in castration-resistant prostate cancer (CRPC) models in one study (Locke et al. 2008) these steroid fluxes have not been confirmed quantitatively to date in either *in vitro* or *in vivo* models.

Another key enzyme in the synthesis of biologically active androgens and oestrogens is 3-betahydroxysteroid dehydrogenase (3β-HSD) which converts dehydroepiandrosterone and androstenediol to androstenedione and testosterone, respectively (White, et al. 2013). 3β-HSD is expressed in the normal human prostate, with immunoblotting revealing that the highest concentrations are found in basal epithelial cells (Luu-The, et al. 2008). Certainly, in mouse xenograft studies using the CRPC LAPC4 cell line, expression of 3β-HSD is increased within the tumour in addition to AKR1C3 and 17β-HSD3 (Chang, et al. 2011), although its mRNA expression almost completely mutually excludes that of CYP17A1 (Hofland et al. 2010).

Inhibitors of 3β-HSD have been explored as an androgen deprivation technique as they are effective in decreasing proliferation in androgen sensitive LNCaP or CRPC cell lines 22Rv1, VCaP and PC346C *in vitro* (Evaul, et al. 2010; Kumagai, et al. 2013). Furthermore, abiraterone was found to inhibit 3β-HSD activity in addition to CYP17A1 in prostate cancer cell lines and isolated yeast microsomes (Li, et al. 2012). This mechanism might rely on abiraterone being converted to the more active Δ(4)-abiraterone (D4A) within the prostate gland by 3β-HSD itself (Li, et al. 2015b). Further research into 3β-HSD inhibition are currently being pursued, however alternative pathways which bypass androstenedione synthesis exist and so 3β-HSD function is not strictly necessary.

An alternative pathway has been demonstrated by which synthesis of DHT within the prostate may bypass testosterone and instead be synthesised by reduction of androstenedione by 5α-reductase SRD5A1 to 5α-androstanedione which is converted to DHT by 17β-HSD5. Mass spectrometry has shown that even in patients on anti-androgen therapy with very low serum testosterone levels, intratumoral DHT concentrations remain at the pre-treatment level (Chang et al. 2011; Sharifi and Auchus 2012). 17β-HSD-5, also known as AKR1C3, appears to be the key enzyme responsible for intratumoural androgen production in CRPC. Its expression in LNCaP, DU145 and PC3 cellsare potently stimulated by androgen deprivation *in vitro* and in humans *in vivo* (Ellem and Risbridger 2009; Ellem, et al. 2004) and this secures continued production of testosterone and DHT from circulating adrenal androgens. Local growth factor activin A was shown to be a key intermediate in the castration-induced rise of *AKR1C3* expression levels and intratumoural testosterone production as observed in

LNCaP, VCaP and PC3 cells. The concentration of activin A and testosterone were also shown to be increased in the cultured supernatants, as measured by ELISA and mass spectrometry (Hofland, et al. 2011). 17β-HSD-5 has also been implicated in ezalutamide resistance to anti-androgen therapy. Knockdown of 17β-HSD-5 using shRNA or inhibition with indomethacin has shown to resensitise enzalutamide-resistant cells *in vitro* and *in vivo* (Liu, et al. 2015).

Peripheral Oestrogen Metabolism in Prostate Cancer

As mentioned previously, aromatase is a key enzyme required for oestrogen synthesis from androgen precursors. Aromatase converts androstenedione and testosterone to E_1 and E_2, respectively (White et al. 2013). The local synthesis of E_2 within the prostate has previously been debated as not all experiments have identified aromatase expression in normal prostate tissue (Ellem et al. 2004). However, it has been demonstrated in human samples by substrate conversion assays and mass spectrometry that E_2 synthesis does occur in prostate cancer cells (and benign prostatic hyperplasia) via aromatisation (Ellem and Risbridger 2009; Härkönen and Mäkelä 2004). In normal prostate, aromatase is expressed by the stromal tissue but not the epithelial cells, however once malignant, epithelial cells also express aromatase (Ellem and Risbridger 2007). Aberrant expression and activity of aromatase is crucial in the pathophysiology of endometrial and breast cancers where an imbalance of oestrogen is a key factor in tumour growth (Chen 1998; Cunha 1994). As with the developmental similarities between breast and prostate tissues (Ellem and Risbridger 2010), abnormal aromatase activity also plays a major role in breast and prostate tumourigenesis (Ellem and Risbridger 2010). Tumourigenic growth factors including epidermal growth factor and transforming growth factor-1 can modulate aromatase activity in androgen-sensitive LNCaP cells lines leading to decreased oestrogen synthesis (Block, et al. 1996). Furthermore, the expression of aromatase is up to 30-fold greater in metastatic prostate cancer compared to primary tumours

(Miftakhova, et al. 2016). In addition, overexpression of aromatase increased the progression of bony metastasis in xenograft experiments where nude mice were injected with PC3 cell lines transfected to overexpress aromatase (Miftakhova, et al. 2016). Consequently, the use of aromatase inhibitors for the treatment of prostate cancer has been investigated many times in patient cohorts. The first generation aromatase inhibitor aminoglutethimide is non-selective and showed poor objective responses including serum PSA levels and disease stability in some studies while showing a significant increase in survival in others (Santen, et al. 1997). One study treated 58 castrated men with advanced prostate cancer resistant to conventional therapy with 500-750mg daily aminoglutethimide; 11 men showed an objective response with a mean remission of 10 months and a further two showed disease stabilisation for a mean seven months (Murray and Pitt 1985). The second generation aromatase inhibitor, 4-hydroxyandrostenedione showed good subjective responses in 18 out of 25 patients with advanced CRPC, particularly alleviation of bone pain in prostate metastases. However the objective responses were still poor with a reduction in tumour volume seen in only three patients and all patients progressed to have skeletal metastasis. (Davies, et al. 1992). A Phase II clinical study looking at the effects of oral letrozole, a third-generation aromatase inhibitor more commonly used in the treatment of hormone-dependent breast cancer, in 43 men with CRPC showed no significant disease regression with serum PSA decreasing by more than 50% in only one patient and decreasing by less than 50% in one further patient (Smith, et al. 2002). A very similar conclusion was drawn from clinical studies looking at anastrazole, another third generation aromatase inhibitor, where out of 14 patients with CRPC none showed a decrease in serum PSA and mild bone pain relief was reported by only two patients (Santen, et al. 2001). While aromatase is of utmost importance in local oestrogen synthesis, it appears as though therapeutic approaches targeting aromatase may be futile in treating prostate cancer. An alternative possibility is that E_2 is not synthesised from androgens within

the prostate but instead is converted from systemic sulphated E_1 within the prostate via steroid sulphatase (STS).

STS is widely expressed in almost all peripheral tissues and is responsible for hydrolysing sulphate moieties off of circulating sulphate-conjugated steroids in order to make them biologically active (Mueller, et al. 2015). Oestrone sulphate (E_1S) is the most abundant circulating oestrogen in adult humans (Muir, et al. 2004) with plasma levels between 2-4nmol/L in men (Mueller et al. 2015) and while oestradiol sulphate also exists, plasma levels are very low. Furthermore, serum E_1S levels have been correlated with increased risk of prostate cancer. In a cohort study of 5995 men aged over 65 where the mean serum E_1S levels in the 275 patients who developed prostate cancer was significantly higher than those who did not develop prostate cancer (Daniels, et al. 2010).

Before sulphated oestrogens can be unconjugated by intracellular STS, transport of sulphated oestrogens into cells requires the expression of organic anion transporter peptides (OATP) (Raftogianis, et al. 2000) and indeed several different OATPs involved in the transport of oestrone sulphate are expressed in prostate cancers (Buxhofer-Ausch, et al. 2013; Giton, et al. 2015; Wright, et al. 2011). STS has been shown to be expressed in normal human prostate tissue (Reed, et al. 2005), prostate cancer cell lines LNCaP, DU-145 and PC3 (Nakamura, et al. 2006) and in primary prostate homogenates (Klein, et al. 1989). Furthermore, one study found that STS is expressed in the majority of localised prostate cancers showing higher expression in malignant tissues compared to benign (Nakamura, et al. 2006). The activity of STS has been proven within the human prostate for the desulphation of dehydroepiandrosterone sulphate (DHEAS) into DHEA, an androgen precursor (Farnsworth

1973). Moreover, E_1 synthesis from desulphation of E_1S within the prostate is putatively 10-fold greater than synthesis via aromatase (Nakamura et al. 2006). The relevance of STS in cancer has been more extensively studied in breast cancer where there is significantly higher expression of STS than in normal breast (Utsumi, et al. 2000). Consequently, several STS inhibitors have been developed for the treatment of breast cancer, some of which have shown early promise (Stanway, et al. 2006). Moreover, first and second generation STS inhibitors have been effective pre-clinically against breast cancer (Foster et al. 2006; Foster et al. 2008; Purohit and Foster 2012). Meanwhile, investigations into the efficacy of STS inhibitors in prostate cancer have been undertaken. It has been observed that middle-aged rats treated with oral STS inhibitor, STX64 decreased conversion of E_1S to E_1 (Giton et al. 2015; Roy, et al. 2013). Neither study presented evidence of STS inhibition affecting any proliferative markers of proliferation, however the latter study did demonstrate that STS inhibition in middle-aged rats prevented increase of prostate mass when treated with E_1S + STX64 *vs* E_1S alone where prostate mass increased (Giton et al. 2015). An alternative conjugate of circulating oestrogens is glucuronide (Raftogianis et al. 2000), however, research into oestrogen glucuronide transport into prostate cells and evidence of glucuronidase enzymes within the prostate is lacking.

Conversion of E_1 to E_2 (and androstenedione to testosterone) requires 17-betahydroxysteroid dehydrogenase (17β-HSD) enzymes (White et al. 2013). 17β-HSDs enzymes are alcohol oxidoreductases which catalyse reduction (E_1 to E_2) and oxidation (E_2 to E_1) at carbon atom 17. There are over 14 different isozymes of 17β-HSDs (17β-HSD *1-14*) and certain 17β-HSDs have a higher propensity to catalyse the reaction in a certain direction, for example 17β-HSD-1 favours reduction whereas 17β-HSD-2 favours oxidation (Lukacik, et al. 2006; Oduwole, et al. 2003). 17β-HSDs play an important role in hormone sensitive cancers.

Increased expression of 17β-HSD-1 in breast cancers of post-menopausal women helps maintain high intratumoural E_2 levels (Lukacik et al. 2006). Moreover, expression of 17β-HSD-2 and 17β-HSD-3 mRNA is significantly higher in malignant prostatic tissues compared to normal prostate tissues (Day, et al. 2013) with one study reporting prostate cancer biopsies showing 30-fold higher mRNA expression than normal. In addition to converting androstenedione to testosterone, 17β-HSD 5 can convert E_1 to E_2. Inhibitors of 17β-HSD 5 have been explored in castration-resistant prostate cancer and breast cancer, in the latter where androgens are not considered to play an important role (Adeniji, et al. 2013). The study found no appreciable decrease in E_2 synthesis in breast cancer cell lines when treated with a 17β-HSD 5 inhibitor and only a moderate decrease in E_2 synthesis in some subpopulations of prostate cancer cell lines. Interestingly, inflammation associated with tumours modulates the expression of 17β-HSD-2 and 17β-HSD-5 (and also 3β-HSD). Treatment of prostate cancer stromal cell lines PrSC with TGFβ1 showed a marked down-regulation in mRNA expression of 17β-HSD-2 and 17β-HSD-5 in a dose-dependent manner (Piao, et al. 2013). The counterintuitive action of TGFβ1 again demonstrates how little is understood about oestrogenic pathways in prostate cancer. Regardless of the mechanisms by which oestrogens become available within the prostate gland, tumour-promoting or tumour-suppressing effects must be mediated by activation of oestrogen receptors (ER).

Oestrogen receptors (ER) in the prostate

The effects of oestrogens on tissues are mediated via activation of oestrogen receptors (ER). There are two well studied ERs; ER alpha (ERα) and ER beta (ERβ) encoded by two separate genes *ESR1* and *ESR2*, respectively. ERα and ERβ are members of the nuclear receptor superfamily (Robinson-Rechavi, et al. 2003). When bound and activated, ERs interact

directly with the genome acting as transcription factors (or activating transcription factors) which act directly on oestrogen response elements (Deblois and Giguere 2013). As well as E_2, ERs can be stimulated by phytoestrogens, and different classes of phytoestrogens have selected preferences for each type of ER. In general, phytoestrogens show agonistic activity towards ERβ at lower concentrations than towards ERα using hamster uterine cells (Takeuchi, et al. 2009). When human cells are examined, the relative binding affinity (RBA) of genistein to ERβ is approximately 20-30 times greater than for ERα as shown in MCF-7 breast cancer cell lines (Pilšáková et al. 2010). The affinity of phytoestrogens for ER widely varies with most molecules having an RBA to ERβ 1000-fold lower than E_2. However, molecules such as genistein and coumesterol have an RBA 100-fold lower than E_2. Genistein and coumesterol are able to activate transcriptional activities of ERα and ERβ at concentrations of 1-10nM compared to physiological E_2 concentrations of 20-40pM in males (Kuiper, et al. 1998; Mueller et al. 2015). Of course, the ability of phytoestrogens to bind to ER also depends on the existing levels of E_1 and E_2 as these molecules are direct competitors with phytoestrogens.

ERs have been studied more extensively in the context of breast cancers, a neoplasm that has been likened as the sister disease to prostate cancer, especially in regards to their hormonal responses and sensitivities (Risbridger, et al. 2010). In breast cancer, activation of ERα promotes tumour growth as it initiates anti-apoptotic (Chaudhri, et al. 2014; Razandi, et al. 2000) and mitogenic effects (Bhatt, et al. 2012; Yamnik and Holz 2010). This anti-apoptotic effect of ERα makes ERα positive breast cancers more likely to metastasise (Ross-Innes, et al. 2012). In fact, a review of ERs in breast and ovarian cancers has found ERα expression correlates with worse prognosis whereas ERβ expression correlates with better clinical

outcomes (Burns and Korach 2012). Generally, ERα activation promotes proliferative pathways whereas ERβ activation leads to apoptotic pathways (Acconcia, et al. 2005).

Expression of ERα and ERβ in the normal prostate has been determined as the role of oestrogens in prostatic development was identified (Ho 2004). Recently it has been reported that prostate progenitor stem cells, while lacking expression of androgen receptor, express ER abundantly. Indeed, the expression of ERβ is putatively 6-fold greater and ERα 125-fold greater in progenitor cells compared to LNCaP mature cells (Di Zazzo, et al. 2016). Although this supports the importance of oestrogens in embryonic and neonatal development of prostate gland, it has been hypothesised that lack of androgen receptor expression could be an imprint which later predisposes to CRPC in the elderly. In non-cancerous prostate ERα is predominantly expressed in the stromal compartment and ERβ is predominantly expressed in basal-epithelial cells. However in prostate cancer, ERα expression is down-regulated in stromal cells and upregulated in the cancerous epithelial cells. ERβ expression is down-regulated in epithelial cells as seen by immunostaining in human prostate tissue (Yeh, et al. 2014). Indeed there is evidence that down-regulation of ERβ promotes activation of NF-κB mediated by hypoxia-inducible factor 1 (HIF-1). In immortalised normal prostate epithelial cell line PNT1a, loss of ERβ using shRNA showed an increase in NF-κB mRNA expression and activity. This mirrors what is seen in high grade, late stage prostate cancer (Mak, et al. 2015). Consequently, it appears that an increase in ERα expression and decrease in ERβ expression is what shifts the balance between protective effects of oestrogens and proliferative effects of oestrogens as has been suggested in other cancers (Barzi, et al. 2013; Burns and Korach 2012). Figure 3 summarises the difference in ERα and ERβ expression between non-cancerous and cancerous prostate tissue. Single nucleotide polymorphisms (SNP) in the ER genes have been investigated and associations have been made between

certain polymorphisms and the risk of prostate cancer (Holt, et al. 2013; Jurečeková, et al. 2015). In both studies, the genomes from histologically confirmed human prostate cancer samples were analysed using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) based analysis and compared to age-matched healthy control subjects. A meta-analysis exploring the results of 24 published studies that include Caucasian, Asian and African participants concluded that *ESR1* rs9340799 polymorphism is allied to increased risk in the general population of Caucasians and Africans whereas ESR2 rs1256049 polymorphisms has been linked to increased risk only in Caucasians (Fu, et al. 2014).

Research into ERβ has been more extensive than in ERα. McPherson et al. (2007) highlighted the potential significance of ERβ manipulation when they treated prostate hyperplasia in oestrogen depleted mice with a selective ERβ agonist and found it to induce apoptosis and shrink the size of the prostate. Hussain et al. (2012) carried forward this research and initial studies have found ERβ agonist treatment with 8β-VE_2 can induce apoptosis in primary human and murine prostatic basal cells, a lineage considered to be the cells of origin for prostate cancers (Taylor, et al. 2012). The mechanism behind how ERβ activation induces apoptosis in prostate cancer cells lines may be via up-regulation of p53-upregulated modulator of apoptosis (PUMA) and consequent intrinsic caspase-9 mechanisms. Dey, et al. overexpressed ERβ in LNCaP, PC3 and 22Rv1 prostate cancer cell lines *in vitro*, the latter which does not express ERβ, and treated with E_2 and agonist 3β-adiol. Immunofluorescence revealed that cells which expressed ERβ were more likely to undergo apoptosis following expression of PUMA independent of p53 (Dey, et al. 2014). (Dey, et al. 2014). It has even been reported that ERβ activation impedes on the epithelial-mesenchymal transition process thereby reducing the risk of invasion and metastasis. In human tissue samples and LNCaP

and PC3 cell lines, treatment with E_2 and high concentration of ERβ1 agonist 3β-adiol resulted in inhibition of VEGF and destabilisation of HIF-1 in vitro thus suppressing the factors that drive epithelial-mesenchymal transition necessary for metastasis. Furthermore, loss of ERβ1 expression by means of shRNA transfection resulted in significant increase in migration and invasion (Mak, et al. 2010). Mounting evidence also suggests that pharmaceutical targeting of ERβ pathways may be effective in treating prostate cancer. However, recently a 'switching roles' theory has been proposed suggesting the effects of ERβ activation switches from protective to proliferative as cancer progresses (Savoy and Ghosh 2013). The theory is based on the observation that castration-resistant prostate cancers have higher expression of ERβ compared to hormone-naïve prostate cancers. It is possible that decreased levels of circulating androgens and up-regulation of androgen receptors may be important in this switch however the actual mechanisms and processes are yet unknown.

Splice variants of ERβ are also important as it has been shown that at least 5 different isoforms exist, many of which are expressed in the prostate (Leung, et al. 2006). Activation of different isoforms may have opposing effects; for example $ER\beta_1$ is tumour-suppressing whereas $ER\beta_2$ is tumour-promoting in LNCaP cells (Chen, et al. 2009). In a study of primary prostate cancer samples from 144 patients who underwent radical prostatectomy, two particular isoforms $ER\beta_2$ and $ER\beta_5$ have been identified to promote invasion and metastasis of prostate cancer and thus correlate with worse outcomes while others continue to be studied (Leung, et al. 2010; Nelson, et al. 2014). Certain ERβ isoforms, such as $ER\beta_2$ and $ER\beta_3$, when activated interact with transcription factors which enable and promote the epithelial mesenchyme transition and hence might be why advanced prostate cancers have higher expression of ERβ (Leung et al. 2010). More research needs to be carried out to understand the mechanisms of the complex downstream pathways of ERβ activation in prostate cancer.

The tumour promoting effects of ERα within the prostate are not as well defined. ERα is expressed in significant quantities in the stromal tissue of prostate cancer where they have been associated with cancer-associated fibroblasts (CAF) (Slavin, et al. 2015). Da, et al. isolated CAF from adenocarcinoma of mouse prostate lentivirally transduced ERα. Conditioned media from ERα+ CAF promoted proliferation of LNCaP, PC3, C4-2 and 22Rv1 cells. Furthermore, in xenograft experiments mice co-implanted with ERα+ CAF showed a higher growth rate of tumour mass compared to injection of prostate cancer cell lines alone (Da, et al. 2015). Activation of ERα on CAFs stimulates the release of tumour-promoting factors which act on prostate epithelia in a paracrine manner. Slug (SNAI2), a transcription factor with anti-apoptotic pathways can repress ERα expression by binding to gene promotor regions and consequently promote epithelial-mesenchymal transition in prostate cancer cells and human breast cancer samples (Li, et al. 2015a). In contrast, downstream pathways of ERα activation can inhibit metastasis by down-regulating expression of matrix metalloproteinase 3 and upregulating expression of thrombospondin 2 as seen in a range of breast cancer cell lines and LNCaP cell line, however this is not evidence in primary human prostate tissue (Li et al. 2015a). This may be an effect of ERα activation which diverts cell resources towards growth of prostate cancer rather than spread and invasion (Hanahan and Weinberg 2011). A study investigating the role of ERα in prostate cancers of PTEN-deficient mice has shown expression of ERα correlates strongly with the expression of Ki67,- a proliferative marker. In addition, inhibition and knockdown of ERα decreases proliferation but has no effect on cell viability thus the tumour mass remained static. This further demonstrates that ERα regulates cell proliferation through PI3K and MAPK signalling (Takizawa, et al. 2015).

Human trials in 1590 men with high grade intraepithelial neoplasia of the prostate has shown no significant decrease in risk of prostate cancer when treated with daily toremifene, a selective oestrogen receptor modulator (SERM) used for the treatment of metastatic breast cancer, compared with placebo. Of the 1467 men who underwent a biopsy during the three-year study, cancer was detected in 34.7% in the placebo group compared to 32.3% in the treatment group (p= 0.39) (Taneja, et al. 2013). Conversely, experimental use of toremifene, in cell lines and nude mice models have suggested that ERα antagonists can repress the tumorigenicity of prostate cancer (Hariri, et al. 2015). Intriguingly, there is recent evidence that abiraterone, used frequently in advanced prostate cancer is able to activate ER. Capper, et al. demonstrated an increase in proliferation of MCF-7 and T47D breast cancer cell lines when treated with abiraterone. The proliferative effects were diminished when the cells were treated with ER antagonist ICI 182,78 (Capper, et al. 2016). ER-mediated progression of prostate cancer might thus constitute a novel mechanism of resistance to abiraterone that warrants further investigation. The signalling mechanisms of ERα and ERβ are summarised in Figure 4.

In addition to the two nuclear ERs, ERα and ERβ, another relatively recently discovered ER exists. G-protein coupled oestrogen receptor (GPER), alternatively known as GPR30, is a membrane-bound receptor discovered in 1998 (O'Dowd, et al. 1998). GPER is found in 50% of breast cancers and is believed to be critically involved in how Tamoxifen (a SERM) resistance is developed (Mo, et al. 2013). Tamoxifen can bind and stimulate GPER in breast cancer (Prossnitz, et al. 2008a) activating downstream cancer promoting pathways. GPER has also been shown to be expressed in various hormone-sensitive tissues in the body including the prostate (Prins and Hu 2013; Prossnitz, et al. 2007) and has very similar affinity for E_2 as ERα and ERβ with almost no interaction with androgens or glucocorticoids (Prossnitz, et al.

2008b). In addition to being activated by endogenous E_2, GPER can also be activated by phytoestrogens with similar RBA as phytoestrogens have to ERβ and elicit an oestrogenic signalling pathways (Thomas and Dong 2006).

Evidence of changes in GPER expression within prostate cancer is scarce, though it has been established with immunofluorescence and immunoblotting that GPER is expressed LNCaP, DU145 and PC3 cellswhich have varying degrees of invasiveness (Maier, et al. 2006). In addition, expression of GPER has been identified by immunohistochemistry and immunoblotting in prostate adenocarcinomas and in pre-neoplastic lesions in 50 patients with confirmed prostate cancer of varying grades of aggressiveness and in 5 patients with benign prostatic disease (Rago, et al. 2016). Naturally, more research has been conducted in aggressive cell lines and primary tissues. In contrast to the effects of GPER activation in breast and ovarian cancers where it promotes growth, it has been identified that treatment of castration-resistant prostate cancer with a specific GPER agonist, G1, actually inhibits the growth of prostate cancer in PC-3, DU145 and LNCaP cell lines *in vitro* and *in vivo* PC3 xenografts (Chan, et al. 2010; Lam, et al. 2014). While most studies only reported tumour inhibition in castration-resistant cell lines, Lam et al. found that G1 treatment has no effect on androgen-sensitive LNCaP cells *in vitro* and *in vivo* xenograft mouse models whereas it had a significant effect on castration-resistant tumours without apparent toxicity to the host (Lam et al. 2014). Furthermore, GPER expression is significantly increased in androgen-deprived environments compared to androgen-replete milieus (Prins and Hu 2013)with increased GPER expression also evident in cells isolated from distant metastases in patients with CRPC CRPC compared to tissue from primary prostate cancers (Lam et al. 2014). Androgen receptor activation downregulates GPER expression thus explaining why expression of GPER is greater in androgen deprived environments (Lam et al. 2014). The mechanisms by

which the GPER agonist G1 has anti-tumour effects has been explored in PC3 cell line *in vitro* and *in vivo* xenograft castrated mice models and is reported to be via up-regulation of p21 and consequent cell cycle arrest at G2 phase (Chan et al. 2010). Although GPER activation inhibits growth of prostate cancer, it increases proliferation of other tissues including testicular germ cells and urothelial cells of the bladder and urinary tract (Chevalier, et al. 2011; Huang, et al. 2015). The fact that GPER activation can have opposing effects in different tissues through the same pathway illustrates the complexity of intracellular oestrogen signalling. Figure 4 grossly summarises GPER signalling pathways that have thus far been identified in prostate cancer.

Conclusion

This review has presented evidence that suggests an imbalance of circulating oestrogens and androgens may be responsible for changes to the development and progression of prostate cancer. In addition to endogenous oestrogen availability, exposure to exogenous oestrogens in the form of phytoestrogens may also have a profound effect. However, there is substantial evidence that intratumoural synthesis of oestrogens, and indeed androgens, plays a significant role as the prostate is endowed with the ability to express key enzymes required for oestrogen synthesis. There is a relationship between stage of disease and level of expression of these enzymes, as is evident from the emergence of resistance to anti-androgen therapy further supports this hypothesis.

Changes in the expression pattern of ERα and ERβ greatly affect whether oestrogens are tumour promoting or tumour suppressing. In normal prostate and during early stages of

prostate cancer where ERβ is the prominent ER, oestrogens may be beneficial as ERβ activation initiates apoptotic pathways. Perhaps this is why a lifetime of increased phytoestrogen consumption can reduce the risk of prostate cancer development. In late stage prostate cancer where ERα is the dominating ER within the prostate, oestrogens are deleterious as ERα activation regulates cell proliferation through PI3K and MAPK signalling. Activation of GPER inhibits growth of prostate cancer however, GPER is not uniformly expressed in all prostate cancer and thus any GPER targeted therapy will be of benefit to a limited number of patients. Figure 5 summarises how the expression of ERs change during the progression of prostate cancer.

Before any definitive conclusions can be drawn over whether oestrogens are good or bad for prostate cancer, further research has to be conducted exploring the signalling pathways of ER within prostate tissue. In addition an understanding of the mechanisms behind abiraterone (Romanel, et al. 2015) and enzalutamide resistance (Claessens, et al. 2014), and whether this is linked to altered androgen and oestrogen metabolism, will be required before the next big step is taken towards development of endocrine therapy for prostate cancer.

References

Acconcia F, Totta P, Ogawa S, Cardillo I, Inoue S, Leone S, Trentalance A, Muramatsu M & Marino M 2005 Survival versus apoptotic 17β-estradiol effect: Role of ERα and ERβ activated non-genomic signaling. *Journal of Cellular Physiology* **203** 193-201.

Adams KF, Chen C, Newton KM, Potter JD & Lampe JW 2004 Soy Isoflavones Do Not Modulate Prostate-Specific Antigen Concentrations in Older Men in a Randomized Controlled Trial. *Cancer Epidemiology Biomarkers & Prevention* **13** 644-648.

Adeniji AO, Chen M & Penning TM 2013 AKR1C3 as a target in castrate resistant prostate cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **137** 136-149.

Adlercreutz H, Mazur W, Bartels P, Elomaa V-V, Watanabe S, Wähälä K, Landström M, Lundin E, Bergh A, Damber J-E, et al. 2000 Phytoestrogens and Prostate Disease. *The Journal of Nutrition* **130** 658.

Allen NE, Appleby PN, Davey GK & Key TJ 2002 Lifestyle and nutritional determinants of bioavailable androgens and related hormones in British men. *Cancer Causes & Control* **13** 353-363.

Allott EH, Masko EM & Freedland SJ 2013 Obesity and Prostate Cancer: Weighing the Evidence. *European Urology* **63** 800-809.

Barazani Y, Katz BF, Nagler HM & Stember DS 2014 Lifestyle, Environment, and Male Reproductive Health. *Urologic Clinics of North America* **41** 55-66.

Barzi A, Lenz AM, Labonte MJ & Lenz H-J 2013 Molecular Pathways: Estrogen Pathway in Colorectal Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **19** 10.1158/1078-0432.CCR-1113-0325.

Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, Iversen P, Bhattacharya S, Carles J, Chowdhury S, et al. 2014 Enzalutamide in Metastatic Prostate Cancer before Chemotherapy. *New England Journal of Medicine* **371** 424-433.

Bhatt S, Xiao Z, Meng Z & Katzenellenbogen BS 2012 Phosphorylation by p38 Mitogen-Activated Protein Kinase Promotes Estrogen Receptor α Turnover and Functional Activity via the SCF(Skp2) Proteasomal Complex. *Molecular and Cellular Biology* **32** 1928-1943.

Block JL, Block NL & Lokeshwar BL 1996 Modulation of aromatase activity by growth factors in an androgen sensitive human prostate cancer cell line, LNCaP. *Cancer Letters* **102** 167-172.

Bosset P-O, Albiges L, Seisen T, de la Motte Rouge T, Phé V, Bitker M-O & Rouprêt M 2012 Current role of diethylstilbestrol in the management of advanced prostate cancer. *BJU International* **110** E826-E829.

Brooke GN & Bevan CL 2009 The Role of Androgen Receptor Mutations in Prostate Cancer Progression. *Current Genomics* **10** 18-25.

Bulun SE, Economos K, Miller D & Simpson ER 1994 CYP19 (aromatase cytochrome P450) gene expression in human malignant endometrial tumors. *The Journal of Clinical Endocrinology & Metabolism* **79** 1831-1834.

Burns KA & Korach KS 2012 Estrogen receptors and human disease: an update. *Archives of Toxicology* **86** 1491-1504.

Buxhofer-Ausch V, Secky L, Wlcek K, Svoboda M, Kounnis V, Briasoulis E, Tzakos AG, Jaeger W & Thalhammer T 2013 Tumor-specific expression of organic anion-transporting polypeptides: transporters as novel targets for cancer therapy. *Journal of drug delivery* **2013** 863539.

Capper CP, Larios JM, Sikora MJ, Johnson MD & Rae JM 2016 The CYP17A1 inhibitor abiraterone exhibits estrogen receptor agonist activity in breast cancer. *Breast Cancer Research and Treatment* 1-8.

Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O & Bray F 2012 International Variation in Prostate Cancer Incidence and Mortality Rates. *European Urology* **61** 1079-1092.

Chan QK, Lam H-M, Ng C-F, Lee AY, Chan ES, Ng H-K, Ho S-M & Lau K-M 2010 Activation of GPR30 inhibits the growth of prostate cancer cells through sustained activation of Erk1/2, c-jun/c-fos-dependent upregulation of p21, and induction of G2 cell-cycle arrest. *Cell Death & Differentiation* **17** 1511-1523.

Chang K-H, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ & Sharifi N 2011 Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. *Proceedings of the National Academy of Sciences* **108** 13728-13733.

Chen CD, Welsbie DS, Tran C, Baek SH, Chen R, Vessella R, Rosenfeld MG & Sawyers CL 2004 Molecular determinants of resistance to antiandrogen therapy. *Nat Med* **10** 33-39.

Chen M, Ni J, Chang H-C, Lin C-Y, Muyan M & Yeh S 2009 CCDC62/ERAP75 functions as a coactivator to enhance estrogen receptor beta-mediated transactivation and target gene expression in prostate cancer cells. *Carcinogenesis* **30** 841-850.

Chen M, Yeh C-R, Chang H-C, Vitkus S, Wen X-Q, Bhowmick NA, Wolfe A & Yeh S 2012 Loss of epithelial oestrogen receptor α inhibits oestrogen-stimulated prostate proliferation and squamous metaplasia via in vivo tissue selective knockout models. *The Journal of Pathology* **226** 17-27.

Chen S 1998 Aromatase and breast cancer. In *Frontiers in bioscience : a journal and virtual library*, pp d922-933.

Chevalier N, Bouskine A & Fenichel P 2011 Role of GPER/GPR30 in tumoral testicular germ cells proliferation. *Cancer biology & therapy* **12** 2-3.

Christoforou P, Christopoulos PF & Koutsilieris M 2014 The Role of Estrogen Receptor β in Prostate Cancer. *Molecular Medicine* **20** 427-434.

Claessens F, Helsen C, Prekovic S, Van den Broeck T, Spans L, Van Poppel H & Joniau S 2014 Emerging mechanisms of enzalutamide resistance in prostate cancer. *Nat Rev Urol* **11** 712-716.

Clemons J, Glodé LM, Gao D & Flaig TW 2013 Low-dose diethylstilbestrol for the treatment of advanced prostate cancer. *Urologic Oncology: Seminars and Original Investigations* **31** 198-204.

Cook LS, Goldoft M, Schwartz SM & Weiss NS 1999 INCIDENCE OF ADENOCARCINOMA OF THE PROSTATE IN ASIAN IMMIGRANTS TO THE UNITED STATES AND THEIR DESCENDANTS. *The Journal of Urology* **161** 152-155.

Cui J, Shen Y & Li R 2013 Estrogen synthesis and signaling pathways during ageing: from periphery to brain. *Trends in molecular medicine* **19** 197-209.

Cunha GR 1994 Role of mesenchymal-epithelial interactions in normal and abnormal development of the mammary gland and prostate. *Cancer* **74** 1030-1044.

Da J, Lu M & Wang Z 2015 Estrogen Receptor Alpha (ERα)-Associated Fibroblasts Promote Cell Growth in Prostate Cancer. *Cell Biochemistry and Biophysics* **73** 793-798.

Daniels NA, Nielson CM, Hoffman AR & Bauer DC 2010 SEX HORMONES AND THE RISK OF INCIDENT PROSTATE CANCER. *Urology* **76** 1034-1040.

Davies JH, Dowsett M, Jacobs S, Coombes RC, Hedley A & Shearer RJ 1992 Aromatase inhibition: 4-hydroxyandrostenedione (4-OHA, CGP 32349) in advanced prostatic cancer. *British Journal of Cancer* **66** 139-142.

Day JM, Foster PA, Tutill HJ, Schmidlin F, Sharland CM, Hargrave JD, Vicker N, Potter BVL, Reed MJ & Purohit A 2013 STX2171, a 17β-hydroxysteroid dehydrogenase type 3 inhibitor, is efficacious in vivo in a novel hormone-dependent prostate cancer model. *Endocrine-Related Cancer* **20** 53-64.

de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, Chi KN, Jones RJ, Goodman OBJ, Saad F, et al. 2011 Abiraterone and Increased Survival in Metastatic Prostate Cancer. *New England Journal of Medicine* **364** 1995-2005.

De Nunzio C, Albisinni S, Freedland SJ, Miano L, Cindolo L, Finazzi Agrò E, Autorino R, De Sio M, Schips L & Tubaro A 2013 Abdominal obesity as risk factor for prostate cancer diagnosis and high grade disease: A prospective multicenter Italian cohort study. *Urologic Oncology: Seminars and Original Investigations* **31** 997-1002.

Deblois G & Giguere V 2013 Oestrogen-related receptors in breast cancer: control of cellular metabolism and beyond. *Nat Rev Cancer* **13** 27-36.

deVere White RW, Hackman RM, Soares SE, Beckett LA, Li Y & Sun B 2004 Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer. *Urology* **63** 259-263.

Dey P, Strom A & Gustafsson JA 2014 Estrogen receptor [beta] upregulates FOXO3a and causes induction of apoptosis through PUMA in prostate cancer. *Oncogene* **33** 4213-4225.

Di Zazzo E, Galasso G, Giovannelli P, Di Donato M, Di Santi A, Cernera G, Rossi V, Abbondanza C, Moncharmont B, Sinisi AA, et al. 2016 Prostate cancer stem cells: the role of androgen and estrogen receptors. *Oncotarget* **7** 193-208.

Ellem SJ & Risbridger GP 2007 Treating prostate cancer: a rationale for targeting local oestrogens. *Nat Rev Cancer* **7** 621-627.

Ellem SJ & Risbridger GP 2009 The Dual, Opposing Roles of Estrogen in the Prostate. *Annals of the New York Academy of Sciences* **1155** 174-186.

Ellem SJ & Risbridger GP 2010 Aromatase and regulating the estrogen:androgen ratio in the prostate gland. *The Journal of Steroid Biochemistry and Molecular Biology* **118** 246-251.

Ellem SJ, Schmitt JF, Pedersen JS, Frydenberg M & Risbridger GP 2004 Local Aromatase Expression in Human Prostate Is Altered in Malignancy. *The Journal of Clinical Endocrinology & Metabolism* **89** 2434-2441.

Evaul K, Li R, Papari-Zareei M, Auchus RJ & Sharifi N 2010 3β-Hydroxysteroid Dehydrogenase Is a Possible Pharmacological Target in the Treatment of Castration-Resistant Prostate Cancer. *Endocrinology* **151** 3514-3520.

Farnsworth WE 1973 Human prostatic dehydroepiandrosterone sulfate sulfatase. *Steroids* **21** 647-664.

Foster PA 2013 Oestrogen and colorectal cancer: mechanisms and controversies. *International Journal of Colorectal Disease* **28** 737-749.

Foster PA, Chander SK, Parsons MF, Newman SP, Woo LW, Potter BV, Reed MJ & Purohit A 2008 Efficacy of three potent steroid sulfatase inhibitors: pre-clinical investigations for their use in the treatment of hormone-dependent breast cancer. *Breast Cancer Res Treat* **111** 129-38.

Foster PA, Newman SP, Chander SK, Stengel C, Jhalli R, Woo LL, Potter BV, Reed MJ & Purohit A 2006 In vivo efficacy of STX213, a second-gegneration steroid sulfatase inhibitor, for hormone-dependent breast cancer therapy. *Clin Cancer Res* **12** 5543-9.

Francis JC, Thomsen MK, Taketo MM & Swain A 2013?-Catenin Is Required for Prostate Development and Cooperates with <italic>Pten</italic> Loss to Drive Invasive Carcinoma. *PLoS Genet* **9** e1003180.

Fu C, Dong W-Q, Wang A & Qiu G 2014 The influence of ESR1 rs9340799 and ESR2 rs1256049 polymorphisms on prostate cancer risk. *Tumor Biology* **35** 8319-8328.

Gautier A, Bonnet F, Dubois S, Massart C, Grosheny C, Bachelot A, Aubé C, Balkau B & Ducluzeau P-H 2013 Associations between visceral adipose tissue, inflammation and sex steroid concentrations in men. *Clinical Endocrinology* **78** 373-378.

Geier R, Adler S, Rashid G & Klein A 2010 The synthetic estrogen diethylstilbestrol (DES) inhibits the telomerase activity and gene expression of prostate cancer cells. *The Prostate* **70** 1307-1312.

Giton F, Sirab N, Franck G, Gervais M, Schmidlin F, Ali T, Allory Y, Taille Adl, Vacherot F, Loric S, et al. 2015 Evidence of estrone-sulfate uptake modification in young and middle-aged rat prostate. *The Journal of Steroid Biochemistry and Molecular Biology* **152** 89-100.

Goetzl MA, VanVeldhuizen PJ & Thrasher JB 2007 Effects of soy phytoestrogens on the prostate. *Prostate Cancer Prostatic Dis* **10** 216-223.

Gooren L & Morgentaler A 2014 Prostate cancer incidence in orchidectomised male-to-female transsexual persons treated with oestrogens. *Andrologia* **46** 1156-1160.

Grönberg H 2003 Prostate cancer epidemiology. *The Lancet* **361** 859-864.

Hanahan D & Weinberg Robert A 2011 Hallmarks of Cancer: The Next Generation. *Cell* **144** 646-674.

Hariri W, Sudha T, Bharali DJ, Cui H & Mousa SA 2015 Nano-Targeted Delivery of Toremifene, an Estrogen Receptor-α Blocker in Prostate Cancer. *Pharmaceutical Research* **32** 2764-2774.

Härkönen PL & Mäkelä SI 2004 Role of estrogens in development of prostate cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **92** 297-305.

Henderson BE, Bernstein L, Ross RK, Depue RH & Judd HL 1988 The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. *British Journal of Cancer* **57** 216-218.

Ho S-M 2004 Estrogens and anti-estrogens: Key mediators of prostate carcinogenesis and new therapeutic candidates. *Journal of Cellular Biochemistry* **91** 491-503.

Hofland J, van Weerden WM, Dits NFJ, Steenbergen J, van Leenders GJLH, Jenster G, Schröder FH & de Jong FH 2010 Evidence of Limited Contributions for Intratumoral Steroidogenesis in Prostate Cancer. *Cancer Research* **70** 1256-1264.

Hofland J, Weerden WMv, Steenbergen J, Dits NFJ, Jenster G & Jong FHd 2011 Activin A regulates local testosterone production and growth in prostate cancer through 17β-hydroxysteroid dehydrogenase. In *13th European Congress of Endocrinology*, p 353. Rotterdam, Netherlands: Endocrine Abstracts, Bioscientifica.

Holt SK, Kwon EM, Fu R, Kolb S, Feng Z, Ostrander EA & Stanford JL 2013 Association of Variants in Estrogen-Related Pathway Genes with Prostate Cancer Risk. *The Prostate* **73** 1-10.

Howell MA 1974 Factor Analysis of International Cancer Mortality Data and per capita Food Consumption. *British Journal of Cancer* **29** 328-336.

Huang W, Chen Y, Liu Y, Zhang Q, Yu Z, Mou L, Wu H, Zhao L, Long T, Qin D, et al. 2015 Roles of ERβ and GPR30 in Proliferative Response of Human Bladder Cancer Cell to Estrogen. *BioMed research international* **2015** 251780.

Huggins C & Hodges CV 1941 Studies on Prostatic Cancer. I. The Effect of Castration, of Estrogen and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate. *Cancer Research* **1** 293-297.

Hussain S, Lawrence MG, Taylor RA, Lo CY-W, BioResource APC, Frydenberg M, Ellem SJ, Furic L & Risbridger GP 2012 Estrogen Receptor ? Activation Impairs Prostatic Regeneration by Inducing Apoptosis in Murine and Human Stem/Progenitor Enriched Cell Populations. *PLoS ONE* **7** e40732.

Jacob M & Henrik M 2006 One, Five and Ten Year Cancer Prevalence by Cancer Network, UK, 2006. In *One, Five and Ten Year Cancer Prevalence by Cancer Network, UK*. London, UK: National Cancer Intelligence Network.

Jasuja GK, Travison TG, Davda M, Murabito JM, Basaria S, Zhang A, Kushnir MM, Rockwood AL, Meikle W, Pencina MJ, et al. 2013 Age Trends in Estradiol and Estrone Levels Measured Using Liquid Chromatography Tandem Mass Spectrometry in Community-Dwelling Men of the Framingham Heart Study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **68** 733-740.

Jurečeková J, Babušíková E, Kmeťová M, Kliment J & Dobrota D 2015 Estrogen receptor alpha polymorphisms and the risk of prostate cancer development. *Journal of Cancer Research and Clinical Oncology* **141** 1963-1971.

Khurana I 2008 *Essentials of Medical Physiology*. India: Elsevier India Pvt. Limited.

Kimbro K & Simons J 2006 Hypoxia-inducible factor-1 in human breast and prostate cancer. *Endocrine-Related Cancer* **13** 739-749.

King KJ, Nicholson HD & Assinder SJ 2006 Effect of increasing ratio of estrogen: Androgen on proliferation of normal human prostate stromal and epithelial cells, and the malignant cell line LNCaP. *The Prostate* **66** 105-114.

Klein H, Molwitz T & Bartsch W 1989 Steroid sulfate sulfatase in human benign prostatic hyperplasia: Characterization and quantification of the enzyme in epithelium and stroma. *Journal of Steroid Biochemistry* **33** 195-200.

Knochenhauer ES, Boots LR, Potter HD & Azziz R 1998 Differential binding of estradiol and testosterone to SHBG. Relation to circulating estradiol levels. *The Journal of reproductive medicine* **43** 665-670.

Koh E, Noda T, Kanaya J & Namiki M 2002 Differential expression of 17β-hydroxysteroid dehydrogenase isozyme genes in prostate cancer and noncancer tissues. *The Prostate* **53** 154-159.

Kolonel LN, Altshuler D & Henderson BE 2004 The multiethnic cohort study: exploring genes, lifestyle and cancer risk. *Nat Rev Cancer* **4** 519-527.

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, Saag PTvd, Burg Bvd & Gustafsson J-Å 1998 Interaction of Estrogenic Chemicals and Phytoestrogens with Estrogen Receptor β. *Endocrinology* **139** 4252-4263.

Kumagai J, Hofland J, Erkens-Schulze S, Dits NFJ, Steenbergen J, Jenster G, Homma Y, de Jong FH & van Weerden WM 2013 Intratumoral conversion of adrenal androgen precursors drives androgen receptor-activated cell growth in prostate cancer more potently than de novo steroidogenesis. *The Prostate* **73** 1636-1650.

Labrie F, Bélanger A, Luu-The V, Labrie C, Simard J, Cusan L, Gomez J & Candas B 2005 Gonadotropin-Releasing Hormone Agonists in the Treatment of Prostate Cancer. *Endocrine Reviews* **26** 361-379.

Lam H-M, Ouyang B, Chen J, Ying J, Wang J, Wu C-L, Jia L, Medvedovic M, Vessella RL & Ho S-M 2014 Targeting GPR30 with G-1: a new therapeutic target for castration-resistant prostate cancer. *Endocrine-Related Cancer* **21** 903-914.

Lee J, Demissie K, Lu S & Rhoads GG 2007 Cancer incidence among Korean-American immigrants in the United States and native Koreans in South Korea. *Cancer Control* **14** 78.

Leung Y-K, Lam H-M, Wu S, Song D, Levin L, Cheng L, Wu C-L & Ho S-M 2010 Estrogen receptor β2 and β5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion. *Endocrine-Related Cancer* **17** 675-689.

Leung Y-K, Mak P, Hassan S & Ho S-M 2006 Estrogen receptor (ER)-β isoforms: A key to understanding ER-β signaling. *Proceedings of the National Academy of Sciences of the United States of America* **103** 13162-13167.

Li R, Evaul K, Sharma KK, Chang K-H, Yoshimoto J, Liu J, Auchus RJ & Sharifi N 2012 Abiraterone Inhibits 3β-Hydroxysteroid Dehydrogenase: A Rationale for Increasing Drug Exposure in Castration-Resistant Prostate Cancer. *Clinical Cancer Research* **18** 3571-3579.

Li Y, Wu Y, Abbatiello TC, Wu WL, Kim JR, Sarkissyan M, Sarkissyan S, Chung SS, Elshimali Y & Vadgama JV 2015a Slug contributes to cancer progression by direct regulation of ERα signaling pathway. *International Journal of Oncology* **46** 1461-1472.

Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, Liu J, Upadhyay SK, Auchus RJ & Sharifi N 2015b Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* **523** 347-351.

Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, Evans CP & Gao AC 2015 Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. *Cancer Research* **75** 1413-1422.

Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, Ettinger SL, Gleave ME & Nelson CC 2008 Androgen Levels Increase by Intratumoral De novo Steroidogenesis during Progression of Castration-Resistant Prostate Cancer. *Cancer Research* **68** 6407-6415.

Lukacik P, Kavanagh KL & Oppermann U 2006 Structure and function of human 17β-hydroxysteroid dehydrogenases. *Molecular and Cellular Endocrinology* **248** 61-71.

Luu-The V, Bélanger A & Labrie F 2008 Androgen biosynthetic pathways in the human prostate. *Best Practice & Research Clinical Endocrinology & Metabolism* **22** 207-221.

Maier KG, Brandy K, Kittur D, Kort K & LaSpina M 2006 GPR30: A novel estrogen receptor expressed in aggressive prostate cancer. *The FASEB Journal* **20** A221.

Mak P, Leav I, Pursell B, Bae D, Yang X, Taglienti CA, Gouvin LM, Sharma VM & Mercurio AM 2010 ERβ Impedes Prostate Cancer EMT by Destabilizing HIF-1α and Inhibiting VEGF-Mediated Snail Nuclear Localization: Implications for Gleason Grading. *Cancer cell* **17** 319-332.

Mak P, Li J, Samanta S & Mercurio AM 2015 ERβ regulation of NF-κB activation in prostate cancer is mediated by HIF-1. *Oncotarget* **6** 40247-40254.

Malkowicz SB 2001 The role of diethylstilbestrol in the treatment of prostate cancer. *Urology* **58** 108-113.

Marcelli M, Ittmann M, Mariani S, Sutherland R, Nigam R, Murthy L, Zhao Y, DiConcini D, Puxeddu E, Esen A, et al. 2000 Androgen Receptor Mutations in Prostate Cancer. *Cancer Research* **60** 944-949.

McPherson SJ, Ellem SJ, Simpson ER, Patchev V, Fritzemeier K-H & Risbridger GP 2007 Essential Role for Estrogen Receptor β in Stromal-Epithelial Regulation of Prostatic Hyperplasia. *Endocrinology* **148** 566-574.

McTernan P, Anwar A, Eggo M, Barnett A, Stewart P & Kumar S 2000 Gender differences in the regulation of P450 aromatase expression and activity in human adipose tissue. *International journal of obesity* **24** 875-881.

Miftakhova R, Hedblom A, Semenas J, Robinson B, Simoulis A, Malm J, Rizvanov A, Heery DM, Mongan NP, Maitland NJ, et al. 2016 Cyclin A1 and P450 Aromatase Promote Metastatic Homing and Growth of Stem-like Prostate Cancer Cells in the Bone Marrow. *Cancer Research* **76** 2453-2464.

Mo Z, Liu M, Yang F, Luo H, Li Z, Tu G & Yang G 2013 GPR30 as an initiator of tamoxifen resistance in hormone-dependent breast cancer. *Breast Cancer Research* **15** 1-15.

Mohler JL, Gregory CW, Ford OH, Kim D, Weaver CM, Petrusz P, Wilson EM & French FS 2004 The Androgen Axis in Recurrent Prostate Cancer. *Clinical Cancer Research* **10** 440-448.

Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD & Nelson PS 2008 Maintenance of Intratumoral Androgens in Metastatic Prostate Cancer: A Mechanism for Castration-Resistant Tumor Growth. *Cancer Research* **68** 4447-4454.

Montico F, Hetzl AC, Cândido EM & Cagnon VHA 2013 Angiogenic and Tissue Remodeling Factors in the Prostate of Elderly Rats Submitted to Hormonal Replacement. *The Anatomical Record* **296** 1758-1767.

Mueller JW, Gilligan LC, Idkowiak J, Arlt W & Foster PA 2015 The Regulation of Steroid Action by Sulfation and Desulfation. *Endocrine Reviews* **36** 526-563.

Muir M, Romalo G, Wolf L, Elger W & Schweikert H-U 2004 Estrone Sulfate Is a Major Source of Local Estrogen Formation in Human Bone. *The Journal of Clinical Endocrinology & Metabolism* **89** 4685-4692.

Murray R & Pitt P 1985 Treatment of advanced prostatic cancer, resistant to conventional therapy with aminoglutethimide. *European Journal of Cancer and Clinical Oncology* **21** 453-458.

Nakamura Y, Suzuki T, Fukuda T, Ito A, Endo M, Moriya T, Arai Y & Sasano H 2006 Steroid sulfatase and estrogen sulfotransferase in human prostate cancer. *The Prostate* **66** 1005-1012.

Nelson AW, Tilley WD, Neal DE & Carroll JS 2014 Estrogen receptor beta in prostate cancer: friend or foe? *Endocrine-Related Cancer* **21** T219-T234.

O'Dowd BF, Nguyen T, Marchese A, Cheng R, Lynch KR, Heng HHQ, Kolakowski Jr LF & George SR 1998 Discovery of Three Novel G-Protein-Coupled Receptor Genes. *Genomics* **47** 310-313.

Oduwole OO, Mäkinen MJ, Isomaa VV, Pulkka A, Jernvall P, Karttunen TJ & Vihko PT 2003 17β-Hydroxysteroid dehydrogenase type 2: independent prognostic significance and evidence of estrogen protection in female patients with colon cancer. *The Journal of Steroid Biochemistry and Molecular Biology* **87** 133-140.

Parkin DM, Bray FI & Devesa SS 2001 Cancer burden in the year 2000. The global picture. *European Journal of Cancer* **37, Supplement 8** 4-66.

Parkin DM, Whelan SL, J F, L T & DB T 1997 Cancer Incidence in Five Continents. . Lyon: International Agency for Research on Cancer Scientific Publications.

Piao Y-s, Wiesenfeld P, Sprando R & Arnold JT 2013 TGFβ1 alters androgenic metabolites and hydroxysteroid dehydrogenase enzyme expression in human prostate reactive stromal primary cells: Is steroid metabolism altered by prostate reactive stromal microenvironment? *The Journal of Steroid Biochemistry and Molecular Biology* **138** 10.1016/j.jsbmb.2013.1005.1016.

Pilšáková L, Riečanský I & Jagla F 2010 The physiological actions of isoflavone phytoestrogens. *Physiological research / Academia Scientiarum Bohemoslovaca* **59** 651-664.

Polari L, Yatkin E, Martínez Chacón MG, Ahotupa M, Smeds A, Strauss L, Zhang F, Poutanen M, Saarinen N & Mäkelä SI 2015 Weight gain and inflammation regulate aromatase expression in male adipose tissue, as evidenced by reporter gene activity. *Molecular and Cellular Endocrinology* **412** 123-130.

Prins GS & Hu W-Y 2013 *Stem Cells and Prostate Cancer*. New York: Springer New York.

Prins GS, Huang L, Birch L & Pu Y 2006 The Role of Estrogens in Normal and Abnormal Development of the Prostate Gland. *Annals of the New York Academy of Sciences* **1089** 1-13.

Prossnitz ER, Arterburn JB & Sklar LA 2007 GPR30: a G protein-coupled receptor for estrogen. *Molecular and Cellular Endocrinology* **265-266** 138-142.

Prossnitz ER, Oprea TI, Sklar LA & Arterburn JB 2008a The ins and outs of GPR30: a transmembrane estrogen receptor. *The Journal of Steroid Biochemistry and Molecular Biology* **109** 350-353.

Prossnitz ER, Sklar LA, Oprea TI & Arterburn JB 2008b GPR30: a novel therapeutic target in estrogen-related disease. *Trends in Pharmacological Sciences* **29** 116-123.

Purohit A & Foster PA 2012 Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers. *Journal of Endocrinology* **212** 99-110.

Quaresma M, Coleman MP & Rachet B 2015 40-year trends in an index of survival for all cancers combined and survival adjusted for age and sex for each cancer in England and Wales, 1971–2011: a population-based study. *The Lancet* **385** 1206-1218.

Raftogianis R, Creveling C, Weinshilboum R & Weisz J 2000 Chapter 6: Estrogen Metabolism by Conjugation. *JNCI Monographs* **2000** 113-124.

Rago V, Romeo F, Giordano F, Ferraro A & Carpino A 2016 Identification of the G protein-coupled estrogen receptor (GPER) in human prostate: expression site of the estrogen receptor in the benign and neoplastic gland. *Andrology* **4** 121-127.

Rajfer J & Coffey DS 1978 Sex steroid imprinting of the immature prostate. Long-term effects. *Investigative urology* **16** 186-190.

Reed MJ, Purohit A, Woo LWL, Newman SP & Potter BVL 2005 Steroid Sulfatase: Molecular Biology, Regulation, and Inhibition. *Endocrine Reviews* **26** 171-202.

Reger M, Zollinger T, Liu Z, Jones J & Zhang J 2015 Abstract 1884: Dietary intake of phytoestrogens and the risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Cancer Research* **75** 1884.

Risbridger GP, Davis ID, Birrell SN & Tilley WD 2010 Breast and prostate cancer: more similar than different. *Nat Rev Cancer* **10** 205-212.

Robinson-Rechavi M, Garcia HE & Laudet V 2003 The nuclear receptor superfamily. *Journal of Cell Science* **116** 585-586.

Romanel A, Tandefelt DG, Conteduca V, Jayaram A, Casiraghi N, Wetterskog D, Salvi S, Amadori D, Zafeiriou Z, Rescigno P, et al. 2015 Plasma AR and abiraterone-resistant prostate cancer. *Science Translational Medicine* **7** 312re310-312re310.

Ronde Wd, Schouw YTvd, Muller M, Grobbee DE, Gooren LJG, Pols HAP & Jong FHd 2005 Associations of Sex-Hormone-Binding Globulin (SHBG) with Non-SHBG-Bound Levels of Testosterone and Estradiol in Independently Living Men. *The Journal of Clinical Endocrinology & Metabolism* **90** 157-162.

Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR, et al. 2012 Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. *Nature* **481** 389-393.

Roy J, Lefebvre J, Maltais R & Poirier D 2013 Inhibition of dehydroepiandosterone sulfate action in androgen-sensitive tissues by EM-1913, an inhibitor of steroid sulfatase. *Molecular and Cellular Endocrinology* **376** 148-155.

Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, Fizazi K, Mainwaring P, Piulats JM, Ng S, et al. 2013 Abiraterone in Metastatic Prostate Cancer without Previous Chemotherapy. *New England Journal of Medicine* **368** 138-148.

Sakr WA, Haas GP, Cassin BF, Pontes JE & Crissman JD 1993 The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. *J Urol* **150** 379-385.

Samaras N, Frangos E, Forster A, Lang PO & Samaras D 2012 Andropause: A review of the definition and treatment. *European Geriatric Medicine* **3** 368-373.

Santen RJ, Petroni GR, Fisch MJ, Myers CE, Theodorescu D & Cohen RB 2001 Use of the aromatase inhibitor anastrozole in the treatment of patients with advanced prostate carcinoma. *Cancer* **92** 2095-2101.

Santen RJ, Thibault A, Myers C & Chung L 1997 Meeting summary: Workshop conference on endocrine therapy of advanced prostate cancer, airlie house, November 3–4, 1996. *Urologic Oncology: Seminars and Original Investigations* **3** 31-38.

Sarveswaran S, Gautam SC & Ghosh J 2012 Wedelolactone, a medicinal plant-derived coumestan, induces caspase-dependent apoptosis in prostate cancer cells via downregulation of PKCε without inhibiting Akt. *International Journal of Oncology* **41** 2191-2199.

Savoy RM & Ghosh PM 2013 The changing roles of steroid nuclear receptors with prostate cancer progression. *Endocrine-Related Cancer* **20** C9-C11.

Scher HI, Fizazi K, Saad F, Taplin M-E, Sternberg CN, Miller K, de Wit R, Mulders P, Chi KN, Shore ND, et al. 2012 Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. *New England Journal of Medicine* **367** 1187-1197.

Sharifi N & Auchus RJ 2012 Steroid biosynthesis and prostate cancer. *Steroids* **77** 719-726.

Siegel R, Naishadham D & Jemal A 2012 Cancer statistics, 2012. *CA: A Cancer Journal for Clinicians* **62** 10-29.

Slavin S, Yeh C-R, Da J, Yu S, Miyamoto H, Messing EM, Guancial E & Yeh S 2014 Estrogen receptor α in cancer-associated fibroblasts suppresses prostate cancer invasion via modulation of thrombospondin 2 and matrix metalloproteinase 3. *Carcinogenesis* **35** 1301-1309.

Smith MR, Kaufman D, George D, Oh WK, Kazanis M, Manola J & Kantoff PW 2002 Selective aromatase inhibition for patients with androgen-independent prostate carcinoma. *Cancer* **95** 1864-1868.

Stanway SJ, Purohit A, Woo LWL, Sufi S, Vigushin D, Ward R, Wilson RH, Stanczyk FZ, Dobbs N, Kulinskaya E, et al. 2006 Phase I Study of STX 64 (667 Coumate) in Breast Cancer Patients: The First Study of a Steroid Sulfatase Inhibitor. *Clinical Cancer Research* **12** 1585-1592.

Strom SS, Yamamura Y, Duphorne CM, Spitz MR, Babaian RJ, Pillow PC & Hursting SD 1999 Phytoestrogen intake and prostate cancer: A case-control study using a new database. *Nutrition and Cancer* **33** 20-25.

Szliszka E, Czuba ZP, Mertas A, Paradysz A & Krol W 2013 The dietary isoflavone biochanin-A sensitizes prostate cancer cells to TRAIL-induced apoptosis. *Urologic Oncology: Seminars and Original Investigations* **31** 331-342.

Takeuchi S, Takahashi T, Sawada Y, Iida M, Matsuda T & Kojima H 2009 Comparative Study on the Nuclear Hormone Receptor Activity of Various Phytochemicals and Their Metabolites by Reporter Gene Assays Using Chinese Hamster Ovary Cells. *Biological and Pharmaceutical Bulletin* **32** 195-202.

Takizawa I, Lawrence MG, Balanathan P, Rebello R, Pearson HB, Garg E, Pedersen J, Pouliot N, Nadon R, Watt MJ, et al. 2015 Estrogen receptor alpha drives proliferation in PTEN-deficient prostate carcinoma by stimulating survival signaling, MYC expression and altering glucose sensitivity. *Oncotarget* **6** 604-616.

Taneja SS, Morton R, Barnette G, Sieber P, Hancock ML & Steiner M 2013 Prostate Cancer Diagnosis Among Men With Isolated High-Grade Intraepithelial Neoplasia Enrolled Onto a 3-Year Prospective Phase III Clinical Trial of Oral Toremifene. *Journal of Clinical Oncology* **31** 523-529.

Taylor RA, Toivanen R, Frydenberg M, Pedersen J, Harewood L, Australian Prostate Cancer B, Collins AT, Maitland NJ & Risbridger GP 2012 Human Epithelial Basal Cells Are Cells of Origin of Prostate Cancer, Independent of CD133 Status. *STEM CELLS* **30** 1087-1096.

Thomas P & Dong J 2006 Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: A potential novel mechanism of endocrine disruption. *The Journal of Steroid Biochemistry and Molecular Biology* **102** 175-179.

Usui T 2006 Pharmaceutical Prospects of Phytoestrogens. *Endocrine Journal* **53** 7-20.

Utsumi T, Yoshimura N, Takeuchi S, Maruta M, Maeda K & Harada N 2000 Elevated steroid sulfatase expression in breast cancers. *The Journal of Steroid Biochemistry and Molecular Biology* **73** 141-145.

Vidal AC, Howard LE, Moreira DM, Castro-Santamaria R, Andriole GL & Freedland SJ 2014 Obesity Increases the Risk for High-grade Prostate Cancer: Results from the REDUCE study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **23** 2936-2942.

Wang F, Vihma V, Soronen J, Turpeinen U, Hämäläinen E, Savolainen-Peltonen H, Mikkola TS, Naukkarinen J, Pietiläinen KH, Jauhiainen M, et al. 2013 17β-Estradiol and Estradiol Fatty Acyl Esters and Estrogen-Converting Enzyme Expression in Adipose Tissue in Obese Men and Women. *The Journal of Clinical Endocrinology & Metabolism* **98** 4923-4931.

Wernert N, Kern L, Heitz P, Bonkhoff H, Goebbels R, Seitz G, Inniger R, Remberger K & Dhom G 1990 Morphological and immunohistochemical investigations of the utriculus prostaticus from the fetal period up to adulthood. *The Prostate* **17** 19-30.

White BA, Porterfield SP & Porterfield SP 2013 *Endocrine and reproductive physiology*. Philadelphia, PA: Elsevier/Mosby.

Whittemore AS, Kolonel LN, Wu AH, John EM, Gallagher RP, Howe GR, Burch JD, Hankin J, Dreon DM, West DW, et al. 1995 Prostate Cancer in Relation to Diet, Physical Activity, and Body Size in Blacks, Whites, and Asians in the United States and Canada. *Journal of the National Cancer Institute* **87** 652-661.

Wright JL, Kwon EM, Ostrander EA, Montgomery RB, Lin DW, Vessella R, Stanford JL & Mostaghel EA 2011 Expression of SLCO Transport Genes in Castration-Resistant Prostate Cancer and Impact of Genetic Variation in SLCO1B3 and SLCO2B1 on Prostate Cancer Outcomes. *Cancer Epidemiology Biomarkers & Prevention* **20** 619-627.

Yamnik RL & Holz MK 2010 mTOR/S6K1 and MAPK/RSK signaling pathways coordinately regulate estrogen receptor α serine 167 phosphorylation. *FEBS Letters* **584** 124-128.

Yang M, Kenfield SA, Blarigan ELV, Kasperzyk JL, Sesso HD, Ma J, Stampfer M & Chavarro JE 2014 Abstract 1263: Dietary patterns after prostate cancer diagnosis in relation to disease-specific and total mortality. *Cancer Research* **74** 1263.

Yeh C-R, Da J, Song W, Fazili A & Yeh S 2014 Estrogen receptors in prostate development and cancer. *American Journal of Clinical and Experimental Urology* **2** 161-168.

Yeh S, Miyamoto H, Shima H & Chang C 1998 From estrogen to androgen receptor: a new pathway for sex hormones in prostate cancer. *Proc Natl Acad Sci* **95** 5527-32.

Figure Legends

Figure 1: Molecular similarities between phytoestrogens and E_2. E_2 contains the cyclopenta[α]phenanthrene ring structure common to all steroid molecules. Isoflavonesand coumestans are two common categories of phytoestrogens and have a molecular structure similar to E_2. As a result phytoestrogens can also bind and activate the oestrogen receptors.

Figure 2: Oestrogen and Androgen synthesis pathways.

Intratumoural E_2 can be formed from desulfation and reduction of circulating oestrone-sulphate (E_1S) by steroid sulphatase (STS) and 17β-hydroxysteroid dehydrogenase (HSD). Alternatively, oestrogens can be produced from androstenedione or testosterone by aromatase. Aromatase competes with 5α-reductase (SRD5A1), responsible for potentiating androgens, for these substrates. DHEA, the precursor for androstenedione, is most likely derived from the large pool of circulating DHEAS by STS, as intratumoural synthesis from progestogens remains disputable.

Figure 3: The expression of ERα and ERβ changes during prostate cancer progression. During development of prostate cancer the ERβ isoform is downregulated in epithelial cells. On the other hand, ERα is upregulated in tumour cells as well as the surrounding environment. The remainder of the 'normal' prostate retains its existing expression of ERα and ERβ

Figure 4: Signalling pathways in prostate cancer through ERα, ERβ and GPER. ERα and ERβ bind to the oestrogen response elements (ERE) of DNA and regulate transcription. Activation of ERα induces mitogenic pathways via PI3K which in turn promotes HIF-1α which activates anti-apoptotic pathways; whereas activation of ERβ induces apoptosis, cell cycle arrest and inhibits dedifferentiation pathways. GPER activation in prostate cancer is anti-tumourigenic as it upregulates p21 and induces cell cycle arrest.

Figure 5: The altered expression of ERs during prostate cancer development. Changes in ERα and ERβ have been studied throughout the evolution of prostate cancer; however, expression of GPER in normal prostate and early stages of prostate cancer is currently unknown.

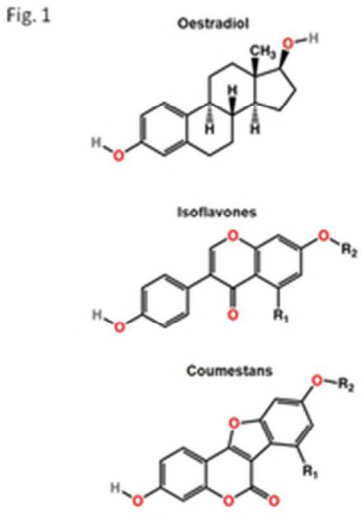

Figure 1
18x13mm (600 x 600 DPI)

Fig. 2

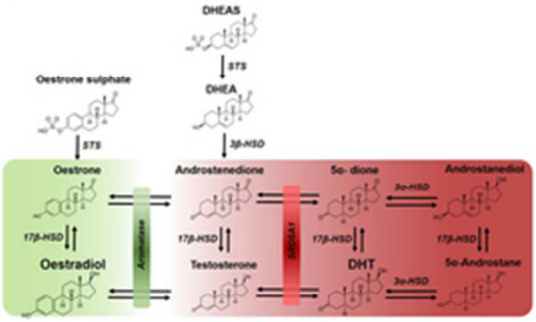

Figure 2
18x13mm (600 x 600 DPI)

Fig. 3

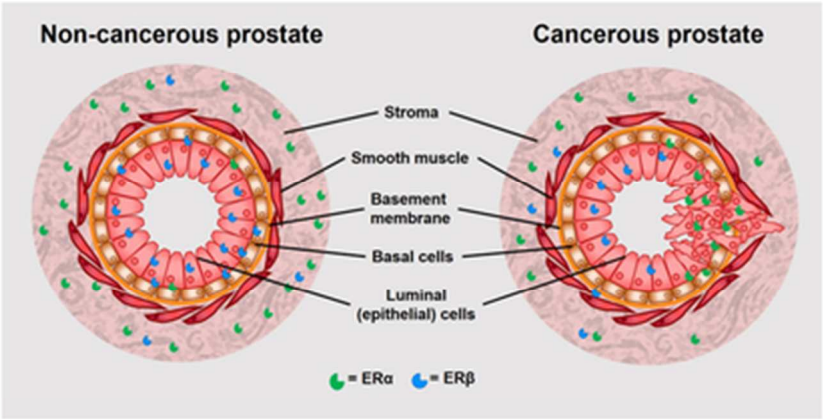

Figure 3
18x13mm (600 x 600 DPI)

Fig. 4

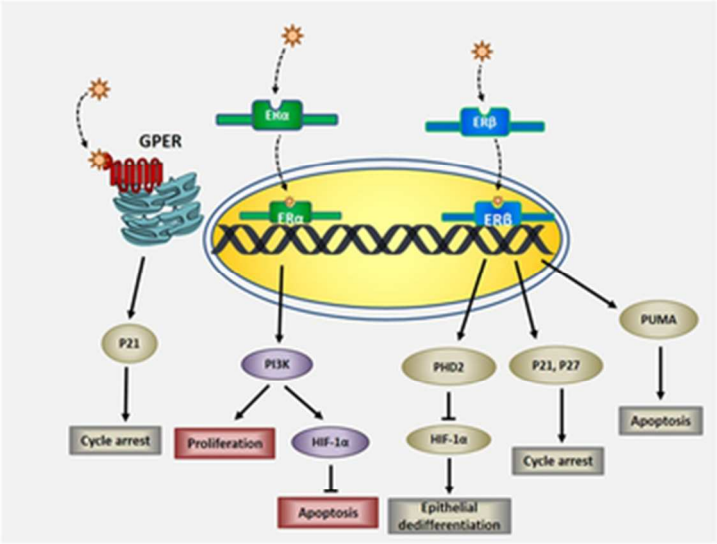

Figure 4
18x13mm (600 x 600 DPI)

Fig. 5

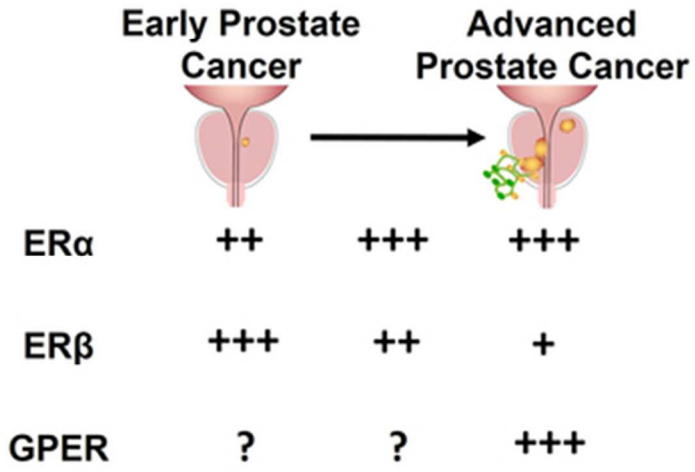

Figure 5
18x13mm (600 x 600 DPI)