

In touch with your feminine side: how oestrogen metabolism impacts prostate cancer

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Abstract

Prostate cancer is the primary cancer in males, with increasing global incidence rates making this malignancy a significant healthcare burden. Androgens not only 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 3 β - and 17 β -hydroxysteroid dehydrogenases. Increased expression of these enzymes in malignant prostate tissue compared with normal prostate indicates that 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.

Key Words

- ▶ oestrogen
- ▶ prostate cancer
- ▶ steroid sulphatase
- ▶ 17beta hydroxysteroid dehydrogenase
- ▶ oestrogen receptor

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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 & 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 traveling 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 & 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 dehydroepiandrosterone 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 and coworkers (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 and coworkers (2004) demonstrated using immunostaining and radioimmunoassays that activation of androgen receptors occurs 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 (de Bono *et al.* 2011, Scher *et al.* 2012, Ryan *et al.* 2013, Beer *et al.* 2014). 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 that 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 & 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 feeds back on the pituitary gland to reduce the secretion of luteinising 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, E₂ can activate both wildtype and, with greater efficacy, mutated (T877A) androgen receptors in LNCaP cells (Veldscholte *et al.* 1992, Yeh *et al.* 1998, Susa *et al.* 2015). 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, 8 tumours out of 38 patients with advanced prostate cancer were found to harbour androgen receptor mutations (Marcelli *et al.* 2000, Brooke & 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 E₂ levels increase and foetal androgen levels decrease. Raised E₂ levels stimulate the developing epithelial cells of the prostate to proliferate and 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 E₂ (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 & 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 with 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 E₂ (Knochenhauer *et al.* 1998), also increases with age which further decreases free serum testosterone relative to free serum E₂ (Samaras *et al.* 2012). Furthermore, there is evidence that E₁ and E₂ 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 & 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 & 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 is 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 (Wang *et al.* 2013, Polari *et al.* 2015). 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 men with a mean age of 53.6 years, where visceral fat was measured using magnetic resonance imaging (Gautier *et al.* 2013).

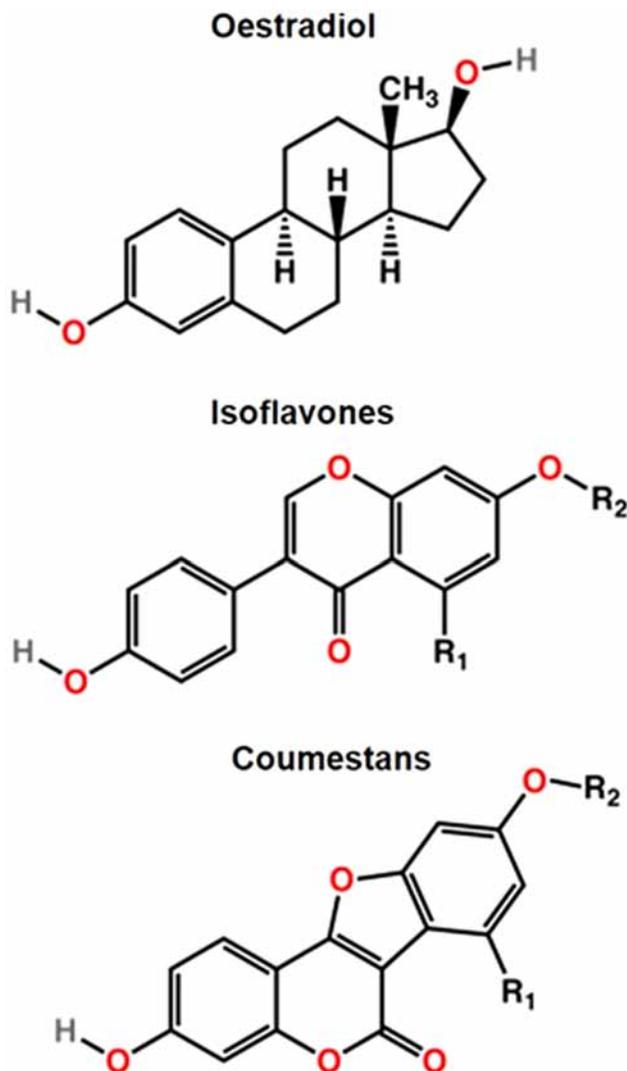
There have been conflicting reports as to whether obesity is a risk factor for prostate cancer, as some suggest it decreases risk while others have found the opposite. Allott and coworkers have summarised the findings published between 1991 and 2012 in their review and conclude obesity is associated 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 is 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 high meat, saturated fat and dairy products has been associated with increased risk of prostate cancer as highlighted by numerous epidemiological studies (Howell 1974, Whittemore *et al.* 1995, Grönberg 2003). 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 with 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, is considerably higher than the Western world (Strom *et al.* 1999, Adlercreutz *et al.* 2000, Goetzl *et al.* 2007). 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 (Pilišáková *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 in 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 syntheses (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

**Figure 1**

Molecular similarities between phytoestrogens and E_2 . E_2 contains the cyclopenta[α]phenanthrene ring structure common to all steroid molecules. Isoflavones and 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. A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0118>.

oestrogen receptors). Isoflavones and coumestans are two main categories of phytoestrogens and are structurally similar to E_2 (Fig. 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 with circa 20% in cancerous cell lines at concentrations of 30 μ M (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 (83 mg/day) or a drink with low isoflavone concentration (3 mg/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 (450 mg/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, and, 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 the 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 (Fig. 2).

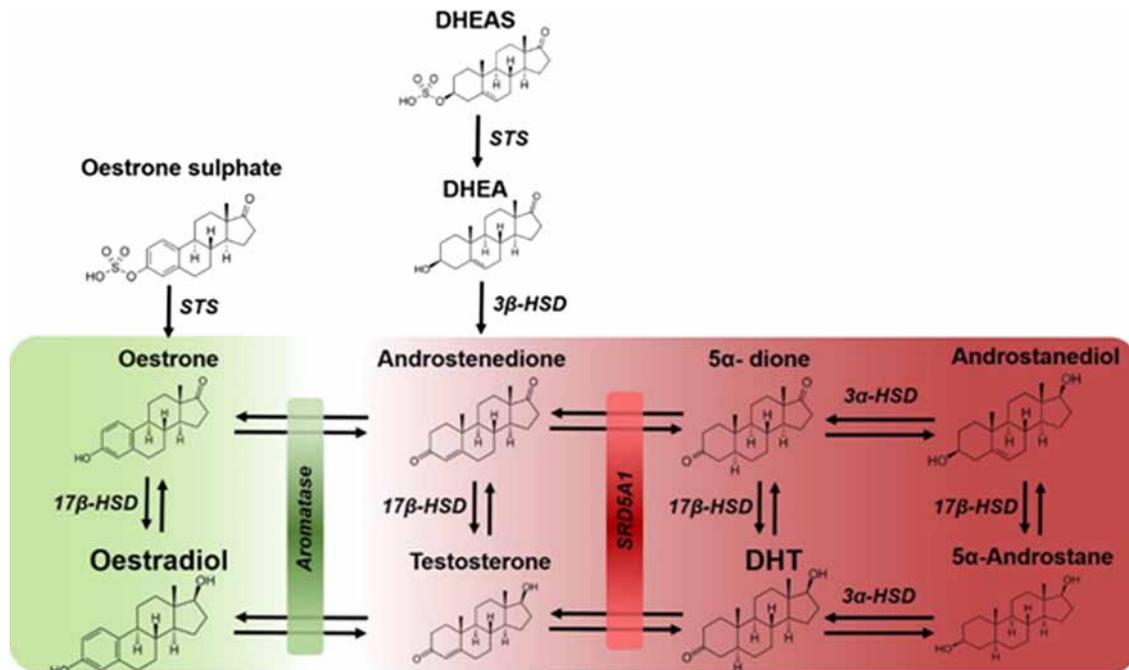


Figure 2

Oestrogen and androgen synthesis pathways. Intra-tumoural E_2 can be formed from desulphation 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 intra-tumoural synthesis from progestogens remains disputable. A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0118>.

There have been conflicting reports on the possibility of prostate cancer to synthesise 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 & 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- β -hydroxysteroid 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 LNCaP 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 *CYP19A1* (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* (Evaull *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 $\Delta(4)$ -abiraterone (D4A) within the prostate gland by 3β -HSD itself (Li *et al.* 2015b). Further research into 3β -HSD inhibition is 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 the reduction of androstenedione by 5α -reductase SRD5A1 to 5α -androstenedione, which is converted to DHT by

17 β -HSD5. Mass spectrometry has shown that even in patients on anti-androgen therapy with very low serum testosterone levels, intra-tumoural DHT concentrations remain at the pre-treatment level (Chang *et al.* 2011, Sharifi & Auchus 2012). 17 β -HSD-5, also known as AKR1C3, appears to be the key enzyme responsible for intra-tumoural androgen production in CRPC. Its expression in LNCaP, DU145 and PC3 cells is potently stimulated by androgen deprivation *in vitro* and in humans *in vivo* (Ellem *et al.* 2004, Ellem & Risbridger 2009), 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 intra-tumoural 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). 17 β -HSD-5 has also been implicated in enzalutamide resistance to anti-androgen therapy. Knockdown of 17 β -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 E₁ and E₂, respectively (White *et al.* 2013). The local synthesis of E₂ 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 E₂ synthesis does occur in prostate cancer cells (and benign prostatic hyperplasia) via aromatisation (Härkönen & Mäkelä 2004, Ellem & Risbridger 2009). In normal prostate, aromatase is expressed by the stromal tissue but not the epithelial cells; however, once malignant, epithelial cells also express aromatase (Ellem & 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 (Cunha 1994, Chen 1998). As with the developmental similarities between breast and prostate tissues (Ellem & Risbridger 2010), abnormal aromatase activity also plays a major role in breast and prostate tumourigenesis (Ellem & 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 with 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 500–750 mg 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 7 months (Murray & 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 anastrozole, 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 E₂ is not synthesised from androgens within the prostate but instead is converted from systemic sulphated E₁ 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 and 4 nmol/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, the mean serum E_1S levels in the 275 patients who developed prostate cancer were 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 E_1S are expressed in prostate cancers (Wright *et al.* 2011, Buxhofer-Ausch *et al.* 2013, Giton *et al.* 2015). 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 with 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, 2008, Purohit & 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 the conversion of E_1S to E_1 (Roy *et al.* 2013, Giton *et al.* 2015). 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 in 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 are lacking.

Conversion of E_1 - E_2 (and androstenedione to testosterone) requires 17 β -hydroxysteroid dehydrogenase (17 β -HSD) enzymes (White *et al.* 2013). 17 β -HSDs enzymes are alcohol oxidoreductases which catalyse reduction (E_1 - E_2) and oxidation (E_2 - E_1) at carbon atom 17. There are over 14 different isozymes of 17 β -HSDs (17 β -HSD 1-14), and certain 17 β -HSDs have a higher propensity to catalyse the reaction in a certain direction; for example, 17 β -HSD-1 favours reduction, whereas 17 β -HSD-2 favours oxidation (Oduwole *et al.* 2003, Lukacik *et al.* 2006). 17 β -HSDs play an important role in hormone-sensitive cancers. Increased expression of 17 β -HSD-1 in breast cancers of post-menopausal women helps maintain high intra-tumoural E_2 levels (Lukacik *et al.* 2006). Moreover, expression of 17 β -HSD-2 and 17 β -HSD-3 mRNA is significantly higher in malignant prostatic tissues compared with 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, 17 β -HSD-5 can convert E_1 to E_2 . Inhibitors of 17 β -HSD-5 have been explored in castration-resistant prostate cancer and breast cancer; in the latter, 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 17 β -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 17 β -HSD-2 and 17 β -HSD-5 (and also 3 β -HSD). Treatment of prostate cancer stromal cell lines PrSC with TGF β 1 showed a marked downregulation in mRNA expression of 17 β -HSD-2 and 17 β -HSD-5 in a dose-dependent manner (Piao *et al.* 2013). The counterintuitive action of TGF β 1 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 (ERs). There are two well-studied ERs: ER alpha (ER α) and ER beta (ER β), encoded by two separate genes *ESR1* and *ESR2*, respectively. ER α and ER β 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 & Giguere 2013). As well as by E₂, 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 ER β at lower concentrations than towards ER α using hamster uterine cells (Takeuchi *et al.* 2009). When human cells are examined, the relative binding affinity (RBA) of genistein to ER β is approximately 20–30 times greater than for ER α as shown in MCF-7 breast cancer cell lines (Pilišáková *et al.* 2010). The affinity of phytoestrogens for ER widely varies with most molecules having an RBA to ER β 1000-fold lower than E₂. However, molecules such as genistein and coumestrol have an RBA 100-fold lower than E₂. Genistein and coumestrol are able to activate transcriptional factors of ER α and ER β at concentrations of 1–10 nM compared with physiological E₂ concentrations of 20–40 pM 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 E₁ and E₂, 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 ER α promotes tumour growth as it initiates anti-apoptotic (Razandi *et al.* 2000, Chaudhri *et al.* 2014) and mitogenic effects (Yamnik & Holz 2010, Bhatt *et al.* 2012). This anti-apoptotic effect of ER α makes ER α -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 ER α expression correlates with worse prognosis, whereas ER β expression correlates with better clinical outcomes (Burns & Korach 2012). Generally, ER α activation promotes proliferative pathways, whereas ER β activation leads to apoptotic pathways (Acconcia *et al.* 2005).

Expression of ER α and ER β 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 ER β is putatively 6-fold greater and ER α is 125-fold greater in progenitor cells compared with 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 the lack of androgen receptor expression could be an imprint which later predisposes to CRPC in the elderly. In non-cancerous prostate, ER α is predominantly expressed in the stromal compartment and ER β is predominantly expressed in basal-epithelial cells. However, in prostate cancer, ER α expression is downregulated in stromal cells and upregulated in the cancerous epithelial cells. ER β expression is downregulated in epithelial cells as seen by immunostaining in human prostate tissue (Yeh *et al.* 2014). Indeed, there is evidence that downregulation of ER β promotes activation of NF- κ B mediated by hypoxia-inducible factor 1 (HIF-1). In immortalised normal prostate epithelial cell line PNT1a, loss of ER β using shRNA showed an increase in NF- κ B 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 ER α expression and decrease in ER β 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 & Korach 2012). Figure 3 summarises the difference in ER α and ER β expression between non-cancerous and cancerous prostate tissue. Single nucleotide polymorphisms (SNPs) 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, Jurečková *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 with 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 polymorphism has been linked to increased risk only in Caucasians (Fu *et al.* 2014).

Research into ER β has been more extensive than in ER α . McPherson and coworkers (2007) highlighted the potential significance of ER β manipulation when

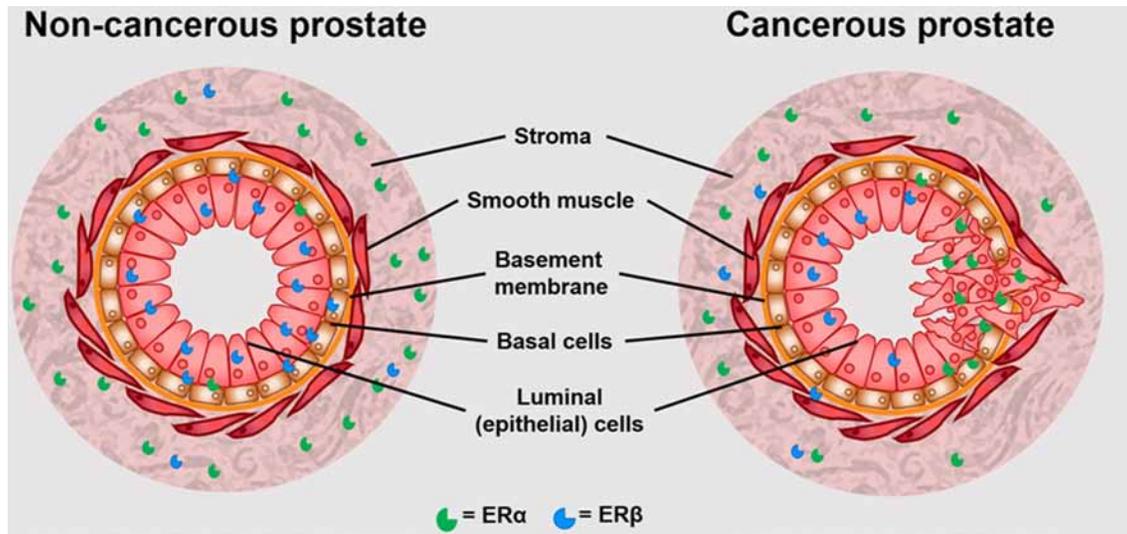


Figure 3

The expression of ER α and ER β changes during prostate cancer progression. During development of prostate cancer, the ER β isoform is downregulated in epithelial cells. On the other hand, ER α is upregulated in tumour cells as well as the surrounding environment. The remainder of the 'normal' prostate retains its existing expression of ER α and ER β . A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0118>.

they treated prostate hyperplasia in oestrogen-depleted mice with a selective ER β agonist and found it to induce apoptosis and shrink the size of the prostate. Hussain and coworkers (2012) carried forward this research, and initial studies have found ER β agonist treatment with 8 β -VE₂ 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 ER β activation induces apoptosis in prostate cancer cells lines may be via upregulation of p53-upregulated modulator of apoptosis (PUMA) and consequent intrinsic caspase-9 mechanisms. Dey and coworkers overexpressed ER β in LNCaP, PC3 and 22Rv1 prostate cancer cell lines *in vitro*, the latter does not express ER β , and treated with E₂ and agonist 3 β -adiol. Immunofluorescence revealed that cells that expressed ER β were more likely to undergo apoptosis following expression of PUMA independent of p53 (Dey et al. 2014). It has even been reported that ER β 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 E₂ and high concentration of ER β 1 agonist 3 β -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 ER β 1 expression by means of shRNA transfection resulted in a significant increase in migration and invasion (Mak et al. 2010). Mounting evidence also

suggests that pharmaceutical targeting of ER β pathways may be effective in treating prostate cancer. However, recently, a 'switching roles' theory has been proposed suggesting that the effects of ER β activation switches from protective to proliferative as cancer progresses (Savoy & Ghosh 2013). The theory is based on the observation that castration-resistant prostate cancers have higher expression of ER β compared with hormone-naïve prostate cancers. It is possible that decreased levels of circulating androgens and upregulation of androgen receptors may be important in this switch; however, the actual mechanisms and processes are yet unknown.

Splice variants of ER β are also important, as it has been shown that at least five 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 β ₁ is tumour-suppressing, whereas ER β 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 β ₂ and ER β 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 ER β isoforms, such as ER β ₂ and ER β ₃, when activated interact with transcription factors which enable and promote the epithelial–mesenchymal transition and hence might be why advanced prostate cancers have higher expression of ER β (Leung et al. 2010). More research needs to be

carried out to understand the mechanisms of the complex downstream pathways of ER β activation in prostate cancer.

The tumour-promoting effects of ER α within the prostate are not as well defined. ER α is expressed in significant quantities in the stromal tissue of prostate cancer, where they have been associated with cancer-associated fibroblasts (CAFs) (Slavin *et al.* 2014). Da and coworkers isolated CAF from adenocarcinoma of mouse prostate lentivirally transduced with ER α . Conditioned media from ER α +CAF promoted proliferation of LNCaP, PC3, C4-2 and 22Rv1 cells. Furthermore, in xenograft experiments, mice co-implanted with ER α +CAF showed a higher growth rate of tumour mass compared with injection of prostate cancer cell lines alone (Da *et al.* 2015). Activation of ER α 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 ER α expression by binding to gene promoter 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 ER α activation can inhibit metastasis by downregulating the expression of matrix metalloproteinase 3 and upregulating the expression of thrombospondin 2 as seen in a range of breast cancer cell lines and LNCaP cells; however, this is not evident in primary human prostate tissue (Li *et al.* 2015a). This may be an effect of ER α activation, which diverts cell resources towards growth of prostate cancer rather than spread and invasion (Hanahan & Weinberg 2011). A study investigating the role of ER α in prostate cancers of PTEN-deficient mice has shown that the expression of ER α correlates strongly with the expression of Ki67, a proliferative marker. In addition, inhibition and

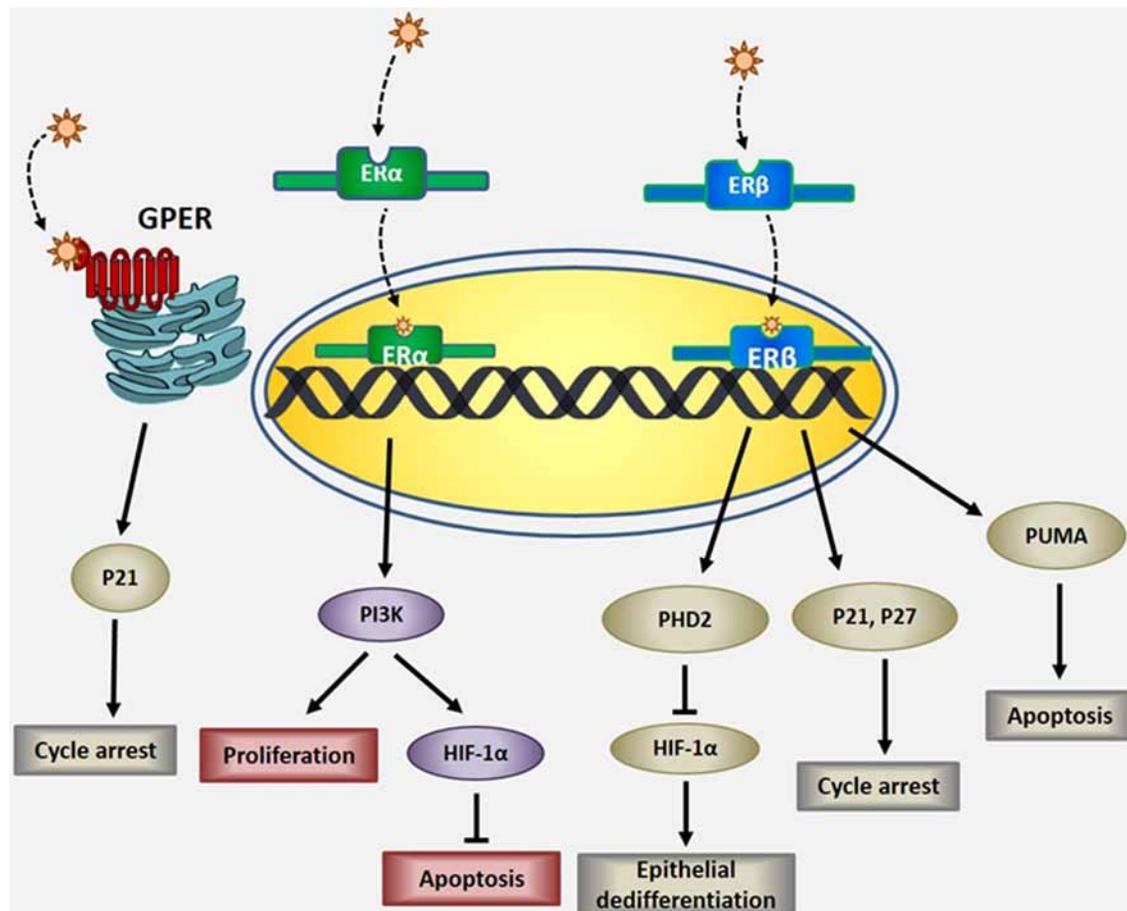


Figure 4

Signalling pathways in prostate cancer through ER α , ER β and GPER. ER α and ER β bind to the oestrogen response elements (EREs) of DNA and regulate transcription. Activation of ER α induces mitogenic pathways via PI3K which in turn promotes HIF-1 α which activates anti-apoptotic pathways, whereas activation of ER β 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. A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0118>.

knockdown of ER α decreases proliferation, but has no effect on cell viability; thus, the tumour mass remained static. This further demonstrates that ER α regulates cell proliferation through PI3K and MAPK signalling (Takizawa *et al.* 2015).

Human trials in 1590 men with high-grade intra-epithelial neoplasia of the prostate has shown no significant decrease in the 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 3-year study, cancer was detected in 34.7% in the placebo group compared with 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 ER α 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.* (2016) 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 ER α and ER β are summarised in Fig. 4.

In addition to the two nuclear ERs, ER α and ER β , 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 (Prossnitz *et al.* 2007, Prins & Hu 2013) and has very similar affinity for E $_2$ as ER α and ER β 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 ER β and can elicit an oestrogenic signalling pathways (Thomas & 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 in LNCaP, DU145 and PC3 cells, which 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 the 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 and coworkers 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 with androgen-replete milieu (Prins & Hu 2013) with increased GPER expression also evident in cells isolated from distant metastases in patients with CRPC compared with tissue from primary prostate cancers (Lam *et al.* 2014). Androgen receptor activation downregulates GPER expression, thus explaining why expression of

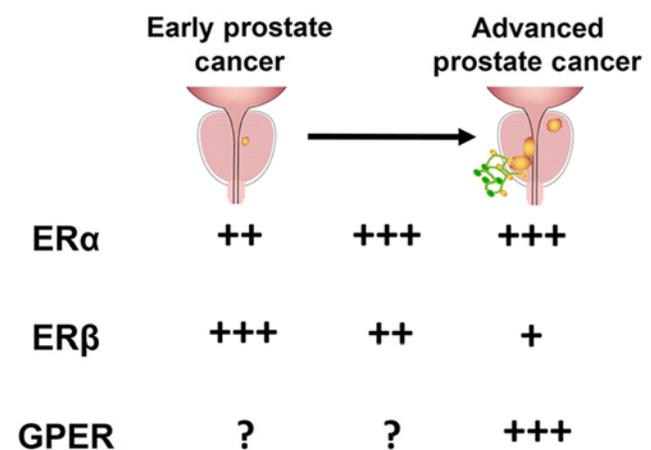


Figure 5

The altered expression of ERs during prostate cancer development. Changes in ER α and ER β 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. A full colour version of this figure is available at <http://dx.doi.org/10.1530/ERC-16-0118>.

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 upregulation 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 intra-tumoural 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 ER α and ER β greatly affect whether oestrogens are tumour promoting or tumour suppressing. In normal prostate and during early stages of prostate cancer where ER β is the prominent ER, oestrogens may be beneficial as ER β 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 ER α is the dominating ER within the prostate, oestrogens are deleterious as ER α 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.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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