

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

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

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## 29    **Abstract**

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

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54     **Introduction**

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

64     Survival statistics from prostate cancer have improved dramatically over the last four decades  
65     which may be attributed to earlier detection and treatment granted by prostate specific  
66     antigen (PSA) testing and transurethral resection of the prostate (TURP). The UK 10-year  
67     survival has improved from 25% when diagnosed in 1970 to 84% in 2010 (Quaresma, et al.  
68     2015). Prostate cancer primarily affects the elderly with 99.9% of patients diagnosed over the  
69     age of 50 and the mean age at diagnosis being 73 (Parkin, et al. 1997). Furthermore, from  
70     autopsy studies of non-cancer-related deaths, there is histological evidence of prostate  
71     neoplasms in more than 50% of men in their 50s (Sakr, et al. 1993). As average male life  
72     expectancy gradually increases, it is foreseeable that men will live longer with the disease  
73     and may experience a poorer quality of life.

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

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

91

## 92 **Sex Steroids and Prostate Cancer**

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

102

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

122 Previously, second-line hormonal therapy has proven to improve survival in patients with  
123 castration-resistant disease, both before and after docetaxel chemotherapy. Both inhibition of  
124 steroidogenic enzyme CYP17A1 using abiraterone and androgen receptor antagonism by  
125 enzalutamide have successfully ablated continued androgen receptor activation and prostate  
126 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<sub>2</sub> can activate both wildtype and, with greater efficacy, mutated (T877A) androgen receptors in LNCaP cells (Susa et al J Cell Physiol 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

151 mutations in the androgen receptor. On the contrary, eight tumours out of 38 patients with  
152 advanced prostate cancer were found to harbour androgen receptor mutations (Marcelli, et al.  
153 2000; Brooke and Bevan 2009). There is, however, mounting evidence that oestrogens may  
154 be involved in the initiation and progression of prostate cancer, although compelling evidence  
155 confirming oestrogen binding affinity to AR is lacking.

156  
**157 Impact of Endogenous Oestrogens in Prostate Cancer**

158 Males are exposed to a high oestrogen/androgen (E/T) ratio twice in their lifetime. The first is  
159 as a foetus, during the third trimester when the maternal E<sub>2</sub> levels increase and foetal  
160 androgen levels decrease. Raised E<sub>2</sub> levels stimulate the developing epithelial cells of the  
161 prostate to proliferate but also cause morphological changes. For example, the prostate glands  
162 of neonatal rats and mice show abnormal proliferation and cell structure when the pregnant  
163 mother is injected with E<sub>2</sub>. (Wernert, et al. 1990). This early exposure may imprint  
164 intracellular changes by modulating expression pathways of steroid enzymes and receptors  
165 as shown in rat models where the response to endogenous androgens and oestrogens becomes  
166 abnormal, thus predisposing the animal to prostate cancer after sexual maturation (Rajfer and  
167 Coffey 1978). Moreover, studies in mice show that when exposed to high levels of oestrogens  
168 in utero, foetal prostate tissue develops abnormalities including intraepithelial neoplasia and  
169 predisposition to carcinogenesis in adult life (Prins, et al. 2006). This hypothesis is supported  
170 by epidemiological evidence obtained from African-American men having twice as high a  
171 risk of developing prostate cancer than comparable Caucasian men which correlates with  
172 African-American women having a higher serum oestrogen level during pregnancy compared  
173 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

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

200

201 **Oestrogen Metabolism in Adipose and Prostate Cancer**

202

203 While in pre-menopausal females the primary source of oestrogens are the ovaries, in males  
204 there is no central organ which produces substantial quantities of E<sub>2</sub>. Instead, peripheral  
205 conversion of oestrogen precursors is the main source of oestrogen in men. Local synthesis of  
206 E<sub>1</sub> and E<sub>2</sub> is regulated by a plethora of enzymes. DHEA secreted from the zona reticularis of  
207 the adrenal glands, and stored in the blood as a reservoir as DHEAS, is the ultimate  
208 precursor. Adipose tissue is another notable source of oestrogen synthesis (Cui, et al. 2013).  
209 White adipose tissues (the predominant type in obesity) express significant quantities of  
210 cytochrome P450 aromatase enzyme (*CYP19A1*) in the abdominal adipose fat of male human  
211 samples, which is the final catalyst in the conversion of androgens to oestrogens (Polari, et al.  
212 2015; Wang, et al. 2013). There is also a positive correlation between the amount of visceral  
213 adipose tissue and serum E<sub>2</sub> levels as shown in a study of 229 man with a mean age of 53.6  
214 years where visceral fat was measured using magnetic resonance imaging (Gautier, et al.  
215 2013).

216

217 There have been conflicting reports as to whether obesity is a risk factor for prostate cancer  
218 as some suggest it decreases risk while others have found the opposite. Allott *et al.* have  
219 summarised the findings published between 1991 to 2012 in their review and conclude  
220 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

245 the Western world (Adlercreutz, et al. 2000; Goetzl, et al. 2007; Strom, et al. 1999). It is  
246 possible that phytoestrogens reduce the risk of prostate cancer through multiple mechanisms.  
247 In rodent models phytoestrogens can upregulate SHBG synthesis in the liver leading to a  
248 higher circulating concentration (Pilšáková, et al. 2010). Increased SHBG is anti-androgenic  
249 as it binds to free testosterone with a higher affinity than oestrogens (Knochenhauer et al.  
250 1998) implementing a net reduction of testosterone relative to E<sub>2</sub> (Ronde, et al. 2005). This  
251 reduction in androgen is thought to be important in the reduction of risk. In addition to  
252 chelation of free testosterone via SHBG, phytoestrogens have a negative feedback effect on  
253 the HPG axis directly leading to reduced secretion of luteinising hormone and consequently  
254 reduced stimulation of androgen and oestrogen synthesis (Goetzl et al. 2007).

255

256 Phytoestrogen compounds are similar enough to endogenous oestrogens to be able to bind to  
257 oestrogen receptors (ER) and evoke ligand-specific intracellular responses (Usui 2006).  
258 Preference for different types of nuclear ER varies between phytoestrogens (see section on  
259 oestrogen receptors). Isoflavones and coumestans are two main categories of phytoestrogens  
260 and are structurally similar to E<sub>2</sub> (Figure 1). The prostate cancer cell lines LNCaP and DU145  
261 are more sensitive to apoptotic factors when treated with isoflavones *in vitro*. A dose-  
262 response relationship between concentration of biochanin A and apoptosis was observed  
263 using cytotoxicity and lactate dehydrogenase release assays, flow cytometry and fluorescence  
264 microscopy (Szliszka, et al. 2013). Coumestans are able to induce caspase-dependent  
265 apoptosis in LNCaP, DU145 and PC3 cells. When treated with wedelolactone, a plant derived  
266 coumestan, there was dose-dependent apoptosis in androgen-sensitive cell lines (LNCaP) and  
267 androgen-independent cell lines (DU145 and PC3). However, normal non-cancerous PrEC  
268 prostate epithelial cells were not affected as harshly showing 90% cell viability compared to  
269 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- $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) which converts dehydroepiandrosterone and androstenediol to androstenedione and testosterone, respectively (White, et al. 2013). 3 $\beta$ -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 3 $\beta$ -HSD is increased within the tumour in addition to AKR1C3 and 17 $\beta$ -HSD3 (Chang, et al. 2011), although its mRNA expression almost completely mutually excludes that of CYP17A1 (Hofland et al. 2010).

318

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

328

329

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

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

### 348 ***Peripheral Oestrogen Metabolism in Prostate Cancer***

349 As mentioned previously, aromatase is a key enzyme required for oestrogen synthesis from  
350 androgen precursors. Aromatase converts androstenedione and testosterone to E<sub>1</sub> and E<sub>2</sub>,  
351 respectively (White et al. 2013). The local synthesis of E<sub>2</sub> within the prostate has previously  
352 been debated as not all experiments have identified aromatase expression in normal prostate  
353 tissue (Ellem et al. 2004). However, it has been demonstrated in human samples by substrate  
354 conversion assays and mass spectrometry that E<sub>2</sub> synthesis does occur in prostate cancer cells  
355 (and benign prostatic hyperplasia) via aromatisation (Ellem and Risbridger 2009; Härkönen  
356 and Mäkelä 2004). In normal prostate, aromatase is expressed by the stromal tissue but not  
357 the epithelial cells, however once malignant, epithelial cells also express aromatase (Ellem  
358 and Risbridger 2007). Aberrant expression and activity of aromatase is crucial in the  
359 pathophysiology of endometrial and breast cancers where an imbalance of oestrogen is a key  
360 factor in tumour growth (Chen 1998; Cunha 1994). As with the developmental similarities  
361 between breast and prostate tissues (Ellem and Risbridger 2010), abnormal aromatase activity  
362 also plays a major role in breast and prostate tumourigenesis (Ellem and Risbridger 2010).  
363 Tumourigenic growth factors including epidermal growth factor and transforming growth  
364 factor-1 can modulate aromatase activity in androgen-sensitive LNCaP cells lines leading to  
365 decreased oestrogen synthesis (Block, et al. 1996). Furthermore, the expression of aromatase  
366 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 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 E<sub>2</sub> is not synthesised from androgens within

392 the prostate but instead is converted from systemic sulphated  $E_1$  within the prostate via  
393 steroid sulphotase (STS).

394

395 STS is widely expressed in almost all peripheral tissues and is responsible for hydrolysing  
396 sulphate moieties off of circulating sulphate-conjugated steroids in order to make them  
397 biologically active (Mueller, et al. 2015). Oestrone sulphate ( $E_1S$ ) is the most abundant  
398 circulating oestrogen in adult humans (Muir, et al. 2004) with plasma levels between 2-  
399 4nmol/L in men (Mueller et al. 2015) and while oestradiol sulphate also exists, plasma levels  
400 are very low. Furthermore, serum  $E_1S$  levels have been correlated with increased risk of  
401 prostate cancer. In a cohort study of 5995 men aged over 65 where the mean serum  $E_1S$  levels  
402 in the 275 patients who developed prostate cancer was significantly higher than those who  
403 did not develop prostate cancer (Daniels, et al. 2010).

404

405 Before sulphated oestrogens can be unconjugated by intracellular STS, transport of sulphated  
406 oestrogens into cells requires the expression of organic anion transporter peptides (OATP)  
407 (Raftogianis, et al. 2000) and indeed several different OATPs involved in the transport of  
408 oestrone sulphate are expressed in prostate cancers (Buxhofer-Ausch, et al. 2013; Giton, et al.  
409 2015; Wright, et al. 2011). STS has been shown to be expressed in normal human prostate  
410 tissue (Reed, et al. 2005), prostate cancer cell lines LNCaP, DU-145 and PC3 (Nakamura, et  
411 al. 2006) and in primary prostate homogenates (Klein, et al. 1989). Furthermore, one study  
412 found that STS is expressed in the majority of localised prostate cancers showing higher  
413 expression in malignant tissues compared to benign (Nakamura, et al. 2006). The activity of  
414 STS has been proven within the human prostate for the desulphation of  
415 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-beta-hydroxysteroid dehydrogenase ( $17\beta$ -HSD) enzymes (White et al. 2013).  $17\beta$ -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  $17\beta$ -HSDs ( $17\beta$ -HSD 1-14) and certain  $17\beta$ -HSDs have a higher propensity to catalyse the reaction in a certain direction, for example  $17\beta$ -HSD-1 favours reduction whereas  $17\beta$ -HSD-2 favours oxidation (Lukacik, et al. 2006; Oduwale, et al. 2003).  $17\beta$ -HSDs play an important role in hormone sensitive cancers.

441 Increased expression of 17 $\beta$ -HSD-1 in breast cancers of post-menopausal women helps  
442 maintain high intratumoural E<sub>2</sub> levels (Lukacik et al. 2006). Moreover, expression of 17 $\beta$ -  
443 HSD-2 and 17 $\beta$ -HSD-3 mRNA is significantly higher in malignant prostatic tissues  
444 compared to normal prostate tissues (Day, et al. 2013) with one study reporting prostate  
445 cancer biopsies showing 30-fold higher mRNA expression than normal. In addition to  
446 converting androstenedione to testosterone, 17 $\beta$ -HSD 5 can convert E<sub>1</sub> to E<sub>2</sub>. Inhibitors of  
447 17 $\beta$ -HSD 5 have been explored in castration-resistant prostate cancer and breast cancer, in  
448 the latter where androgens are not considered to play an important role (Adeniji, et al. 2013).  
449 The study found no appreciable decrease in E<sub>2</sub> synthesis in breast cancer cell lines when  
450 treated with a 17 $\beta$ -HSD 5 inhibitor and only a moderate decrease in E<sub>2</sub> synthesis in some  
451 subpopulations of prostate cancer cell lines. Interestingly, inflammation associated with  
452 tumours modulates the expression of 17 $\beta$ -HSD-2 and 17 $\beta$ -HSD-5 (and also 3 $\beta$ -HSD).  
453 Treatment of prostate cancer stromal cell lines PrSC with TGF $\beta$ 1 showed a marked down-  
454 regulation in mRNA expression of 17 $\beta$ -HSD-2 and 17 $\beta$ -HSD-5 in a dose-dependent manner  
455 (Piao, et al. 2013). The counterintuitive action of TGF $\beta$ 1 again demonstrates how little is  
456 understood about oestrogenic pathways in prostate cancer. Regardless of the mechanisms by  
457 which oestrogens become available within the prostate gland, tumour-promoting or tumour-  
458 suppressing effects must be mediated by activation of oestrogen receptors (ER).

459

#### 460 **Oestrogen receptors (ER) in the prostate**

461 The effects of oestrogens on tissues are mediated via activation of oestrogen receptors (ER).  
462 There are two well studied ERs; ER alpha (ER $\alpha$ ) and ER beta (ER $\beta$ ) encoded by two separate  
463 genes *ESR1* and *ESR2*, respectively. ER $\alpha$  and ER $\beta$  are members of the nuclear receptor  
464 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  $ER\beta$  at lower concentrations than towards  $ER\alpha$  using hamster uterine cells (Takeuchi, et al. 2009). When human cells are examined, the relative binding affinity (RBA) of genistein to  $ER\beta$  is approximately 20-30 times greater than for  $ER\alpha$  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  $ER\beta$  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  $ER\alpha$  and  $ER\beta$  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  $ER\alpha$  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  $ER\alpha$  makes  $ER\alpha$  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  $ER\alpha$  expression correlates with worse prognosis whereas  $ER\beta$  expression correlates with better clinical

outcomes (Burns and Korach 2012). Generally, ER $\alpha$  activation promotes proliferative pathways whereas ER $\beta$  activation leads to apoptotic pathways (Acconcia, et al. 2005).

Expression of ER $\alpha$  and ER $\beta$  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 $\beta$  is putatively 6-fold greater and ER $\alpha$  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 ER $\alpha$  is predominantly expressed in the stromal compartment and ER $\beta$  is predominantly expressed in basal-epithelial cells. However in prostate cancer, ER $\alpha$  expression is down-regulated in stromal cells and upregulated in the cancerous epithelial cells. ER $\beta$  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 ER $\beta$  promotes activation of NF- $\kappa$ B mediated by hypoxia-inducible factor 1 (HIF-1). In immortalised normal prostate epithelial cell line PNT1a, loss of ER $\beta$  using shRNA showed an increase in NF- $\kappa$ 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 $\alpha$  expression and decrease in ER $\beta$  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 ER $\alpha$  and ER $\beta$  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; 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 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 ER $\beta$  has been more extensive than in ER $\alpha$ . McPherson et al. (2007) highlighted the potential significance of ER $\beta$  manipulation when they treated prostate hyperplasia in oestrogen depleted mice with a selective ER $\beta$  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 ER $\beta$  agonist treatment with 8 $\beta$ -VE<sub>2</sub> 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 $\beta$  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 ER $\beta$  in LNCaP, PC3 and 22Rv1 prostate cancer cell lines *in vitro*, the latter which does not express ER $\beta$ , and treated with E<sub>2</sub> and agonist 3 $\beta$ -adiol. Immunofluorescence revealed that cells which expressed ER $\beta$  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 ER $\beta$  activation impedes on the epithelial-mesenchymal transition process thereby reducing the risk of invasion and metastasis. In human tissue samples and LNCaP

539 and PC3 cell lines, treatment with E<sub>2</sub> and high concentration of ERβ1 agonist 3β-adiol  
540 resulted in inhibition of VEGF and destabilisation of HIF-1 in vitro thus suppressing the  
541 factors that drive epithelial-mesenchymal transition necessary for metastasis. Furthermore,  
542 loss of ERβ1 expression by means of shRNA transfection resulted in significant increase in  
543 migration and invasion (Mak, et al. 2010). Mounting evidence also suggests that  
544 pharmaceutical targeting of ERβ pathways may be effective in treating prostate cancer.  
545 However, recently a 'switching roles' theory has been proposed suggesting the effects of ERβ  
546 activation switches from protective to proliferative as cancer progresses (Savoy and Ghosh  
547 2013). The theory is based on the observation that castration-resistant prostate cancers have  
548 higher expression of ERβ compared to hormone-naïve prostate cancers. It is possible that  
549 decreased levels of circulating androgens and up-regulation of androgen receptors may be  
550 important in this switch however the actual mechanisms and processes are yet unknown.

551

552 Splice variants of ERβ are also important as it has been shown that at least 5 different  
553 isoforms exist, many of which are expressed in the prostate (Leung, et al. 2006). Activation  
554 of different isoforms may have opposing effects; for example ERβ<sub>1</sub> is tumour-suppressing  
555 whereas ERβ<sub>2</sub> is tumour-promoting in LNCaP cells (Chen, et al. 2009). In a study of primary  
556 prostate cancer samples from 144 patients who underwent radical prostatectomy, two  
557 particular isoforms ERβ<sub>2</sub> and ERβ<sub>5</sub> have been identified to promote invasion and metastasis  
558 of prostate cancer and thus correlate with worse outcomes while others continue to be studied  
559 (Leung, et al. 2010; Nelson, et al. 2014). Certain ERβ isoforms, such as ERβ<sub>2</sub> and ERβ<sub>3</sub>,  
560 when activated interact with transcription factors which enable and promote the epithelial  
561 mesenchyme transition and hence might be why advanced prostate cancers have higher  
562 expression of ERβ (Leung et al. 2010). More research needs to be carried out to understand  
563 the mechanisms of the complex downstream pathways of ERβ activation in prostate cancer.



564

565 The tumour promoting effects of ER $\alpha$  within the prostate are not as well defined. ER $\alpha$  is  
566 expressed in significant quantities in the stromal tissue of prostate cancer where they have  
567 been associated with cancer-associated fibroblasts (CAF) (Slavin, et al. 2015). Da, et al.  
568 isolated CAF from adenocarcinoma of mouse prostate lentivirally transduced ER $\alpha$ .  
569 Conditioned media from ER $\alpha$ + CAF promoted proliferation of LNCaP, PC3, C4-2 and  
570 22Rv1 cells. Furthermore, in xenograft experiments mice co-implanted with ER $\alpha$ + CAF  
571 showed a higher growth rate of tumour mass compared to injection of prostate cancer cell  
572 lines alone (Da, et al. 2015). Activation of ER $\alpha$  on CAFs stimulates the release of tumour-  
573 promoting factors which act on prostate epithelia in a paracrine manner. Slug (SNAI2), a  
574 transcription factor with anti-apoptotic pathways can repress ER $\alpha$  expression by binding to  
575 gene promotor regions and consequently promote epithelial-mesenchymal transition in  
576 prostate cancer cells and human breast cancer samples (Li, et al. 2015a). In contrast,  
577 downstream pathways of ER $\alpha$  activation can inhibit metastasis by down-regulating  
578 expression of matrix metalloproteinase 3 and upregulating expression of thrombospondin 2  
579 as seen in a range of breast cancer cell lines and LNCaP cell line, however this is not  
580 evidence in primary human prostate tissue (Li et al. 2015a). This may be an effect of ER $\alpha$   
581 activation which diverts cell resources towards growth of prostate cancer rather than spread  
582 and invasion (Hanahan and Weinberg 2011). A study investigating the role of ER $\alpha$  in prostate  
583 cancers of PTEN-deficient mice has shown expression of ER $\alpha$  correlates strongly with the  
584 expression of Ki67,- a proliferative marker. In addition, inhibition and knockdown of ER $\alpha$   
585 decreases proliferation but has no effect on cell viability thus the tumour mass remained  
586 static. This further demonstrates that ER $\alpha$  regulates cell proliferation through PI3K and  
587 MAPK signalling (Takizawa, et al. 2015).

588

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 ER $\alpha$  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 ER $\alpha$  and ER $\beta$  are summarised in Figure 4.

In addition to the two nuclear ERs, ER $\alpha$  and ER $\beta$ , 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<sub>2</sub> as ER $\alpha$  and ER $\beta$  with almost no interaction with androgens or glucocorticoids (Prossnitz, et al.

2008b). In addition to being activated by endogenous E<sub>2</sub>, GPER can also be activated by phytoestrogens with similar RBA as phytoestrogens have to ER $\beta$  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 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 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 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 ER $\alpha$  and ER $\beta$  greatly affect whether oestrogens are tumour promoting or tumour suppressing. In normal prostate and during early stages of

prostate cancer where ER $\beta$  is the prominent ER, oestrogens may be beneficial as ER $\beta$  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 $\alpha$  is the dominating ER within the prostate, oestrogens are deleterious as ER $\alpha$  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.

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## Figure Legends

**Figure 1: Molecular similarities between phytoestrogens and E<sub>2</sub>.** E<sub>2</sub> contains the cyclopenta[ $\alpha$ ]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<sub>2</sub>. As a result phytoestrogens can also bind and activate the oestrogen receptors.

## Figure 2: Oestrogen and Androgen synthesis pathways.

Intratumoural E<sub>2</sub> can be formed from desulfation and reduction of circulating oestrone-sulphate (E<sub>1</sub>S) by steroid sulphotase (STS) and 17 $\beta$ -hydroxysteroid dehydrogenase (HSD). Alternatively, oestrogens can be produced from androstenedione or testosterone by aromatase. Aromatase competes with 5 $\alpha$ -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 ER $\alpha$ and ER $\beta$ changes during prostate cancer progression.

During development of prostate cancer the ER $\beta$  isoform is downregulated in epithelial cells. On the other hand, ER $\alpha$  is upregulated in tumour cells as well as the surrounding environment. The remainder of the 'normal' prostate retains its existing expression of ER $\alpha$  and ER $\beta$ .

**Figure 4: Signalling pathways in prostate cancer through ER $\alpha$ , ER $\beta$  and GPER.** ER $\alpha$  and ER $\beta$  bind to the oestrogen response elements (ERE) of DNA and regulate transcription. Activation of ER $\alpha$  induces mitogenic pathways via PI3K which in turn promotes HIF-1 $\alpha$  which activates anti-apoptotic pathways; whereas activation of ER $\beta$  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 ER $\alpha$  and ER $\beta$  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.

Fig. 1

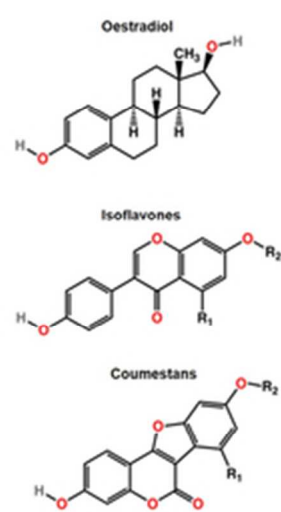


Figure 1  
18x13mm (600 x 600 DPI)

Fig. 2

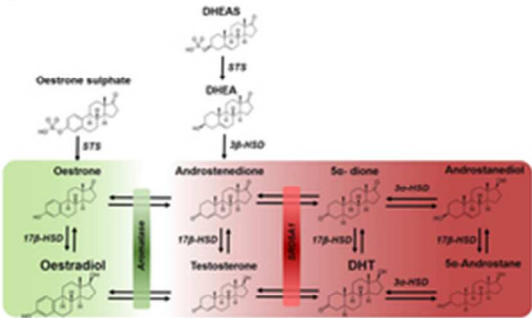


Figure 2  
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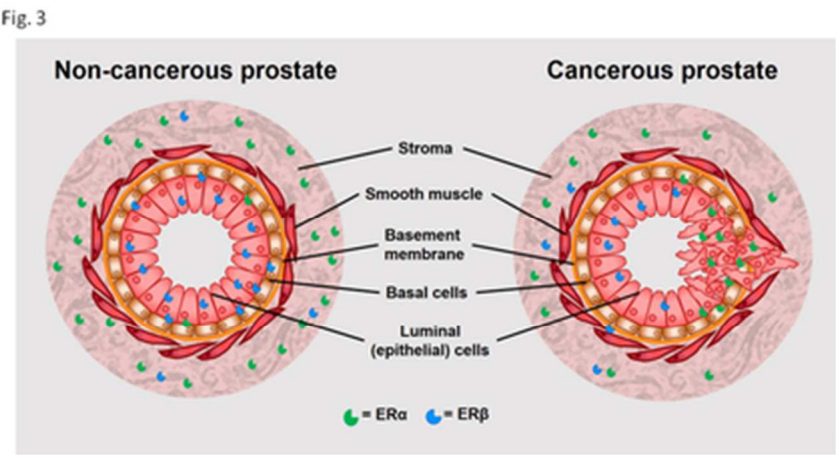


Figure 3  
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Fig. 4

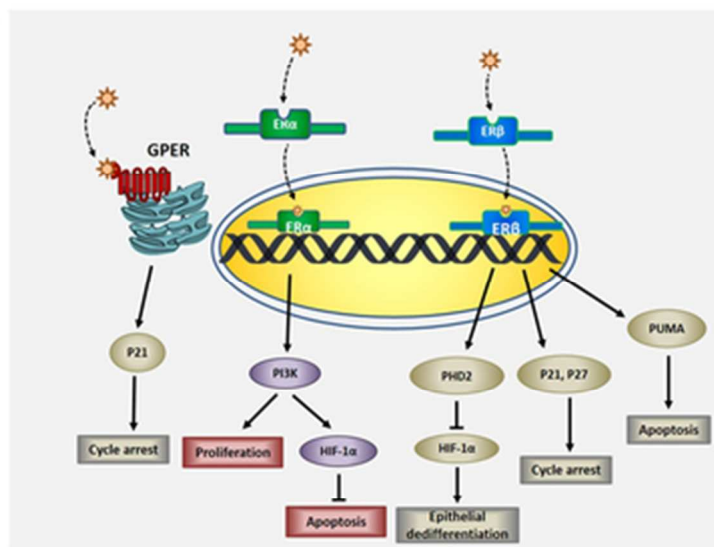


Figure 4  
18x13mm (600 x 600 DPI)

Fig. 5

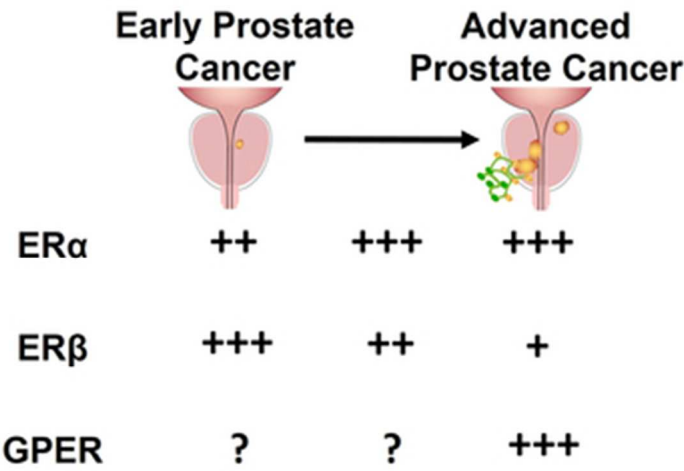


Figure 5  
18x13mm (600 x 600 DPI)