

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 β - and 17 β -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 4th 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 dehydroepiandrostrone 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.

127 2012). However, as with other androgen ablation therapy, resistance to abiraterone and
128 enzalutamide inevitably develops.

129

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

145 Importantly, the interactions of oestrogens on androgen receptors should be considered. For
146 example, E₂ can activate both wildtype and, with greater efficacy, mutated (T877A) androgen
147 receptors in LNCaP cells (Susa et al J Cell Physiol 2015; Yeh et al. 1998; Veldscholte et al
148 J Steroid Biochem Mol Biol. 1992). Mutations of the androgen receptor are uncommon in the
149 early stages of prostate cancer but are much more frequent in late-stage disease. In one study,
150 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 E₂ levels increase and foetal
160 androgen levels decrease. Raised E₂ 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 E₂. (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).

174 The second time men are exposed to a high E/T ratio is during old age when serum
175 testosterone decreases, partly due to a dampened HPG axis and partly due to reduced Leydig
176 cell function in the testes. In addition to this, sex hormone-binding globulin (SHBG), which
177 has a higher affinity to testosterone than E₂ (Knochenhauer, et al. 1998), also increases with
178 age which further decreases free serum testosterone relative to free serum E₂ (Samaras, et al.
179 2012). Furthermore, there is evidence that E₁ and E₂ not only remain at the same level, but in
180 fact increase with age even when accounted for BMI and other metabolic diseases (Jasuja, et
181 al. 2013). While the evidence for an association between serum oestrogen concentration and
182 risk of prostate cancer is unclear and inconsistent, increased serum oestrogen concentrations
183 may stimulate the prostate stroma and epithelia to proliferate and subsequently become
184 neoplastic. Indeed a higher oestrogen:androgen ratio stimulates proliferation of normal
185 prostate stromal (PrSC) and normal epithelial (PrEC) cell lines in vitro (King, et al. 2006).

186

187 Another interesting population which is exposed to a high E/T ratio are transsexual male to
188 female individuals. Often in this group of former males, individuals are orchietomised and
189 then supplemented with anti-androgens to relinquish masculine secondary sex characteristics.
190 They are also supplemented with oestrogens to acquire and enhance feminine characteristics.
191 Their prostates, however, remain unadulterated. A study observing such a cohort of
192 transsexual persons for over 30 years has not identified any increase in risk for prostate
193 cancer (Gooren and Morgentaler 2014). However the study has suggested that when
194 presenting these patients are more likely to be diagnosed with a later stage disease. One
195 limitation admitted by the authors is that the majority of the cohort has not reached the mean
196 age at which prostate cancer is typically diagnosed (Gooren and Morgentaler 2014).
197 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 E₂. Instead, peripheral
205 conversion of oestrogen precursors is the main source of oestrogen in men. Local synthesis of
206 E₁ and E₂ 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 E₂ 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

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

232

233 **Impact of Exogenous Oestrogen on Prostate Cancer**

234 Exogenous oestrogen intake and subsequent availability to the prostate should be considered
235 when determining whether oestrogens affect the development and progression of prostate
236 cancer. A Western diet comprising of high meat, saturated fat, and dairy products has been
237 associated with increased risk of prostate cancer as highlighted by numerous epidemiological
238 studies (Grönberg 2003; Howell 1974; Whittemore, et al. 1995). Additionally, it has been
239 observed that such a Western diet is more likely to cause men diagnosed with prostate cancer
240 to die from the disease when compared to a diet rich in fruits, vegetables, and whole grain
241 cereals (Yang, et al. 2014). Supporting this, it has been widely speculated that dietary
242 oestrogenic compounds from plant sources, termed phytoestrogens, are protective against
243 prostate cancer and are the reason behind lower incidence rates in East Asia where per capita
244 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 E₂ (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 E₂ (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).

270 While in vitro evidence argues that phytoestrogens are protective against prostate cancer,
271 clinical trials looking at the relationship between consumption of dietary phytoestrogens and
272 progression of prostate cancer have been inconclusive (Goetzl et al. 2007). One double blind
273 randomised control trial in which 81 healthy men were either given a soy protein drink with
274 high isoflavone concentration (83mg/day) or a drink with low isoflavone concentration
275 (3mg/day) showed no significant difference in PSA over 12 months (Adams, et al. 2004).
276 Another trial offering men with confirmed prostate cancer who had either failed
277 medical/surgical therapy or had chosen active surveillance a high dose (450mg/day) oral
278 isoflavone supplement for 6 months showed only a clinically insignificant improvement in
279 PSA in the active surveillance group with no difference in the failed therapy group (deVere
280 White, et al. 2004). Furthermore, a study following up 3628 men with diagnosed prostate
281 cancer for a median duration of 11.5 years showed an increased risk of advanced prostate
282 cancer (HR: 1.62) but a reduced risk of non-advanced prostate cancer (HR: 0.88) in the
283 higher dietary intake of isoflavones group. Dietary intake of phytoestrogens was measured
284 using a validated food frequency questionnaire and so exact doses of phytoestrogens are
285 subject to variation (Reger et al. 2015). This preliminary evidence could infer that dietary
286 phytoestrogens might protect against initiation of prostate cancer, however may promote the
287 progression of advanced prostate cancer.

288

289

290

291 **Steroid metabolism in the prostate**

292 *Androgens*

293 The metabolism of oestrogens and oestrogen precursors is important for availability of
294 biologically active E₂ to prostate cancer cells. Oestrogens are synthesised from androgens
295 which themselves are synthesised from progestogens (Khurana 2008). In addition to
296 circulating androgens secreted from the testes, normal prostate tissues have the potential to
297 produce androgens from circulating C19 steroids DHEA and androstenedione (Figure 2).
298 There have been conflicting reports on the possibility of prostate cancer to synthesize
299 androgens *de novo* through the conversion of progestogens via cytochrome P450 17A1 (17-
300 hydroxylase and 17, 20 lyase enzyme [*CYP17A1*]). In prostate cancer, the expression of
301 cytochrome P450 17A1 was reportedly increased in LNCaP and LuCaP cells and human
302 prostate tissue samples ascertained by PCR and immunoblotting (Locke, et al. 2008;
303 Montgomery, et al. 2008); however not all studies support this (Ellem and Risbridger 2009;
304 Hofland, et al. 2010). Although DHT formation from cholesterol was detected using mass
305 spectrometry in castration-resistant prostate cancer (CRPC) models in one study (Locke et al.
306 2008) these steroid fluxes have not been confirmed quantitatively to date in either *in vitro* or
307 *in vivo* models.

308

309 Another key enzyme in the synthesis of biologically active androgens and oestrogens is 3-
310 betahydroxysteroid dehydrogenase (3 β -HSD) which converts dehydroepiandrosterone and
311 androstenediol to androstenedione and testosterone, respectively (White, et al. 2013). 3 β -
312 HSD is expressed in the normal human prostate, with immunoblotting revealing that the
313 highest concentrations are found in basal epithelial cells (Luu-The, et al. 2008). Certainly, in
314 mouse xenograft studies using the CRPC LAPC4 cell line, expression of 3 β -HSD is increased
315 within the tumour in addition to AKR1C3 and 17 β -HSD3 (Chang, et al. 2011), although its
316 mRNA expression almost completely mutually excludes that of CYP17A1 (Hofland et al.
317 2010).

318

319 Inhibitors of 3 β -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 β -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 β -HSD itself (Li, et al. 2015b). Further research into 3 β -HSD inhibition are
326 currently being pursued, however alternative pathways which bypass androstenedione
327 synthesis exist and so 3 β -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 α -reductase SRD5A1 to 5 α -androstanedione which is converted to DHT by 17 β -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 β -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). 17β -HSD-5 has also been implicated in enzalutamide
345 resistance to anti-androgen therapy. Knockdown of 17β -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_1 and E_2 ,
351 respectively (White et al. 2013). The local synthesis of E_2 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_2 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

367 (Miftakhova, et al. 2016). In addition, overexpression of aromatase increased the progression
368 of bony metastasis in xenograft experiments where nude mice were injected with PC3 cell
369 lines transfected to overexpress aromatase (Miftakhova, et al. 2016). Consequently, the use
370 of aromatase inhibitors for the treatment of prostate cancer has been investigated many times
371 in patient cohorts. The first generation aromatase inhibitor aminoglutethimide is non-
372 selective and showed poor objective responses including serum PSA levels and disease
373 stability in some studies while showing a significant increase in survival in others (Santen, et
374 al. 1997). One study treated 58 castrated men with advanced prostate cancer resistant to
375 conventional therapy with 500-750mg daily aminoglutethimide; 11 men showed an objective
376 response with a mean remission of 10 months and a further two showed disease stabilisation
377 for a mean seven months (Murray and Pitt 1985). The second generation aromatase inhibitor,
378 4-hydroxyandrostenedione showed good subjective responses in 18 out of 25 patients with
379 advanced CRPC, particularly alleviation of bone pain in prostate metastases. However the
380 objective responses were still poor with a reduction in tumour volume seen in only three
381 patients and all patients progressed to have skeletal metastasis. (Davies, et al. 1992). A Phase
382 II clinical study looking at the effects of oral letrozole, a third-generation aromatase inhibitor
383 more commonly used in the treatment of hormone-dependent breast cancer, in 43 men with
384 CRPC showed no significant disease regression with serum PSA decreasing by more than
385 50% in only one patient and decreasing by less than 50% in one further patient (Smith, et al.
386 2002). A very similar conclusion was drawn from clinical studies looking at anastrozole,
387 another third generation aromatase inhibitor, where out of 14 patients with CRPC none
388 showed a decrease in serum PSA and mild bone pain relief was reported by only two patients
389 (Santen, et al. 2001). While aromatase is of utmost importance in local oestrogen synthesis, it
390 appears as though therapeutic approaches targeting aromatase may be futile in treating
391 prostate cancer. An alternative possibility is that E₂ is not synthesised from androgens within

392 the prostate but instead is converted from systemic sulphated E₁ within the prostate via
393 steroid sulphatase (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 (E₁S) 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 E₁S 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 E₁S 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

416 1973). Moreover, E_1 synthesis from desulphation of E_1S within the prostate is putatively 10-
417 fold greater than synthesis via aromatase (Nakamura et al. 2006). The relevance of STS in
418 cancer has been more extensively studied in breast cancer where there is significantly higher
419 expression of STS than in normal breast (Utsumi, et al. 2000). Consequently, several STS
420 inhibitors have been developed for the treatment of breast cancer, some of which have shown
421 early promise (Stanway, et al. 2006). Moreover, first and second generation STS inhibitors
422 have been effective pre-clinically against breast cancer (Foster et al. 2006; Foster et al. 2008;
423 Purohit and Foster 2012). Meanwhile, investigations into the efficacy of STS inhibitors in
424 prostate cancer have been undertaken. It has been observed that middle-aged rats treated with
425 oral STS inhibitor, STX64 decreased conversion of E_1S to E_1 (Giton et al. 2015; Roy, et al.
426 2013). Neither study presented evidence of STS inhibition affecting any proliferative markers
427 of proliferation, however the latter study did demonstrate that STS inhibition in middle-aged
428 rats prevented increase of prostate mass when treated with E_1S + STX64 vs E_1S alone where
429 prostate mass increased (Giton et al. 2015). An alternative conjugate of circulating oestrogens
430 is glucuronide (Raftogianis et al. 2000), however, research into oestrogen glucuronide
431 transport into prostate cells and evidence of glucuronidase enzymes within the prostate is
432 lacking.

433

434 Conversion of E_1 to E_2 (and androstenedione to testosterone) requires 17-betahydroxysteroid
435 dehydrogenase (17β -HSD) enzymes (White et al. 2013). 17β -HSDs enzymes are alcohol
436 oxidoreductases which catalyse reduction (E_1 to E_2) and oxidation (E_2 to E_1) at carbon atom
437 17. There are over 14 different isozymes of 17β -HSDs (17β -HSD 1-14) and certain 17β -
438 HSDs have a higher propensity to catalyse the reaction in a certain direction, for example
439 17β -HSD-1 favours reduction whereas 17β -HSD-2 favours oxidation (Lukacik, et al. 2006;
440 Oduwole, et al. 2003). 17β -HSDs play an important role in hormone sensitive cancers.

441 Increased expression of 17 β -HSD-1 in breast cancers of post-menopausal women helps
442 maintain high intratumoural E₂ levels (Lukacik et al. 2006). Moreover, expression of 17 β -
443 HSD-2 and 17 β -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 β -HSD 5 can convert E₁ to E₂. Inhibitors of
447 17 β -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 E₂ synthesis in breast cancer cell lines when
450 treated with a 17 β -HSD 5 inhibitor and only a moderate decrease in E₂ synthesis in some
451 subpopulations of prostate cancer cell lines. Interestingly, inflammation associated with
452 tumours modulates the expression of 17 β -HSD-2 and 17 β -HSD-5 (and also 3 β -HSD).
453 Treatment of prostate cancer stromal cell lines PrSC with TGF β 1 showed a marked down-
454 regulation in mRNA expression of 17 β -HSD-2 and 17 β -HSD-5 in a dose-dependent manner
455 (Piao, et al. 2013). The counterintuitive action of TGF β 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 α) and ER beta (ER β) encoded by two separate
463 genes *ESR1* and *ESR2*, respectively. ER α and ER β are members of the nuclear receptor
464 superfamily (Robinson-Rechavi, et al. 2003). When bound and activated, ERs interact

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

480

481 ERs have been studied more extensively in the context of breast cancers, a neoplasm that has
482 been likened as the sister disease to prostate cancer, especially in regards to their hormonal
483 responses and sensitivities (Risbridger, et al. 2010). In breast cancer, activation of $ER\alpha$
484 promotes tumour growth as it initiates anti-apoptotic (Chaudhri, et al. 2014; Razandi, et al.
485 2000) and mitogenic effects (Bhatt, et al. 2012; Yamnik and Holz 2010). This anti-apoptotic
486 effect of $ER\alpha$ makes $ER\alpha$ positive breast cancers more likely to metastasise (Ross-Innes, et
487 al. 2012). In fact, a review of ERs in breast and ovarian cancers has found $ER\alpha$ expression
488 correlates with worse prognosis whereas $ER\beta$ expression correlates with better clinical

489 outcomes (Burns and Korach 2012). Generally, ER α activation promotes proliferative
490 pathways whereas ER β activation leads to apoptotic pathways (Acconcia, et al. 2005).

491

492 Expression of ER α and ER β in the normal prostate has been determined as the role of
493 oestrogens in prostatic development was identified (Ho 2004). Recently it has been reported
494 that prostate progenitor stem cells, while lacking expression of androgen receptor, express ER
495 abundantly. Indeed, the expression of ER β is putatively 6-fold greater and ER α 125-fold
496 greater in progenitor cells compared to LNCaP mature cells (Di Zazzo, et al. 2016). Although
497 this supports the importance of oestrogens in embryonic and neonatal development of
498 prostate gland, it has been hypothesised that lack of androgen receptor expression could be an
499 imprint which later predisposes to CRPC in the elderly. In non-cancerous prostate ER α is
500 predominantly expressed in the stromal compartment and ER β is predominantly expressed in
501 basal-epithelial cells. However in prostate cancer, ER α expression is down-regulated in
502 stromal cells and upregulated in the cancerous epithelial cells. ER β expression is down-
503 regulated in epithelial cells as seen by immunostaining in human prostate tissue (Yeh, et al.
504 2014). Indeed there is evidence that down-regulation of ER β promotes activation of NF- κ B
505 mediated by hypoxia-inducible factor 1 (HIF-1). In immortalised normal prostate epithelial
506 cell line PNT1a, loss of ER β using shRNA showed an increase in NF- κ B mRNA expression
507 and activity. This mirrors what is seen in high grade, late stage prostate cancer (Mak, et al.
508 2015). Consequently, it appears that an increase in ER α expression and decrease in ER β
509 expression is what shifts the balance between protective effects of oestrogens and
510 proliferative effects of oestrogens as has been suggested in other cancers (Barzi, et al. 2013;
511 Burns and Korach 2012). Figure 3 summarises the difference in ER α and ER β expression
512 between non-cancerous and cancerous prostate tissue. Single nucleotide polymorphisms
513 (SNP) in the ER genes have been investigated and associations have been made between

514 certain polymorphisms and the risk of prostate cancer (Holt, et al. 2013; Jurečková, et al.
515 2015). In both studies, the genomes from histologically confirmed human prostate cancer
516 samples were analysed using polymerase chain reaction restriction fragment length
517 polymorphism (PCR-RFLP) based analysis and compared to age-matched healthy control
518 subjects. A meta-analysis exploring the results of 24 published studies that include
519 Caucasian, Asian and African participants concluded that *ESR1* rs9340799 polymorphism is
520 allied to increased risk in the general population of Caucasians and Africans whereas *ESR2*
521 rs1256049 polymorphisms has been linked to increased risk only in Caucasians (Fu, et al.
522 2014).

523

524 Research into ER β has been more extensive than in ER α . McPherson et al. (2007) highlighted
525 the potential significance of ER β manipulation when they treated prostate hyperplasia in
526 oestrogen depleted mice with a selective ER β agonist and found it to induce apoptosis and
527 shrink the size of the prostate. Hussain et al. (2012) carried forward this research and initial
528 studies have found ER β agonist treatment with 8 β -VE₂ can induce apoptosis in primary
529 human and murine prostatic basal cells, a lineage considered to be the cells of origin for
530 prostate cancers (Taylor, et al. 2012). The mechanism behind how ER β activation induces
531 apoptosis in prostate cancer cells lines may be via up-regulation of p53-upregulated
532 modulator of apoptosis (PUMA) and consequent intrinsic caspase-9 mechanisms. Dey, et al.
533 overexpressed ER β in LNCaP, PC3 and 22Rv1 prostate cancer cell lines *in vitro*, the latter
534 which does not express ER β , and treated with E₂ and agonist 3 β -adiol. Immunofluorescence
535 revealed that cells which expressed ER β were more likely to undergo apoptosis following
536 expression of PUMA independent of p53 (Dey, et al. 2014). (Dey, et al. 2014). It has even
537 been reported that ER β activation impedes on the epithelial-mesenchymal transition process
538 thereby reducing the risk of invasion and metastasis. In human tissue samples and LNCaP

539 and PC3 cell lines, treatment with E₂ 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 ERβ₁ is tumour-suppressing
555 whereas ERβ₂ 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 ERβ₂ and ERβ₅ 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 ERβ₂ and ERβ₃,
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 α within the prostate are not as well defined. ER α 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 α .
569 Conditioned media from ER α + CAF promoted proliferation of LNCaP, PC3, C4-2 and
570 22Rv1 cells. Furthermore, in xenograft experiments mice co-implanted with ER α + 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 α 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 α 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 α 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 α
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 α in prostate
583 cancers of PTEN-deficient mice has shown expression of ER α correlates strongly with the
584 expression of Ki67,- a proliferative marker. In addition, inhibition and knockdown of ER α
585 decreases proliferation but has no effect on cell viability thus the tumour mass remained
586 static. This further demonstrates that ER α regulates cell proliferation through PI3K and
587 MAPK signalling (Takizawa, et al. 2015).

588

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

604

605 In addition to the two nuclear ERs, ER α and ER β , another relatively recently discovered ER
606 exists. G-protein coupled oestrogen receptor (GPER), alternatively known as GPR30, is a
607 membrane-bound receptor discovered in 1998 (O'Dowd, et al. 1998). GPER is found in 50%
608 of breast cancers and is believed to be critically involved in how Tamoxifen (a SERM)
609 resistance is developed (Mo, et al. 2013). Tamoxifen can bind and stimulate GPER in breast
610 cancer (Prossnitz, et al. 2008a) activating downstream cancer promoting pathways. GPER has
611 also been shown to be expressed in various hormone-sensitive tissues in the body including
612 the prostate (Prins and Hu 2013; Prossnitz, et al. 2007) and has very similar affinity for E₂ as
613 ER α and ER β with almost no interaction with androgens or glucocorticoids (Prossnitz, et al.

614 2008b). In addition to being activated by endogenous E₂, GPER can also be activated by
615 phytoestrogens with similar RBA as phytoestrogens have to ERβ and elicit an oestrogenic
616 signalling pathways (Thomas and Dong 2006).

617

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

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

648

649 **Conclusion**

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

659

660 Changes in the expression pattern of ER α and ER β greatly affect whether oestrogens are
661 tumour promoting or tumour suppressing. In normal prostate and during early stages of

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

671

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

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Figure Legends

Figure 1: Molecular similarities between phytoestrogens and E₂. E₂ 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 E₂. As a result phytoestrogens can also bind and activate the oestrogen receptors.

Figure 2: Oestrogen and Androgen synthesis pathways.

Intratumoural E₂ can be formed from desulfation and reduction of circulating oestrone-sulphate (E₁S) by steroid sulphatase (STS) and 17 β -hydroxysteroid dehydrogenase (HSD). Alternatively, oestrogens can be produced from androstenedione or testosterone by aromatase. Aromatase competes with 5 α -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 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

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 (ERE) 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.

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.

Fig. 1

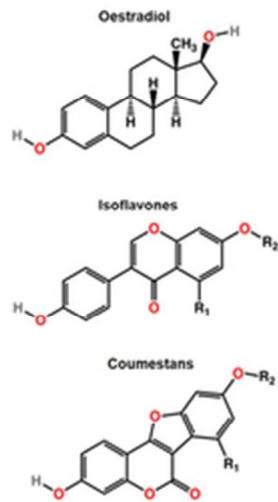


Figure 1
18x13mm (600 x 600 DPI)

Fig. 2

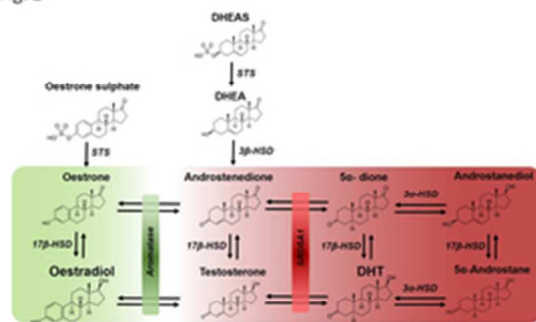


Figure 2
18x13mm (600 x 600 DPI)

Fig. 3

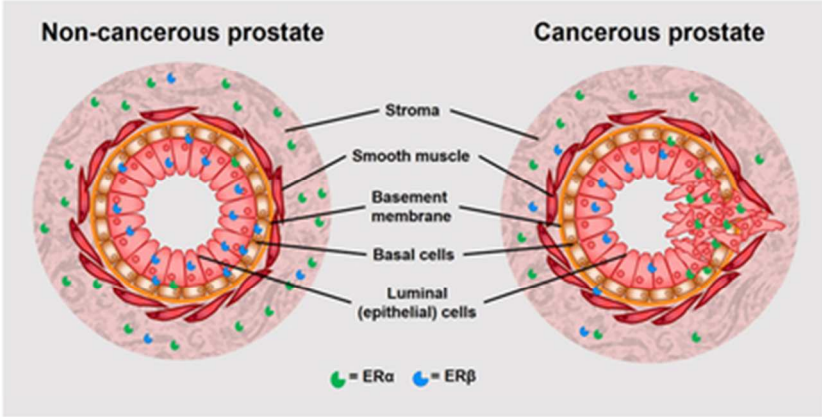


Figure 3
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Fig. 4

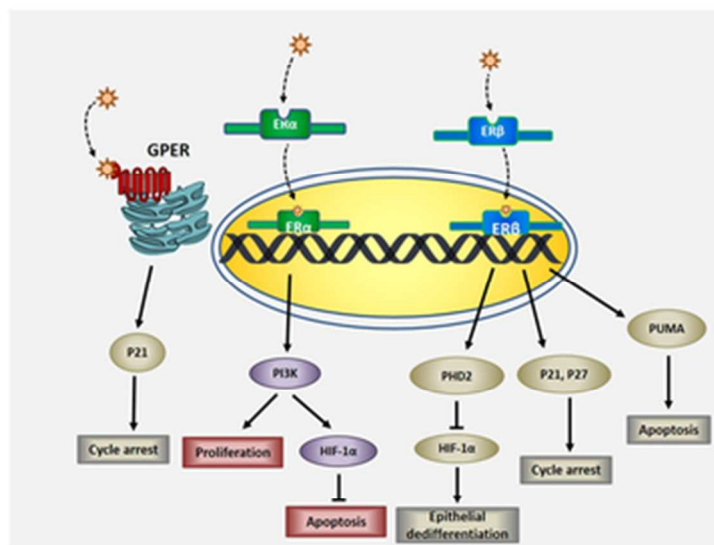


Figure 4
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Fig. 5

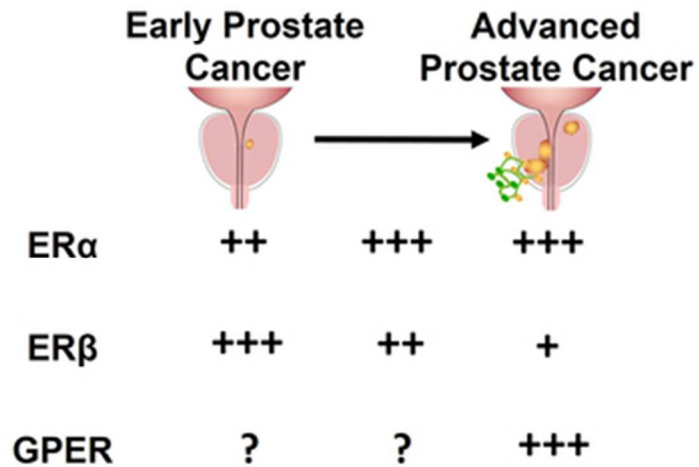


Figure 5
18x13mm (600 x 600 DPI)