EUROPEAN JOURNAL OF CANCER 44 (2008) 928–936

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Review

A potential paradox in prostate adenocarcinoma progression: Oestrogen as the initiating driver

Paras B. Singh[a,b], *Shyam S. Matanhelia*[b], *Francis L. Martin*[a,*]

[a]*Department of Biological Sciences, Lancaster University, Lancaster, UK*
[b]*Lancashire Teaching Hospitals NHS Foundation Trust, Fulwood, Preston, UK*

ARTICLE INFO

Article history:
Received 5 February 2008
Received in revised form
26 February 2008
Accepted 28 February 2008
Available online 1 April 2008

Keywords:
Anti-oestrogen
CYP1B1
Chemoprevention
Growth-promoting
Initiation
17β-Oestradiol
Prostate adenocarcinoma
Testosterone

ABSTRACT

One in 10 men in the developed world will present with prostate cancer (CaP), and in an ageing population developing strategies for its chemoprevention or treatment is of significance. For decades, androgen ablation has remained the frontline treatment for CaP that is no longer organ-confined and thus deemed surgically inoperable. Orchidectomy or drug-induced reduction of serum testosterone levels with the consequent removal of growth-promoting effects in the prostate is the driving rationale for this regimen. However, resistance often develops within a few months to years and androgen-insensitive tumours develop. In recent years, there has been an increasing focus on chemoprevention with agents such as finasteride being employed to reduce the risk of developing CaP. Significantly, such chemoprevention strategies are also based on 5α-reductase inhibition thus reducing intraprostatic dihydrotestosterone levels. Although there may be an overall reduction in CaP incidence in cohorts using such chemoprevention, in a subset of users who do develop this pathology there results a more aggressive, higher-grade disease. There have also been suggestions regarding the protective role of androgens against high-grade CaP. This leads to the intriguing notion that 17β-oestradiol (E_2) may be an initiating driver of CaP; in fact, in old studies in which CaP was induced in rodents, E_2 often accelerated the effect of the carcinogen. Might certain chemoprevention strategies or androgen ablation result in a systemic feedback loop in hormone synthesis or metabolism? If so, elevated serum E_2 levels could result in its increased conversion to genotoxic catechol oestrogens in target tissues such as the prostate. Paradoxically, if E_2 were to be an initiating factor in CaP, anti-oestrogens might be an overlooked treatment or chemoprevention strategy.

1. Introduction

The hormone-responsive prostate gland is the most common site of pathology in the human male; the most commonly occurring malignancy at this site is prostate adenocarcinoma (CaP). For CaP that is no longer organ-confined and thus deemed surgically inoperable, androgen ablation has remained the frontline treatment. However, within a few months to years post-surgery or after chemical castration, androgen-insensitive tumours often arise. Chemoprevention strategies, often based on 5α-reductase inhibition, to reduce the risk of developing CaP are currently being examined. There are, however,

* *Corresponding author:* Tel.: +44 1524 594505; fax: +44 1524 593192.
E-mail address: f.martin@lancaster.ac.uk (F.L. Martin).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2008.02.051

feasible mechanisms through which androgen ablation or certain chemoprevention strategies could result in elevated serum 17β-oestradiol (E_2) levels. In hormone-responsive target tissues such as the prostate gland that express cytochrome P450 (CYP) enzymes like CYP1B1,[1] this might result in increased conversion to genotoxic catechol oestrogens. This review raises the intriguing notion that E_2 is an initiating driver in CaP. Paradoxically, the use of anti-oestrogens might be an overlooked treatment or chemoprevention strategy in CaP. To understand prostate carcinogenesis, it is important to analyse the evidence regarding the role of androgens and oestrogens in normal prostatic morphogenesis and development, and their role in cancer. This review will focus primarily on whether there is evidence that oestrogens play a role in CaP and if this lends support for a different chemopreventive strategy for this disease.

2. Incidence of CaP

CaP is the most commonly diagnosed non-cutaneous cancer in men in developed countries. Worldwide there were 679,000 new cases in 2002 making it the 2nd most common male cancer. There is approximately an 80-fold difference in incidence and a 16-fold difference in mortality demographically between highest-incidence (e.g. US) and lowest-incidence regions (e.g. China).[2] However, with increasing affluence and westernisation there appears to be an elevation in CaP incidence and mortality in traditionally low-incident Asian countries.[3,4] Despite much research, the aetiology of CaP remains obscure. The only known risk factors, all un-modifiable, for this disease are age, family history and ethnicity. Age is possibly the strongest risk factor and 75% of CaP occurs in men over 65 years.[2] The world population of men in this age bracket is projected to quadruple by 2050; based on current estimates, this will result in a significant rise in the burden of this disease on society.[5]

A positive family history of CaP is an independent risk factor, and various studies have pointed to predisposing heritable factors.[6,7] The Nordic twin study comparing concordance rates of different cancers in monozygotic and dizygotic twins suggested that up to 42% of CaP risk can be explained by heritable factors.[8] Despite this suggested heritable component, linkage analysis and genetic polymorphism studies have mostly failed to demonstrate compelling gene-associated risks.[7] Recently, genome-wide association studies and admixture mapping have implicated an 8q24 variant for the increased risk and incidence of CaP seen in younger African-American men as compared to those with American-European ancestry.[9,10] Recall bias for family members with the disease, shared environment, life-style, mere chance due to the high prevalence of latent disease and widespread PSA testing can confound inheritance studies of CaP as these may result in familial clustering effects.[11–13]

Although there are marked differences in the levels of clinically detected CaP amongst different ethnicities and geographical regions of the world, the prevalence of latent disease may be more uniform. In fact, recent studies suggest that there might be a lower prevalence of latent disease in countries where screening with prostate specific antigen (PSA) is widespread.[14,15] What driver makes latent CaP manifest itself clinically in some populations and not in others? Environmental factors and/or hormonal effects may have a role in this.

3. Prostate development

Normal prostate organogenesis and development occur under hormonal control. In the foetus, circulating androgens produced by the foetal testis interact with the urogenital mesenchymal androgen receptor (AR) to induce epithelial-cell differentiation and prostate bud growth.[16–18] Testosterone can exert its action directly through the AR or effects may be mediated following its irreversible conversion by the enzyme 5α-reductase type II to the 10-fold more potent dihydrotestosterone (DHT). There is a profound reduction in prostatic morphogenesis and development in humans and mice that lack 5α-reductase type II enzyme.[19,20] Epithelial-cell differentiation including ductal branching, canalisation and basal cell and luminal cell formation is regulated by paracrine influences from the urogenital mesenchyme.[18] Conversely, the presence of prostate epithelium plays a crucial role in the differentiation of mesenchymal cells into periductal smooth muscle cells.[21] During adult life, androgens act upon the stromal ARs to maintain a fully differentiated, growth-quiescent gland while epithelial-cell ARs maintain the secretion functions in the mature prostate.[18]

4. Androgens and CaP

Androgens are required for the normal growth and differentiation of the prostate. Though there is evidence implicating androgens in prostatic carcinogenesis,[22,23] the epidemiological data confirming this are sparse (Table 1). A meta-analysis of 10 prospective studies by Eaton and colleagues[24] found no differences in pre-diagnostic serum levels of endogenous androgens and their metabolites in men who subsequently went on to develop CaP compared to those who remained disease-free.[24] The serum level of androstanediol glucuronide (3α-diol-g), a downstream product of DHT metabolism, was found to be 5% higher in CaP cases suggesting higher intraprostatic androgenic activity. More recent studies have also failed to show any positive association between circulating androgens and CaP (Table 2).[25–35] There have also been suggestions that higher androgen serum levels may be protective against aggressive forms of CaP.[26]

There is only a weak correlation between serum androgen levels and tissue concentrations.[28,36–38] Additionally, testosterone is the major circulating androgen whereas it is mainly DHT that is found in tissues. The enzyme aromatase is also expressed in the prostate and this may convert testosterone to E_2.[39–42] Intraprostatic hormone levels may more directly influence growth and carcinogenesis; hence, measuring tissue concentrations of steroid hormones may be a more sensitive indicator of risk. Such studies that examined intraprostatic hormone levels found higher androgen (testosterone, DHT and 3α-diol-g) levels in prostates of patients with CaP compared with controls.[28,43]

With the apparent ethnic differences in CaP incidence and mortality, several studies have investigated whether differences in androgen levels are responsible. For instance, lower testosterone levels were found in Japanese men compared

Table 1 – Population studies comparing the levels of steroid hormones

Study	Comparison	Hormones	Results
Henderson et al.[50]	Pregnant American black ($n = 20$) and white ($n = 20$) women	T	48% higher in blacks[a]
		E_2 (total, free, %free)	Total E_2 – 37% higher in blacks[a]
Ross et al.[51]	American black ($n = 50$) and white ($n = 50$) college students	T (total, free, %free)	Free T and mean total T higher in blacks[a]
		E_1	Higher in blacks[a]
		E_2, SHBG	No significant difference
Ross et al.[52]	Japanese (n = 54), American white ($n = 50$) and black ($n = 50$) students	T	No significant difference
		3α-diol-g	Higher levels in whites and blacks[a]
Gapstur et al.[53]	Young American black ($n = 483$) and white ($n = 695$) men	T (total, free), SHBG	No significant difference. Age related decrease in T and increase in SHBG seen from 3rd decade onwards
Litman et al.[46]	Black ($n = 538$), Hispanic ($n = 651$) and white ($n = 710$) men	T, SHBG	No significant difference
		DHT, DHT/T ratio	Significantly higher in blacks after adjustments
Rohrmann et al.[54]	Non-hispanic black ($n = 363$), white ($n = 674$) and Mexican-American ($n = 376$) men	T (total, free)	Higher in Mexican-Americans
		3α-diol-g	Higher in whites
		SHBG, E_2	Higher in blacks[a]

T – testosterone, E_1 – oestrone, E_2 – 17β-oestradiol, DHT – dihydrotestosterone, SHBG – sex hormone binding globulin, 3α-diol-g – androstanediol glucuronide.
a Statistically significant.

Table 2 – Case–control studies comparing the levels of circulating steroid hormones

Study	Comparison	Hormones	Results
Nomura et al.[29ac]	Japanese-Americans, cases ($n = 98$) and controls ($n = 98$)	T, DHT	Higher in controls[e]
		E_2	Higher in cases[e]
Barrett-Connor et al.[30ab]	American men, cases ($n = 57$) and controls ($n = 951$)	T	No significant difference
		Androstenedione	Significant risk[d]
		E_2	No significant difference
Nomura et al.[31ac]	Japanese-Americans, cases ($n = 141$) and controls ($n = 141$)	T, DHT, Androstenedione	No significant difference
Gann et al.[32ac]	American white men, cases ($n = 222$) and controls ($n = 390$)	T	Increasing risk with higher levels[d]
		DHT	No association
		3α-diol-g	Weak association with risk[e]
		E_2	Inverse (non-linear) association[d]
Vatten et al.[33ac]	Norwegian men, cases ($n = 59$) and controls ($n = 180$)	T, DHT, 3α-diol-g	No significant difference
Chen et al.[25c]	American men (94% Caucasians), cases ($n = 300$) and controls ($n = 300$)	T	No association with risk
		3α-diol-g	Weak association with risk[e]
		E_2	Inverse risk with higher levels[d]
Stattin et al.[34c]	Nordic men, cases ($n = 708$) and controls ($n = 2242$)	T	Inverse risk with higher levels[d]
		Free T, SHBG	Decreased risk[e]
Platz et al.[35c]	US men, cases (n = 460) and controls (n = 460)	T	No overall difference (positive association with low grade[d]; inverse association with high grade[d])
		DHT, SHBG, E_2	No significant difference
Severi et al.[26c]	Australian men, cases ($n = 524$) and controls ($n = 1859$)	T, Androstenodione SHBG 3α-diol-g, E_2	No significant difference. Inverse association with aggressive CaP. [e]
Wiren et al.[27]	Swedish men, cases ($n = 392$) and controls ($n = 392$)	T, 3α-diol-g, SHBG	No significant difference
Heracek et al.[28]	Czech men ($n = 178$; BPH – 57, CaP – 121)	T, Free T, DHT, SHBG	No significant difference

T – testosterone, E_1 – oestrone, E_2 - 17β-oestradiol, DHT – dihydrotestosterone, SHBG – sex hormone binding globulin, 3α-diol-g - androstanediol glucuronide, BPH – benign prostatic hyperplasia, CaP – prostate cancer.
a Reviewed by Eaton et al.[24].
b Prospective cohort study.
c Nested case–control study.
d Statistically significant.
e Not statistically significant.

to a Dutch cohort.[44] When serum levels of testosterone, DHT and sex hormone binding globulin (SHBG), and DHT/testosterone ratios were compared in Oriental-Americans, whites and African-Americans, significantly higher testosterone levels but significantly lower DHT/testosterone ratios were found in Oriental-Americans.[45] Similar findings were reported in a

more recent study comparing serum androgens in Black, Hispanic and Caucasian cohorts; serum testosterone levels were similar between the groups, but black males showed a higher DHT and DHT/testosterone ratio (Table 1).[46]

CaP has a long natural history with the vast majority of the disease remaining latent in one's lifetime. Autopsy studies have shown a high prevalence of high-grade prostatic intraepithelial neoplasia (HGPIN) and latent CaP in the 3rd to 4th decades of life.[47,48] A single measure taken at the 5th or 6th decade or later may not reflect the overall lifetime exposure to androgens; furthermore, one might speculate that such measurements at this point in life may not allow for relevant extrapolation to hormonal carcinogenesis.[49] In utero exposure of African-American males to higher maternal E_2 and testosterone levels has also been a speculated cause for higher CaP risk later in life.[50] One study found higher concentrations of total testosterone in young black men compared to a matched Caucasian cohort;[51] however, subsequent studies have failed to show such differences (Table 1).[52–54]

5. 5α-Reductase and CaP

5α-Reductase converts testosterone to DHT and exists in 2 forms; type I, which is found in skin and liver, and type II, which is predominantly located in the prostate and genital skin.[55] 5α-Reductase type II enzyme is encoded by *SRD5A2* located at chromosome 2p23.[56] Just as DHT is important for prostate growth and development, it may play a role in CaP. Male pseudohermaphrodites with a 46XY genotype have a congenital deficiency of the 5α-reductase type II enzyme. They possess a small, rudimentary prostate and do not develop benign prostatic hyperplasia (BPH) or CaP.[57] It has been suggested that there is an increased 5α-reductase activity in the prostates of higher-risk Caucasian as compared to low-risk Asian populations;[52,58] however, similar levels of prostatic 5α-reductase activity between Caucasian and Chinese cohorts have also been reported.[59]

5α-Reductase inhibitors seem to be attractive candidates for CaP prevention. Finasteride is a competitive 5α-reductase type II inhibitor that is used in the treatment of BPH. Its administration in men results in rapid reduction in DHT levels, reduction in prostatic volumes and marked involution of prostatic epithelium.[60,61] The Prostate Cancer Prevention Trial (PCPT) established a large-scale phase III, double-blind, placebo-controlled randomized trial looking into the efficacy of finasteride in reducing the prevalence of CaP over a 7 year period. During the trial period, there was a 25% risk reduction in prevalence in the finasteride group compared to placebo; however, there was also a 1.3% rise in the cases of high-grade CaP in the group receiving the drug.[62] As finasteride usage decreased prostate volume by 24%, it was suggested that an increased proportion of tissue was biopsied in users and thus, the actual risk reduction may be even greater than that observed. [63] Regarding the increased detection of high-grade CaP in the finasteride arm, it has been argued that androgen deprivation due to usage may result in changes mimicking high-grade disease.[62] It has also been suggested that it may be a consequence of PSA under-correction resulting in higher PSA velocity at biopsy in men on finasteride.[64] Criticisms regarding the findings of the PCPT study have been that as it looked at the period prevalence of CaP by both for-cause and end-of-study biopsies, the risk reduction is an overestimation and the actual reduction may actually be 10% when only for-cause biopsies are considered.[65] Reduced DHT environment in the prostate of patients on finasteride may induce high-grade CaP or may preferentially promote its growth as these tumours may be androgen insensitive.[62] In patients treated with finasteride, tissue levels of testosterone may rise 10-fold and this in itself may be carcinogenic;[65] another view is that increased E_2 levels are formed following testosterone aromatisation and this may promote the development of high-grade CaP.[66] Decreased DHT levels in Japanese men with high-grade CaP have recently been reported.[67]

6. Role of oestrogen in CaP

Traditionally, oestrogen has been considered protective against CaP and it has been used for the treatment of advanced disease.[68] Its protective activity is primarily associated with a negative feedback on the hypothalamo–pituitory–gonadal axis leading to a state of chemical castration. Most studies investigating the role of hormones in CaP have examined serum E_2 levels, which may not reflect intraprostatic concentrations. Through the catalytic activity of aromatase, E_2 is produced from testosterone locally in the prostate.[39–42,69] The earliest evidence of oestrogens acting as pro-carcinogens in the prostate comes from a study in Noble (Nb) rats where it was shown that CaP developed more rapidly when oestrogen was administered in addition to testosterone.[22] Significant dysplasia (and one case of adenocarcinoma) in the dorso-lateral region of the prostate of Nb rats occurred when they were treated with testosterone and E_2; prolonged treatment with DHT only resulted in prostatic enlargement.[70] Aromatase-knockout mice cannot produce E_2 locally in the prostate, but show elevated levels of testosterone and DHT; with age, they are prone to developing BPH but do not develop CaP.[71] Such studies suggest that there may be a complex interaction between oestrogen and testosterone in the development of prostate carcinogenesis.

Prenatal and/or neonatal exposures to low levels of oestrogenic agents in rodent models result in the induction of significant effects in normal prostatic morphogenesis and development. Such effects are described as neonatal oestrogenisation or imprinting and seem to increase the sensitivity of the subsequent adult prostate to testosterone, up-regulates the oestrogen receptor (ER) in the prostate and enhances inflammation, epithelial hyperplasia and dysplasia.[72–74] Currently, there is no direct evidence to support such neonatal imprinting in humans. In utero exposure to higher levels of oestrogen has been suggested as a causative factor for the higher incidence of CaP in blacks compared to Caucasians (Table 1).[50] Prior to 1971 in the US and until the 1980s in some European countries, diethylstilbestrol (DES) was administered to pregnant mothers leading to elevated foetal oestrogen exposure. Although no study to date has shown any positive risk of CaP in male offspring pre-natally exposed to DES, these men may only now be entering the susceptible age for this disease.[75–77]

7. ER and CaP

Oestrogens mediate their effects through nuclear ER, and two receptor subtypes have been isolated in the prostate; ERα is mainly expressed in the stromal compartment and ERβ is expressed in the epithelium.[78–80] These isoforms are distinct products of two different genes; ERα is encoded by chromosome 6q25.1[81] and ERβ is encoded by chromosome 14q22-24.[82]

E_2 controls both ERα and ERβ expressions in the prostate. ERα appears to play a more important role in prostate growth and carcinogenesis. Studies in ERα-knockout mice suggest that they are only prone to develop BPH and not HGPIN or CaP, as opposed to wild-type mice treated with testosterone and E_2 for 4 months.[83] Neonatal imprinting may be mediated by ERα.[84] ERβ seems to inhibit ERα activation and may promote differentiation and cellular homeostasis of the prostatic epithelium.[85–87] ERβ may be under androgenic regulation in the prostate as suggested by its down regulation in castrated rats, which is reversed by subsequent testosterone administration.[88] In light of the age-associated declines in testosterone levels in humans (see later), this may be a possible modulating factor in the regulatory role of ERβ in ageing men and may have important implications.

ERβ is highly expressed in normal human prostate; however, there seems to be some loss of expression of this receptor in BPH and up to 3/4 of CaP cases do not show ERβ expression.[89] This loss of ERβ expression in CaP implies a regulatory role in normal prostatic growth. Paradoxically, ERβ exhibits elevated expression in metastatic CaP.[80] Cancers that are ERβ-positive are also associated with a higher relapse rate.[89] ERβ may be protective against abnormal epithelial-cell proliferation and carcinogenesis, and a reduction in expression may be required in oestrogen-dependent CaP progression.[90]

8. E_2-mediated genotoxicity and CaP

Oestrogens or endocrine-active agents may also act as genotoxic agents; it may be that both receptor-mediated hormonal effects and genotoxic activity through DNA-adduct formation and the induction of single-strand breaks (SSBs) may contribute to carcinogenesis.[91–93] CYP enzymes may play an important role in hormone genotoxicity, particularly CYP1B1 that catalyses the conversion of E_2 to catechol oestrogens.[94] CYP1B1 is a phase I metabolising enzyme that is involved in the monooxygenation of a variety of endogenous and exogenous compounds including steroids and xenobiotics. It is a constitutively expressed, inducible enzyme that is present in a variety of extra-hepatic tissues that are hormone responsive. In benign human prostate, its expression has been found to be higher in the cancer-susceptible peripheral zone compared to the transition zone.[1,95] This has led to suggestions that anti-oestrogen activity may be a novel chemotherapeutic approach,[96] something that has recently been lent support by findings that there is a strong association between polymorphisms in this gene and CaP risk.[97] Such genetic variants may influence DNA-adduct formation and tumour-cell differentiation whilst playing a role in oestrogen-mediated mechanisms in CaP.[98–100]

Amongst the CYPs, CYP1B1 is the most efficient oestrogen hydroxylase involved in the extra-hepatic hydroxylation of E_2 to 4-OH E_2.[101] The 4-OH E_2 metabolite may be inactivated by catechol-O-methyltransferase (COMT) or may undergo redox cycling to reactive quinines or semiquinones that may give rise to DNA adducts or SSBs.[91,92] *CYP1B1* expression may be ERα -regulated by E_2 although it is also inducible by a variety of environmental and/or dietary (pro-)carcinogens acting as exogenous ligands.[102,103] Differential *CYP1B1* expression may modulate both exogenous and endogenous (pro-)carcinogen metabolism, and consequently the susceptibility of target prostate epithelial cells to DNA-damaging mechanisms.[104,105]

9. Ageing, environmental oestrogenisation and obesity

Circulating serum testosterone levels decline gradually with increasing age; this decline is more marked for free and bio-available testosterone. Mean serum testosterone levels at 75 years of age are about 66% of that of a 25-year-old, while free and bio-available testosterone is only 50% of that of a younger man.[106] Although there is also a moderate decline in the levels of free E_2, overall there appears to be an increase in E_2/testosterone ratio with increasing age. Also, with ageing there is an increase in intra-prostatic E_2 and E_2/androgen ratio, particularly in prostatic stroma of BPH or normal prostate.[107] Declining androgenic influences with increasing age in the face of persistent oestrogenic stimulation may enhance CaP growth.

Migration studies suggest that environment plays an important role in CaP development.[108] Migrants from a low-incident country to a high-incident country acquire a similar level of CaP incidence as the host population.[109] There is some evidence of increased oestrogenising environmental effects,[110–112] and this may be one of the contributing factors for the elevated prevalence of clinically significant CaP in the Western world. Also, the global burden of obesity is rising.[113] In an obese state, there is increased aromatase activity resulting in higher peripheral conversion of testosterone to E_2. This may also give rise to a higher incidence of CaP in the developed world and the rising incidence in newly industrialized countries. Though the endocrine changes associated with obesity provide a plausible scientific mechanism, epidemiological data linking CaP to obesity are conflicting.[114–116]

10. Phytoestrogens and SERMs

Phytoestrogens are weak oestrogenic compounds primarily found in soy products but, also, in fruits and vegetables. They may be protective against CaP through a number of effects: (1) by competing for the same ER, they may prevent the action of more potent oestrogens,[117] (2) by inhibiting aromatase, they may thus decrease local tissue E_2 concentrations,[118] (3) by inhibiting 5α-reductase,[119] and (4) by inhibiting tyrosine kinases,[120] which may also play a role in CaP.[121]

Like phytoestrogens, selective oestrogen receptor modulators (SERMs) have weak oestrogenic activity. Depending upon the profile of co-regulatory proteins present in a particular tissue, they may act as ER agonists or antagonists. Also, different

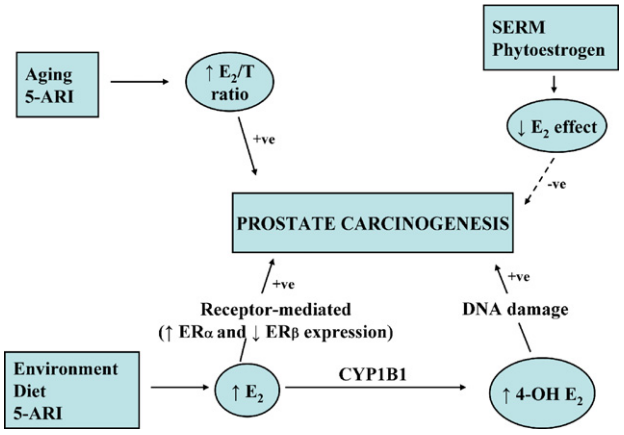

Fig. 1 – A central role for oestrogenic mechanisms in prostate carcinogenesis. The dashed arrow represents a possible negative effect (–ve) on CaP development; other arrows are indicated to represent mechanisms inducing a positive effect (+ve). *Abbreviations:* 5-ARI, 5α-reductase inhibitor; E_2, 17β -oestradiol; SERM, selective oestrogen receptor modulator; T, testosterone.

SERMs preferentially interact in an agonist or antagonist fashion with a particular ER subtype.[122] In the past, the 1st-generation SERM tamoxifen has been used in the treatment of advanced CaP with mixed results.[123,124] However, as epidemiological and experimental data increasingly point towards a role for oestrogen in prostate carcinogenesis, the use of SERMs may be a promising CaP-chemoprevention strategy. SERMs do not alter androgen levels and thus have no sexual side-effects. Tamoxifen may be capable of DNA-adduct formation.[125,126] Newer SERMs such as toremifene may exhibit less or no genotoxicity,[127,128] although others have suggested the contrary.[129] Also, long-term data on toremifene are lacking although it was noted to prevent CaP in the TRAMP mouse *via* ER-signalling pathways.[130] It has also been observed to reduce progression to CaP in patients with HGPIN.[131] Development of newer SERMs with prostate specific action may play a vital role in CaP chemoprevention. Unlike 5α-reductase inhibitors, these may be better accepted due to fewer sexual side-effects. Also, by selectively inhibiting E_2 activity at the target organ they may also permit the systemically beneficial actions of E_2 such as the maintenance of cognitive function, bone mineralisation and prevention of cerebro-vascular and cardiac events.[96,132]

11. Conclusions

There is a plausible argument that oestrogenic mechanisms play a central role in prostate carcinogenesis (Fig. 1). Factors such as increasing age modify oestrogenic status in human males resulting in a reduction in the bio-available testosterone whilst increasing the E_2/testosterone ratio. Coupled with the facts that altering the E_2/testosterone ratio is a mechanism of inducing rodent prostate carcinogenesis and that there is an altered ER profile in human prostate carcinogenesis, there is a compelling evidence that paradoxically oestrogen may be an initiating driver in CaP progression. Whether such effects might be *via* receptor-mediated mechanisms and/or non-receptor-mediated mechanisms (e.g. DNA damaging) in the presence or in the absence of other (pro-)carcinogens remains to be ascertained. In the ageing population of the developed world, there appears to be an increasingly relevant endocrine influence in disease susceptibility. The potential role of SERMs or other oestrogen-intervention strategies is an important area of future investigation in CaP chemoprevention.

Conflict of interest statement

None declared.

Acknowledgement

Research in F.L.M.'s laboratory into the mechanisms of prostate carcinogenesis is funded by Rosemere Cancer Foundation.

REFERENCES

1. Ragavan N, Hewitt R, Cooper LJ, et al. *CYP1B1* expression in prostate is higher in the peripheral than in the transition zone. *Cancer Lett* 2004;**215**:69–78.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005;**55**:74–108.
3. Hsing AW, Devesa SS. Trends and patterns of prostate cancer: what do they suggest? *Epidemiol Rev* 2001;**23**:3–13.
4. Sim HG, Cheng CW. Changing demography of prostate cancer in Asia. *Eur J Cancer* 2005;**41**:834–45.
5. Lunenfeld B. The ageing male: demographics and challenges. *World J Urol* 2002;**20**:11–6.
6. Lesko SM, Rosenberg L, Shapiro S. Family history and prostate cancer risk. *Am J Epidemiol* 1996;**144**:1041–7.
7. Schaid DJ. The complex genetic epidemiology of prostate cancer. *Hum Mol Genet* 2004;**13**(1):R103–21.
8. Lichtenstein P, Holm NV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med* 2000;**343**:78–85.
9. Amundadottir LT, Sulem P, Gudmundsson J, et al. A common variant associated with prostate cancer in European and African populations. *Nat Genet* 2006;**38**:652–8.
10. Freedman ML, Haiman CA, Patterson N, et al. Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. *Proc Natl Acad Sci USA* 2006;**103**:14068–73.
11. Ostrander EA, Stanford JL. Genetics of prostate cancer: too many loci, too few genes. *Am J Hum Genet* 2000;**67**:1367–75.
12. Grönberg H. Prostate cancer epidemiology. *Lancet* 2003;**361**:859–64.
13. Ragavan N, Grover PL, Balasubramanian SP, et al. An observational study of cancers among female partners of UK-resident prostate cancer patients. *Cancer Lett* 2006;**242**:88–94.
14. Konety BR, Bird VY, Deorah S, Dahmoush L. Comparison of the incidence of latent prostate cancer detected at autopsy before and after the prostate specific antigen era. *J Urol* 2005;**174**:1785–8.
15. Delongchamps NB, Singh A, Haas GP. The role of prevalence in the diagnosis of prostate cancer. *Cancer Control* 2006;**13**:158–68.

16. Takeda H, Nakamoto T, Kokontis J, Chodak GW, Chang C. Autoregulation of androgen receptor expression in rodent prostate: immunohistochemical and in situ hybridization analysis. *Biochem Biophys Res Commun* 1991;**177**:488–96.
17. Cooke PS, Young P, Cunha GR. Androgen receptor expression in developing male reproductive organs. *Endocrinology* 1991;**128**:2867–73.
18. Cunha GR, Ricke W, Thomson A, et al. Hormonal, cellular, and molecular regulation of normal and neoplastic prostatic development. *J Steroid Biochem Mol Biol* 2004;**92**:221–36.
19. Anderson S, Berman DM, Jenkins EP, Russell DW. Deletion of steroid 5α-reductase 2 gene in male pseudohermaphroditism. *Nature* 1991;**354**:159–61.
20. Deslypere JP, Young M, Wilson JD, McPhaul MJ. Testosterone and 5α-dihydrotestosterone interact differently with the androgen receptor to enhance transcription of the MMTV-CAT reporter gene. *Mol Cell Endocrinol* 1992;**88**:15–22.
21. Hayward SW, Haughney PC, Rosen MA, et al. Interactions between adult human prostatic epithelium and rat urogenital sinus mesenchyme in a tissue recombination model. *Differentiation* 1998;**63**:131–40.
22. Noble RL. The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration. *Cancer Res* 1977;**37**:1929–33.
23. Henderson BE, Ross RK, Pike MC, Casagrande JT. Endogenous hormones as a major factor in human cancer. *Cancer Res* 1982;**42**:3232–9.
24. Eaton NE, Reeves GK, Appleby PN, Key TJ. Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. *Br J Cancer* 1999;**80**:930–4.
25. Chen C, Weiss NS, Stanczyk FZ, et al. Endogenous sex hormones and prostate cancer risk: a case–control study nested within the carotene and retinol efficacy trial. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:1410–6.
26. Severi G, Morris HA, MacInnis RJ, et al. Circulating steroid hormones and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:86–91.
27. Wiren S, Stocks T, Rinaldi S, et al. Androgens and prostate cancer risk: a prospective study. *Prostate* 2007;**67**:1230–7.
28. Heracek J, Richard H, Martin H, et al. Tissue and serum levels of principal androgens in benign prostatic hyperplasia and prostate cancer. *Steroids* 2007;**72**:375–80.
29. Nomura A, Heilbrun LK, Stemmermann GN, Judd HL. Prediagnostic serum hormones and the risk of prostate cancer. *Cancer Res* 1988;**48**:3515–7.
30. Barrett-Connor E, Garland C, McPhillips JB, Khaw KT, Wingard DL. A prospective, population-based study of androstenedione, estrogens, and prostatic cancer. *Cancer Res* 1990;**50**:169–73.
31. Nomura AM, Stemmermann GN, Chyou PH, Henderson BE, Stanczyk FZ. Serum androgens and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 1996;**5**:621–5.
32. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. *J Natl Cancer Inst* 1996;**88**:1118–26.
33. Vatten LJ, Ursin G, Ross RK, et al. Androgens in serum and the risk of prostate cancer: a nested case–control study from the Janus serum bank in Norway. *Cancer Epidemiol Biomarkers Prev* 1997;**6**:967–9.
34. Stattin P, Lumme S, Tenkanen L, et al. High levels of circulating testosterone are not associated with increased prostate cancer risk: a pooled prospective study. *Int J Cancer* 2004;**108**:418–24.
35. Platz EA, Leitzmann MF, Rifai N, et al. Sex steroid hormones and the androgen receptor gene CAG repeat and subsequent risk of prostate cancer in the prostate-specific antigen era. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:1262–9.
36. Montie JE, Pienta KJ. Review of the role of androgenic hormones in the epidemiology of benign prostatic hyperplasia and prostate cancer. *Urology* 1994;**43**:892–9.
37. Hsing AW. Hormones and prostate cancer: where do we go from here? *J Natl Cancer Inst* 1996;**88**:1093–5.
38. Page ST, Lin DW, Mostaghel EA, et al. Persistent intraprostatic androgen concentrations after medical castration in healthy men. *J Clin Endocrinol Metab* 2006;**91**:3850–6.
39. Stone NN, Fair WR, Fishman J. Estrogen formation in human prostatic tissue from patients with and without benign prostatic hyperplasia. *Prostate* 1986;**9**:311–8.
40. Matzkin H, Soloway MS. Immunohistochemical evidence of the existence and localization of aromatase in human prostatic tissues. *Prostate* 1992;**21**:309–14.
41. Ellem SJ, Schmitt JF, Pedersen JS, Frydenberg M, Risbridger GP. Local aromatase expression in human prostate is altered in malignancy. *J Clin Endocrinol Metab* 2004;**89**:2434–41.
42. Takase Y, Lévesque MH, Luu-The V, El-Alfy M, Labrie F, Pelletier G. Expression of enzymes involved in estrogen metabolism in human prostate. *J Histochem Cytochem* 2006;**54**:911–21.
43. Hammond GL. Endogenous steroid levels in the human prostate from birth to old age: a comparison of normal and diseased tissues. *J Endocrinol* 1978;**78**:7–19.
44. de Jong FH, Oishi K, Hayes RB, et al. Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese case–control study. *Cancer Res* 1991;**51**:3445–50.
45. Wu AH, Whittemore AS, Kolonel LN, et al. Serum androgens and sex hormone-binding globulins in relation to lifestyle factors in older African-American, white, and Asian men in the United States and Canada. *Cancer Epidemiol Biomarkers Prev* 1995;**4**:735–41.
46. Litman HJ, Bhasin S, Link CL, Araujo AB, McKinlay JB. Serum androgen levels in black, Hispanic, and white men. *J Clin Endocrinol Metab* 2006;**91**:4326–34.
47. Yatani R, Shiraishi T, Nakakuki K, et al. Trends in frequency of latent prostate carcinoma in Japan from 1965–1979 to 1982–1986. *J Natl Cancer Inst* 1988;**80**:683–7.
48. Sakr WA, Grignon DJ, Crissman JD, et al. High grade prostatic intraepithelial neoplasia (HGPIN) and prostatic adenocarcinoma between the ages of 20–69: an autopsy study of 249 cases. *In Vivo* 1994;**8**:439–43.
49. Hsing AW. Hormones and prostate cancer: what's next? *Epidemiol Rev* 2001;**23**:42–58.
50. Henderson BE, Bernstein L, Ross RK, Depue RH, Judd HL. The early in utero oestrogen and testosterone environment of blacks and whites: potential effects on male offspring. *Br J Cancer* 1988;**57**:216–8.
51. Ross R, Bernstein L, Judd H, Hanisch R, Pike M, Henderson B. Serum testosterone levels in healthy young black and white men. *J Natl Cancer Inst* 1986;**76**:45–8.
52. Ross RK, Bernstein L, Lobo RA, et al. 5α-reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet* 1992;**339**:887–9.
53. Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K. Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity, and race. The CARDIA male hormone study. *Cancer Epidemiol Biomarkers Prev* 2002;**11**:1041–7.
54. Rohrmann S, Nelson WG, Rifai N, et al. Serum estrogen, but not testosterone, levels differ between black and white men in a nationally representative sample of Americans. *J Clin Endocrinol Metab* 2007;**92**:2519–25.
55. Thigpen AE, Silver RI, Guileyardo JM, Casey ML, McConnell DW, Russell DW. Tissue distribution and ontogeny of steroid

5α-reductase isozyme expression. *J Clin Invest* 1993;**92**:903–10.
56. Russell DW, Wilson JD. Steroid 5α-reductase: two genes/two enzymes. *Annu Rev Biochem* 1994;**63**:25–61.
57. Wilson JD, Griffin JE, Russell DW. Steroid 5α-reductase 2 deficiency. *Endocr Rev* 1993;**14**:577–93.
58. Lookingbill DP, Demers LM, Wang C, Leung A, Rittmaster RS, Santen RJ. Clinical and biochemical parameters of androgen action in normal healthy Caucasian versus Chinese subjects. *J Clin Endocrinol Metab* 1991;**72**:1242–8.
59. Santner SJ, Albertson B, Zhang GY, et al. Comparative rates of androgen production and metabolism in Caucasian and Chinese subjects. *J Clin Endocrinol Metab* 1998;**83**:2104–9.
60. Gormley GJ, Stoner E, Bruskewitz RC, et al. The effect of finasteride in men with benign prostatic hyperplasia. The Finasteride Study Group. *N Engl J Med* 1992;**327**:1185–91.
61. Marks LS, Partin AW, Dorey FJ, et al. Long-term effects of finasteride on prostate tissue composition. *Urology* 1999;**53**:574–80.
62. Thompson IM, Goodman PJ, Tangen CM, et al. The influence of finasteride on the development of prostate cancer. *N Engl J Med* 2003;**349**:215–24.
63. Klein EA, Tangen CM, Goodman PJ, Lippman SM, Thompson IM. Assessing benefit and risk in the prevention of prostate cancer: the prostate cancer prevention trial revisited. *J Clin Oncol* 2005;**23**:7460–6.
64. D'Amico AV, Barry MJ. Prostate cancer prevention and finasteride. *J Urol* 2006;**176**:2010–2 [discussion 2012–2013].
65. Walsh PC. Editorial comment. *J Urol* 2004;**171**:506–7.
66. Pitts Jr WR. Validation of the Pitts unified theory of prostate cancer, late-onset hypogonadism and carcinoma: the role of steroid 5α-reductase and steroid aromatase. *BJU Int* 2007;**100**:254–7.
67. Nishiyama T, Ikarashi T, Hashimoto Y, Suzuki K, Takahashi K. Association between the dihydrotestosterone level in the prostate and prostate cancer aggressiveness using the Gleason score. *J Urol* 2006;**176**:1387–91.
68. Huggins C, Hodges CV. Studies on prostatic cancer: I. The effect of castration, of estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *CA Cancer J Clin* 1972;**22**:232–40.
69. Risbridger GP, Bianco JJ, Ellem SJ, McPherson SJ. Oestrogens and prostate cancer. *Endocr Relat Cancer* 2003;**10**:187–91.
70. Leav I, Ho SM, Ofner P, Merk FB, Kwan PW, Damassa D. Biochemical alterations in sex hormone-induced hyperplasia and dysplasia of the dorsolateral prostates of Noble rats. *J Natl Cancer Inst* 1988;**80**:1045–53.
71. McPherson SJ, Wang H, Jones ME, et al. Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland. *Endocrinology* 2001;**142**:2458–67.
72. Rajfer J, Coffey DS. Sex steroid imprinting of the immature prostate. Long-term effects. *Invest Urol* 1978;**16**:186–90.
73. Prins GS, Birch L. Neonatal estrogen exposure up-regulates estrogen receptor expression in the developing and adult rat prostate lobes. *Endocrinology* 1997;**138**:1801–9.
74. Chang WY, Wilson MJ, Birch L, Prins GS. Neonatal estrogen stimulates proliferation of periductal fibroblasts and alters the extracellular matrix composition in the rat prostate. *Endocrinology* 1999;**140**:405–15.
75. Strohsnitter WC, Noller KL, Hoover RN, et al. Cancer risk in men exposed in utero to diethylstilbestrol. *J Natl Cancer Inst* 2001;**93**:545–51.
76. Schrager S, Potter BE. Diethylstilbestrol exposure. *Am Fam Physician* 2004;**69**:2395–400.
77. Harkonen PL, Makela SI. Role of estrogens in development of prostate cancer. *J Steroid Biochem Mol Biol* 2004;**92**:297–305.
78. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996;**93**:5925–30.
79. Lau KM, Leav I, Ho SM. Rat estrogen receptor-α and -β, and progesterone receptor mRNA expression in various prostatic lobes and microdissected normal and dysplastic epithelial tissues of the Noble rats. *Endocrinology* 1998;**139**:424–7.
80. Leav I, Lau KM, Adams JY, et al. Comparative studies of the estrogen receptors β and α and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am J Pathol* 2001;**159**:79–92.
81. Menasce LP, White GR, Harrison CJ, Boyle JM. Localization of the estrogen receptor locus (ESR) to chromosome 6q25.1 by FISH and a simple post-FISH banding technique. *Genomics* 1993;**17**:263–5.
82. Enmark E, Pelto-Huikko M, Grandien K, et al. Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab* 1997;**82**:4258–65.
83. Cunha GR, Wang YZ, Hayward SW, Risbridger GP. Estrogenic effects on prostatic differentiation and carcinogenesis. *Reprod Fertil Dev* 2001;**13**:285–96.
84. Prins GS, Birch L, Couse JF, Choi I, Katzenellenbogen B, Korach KS. Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor α: studies with αERKO and βERKO mice. *Cancer Res* 2001;**61**:6089–97.
85. Paech K, Webb P, Kuiper GG, et al. Differential ligand activation of estrogen receptors ERα and ERβ at AP1 sites. *Science* 1997;**277**:1508–10.
86. Maruyama S, Fujimoto N, Asano K, Ito A. Suppression by estrogen receptor β of AP-1 mediated transactivation through estrogen receptor α. *J Steroid Biochem Mol Biol* 2001;**78**:177–84.
87. Imamov O, Morani A, Shim GJ, et al. Estrogen receptor β regulates epithelial cellular differentiation in the mouse ventral prostate. *Proc Natl Acad Sci USA* 2004;**101**:9375–80.
88. Asano K, Maruyama S, Usui T, Fujimoto N. Regulation of estrogen receptor α and β expression by testosterone in the rat prostate gland. *Endocr J* 2003;**50**:281–7.
89. Horvath LG, Henshall SM, Lee CS, et al. Frequent loss of estrogen receptor-β expression in prostate cancer. *Cancer Res* 2001;**61**:5331–5.
90. Carruba G. Estrogens and mechanisms of prostate cancer progression. *Ann N Y Acad Sci* 2006;**1089**:201–17.
91. Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents – DNA adducts and mutations. *J Natl Cancer Inst Monogr* 2000;**27**:75–93.
92. Yared E, McMillan TJ, Martin FL. Genotoxic effects of oestrogens in breast cells detected by the micronucleus assay and the Comet assay. *Mutagenesis* 2002;**17**:345–52.
93. Kalantzi OI, Hewitt R, Ford KJ, et al. Low dose induction of micronuclei by lindane. *Carcinogenesis* 2004;**25**:613–22.
94. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. *J Natl Cancer Inst Monogr* 2000;**27**:67–73.
95. Sissung TM, Price DK, Sparreboom A, Figg WD. Pharmacogenetics and regulation of human cytochrome P450 1B1: implications in hormone-mediated tumor metabolism and a novel target for therapeutic intervention. *Mol Cancer Res* 2006;**4**:135–50.
96. Singh MN, Stringfellow HF, Paraskevaidis E, Martin-Hirsch FL, Martin FL. Tamoxifen: important considerations of a multi-functional compound with organ-specific properties. *Cancer Treat Rev* 2007;**33**:91–100.
97. Cussenot O, Azzouzi AR, Nicolaiew N, et al. Combination of polymorphisms from genes related to estrogen metabolism and risk of prostate cancers: the hidden face of estrogens. *J Clin Oncol* 2007;**25**:3596–602.

98. Nock NL, Tang D, Rundle A, et al. Associations between smoking, polymorphisms in polycyclic aromatic hydrocarbon (PAH) metabolism and conjugation genes and PAH-DNA adducts in prostate tumors differ by race. *Cancer Epidemiol Biomarkers Prev* 2007;**16**:1236–45.
99. Tang D, Liu JJ, Bock CH, et al. Racial differences in clinical and pathological associations with PhIP-DNA adducts in prostate. *Int J Cancer* 2007;**121**:1319–24.
100. Nock NL, Cicek MS, Li L, et al. Polymorphisms in estrogen bioactivation, detoxification and oxidative DNA base excision repair genes and prostate cancer risk. *Carcinogenesis* 2006;**27**:1842–8.
101. Badawi AF, Cavalieri EL, Rogan EG. Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16α-hydroxylation of 17β-estradiol. *Metabolism* 2001;**50**:1001–3.
102. Tsuchiya Y, Nakajima M, Kyo S, Kanaya T, Inoue M, Yokoi T. Human *CYP1B1* is regulated by estradiol via estrogen receptor. *Cancer Res* 2004;**64**:3119–25.
103. Shimada T, Hayes CL, Yamazaki H, et al. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res* 1996;**56**:2979–84.
104. Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. *Carcinogenesis* 2000;**21**:1683–9.
105. Martin FL, Cole KJ, Muir GH, et al. Primary cultures of prostate cells and their ability to activate carcinogens. *Prostate Cancer Prostatic Dis* 2002;**5**:96–104.
106. Kaufman JM, Vermeulen A. The decline of androgen levels in elderly men and its clinical and therapeutic implications. *Endocr Rev* 2005;**26**:833–76.
107. Krieg M, Nass R, Tunn S. Effect of aging on endogenous level of 5α-dihydrotestosterone, testosterone, estradiol, and estrone in epithelium and stroma of normal and hyperplastic human prostate. *J Clin Endocrinol Metab* 1993;**77**:375–81.
108. Grover PL, Martin FL. The initiation of breast and prostate cancer. *Carcinogenesis* 2002;**23**:1095–102.
109. Muir CS, Nectoux J, Staszewski J. The epidemiology of prostatic cancer. Geographical distribution and time-trends. *Acta Oncol* 1991;**30**:133–40.
110. McLachlan JA. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. *Endocr Rev* 2001;**22**:319–41.
111. Davis C, Bhana S, Shorrocks AJ, Martin FL. Oestrogens induce G_1 arrest in benzo[*a*]pyrene-treated MCF-7 breast cells whilst enhancing genotoxicity and clonogenic survival. *Mutagenesis* 2002;**17**:431–8.
112. Hewitt R, Forero A, Luncsford PJ, et al. Enhanced micronucleus formation and modulation of Bcl-2:Bax in MCF-7 cells following exposure to binary mixtures. *Environ Health Perspect* 2007;**115**(Suppl. 1):129–36. doi:10.1289/ehp.9361.
[113]. WHO Global Strategy on Diet, physical activity and health. <http://www.who.int/dietphysicalactivity/media/en/gsfs_obesity.pdf>.
114. Engeland A, Tretli S, Bjorge T. Height, body mass index, and prostate cancer: a follow-up of 950000 Norwegian men. *Br J Cancer* 2003;**89**:1237–42.
115. Giovannucci E, Rimm EB, Liu Y, et al. Body mass index and risk of prostate cancer in U.S. health professionals. *J Natl Cancer Inst* 2003;**95**:1240–4.
116. Porter MP, Stanford JL. Obesity and the risk of prostate cancer. *Prostate* 2005;**62**:316–21.
117. Martin PM, Horwitz KB, Ryan DS, McGuire WL. Phytoestrogen interaction with estrogen receptors in human breast cancer cells. *Endocrinology* 1978;**103**:1860–7.
118. Adlercreutz H, Bannwart C, Wahala K, et al. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* 1993;**44**:147–53.
119. Evans BA, Griffiths K, Morton MS. Inhibition of 5 alpha-reductase in genital skin fibroblasts and prostate tissue by dietary lignans and isoflavonoids. *J Endocrinol* 1995;**147**:295–302.
120. Akiyama T, Ishida J, Nakagawa S, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987;**262**:5592–5.
121. Carey AM, Pramanik R, Nicholson LJ, et al. Ras-MEK-ERK signaling cascade regulates androgen receptor element-inducible gene transcription and DNA synthesis in prostate cancer cells. *Int J Cancer* 2007;**121**:520–7.
122. Riggs BL, Hartmann LC. Selective estrogen-receptor modulators – mechanisms of action and application to clinical practice. *N Engl J Med* 2003;**348**:618–29.
123. Glick JH, Wein A, Padavic K, Negendank W, Harris D, Brodovsky H. Tamoxifen in refractory metastatic carcinoma of the prostate. *Cancer Treat Rep* 1980;**64**:813–8.
124. Glick JH, Wein A, Padavic K, Negendank W, Harris D, Brodovsky H. Phase II trial of tamoxifen in metastatic carcinoma of the prostate. *Cancer* 1982;**49**:1367–72.
125. Han XL, Liehr JG. Induction of covalent DNA adducts in rodents by tamoxifen. *Cancer Res* 1992;**52**:1360–3.
126. Umemoto A, Monden Y, Lin CX, et al. Determination of tamoxifen-DNA adducts in leukocytes from breast cancer patients treated with tamoxifen. *Chem Res Toxicol* 2004;**17**:1577–83.
127. Kim SY, Suzuki N, Laxmi YR, Umemoto A, Matsuda T, Shibutani S. Antiestrogens and the formation of DNA damage in rats: a comparison. *Chem Res Toxicol* 2006;**19**:852–8.
128. Shibutani S, Ravindernath A, Terashima I, et al. Mechanism of lower genotoxicity of toremifene compared with tamoxifen. *Cancer Res* 2001;**61**:3925–31.
129. Liu X, Pisha E, Tonetti DA, et al. Antiestrogenic and DNA damaging effects induced by tamoxifen and toremifene metabolites. *Chem Res Toxicol* 2003;**16**:832–7.
130. Raghow S, Hooshdaran MZ, Katiyar S, Steiner MS. Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. *Cancer Res* 2002;**62**:1370–6.
131. Price D, Stein B, Sieber P, et al. Toremifene for the prevention of prostate cancer in men with high grade prostatic intraepithelial neoplasia: results of a double-blind, placebo controlled, phase IIB clinical trial. *J Urol* 2006;**176**:965–70 [discussion 970-1]..
132. Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest* 2006;**116**:561–70.