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A PERSPECTIVE ON THE ROLE OF ESTROGEN IN HORMONE-INDUCED PROSTATE CARCINOGENESIS

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Abstract

Androgens are thought to cause prostate cancer, but the precise mechanisms by which they do so are unclear. Data, mostly from animal studies, suggest that for androgens to cause prostate cancer they must be aromatized to estrogen and act in concert with these estrogen metabolites. Androgen-receptor mediated activity of androgens and estrogen receptor-mediated effects of estrogen metabolites are likely to be necessary, but estrogen genotoxicity appears to be a probable critical factor as well. Only when all these mechanisms are active, may prostate carcinogenesis result. Convincing proof-of-concept studies are needed to definitively test this concept which, if proven, may lead to clinically feasible chemoprevention approaches interfering with these mechanisms.

Keywords

Prostate cancer; Estrogens; Androgens; Hormonal Carcinogenesis

1. Introduction

Prostate cancer is the leading non-skin malignancy detected in US males and the second cause of death due to male cancer in the US [1]. The causes of this major male malignancy are not entirely clear, but the idea that androgenic hormones play a major causative role in prostate carcinogenesis has been around for decades [2]. The basis for this assumption is that the prostate gland is an androgen-dependent tissue and that prostate cancer is an androgen-dependent malignancy [2]. The underlying mechanism has been postulated to be androgenic stimulation of cell proliferation resulting in an increased risk of oncogenic genetic alterations [3]. However, the human and biological evidence for this is indirect and very limited at best. There is no evidence that androgens cause sustained cell proliferation in the prostate. This is illustrated in rats that are surgically castrated, which causes involution of the prostate gland by apoptosis and cessation of secretory activity, and after a couple of

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Conflict of Interest Statement
None

weeks are given androgen back at physiological levels; this treatment causes a few waves of cell proliferation in the prostate, but after about four days, cell proliferation returns to levels found in intact control rats [4]. The further growth of the prostate upon continued androgen treatment is caused by increased secretion, not cell proliferation [4; 5]. There are no human data of the effects of androgen treatment on prostatic cell proliferation; this would be extremely difficult to investigate. There are only data on the effects of androgen treatment on serum levels of prostate specific antigen (PSA), but these do not necessarily reflect cell proliferation and are more likely to indicate effects at the level of PSA production by the prostate and prostate cancer cells [6]. Thus, if androgens indeed cause prostate cancer, the mechanisms by which they do this are currently not understood.

2. Androgens

There is no evidence that circulating hormone levels are associated with later risk of prostate cancer [7; 8]. Serum hormone levels provide no information about hormone concentrations in prostate tissue, which are controlled by intraprostatic metabolism of androgens [9; 10]. There is also no convincing evidence that functional polymorphisms in genes involved in intraprostatic metabolism of androgens are associated with risk of prostate cancer [11; 12; 13; 14; 15; 16; 17; 18; 19]. However, these genetic studies also do not address potentially important intra-prostatic factors affecting androgen metabolism and hormone concentrations in prostate tissue. Studies of genetic factors and serum hormone levels also do not reflect in which epithelial or stromal cell type androgens are metabolized or act on androgen receptors (AR) [9; 10].

Indirect evidence that androgens are involved in prostate carcinogenesis is derived from human studies with 5α-reductase inhibitors which reduce the formation of 5α-dihydrotestosterone (DHT) from testosterone (T) by this enzyme in the prostate and peripheral fat tissue. The 5α-reductase-type 2 inhibitor finasteride and dual 5α-reductase-type 1 & 2 inhibitor dutasteride have been tested in large clinical trials [20; 21] and both reduced risk of developing prostate cancer by 23-24% over a 4-7 year intervention period [22; 23]. Although these studies provide evidence in support of androgen action as an important factor of prostate cancer development, the duration of the intervention was short in view of the known slow growth of prostate cancer and the study subjects were middle-aged men who have a high frequency of small cancers in their prostates [24]. Thus, these studies are unlikely to provide much insight in whether androgens are involved in the process of carcinogenesis as such or only influence growth and progression of pre-existing cancer. It is not clear whether treatment of aging men with T to ameliorate effects of declining androgen levels increases risk of prostate cancer [25; 26]. Although meta-analyses of T-treated men did not indicate elevated risk [27; 28], there was a significant increased risk of any prostate-related problems identified in one of these studies [28]. It is important to note that the sample sizes of the studies included in these meta-analyses were small and the treatment duration short. Thus, the observed lack of elevated risk of prostate cancer in T-treated aging men should be considered very preliminary [6; 27; 28]. There are no adequate studies of exposure to anabolic steroids and prostate cancer risk; only some case reports of prostate cancer in anabolic steroid users exist [29].

The most direct and convincing evidence that androgens can cause prostate cancer comes from experiments with rats treated with T. Treatment of the inbred NBL rat strain (also known as Noble or Nb rats) with subcutaneously implanted cholesterol pellets containing T propionate at 6-8 week intervals caused grossly visible prostate adenocarcinomas in 19% of animals [30]. We extended this observation in an experiment with NBL rats treated with subcutaneously placed Silastic tubing implants containing T (not testosterone propionate often used by others) which hardly elevated circulating T and found that 11 of 30 rats (37%) developed histologically confirmed adenocarcinomas in the dorsolateral prostate [unpublished data]. We also applied the same treatment to outbred Wistar Cpb:WU rats and 18% developed prostate tumors [unpublished data]. Subcutaneous Silastic tubing implants containing T propionate (not T) induced prostate cancer in 7-15% of Lobund Wistar (LW) rats and some other rat strains [31; 32; 33; 34; 35]. T propionate is fairly rapidly released from Silastic tubing implants and results initially in high circulating T levels that later decline [33], while for unknown reasons T is far less rapidly released from Silastic implants and a sustained stable marginal elevation in circulating T is possible and has been used by us [36]. Thus, chronic T treatment, even when elevating circulating androgen levels only slightly, results in development of prostate adenocarcinomas in at least five different rat strains in incidences ranging from 7 to 37%, with the NBL rat being the most sensitive.

If androgen administration described above is preceded by treatment with a prostate-targeted chemical carcinogen, high prostate cancer incidences can be induced in rats, demonstrating that T is a strong tumor promoter [35; 36; 37; 38]. This tumor promoting effect of T in rats is evident even at circulating T concentrations that are well within the physiological range [31; 36; 39] and may be a significant factor in the carcinogenic activity of T by itself for the rat prostate summarized above.

3. Estrogens

T can be converted to 17β-estradiol (E2) by the enzyme aromatase (CYP 19), which is expressed in fat tissue and in the human and rodent prostate [40]. Therefore, estrogen may be involved in the aforementioned induction of prostate cancer by T in rat models. We have shown that when T treatment of NBL rats is combined with E2, prostate cancer incidence is increased from 35–40% with androgen alone to 90–100% [41; 42]. Even a short course of estrogen treatment is sufficient to result in a high incidence of prostate cancer in NBL rats if chronic low-dose T treatment is given, while the T metabolite DHT cannot be aromatized to estrogen and does not induce prostate cancer [unpublished data]. These results indicate that estrogen plays a critical role in prostate carcinogenesis, at least in the rat. Of note, estrogen treatment alone results in shutdown of luteinizing hormone (LH) production and endogenous androgen production, resulting in prostatic atrophy.

Interestingly, T plus E2 also induces cancer in BPH-1 human prostate epithelial cells that are grafted under the renal capsule of nude mice together with inductive rat or mouse urogenital sinus mesenchyme [43; 44]. These BPH-1 cells are immortalized by SV40-T-antigen, but are by themselves not tumorigenic (with or without the inductive mesenchyme) [43; 45]. The cancers that are induced by T plus E2 in these human prostate cells are capable of metastasis [43]. Besides the T+E2-treated NBL rat model, this is the only other model in

which these two steroid hormones in concert have been shown to cause cancer in prostate tissue.

Aromatase knockout mice [46] and mice overexpressing aromatase [47; 48] suffer from androgen metabolism abnormalities that limit their potentially interesting use for carcinogenesis studies [49]. Aromatase knockout mice lack estrogen production, but have elevated circulating T levels and their prostate is enlarged but does not develop cancer [46]. In aromataseoverexpressing mice estrogen production is elevated, while T levels are considerably reduced and no neoplastic or preneoplastic prostate lesions develop [26; 50]. These observations are consistent with the idea that both hormones are necessary for prostate carcinogenesis.

In humans, however, there is no direct evidence of an association between circulating estrogens levels and risk of prostate cancer [7; 8; 51], with the possible exception of African American men [52]. There is also no evidence of an association of risk with single nucleotide polymorphisms (SNPs) in the aromatase (CYP19A1) gene that are associated with altered serum levels of total and free E2 [53]. Interestingly, the ratio of E2 to T increases with age in parallel with a decrease in T levels and an increasing prevalence of prostate cancer in men, which has been suggested to point to a role of estrogen in prostate carcinogenesis [54].

Both estrogen receptors (ER)-α and ER-β are expressed in the rat and human prostate and they may mediate some or all of the prostatic effects of estrogens [55; 56; 57]. Treatment of NBL rats with the antiestrogen ICI182,780 inhibits the induction by T plus E2 of development of prostatic dysplasia (a putative preneoplastic lesion comparable to human prostatic intraepithelial neoplasia or PIN) [58]. In contrast, the antiestrogen tamoxifen did not affect prostate cancer yield in rats treated with low-dose T after exposure to a prostate-targeted carcinogen [59] , but the effect of tamoxifen has not been examined in rats treated with T plus E2. Of note, the dysplasia in NBL rats treated with E2 plus T occurs in a different region of the prostate (dorsolateral prostate) than where carcinomas are found which originate from the periurethral prostatic ducts [41] and this dysplasia rarely progresses to cancer [unpublished data]. Mice lacking the ER-β have been reported to develop enlargement and focal hyperplasia of the ventral prostate [60; 61], but this has not been confirmed in other studies [49; 62; 63] and prostate enlargement by itself is not associated with prostate carcinogenesis [5]. We observed in immunohistochemical studies that the regions of the NBL rat prostate which are most susceptible to the carcinogenic effects of $T+E_2$ have relatively low ER-α expression and very high ER-β expression [unpublished data]. Overall, these data suggest that estrogen receptors may play a role in the hormonal induction of prostate cancer in rats, but conclusive studies are lacking at present. In contrast, it has been suggested that in the human prostate ER-β, which is selectively expressed in epithelial cells, may mediate inhibition of the progression of cancer [28; 64], but this is not a generally accepted or validated concept. There are some studies suggesting associations between SNPs in the ER-α and ER-β genes [65], but their results still need confirmation.

4. Estrogens as Chemical Carcinogens

Evidence has been reported of enzymatic conversion of E2 and estrone to 2- and 4-hydroxyestradiol and -estrone mediated by CYP1A1 and CYP1B1 from studies of rodent [66] and human prostate tissue [E. Cavalieri & E. Rogan, personal communication] and analyses of levels of estrogen metabolites and adducts in the urine of men with or without prostate cancer [67]. These so-called catecholestrogens can be converted to highly reactive estrogen semiquinones and estrogen quinones by the process of redox cycling. These reactive intermediates can adduct DNA and redox cycling itself causes generation of reactive oxygen species (ROS) which, in turn, cause lipid peroxidation resulting in the formation of lipid hydroperoxides. Both ROS and reactive lipid hydroperoxides can also damage DNA and potentially lead to the formation of mutations [68]. The 4-hydroxyestradiol (4OH-E2)-quinone-DNA adducts rapidly depurinate, resulting in apurinic sites in the DNA. These apurinic sites can potentially lead to the mutations when repaired by error-prone DNA repair mechanisms [69], although such mutations have not been definitively demonstrated [68; 70]. A summary of this mechanism is provided in Figure 1 and for details of this complex mechanism, the reader is referred to Cavalieri *et al*. [66; 68] and Bolton and Thatcher [70]. Detection of 4OH-E2-quinone-DNA adducts has been problematic, because these are depurinating with a very short half-live leading to apurinic sites which are difficult to detect, but with highly sensitive analytical methods (LC-MS/MS) formation of such adducts has conclusively been demonstrated after estrogen treatment of DNA, cells, and tissues [70; 71; 72; 73; 74].

We have shown that these reactions can take place in the rat prostate in experiments in which we injected animals with 4OH-E2 or 4OH-E2-quinone and measured prostate tissue levels of E2, 4OH-E2, and detoxified methylated and glutathione conjugated 4OH-E2 metabolites [66]. Following treatment of NBL rats for 16 weeks with T plus E2, we identified a major DNA adduct by ^{32}P-postlabeling selectively in the periurethral area of their prostates, the site of later cancer development [75]. A low level of this adduct was also found at this location in control animals, perhaps indicating the sensitivity of this tissue for DNA damage. Treatment of rats with only T caused moderately elevated levels of this adduct in the periurethral prostate [unpublished data]. Ho and Roy reported that T plus E2 treatment induced DNA strand breaks, and fluorescent lipid peroxidation products in the dorsolateral, but not ventral, prostate of NBL rats [76]. We measured the formation of (a) 8-hydroxydeoxyguanosine (8-OHdG), an indicator of oxidative DNA damage, and (b) DNA damaging lipid hydroperoxides in the prostate of NBL rats after treatment with T plus E2 for 16 weeks ; the highest levels of 8-OHdG and lipid hydroperoxides were found in the periurethral area of the prostate where cancer develops [68]. However, the contributions of oxidative DNA damage and lipid peroxidation to prostate carcinogenesis by T plus E2 are not clear, because dietary treatment with α-tocopherol and selenomethionine did not reduce the induction of prostate carcinomas [42].

Nevertheless, the above summarized data provide evidence indicating that estrogen treatment causes DNA damage in the NBL rat prostate and that this occurs prior to cancer development and at the exact same site within the rat prostate where carcinomas develop after treatment with T plus E2 [66; 68; 75]. We have also developed evidence that enzymes

that provide protection against reactive estrogen metabolites, such as catechol-*O*-methyltransferase and glutathione reductase, are more active in the dorsolateral prostate region, which does not develop cancer in NBL rats treated with T plus E2, and less active in the periurethral prostate area, where carcinomas do develop [66].

6. Conclusions: Estrogenicity, Estrogen-Genotoxicity, and Androgenic Stimulation May Act in Concert in Hormonal Prostate Carcinogenesis

Collectively, the data, mostly from animal studies, summarized in this paper suggest that for androgens to cause prostate cancer they must be aromatized to estrogen and act in concert with these metabolites. Androgen-receptor mediated activity of the androgens and estrogen receptor-mediated effects of the estrogen metabolites are likely to be necessary, but estrogen genotoxicity appears to be a probable critical factor as well. Only when all these mechanisms are active, prostate carcinogenesis may be the result, at least in the NBL rat model. To explore whether this hypothesis holds true for human prostate carcinogenesis will require extensive tissue-based epidemiologic studies, but there is some experimental evidence that T plus E2 can induce malignant transformation of human prostate cells in xenograft experiments in nude mice mentioned earlier [44; 45; 56]. If all these factors, including aromatization of androgens, are required for androgenic hormones to be carcinogenic for the prostate, interference with any of these might be sufficient to yield a preventive effect and interference with a combination of these factors might have an even stronger preventive effect. With the NBL rat model available, it should be possible to critically test this idea as a first step towards developing new preventive strategies for prostate cancer that can be evaluated in clinical trials with agents that are very well tolerated and bioavailable upon oral administration at clinically feasible doses. However, convincing proof-of-concept studies using this model are needed to demonstrate conclusively that joint androgen-estrogen action and receptor-mediated and genotoxic effects are indeed all required for prostate carcinogenesis and that these mechanisms can be interfered with using chemopreventive treatments. While this challenge can be met with the NBL rat model, other preclinical models are desirable but not available at present and translation to human application will entail considerable multidisciplinary efforts and clinical trials.

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Abbreviations used

4OH-E2	4-hydroxyestradiol
8-OHdG	8-hydroxydeoxyguanosine
AR	androgen receptors
DHT	5α-dihydrotestosterone
E2	17β-estradiol

ER	estrogen receptor
LH	luteinizing hormone
PSA	prostate specific antigen
ROS	reactive oxygen species
SNP	single nucleotide polymorphism
T	testosterone

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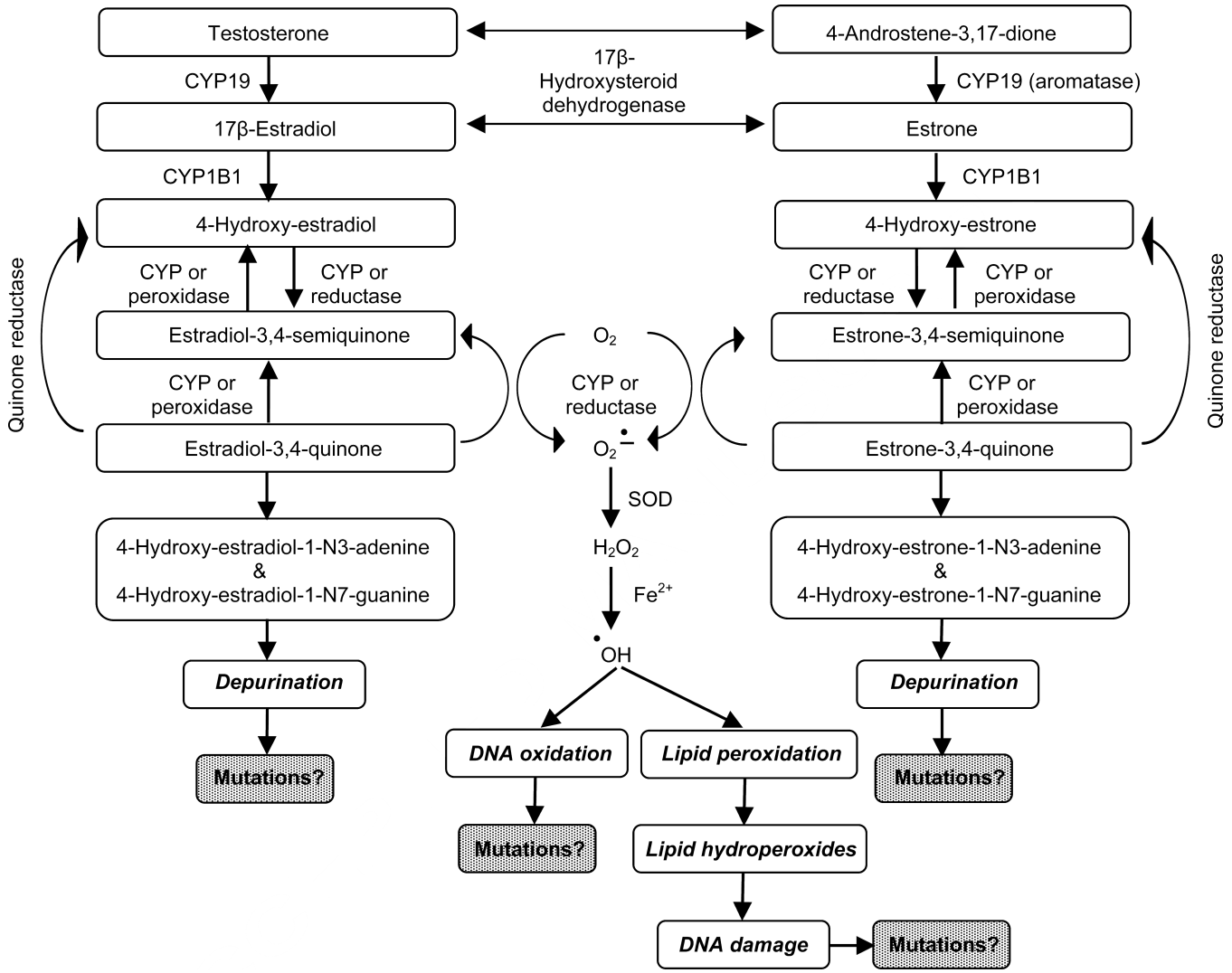

Fig. 1.

Summary of the metabolism of androgens and estrogens to reactive estrogen intermediates and the damage to DNA and lipids they can cause. (SOD = superoxide dismutase; CYP = a cytochrome P450 enzyme)