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. Androgenic hormones are generally believed to be causatively associated with prostate carcinogenesis, but human evidence, mostly epidemiological, for this is minimal. Circulating hormone levels are not associated with the risk of prostate cancer and neither are polymorphisms in various genes encoding the androgen metabolizing enzymes or androgen receptors. Evidence in support of the involvement of androgens in prostate cancer development is derived from clinical trials with 5α-reductase inhibitors, which reduced the risk by approximately 25%. Animal studies using rat models, however, provide clear evidence that testosterone can induce prostate cancer and can act as a strong tumor promoter in concert with genotoxic carcinogens. One such genotoxic factor may be 17β-estradiol, which is generated from testosterone by the aromatase enzyme. Estradiol can be converted to catecholestrogens, which through redox cycling, generate reactive metabolites that can adduct the DNA and potentially lead to mutations. Animal studies and limited human evidence suggest that estrogens can be involved in prostate carcinogenesis by such a genotoxic mechanism. However, how androgens exert their tumor-promoting effect is not clear. It is likely that hormonal and non-hormonal factors as well as genetic and non-genetic (environmental) factors interact in a highly complex and poorly understood manner to determine the risk of prostate cancer.

Keywords: Androgens, estrogens, hormonal carcinogenesis, prostate cancer, steroid hormones

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 malignancy are not entirely certain, but it is generally assumed that androgenic hormones play a major role in prostate carcinogenesis.[2] The author has previously reviewed the evidence for causative involvement of steroid hormones in prostate cancer, particularly androgens.[3] The purpose of the current article is to summarize more recent information regarding the role of steroid hormones in prostate carcinogenesis and critically put these data into context. Data on the emerging role of estrogens in prostate carcinogenesis, in particular, will be discussed, as well as recent data indicating novel mechanisms by which androgens can interact with estrogens and cause molecular changes leading to prostate cancer.

The basis for the assumption that androgenic hormones play a causative role in prostate carcinogenesis is that the prostate gland is an androgen-dependent tissue and prostate cancer is an androgen-dependent malignancy.[2] The underlying
mechanism has been postulated to be the androgenic stimulation of cell proliferation, resulting in an increased risk of oncogenic genetic alterations.\[9\] However, the evidence for all this is indirect and very limited at best.

There is no evidence that androgens cause sustained cell proliferation in the prostate. A classical experiment is to surgically castrate rats which causes involution of the prostate gland by apoptosis and cessation of secretory activity, and give androgen back at physiological levels after a couple of weeks; this will cause a few waves of cell proliferation in the prostate, but after a few days, cell proliferation returns to levels found in intact control rats.\[14\] Further growth of the prostate upon continued androgen treatment is caused by increased secretion, not cell proliferation.\[14,15\] There are no human data of the effects of androgen treatment and prostatic cell proliferation, as these are extremely difficult to obtain, and data only exists on the effects of androgen treatment on serum levels of prostate specific antigen (PSA), which do not necessarily reflect cell proliferation and may indicate effects on the level of PSA production by the prostate and prostate cancer cells.\[10\] A study of intermittent androgen suppression with serial tumor biopsies of advanced prostate cancer patients was not informative, because it indicated a highly variable cell proliferation (Ki-67 staining) response to the cessation of androgen ablation, although serum PSA responded with the expected rebound after cessation of hormone ablation.\[7\]

In the following sections, the current knowledge about the role of steroid hormones in prostate carcinogenesis will be summarized, first focusing on studies of androgens in human populations and clinical trials, followed by laboratory animal model research. Subsequently, the focus will shift to the role of estrogens followed by a final summary of current hypotheses.

**CIRCULATING ANDROGEN LEVELS AND RISK OF PROSTATE CANCER**

There have been many attempts to determine the association between the levels of androgens and other sex steroid hormones and the risk of prostate cancer later in life. A meta-analysis, in 1999, of eight such studies, did not find any evidence for such an association of risk with total and free testosterone, 5α-dihydrotestosterone (DHT), androstenedione, dehydroepiandrosterone (DHEA) sulfate, estrone, estradiol, sex hormone binding globulin (SHBG), luteinizing hormone (LH) or prolactin; but for androstenediol glucuronide there was a very weak positive association with a risk (risk ratio 1.05; 95% confidence interval (CI) 1.00 – 1.11).\[8\] In a more recent pooled analysis of these and other prospective studies (n = 18), including as many as 3886 prostate cancer cases and 6438 controls, there was also no evidence of any association of sex steroid hormones levels with subsequent prostate cancer risk, with the exception of SHBG, for which there was a modest negative association (relative risk 0.86; 95% CI 0.75 – 0.98), but this was not reflected in a positive association for either free testosterone or free estradiol.\[10\] Thus, overall, there is no evidence that circulating levels of sex steroid hormones were associated with risk of prostate cancer. A major problem with these studies is that they involved only a single hormone measurement, which does not reflect variations over time. Most importantly, serum hormone levels provide little information about hormone concentrations in prostate tissue, which are controlled by intraprostatic metabolism of androgens, and are not reflective of the epithelial or stromal cell type in which the androgens are metabolized or act on the androgen receptor.\[10,11\] In older studies of populations that differed in the risk of prostate cancer, higher levels of 3α,17β-androstanediol glucuronide were found in the higher risk populations (such as US males) than in men at lower risk (such as Japanese males) leading to the hypothesis that the risk of prostate cancer is associated with the activity of 5α-reductase, converting testosterone into DHT, which is catabolized to 3α,17β-androstanediol by 3α-hydroxysteroid dehydrogenase type 3 (or AKR1C2).\[2,12\] However, the above-mentioned pooled analysis of 18 prospective studies did not yield any support for this idea.\[13\] Androstanediol glucuronide reflects the overall catabolism of DHT, but this happens in both the prostate and liver. The other main DHT metabolite, 3β,17β-androstanediol formed by the AKR1C1 enzyme, has a weak estrogenic activity, which could be inhibitory on tumor growth via acting on the estrogen receptor-β.\[13\]

**POLYMORPHISMS IN GENES RELATED TO ANDROGEN ACTION OR METABOLISM AND PROSTATE CANCER RISK**

To obtain information about steroid hormone metabolism and prostate cancer risk, hypotheses were developed from a multigenic model on the role of these hormones in prostate carcinogenesis, focusing on the activity of enzymes involved in androgen biosynthesis (steroid 17α-hydroxylase / 17,20-lyase [CYP17], 17β-hydroxysteroid dehydrogenase [HSD17B], and 3β-hydroxysteroid dehydrogenase [HSD3B]), androgen activation (5α-reductase [SRD5A2 and SRD5A1]) and androgen catabolism / clearance (3β- and 3α-hydroxysteroid dehydrogenase [HSD3A and HSD3] eliminating DHT and cytochrome P450 3A [CYP3A4 and CYP3A5] clearing testosterone).\[14-17\] However, despite considerable effort in examining the genetic variation in these genes which affects their activity, in several studies (some of which were large and included haplotype analyses), no convincing
evidence emerged that functional polymorphisms in the CYP17, HSD17B1, HSD3B, SRD5A2, and CYP3A4 genes are associated with risk of prostate cancer.[16,18-25] However, in some studies significant associations with prostate cancer risk were found. For example, in one study an association was found between prostate cancer risk and polymorphisms in the SRD5A1 and HSD3B1 genes that are of uncertain functional significance.[26] In a meta-analysis of the V89L polymorphism in the SRD5A2 gene an association was found between elevated risk and the L allele, but only for men with a European heritage and men younger than 65 years,[27] even though the L allele confers lower enzyme activity.[28] A CYP17 polymorphism was associated with the risk of prostate cancer, but only in African–American men.

Thus, age and race are probably important factors, but have not always been addressed, as is also the case with the critical issue of functionality of polymorphisms. Although some studies have found significant associations with risk, overall the studies reported to date all suffer, to some degree, from issues related to insufficiently large sample sizes, study population differences, insufficient across-laboratory standardization, and quality control of the methods used for genotyping.[9,21] Of course these studies cannot address potentially important gene-environment and intraprostatic factors affecting androgen metabolism and gene–gene interactions have hardly been addressed.[9]

The androgen receptor (AR) gene contains a polymorphic region in exon 1 that consists of variable CAG microsatellite repeats that are associated with differences in AR activity. The normal range of these repeats is between eight and thirty-five and the mean is about twenty CAG repeats. The shorter the CAG repeat length, the higher the activity of the AR.[29,30] Several initial studies have suggested an association between shorter CAG repeat lengths and increased risk of prostate cancer.[31] However, larger, more recent studies do not confirm this.[31-33] Furthermore, interactions between genes and the stage (early versus advanced stage) or aggressiveness of the disease may be important as suggested, for example, by a study of polymorphisms in the AR and PSA genes.[34]

**CLINICAL TRIALS WITH 5α-REDUCTASE INHIBITORS**

Indirect evidence that androgens are involved in prostate carcinogenesis is derived from human studies with 5α-reductase inhibitors, which reduce the formation of DHT from testosterone via this enzyme in the prostate and the periphery, mainly the fat tissue. The 5α-reductase-type 2 inhibitor, finasteride, and dual 5α-reductase-type 1 and 2 inhibitor, dutasteride, have been studied in large clinical trials for their ability to reduce the risk of prostate cancer.[35,36] A reduced risk of developing prostate cancer by 23 – 24%, over a four-to-seven-year intervention period, was found in both of these studies, in men at average risk for prostate cancer or high-risk men.[37] Both agents exerted the strongest preventive effect for low-grade prostate cancer, but for more clinically significant high-grade cancer there was no protective effect in the dutasteride trial, for cancers with a Gleason score of 7 or higher, and a small but significantly increased risk in the finasteride study for tumors with a Gleason score of 8 or higher. These studies provide evidence in support of androgen action as an important factor for prostate cancer development. However, the duration of the intervention in these two trials was short, in view of the known slow growth of prostate cancer. Moreover, the study subjects were middle-aged men who are known to have a high frequency of small cancers in their prostates.[39] Thus, results of these studies are unlikely to provide much insight into whether androgens are involved in the process of carcinogenesis or only influence the growth and progression of pre-existing cancers.

**TESTOSTERONE TREATMENT, ANABOLIC STEROIDS, AND RISK OF PROSTATE CANCER**

There has been a marked increase in recent years of treatment of aging men with testosterone, to ameliorate the effects of declining androgen levels with aging.[40] Whether this treatment increases the risk of prostate cancer has been subject to much discussion.[41,42] Recent meta-analyses of the effect of testosterone treatment on prostate cancer development did not indicate elevated risk,[43,44] although there was a significant increased risk of any prostate-related problems identified in one of these studies.[44] However, the sample sizes in the studies involved in these meta-analyses were low and the treatment duration short, and the lack of elevated risk of prostate cancer should be considered very preliminary and studies including many more subjects and much longer treatment periods are needed.[46,43,44] Studies relying on the effects of testosterone treatment of men on their serum PSA levels are not relevant to assess the risk of prostate cancer.[6]

Anabolic steroid abuse has become almost epidemic in the US and is not limited to athletes.[45-47] Although increased risk of prostate cancer has been suggested, no epidemiological studies have been conducted, and there are only some case reports of prostate cancer in anabolic steroid users.[48]
ANDROGENS AND PROSTATE CANCER PROGRESSION

The vast majority of prostate cancers initially respond to androgen ablation therapy with regression and reduction of PSA, but in virtually all cases, the tumors become hormone refractory and are currently referred to as castrate-resistant prostate cancer. [49,50] In most castrate-resistant prostate cancers, the androgen receptor (AR) remains strongly expressed and active in the absence of androgens. [50,51] The mechanisms identified for this phenomenon are AR gene amplification, altered expression and function of AR co-activators, and ligand-independent AR activation through stimulation of alternate signal transduction pathways, [49,52,53] as well as local production of androgen within the castration-resistant tumor. [54,55] Although this has not been observed in all studies. [56] The AR can also acquire mutations that make the receptor either hypersensitive to androgen, facilitate AR function because of altered interactions with AR co-regulators, or expand AR ligand specificity when these mutations occur in the ligand-binding domain, so that the receptor can be promiscuously activated by a broad group of steroids, including estrogens, progestins, adrenal steroids, and even anti-androgens. [57-59] The AR of the commonly used LNCaP cell line contains such a mutation (T877A). [60] Most of these mutations do not appear to affect the receptor-binding affinity for androgens, such as DHT, but can still affect the transcriptional activity of the receptor, in response to these androgens. AR mutations appear to be rare in the early stages of prostate cancer (0 – 4%), but become more frequent in more advanced tumors or recurrent tumors. [59,61-63] AR mutations have been detected in 10 to well over 30% of patients with castrate-resistant tumors which have failed anti-androgen therapy, suggesting that the anti-androgen therapy causes a natural selection of cells that have acquired mutations. [64,65] The fact that the AR remains active, and probably essential, during prostate cancer progression and in the absence of circulating androgens puts further emphasis on the central role of androgen action in prostate carcinogenesis.

ANIMAL STUDIES OF ANDROGENS AND PROSTATE CARCINOGENESIS

The most direct and convincing evidence that androgens can cause prostate cancer comes from experiments with rats treated with testosterone. Robert Noble treated an inbred rat strain (designated NBL or Nb), which was probably of Long Evans origin, [60] with subcutaneously implanted cholesterol pellets containing testosterone propionate at six-to-eight-week intervals and found that 16 of the 85 treated rats (19%) developed grossly visible prostate adenocarcinomas. [67] We extended this observation with an experiment with NBL rats in which we subcutaneously placed Silastic tubing implants containing testosterone (not testosterone propionate), which hardly elevated circulating testosterone, and found that 11 out of 30 rats (37%) developed histologically confirmed adenocarcinomas in the dorsolateral prostate. [68] The same treatment of outbred Wistar Cpb:WU rats caused an 18% incidence of prostate cancer. [68] Morris Pollard applied subcutaneous Silastic tubing implants containing testosterone propionate (not testosterone) to a nearly inbred Wistar rat strain he maintained at Notre Dame, the Lobund Wistar (LW) rat, and reported an incidence of grossly visible prostate carcinomas of 0, [69] 14, [70] and 15%. [71] This was essentially reproduced by Hoover et al., [72] who reported three prostate carcinomas in 42 LW rats (7%). Previously Pollard found a prostate tumor in one of twenty-five (4%) similarly treated Sprague Dawley rats. [73] Parviz Pour treated Wistar rats (MRC) maintained at the Eppley Institute with testosterone propionate in subcutaneous Silastic tubing implants, and induced prostate adenocarcinomas in two of the sixteen (13%) treated rats. [74] Testosterone propionate is fairly rapidly released from Silastic tubing implants, [75] while for unknown reasons testosterone is far less rapidly released and a sustained stable marginal elevation in circulating testosterone is possible. [68] Thus, chronic testosterone treatment results in the development of prostate adenocarcinomas in five different rat strains in incidences ranging from 4 to 37%.

If androgen administration described earlier is preceded by treatment with a chemical carcinogen, high prostate cancer incidences can be induced in rats, demonstrating that testosterone is a strong tumor promoter, which may be a factor in the carcinogenic activity of testosterone in the rat prostate summarized earlier. Carcinogens that are targeted to the prostate because of prostate-specific activation of a pro-carcinogen or stimulation of cell proliferation in the prostate at the time of administration of a chemical carcinogen, such as methylnitrosourea, appear to be most effective. [62,74-76] In the LW rat strain, an injection of methylnitrosourea that is not targeted to the prostate also functions as a very effective tumor initiating factor when followed by chronic androgen treatment. [18,71] This tumor promoting effect of testosterone in rats is evident even at circulating androgen concentrations that are well within the physiological range. [62,70,77]

Treatment of rats with the 5α-reductase inhibitor finasteride or the antiandrogen casodex concomitant with administration of testosterone propionate via Silastic implants, after treatment with the prostate-targeted carcinogen 3,2’-dimethyl-4-aminobiphenyl (DMAB), inhibited carcinogenesis in the dorsolateral prostate. [77] In contrast, when DMBA was given without testosterone propionate treatment, these
antiandrogens enhanced rather than inhibited the induction of ventral prostatic neoplasms. Another 5α-reductase inhibitor inhibited the development of spontaneous carcinomas in the ventral prostate of the ACI rat, but only at low and not high doses. Other studies with agents interfering with androgen action, particularly the androgen receptor blocker flutamide, have also been very effective in preventing prostate cancer development in rats treated sequentially with methyltritrosourea targeted to the prostate, followed by chronic low-dose testosterone administration via Silastic implants.

These studies appear to be consistent with the results of the clinical trials with finasteride or dutasteride summarized earlier in the text and strongly support the idea that androgens are causatively involved in prostate carcinogenesis. The precise mechanisms by which androgens act in this fashion are, however, not very clear and may differ depending on which phase of carcinogenesis the androgens are involved in and may be influenced by their interactions with other potentially important factors, such as prostatic inflammation, diet, and genetic predisposition. One emerging mechanism is related to the discovery that androgen can induce topoisomerase-2B-mediated double-strand breaks in the DNA, resulting in gene rearrangements leading to TMPRSS2:ERG gene fusions in prostate cancer cells (LNCaP and LAPC-4) and SV40-immortalized prostate epithelial cells. This fusion of the androgen-regulated TMPRSS2 gene to ERG, which encodes an ETS transcription factor, is the most frequent gene fusion in prostate cancers, found in approximately 50% of cases; upregulation of this fusion gene by androgen is postulated to be involved in prostate carcinogenesis. The studies of androgen induction of this fusion gene have been conducted in cell cultures and it remains to be seen whether this occurs in vivo in the human prostate.

Two studies of genetically modified mice demonstrate the importance of AR activity in prostate carcinogenesis. Transgenic mice that overexpress AR in the prostate by targeting additional AR cDNA under control of the probasin promoter, developed dysplastic lesions in 45% of the mice that had morphological similarities with human prostatic intraepithelial neoplasia or PIN, but no cancer was observed. Transgenic mice (n = 5) with a mutation in the AR (E231G) that caused a ligand-independent activity of the receptor developed metastatic adenocarcinomas in the ventral prostate lobe. Although these studies clearly suggest that just increased AR activity may lead to prostate cancer, there are no publications, using these two models, after the initial reports.

ROLE OF ESTROGENS IN PROSTATE CARCINOGENESIS: ANIMAL, AND HUMAN STUDIES

Testosterone can be converted to 17β-estradiol by the enzyme aromatase, which is expressed in the fat tissue and in human and rodent prostates. Therefore, estrogen may be involved in the induction of prostate cancer by testosterone in rat models (see earlier text). When estradiol is combined with the testosterone treatment of these NBL rats, prostate cancer incidence is increased from 35 – 40% with androgen alone to 90 – 100%. 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 testosterone treatment is given, while the testosterone metabolite DHT which cannot be aromatized to estrogen does not induce prostate cancer. These data indicate that estrogen plays a critical role in prostate carcinogenesis. Of note, estrogen treatment alone results in the shutdown of LH production and endogenous androgen production, resulting in prostatic atrophy. Aromatase knockout mice (and aromatase overexpressing mice, suffer from androgen metabolism abnormalities which limits their potentially interesting use for carcinogenesis studies. Aromatase knockout mice lack estrogen production and have elevated circulating testosterone levels and their prostates are enlarged, but they do not develop cancer. In aromatase-overexpressing mice, estrogen production is elevated, while testosterone levels are considerably reduced, but no neoplastic or preneoplastic prostate lesions develop. These observations are consistent with the idea that both hormones are necessary for prostate carcinogenesis. In humans, however, there is no evidence of an association between circulating estradiol levels and risk of prostate cancer with the possible exception of African–American men, in whom the serum estrogen level and the ratio of estradiol-to-testosterone has been positively associated with risk in a large NHANES III-based study. There has also been 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 estradiol and even free (but not total) testosterone. On the other hand, evidence of an association between risk and TTTA repeat length polymorphisms in the estrogen receptor has been found, but is not biologically plausible. The low number of cases in several of these studies may account for inconsistencies in their results.

Both estrogen receptors-α and -β are expressed in the rat and human prostate, and they may mediate some or all the prostatic effects of estrogens. When NBL rats are treated with estradiol plus testosterone in combination with the antiestrogen ICI182,780, development of prostatic dysplasia (a
An association was found of conversion of estradiol and estrone to 2- and 4-hydroxyestradiol and -estrone. These catecholestrogens 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 ventral prostate region, which does not develop cancer in NBL rats treated with estradiol plus testosterone, and less active in the periurethral prostate area, where carcinomas do develop.\[108\]

CONCLUDING REMARKS

It is attractive and biologically plausible to postulate that endogenous factors present in every man, namely androgens, are responsible for the high frequency of prostate cancer in aging men. However, the data summarized here indicate that the human evidence for this is weak at best, and limited by the fact that adequate access to the tissue of interest, for detailed studies of intraprostatic factors and mechanisms, is extremely difficult if not impossible to attain. In contrast, animal model data indicate that testosterone is carcinogenic for the rat prostate and acts as a strong tumor promoter. The presence of weak genotoxic factors within the prostate can then conceivably result in carcinogenesis promoted by even moderate levels of androgens. One such genotoxic factor may be 17\( \beta \)-estradiol, the aromatase metabolite of testosterone. Other carcinogens may also be involved, as suggested by animal studies with a range of carcinogens that target or are made to target the prostate.\[74,76,114\] Such exposures to genotoxic carcinogens may be sufficient to cause prostate cancer at high prevalence in humans, just as 17\( \beta \)-estradiol appears to do in the testosterone-treated NBL rat\[87\] and other carcinogens in androgen-treated rat models.\[71,72,74-76\] The increase in estrogen-to-androgen ratio that occurs in aging men\[115\] can be viewed as support for the notion that estrogens, in addition to androgens, are critical factors in prostate carcinogenesis. This hypothesis that estrogens, which are ubiquitously and continuously present in the prostate, can induce mutations may explain why prostate cancer is so common. However, this hypothesis does not explain why some tumors progress to be clinically evident and aggressive, while others remain apparently indolent. It also does not explain why this process of progression appears to be far more common in Western countries, particularly among African–American men, than in most Asian countries, while migrants from low-risk to high-risk countries acquire the risk of their new environment.\[2,116\] Clearly, there are other factors than hormones per se that play a critically decisive role. There is no doubt that there are environmental factors, which are critical determinants of risk of clinically evident prostate cancer,
explaining the changes in prostate cancer risk in migrants from low- to high-risk countries, but these factors have not been identified. It is likely that there are genetic, hereditary factors as well as environmental factors that determine (i) the sensitivity of the androgen receptor for DHT, (ii) critical steps in the metabolism of androgens and estrogens, and (iii) the activity of enzymes involved in the generation of and protection against reactive estrogen metabolites and DNA repair. These various genetic and non-genetic, hormonal and non-hormonal factors probably interact in highly complex and poorly understood ways, to modify risk of prostate cancer. The notion of such a complexity of prostate carcinogenesis is consistent with the great complexity in the molecular alterations in prostate cancers that has emerged over the past decade. Nonetheless, the idea that androgens are central to prostate carcinogenesis remains fully viable, even though epidemiological studies have not provided support for this, and the precise mechanisms by which they may act in the initial stages of this process are unclear. This lack of understanding impedes the development of better preventive approaches than those provided by 5α-reductase inhibitors. The critical role of the androgen receptor and androgen metabolism during prostate cancer progression, on the other hand, is better understood and has resulted in improved treatments.

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REFERENCES

68. Bosland MC. Unpublished data.


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