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Estrogen and Prostate Cancer: An Eclipsed Truth in an Androgen-Dominated Scenario

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Abstract Prostate cancer is the commonest non-skin cancer in men. Incidence and mortality rates of this tumor vary strikingly throughout the world. Although several factors have been implicated to explain this remarkable variation, lifestyle and dietary factors may play a dominant role, with sex hormones behaving as intermediaries between exogenous factors and molecular targets in development and progression of prostate cancer. Human prostate cancer is generally considered a paradigm of androgen-dependent tumor; however, estrogen role in both normal and malignant prostate appears to be equally important. The association between plasma androgens and prostate cancer remains contradictory and mostly not compatible with the androgen hypothesis. Similar evidence apply to estrogens, although the ratio of androgen to estrogen in plasma declines with age. Apart from methodological problems, a major issue is to what extent circulating hormones can be considered representative of their intraprostatic levels. Both nontumoral and malignant human prostate tissues and cells are endowed with key enzymes of steroid metabolism, including 17βhydroxysteroid dehydrogenase (17βHSD), 5β-reductase, 3α/3βHSD, and aromatase. A divergent expression and/or activity of these enzymes may eventually lead to a differential prostate accumulation of steroid derivatives having distinct biological activities, as it occurs for hydroxylated estrogens in the human breast. Locally produced or metabolically transformed estrogens may differently affect proliferative activity of prostate cancer cells. Aberrant aromatase expression and activity has been reported in prostate tumor tissues and cells, implying that androgen aromatization to estrogens may play a role in prostate carcinogenesis or tumor progression. Interestingly, many genes encoding for steroid enzymes are polymorphic, although only a few studies have supported their relation with risk of prostate cancer. In animal model systems estrogens, combined with androgens, appear to be required for the malignant transformation of prostate epithelial cells. Although the mechanisms underlying the hormonal induction of prostate cancer in experimental animals remain uncertain, there is however evidence to support the assumption that long term administration of androgens and estrogens results in an estrogenic milieu in rat prostates and in the ensuing development of dysplasia and cancer. Both androgen and estrogen have been reported to stimulate proliferation of cultured prostate cancer cells, primarily through receptor-mediated effects. As for estrogens, the two major receptor types, ERα and ERβ, are expressed in both normal and diseased human prostate, though with a different cellular localization. Since these two receptors are different in terms of ligand binding, heterodimerization, transactivation, and estrogen response element activity, it is likely that an imbalance of their expression may be critical to determine the ultimate estrogen effects on prostate cancer cells. In prostate cancer, ERβ activation appears to limit cell proliferation directly or through ERα inhibition, and loss of ERβ has been consistently associated with tumor progression. Several splicing variants of both ERα and ERβ exist. Little is known about their expression and function in the human prostate, although reciprocal regulation and interaction with gene promoter both warrant further investigation. In summary, although multiple consistent evidence suggests that estrogens are critical players in human prostate cancer, their role has been only recently reconsidered, being eclipsed for years by an androgen-dominated interest. J. Cell. Biochem. 102: 899–911, 2007.

Key words: estrogen; prostate cancer; plasma hormones; tissue hormones; metabolism; receptors; androgen

Prostate cancer represents a common cause of morbidity and mortality in men in Western countries. Despite the most recent advances in both basic and transnational research, the molecular basis of prostate cancer remains mostly obscure. Endogenous sex steroids along with genetic factors, environmental factors (including diet) and host immune and inflammatory

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responses are likely to concur in the pathogenesis of this disease.

It is noteworthy that environmental and, especially, dietary factors are supposed to induce significant changes in endogenous hormones and their metabolism, eventually leading to prostate cancer development and/or progression [Kolonel et al., 2004]. In this respect, sex steroids may be regarded as intermediaries between exogenous effectors, either environmental or nutritional, and molecular targets in the process of initiation, promotion and progression of prostate cancer.

Although estrogen regulation of prostate development, growth and differentiation is well established, the potential role of estrogens in prostate cancer has been only recently reconsidered. It has been known for decades, but lately recognized, that neither androgens nor estrogens have a sexual specificity, the former being implicated in breast and the latter in prostate, either normal or malignant, cell growth. This concept is nicely pointed out in a paper by Kuiper et al. [1998], where estrogen is described as a male and female hormone.

Interestingly, breast and prostate cancer share many similarities, in terms of geographical distribution, risk factors, biomolecular determinants, and natural history of disease. In this respect, cancer of the human prostate and breast can be viewed as brother and sister tumors, where dietary factors and hormones, notably estrogens, are crucial and interactive players in many biological and pathological processes. If this is true, then prostate and breast cancer may be primarily considered, as elegantly proposed by Coffey [2001], an acquired nutritional disease that ought to be prevented through changes of lifestyle and dietary habits.

EPIDEMIOLOGIC EVIDENCE

Prostate cancer is the most frequently diagnosed non-skin tumor and the second leading cause of death for cancer in men in the United States, with an estimate of 218,890 new cases and 27,050 deaths from this disease expected in the year 2007 [American Cancer Society, 2007]. In the United States, but also in many other Western countries, human prostate cancer has exhibited striking changes of incidence over the last two decades, mostly because of the introduction of prostate specific antigen (PSA) blood test as a diagnostic tool for prostate cancer screening. Recently, incidence rates have leveled off in men aged 65 years and older, while mortality rates are consistently declining since 1990. In spite of this, but notably because of the fact that mechanisms underlying prostate cancer development and/or progression are poorly understood, this neoplasm remains today a major healthcare problem having high socioeconomic impact.

It is well recognized that both incidence and mortality rates for prostate cancer vary considerably throughout the world [Parkin et al., 2005]. There is, in fact, a nearly 40-fold and a 12-fold difference, respectively, in incidence and mortality rates from prostate cancer between African American men and men in Hong Kong and Japan. In Europe, incidence rates for prostate cancer are markedly higher in Northern countries (80.1/100,000) than in Southern Europe (44.7/100,000); in particular, Sweden has the highest rates (139.3/100,000) and Greece the lowest (43.4/100,000), with a cumulative risk that ranges from 0.5 up to 2.2 across European countries.

Although the factors that might contribute to explain this large geographic variation remain largely unknown, there is an overall consensus that lifestyle and diet play a primary role, while genetic and environmental factors might account only for a limited proportion (likely less than 10%) of prostate cancer cases.

A minor proportion (5–9%) of prostate cancer can be based on heritable genetic defects, while familial forms of prostate cancer may account for nearly 20% of cases [Cancel-Tassin and Cussenot, 2005]. However, the effects of environment and lifestyle appear to be essential for the manifestation of disease, even in men carriers of strong cancer-susceptibility genes.

Sedentary lifestyle and high fat diet have been related to an increase in prostate cancer risk [Giovannucci et al., 1998; Kolonel et al., 1999]. Furthermore, some studies have found an association between high protein and energy intake, as well as low intake of fiber and complex carbohydrates and an increased risk of developing prostate cancer [Kolonel, 1996]. However, the significance of the above association is admittedly low and age, ethnicity, and family history remain the few, well-established risk factors for prostate cancer [Hsing and Chokkalingam, 2006].

Two major findings support the view that lifestyle and dietary factors play a dominant

role in prostate cancer development. In the first place, studies on migrants who move from countries having low incidence/mortality rates of prostate cancer (e.g., China or Japan) to countries with higher prostate cancer rates (Unites States) show, within a generation, a significant increase in prostate cancer incidence/mortality as compared with their peers in the countries of origin [Wynder et al., 1991; Cook et al., 1999]. Secondly, prostate cancer incidence is rising rapidly in Asian countries, including Japan, as Asians gradually adopt westernized diet and lifestyle [Sim and Cheng, 2005].

Several authors have hypothesized that phytochemicals contained in Asian diets, notably the phytoestrogens from soy products, might act as natural anticancer agents to prevent prostate cancer. However, a recent review [Ganry, 2005] of analytical epidemiological studies on the association of soy and other nutrients containing phytoestrogens with the risk of prostate cancer showed inconsistent results, with only a few studies reporting a decrease of prostate cancer risk in relation to the intake of legumes, soy food, and isoflavones. On the other hand, after World War II drastic changes in lifestyle and dietary habits have occurred in Asian countries, especially Japan. As pointed out by Ganmaa et al. [2003], these changes have been paralleled by a marked increase of both testicular and prostatic cancers. The authors claim that the 20-fold increase in the consumption of milk seen in the Japanese population after the war should be considered to at least partly explain the concurrent increase of prostate cancer incidence and mortality rates. This because the introduction of milk in an essentially no-meat/no-milk culture has provided a considerable and unprecedented source of saturated fats and estrogens that may, in turn, have an impact on prostate cancer development and progression.

An explanation of the linkage between lifestyle and/or dietary factors and prostate cancer risk might be the influence of these factors on sex steroids, particularly estrogens, with a special emphasis on circulating steroids and their intraprostatic levels and biotransformation patterns.

CIRCULATING STEROIDS

The concept that human prostate cancer represents a prototype of age-related, androgen-dependent tumor is widely accepted. Paradoxically, however, both total and bioavailable serum testosterone significantly decline with age, eventually leading to an inverse relationship between circulating testosterone and the risk of developing prostate cancer.

Many epidemiologic studies have investigated the association between circulating androgens and prostate cancer, but the resulting data have been inconsistent and largely inconclusive. A prospective study conducted in Rancho Bernardo, in California, revealed an association of elevated plasma estradiol and estrone with an increased risk of prostate cancer [Barrett-Connor et al., 1990]. Conversely, the Physician's Health Study [Gann et al., 1996] reported a significant trend of increasing prostate cancer risk with increasing plasma testosterone and an inverse association of estradiol with prostate cancer risk after adjusting for reciprocal levels and sex hormone binding globulin (SHBG). A subsequent meta-analysis by Eaton et al. [1999], presenting a quantitative review of the data from eight prospective epidemiological studies, clearly indicated that there are no significant differences in circulating hormones, either androgens or estrogens, between men who subsequently develop prostate cancer and those who remain free of disease. Two more recent nested case-control studies on plasma levels of both androgens and estrogens failed to show any association with risk of prostate cancer [Platz et al., 2005; Wirén et al., 2007]. Interestingly enough, one of the two studies has reported a positive association of plasma total testosterone with low-grade disease and an inverse association with high-grade disease [Platz et al., 2005].

Overall, the relationship between androgens and prostate cancer is generally considered highly likely based on the fact that a high proportion of patients having locally advanced prostate tumors initially respond to endocrine treatment, while they frequently acquire an androgen-refractory state after a variable time length (usually within 2 years from presentation). Hence, several authors have raised the question why has it been so difficult to prove that circulating androgens are associated with the risk of developing prostate cancer? The most obvious answer to this question is that circulating androgens are simply not associated with prostate cancer risk.

There is no doubt however that several issues related to measurement of plasma steroids, both androgens and estrogens, could be considered to explain this large inconsistency of data. In the first place, it is unlikely that a single assay of plasmatic androgens can be considered representative of average androgen levels over an etiologically relevant time of life. In this respect, it ought to be emphasized that the time scale of prostate carcinogenesis and cancer progression can span 35–40 years or longer. Therefore, the timing for the carcinogenetic impact of androgen and/or estrogen on human prostate should be allocated back to 20–30 years (or even earlier) prior to the clinical manifestation of the tumor, when serum androgens are higher and hence potentially relevant. On the other hand, there is consistent evidence that exposure of prostate cells to elevated estrogens early in uterine or perinatal life (a process referred to as developmental estrogenization or estrogen imprinting) may be responsible for permanent perturbations of prostate development that may eventually result in a propensity of prostate to develop precancerous or malignant lesions [McLachlan, 2001; Prins et al., 2006, 2007]. In addition, perinatal or neonatal exposure of prostate gland to endogenous estrogen and/or environmental estrogen-mimickers may directly affect androgen-driven prostate development or result in functional and morphological prostate alteration that may in turn predispose the tissue to an earlier onset of disease, including cancer [Jarred et al., 2000; Maffini et al., 2006]. One could speculate that developmental estrogenization induce significant changes in some embryonic stem cells that may, in turn, generate a population of adult imprinted prostate stem cells having a high susceptibility of developing cancer. All other things being equal, an increased adult prostate stem cell pool would elevate the risk that one stem cell might be initiated [Trosko, 2007].

In men, the balance between circulating levels of androgens and estrogens changes significantly upon aging [Vermeulen et al., 2002]. Plasma androgen levels decline whereas estrogen levels remain fairly constant, eventually leading to a decrease of androgen to estrogen ratio with age, and suggesting that estrogens may also have a role in prostate cancer.

All the above issues might contribute to justify, at least in part, the inconsistency of data on the association of plasma androgens and prostate cancer risk. However, a major problem remains to what extent levels of circulating steroids can be considered representative of the respective intraprostatic concentrations. Levels of sex steroids in peripheral target tissues, including breast and prostate, have been reported to be strikingly greater (10- to 100-fold) than the respective plasma values [van Landeghem et al., 1985; Castagnetta et al., 2002]. Furthermore, either normal or malignant steroid target tissues are equipped with key enzymes of steroid metabolism, including several hydroxysteroid dehydrogenases, 5α-reductases, hydroxylases, sulfotransferases, sulfatases, and aromatase. Therefore, a different expression and/or activity of these enzymes may produce a differential accumulation of biologically active metabolites, eventually leading to profiles of intratissue steroids that may substantially diverge from their plasmatic counterpart. Simpson and colleagues [Simpson et al., 2005] have pointed out that estrogens circulating in men and in postmenopausal women are not the drivers of estrogen action; they instead represent a reflection of estrogen uptake and biotransformation at extragonadal sites, including prostate. In other words, they are reactive rather than proactive [Labrie et al., 2003].

Early studies of our own group have suggested that urinary profiles of estrogens may be used to better categorize breast cancer patients in relation to their prognosis and response to treatment [Castagnetta et al., 1981]. In this respect, metabolic profiles of estrogens in urine appear to be comparable to those obtained by measurement of their intratissue concentrations, and can be potentially used as an indirect indication of endogenous estrogens. We have recently reported [Muti et al., 2002] that a lower risk of developing prostate cancer is associated to a higher ratio of 2-hydroxyestrone [that has been originally proposed to act as anticancer estrogen and accordingly named the good estrogen: Bradlow et al., 1996] to 16α-hydroxyestrone [that has been claimed to be genotoxic: Bradlow et al., 1985] in urine.

LOCAL STEROID BIOSYNTHESIS AND METABOLISM

As emphasized above, the balance between androgens and estrogens in individual target tissues may differ significantly from that in the

plasma, being strictly dependent upon the expression and/or the activity of steroid metabolizing enzymes, including 5α-reductase and aromatase. In particular, the assessment of local synthesis and metabolism of sex steroids has become increasingly important in the understanding of both breast and prostate cancer, also because abnormal levels of estradiol and/or estrone and, especially, of some of their hydroxylated derivatives have been implicated in tumor development and progression [Yager and Davidson, 2006].

As compared to breast, only a few early studies have assessed intraprostatic levels of sex hormones [Farnsworth and Brown, 1976; Geller et al., 1978]. Although these studies present some interesting preliminary observation on how prostate cells, either epithelial or stromal, metabolize androgens, they are largely insufficient and not significant enough to draw any conclusive inference.

Cavalieri et al. [1997] have recently reviewed experimental evidence in support of their hypothesis that locally produced estrogen metabolites, precisely catechol estrogen-3,4-quinones, may react with DNA to form depurinating adducts. After adduct release from DNA, error-prone base excision repair of the resulting apurinic sites may eventually lead to mutations that can initiate several types of human solid tumors, including prostate.

Estrogen patterns in target tissues and cells are far more complex than one could expect on the basis of circulating estrogen species. The two major plasma estrogens, estradiol (E2) and estrone (E1), are readily interconverted in the tissue through the action of different 17βhydroxysteroid dehydrogenase enzymes (17βHSDs). Both these classical estrogens can be hydroxylated at the C-2/C-4 positions, giving rise to the so-called catecholestrogens (CCE), namely the 2-hydroxy and 4-hydroxy estrogens. The CCE can be further metabolized by catechol-*O*-methyltransferase to their methoxy derivatives. Two mutually exclusive pathways, the 16α- and the 16β-hydroxylation, may act to produce a series of additional metabolites of either E2 or E1, of variable or as yet unexplored biological activity. In particular, 16αOHE1, along with other oxidative estrogen metabolites, has been repeatedly implicated in human breast carcinogenesis [Yager and Davidson, 2006]. Recently, estrogen derivatives of both 16α-hydroxylation (e.g., 16αOHE1 and 17epiestriol) and 16β-hydroxylation (e.g., 16βOHE1) pathways have been reported to be tumorigenic in endometrium of young adult mice [Takahashi et al., 2004]. Interestingly, in a recent randomized, dietary intervention study (the MeDiet study), we have indicated that a traditional Mediterranean diet markedly reduces (by more than 40%) urinary estrogen levels in healthy postmenopausal women [Carruba et al., 2006]. It should be noted that, in this study, the majority of estrogens in urine was represented by hydroxy and methoxy derivatives of either E2 or E3 (notably 2OHE2, 17epiestriol, and 16ketoE2), while classical estrogens (namely E2 and E1) accounted for a mere 0.5% of total urinary estrogens as a sum. This picture is cognate to what we have observed by measuring tissue levels of estrogens in both nontumoral and malignant human breast, where hydroxylated estrogens accounted for over 80% of all estrogens in either condition [Castagnetta et al., 2002]. This similarity reinforces the view that urinary estrogens can be used as indirect indicators of patterns of intratissue estrogens. Unfortunately, no direct, unequivocal evaluation of estrogen intraprostatic levels as been so far provided.

In vitro studies have explored expression and activity of steroid enzymes in human prostate cancer cells to appraise the impact of local metabolism on the bioavailability of active hormones to malignant prostate cells. Vihko et al. [2006], using both androgen-sensitive and androgen-independent LNCaP prostate cancer cells as a model system, have reported a marked decrease of oxidative activity and a corresponding increase of reductive activity of 17βHSD in the progression towards an androgen-refractory state. This would result in the accumulation of bioactive estrogen (e.g., estradiol) in androgen-independent cells, while oxidized estrogens (e.g., estrone) would be prevalent in androgen-sensitive cells. We have previously inspected rates and direction of sex steroid metabolism in human prostate cancer cells using an original approach that allows the simultaneous measurement of several enzyme activities in *intact* cultured cells [Castagnetta et al., 1994]. In brief, androgen-responsive LNCaP cells show consistent formation of the bioactive androgen dihydrotestosterone (DHT) and its derivatives, 3α/3β-androstanediol, along with estrogen while androgen-resistant PC3 cells exhibit a largely dominant 17βoxidation,

leading to the production of oxidized androgen (androstenedione) and estrogen (estrone) derivatives. We have revealed that these highly divergent patterns of sex steroid metabolism are a reflection of a differential expression and activity several steroid enzymes, including 17βHSDs, 3α/3βHSDs, and 5α-reductase, in the two cell lines [Carruba et al., 1997]. This finding is of outmost importance, since it underpins the concept that local steroid formation and metabolism is crucial to determine the overall biological impact of steroid hormones in individual target (cancer) tissues and cells.

Local estrogen biosynthesis may occur via aromatization of androgens through the aromatase enzyme. In this respect, aromatase may act as a critical regulator of the balance between androgens and estrogens at both tissue and plasma level. While there is consistent evidence that aberrant aromatase may play a key role in development and/or progression of human breast cancer, aromatase expression and activity in either nontumoral or malignant human prostate remain equivocal. As evidence is accumulating that the prostate gland is a primary target for direct estrogenic activity and that local synthesis of estrogen may be significant in prostate cancer, it might important to determine whether or not aromatase is locally expressed and to identify any change that may occur with prostatic disease.

Our in vitro studies have revealed that LNCaP prostate cancer cells contain aromatase activity, even though to a significantly lower extent than that observed in MCF7 human mammary carcinoma cells [Castagnetta et al., 1997]. In a more recent work, Ellem and Risbridger [2006] have assessed aromatase RNA, protein, and enzyme activity in benign and malignant human prostate tissues, as well as in human prostate cancer cell lines. The authors found that, aromatase expression was confined to the stroma in nonmalignant prostate tissues, while it was expressed throughout microdissected epithelial tumor cells and prostate cancer cell lines. This evidence provides a basis for a better understanding of estrogen role in human prostate cancer and for the potential development of alternative strategies for both prevention and treatment of this disease.

It is noteworthy that polymorphisms of genes encoding for key steroid enzymes, along with their epigenetic silencing or structural alteration, may result in a profound perturbation of enzyme expression and activity. To date, however, a relatively small number of studies have been conducted to address this issue and results have been often contradictory.

Several polymorphisms of the SRD5A2, CYP1A1, CYP1B1, CYP3A4, CYP17, CYP19, HSD3B1, HSD3B2, HSB17B3 genes have been studied. At least three different polymorphisms in the CYP1A1 gene, encoding the 2-hydroxylase enzyme, have been associated with an increased risk of developing prostate cancer, with the exception of one single nucleotide polymorphisms (SNP) that has been reported to have an opposite impact on prostate cancer risk in Japanese and a Caucasian American population [Murata et al., 2001; Chang et al., 2003]. Comparable finding was obtained for the CYP1B1 gene that encodes for the 4-hydroxylase enzyme [Nock et al., 2006]. It is worth noting that these polymorphisms usually induce a prolonged half-life and activity of both enzymes, and, hence, a sustained carcinogenetic potential of their products, respectively 2- and 4-hydroxy estradiol. The gene SRD5A2, encoding for the 5α-reductase type 2 enzyme, contains several polymorphic regions. Of course the effect of these polymorphisms on prostate cancer risk has been inspected with interest in some studies, as this enzyme governs the conversion of testosterone into its biologically active derivative DHT in the prostate. However, the resulting evidence indicates a weak to modest increase of risk at its best and therefore does not apparently support a pivotal role of DHT in prostate cancer development and progression [Cunningham et al., 2007]. Other variants of candidate genes along the androgen metabolic pathways have been proposed to affect prostate cancer risk. In a recent paper, Mononen et al. [2006] have reported a novel SNP in the CYP19A1 aromatase gene, which is mildly though significantly associated with prostate cancer risk, suggesting that this SNP may encode for a variant enzyme having higher activity and, hence, resulting in lower androgen levels.

The functional significance of these polymorphisms is not clarified and remains fairly speculative. Many aspects concur to make the results of these studies inconsistent, but probably the most important issue is how these polymorphic genes relate to each other and to levels of circulating hormones. In this respect, the evaluation of haplotypes and diplotypes

is being exploited to determine the impact of polymorphic genes on the production and/or activity of steroid enzymes associated with individual risk of prostate cancer.

ESTROGEN AND PROSTATE TUMOR DEVELOPMENT AND GROWTH

Experimental Animals

It is known that, of the thousands of mammals with prostates, only humans and dogs have a considerable incidence of spontaneous prostate cancer. Coffey [2001], this peculiar brotherhood gets back to approximately 12,000 years ago when humans brought the dog into their society to increase their hunting ability. As a consequence, dogs were easily domesticated and shared with humans their diet, marking a major shift in eating patterns from fresh vegetables and fruits to stored meat and dairy products. This would imply that dietary factors may have acted through several thousand years to determine the present propensity of humans' and dogs' prostate to spontaneously develop cancer. As already pointed out, this could have occurred because dietary components may have induced profound modifications in endogenous hormones, notably androgens and estrogens, through dramatic changes in their local metabolism and circulating levels.

Early studies reported that long-term administration of testosterone to rats results in the induction of prostate tumors, though in a limited proportion of cases and in some but not all rat strains [Noble, 1982; Pollard et al., 1982; Bosland, 1992]. These data would suggest that testosterone act as a complete carcinogen on the rat prostate.

In the Noble rats, however, the administration of testosterone and estradiol, either in sequence or combined, induces the appearance of both ductal and acinar epithelial dysplasia, a precancerous lesion similar to human intraepithelial neoplasia (PIN), followed by the development of adenocarcinomas of the dorsolateral prostate in 100% of the animals [Leav et al., 1989; Bosland et al., 1995]. The mechanisms underlying the hormonal induction of prostate cancer in rats remain as yet undefined. As far as estrogens are concerned, there is indication that both receptor-mediated and nonreceptor effects may be involved. Interestingly, the pure antiestrogen ICI-182,780 completely prevented the development of dysplasia in dorsolateral prostate of rats exposed for 16 weeks to a combination of testosterone and estradiol [Thompson et al., 2002]. Although this finding would suggest that the use of this estrogen antagonist abrogates receptor-mediated estrogen effects, ICI-182,780 also induces a block of hyperprolactinemia in treated rats and, therefore, the significance of this evidence could not be clearly dissected. Other studies have revealed that Noble rats treated with testosterone and estradiol or with testosterone and the synthetic estrogen diethylstilbestrol (DES) for 16 weeks accumulate estradiol and the estrogenic androgen 5α-androstane-3β,17β-diol (3α-androstanediol, 3α-diol), respectively, in dorsolateral or ventral prostate [Leav et al., 1989; Ofner et al., 1992]. This combined evidence strongly supports the view that androgen and estrogen treatment of animals creates an estrogenic milieu in rat prostates, eventually leading to the development of epithelial dysplasia and adenocarcinoma in the Noble rat model. In an elegant model, Wang et al. [2000] rescued rudimental pelvic organs of a Rb KO mice and grafted them into male adult nude mice to develop functional prostatic tissue. The authors indicated that tissue recombinants of Rb −/− prostate epithelium and wild type urogenital mesenchyme developed dysplastic and malignant lesions 5–8 weeks after host animals received silastic implants of testosterone and estradiol.

Notwithstanding, it should be considered that the rodent prostate is by no mean equivalent to human or dog prostate, being composed of paired dorsal, lateral, ventral and anterior lobes. Consequently, results of studies on the hormonal induction of prostate cancer in rat models should be taken cautiously. The administration of pharmacological doses of estradiol, alone or in combination with the estrogenic androgen 3α-diol, to castrated dogs results in the development of a well-defined proliferative prostatic lesion, referred to as squamous metaplasia [Merk et al., 1986]. This implies that atrophic canine prostate maintains the ability to respond to sex hormones, especially estrogen.

In Vitro Studies

Despite numerous previous studies have assessed the proliferative effects of sex hormones in cultured prostate cancer cells, the resulting

data are still featured by some inconsistency. Several reports have indicated that androgens markedly stimulate prostate cancer cell growth [Sonnenschein et al., 1989; Iguchi et al., 1990]. However, unequivocal evidence for a direct increase of DNA synthesis in cultured prostate tumor cells in response to bioactive androgens is surprisingly rare. In addition, findings obtained in cell model systems are difficult to interpret also because many variables, including experimental conditions, age of cells in culture, and presence of endogenous hormones, may considerably affect the results. We have previously reported that physiological estrogen may either stimulate or decrease proliferative activity of androgen-sensitive LNCaP and androgen-refractory PC3 prostate cells, respectively, and that these effects are predominantly receptor-mediated being completely abolished by the simultaneous addition of the pure estrogen antagonist ICI-182,780 [Carruba et al., 1994; Castagnetta et al., 1995]. This would suggest that estrogen may regulate prostate tumor cell growth albeit the cells have become androgen-resistant, as also indicated by the significant response rates to the systemic administration of estrogens observed in prostate cancer patients having a metastatic, androgen-refractory disease [Ockrim et al., 2006]. Given that proliferative effects of estrogens on human prostate cancer cells in culture appear to be mostly receptor-mediated, it would be important to assess sex steroid receptor content and the balanced expression of different steroid receptors and their variants.

ESTROGEN RECEPTORS AND PROSTATE CANCER

It is well established that estrogens are important regulators not only of development and function of the reproductive system and the mammary gland, but also of many other "non-classical" human target tissues, including brain, liver, bone, cardiovascular system, and adipose tissue [Mueller and Korach, 2001]. Studies conducted either in vivo or in vitro have consistently indicated that classical effects of sex hormones are mediated through specific intracellular receptors that belong to the superfamily of nuclear receptors [Escriva et al., 2004]. On the other hand, evidence is accumulating that estrogens and their receptors may combine or act unconnectedly to exploit an amazing array of both genomic and nongenomic, either ligand dependent or independent, actions [Levin, 2001].

Two major estrogen receptor (ER) types have been identified, the classical ERα and the recently discovered ERβ. They are encoded by separate genes, *ESR1* and *ESR2*, which are located at different chromosomal sites. There is increasing evidence that several splicing variants and mutants exist for each receptor in both normal and diseased tissues, although these variants are frequently coexpressed with the wild type receptors and, hence, their function is difficult to dissect [Herynk and Fuqua, 2004].

The ERα and ERβ have distinct tissue-specific expression and exploit a variety of physiological activities in several human tissues [Gustafsson, 2003]. Both ERs typically act as nuclear transcription factors, with their respective patterns of gene regulation and function being strictly dependent on their expression levels and balance in individual tissues. Differences in ligand binding, heterodimerization, transactivation, and estrogen response element (ERE) activity, may determine the ultimate direct effects of estrogen in target tissues and cells. In this respect, an alteration of ERα and ERβ balance may be primarily implicated in the etiology of various diseases, including cancer.

Both ERα and ERβ are expressed in the adult human prostate, although ERβ is localized predominantly to the basal epithelial compartment and, to a lesser extent, to stromal cells, while ERα appears to be generally located in the stromal compartment. Recent studies with estrogen receptor knockout (ERKO) mice have helped to better understand the function of either receptor and to unravel their role in both normal and diseased tissues. In particular, the onset of prostatic epithelial hyperplasia has been reported in the adult ERβ knockout (βERKO), while it has not been observed in the ERα knockout (αERKO) mice [Weihua et al., 2001]. This evidence suggests that ERβ may play a protective role against abnormal proliferation of prostate epithelial cells. Interestingly enough, both synthetic antiestrogen (toremifene) and natural phytoestrogen (genistein) prevent development of prostate cancer in the transgenic adenocarcinoma mouse prostate (TRAMP) mouse model acting as ERβ agonists [Mentor-Marcel et al., 2001; Raghow et al., 2002].

The expression of ERα and ERβ (at both transcript and protein level) has been

scrutinized by different studies in *nontumoral*, hyperplastic, and malignant human prostate tissues. The majority of studies have consistently revealed a substantial decrease of ERβ expression in the malignant prostate as compared with benign or normal tissues, while ERα expression remained unchanged or even increased [reviewed by Bardin et al., 2004]. There is indirect evidence to suggest that the two receptors are reciprocally regulated and that the protective role of ERβ may be based on direct (ERβ-specific) and/or indirect (through regulation of ERα) effects limiting cell proliferation. Therefore, loss of ERβ expression may represent a crucial step in an estrogen-driven prostate cancer progression.

Recently, selective ERα and ERβ ligands, whose agonist or antagonist activity depends specifically on cellular context and promoter sequences of regulated genes, have been identified for each receptor and designated as selective estrogen receptor modulators (SERMs). SERMs, along with synthetic estrogens and antagonists have recently emerged as promising agents in both prevention and treatment of human prostate cancer [Ho, 2004].

Unfortunately little is known about the expression and the functional meaning of splicing variants of either receptor in the human prostate. It has been shown that two ERα variants, the hERα46 and hERα36, are powerful inhibitors of wild type hERα66 transactivation, the former being located almost exclusively in cell nuclei and the latter being predominantly associated to the plasma membrane where it transduces both estrogen and antiestrogen signaling, including activation of mitogen-activated protein kinase [Penot et al., 2005; Wang et al., 2006]. On the other hand, several relatively abundant ERβ isoforms have been described, with two of them, the hERβ2 and hERβ5, being reported to inhibit the transcriptional activity of ERα [Peng et al., 2003]. Overall, this finding suggests that differentially expressed variants of both ERα and ERβ may eventually modulate estrogen action in target tissues, including prostate.

Epigenetic alterations of genes encoding both ERα and ERβ have been described in human prostate cancer. In particular, hypermethylation of the promoter region and silencing of the gene have been reported to occur for both receptors in prostate cancer tissues and cells [Li et al., 2004]. In addition, direct acetylation on a specific motif of ERα has been associated to the promotion of contact-independent growth in cancer cells [Leader et al., 2006; Popov et al., 2007]. These changes may also have a role in determining the net biological effects of estrogen in either normal or diseased prostate gland.

CONCLUSIONS

Doubtlessly, since the pioneering work of Charles Huggins, prostate cancer has become a paradigm of androgen-dependent tumor, with androgens being universally considered critical regulators of normal prostatic function and inducers of malignant prostate growth. This general concept has endured against a bulk of experimental evidence suggesting that estrogen and other growth factors may play a role in the development and/or progression of human prostate cancer. Presently, the view of androgens as all-seasoned and sole determinants of prostate tumor development and progression appears to be a never-ending persuasion that has, faultily, lead to neglect different areas of research with promising perspectives for both treatment and prevention of this disease.

As already emphasized in this article, circulating androgen can be locally transformed into estrogen through the activity of the aromatase enzyme, implying that androgen aromatization may be in part responsible for androgen action in nontumoral and malignant prostate. Interestingly, lifelong exposure of aromatase knockout (ArKO) mouse to elevated androgens resulted in the development of prostatic hyperplasia, although no malignant changes could be detected in the prostate at any time [McPherson et al., 2001].

The assumption that androgen effects in the prostate may be mediated in part through their aromatization to estrogens is corroborated by some data from spare, unrelated studies. Firstly, in the Prostate Cancer Prevention Trial the arm receiving finasteride, a 5α-reductase inhibitor, revealed a significantly greater incidence of high-grade (Gleason score $\geq$7) prostate tumor than in the placebo group [Thompson et al., 2003]. This unexpected finding could be a result of locally elevated estrogen produced through aromatization of testosterone that has accumulated in prostatic tissues as a consequence of the 5α-reductase inhibition. In second place, clinical studies of non-metastatic prostate cancer have shown that higher Gleason

score is associated with lower levels of either total or free testosterone [Schatzl et al., 2001]. In a recent paper, Platz et al. [2005] revealed a positive association of plasma total testosterone and free testosterone with low grade prostate cancer and an inverse association with high-grade prostate cancer. Lastly, obese men with prostate cancer, aged 50 years or less, have a significantly higher risk of high-grade disease [Rohrmann et al., 2003]. Lower levels of circulating androgen may be associated to low intraprostatic concentrations of testosterone induced by abnormally high aromatization to estrogens, as it occurs in obesity. Overall, this finding supports the conception that locally elevated androgen aromatization into estrogen may be implicated in prostate malignancies. It is intriguing to speculate that the inverse relationship of androgen and prostate tumor grade may also be explained by the assumption that androgens act as differentiating agents for prostate cancer stem cells, with low androgen levels being permissive of symmetrical cancer stem cell division and eventually of aberrant tumor cell growth and high-grade prostate cancer.

Several hypothetical models have been proposed to explain the role of sex steroids, both androgens and estrogens, in the prostate carcinogenesis and tumor progression.

Bosland [2000] has presented a multifactorial model where androgens act as strong tumor promoters through receptor-mediated mechanisms to complete the malignant transformation initiated by potent endogenous genotoxic compounds (notably estrogens) and/or as yet unidentified weak environmental carcinogens. Although this hypothesis is founded on solid experimental grounds, it however does not take into account the potential role of estrogen in both the promotion and progression phases of prostate cancer, whereby these hormones may induce significant alteration of either genome or epigenome.

In another theoretical model, it is hypothesized that estradiol is essential for inducing prostate cancer cell growth through the formation of telomeres, while testosterone upregulates proapoptotic proteins, a process that is counteracted or prevented by the action of DHT (Friedman, 2005). In this model estrogen-induced telomerase is a result of estradiol binding to an ERα/ERβ heterodimer, while testosterone-driven apoptosis and DHT-dependent prostate tumor cell growth are accomplished by binding respectively to a membrane and an intracellular androgen receptor. While this model does not address mechanisms implicated in the genetic mutations that initiate prostate carcinogenesis, it however delineates growth regulation of prostate cancer.

We have recently proposed a model for prostate cancer progression [Carruba, 2006] where, after an initial hormone-responsive phase, tumor cells become androgen-resistant as a consequence of AR mutation or alteration of a diverse androgen signaling. The concurrent loss of ERβ, possibly induced by hypermethylation in the gene promoter region, creates an estrogen-sensitive condition where growth of cancer cells is stimulated by estrogen that is produced locally by aromatase and that acts through ERα. Should this speculative model be confirmed, it may represent a basis to develop new strategies for both prevention and treatment of this malignancy based on the use of aromatase inhibitors, ERα antagonists, and ERβ-specific ligands, alone or in combination, depending on the estrogen sensitive status of individual cancer tissues.

Although significant advances have been attained in prostate cancer research, there is no cogent and comprehensive model to elucidate biomolecular processes responsible for initiation, promotion and progression of human prostate. Several interdependent factors, including the interplay of estrogen and androgen, changes and polymorphisms in biosynthesis and transformation of intraprostatic hormones, alteration of androgen signaling or local balance between estrogen receptor isoforms and their splicing variants, all being markedly affected by lifestyle (diet) factors and genetic determinants, are critical players in prostate tumor development and progression.

When we are unable of placing and integrating distinct experimental data in a networked context, while keeping an eye on the whole picture, we would not get significant insight from our research. In this respect, estrogen represents a pointed example of how a key factor in prostate cancer has received little attention for years being eclipsed by an androgen-dominated interest.

REFERENCES

American Cancer Society. 2007. Cancer facts and figures 2007. Atlanta: American Cancer Society.

Bardin A, Boulle N, Lazennec G, Vignon F, Pujol P. 2004. Loss of ERβ expression as a common step in estrogen-dependent tumor progression. Endocr Related Cancer 11: 537–551.

Barrett-Connor E, Garland C, McPhillips JB, Khaw KT, Wingard DL. 1990. A prospective, population-based study of androstenedione, estrogens, and prostatic cancer. Cancer Res 50:169–173.

Bosland MC. 1992. Animal models for the study of prostate carcinogenesis. J Cell Biochem Suppl 16H:89–98.

Bosland MC. 2000. The role of steroid hormones in prostate carcinogenesis. J Natl Cancer Inst Monogr 27:39–66.

Bosland MC, Ford H, Horton L. 1995. Induction at high incidence of ductal prostate adenocarcinomas in NBL/Cr and Sprague–Dawley Hsd:SD rats treated with a combination of testosterone and estradiol-17β or diethylstilbestrol. Carcinogenesis 16:1311–1317.

Bradlow HL, Hershcopf RJ, Martucci CP, Fishman J. 1985. Estradiol 16α-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: A possible model for the hormonal etiology of breast cancer in humans. Proc Natl Acad Sci USA 82:6295–6299.

Bradlow H, Telang N, Sepkovic D, Osborne M. 1996. 2-Hydroxyestrone: The "good" estrogen. J Endocrinol 150 (Suppl):S259–S265.

Cancel-Tassin G, Cussenot O. 2005. Genetic susceptibility to prostate cancer. Br J Urol Int 96:1380–1385.

Carruba G. 2006. Estrogen and mechanisms of prostate cancer progression. Ann NY Acad Sci 1089:201–217.

Carruba G, Pfeffer U, Fecarotta E, Coviello D, D'Amato E, Lo Casto M, Vidali G, Castagnetta L. 1994. Estradiol inhibits growth of hormone non responsive PC3 human prostate cancer cells. Cancer Res 54:1190–1193.

Carruba G, Adamski J, Calabrò M, Miceli MD, Cataliotti A, Bellavia V, Lo Bue A, Polito L, Castagnetta L. 1997. Molecular expression of 17βhydroxysteroid dehydrogenase types in relation to their activity in human prostate cancer cells. Mol Cell Endocrinol 135:51–57.

Carruba G, Granata OM, Pala V, Campisi I, Agostara B, Cusimano R, Ravazzolo B, Traina A. 2006. A traditional mediterranean diet decreases endogenous estrogens in healthy postmenopausal women. Nutr Cancer 56:253–259.

Castagnetta L, D'Agostino C, Lo Casto M, Traina A, Leake RE. 1981. Breast cancer: A comparison of response to endocrine therapy and oestrogen excretion patterns including unusual metabolites. Br J Cancer 44:670–674.

Castagnetta L, Granata OM, Polito L, Blasi L, Cannella S, Carruba G. 1994. Different conversion metabolic rates of testosterone are associated to hormone-sensitive status and -response of human prostate cancer cells. J Steroid Biochem 49:351–357.

Castagnetta L, Miceli MD, Sorci C, Pfeffer U, Farruggio R, Oliveri G, Calabrò M, Carruba G. 1995. Growth of LNCaP human prostate cancer cells is stimulated by estradiol via its own receptor. Endocrinology 136:2309–2319.

Castagnetta L, Granata OM, Bellavia V, Amodio R, Scaccianoce E, Notarbartolo M, Follari MR, Miceli MD, Carruba G. 1997. Product of aromatase activity in intact LNCaP and MCF7 human cancer cells. J Steroid Biochem Mol Biol 61:287–292.

Castagnetta L, Granata OM, Traina A, Ravazzolo B, Amoroso M, Miele M, Bellavia V, Agostara B, Carruba G. 2002. Tissue content of hydroxyestrogens in relation to survival of breast cancer patients. Clin Cancer Res 8: 3146–3155.

Cavalieri E, Stack D, Devanesan P, Todorovic R, Dwivedy I, Higginbotham S, Johansson S, Patil K, Gross M, Gooden J, Ramanathan R, Cerny R, Rogan E. 1997. Molecular origin of cancer: Catechol estrogen-3, 4-quinones as endogenous tumor initiators. Proc Natl Acad Sci USA 94:10937–10942.

Chang BL, Zheng SL, Isaacs SD, Turner A, Hawkins GA, Wiley KE, Bleecker ER, Walsh PC, Meyers DA, Isaacs WB, Xu J. 2003. Polymorphisms in the CYP1A1 gene are associated with prostate cancer risk. Int J Cancer 106: 375–378.

Coffey DS. 2001. Similarities of prostate and breast cancer: Evolution, diet, and estrogens. Urology 57 (Suppl 4A): 31–38.

Cook LS, Goldoft M, Schwartz SM, Weiss NS. 1999. Incidence of adenocarcinoma of the prostate in Asian immigrants to the United States and their descendants. J Urol 161:152–155.

Cunningham JM, Hebbring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN. 2007. Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. Cancer Epidemiol Biomarkers Prev 16: 969–978.

Eaton NE, Reeves GK, Appleby PN, Key TJ. 1999. Endogenous sex hormones and prostate cancer: A quantitative review of prospective studies. Br J Cancer 80:930–934.

Ellem SJ, Risbridger GP. 2006. Aromatase and prostate cancer. Minerva Endocrinol 31:1–12.

Escriva H, Bertrand S, Laudet V. 2004. The evolution of the nuclear receptor superfamily. Essays Biochem 40:11–26.

Farnsworth WE, Brown JR. 1976. Androgen of the human prostate. Endocr Res Commun 3:105–117.

Friedman AE. 2005. The estradiol-dihydrotestosterone model of prostate cancer. Theor Biol Med Model 2(1):10.

Ganmaa D, Li XM, Qin LQ, Wang PY, Takeda M, Sato A. 2003. The experience of Japan as a clue to the etiology of testicular and prostatic cancers. Med Hypotheses 60: 724–730.

Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. 1996. Prospective study of sex hormone levels and risk of prostate cancer. J Natl Cancer Inst 88:1118–1126.

Ganry O. 2005. Phytoestrogens and prostate cancer risk. Prev Med 41:1–6.

Geller J, Albert J, de la Vega D, Loza D, Stoeltzing W. 1978. Dihydrotestosterone concentration in prostate cancer tissue as a predictor of tumor differentiation and hormonal dependency. Cancer Res 38:4349–4352.

Giovannucci E, Leitzman M, Speigelman D, Rimm EB, Colditz GA, Stampfer MJ, Willett WC. 1998. A prospective study of physical activity and prostate cancer in male health professionals. Cancer Res 58:5117–5122.

Gustafsson JA. 2003. What pharmacologists can learn from recent advances in estrogen signalling. Trends Pharmacol Sci 24:479–495.

Herynk MH, Fuqua SAW. 2004. Estrogen receptor mutations in human disease. Endocr Rev 25:869–898.

Ho SM. 2004. Estrogens and antiestrogens: Key mediators of prostate carcinogenesis and new therapeutic candidates. J Cell Biochem 91:491–503.

Hsing AW, Chokkalingam AP. 2006. Prostate cancer epidemiology. Front Biosci 11:1388–1413.

Iguchi T, Fukazawa Y, Tani N, Sato T, Ozawa S, Takasugi N, Shuin T, Kubotal Y, Petrov V. 1990. Effect of some hormonally active steroids upon the growth of LNCaP human prostate tumour cells *in vitro*. Cancer J 3:184–191.

Jarred RA, Cancilla B, Prins GS, Thayer KA, Cunha GR, Risbridger GP. 2000. Evidence that estrogens directly alter androgen-regulated prostate development. Endocrinology 141:3471–3477.

Kolonel LN. 1996. Nutrition and prostate cancer. Cancer Causes Control 7:83–94.

Kolonel LN, Nomura AM, Cooney RV. 1999. Dietary fat and prostate cancer: Current status. J Natl Cancer Inst 91: 414–428.

Kolonel LN, Altshuler D, Henderson BE. 2004. The multiethnic cohort study: Exploring genes, lifestyle and cancer risk. Nat Rev Cancer 4:519–527.

Kuiper GGJM, Carlquist M, Gustafsson JA. 1998. Estrogen is a male and female hormone. Sci Med 5:36–45.

Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX, Pellitier G. 2003. Endocrine and intracrine sources of androgens in women: Inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev 24:152–182.

Leader JE, Wang C, Popov VM, Fu M, Pestell RG. 2006. Epigenetics and the estrogen receptor. Ann NY Acad Sci 1089:73–87.

Leav I, Merk FB, Kwan PW, Ho SM. 1989. Androgen supported estrogen-enhanced epithelial proliferation in the prostates of intact Noble rats. Prostate 15:23–40.

Levin ER. 2001. Cell Localization, physiology and nongenomic actions of estrogen receptors. J Appl Physiol 91:1860–1867.

Li LC, Okino ST, Dahiya R. 2004. DNA methylation in prostate cancer. Biochim Biophys Acta 1704:87–102.

Maffini MV, Rubin BS, Sonnenschein C, Soto AM. 2006. Endocrine disruptors and reproductive health: The case of bisphenol-A. Mol Cell Endocrinol 254–255:179–186.

McLachlan JA. 2001. Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. Endocr Rev 22:319–341.

McPherson SJ, Wang H, Jones ME, Pedersen J, Iismaa TP, Wreford N, Simpson ER, Risbridger GP. 2001. Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland. Endocrinology 142:2458–2467.

Mentor-Marcel R, Lamartiniere CA, Eltoum IE, Greenberg NM, Elgavish A. 2001. Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). Cancer Res 61:6777–6782.

Merk FB, Warhol MJ, Kwan PW, Leav I, Alroy J, Ofner P, Pinkus GS. 1986. Multiple phenotypes of prostatic glandular cells in castrated dogs after individual or combined treatment with androgen and estrogen. Morphometric, ultrastructural, and cytochemical distinctions. Lab Invest 54:442–456.

Mononen N, Seppala EH, Duggal P, Autio V, Ikonen T, Ellonen P, Saharinen J, Saarela J, Vihinen M, Tammela TL, Kallioniemi O, Bailey-Wilson JE, Schleutker J. 2006. Profiling genetic variation along the androgen biosynthesis and metabolism pathways implicates several single nucleotide polymorphisms and their combinations as prostate cancer risk factors. Cancer Res 66:743–747.

Mueller SO, Korach SK. 2001. Estrogen receptors and endocrine diseases: Lessons from estrogen receptor knockout mice. Curr Opin Pharmacol 1:613–619.

Murata M, Watanabe M, Yamanaka M, Kubota Y, Ito H, Nagao M, Katoh T, Kamataki T, Kawamura J, Yatani R, Shiraishi T. 2001. Genetic polymorphisms in cytochrome P450 (CYP) 1A1. CYP1A2, CYP2E1, glutathione *S*-transferase (GST) M1 and GSTT1 and susceptibility to prostate cancer in the Japanese population. Cancer Lett 165:171–177.

Muti P, Westerlind K, Wu T, Grimaldi T, De Berry J III, Schunemann H, Freudenheim JL, Hill H, Carruba G, Bradlow L. 2002. Urinary estrogen metabolites and prostate cancer: A case–control study in the United States. Cancer Causes Control 13:947–955.

Noble RL. 1982. Prostate carcinoma of the Nb rat in relation to hormones. Int Rev Exp Pathol 23:113–159.

Nock NL, Cicek MS, Li L, Liu X, Rybicki BA, Moreira A, Plummer SJ, Casey G, Witte JS. 2006. Polymorphisms in estrogen bioactivation, detoxification and oxidative DNA base excision repair genes and prostate cancer risk. Carcinogenesis 27:1842–1848.

Ockrim J, Lalani E-N, Aubel P. 2006. Therapy insight: Parenteral estrogen treatment for prostate cancer—A new dawn for an old therapy. Nat Clin Pract Oncol 3: 552–563.

Ofner P, Bosland MC, Vena RL. 1992. Differential effects of diethylstilbestrol and estradiol-17β in combination with testosterone on rat prostate lobes. Toxicol Appl Pharmacol 112:300–309.

Parkin DM, Bray F, Ferlay J, Pisani P. 2005. Global cancer statistics, 2002. CA Cancer J Clin 55:74–108.

Peng B, Lu B, Leygue E, Murphy LC. 2003. Putative functional characteristics of human estrogen receptor-beta isoforms. J Mol Endocrinol 30:13–29.

Penot G, Le Péron C, Mérot Y, Grimaud-Fanouillère E, Ferrière F, Boujrad N, Kah O, Saligaut C, Ducouret B, Métivier R, Flouriot G. 2005. The human estrogen receptor-α isoform hERα46 antagonizes the proliferative influence of hERα66 in MCF7 breast cancer cells. Endocrinology 146:5474–5484.

Platz EA, Leitzmann MF, Rifai N, Kantoff PW, Chen YC, Stampfer MJ, Willett WC, Giovannucci E. 2005. 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 14:1262–1269.

Pollard M, Luckert PH, Schmidt MA. 1982. Induction of prostate adenocarcinomas in Lobund Wistar rats by testosterone. Prostate 3:563–568.

Popov VM, Wang C, Shirley LA, Rosenberg A, Li S, Nevalainen M, Fu M, Pestell RG. 2007. The functional significance of nuclear receptor acetylation. Steroids 72: 221–230.

Prins GS, Huang L, Birch L, Pu Y. 2006. The role of estrogens in normal and abnormal development of the prostate gland. Ann NY Acad Sci 2006. 1089:1–13.

Prins GS, Birch L, Tang WY, Ho SM. 2007. Developmental estrogen exposures predispose to prostate carcinogenesis with aging. Reprod Toxicol 23:374–382.

Raghow S, Hooshdaran MZ, Katiyar S, Steiner MS. 2002. Toremifene prevents prostate cancer in the transgenic

adenocarcinoma of mouse prostate model. Cancer Res 62: 1370–1376.

Rohrmann S, Roberts WW, Walsh PC, Platz EA. 2003. Family history of prostate cancer and obesity in relation to high-grade disease and extraprostatic extension in young men with prostate cancer. Prostate 55:140–146.

Schatzl G, Madersbacher S, Thurridl T, Waldmuller J, Kramer G, Haitel A, Marberger M. 2001. High-grade prostate cancer is associated with low serum testosterone levels. Prostate 47:52–58.

Sim HG, Cheng CW. 2005. Changing demography of prostate cancer in Asia. Eur J Cancer 41:834–845.

Simpson ER, Misso M, Hewitt KN, Hill RA, Boon WC, Jones ME, Kovacic A, Zhou J, Clyne CD. 2005. Estrogen—The good, the bad, and the unexpected. Endocr Rev 26:322–330.

Sonnenschein C, Olea N, Pasanen ME, Soto AM. 1989. Negative controls of cell proliferation: Human prostate cancer cells and androgens. Cancer Res 49:3474–3481.

Takahashi M, Shimomoto T, Miyajima K, Yoshida M, Katashima S, Uematsu F, Maekawa A, Naka D. 2004. Effects of estrogens and metabolites on endometrial carcinogenesis in young adult mice initiated with *N*-ethyl-*N*0-nitro-*N*-nitrosoguanidine. Cancer Lett 211: 1–9.

Thompson CJ, Tam NN, Joyce JM, Leav I, Ho SM. 2002. Gene expression profiling of testosterone and estradiol-17 beta-induced prostatic dysplasia in Noble rats and response to the antiestrogen ICI 182,780. Endocrinology 143:2093–2105.

Thompson IM, Goodman PJ, Tangen CM, Lucia MS, Miller GJ, Ford LG, Lieber MM, Cespedes RD, Atkins JN, Lippman SM, Carlin SM, Ryan A, Szczepanek CM, Crowley JJ, Coltman CA, Jr. 2003. The influence of finasteride on the development of prostate cancer. N Engl J Med 349:215–224.

Trosko JE. 2007. Stem cells and cell-cell communication in the understanding of the role of diet and nutrients in human diseases. J Fd Hyg Safety 22:1–14.

van Landeghem AAJ, Poortman J, Nabuurs M, Thijssen JHH. 1985. Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. Cancer Res 45:2900–2906.

Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I. 2002. Estradiol in elderly men. Aging Male 5:98–102.

Vihko P, Herrala A, Harkonen P, Isomaa V, Kaija H, Kurkela R, Pulkka A. 2006. Control of cell proliferation by steroids: The role of 17HSDs. Mol Cell Endocrinol 248:141–148.

Wang Y, Hayward SW, Donjacour AA, Young P, Jacks T, Sage J, Dahiya R, Cardiff RD, Day ML, Cunha GR. 2000. Sex hormone-induced carcinogenesis in Rb-deficient prostate tissue. Cancer Res 60:6008–6017.

Wang ZY, Zhang XT, Shen P, Loggie BW, Chang YC, Deuel TF. 2006. A variant of estrogen receptor-α, hER-α36: Transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signalling. Proc Natl Acad Sci USA 103:9063–9068.

Weihua Z, Makela S, Andersson LC, Salmi S, Saji S, Webster JI, Jensen EV, Nilsson S, Warner M, Gustafsson JA. 2001. A role for estrogen receptor beta in the regulation of growth of the ventral prostate. Proc Natl Acad Sci USA 98:6330–6335.

Wirén S, Stocks T, Rinaldi S, Hallmans G, Bergh A, Stenman UH, Kaaks R, Stattin P. 2007. Androgens and prostate cancer risk: A prospective study. Prostate 67: 1230–1237.

Wynder EL, Fujita Y, Harris RE, Hirayama T, Hiyama T. 1991. Comparative epidemiology of cancer between the United States and Japan. A second look. Cancer 67:746–763.

Yager JD, Davidson NE. 2006. Estrogen carcinogenesis in breast cancer. N Engl J Med 354:270–282.