Estrogens in Prostate Cancer

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Introduction

Although prostate cancer represents a major health issue in men in Western countries, being a common cause of morbidity and mortality after the age of 50, it ought to be preventable and curable. Notwithstanding, despite the most recent advances in both basic and translational research, the molecular basis of prostate cancer remains poorly understood. In particular, the mechanisms underlying development and progression of this neoplasm appear to be complex: genetic and environmental factors (notably lifestyle and diet), along with endogenous sex hormones and host immune and inflammatory response, are likely to be interconnected in the pathogenesis of the disease.

As for breast cancer, dietary factors are thought to profoundly affect levels of endogenous hormones and their metabolism, eventually leading to prostate cancer development and/or progression [1]. In this context, sex hormones may act as intermediaries between exogenous factors, either environmental or nutritional, and biomolecular targets in both development and progression of prostate malignancies. Fascinatingly, breast and prostate cancer share many similarities, in terms of geographical distribution, risk factors, biomolecular determinants, and natural history. In a figurative way, cancer of the human prostate and breast can be viewed as brother and sister tumors, where dietary factors and hormones, especially estrogens, represent key interrelated players in many biological and pathological processes. In this framework, both breast and prostate cancer may be primarily considered, as elegantly proposed by Coffey [2], an acquired nutritional disease that could be prevented through changes of lifestyle and dietary habits.

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Although estrogen regulation of prostatic development, growth and function is generally recognized, the potential role of estrogens in human prostate cancer has been mistakenly neglected for decades and only recently reconsidered [3]. It has been long time remarked, but lately acknowledged, that neither androgens nor estrogens have a sexual selectivity, the former being implicated in breast and the latter in prostate, either normal or malignant, cell growth. This concept is nicely presented in a paper by Kuiper and colleagues where estrogen is described as a male and female hormone [4].

Epidemiological Studies

Prostate cancer is the commonest non-skin tumor and the second leading cause of cancer death in men in the United States, with an estimate of 241,740 new cases and 28,170 deaths from this disease expected in the year 2012 [5]. Between the late 1980s and 1990s, incidence rates of prostate cancer have increased dramatically in USA, Europe, and in many other Westernized countries with a peak in 1992 as a consequence of the introduction of prostate-specific antigen (PSA) blood test as a diagnostic tool for prostate cancer screening. The causes of the subsequent decline of prostate cancer incidence, that is present solely in men aged 65 years and older, remain indefinite. In addition, mortality rates of prostate cancer have been consistently decreasing in Western countries since the late 1990s. Notwithstanding, human prostate carcinoma continues to represent a major health and socioeconomic issue especially because mechanisms underpinning prostate carcinogenesis and tumor progression are largely unclear and, hence, new strategies for prevention, early diagnosis, and personalized treatment have only been rarely developed and implemented in clinical practice.

Both incidence and mortality rates for prostate cancer vary greatly worldwide, with as much as 50-fold and a 12-fold [6] difference, respectively, between African American and Caribbean men and men in Eastern Asia (China, Korea) and Africa (Egypt, Somalia). In European

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countries, incidence of prostate cancer is markedly higher in Northern (80.1/100,000) than in southern Europe (44.7/100,000), with Sweden having the highest rates (139.3/100,000) and Greece the lowest (43.4/100,000).

Although several aspects may account for these large geographic variations, there is an overall consensus that lifestyle and, notably, diet play a key role, while environmental and genetic factors may only have a limited impact on prostate cancer incidence. A small proportion (5–9%) of prostate cancer cases can be in fact associated with heritable genetic defects, while familial prostate cancer may represent up to 20% of cases [7]. However, even in men who carry strong cancer-susceptibility genes, the contribution of environment and lifestyle appears to be critical for the manifestation of disease.

An increased risk of developing prostate cancer has been reported in relation to a high fat diet, high protein and energy intake, low intake of fiber and complex carbohydrates, and a sedentary lifestyle [8–10]. However, the statistical significance of this association is low, and age, ethnicity, and family history remain the few, well-established risk factors for prostate cancer [11].

Previous studies on migrant populations who moved from countries with low incidence/mortality rates of prostate cancer (i.e., China or Korea) to countries with higher prostate cancer rates (Unites States) showed, within a generation, a significant increase in prostate cancer incidence/mortality as compared with their peers in the countries of origin [12, 13]. On the other hand, prostate cancer incidence is rising rapidly in countries that have been historically characterized by low rates especially Asian countries such as China and Japan, as oriental populations gradually adopt Western diet and lifestyle. This evidence suggests that environmental and, especially, lifestyle factors play a dominant role in prostate cancer development.

Several studies have hypothesized that plant hormones contained in Asian diets, particularly the phytoestrogens present in soy products, might act as natural hormone antagonists and anticancer agents and that their intake could be associated with a decrease of prostate cancer risk. A recent review [14] of epidemiological studies on the association of soy and other nutrients containing phytoestrogens with the risk of developing prostate cancer showed contradictory results with only a few studies reporting a risk reduction associated with the intake of soy food, legumes, and isoflavones. In a meta-analysis of eight epidemiological studies, Yan and Spitznagel indicated that the consumption of soy food was related to a nearly 30% reduction of prostate cancer risk, despite only three studies in the analysis showed statistically significant lower risk of prostate cancer [15]. Several studies in Asian men have also reported a trend toward decreased prostate cancer risk with increased equol (a gut bacterial product of the isoflavone daidzein). In addition, lower equol concentrations or a lower prevalence of equol producers have been observed in Asian populations among men with prostate cancer compared with controls, whereas studies in European populations have reported no association [16].

Interestingly, after World War II, lifestyle and dietary habits in Asian countries, especially Japan, have drastically changed, and these changes have been accompanied by a marked increase of both testicular and prostatic tumors [17]. In particular, the introduction of milk in a no-meat/no-milk dietary culture produced a significant, unprecedented source of saturated fats and estrogens that could have, in turn, favored prostate cancer development and progression. In this respect, Ganmaa and colleagues claim that the 20-fold increase of milk consumption seen in Japan after the war should be taken into account to explain, at least in part, the increase of prostate cancer incidence and mortality that has recently occurred in this country.

An explanation of the linkage between environmental and/or lifestyle factors and prostate cancer risk may lie in the potential impact of these factors on both levels and biotransformation of endogenous sex steroids, particularly estrogens. It is noteworthy that environmental and dietary factors are highly likely to induce significant changes in circulating hormones, their intraprostatic levels and metabolic patterns, eventually leading to prostate cancer development and/or progression.

Circulating Sex Steroids

Doubtlessly, the human prostate gland is dependent upon androgen for its development, function, and homeostasis. On the other hand, the potential implication of androgens in prostate carcinogenesis and tumor progression remains a common assumption (the “androgen hypothesis”), to such a point that prostate cancer is universally recognized as a prototype of age-related, androgen-dependent tumor. This assumption is based also on the fact that a high proportion of patients having locally advanced prostate tumors initially respond to hormone treatment, while they frequently develop an androgen-refractory condition after a relatively short time (usually within 2 years from presentation).

Both total and free serum testosterone significantly decline with age, eventually leading to an inverse relationship between testosterone levels in blood and prostate cancer risk. Thus, we are facing a seeming paradox whereby the higher the circulating testosterone, the lower the risk of developing prostate cancer.

In men, the balance between circulating levels of androgens and estrogens changes significantly with age [18]. In the aging male, a reduced production by the testes and increased levels of sex hormone-binding globulin (SHGB)

combine to lower free circulating testosterone. While plasma androgens decline, estrogen levels remain fairly constant, also as a consequence of an age-related increase of adipose tissue where estrogens are produced through the aromatization of androgens [19]. The ultimate result is a marked increase of the estrogen to androgen ratio and, hence, a potential increase of estrogenic activity on prostate gland that may eventually lead to abnormal growth and subsequent malignant transformation [20].

Apart from aging, males are exposed to relatively higher levels of circulating estrogens solely during in utero development. Several studies have indicated exposure of prostate cells to elevated estrogens early in uterine or perinatal life (a process referred to as developmental estrogenization or estrogen imprinting) may induce permanent disorders of prostate development that may in turn result in a higher propensity of prostate to develop precancerous or malignant lesions [21–23]. In addition, perinatal or neonatal exposure of prostate gland to endogenous estrogen and/or environmental estrogen-like endocrine disruptors may directly impair androgen-driven prostate development or result in functional and morphological prostate alterations that may in turn predispose the tissue to an earlier onset of disease, including cancer [24, 25]. One could speculate that developmental estrogenization generates important changes in the pool of embryonic stem cells that may, in turn, give rise to 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 become initiated [26].

The association between circulating androgens and prostate cancer risk has been explored by several studies, but the resulting data have been inconsistent and largely conflicting the "androgen hypothesis." None of the numerous prospective studies that have investigated the relationship between absolute plasma levels of testosterone and the risk of developing prostate cancer have shown any significant association. The subsequent meta-analyses by Eaton and colleagues [27] and Hsing et al. [28], respectively presenting quantitative reviews of the data from 8 and 12 available prospective studies, clearly revealed no significant differences in circulating hormones, either androgens or estrogens, between men who subsequently develop prostate cancer and those who remain free of disease. Only one study, the Physician's Health Study [29], reported a significant rise of prostate cancer risk with increasing plasma testosterone levels and an inverse association of estradiol with risk after adjusting for reciprocal levels and sex hormone-binding globulin (SHBG). However, this study found no significant difference in the risk of prostate cancer between men in the highest and the lowest quartiles of serum total testosterone.

Only a few earlier studies have investigated the correlation of serum levels of free testosterone and the risk of prostate cancer. Again, no significant association has been reported when the free fraction of this androgen was measured directly [30, 31].

The Rancho Bernardo study, conducted in California, revealed an association of elevated plasma estradiol and estrone with an increased risk of prostate cancer [32]. Two more recent nested case-control studies on serum levels of both androgens and estrogens failed to show any association with prostate cancer risk [33, 34]. 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 [33].

Recently, a limited but significant decrease of prostate cancer risk has been associated with increasing serum levels of total testosterone [35]. In a study on hypogonadal men, Morgentaler and colleagues [36] reported that subjects with PSA levels <4.0 ng/mL had a 15% overall rate of prostate biopsies positive for cancer. Interestingly, subjects with plasma levels of testosterone <250 ng/dL had a prostate cancer rate of 21% as opposed to 12% for men with a testosterone level >250 ng/dL. Furthermore, the probability of cancer in men in the lowest tertile was over twice as much as that in men in the highest tertile of both total and free testosterone.

Several studies have scrutinized the relationship between pretreatment serum levels of testosterone with clinical stage of prostate cancer and patient survival, suggesting that low serum testosterone could be used as a negative prognostic predictor for this neoplasia. In the last decade or so, a number of papers have emphasized that low serum testosterone is associated with prognostically adverse characteristics of prostate cancer, including high-grade [37, 38], poor clinical outcome [39], advanced pathological stage at surgery [40, 41], and shorter survival [42].

Based on the above inconsistency, investigators have raised the question why it has been so problematical to demonstrate that plasmatic androgens are related to 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.

It should be taken into consideration, however, that several issues related to measurement of plasma steroids, both androgens and estrogens, could be contemplated to explain this large inconsistency of data. They include the low statistical strength of most studies, the limited number of incident cases in prospective studies, the minor differences in sex steroid serum levels between cases and controls, and the rather large intra- and inter-assay laboratory variations of serum hormone measurements [43]. On the other hand, several other variables, including obesity, physical activity, diabetes, metabolic syndrome, and benign prostatic hyperplasia, that might have an impact on serum levels of hormones and have been related to prostate cancer have not been adjusted for in previous nested case-control studies [44].

In any case, it is unlikely that a single assay of plasmatic androgens can be regarded as descriptive of average androgen levels over an etiologically relevant period of life. In this respect, since the length of prostate carcinogenesis and tumor progression can span 35–40 years or longer, the timing for the carcinogenetic activity of androgen and/or estrogen on human prostate should be counted 20–30 years (or even earlier) prior to the clinical manifestation of the disease, when serum androgens are higher and, hence, could be biologically relevant.

All the above issues might contribute to justify, at least in part, the inconsistency of data on the association of plasmatic androgens and prostate cancer risk. However, a major problem remains whether or not plasma levels of steroids can be considered representative of the respective intraprostatic concentrations. Intratissue levels of sex steroids in target organs, including breast and prostate, have been reported to be markedly greater (10- to 100-fold) than the respective values in plasma [45, 46]. Furthermore, both normal and malignant steroid target tissues are equipped with a repertoire of enzymes of steroid metabolism, including a superfamily of hydroxysteroid dehydrogenases, two 5α-reductases, several hydroxylases, sulfotransferases, sulfatases, and aromatase. A different expression and/or activity of these enzymes may result in a different accumulation of bioactive metabolites, eventually leading to patterns of intratissue steroids that may substantially diverge from the plasmatic figure. Simpson and colleagues [47] have emphasized that estrogens circulating in men and in postmenopausal women are not the drivers of estrogen action, but they represent a reflection of estrogen uptake and biotransformation at extragonadal sites, including prostate. In other words, they are *reactive* rather than *proactive* [48].

Tissue Biosynthesis and Metabolism

As pointed out above, the balance between androgens and estrogens in individual target tissues may be significantly different from that in plasma, being dependent on several factors including uptake from the circulation, binding to steroid receptors and cofactors, and, notably, expression and/or activity of steroid enzymes, including 5α-reductase and aromatase. In this context, the ultimate biological impact of parent sex steroids and their derivatives could be assessed only through the evaluation of their local biosynthesis and metabolism. This issue has become increasingly important for a better understanding of the potential role of estrogens in breast and prostate cancer, also because abnormal levels of estradiol and/or estrone and, especially, of some of their hydroxylated tissue derivatives have been implicated in tumor development and progression [49].

As compared to breast, only a few early studies have assessed intraprostatic levels of sex hormones [50, 51]. 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.

In androgen target tissues, such as skin and prostate, testosterone is converted into its bioactive metabolite dihydrotestosterone (DHT). DHT binds in turn to androgen receptors and localizes in the nuclei of prostate epithelial cells as a dimer to regulate transcriptional activity of androgen-sensitive genes and DNA synthesis. The extent of DHT formation, that is governed by the 5α-reductase enzyme(s), produces DHT tissue levels markedly higher than those of testosterone, leading to a totally reversed testosterone:DHT ratio with respect to plasma (1:6 vs. 10:1, respectively) [52, 53]. In humans, two isozymes (type I and II) of the 5α-reductase exist, having distinct enzyme kinetics and tissue distribution. The type 1 isoform (encoded by *SRD5A1*) is expressed predominantly in skin and hair, while the type 2 enzyme (encoded by *SRD5A2*) is located primarily in androgen target tissues, including skin and prostate [54].

Results of the Prostate Cancer Prevention Trial (PCPT) indicate that the use of finasteride, a 5α-reductase inhibitor, for chemoprevention of prostate cancer results in a decrease of the overall number of incident cases but increases the proportion of high-grade prostate tumors [55]. Correspondingly, Nishiyama et al. [56] reported significantly lower levels of intraprostatic DHT in men with prostate cancer having a 7–10 Gleason score (GS) as compared with prostate cancer of ≤6 GS, suggesting that locally advanced, aggressive disease can progress even in a low-androgen environment. The authors also found no correlation between plasma levels of testosterone and/or DHT and intraprostatic levels of DHT. Indeed, Freedland and associates [57] have reported that circulating testosterone could not be mirroring intraprostatic androgenicity, and, hence, comparison of men having low and high testosterone levels could not be useful for a better understanding of the association between low androgen and aggressive prostate tumors.

Tissue estrogen biosynthesis occurs primarily through androgen aromatization. Since results of several studies suggest that human prostate gland is a primary target for estrogen action and that local synthesis of estrogen may be significant in prostate cancer, it would be important to determine whether or not aromatase is expressed in prostate tissues and to investigate the association between aromatase alteration and prostatic disease(s), including cancer. In this respect, the aromatase enzyme may act as a critical regulator of the balance between androgens and estrogens in target tissues and plasma. In the last decades, consistent evidence has accumulated to support the hypothesis that abnormal aromatase may play a critical role in development and/or

progression of human breast cancer. The *normal* prostate expresses aromatase in the stromal compartment, while aromatase expression is induced in malignant prostate through an abnormal promoter utilization, eventually leading to an altered T:E ratio that is associated with the development of disease [58]. 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, supporting a pivotal role of local estrogen biosynthesis in prostate cancer development [59]. In addition, significant expression and activity of aromatase have been detected in LNCaP, DU145, PC3 prostate cancer cells, and microdissected prostate epithelial tumor cells, while the enzyme could not be detected in *nonmalignant* prostate epithelial cells [60]. However, the potential implication of aromatase in either nontumoral or malignant human prostate remains today equivocal.

Estrogen patterns in target tissues and cells are much more assorted than one could expect on the basis of circulating estrogen profiles. The two major plasmatic estrogens, estradiol (E2) and estrone (E1), are readily interconverted in the tissue through the action of a superfamily of 17β-hydroxysteroid dehydrogenase enzymes (17β-HSDs) having distinct catalytic preferences and tissue distributions [61]. The hydroxylation of these "classical" estrogens at the C2/C4 positions through cytochrome P450 enzymes encoded by the CYP1A1 and CYP1B1 genes generates the so-called catecholestrogens (CCE), namely, the 2-hydroxy and 4-hydroxy derivatives of E2 and E1. Until are further metabolized by the catechol-O-methyltransferase enzyme into inactive methoxy derivatives, CCE may produce reactive oxygen species (ROS) that are in turn responsible of oxidative DNA damage.

Two mutually exclusive pathways, the 16α- and the 16β-hydroxylation, may act to produce a series of additional metabolites of either of as yet undefined biological activity. In particular, 16α-OHE1, along with other hydroxylated estrogens, has been repeatedly implicated in human breast carcinogenesis [49]. In a recent study, estrogen derivatives produced through either 16α-hydroxylation (e.g., 16α-OHE1 and 17-epiestriol) or 16β-hydroxylation (e.g., 16β-OHE1) have been reported to be comparable to classic E2 or E1 not only in terms of estrogenic potency but also for tumorigenicity in young adult mice [62].

Unfortunately, however, no direct unequivocal evaluation of estrogen intraprostatic levels has been so far provided.

In a recent randomized, dietary intervention study (the MeDiet study), we have ascertained that a traditional Mediterranean diet markedly reduces (over 40%) urinary levels of estrogens in healthy postmenopausal women [63]. It is of interest to note that, in this study, the majority of urinary estrogens was represented by hydroxy and methoxy derivatives of either E2 or E3 (notably 2-OHE2, 17-epiestriol, and 16-ketoE2), while *classical* estrogens (namely, E2 and E1) accounted for a mere 0.5% of total endogenous estrogens in urine. This pattern is cognate to what we have found by measuring intratissue levels of estrogens in both *nontumoral* and malignant human breast, whereby hydroxy estrogens accounted for the majority (more than 80%) of all estrogen metabolites in either condition [46]. In other words, metabolic profiles of estrogens in urine appear to be comparable to those obtained by measurement of their intratissue concentrations. This, incidentally, reinforces the suggestion that urinary estrogens can be used as indirect indicators of patterns of intratissue estrogens. In this respect, we have reported that a lower risk of developing prostate cancer is associated with a higher ratio of 2-hydroxyestrone (that has been originally proposed to act as anticancer estrogen and named accordingly *the good estrogen*, [64]) to 16α-hydroxyestrone (that has been claimed to be genotoxic, [65]) in urine [66].

Aiming to determine the impact of local metabolism on the distribution of bioactive steroids to malignant prostate cells, a few studies have assessed both expression and activity of key steroid enzymes in cultured human prostate cancer cells.

Recently, Vihko and colleagues [67], using both androgen-sensitive and androgen-independent LNCaP prostate cancer cells as a model system, have suggested that progression of prostate cancer to an androgen-refractory state is associated with a significant decrease of oxidative activity and a corresponding increase of reductive activity of the 17β-HSD enzyme(s). As a consequence, reduced bioactive estrogen (namely, estradiol) would accumulate in androgen-independent cells, while oxidized estrogen (namely, estrone) would become prevalent in androgen-sensitive cells.

We have originally established and optimized a rapid, simple approach to measure simultaneously the activity of several steroid enzymes in *intact* cultured cells [68]. Using this approach, we have assessed rates and direction of androgen metabolism in human prostate cancer cells [69]. Shortly, androgen-responsive LNCaP cells show consistent conversion of testosterone into the bioactive androgen DHT and its derivatives, 3α/3β-androstanediol, along with 17-βreduction of E1 to E2, while androgen-resistant PC3 cells exhibit a massive 17β-oxidation, leading to the predominance of oxidized androgen (androstenedione) and estrogen (estrone) derivatives. We have subsequently revealed that these highly divergent metabolic patterns are a consequence of a different expression and activity of several steroid enzymes, including 17β-HSDs, 3α-/3β-HSDs, and 5α-reductase, in the two cell lines [70]. This finding is of outmost importance since it corroborates the view that local steroid formation and metabolism is critical to determine the respective amounts of individual bioactive metabolites and, hence, the ultimate biological impact of sex steroids in target tissues and cells.

Our previous studies have revealed that aromatase activity is present in LNCaP prostate cancer cells even though to a significantly lesser extent than that observed in MCF7 human mammary carcinoma cells [71]. More recently, Ellem and Risbridger [72] have assessed aromatase RNA, protein, and enzyme activity in benign and malignant human prostate tissues, as well as in human prostate cancer cell lines. While aromatase was expressed solely in the stromal compartment of nontumoral prostate tissues, it was detected in microdissected epithelial tumor cells and prostate cancer cell lines.

Genes encoding for steroid enzymes are highly polymorphic in nature. Gene polymorphisms, along with epigenetic silencing or structural alteration, may all be associated with an increased risk of prostate cancer. To date, however, a relatively finite number of epidemiologic studies have been conducted to address this issue, and only limited, inconsistent evidence of the association between prostate cancer risk and gene polymorphisms has been provided.

As far as androgen metabolism is concerned, polymorphisms of genes involved in androgen biosynthesis (CYP11A1, CYP17A1, CYP19A1, CYP17A2), DHT formation (SRD5A2), and androgen inactivation and excretion (CYP3A4, HSD3B1, HSD3B2) have all been related to risk of developing prostate cancer [73–79]. In particular, several polymorphic regions are present in the SRD5A2 gene that encodes for the type 2 5α-reductase enzyme. Polymorphisms of this gene have been studied with special interest since its enzyme product presides over DHT formation in prostatic tissues. However, the present evidence indicates only a weak to modest increase of prostate cancer risk and, hence, does not apparently support the implication of DHT in prostate cancer development and progression [80].

As for estrogen metabolism, three different polymorphisms of the CYP1A1 gene, encoding the 2-hydroxylase enzyme, have been associated with an increased risk of prostate cancer, while only one single nucleotide polymorphism (SNP) has been reported to have an opposite impact by reducing prostate cancer risk in Japanese and a Caucasian-American population [81, 82]. Comparable finding was obtained for the CYP1B1 gene that encodes the 4-hydroxylase enzyme [83]. It is noteworthy that these polymorphisms result in a prolonged half-life and activity of either enzyme, and, hence, produce a sustained exposure of prostate cells to their products, respectively, 2- and 4-hydroxy estradiol, amplifying their carcinogenetic potential.

In a recent paper, Mononen and colleagues have identified a novel SNP of the CYP19A1 gene that encodes for a variant aromatase enzyme having higher activity and that is significantly associated with prostate cancer risk [79]. The reported evidence implies that this SNP results in lower androgen levels and greater amounts of tissue estrogens, supporting the potential implication of estrogen in prostate cancer development and growth.

Although some of these gene polymorphisms could be relevant in prostate carcinogenesis and tumor progression, their significance is still unclear and remains fairly speculative. Many issues may concur to make results of these studies inconsistent, but probably the most important is the lack of information on the combined effect of these polymorphic genes on prostate cancer risk [84]. Further studies on haplotypes and diplotypes are being conducted to determine the ultimate effects of polymorphic genes on the production and/or activity of steroid enzymes in relation to individual risk of prostate cancer.

Estrogens in Prostate Tumor Development and Growth

Since the pioneering work of Charles Huggins, the concept that human prostate cancer represents a paradigm of androgen-dependent tumor has endured for decades against a bulk of experimental evidence suggesting that estrogens and other growth factors may be at least equally important in prostate carcinogenesis and tumor progression (reviewed in 3).

Estrogens in Tumor Initiation

Recent experimental evidence suggests that prostate cancer originates from precancerous lesions, such as chronic proliferative inflammatory atrophy (PIA), as a consequence of prostate tissue injury [85]. Normally, in response to tissue injury, the prostate stem cell niche, that represents a minority (1–3%) of basal epithelial cells and has been located at the basement membrane of the prostatic glandular epithelium, would give rise a population of transit-amplifying/intermediate cells that would, in turn, terminally differentiate and generate luminal secretory and neuroendocrine epithelial cell types. It is speculated that tumor-initiating cells could arise during the prostate regeneration process within the pool of prostate stem cells when their differentiation ability is somehow impaired by a mutation activating oncogenic and/or abrogating tumor-suppressor signaling pathways [86]. The resulting progeny of cells would clonally expand and undergo the promotion and progression phases of the multistep carcinogenetic process, eventually leading to create a population of cancer stem cells featured by unrestricted replicative potential and reduced apoptosis. In this context, estrogens have been reported to upregulate both expression and activity of telomerase in human prostate epithelial cell lines, an event that is generally associated with unlimited cell proliferation [87].

Cavalieri and Rogan [88] have produced consistent experimental evidence in support of their hypothesis that selected tissue estrogen metabolites, notably the electrophilic catechol

estrogen-3,4-quinones, may react with DNA and generate depurinating estrogen-DNA adducts. After adducts are released from DNA, error-prone base excision repair of the resulting apurinic sites may eventually lead to mutations that can be critical to initiate breast, prostate, and several other human cancers.

Experimental Animals

Early studies have reported that long-term administration of testosterone to rats induces the development of prostate tumors, suggesting that testosterone act as a complete carcinogen on the rat prostate, though in a limited proportion of cases and in some but not all rat strains [89–91]. However, when Noble rats were used as model system, the administration of testosterone and estradiol, in sequence or combined, resulted in the occurrence of both ductal and acinar epithelial dysplasia, followed within 1 year by the development of adenocarcinomas of the dorsolateral prostate in 90–100% of the animals [92]. If rats were treated with androgen alone, the incidence of prostate cancer dropped to 35–40% [93].

The mechanisms underpinning the hormonal carcinogenesis in the rat prostate remain largely undefined, but there is evidence to suggest that both receptor-mediated and *nonreceptor* effects may be implicated. As far as estrogens are concerned, the development of dysplastic lesions in the dorsolateral prostate of rats exposed for 16 weeks to a combination of testosterone and estradiol was almost completely abrogated by the simultaneous administration of the pure antiestrogen ICI-182,780 [94]. However, since ICI-182,780 also induces a block of the hyperprolactinemia produced in rats by estrogen treatment, it is difficult to establish whether the effects of this estrogen antagonist are a consequence of binding to estrogen receptor or not.

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 and ventral prostate [92, 95]. This evidence suggests that androgen and estrogen treatment of animals creates an estrogenic milieu in the rat prostates, eventually leading to the development of epithelial dysplasia and adenocarcinoma in the Noble rat prostate model. In an elegant study, Wang et al. [96] rescued pelvic organ rudiments of Rb KO mice and grafted them under the renal capsule of male adult nude mice to develop functional prostatic tissue. When Rb-/-prostate epithelium was combined with wild-type urogenital mesenchyme to construct chimeric tissue recombinants, dysplastic and malignant lesions occurred 5–8 weeks after host animals received silastic implants containing testosterone (25 mg) and estradiol (2.5 mg).

Although most studies on hormonal carcinogenesis of the prostate have been conducted on rodents, it ought to be emphasized that the rat prostate, consisting of dorsal, lateral, ventral, and anterior lobes, has embryology and anatomy distinct from human and dog prostates. Therefore, results of these studies should be interpreted with caution.

Endocrine Disruptors

Accumulating evidence from both epidemiological and animal studies suggests that environmental exposure to endocrine-disrupting chemicals may be important for development or progression of human prostate cancer. These compounds may disturb estrogen signaling by interfering with either ER or enzymes of steroid metabolism, eventually leading to significant changes of levels of individual estrogen derivatives having distinct biological activity. Endocrine disruptors include pesticides, polychlorinated biphenols (PCBs), polyhalogenated aromatic hydrocarbons (such as bisphenol A, BPA), phthalates, arsenic, cadmium, and UV filters. Most of them have estrogen-like activity and are also referred to as xenoestrogens; many have been associated to an increase of prostate cancer risk (reviewed in [97]). The accumulation and the assortment of xenoestrogens in the environment have enormously increased in recent years, and this has also been related to the persistent increase of estrogen-related diseases, including breast and prostate cancer, neurodegenerative disorders, endometriosis, premature puberty, cryptorchidism, and many others. It is important to note that sensitivity of prostate tissues to endocrine disruptors appears to be prominent through critical developmental phases, notably in uterine life, at birth, and during puberty. A sustained exposure to xenoestrogens during these periods may be responsible for an increased susceptibility to develop prostate cancer later in life.

In Vitro Studies

Both epidemiological and experimental evidences presented herein support the view that prostate cancer arises in the aging male in an estrogenic environment. However, the ultimate biological impact of sex steroids, particularly estrogen, on prostate cancer cells is difficult to dissect as it is strictly dependent upon several variables, including the estrogen:androgen ratio in both plasma and prostate, the expression and activity of steroid enzymes, the binding to intracellular and/or membrane receptors, the exploitation of genomic and/or nongenomic mechanism(s) of action.

Previous studies have assessed the proliferative effects of sex hormones in cultured prostate cancer cells. Although several reports have shown that androgens markedly stimulate

prostate cancer cell growth [98, 99], unequivocal evidence for a direct increase of DNA synthesis brought about by bioactive androgens in prostate tumor cell lines is surprisingly rare and often conflicting. The inconsistency of the results obtained in cell model systems does not allow to draw any truthful interpretation also because different variables including culture and experimental conditions, age of cultured cells, and exposure to endogenous hormones and growth factors may considerably affect the results.

Various in vitro studies carried out on LNCaP cells have indicated that both androgen and antiandrogen stimulate growth of these cells [100]. We have previously reported that exposure to physiological estrogen concentrations may either stimulate or decrease growth of androgen-responsive LNCaP or androgen-refractory PC3 prostate cells, respectively, and that these effects are predominantly receptor-mediated being completely abrogated by the simultaneous addition of the pure estrogen antagonist ICI-182,780 [101, 102]. This evidence implies that estrogen may affect proliferative activity of prostate cancer cells even if the cells have become androgen resistant. This finding is also corroborated by the significant rates of clinical response to the systemic administration of estrogens observed in prostate cancer patients having a metastatic, androgen-refractory disease [103]. Other authors have revealed that tamoxifen (mixed antiestrogen) and ICI-182,780 (pure antiestrogen) inhibit growth of both DU145 and PC3 prostate cancer cell lines and have cytotoxic effect on DU145 cells. This latter effect could be prevented by the pretreatment of cells with an estrogen receptor (ER) β antisense oligonucleotide, suggesting that antiestrogens may accomplish their antitumor effects also through this type of ER [104]. Based on the finding that the proliferative effects of estrogens on human prostate cancer cells in culture appear to be typically receptor-mediated, it would be important to assess ER content and the balanced expression of different ER types and their variants.

Estrogen Receptors and Prostate Cancer

Several in vivo or in vitro studies have repeatedly pointed out that classical effects of sex steroids are mediated through specific intracellular receptors that belong to the superfamily of nuclear receptors [105]. However, there is accumulating evidence that estrogens and their receptors may combine or act unconnectedly to exploit an amazing array of both genomic and nongenomic, either ligand-dependent or ligand-independent, activities [106].

Two major ER types, the classical ERα and the more recently discovered ERβ, have been identified. The two receptor types are encoded by separate genes, respectively *ESR1* and *ESR2*, located on different chromosomes. In addition, several ERα and ERβ splicing variants and deletion mutants have been isolated in both *nontumoral* and diseased target tissues and cells [107]. However, these ER species are habitually coexpressed with wild-type receptors, and, hence, their potential role in either physiological or pathological processes is difficult to dissect.

The ERα and ERβ are characterized by tissue-specific distribution and exploit a variety of physiological activities in several human tissues [108]. Both receptors typically act as nuclear transcription factors with the ultimate biomolecular effect of estrogen on target cells being dependent on their respective expression levels and balance in individual tissues, ligand binding, heterodimerization, transactivation, and estrogen response element (ERE) activity. In this respect, an alteration of ERα and ERβ balance may be implicated in the etiology of various diseases, including prostate cancer.

Both ERα and ERβ are expressed in the adult human prostate, although ERα is generally located in the stromal compartment, while ERβ is located predominantly in the basal cell layer of the glandular epithelium. Various studies have inspected the expression of ERα and ERβ (at both transcript and protein level) in *nontumoral*, hyperplastic, and malignant human prostate tissues and cells. The resulting data have consistently revealed a marked decrease of ERβ expression in the malignant prostate as compared with benign (hyperplastic) or normal tissues, while ERα expression remains unchanged or even increased. There is convincing evidence that the two receptors are mutually regulated, with ERβ limiting cell proliferation by direct (ERβ-specific) effects on gene transcription and/or indirect activity through modulation of ERα. In this respect, loss of ERβ expression may represent a crucial step in estrogen-related mechanisms of prostate cancer progression (reviewed in [109]).

Previous studies based on estrogen receptor knockout (ERKO) mice model systems have provided important insights for a better understanding of ER role in both normal and diseased prostate. In particular, the adult ERβ knockout (βERKO) mouse has been associated with the onset of prostatic epithelial hyperplasia, while no prostatic alteration could be observed in the ERα knockout (αERKO) mice [110]. This evidence reinforces the assumption that ERβ may play a protective role against prostate malignant cell growth. Interestingly, both synthetic antiestrogen (toremifene) and natural phytoestrogen (genistein) prevent prostate cancer development in the transgenic adenocarcinoma mouse prostate (TRAMP) model acting as ERβ agonists [111, 112].

Cancer progression is hallmarked by the acquisition of genetic and epigenetic changes that eventually lead to the generation of a phenotypically diverse progeny of cancer cells. In this framework, hypermethylation of CpG islands in the promoter region of tumor-suppressor genes is a common mechanism of gene silencing during tumor progression. Loss of ERβ expression has been reported in both primary cultures

of human prostate cancer cells and prostate carcinoma tissues [113, 114]. Conversely, metastatic lesions of prostatic carcinomas frequently display high expression levels of ERβ [115]. This combined evidence apparently indicates that while loss of ERβ is an important event in prostate carcinogenesis, its re-expression in metastatic disease could even provide some survival advantage to prostate malignant cells. However, hypermethylation of the promoter region and silencing of the genes have been reported to occur for both ERα and ERβ in prostate cancer tissues and cells [116]. In addition, direct acetylation of ERα by the coactivator p300 at well-conserved lysine residues in the hinge/ligand domain of the receptor has been associated with both hypersensitivity to estradiol and contact-independent growth in cancer cells [117, 118]. All the above changes may be crucial in determining the net biological effects of estrogen in either normal or diseased prostate gland.

A few studies have investigated polymorphisms of both AR and ER genes in relation to prostate cancer risk. It has been experimentally observed that the length of the polymorphic glutamine (CAG) trinucleotide repeat in the AR gene affects both transactivation of AR and transcriptional activity of androgen target genes, hence having a potential on prostate cancer development and growth [119, 120]. In contrast, Platz and colleagues [33] revealed that neither circulating steroids nor length of the AR gene CAG repeat is associated with prostate cancer, supporting the view that these factors do not significantly contribute to prostate carcinogenesis and tumor progression.

A positive association with prostate cancer of the T/T variant of the PvuII site in the ERα gene and the TC or CC variant alleles of ERβ has been reported in case-control studies [121, 122].

Little is known about the expression and the functional meaning of splice or deletion variants of ER in the human prostate. There is evidence that two ERα splice variants, hERα46 and hERα36, are potent inhibitors of the wild type hERα66 transactivation. In particular, hERα46 is located almost exclusively in cell nuclei, while hERα36 is predominantly associated to the plasma membrane where it transduces both estrogen and antiestrogen signaling, including activation of mitogen-activated protein kinase [123, 124]. On the other hand, several relatively abundant ERβ isoforms have been described. In particular, hERβ2 and hERβ5 have been reported to inhibit transcriptional activity of ERα [125]. Presently, no ERα variant has been described in prostatic tissues, while both hERβ2 and hERβ5 have been detected in prostate cancer, with the combined expression of the two receptor variants being a prognostic indicator of patients having shorter disease-free survival [126]. In a recent report, Taylor and colleagues have indicated that expression of the ERαΔ5 deletion variant is significantly greater in tumor-adjacent prostate samples as compared to benign tissues [127].

Results of further studies on ER variants in both normal and malignant human prostate are awaited with interest to provide important insight into the role of ER and estrogen signaling in prostate cancer development and progression.

In the recent years, selective estrogen and androgen receptor modulators have attracted interest for their potential use in the management of various human diseases (reviewed in [128]). In particular, selective estrogen receptor modulators (SERMs) have been used to prevent bone fractures, to treat ER-positive postmenopausal breast cancer patients, and to induce ovulation in infertile women [129]. On the other hand, selective androgen receptor modulators (SARMs), a newcomer category of agents that are currently being investigated mostly at basic and preclinical level, have been proposed for both prevention and treatment of human prostate cancer [130]. SERMs, including raloxifene, lasofoxifene, and arzoxifene, selectively bind ERα and ERβ to accomplish either estrogenic or antiestrogenic activities in a variety of human tissues. It has been suggested that SERMs induce a conformational change of ER, dissociate the receptor from the heat-shock protein complexes, release ER in a monomeric form, and permit its translocation to nuclei of cells where it binds as a homodimer to the regulatory sequences of target genes to either initiate or suppress transcription [131]. Today, research on SARMs is largely in its infancy, with no SARM approved for clinical use and a few agents completing phase I and II trials. Their potential efficacy in prostate cancer remains to be established and again based on an "androgen hypothesis" that has been by far disputed more than convincing. On the other hand, the loss of ERβ expression during prostate cancer progression and its re-expression in metastatic prostate cancer cells raise the possibility of using ERβ-specific ligands in triggering cell death in these malignant cells. In this context, SERMs, along with synthetic estrogen receptor ligands and antagonists, have recently emerged as promising agents in both prevention and treatment of human prostate cancer [132].

Perspectives

In spite of the recent, significant advances in the research on prostate cancer, mechanisms underpinning development and progression of the malignant prostate remain undefined. Several networked factors, including the balance of estrogen and androgen, changes and polymorphisms in the enzymes responsible for biosynthesis and transformation of intraprostatic hormones, alteration of hormone signaling or local balance between estrogen receptor types and variants, are all markedly affected by lifestyle factors (notably diet), genetic determinants, and exposure to environmental chemicals and may play a critical role in human prostate cancer.

Presently, the lasting conception that androgens are the key determinants in prostate carcinogenesis and tumor progression appears to be a never-ending persuasion that has, faultily, led to neglect different areas of research with promising perspectives for both treatment and prevention of this disease.

In particular, steroidogenic enzyme inhibitors [133], ER subtype-selective agonists/antagonists or SERMs [134, 135], have been in turn proposed as potential agents for both chemoprevention and treatment of prostate cancer.

In a recent intriguing paper, Williams [136] has combined apparently distant evidence from epidemiology, basic research, animal model systems, and clinics to design a unifying hypothesis for the increasing prevalence of global disease worldwide. The controversial breakthrough presented by the author proposes that several distinct factors may significantly affect hormone balance in the organism through upregulation of the P450 aromatase enzyme and the resulting unopposed excess of endogenous estrogen, alteration of insulin receptor machinery and leptins, and exposure to elevated environmental xenoestrogens. This unbalanced hormonal milieu may represent a common condition for development of life-threatening diseases, including cancer, diabetes, obesity, Alzheimer's disease, that are currently pandemic.

In this respect, we have been forerunner in approaching and emphasizing the potential implication of estrogen not only in endocrine-related tumors, including prostate cancer, but also in several other human diseases [137]. A better understanding of estrogen-driven mechanisms in different processes related to health and disease would be of primary importance to design and exploit original preventive and therapeutic strategies also in prostatic carcinoma.

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