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Estrogens and Anti-Estrogens: Key Mediators of Prostate Carcinogenesis and New Therapeutic Candidates

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Abstract Despite the historical use of estrogens in the treatment of prostate cancer (PCa) little is known about their direct biological effects on the prostate, their role in carcinogenesis, and what mechanisms mediate their therapeutic effects on PCa. It is now known that estrogens alone, or in synergism with an androgen, are potent inducers of aberrant growth and neoplastic transformation in the prostate. The mechanisms of estrogen carcinogenicity could be mediated via induction of unscheduled cell proliferation or through metabolic activation of estrogens to genotoxic metabolites. Age-related changes and race-/ethnic-based differences in circulating or locally formed estrogens may explain differential PCa risk among different populations. Loss of expression of estrogen receptor (ER)-β expression during prostate carcinogenesis and prevention of estrogen-mediated oxidative damage could be exploited in future PCa prevention strategies. Re-expression of ER-β in metastatic PCa cells raises the possibility of using ER-β-specific ligands in triggering cell death in these malignant cells. A variety of new estrogenic/anti-estrogenic/selective estrogen receptor modulator (SERM)-like compounds, including 2-methoxyestradiol, genistein, resveratrol, licochalcone, Raloxifene, ICI 182,780, and estramustine are being evaluated for their potential in the next generation of PCa therapies. Increasing numbers of patients self-medicate with herbal formulations such as PC-SPES. Some of these compounds are selective ER-β ligands, while most of them have minimal interaction with ER-α. Although many may inhibit testosterone production by blockade of the hypothalamal–pituitary–testis axis, the most effective agents also exhibit direct cytostatic, cytotoxic, or apoptotic action on PCa cells. Some of them are potent in interfering with tubulin polymerization, blocking angiogenesis and cell motility, suppressing DNA synthesis, and inhibiting specific kinase activities. Further discovery of other compounds with potent apoptotic activities but minimal estrogen action should promote development of a new generation of effective PCa preventive or treatment regimens with few or no side-effects due to estrogenicity. Further advancement of our knowledge of the role of estrogens in prostate carcinogenesis through metabolic activation of estrogens and/or ER-mediated pathways will certainly result in better preventive or therapeutic modalities for PCa. J. Cell. Biochem. 91: 491–503, 2004.

Key words: estrogen receptor; anti-estrogens; selective estrogen receptor modulator (SERM); estrogen receptor relates; estrogen receptor co-regulators; oxidative stress; genomic damages; prostate cancer risk; hormone refractory disease; hormonal therapy

Historically, androgens have been considered to be the major sex hormones that regulate normal and malignant growth of the prostate [Ho et al., 1997; Bosland, 2000; Prins, 2000; Taplin and Ho, 2001; Risbridger et al., 2003]. However, recent evidence suggests that estrogens significantly contribute to normal prostatic functions as well as to the genesis of prostate cancer (PCa). Paradoxically, diethylstilbestrol (DES), a xenoestrogen, was used as the first standard therapy for PCa treatment [Huggins and Hodges, 1941]. Recently, novel phytoestrogens [Castle and Thrasher, 2002; Morrissey and Watson, 2003] and synthetic estrogens/anti-estrogens/selective estrogen receptor modulators (SERMs) [Steiner et al., 2001; Kim et al., 2002; Steiner and Raghow, 2003] have emerged as promising PCa preventive and treatment agents. The primary objective of this review is to address the mechanisms of action of estrogen carcinogenicity in the prostate in order to enrich our understanding of the roles played by these hormones in the pathogenesis of PCa. The review is also

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intended to evaluate the potential of a new generation of estrogenic and anti-estrogenic compounds as therapies for the prevention and treatment of PCa.

Estrogens/Anti-Estrogens as Contributing Factors to the Genesis and Progression of PCa

It is well established that estrogens play important roles in normal growth, differentiation, and development of the prostate [Ho et al., 1997; Bosland, 2000; Prins, 2000; Taplin and Ho, 2001; Risbridger et al., 2003]. However, any significant contribution by these steroids to the development of prostatic diseases, including PCa in men and animals, is not yet apparent. Estrogens alone, or in synergism with androgens, are potent inducers of aberrant growth and neoplastic transformation in the prostate. Administration of pharmacological doses of estrogen induces a well-known proliferative lesion, termed squamous metaplasia, in the prostates of various mammalian species, including humans [Leav et al., 1978; Merk et al., 1982, 1986; Goodwin and Cummings, 1984; Sugimura et al., 1988; Levine et al., 1991; Yonemura et al., 1995; Cunha et al., 2001; Risbridger et al., 2001]. The metaplastic change is initiated by the proliferation of basal epithelial cells, which subsequently differentiate into squamous cells. In Noble rats, administration of estrogens in conjunction with an androgen leads to the development of a pre-cancerous lesion similar to human prostatic intraepithelial neoplasia (PIN) and a high incidence of full blown prostate adenocarcinoma [Noble, 1977, 1980; Drago et al., 1979; Leav et al., 1988, 1989; Ofner et al., 1992; Bosland et al., 1995; Ho and Lane, 1996; Wong et al., 1998; Tam et al., 2000]. The combined hormonal treatment also initiated and promoted malignant transformation in Rb-deficient prostatic tissues grafted under the kidney capsules of immune-deficient mice [Wang et al., 2000].

Epidemiological findings reveal that African–Americans, who have the highest incidence of PCa in the USA, also have significantly higher levels of serum estrogens, even at a young age, when compared with their Caucasian–American peers [Hill et al., 1984; Ross et al., 1986; Henderson et al., 1988; de Jong et al., 1991; Bosland, 2000]. In contrast, lower circulating estrogen levels have been noted in Japanese men, who are known to have a low risk of PCa when compared with a higher-risk group, Caucasian–Dutch men [de Jong et al., 1991]. Furthermore, the incidence of PCa rises exponentially in elderly men, in whom the ratio of estrogen to androgen may increase by up to 40%. This age-related hormonal shift is due primarily to a decline in testicular function and an increase in aromatization of adrenal androgens by peripheral adipose tissue during aging [Vermeulen et al., 1972; Zumoff et al., 1982; Gray et al., 1991a,b; Griffiths, 2000]. At the cellular level, significant declines in 5α-dihydrotestosterone (DHT) levels and increases in estrogen (E2 and estrone) levels have been observed in prostatic epithelial cells [Krieg et al., 1993]. Collectively, these endocrine changes at mid-life, termed andropause, may significantly augment estrogenic stimulation of the prostatic epithelium leading to reactivation of growth and subsequent neoplastic transformation. Several theories have been advanced to explain the mechanism of action of estrogen carcinogenicity in the prostate. The first suggests that enhancement of unscheduled cell proliferation under rising estrogenic influence may lead to the accumulation of spontaneous genetic errors in progenitor cells [Henderson and Feigelson, 2000]. Others propose direct and/or indirect estrogen- or estrogen metabolite-mediated genotoxicity as causative factors of neoplastic transformation of the prostatic epithelium.

Estrogen Metabolism as a Determinant of Prostatic Carcinogenesis

In female tissues like the breast and endometrium, estrogens induce tumors partially via metabolic activation of the natural estrogens to genotoxic metabolites, such as catechol estrogens and quinone/semiquinone intermediates [Cavalieri et al., 2000; Yager, 2000]. These metabolites induce genomic damage directly by formation of DNA adducts or indirectly via formation of reactive oxygen species (ROS), which can cause genomic and protein damages and lipid peroxidation. Conversion of estrogens to 2-hydroxy (OH)-estrogens is primarily mediated by the enzyme cytochrome P450 1A1 (gene: *CYP1A1*) and into 4-OH-estrogens by cytochrome P450 1B1 (gene: *CYP1B1*) [Yager and Liehr, 1996]. As an important cellular defensive mechanism, these highly reactive catechol estrogens are inactivated quickly by the enzyme catechol-O-methyl-transferase (COMT). Additionally, cells are equipped with several

isoforms of glutathione-*S*-transferases (GSTs) that detoxify ROS and other electrophilic moieties by conjugative reactions. Genetic polymorphisms that alter enzyme activity and DNA hypermethylation- or mutation-mediated silencing of key estrogen metabolizing genes have been associated with higher or lower risks for breast and endometrial cancer [Yager, 2000]. In other words, the levels and activities of these enzymes collectively influence the magnitude and the duration of lifetime exposure to these potentially transforming estrogen metabolites. Although many of the aforementioned pathways are well characterized in female estrogen-sensitive organs, few have been investigated for their contribution to prostate carcinogenesis. Dorsolateral prostate carcinomas arise in 100% of Noble rats treated with estradiol and testosterone. It was demonstrated that the combined hormone treatment induced specific DNA adduct formation in prostatic epithelial cells of the proximal ducts, where malignant lesions are believed to originate [Han et al., 1995]. Increased DNA single-strand breaks [Ho and Roy, 1994], elevated lipid peroxidation [Ho and Roy, 1994], accumulation of 8-oxo-2(-deoxyguanosine and nitrated proteins [Tam et al., unpublished data], down regulation of antioxidant defense enzyme activities [Tam et al., 2003], and accumulation of 4-OH-estradiol and its quinone derivatives [Cavalieri et al., 2002] were also noted in the cancer-susceptible dorsolateral prostates of animals treated with estrogen plus testosterone. Importantly, evidence of reduced activities of COMT and quinone reductase observed in the dorsolateral prostates of the treated rats might explain the preferential accumulation of these transforming estrogen metabolites in the rat prostate.

Recent molecular epidemiological data indicate that Japanese men homozygous for the G allele of SNP2455A > G of *CYP1A1*, a polymorphism that confers higher enzyme activity, were more susceptible to PCa development [Murata et al., 2001]. However, in an American population that was 86% Caucasian, this polymorphism was associated with a lower PCa risk [Chang et al., 2003]. The discrepancy could be due to ethnic/racial differences or other factors yet to be identified. In the same population, three other polymorphisms in the *CYP1A1* gene were found to be associated with increased PCa risk [Chang et al., 2003]. Considering the important roles played by CYP1A1 in the oxidative metabolism of estrogen, the impact of *CYP1A1* polymorphisms on CaP risk is worthy of further investigation. Of equal importance is a recent finding of higher PCa risk in Japanese men carrying a polymorphism at codon 119 (homozygous T/T compared to G/G 453) of *CYP1B1* [Tanaka et al., 2002]. This polymorphism is believed to confer longer half-life to the enzyme CYP1B1, which converts estrogens into 4-OH estrogens. It has been postulated that 4-OH-estrogens are more carcinogenic than 2-OH-estrogens in female organs such as the breast [Yager, 2000]. A similar mechanism has yet to be identified in the prostate. Another gap in our understanding of the connection between estrogen metabolism and the pathogenesis of PCa is our lack of data on the enzyme COMT, which can convert 2-OH-catecholestrogens to 2-methoxyestradiol. Since 2-methoxyestradiol has recently been shown to exhibit potent apoptotic activity against rapidly growing PCa cells [Lakhani et al., 2003], it would be of importance to correlate expression levels and polymorphisms of *COMT* to PCa risk in future epidemiological studies. Similarly, in situ production of estrogen from androgen via the activity of the enzyme aromatase has not been fully investigated as a risk factor modulator of PCa. Both aromatase protein and its enzyme activity have been demonstrated in PCa and benign prostatic hyperplasia (BPH) [Matzkin and Soloway, 1992; Di Salle et al., 1994; Hiramatsu et al., 1997]. Unfortunately, most clinical trials testing aromatase inhibitors have focused on their efficacy in treating BPH [Radlmaier et al., 1996; Suzuki et al., 1998] and attention has not been given to their use in PCa prevention or treatment. Studies of transgenic mice overexpressing aromatase (AROM+) [Li et al., 2001] and aromatase knockout mice (ArKO) [McPherson et al., 2001] have yielded only limited insights into the relationship between in situ estrogen formation and PCa risk. This is largely due to the fact that numerous endocrine factors are altered in these animals, which makes data interpretation difficult. Since it has been shown that alternative use of promoter to transactivate aromatase is an important determinant of breast carcinogenesis [Harada et al., 1993; Chen et al., 1999], studies focusing on establishing a similar scenario should be conducted for PCa. Finally, a recent observational study has reported that obesity,

commonly associated with more aromatization of androgens to estrogen, is associated with a poorer histology in young men with PCa, especially men younger than 50 years of age [Rohrmann et al., 2003]. This finding is in concordance with the hypothesis that in situ production of estrogen via aromatization may contribute to tumor initiation and progression in the prostate.

Estrogen Receptors (ERs) as Functionally Divergent Mediators of Estrogen Action

Until recently the effects of estrogens on all tissues, including the prostate, were believed to be mediated by a steroid hormone receptor, now termed ER-α [Nilsson and Gustafsson, 2000; Pettersson and Gustafsson, 2001]. In 1996, another ER subtype, referred to as ER-β, was identified in rats [Kuiper et al., 1996] and in humans [Mosselman et al., 1996]. The two ER subtypes are structurally similar, consisting of the six common domains (A–F) found in all steroid hormone receptors. The DNA-binding domain (DBD) of ER-β, e.g., differs from ER-α by only three amino acids. In contrast, the N-terminal A/B domain of both receptors share only a 15.5% homology, the ligand binding domains (LBDs) are 58% homologous, and the F domains are distinctly different [Kuiper et al., 1996; Mosselman et al., 1996; Nilsson and Gustafsson, 2000; Pettersson and Gustafsson, 2001]. These structural dissimilarities are thought to reflect functional differences between the two receptors. ER-α and ER-β have been shown to bind to the same ligand with different affinities [Kuiper et al., 1997]. For example, genistein and some SERMs are known to bind ER-β with higher affinities than to ER-α. In addition, after binding to estrogens/antiestrogens/SERMs, the two ERs may use different enhancer elements such as the estrogen responsive element (ERE), the activating protein-1 (AP-1) site, the cyclic AMP-response element, the antioxidant responsive element, and the NF-kappa B site in the promoter regions of various genes [Paech et al., 1997; Cerillo et al., 1998; Montano et al., 1998; Pelzer et al., 2001; Weatherman et al., 2001; Liu et al., 2002]. It is now believed that the two ER-subtypes could mediate diverse biological functions through interaction with specific ligands and activation of different enhancer elements/transcriptional factor binding sites. Adding to the complexity of mechanisms mediating ER action is the involvement of co-regulators that are required for ER signaling. The possibility exists that the two ER subtypes, depending on their binding ligand, interact with different coactivators, corepressors, and integrators to achieve the broadest degree of functional diversity and specificity [Osborne et al., 2001; Tremblay and Giguere, 2002].

Expression of ER Subtypes in Normal and Malignant Prostates: Implications in Tumor Initiation and Progression

The precise biological functions of the two ER subtypes in the prostate are currently undefined and their possible role in PCa initiation and progression is likewise unknown. Several studies have described the expression of the receptors, at both mRNA and protein levels, in the epithelial and stromal compartments of the normal and malignant adult human prostate [Horvath et al., 2001; Latil et al., 2001; Leav et al., 2001; Royuela et al., 2001; Torlakovic et al., 2002; Weihua et al., 2002; Fixemer et al., 2003; Tsurusaki et al., 2003]. ER-β is localized predominantly to the basal epithelial cell compartment of the normal human prostate, where ER-α is rarely found. ER-α is mainly expressed in the stroma of the normal gland. Results from these studies suggest that ER-β may exert a protective effect against aberrant cell proliferation and/or carcinogenesis [Chang and Prins, 1999; Poelzl et al., 2000; Signoretti and Loda, 2001]. Furthermore, findings in ER-β knockout mice indicate that these animals develop prostatic hyperplasia in old age, a phenomenon that does not occur in ER-α knockout mice [Krege et al., 1998]. A protective role of ER-β against proliferative lesions is also suggested by the studies of Prins [Chang and Prins, 1999]. Neonatal exposure of rats to estrogen causes downregulation of ER-β, upregulation of ER-α, and development of hyperplastic, dysplastic, and neoplastic lesions in the adult ventral prostates. Other suggested functions of ER-β in the normal prostate are related to its role in protecting tissues against oxidative injury. It has been shown that the ER-β is more effective than ER-α in transactivation of the electrophile/antioxidant response element and therefore is a better inducer of antioxidant enzymes such as quinone reductase and GST [Montano and Katzenellenbogen, 1997; Montano et al., 2000]. This action of the ER-β, though not yet demonstrated in the prostate, is known to mediate

artheroprotective [Dimitrova et al., 2002] and neuroprotective [Treuter et al., 2000; Mize et al., 2003] effects of estrogens.

In a comprehensive study [Leav et al., 2001] the expression of androgen receptor (AR), ER-α, and ER-β in dyplastic lesions, primary carcinomas and in metastases of PCa was compared with that in normal glands from archival specimens. In the peripheral zone of the normal prostate, ER-β was overwhelmingly localized in the nuclei of basal cells, the purported proliferative compartment of the human prostate [De Marzo et al., 1998]. In the stroma of the peripheral zone, both ER-α and ER-β were expressed, while AR was present in both stromal and differentiated secretory cells. Compared with the normal epithelium, downregulation of ER-β was observed in high-grade PIN lesions, while the expression of ER-α or AR in these lesions remained unchanged. Interestingly, ER-β was strongly expressed in grade 3 carcinoma but was markedly diminished in carcinomas that were Gleason grade 4/5. Additional studies [Horvath et al., 2001; Latil et al., 2001; Pasquali et al., 2001a,b; Tsurusaki et al., 2003] have confirmed a loss or reduced expression of ER-β as PCa progresses in the primary site. These findings suggest a role for ER-β in limiting basal cells proliferation [McNeal, 1988; Bonkhoff et al., 1994; McNeal et al., 1995], which may help preserve genomic integrity [Signoretti and Loda, 2001; Weihua et al., 2002]. In addition, the proposed involvement of ER-β in regulating antioxidant enzyme expression [Montano and Katzenellenbogen, 1997; Montano et al., 2000] may protect basal cells from oxidative damage, which is a risk factor for neoplastic transformation. In this regard, the strong expression of ER-β in basal epithelial cells may be necessary to counteract androgen's stimulation of continuous growth [Lee, 1997] and/or its pro-oxidant action [Ripple et al., 1997, 1999]. Disruption of this equilibrium may trigger aberrant growth and transformation in the progenitor cells within the prostate basal epithelial layer, which has been postulated to harbor susceptible cell populations that give rise to PCa [Garraway et al., 2003].

In marked contrast, ER-β protein but not ER-α protein was dramatically expressed in the majority of metastases of PCa in bone and regional lymph nodes [Leav et al., 2001]. It is currently unclear why ER-β expression is elevated in metastases. Either local factors in the distant metastatic sites may induce ER-β expression or a clonal selection process may occur in primary tumors. The latter scenario would argue that PCa cells that fail to loss ER-β expression (for unknown reasons) have higher matastatic potentials and, therefore, escape to distant sites. Adding to the complexity of understanding the ER-β regulation of PCa development, a recent study [Fujimura et al., 2001] has found that while wild-type ER-β expression was significantly lower in the cancers than in the benign epithelium, ER-βcx (a C-terminal truncated splice variant of ER-β) was expressed at significantly higher levels in high-grade cancers (83%) compared with the low-grade tumors (22%) and the benign tissues. Although the functional roles of ER-βcx remain unclear, these findings raise the possibility that wild-type ER-β and ER-βcx are independent predictors of PCa. All in all, it has become apparent that ER-β is a key determinant of the genesis, progression and metastasis of PCa. Additional research is needed to fully unveil its contribution to the initiation and progression of PCa.

Finally, ER-α, expressed mainly in the stromal compartment of the prostate, may also contribute to the pathogenesis of PCa. In a study of the genotypic and allelic frequencies of the six different polymorphic loci of *ER-α* in a Japanese population, polymorphism in codon 10 of *ER-α* was found to be a possible risk factor for PCa [Tanaka et al., 2003].

Estrogen Imprinting and PCa Risks

Experimental and epidemiological evidence suggest that PCa risk in adulthood could be determined by exposure to estrogens during embryonic, perinatal/neonatal, or peripubertal development, a phenomenon referred to as "estrogen imprinting" [Rajfer and Coffey, 1978]. Perinatal and neonatal exposure of rats [Arai et al., 1977, 1983; Vorherr et al., 1979; Prins, 1992] or mice [McLachlan et al., 1975; McLachlan, 1977; Pylkkanen et al., 1991] to estrogens or estrogen mimics induces development of various proliferative lesions in the adult gland. These changes may be partially explained by permanent changes in androgen receptor levels, ER statuses, interacinar stromal tissue volume, and proliferative potential of the prostatic epithelium [Prins, 1992; vom Saal et al., 1997; Prins et al., 1998; Prins et al., 2001]. The "estrogenized" adult glands also demonstrate a lack of differentiation, disorganization

of the acini, and excessive luminal sloughing. Analogous to these animal studies, it has recently been reported that the fetal human prostate does not express ER-α at the protein level but does strongly express ER-β [Adams et al., 2002]. This finding indicates that ER-β is the sole transducer of estrogen action in the fetal human prostate during in utero development. Observation studies provide additional support for a fetal base determination of adult PCa risk. It has been shown that circulating E2 levels of pregnant African–American women are 37% higher than those of European–American women [Henderson et al., 1988]. Furthermore, Ekbom and associates [Ekbom et al., 1996, 2000] have reported that indicators of high levels of endogenous pregnancy hormones, such as high birth weight and jaundice in the offspring, are associated with increased risk for PCa, while indicators of low levels, like pre-eclampsia, are associated with decreased risk [Ekbom et al., 1996]. If intrauterine or perinatal environments are important for PCa risk in adulthood, cancer prevention strategies should be directed to these early developmental stages.

SERMs, Phytoestrogens, and new Estrogenic/Anti-Estrogenic Agents as Potential Therapies for PCa

The hypothesis that estrogen inhibits growth of PCa originates in the classic studies of Huggins and Hodges [1941] who pioneered the use of a potent xenoestrogen, DES, to treat men with PCa. Castration combined with DES was proven to be an affordable, highly efficient treatment, but it quickly fell out of favor due to cardiac toxicity [Cox and Crawford, 1995; Denis and Griffiths, 2000; Taplin and Ho, 2001]. A few clinical trials have found that the first generation of anti-estrogens/SERMs, such as tamoxifen and toremifene, as alternatives to DES, are not effective therapies [Bergan et al., 1999; Stein et al., 2001]. Despite these early set-backs, the likelihood of developing more effective and less toxic SERMs/estrogen-like compounds to treat PCa treatment has improved substantially, coincident with our growing knowledge on the differential expression of ER subtypes and differential action of estrogen/anti-estrogen/SERM in normal and malignant prostatic epithelial cells.

Traditionally, the primary mechanism by which estrogens are believed to induce PCa regression is through action on the hypothalamic-pituitary axis, thereby inhibiting testosterone synthesis [El Rayes and Hussain, 2002]. However, it is now known that many estrogens/anti-estrogens/phytoestrogens/SERMs including DES, 2-methoxy-E_2, genistein, resveratrol, licochalcone, Raloxifene, ICI 182,780, and estramustine have antitumor effects independent of this pathway. Their ability to suppress PCa cell growth has been attributed to a broad range of actions including direct cytotoxicity [Schulz et al., 1988], interruption of cell cycle progression [Kumar et al., 2001; Qadan et al., 2001], induction of apoptosis [Qadan et al., 2001; Kim et al., 2002; Shimada et al., 2003], depolymerization of microtubules [Dahllof et al., 1993], inhibition of DNA synthesis [Kuwajerwala et al., 2002], inhibition of topoisomerase II [Matsukawa et al., 1993], blockade of tyrosine kinase [Matsukawa et al., 1993; Misra et al., 2002], disruption of apoptotic regulators such as bcl-2 [Rafi et al., 2000], and activation of death domain receptors [Mor et al., 2000; LaVallee et al., 2003]. The ER subtype responsible for mediating the anti-tumor action of the pure anti-estrogen ICI-182,780 [Lau et al., 2000], the SERM Raloxifene [Kim et al., 2002], the phytoestrogen genistein, and others [An et al., 2001; Boue et al., 2003] is likely ER-β and not ER-α.

In addition to their ability to induce cell cycle arrest/apoptosis in PCa cells, some of these estrogenic/anti-estrogenic compounds are also potent inhibitors of angiogenesis and metastasis [Li and Sarkar, 2002]. Genistein was shown to upregulate expression of genes with anti-angiogenesis or anti-metastasis properties [Li and Sarkar, 2002] and to activate the cell adhesion-associated signaling via focal adhesion kinase [Liu et al., 2000] in PCa cells. Raloxifene, a SERM, administered to tumor-bearing male Lobund–Wistar (LW) rats, was shown to produce marked reduction in PCa metastasis to lymphatic and pulmonary tissues [Neubauer et al., 1995].

Estramustine, when combined with a variety of microtubular inhibitors, has clinically been proven highly active in treating hormone-refractory PCa [Gilligan and Kantoff, 2002]. Several new estrogenic/anti-estrogenic compounds are now in clinical phase I and phase II studies for PCa prevention or treatment. These include 2-methoxyestradiol [Lakhani et al., 2003], genistein [Miltyk et al., 2003],

clover-derived dietary isoflavones [Jarred et al., 2002], and two new SERMs, GTx-006 and arzoxifene [Steiner et al., 2001]. The hope is that these novel estrogens/anti-estrogens, phytoestrogens or SERMs can selectively activate apoptosis or cell cycle arrest in rapidly growing PCa cells, via non-ER-α and/or -ER-β signaling pathways, offering highly effective therapies for PCa while avoiding some of the undesirable side-effects believed to be caused by the "classical" estrogen action.

Herbal Estrogenic Formulations in Prevention and Treatment of PCA

Complementary and alternative medicines (CAMs) are estimated to be used by 7–64% of PCa patients, with younger and more educated men more likely to use them [Kao and Devine, 2000; Porterfield, 2000; Steginga et al., 2001]. Among CAM therapies for PCa, PC-SPES is by far the most widely used due to its potency [Kao and Devine, 2000; Yip et al., 2003]. It has recently been recalled by the US Food and Drug Administration because of contamination with the synthetic estrogen DES [Guns et al., 2002]. PC-SPES is an herbal formulation previously available as a dietary supplement sold in 'natural food' or 'health food' stores in the US. It is comprised of extracts from eight different plants: chrysanthemum, *Isatis indigotica*, *Glycyrrhiza glabra*, *Ganoderma lucidum*, *Panax pseudo-ginseng*, *Rabdosia rebescens*, saw palmetto, and *scutellaria baicalensis* [Thomson et al., 2002]. A handful of clinical studies indicate that the formulation has good clinical efficacy and benefits most patients (97%) who have hormonally-responsive PCa [Small et al., 2000; Pirani, 2001; Oh and Small, 2002]. It also has activity against a fraction of hormone-independent PCa [de la et al., 2000; Pfeifer et al., 2000; Small et al., 2000; Oh et al., 2001]. Experimental studies have documented in vitro and in vivo efficacy of the formulation and described possible estrogenic mechanisms of action [Hsieh et al., 1997, 1998; DiPaola et al., 1998; Kubota et al., 2000]. Biochemical studies indicated that PC-SPES has potent estrogenic activity similar to 1 nM estradiol. However, mass spectrometry analysis indicated that the estrogenic effect was not due to DES, estrone, or E2 [Hsieh et al., 1997, 1998; Darzynkiewicz et al., 2000]. The unsupervised use of herbal CAM by so many has raised serious concerns. First, the specific active estrogenic agents responsible for the therapeutic efficacy have not been isolated or characterized. Secondly, there is a paucity of data to demonstrate convincingly the safety, efficacy, effectiveness, and mechanisms of the effects of these compounds. Most importantly, up to 72% of PCa patients do not inform their physicians [Lippert et al., 1999] when they are using these therapies, while they are also receiving standard treatments for PCa. This may adversely affect or confound statistics surrounding the outcomes of standard treatments [Kao and Devine, 2000]. Finally, the difference in philosophies between Western medicine and herbal medicine may make evaluation of herbal medicine difficult when the same standards established to test synthetic pharmaceutical compounds were to be used as criteria. In order to advance CAM usage, well-designed clinical trials, perhaps using a set of modified standards, are necessary to assess the efficacy and safety of CAMs in PCa treatment [Yip et al., 2003]. Unfortunately, such trials are expected to be highly complex and expensive, and will require lengthy follow-up. Until such trials are completed, our knowledge and understanding of the mechanisms of action and efficacies of CAMs in PCa prevention and treatment will continue to be sketchy [Wilkinson and Chodak, 2003].

Overall Conclusions

The central role of estrogens in PCa development has been well supported by epidemiological findings and experimental animal data. Although it has been demonstrated in female estrogen-sensitive organs that estrogens can act as genotoxic carcinogens through metabolic activation, the importance of estrogen metabolites in prostate carnogenesis has thus far received very little attention. Data from animal studies support the hypothesis that formation of genotoxic metabolites and reactive intermediates as a probable cause of PCa initiation. Individuals expressing specific polymorphisims of estrogen metabolizing enzymes and local production of estrogen via aromatization probably determine the lifetime exposure and influence PCa risks among different ethnic/racial/geographical groups. The recent discovery of ER-β, in addition to ER-α, provides new insights to the mechanism(s) of ER-mediated events associated with PCa initiation and progression. Availability of a host of new estrogenic/anti-estrogenic/SERM-like

compounds and unearthing of novel anticancer actions afforded by these compounds on PCa cells open avenues for the development of a new generation of PCa therapies with high antitumor potency and minimal systemic side-effects caused by their innate estrogenicity.

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