The Role of Estrogens in Prostate Carcinogenesis: A Rationale for Chemoprevention

Maarten C. Bosland, DVSc, PhD

Departments of Environmental Medicine and Urology, New York University School of Medicine, New York, NY

Estrogens as hormonal therapy, particularly diethylstilbestrol, are effective against androgen-dependent prostate cancer, but paradoxically estrogens might also be involved in the causation of this malignancy. Therefore, antiestrogens have been suggested as both a chemopreventive and chemotherapeutic treatment, thereby inhibiting the development and progression of prostate cancer. This review addresses the role of estrogens in prostate carcinogenesis and prostate cancer progression and examines the rationale for using antiestrogenic agents in chemoprevention of prostate cancer. [Rev Urol. *2005;7(suppl 3):S4-S10*]

Key words: Prostate cancer • Estrogen • Antiestrogen • Carcinogenesis • Chemoprevention

Estrogens as hormonal therapy, particularly diethylstilbestrol (DES), are effective against androgen-dependent prostate cancer, but paradoxically estrogens might also be involved in the causation of this malignancy.[1] Therefore, antiestrogens have been suggested as chemopreventive agents to inhibit the development and progression of prostate cancer, and they might even have a therapeutic benefit.[2,3] This review addresses the role of estrogens in prostate carcinogenesis and examines the rationale for using antiestrogenic agents in chemoprevention of prostate cancer.

Estrogens as Hormonal Therapy for Prostate Cancer

DES was the first hormonal therapy for cancer, developed by Huggins and Hodges in 1941. Low-dose DES treatment is still used today as first-line hormonal therapy, albeit not in the United States, and can provide benefit as second-line hormonal therapy.[4-6] DES treatment ultimately leads to androgen-refractory prostate cancer, just as androgen-targeted hormonal therapy does. DES is a strong estrogen that suppresses pituitary secretion of luteinizing hormone (LH) and consequently inhibits testicular testosterone production, acting as an antiandrogen; it might also increase serum sex hormone-binding globulin, thereby reducing the amount of free testosterone in the circulation. DES also has direct effects on the prostate, being cytotoxic (eg, as DES-phosphate) and inhibiting proliferation and inducing apoptosis in prostate cancer cells in tissue culture. The mechanism by which DES acts directly on prostate cancer cells is not known with certainty, but it might involve up-regulation of transforming growth factor β, apoptosis, and cell–cell interactions. Whether an estrogen receptor (ER)-mediated action of DES is involved, and if so, by which of the currently known ER subtypes, ER-α or ER-β, is not known. Also unknown is whether estrogens other than DES have similar effects.

Possible Role of Estrogens in Prostate Carcinogenesis

There are multiple lines of evidence to suggest that estrogens are involved in prostate carcinogenesis.[1] For example, genetic polymorphisms of genes in the estrogen metabolism pathway were associated significantly with familial prostate carcinoma risk.[7] Moreover, one study[8] suggests that a polymorphism in codon 10 of ER-α might be a risk factor for prostate cancer, and another[9] suggests that variants of the GGGA polymorphism from the ER-α gene might be associated with an increased risk of developing prostate cancer. Both ER-α and ER-β are expressed in the normal prostate: ER-α is found in the stromal cells of the human prostate, and ER-β is present in the basal cells. However, ER expression status is disrupted in the malignant prostate, where ER-β expression is decreased during malignancy progression owing to methylation of CpG dinucleotides in the promoter of the gene.[10-13] This suggests that ER-β has a function in tumor suppression. Indeed, restoration of ER-β by adenoviral delivery inhibits the invasiveness and growth of prostate cancer cells.[14] Moreover, cells overexpressing ER-β undergo apoptosis,[14] and ventral prostates of ER-β knock-out mice exhibit decreased apoptosis,[15] suggesting that ER-β acts as a tumor suppressor by its antiproliferative, anti-invasive, and pro-apoptotic properties. As prostate cancer risk increases with age, circulating androgen levels decrease but estrogen levels remain more constant, resulting in a decrease in the androgen/estrogen ratio in the serum. Weak associations between prostate cancer risk and prediagnostic serum estrogen levels have been found in some (but not all) epidemiologic studies. In addition, circulating levels of estradiol, and particularly the estradiol precursor estrone, are slightly higher in African American men than in Caucasian men, whereas African Americans have twice the prostate cancer risk of Caucasians.[16] Collectively, these observations lead to the hypothesis that increased circulating estrogens might elevate prostate cancer risk and act through the ER.

This idea is strongly supported by the finding that estrogens enhance androgen-induced prostate cancer in an animal model.[1,17] When testosterone is chronically administered to rats at low doses, prostate cancer is found in several rat strains, in most cases at low incidence (5%-10%), but in the NBL rat strain (also known as the Noble rat) the incidence is as high as 35% to 40%.[1] When estradiol is given together with low-dose testosterone, the incidence of prostate carcinomas increases to nearly 100%.[17] Most of these estradiol-plus-testosterone-induced tumors are located in the periurethral prostatic ducts. Estradiol alone chemically castrates the rats because it eliminates testicular testosterone production through suppression of LH secretion, as does DES. In fact, DES-plus-testosterone treatment also causes prostate cancer in these rats, and strains other than the NBL rat might also respond with increased prostate cancer rates if testosterone treatment is combined with estrogens.[17] In summary, estrogens are required for a maximal carcinogenic response to androgens, at least in rat models.

Weak associations between prostate cancer risk and prediagnostic serum estrogen levels have been found in some (but not all) epidemiologic studies.

Androgens as Precursors of Estrogen in Prostate Carcinogenesis

A causative role of androgens in prostate cancer is generally accepted for a number of reasons. The prostate is an androgen-dependent tissue, and early-stage prostate cancer is androgen dependent. Hormonal therapy eventually results in androgen-refractory prostate cancer, but these

cancers still express androgen receptors (ARs), which has been attributed to a poorly understood ligand-independent activation of these receptors. Serum androgen levels tend to be elevated in high-risk populations, such as African American men, in some studies; however, androgen levels are not lower in low-risk Japanese men than in Caucasians.[18] Furthermore, associations between risk and prediagnostic serum androgen levels are only weak at best and not found in most studies.[19] The only consistent finding is that 5α-reduced metabolites are associated with risk in prediagnostic serum studies, and comparisons among low- and high-risk populations suggest that 5α-reductase activity is a critical factor.[16,18,19] The strongest direct evidence for androgens causing prostate cancer comes from studies in which androgens induce prostatic carcinomas in several rat strains, as indicated earlier, and also strongly enhance prostate carcinogenesis after treatment with chemical carcinogens.[1]

It is less well appreciated that testosterone is by far the most significant precursor of estradiol-17-β (estradiol) in men; the other (minor) estradiol precursor is estrone formed from androstenedione, which is produced by the adrenals and to a lesser extent by the testes. The conversion of testosterone to estradiol is mediated by the enzyme aromatase, which is most highly expressed in fat tissue and might also be present in the prostate, although this remains controversial. This raises the possibility that the prostate cancer induced by testosterone in the rat prostate is, at least in part, due to estrogen effects.

It is possible that the prostate cancer induced by testosterone in the rat prostate is, at least in part, due to estrogen effects.

Mechanisms of Estrogen Carcinogenesis: Receptor Mediation

The AR is almost certainly involved in androgen-induced prostate cancer. Combined treatment of a rat model with testosterone and the AR-blocking antiandrogen flutamide almost entirely eliminates the induction of prostate cancer by the androgen after

Supporting this notion, when 5α-dihydrotestosterone (DHT), which cannot be converted to estradiol by aromatase, was given to NBL rats, prostate cancer developed in less than 5% of animals (Bosland and colleagues, unpublished data). However, when estradiol was combined with DHT, tumor incidence increased only to 15%. There are no studies of aromatase inhibitors in this rat model. Thus, precisely how estrogen interacts with androgen in producing prostate cancer in rats is complex and remains unclear. Evidence for such an interaction has come recently from experiments showing that a metabolite of DHT, 5α-androstane-3β, 17β-diol (3β-androstanediol), has the characteristics of the natural ligand for ER-β.[20] Although it has a lower affinity than estradiol for ER-β, it is present in the prostate at a sufficient level to activate ER-β.[15] These findings, together with the finding that testosterone up-regulates ER-β messenger ribonucleic acid expression,[21] suggest that there is a close interaction between the estrogen and androgen pathways in the prostate.

In the NBL rat treated with estradiol and testosterone, the pure antiestrogen ICI 182,780 inhibited development of dysplasia

administration of a chemical carcinogen (Bosland and colleagues, unpublished data). Whether the ER is involved is less clear, although co-administration of the ER antagonist tamoxifen and testosterone did not affect induction of prostate cancer in a rat model by androgen after administration of a chemical carcinogen (Bosland and colleagues, unpublished data). In the NBL rat treated with estradiol and testosterone, the pure antiestrogen ICI 182,780 inhibited development of dysplasia,[22] a prostatic intraepithelial neoplasia (PIN)-like lesion that does not seem to progress to cancer (Bosland and colleagues, unpublished data), but effects of antiestrogens on cancer induction are not known. This anti-dysplasia effect of ICI 182,780 in the NBL rat model suggests that ER mediation is involved. Alternatively, this antiestrogen might inhibit the prolactin-elevating effect of estradiol in this model and thereby inhibit induction of inflammation and inflammation-related dysplasia in the rat prostate, which is known to be elicited by elevated prolactin levels.[23] Of note, preliminary findings in studies by the author suggest that inflammation is probably not involved in periurethral prostate carcinogenesis, although it is commonly found in association with prostatic dysplasia induced by estradiol and testosterone. In conclusion, it is not clear at present whether the ER mediates the induction of prostate carcinomas by estradiol and testosterone in rats.

There are genetic polymorphisms that result in more activity of those enzymes that increase levels of androgens (eg, 5α-reductase) or estrogens (eg, aromatase), but whether these are

associated with prostate cancer risk has not been established to date. Similarly, there are genetic polymorphisms resulting in more receptor activity for androgens or estrogens (ie, AR or ER single nucleotide polymorphisms), but their association with risk of prostate cancer remains unclear, with one exception. The number of CAG repeats in the AR promotor has been found to be associated with risk in most but not all studies. Short CAG repeat lengths are associated with increased AR transactivation and higher risk compared with longer repeat lengths.

Mechanisms of Estrogen Carcinogenesis: Genotoxic Activity of Estrogen Metabolites

There is a growing body of evidence to suggest that estrogens might act as carcinogenic factors not only through the ER but also through a genotoxic (deoxyribonucleic acid [DNA]-damaging) mechanism.[24] Estradiol (and DES) can be converted to so-called catecholestrogen metabolites by P450-mediated hydroxylation.[25] Unless they are detoxified, these catecholestrogens can undergo a process called *redox-cycling*. This process leads to the formation of reactive oxygen species that can lead to DNA damage and lipid peroxidation, as well as to the formation of reactive intermediates that can directly adduct to DNA, which might lead to mutagenesis.[24] There is evidence that this happens in tissue culture and in animal models of estrogen-induced cancer, as well as a limited amount of evidence from studies with human breast cancer tissue.[24,25]

For prostate cancer, there is evidence from studies with the NBL rat model. Preceding cancer formation in this model, at the exact site of tumor development (the periurethral ducts), there is DNA adduction, oxidative DNA damage, and lipid peroxidation in response to combined estrogen–androgen treatment.[24,26] In addition, there is limited evidence for the presence in the NBL rat prostate of aromatase, the P450 enzymes necessary for the formation of catecholestrogens, and the conditions necessary for redox-cycling.[27] Finally, there are also indications that the sites of cancer development (the periurethral ducts) and dysplasia (dorsolateral prostate acini) in the NBL rat have lower levels of enzymes that protect against catecholestrogens and redox-cycling.[27] In conclusion, there is evidence from animal studies that estrogens can have genotoxic activity in the rat prostate and that this might be related to induction of cancer by estradiol plus testosterone. There are at present no human data in this regard.

The therapeutic advantage of SERMs is their potential for exhibiting agonistic effects in some tissues (eg, enhancing the beneficial effects of estrogens on bone tissues) while exerting antagonistic effects in others.

Estrogen Ablation Therapy in Prostate Cancer

Estrogen ablation therapy has been proposed for prostate cancer treatment on the basis of observations in preclinical studies, mostly with cells in culture. Antiestrogens, which are selective in their antiestrogenic effects on different tissues, are often referred to as *selective ER modulators* (SERMs). SERMs have the potential to act as ER agonists in some tissues but as ER antagonists in others.[28] Indeed, the therapeutic advantage of SERMs is their potential for exhibiting agonistic effects in some tissues (eg, enhancing the beneficial effects of estrogens on bone tissues) while exerting antagonistic effects in others (eg, inhibiting the harmful effects of estrogens on prostate cell proliferation). Many of the selective effects of SERMs seem to be mediated by their influence on the interaction between the ER and ER-coregulatory proteins (coactivators and corepressors). Thus, the binding of a SERM to the ER will induce a conformational change in the receptor, such that the ER will interact to a greater or lesser extent with the coregulators available. Because the amount of a given coregulator differs in different tissues, the ER can be either activated or inactivated, depending on the availability and/or concentration of coactivators or corepressors. For example, tetrahydrochrysene (THC) acts as an ERα agonist by stabilizing a conformation of ERα ligand-binding domain that permits coactivator association.[29] In contrast, THC acts as an ERβ antagonist by preventing coactivator association. SERMs also influence the stability of ER and thus might affect the activity of the ER by modulating the half-life of the receptor in a cell.[30]

Tamoxifen, the first clinically used antiestrogen SERM, inhibits proliferation of PC-3 and DU-145 prostate cancer cells[31] and induces apoptosis in LNCaP cells.[32] It also inhibits in vivo growth of the CWR22 prostate cancer xenograft in nude mice.[33] Tamoxifen has been studied in phase II clinical trials with prostate cancer patients, but therapeutic efficacy was uncertain.[34,35] A major problem with tamoxifen is its genotoxic effects on the uterus and other tissues, where DNA adducts have been found, and its use has been associated with an elevated risk of uterine cancer in women and rats.[36] In addition, compounds such as tamoxifen have mixed antagonist and agonist (= estrogenic) effects. It is not

clear which of these 2 modes of action is responsible for the beneficial effects of tamoxifen found in preclinical studies.

The anti-prostatic tumor effect of raloxifene, a more recently developed antiestrogenic SERM, has been investigated in several tumor models. Neubauer and associates[37] reported that raloxifene reduced the development of pulmonary metastasis in a dose dependent manner in the PAIII prostatic adenocarcinoma model in Lobund-Wistar (LW) rats. Kim and colleagues[38] have shown that raloxifene also induces apoptosis in LNCaP cells.[38] In women, raloxifene mimics the beneficial effects of estrogen on bone density without some of the risks associated with estrogen.[39] A recent study in men receiving a gonadotropin-releasing hormone agonist showed that raloxifene significantly increases bone mineral density of the hip and tends to increase bone mineral density of the spine.[40]

The anti-prostatic cancer effects of other SERMs including trioxifene, ICI 182,780 and toremifene have also been characterized. The antiestrogen trioxifene inhibits in vivo growth and progression of a transplantable rat prostate tumor.[41] The pure antiestrogen ICI 182,780 and the SERM antiestrogen toremifene inhibit proliferation of PC-3 cells.[42] Toremifene has similar genotoxic effects as tamoxifen but at a much reduced level, and there is no evidence of an association with an elevated uterine cancer risk in women and rats. It is therefore considered a much safer drug.[43]

Natural "phytoestrogens," such as genistein, indole-3-carbinol, and resveratrol can inhibit proliferation of prostate cancer cells (see, for example, Morris and colleagues,[44] Chinni and Sarkar,[45] and Davis and colleagues[46]). These "nutriceutical" compounds might offer alternatives to pharmaceutical antiestrogens for estrogen ablation therapy. In conclusion, there are experimental observations suggesting that antiestrogens might have therapeutic efficacy in prostate cancer, but the limited studies with tamoxifen do not support this concept in hormone refractory disease.

The concept of chemoprevention needs to be widened to include prevention of growth and progression of already-existing cancer.

Prostate Cancer Chemoprevention

Chemoprevention is generally defined as the inhibition of development of premalignant lesions or of their progression to cancer by pharmaceutical agents or nutriceutical compounds. For prostate cancer, however, this concept needs to be widened to include prevention of growth and progression of already-existing cancer.[47] This shift in thinking about chemoprevention when applied to prostate cancer is based on the observation that histologic prostate cancer is common even in younger men. For example, Sakr and colleagues[48] reported that approximately 30% of men between the ages of 30 and 50 years have histologic cancers in their prostate. In this elegant study histologic cancers were found more often than high-grade PIN (HGPIN) in young men, casting some doubt about the notion that HGPIN is the main or only precursor of prostate cancer.[48] However, the results of other studies quantifying the prevalence of HGPIN and histologic cancer in autopsy and radical prostatectomy surgical specimens obtained from African American and Caucasian men suggest that HGPIN is an important, but not the only, precursor of clinically relevant prostate cancers. Consequently, the development and progression of both HGPIN and histologic cancer should be the objective of prostate cancer chemoprevention.[47] In addition, there is evidence to suggest that there are many different molecular pathways and mechanisms that result in prostate cancer in humans. One of these mechanisms might be oxidative damage, such as found in the aforementioned NBL rat model and likely to occur in the human prostate in association with prostatitis. Thus, it is probably important that chemoprevention agents have multiple anticancer activities. Possibly important in this regard is that many SERMs have mixed agonist and antagonist action at the ER, as well as other anticancer activities. This might provide them with a broader spectrum of chemopreventive activity than pure antiestrogenic agents. At the same time, it is critical that SERMs used as chemopreventive agents are very safe and have no genotoxic or other adverse properties.

Estrogen Ablation in Prostate Cancer Prevention

Preclinical therapeutic efficacy studies suggest preventive activity of phytoestrogens and SERMs. Therefore, the potential of these agents to prevent prostate cancer has been explored in animal studies and in one human clinical trial. The SERM antiestrogen toremifene inhibited in vivo development and growth of prostate tumors in the transgenic mouse prostate cancer model.[49] Toremifene had suggestive protective activity in men with HGPIN, in whom reversal of HGPIN lesions was observed, and it was well tolerated in a phase II clinical trial.[50]

In one study, tamoxifen significantly decreased the risk of developing androgen-induced tumors in the LW rat.[51] The pure antiestrogen ICI 182,780 also inhibited development

of prostatic dysplasia in the NBL rat treated with estradiol plus testosterone.[22] In contrast, tamoxifen did not prevent prostate cancer in rats treated with testosterone after administration with a chemical carcinogen, but in this model there is no clear evidence that the cancers were induced via an estrogenic mechanism.

Summary and Conclusions

Estrogens and ERs are clearly linked to the development and progression of prostate cancer. These activities of estrogens might be associated not only with their ER-mediated effects but also with the DNA-damaging and potentially mutagenic activity of some estrogenic compounds. ER-mediated activity of estrogens that are carcinogenic to the prostate in animal models provides a rationale for the exploration of using antiestrogens that block the ER as preventive agents. The results of one clinical trial suggest that this is a realistic proposition. In addition, there is some evidence to suggest that antiestrogens can have therapeutic benefit as well, but this has not yet been explored clinically. Because chemoprevention of prostate cancer should target not only the development and progression of HGPIN but also histologic prostate cancer, discovering drugs or natural substances that interfere with the development of both HGPIN and cancer and progression of HGPIN to cancer will be very important. Because prostate carcinogenesis involves androgens and oxidative stress, which are associated with inflammation and possibly estrogen effects, mixed antiestrogenic, anti-androgenic, and antioxidant activities of agents or combinations of agents are probably critical to the success of chemoprevention of prostate cancer. Successful agents must also be very safe because of the very long duration of their application in men at risk for prostate cancer. Thus, SERMs offer the possibility of prevention and/or treatment of prostate cancer. Several approaches to prostate cancer chemoprevention and treatment are under development. ■

Acknowledgements

This work was supported in part by National Institutes of Health (NIH) grants CA49210, CA75293, CA76426, and CA104334; NIH Center Grants CA13343 and ES00260, and US Department of Defense grant DAMD17-02-1-0660. The author thanks his collaborators Ercole Cavalieri, DSc, Elli Rogan, PhD, Shuk-mei Ho, PhD, David McCormick, PhD, and William Johnson, PhD.

References

1. Bosland MC. The role of steroid hormones in prostate carcinogenesis. *J Natl Cancer Inst Monogr*. 2000;27:39-66.
2. Ho SM. Estrogens and anti-estrogens: key mediators of prostate carcinogenesis and new therapeutic candidates. *J Cell Biochem*. 2004;91: 491-503.
3. Steiner MS, Raghow S. Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk. *World J Urol*. 2003;21: 31-36.
4. Malkowicz SB. The role of diethylstilbestrol in the treatment of prostate cancer. *Urology*. 2001;58(2 suppl 1):108-113.
5. Burns-Cox N, Basketter V, Higgins B, Holmes S. Prospective randomised trial comparing diethylstilboestrol and flutamide in the treatment of hormone relapsed prostate cancer. *Int J Urol*. 2002;9:431-434.
6. Smith DC, Redman BG, Flaherty LE, et al. A phase II trial of oral diethylstilbestrol as a second-line hormonal agent in advanced prostate cancer. *Urology*. 1998;52:257-260.
7. Suzuki K, Nakazato H, Matsui H, et al. Genetic polymorphisms of estrogen receptor alpha, CYP19, catechol-O-methyltransferase are associated with familial prostate carcinoma risk in a Japanese population. *Cancer*. 2003;98: 1411-1416.
8. Tanaka Y, Sasaki M, Kaneuchi M, et al. Polymorphisms of estrogen receptor alpha in prostate cancer. *Mol Carcinogenesis*. 2003;37:202-208.
9. Cancel-Tassin G, Latil A, Rousseau F, et al. Association study of polymorphisms in the human estrogen receptor alpha gene and prostate cancer risk. *Eur Urol*. 2003;44:487-490.
10. Leav I, Lau KM, Adams JY, et al. Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am J Pathol*. 2001; 159:79-92.
11. Zhu X, Leav I, Leung YK, et al. Dynamic regulation of estrogen receptor-beta expression by DNA methylation during prostate cancer development and metastasis. *Am J Pathol*. 2004; 164:2003-2012.
12. Latil A, Bieche I, Vidaud D, et al. Evaluation of androgen, estrogen (ER alpha and ER beta), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. *Cancer Res*. 2001;61:1919-1926.
13. Ji Q, Liu PI, Elshimali Y, Stolz A. Frequent loss of estrogen and progesterone receptors in human

Main Points

- Observations from several studies led to the hypothesis that increased circulating estrogens might elevate prostate cancer risk and act through the estrogen receptor; this idea is strongly supported by the finding that estrogens enhance androgen-induced prostate cancer in an animal model.
- Evidence from animal studies suggests that estrogens can have genotoxic activity in the rat prostate and that this might be related to induction of cancer by estradiol plus testosterone; there are at present no human data in this regard.
- Experimental observations suggest that antiestrogens might have therapeutic or preventive efficacy in prostate cancer. Studies with tamoxifen do not support the concept of therapeutic benefit, but there are no completed human studies exploring this idea with other agents. There is some support from a human study for the notion that antiestrogens might have preventive efficacy against prostate cancer.

prostatic tumors determined by quantitative real-time PCR. *Mol Cell Endocrinol*. 2005;229: 103-110.
14. Cheng J, Lee EJ, Madison LD, Lazennec G. Expression of estrogen receptor beta in prostate carcinoma cells inhibits invasion and proliferation and triggers apoptosis. *FEBS Lett*. 2004;566: 169-172.
15. Imamov O, Morani A, Shim GJ, et al. Estrogen receptor beta regulates epithelial cellular differentiation in the mouse ventral prostate. *Proc Natl Acad Sci USA*. 2004;101:9375-9380.
16. Ross R, Bernstein L, Judd H, et al. Serum testosterone levels in healthy young black and white men. *J Natl Cancer Inst*. 1986;76:45-48.
17. Bosland MC, Ford H, Horton L. Induction of a high incidence of ductal prostate adenocarcinomas in NBL and Sprague Dawley rats treated with estradiol-17β or diethylstilbestrol in combination with testosterone. *Carcinogenesis*. 1995; 16:1311-1317.
18. Ross RK, Bernstein L, Lobo RA, et al. 5-alpha-reductase activity and risk of prostate cancer among Japanese and US white and black males. *Lancet*. 1992;339:887-889.
19. Eaton NE, Reeves GK, Appleby PN, Key TJ. Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. *Br J Cancer*. 1999;80:930-934.
20. Weihua Z, Lathe R, Warner M, Gustafsson JA. An endocrine pathway in the prostate, ERbeta, AR, 5alpha-androstane-3beta,17beta-diol, and CYP7B1, regulates prostate growth. *Proc Natl Acad Sci USA*. 2002;99:13589-13594.
21. Asano K, Maruyama S, Usui T, Fujimoto N. Regulation of estrogen receptor alpha and beta expression by testosterone in the rat prostate gland. *Endocr J*. 2003;50:281-287.
22. Thompson CJ, Tam NN, Joyce JM, et al. Gene expression profiling of testosterone and estradiol-17 beta-induced prostatic dysplasia in Noble rats and response to the antiestrogen ICI 182,780. *Endocrinology*. 2002;143:2093-2105.
23. Lane KE, Leav I, Ziar J, et al. Suppression of testosterone and estradiol-17beta-induced dysplasia in the dorsolateral prostate of Noble rats by bromocriptine. *Carcinogenesis*. 1997; 18:1505-1510.
24. Cavalieri E, Frenkel K, Liehr JG, et al. Estrogens as endogenous genotoxic agents—DNA adducts and mutations. *J Natl Cancer Inst Monogr*. 2000; 27:75-93.
25. Jefcoate CR, Liehr JG, Santen RJ, et al. Tissue-specific synthesis and oxidative metabolism of estrogens. *J Natl Cancer Inst Monogr*. 2000;27:95-112.
26. Han X, Liehr JG, Bosland MC. Induction of a DNA adduct detectable by ^{32}P-postlabeling in the dorsolateral prostate of NBL/Cr rats treated with estradiol-17β and testosterone. *Carcinogenesis*. 1995;16:951-954.
27. Cavalieri EL, Devanesan PD, Bosland MC, et al. Catechol estrogen metabolites and conjugates in different regions of the prostate of Noble rats treated with 4-hydroxyestradiol: implications for estrogen-induced initiation of prostate cancer. *Carcinogenesis*. 2002;23:329-333.
28. Jordan VC. Selective estrogen receptor modulation: concept and consequences in cancer. *Cancer Cell*. 2004;5:207-213.
29. Shiau AK, Barstad D, Radek JT, et al. Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism. *Nat Structural Biol*. 2003;9:359-364.
30. Wijayaratne AL, McDonnell DP. The human estrogen receptor-alpha is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. *J Biol Chem*. 2001;276: 35684-35692.
31. Rohlff C, Blagosklonny MV, Kyle E, et al. Prostate cancer cell growth inhibition by tamoxifen is associated with inhibition of protein kinase C and induction of p21(waf1/cip1). *Prostate*. 1998;37:51-59.
32. El Etreby MF, Liang Y, Lewis RW. Induction of apoptosis by mifepristone and tamoxifen in human LNCaP prostate cancer cells in culture. *Prostate*. 2000;43:31-42.
33. Ma ZS, Huynh TH, Ng CP, et al. Reduction of CWR22 prostate tumor xenograft growth by combined tamoxifen-quercetin treatment is associated with inhibition of angiogenesis and cellular proliferation. *Int J Oncol*. 2004;24: 1297-1304.
34. Glick JH, Wein A, Padavic K, et al. Phase II trial of tamoxifen in metastatic carcinoma of the prostate. *Cancer*. 1982;49:1367-1372.
35. Bergan RC, Reed E, Myers CE, et al. A Phase II study of high-dose tamoxifen in patients with hormone-refractory prostate cancer. *Clin Cancer Res*. 1999;5:2366-2373.
36. Kim SY, Suzuki N, Laxmi YR, Shibutani S. Genotoxic mechanism of tamoxifen in developing endometrial cancer. *Drug Metab Rev*. 2004;36:199-218.
37. Neubauer BL, Best KL, Counts DF, et al. Raloxifene (LY156758) produces antimetastatic responses and extends survival in the PAIII rat prostatic adenocarcinoma model. *Prostate*. 1995; 27:220-229.
38. Kim IY, Seong do H, Kim BC, et al. Raloxifene, a selective estrogen receptor modulator, induces apoptosis in androgen-responsive human prostate cancer cell line LNCaP through an androgen-independent pathway. *Cancer Res*. 2002;62:3649-3653.
39. Reginster JY, Sarkar S, Zegels B, et al. Reduction in PINP, a marker of bone metabolism, with raloxifene treatment and its relationship with vertebral fracture risk. *Bone*. 2004;34:344-351.
40. Smith MR, Fallon MA, Lee H, Finkelstein JS. Raloxifene to prevent gonadotropin-releasing hormone agonist-induced bone loss in men with prostate cancer: a randomized controlled trial. *J Clin Endocrinol Metab*. 2004;89: 3841-3846.
41. Neubauer BL, McNulty AM, Chedid M, et al. The selective estrogen receptor modulator trioxifene (LY133314) inhibits metastasis and extends survival in the PAIII rat prostatic carcinoma model. *Cancer Res*. 2003;63:6056-6062.
42. Kawashima H, Tanaka T, Cheng JS, et al. Effect of anti-estrogens on the androgen receptor activity and cell proliferation in prostate cancer cells. *Urol Res*. 2004;32:406-410.
43. Shibutani S, Ravindernath A, Terashima I, et al. Mechanism of lower genotoxicity of toremifene compared with tamoxifen. *Cancer Res*. 2001;61: 3925-3931.
44. Morris GZ, Williams RL, Elliott MS, Beebe SJ. Resveratrol induces apoptosis in LNCaP cells and requires hydroxyl groups to decrease viability in LNCaP and DU 145 cells. *Prostate*. 2002;52: 319-329.
45. Chinni SR, Sarkar FH. Akt inactivation is a key event in indole-3-carbinol-induced apoptosis in PC-3 cells. *Clin Cancer Res*. 2002;8:1228-1236.
46. Davis JN, Singh B, Bhuiyan M, Sarkar FH. Genistein-induced upregulation of p21WAF1, downregulation of cyclin B, and induction of apoptosis in prostate cancer cells. *Nutr Cancer*. 1998;32:123-131.
47. Bosland MC, McCormick DL, Melamed J, et al. Chemoprevention strategies for prostate cancer. *Eur J Cancer Prev*. 2002;11(suppl 2):S18-S27.
48. Sakr WA, Grignon DJ, Crissman JD, et al. High grade prostatic intraepithelial neoplasia (HGPIN) and prostatic adenocarcinoma between the ages of 20-69: an autopsy study of 249 cases. *In Vivo*. 1994;8:439-443.
49. Raghow S, Hooshdaran MZ, Katiyar S, Steiner MS. Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. *Cancer Res*. 2002;62:1370-1376.
50. Steiner MS, Pound CR. Phase IIA clinical trial to test the efficacy and safety of Toremifene in men with high-grade prostatic intraepithelial neoplasia. *Clin Prostate Cancer*. 2003;2:24-31.
51. Lucia MS, Anzano MA, Slayter MB, et al. Chemopreventive activity of tamoxifen, N-(4-hydroxyphenyl) retinamide, and the vitamin D analogue Ro24-5531 for androgen-promoted carcinomas of the rat seminal vesicle and prostate. *Cancer Res*. 1995;55:5621-5627.