Inhibition of Androgen-independent Growth of Prostate Cancer Xenografts by 17β-Estradiol

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Abstract

Purpose: Estrogen treatment has long been known to be of benefit in prostate cancer (CaP), but its mechanism was thought to involve merely a reduction in androgen levels. However, new evidence indicates that estrogen may exert effects on CaP cells in the absence of androgens.

Experimental Design: Implantation of CaP xenografts (LuCaP 35, LuCaP 49, LuCaP 58, LuCaP 73, PC-3, and LNCaP) into intact and ovariectomized female mice was done to characterize growth and take rates in the absence of androgens. Ovariectomized female mice were supplemented with 17β-estradiol, and LuCaP 35 CaP xenograft take and growth rates were determined. Reverse transcription-PCR was used to evaluate the presence of the estrogen receptor messages in CaP xenografts.

Results: We have observed significant inhibition of CaP growth in intact versus ovariectomized female animals in five of six CaP xenograft lines. 17β-Estradiol supplements given to ovariectomized female mice led to inhibition of tumor establishment and diminished growth of LuCaP 35 similar to that observed in intact female mice. Using reverse transcription-PCR, we have shown that these xenografts express the estrogen receptor β message.

Conclusions: We have determined that 17β-estradiol supplementation causes inhibition of CaP growth in an animal model by mechanisms that are independent of androgen action. This gives rise to the possibility that estrogen therapy may be of potential use with hormone-refractory cancers. The xenograft models we describe herein may be useful as well in elucidating the pathways mediating the androgen-independent effects of estrogen on CaP.

Introduction

The beneficial effects of androgen withdrawal on advanced CaP were established as early as 1941. In 1946, Nesbit and Plumb (1) reported prolonged survival of CaP patients who had undergone orchietomy or estrogen therapy, with slight advantages for estrogen therapy. It was believed that the response to estrogen therapy in hormone-sensitive CaP was mediated primarily via suppression of the hypothalamo-hypophyseal axis and consequent reduction in testosterone. In an evaluation of DES (a synthetic compound with estrogenic effects), the Veterans Administration Cooperative Urological Research Group concluded that DES was effective in controlling advanced CaP, but that this therapy should be withheld because of the risk of cardiovascular complications until symptoms of metastatic disease appeared (2). The advent of luteinizing hormone-releasing hormone analogues, which are now mainly used as a means of chemical castration, essentially ended the era of estrogen treatment of CaP. In 1986, de la Monte et al. (3) reported that patients treated with estrogen appeared to survive somewhat longer than patients who had undergone surgical castration (0.05 < P < 0.1). In 1988, Byar and Corle (4) commented that no form of endocrine therapy had been proved superior to 1 mg of DES daily. In 1976, Mangan et al. (5) postulated direct action of estrogen on CaP via ERs. Experimental data published by Ferro (6) indicated that estrogens may exert a direct cytotoxic effect on CaP tumors. If there is a direct effect of estrogens that inhibits CaP progression or prolongs patient survival, then the use of estrogens at low levels may prove to be justified.

The presence of ERs in prostatic tissues has long been controversial (7–9). Radioigand-binding assays with normal prostate, benign prostatic hypertrophy, and CaP have demonstrated the presence of E2 binding sites (7). In contrast, immunohistochemical studies have only rarely demonstrated the presence of ERs in these tissues (8–11). The discrepancies relating to the expression of ERs may largely be attributable to the existence of two receptor types, ERα (12) and ERβ (13); most of these reports were published before the discovery of ERβ.

Evidence of the presence of ERα and ERβ messages in prostatic tissues has appeared recently (14–16). Bonkhoff et al. (17) reported detection of ERα protein, but not ERβ, in prostate tissues. Horvath et al. (18) reported detection of ERβ in normal prostate epithelium but significantly decreased expression of ERβ in prostatic carcinoma. Pasquali et al. (19) detected ERβ in normal prostate but not in samples of CaP. Interestingly, Leav et al. (20) reported decreased immunoreactivity of ERβ in high-level dysplasia but reapparance of this protein in high-grade carcinoma and in CaP metastases. Renewed interest in the

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3 The abbreviations used are: CaP, prostate cancer; DES, diethylstilbestrol; ER, estrogen receptor; E2, 17β-estradiol; IF, intact female; AR, androgen receptor; OVXF, ovariectomized female; PSA, prostate-specific antigen; RT-PCR, reverse transcription-PCR.
presence of ER in prostatic tissues and the potential benefits of estrogen therapy in CaP is indicated by recent reviews (21–23).

This study was undertaken to evaluate differences in growth of CaP xenografts in IF mice and OVXF mice to determine whether E2 has inhibitory effects on CaP growth. We report that the growth of CaP xenografts was inhibited in IF mice compared with OVXF mice, and that E2 supplementation restored growth inhibition similar to that observed in IF animals.

Materials and Methods

Tissue Culture. PC-3 and LNCaP CaP cells were obtained from the American Type Culture Collection and maintained in RPMI 1640 with 10% FCS under standard conditions.

CaP Xenografts. LuCaP 35 originated from a lymph node CaP metastasis. It is hormone sensitive and expresses PSA and a wild-type AR. LuCaP 49 was established from an omental mass (a small cell carcinoma) and is hormone insensitive with no expression of PSA and AR. LuCaP 58 originated from a lymph node CaP metastasis, and LuCaP 73 originated from a prostatic pelvic mass CaP metastasis. These xenografts are hormone sensitive and express PSA and AR, but as yet it is not known whether they express wild-type or mutated AR (24). All xenografts are maintained by passaging small pieces of tumors in male BALB/c nu/nu mice.

Animal Studies. All procedures were performed in compliance with the University of Washington Institutional Animal Care and Use Committee and NIH guidelines. Twelve groups of 15 female BALB/c nu/nu mice were used. Half of the animals were ovariectomized at 8 weeks of age, and all animals were implanted with tumors at least 2 weeks after surgery. LuCaP 35, 41, 49, 58, and 73 tumor bits were implanted s.c.; 2×10⁶ PC-3 cells or LNCaP cells mixed 1:1 with Matrigel were injected s.c.

E2 Supplementation. Three groups of 15 female BALB/c nu/nu mice were used. Group 1 consisted of OVXF mice with LuCaP 35 supplemented with E2 by s.c. implantation of 90-day slow-release pellets (Innovative Research of America, Sarasota, FL). Group 2 consisted of IF mice with LuCaP 35 and placebo pellets, and group 3 consisted of OVXF mice with placebo pellets. Tumor growth was monitored by measuring tumor volume twice per week. Tumor volume was calculated as length × height × width × 0.5236.

Statistical Analysis. The significance of differences in tumor growth rate in IF versus OVXF mice was tested using the log-rank statistic, where the significance level (P) was determined using the permutation method. To apply the test within each experiment, the maximum tumor weight achieved by at least 90% of the tumors in that experiment was set to be the end point for the test. A contingency table was used to determine the significance of differences in tumor take rates.

RT-PCR. RT-PCR was performed using total RNA as described previously (25). β2-Microglobulin was used as a control for RNA quality and RT performance. Primers used were: for B2-microglobulin (M17987) 5′-CGC ATT TCC CCT CAT CCC TGT CCA G. RNA from MCF7, a breast cancer cell line, was used as a positive control.

Results

The take rates of CaP xenografts were generally lower in IF versus OVXF mice (five of six); however, the differences were statistically significant only for LuCaP 35 and LuCaP 73 (Table 1). Androgen-sensitive xenografts LuCaP 35, LuCaP 58, LuCaP 73, and LNCaP exhibited significant growth inhibition (i.e., smaller tumors) in IF versus OVXF mice (Fig. 1, A, C, D, and E). We also observed significant growth inhibition of the androgen-insensitive CaP xenograft LuCaP 49, but not the androgen-insensitive PC-3 xenograft, in IF versus OVXF mice (Fig. 1, B and F).

The observation of growth inhibition in IF mice was intriguing and supportive of the hypothesis that estrogen exerts inhibitory effects on CaP that are independent of androgen suppression. To confirm that the observed inhibition was caused by E2, rather than by another factor (or factors) secreted by the ovaries, we tested E2 supplementation of OVXF mice. The take rate of LuCaP 35 in OVXF mice supplemented with E2 was zero at day 90, compared with take rates of 43% in LuCaP 35 OVXF animals with placebo, and 0%, as expected, in the IF group (Fig. 2).

Only samples that were positive for the B2-microglobulin control were used for analysis of expression of ERβ messages. We detected ERβ messages in all of the CaP xenografts tested, with positive rates of about 30–90%. On the basis of our RT-PCR results, the levels of ERβ message were rather low, requiring 40–45 cycles for detection of the signal. The signal was much weaker than the ERβ amplicon from MCF7 breast cancer cells.

Discussion

In the course of our characterization of the LuCaP 23.1 xenografts, we observed inhibition of growth rate in IF mice compared with castrated male mice (26). These results suggested to us that there was a factor present in IF mice that actively suppressed CaP growth. Subsequent examination of six additional CaP xenografts in IF and OVXF mice confirmed the existence of a strong suppressive effect on CaP originating from
the ovaries; administration of E2 to OVXF mice supported our hypothesis that E2 was the source of the effect.

Two hypothetical mechanisms may be invoked to explain how E2 can affect CaP tumor growth in female mice in the absence of androgens: (a) E2 exerts direct inhibitory effects via ER expressed on CaP cells or via other, as yet unidentified, mechanisms; and (b) E2 exerts effects on other cells, which then secrete signaling molecules that inhibit CaP growth (Fig. 3). The presence of ERβ messages in CaP xenografts in this study supports the hypothesis that the observed inhibition of CaP growth may be attributable to direct effects of estrogens via ERβ. The connection between ERβ and suppression of prostate epithelium proliferation is also supported by findings in ERβ knockout mice, which develop prostate hypertrophy with aging (27). Recently, Weihua et al. (28) reported that the prostatic epithelium in ERβ knockout mice proliferates more rapidly than in wild-type mice. The decrease in expression of ERβ in CaP compared with normal prostatic epithelium is also consistent with a proliferation-regulatory role for ERβ (14), although the data of Leav et al. (20) must be kept in mind until additional studies settle the question of potential up-regulation of ERβ in metastasis. Growth inhibition by estrogens has also been observed in colon and colorectal cancers (29, 30). Despite the non-endocrine nature of these tissues, there is an additional parallel between colorectal and prostate tissues because levels of ERβ message are lower in cancer than in normal samples in both cases; yet this does not appear to allow escape from the growth-regulatory effects of estrogen in either case.

Fig. 1 Growth rates of CaP xenografts in female mice. A, LuCaP 35; B, LuCaP 49; C, LuCaP 58; D, LuCaP 73; E, LNCaP; F, PC-3. Means of tumor volumes (mm³) are plotted against time after implantation (days); bars, SE.
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Paradoxically, estrogens have been implicated in prostate proliferation in certain scenarios. E2 was reported to cause a dose-dependent stimulation of prostate growth in castrated beagle dogs (31). Increasing levels of E2 cause stromal hyperproliferation in animal models. Risbridger et al. (32) reported recently that DES exhibits stimulatory effects on proliferation of prostate basal epithelium in mice, but these processes were associated with ERα. A role for ERα in development of CaP was also described by Wang et al. (33), who reported that E2 in combination with testosterone caused development of hyperplasia and CaP in a murine model, but when ERα knockout mice were used, hyperplasia without cancer was observed. We therefore consider that the differences in E2 action could be attributable to the presence of a different subtype of ER in stroma and basal epithelium (likely ERα versus luminal epithilium (ERβ); reviewed in Ref. 22).

There is mounting evidence that other mechanisms of estrogen action are elicited independently of ER; however, these processes are not yet well understood. Das et al. (34) reported that rapid action by estrogens involves interactions with an unidentified receptor. Das et al. (35) reported also that E2 targeted genes involved in protein processing, calcium homeostasis, and Wnt signaling independently of signaling via ERs.

The major alternative hypothesis (Ref. 2; indirect effect) holds that E2 acts to suppress CaP growth by stimulating other cells to secrete signaling molecules. This hypothesis is supported by negative evidence from in vitro studies of estrogen effects, in both our own and in other laboratories. We saw no direct inhibitory effect of physiologic levels of E2 on in vitro proliferation of LNCaP (data not shown). However, these results do not rule out possible direct effects of E2 on CaP cells, because the in vitro evaluations lack interactions between stromal and epithelial cells, which could be involved in ERβ activation. Alternatively, factors necessary for ER transcriptional activation by E2 or for other aspects of signal transduction may be missing in the cell lines grown in vitro. Our results with PC-3 xenografts in vivo also support the indirect versus the direct (ERβ-mediated) hypothesis, because growth of this xenograft, which expresses ERβ, was not different in IF versus OVXF mice. However, the effect observed could be attributable to absence of some additional necessary factors. PC-3 cells originated from a CaP bone metastasis, but these cells do not express prostatic markers such as PSA, prostate acid phosphatase, and AR; therefore, it is not unlikely that some other unidentified factor involved in estrogen responses is also missing.

The possibility that AR is involved in the inhibition observed was also considered, because PC-3, the only cell line exhibiting resistance to the E2 inhibition, does not express AR. However, LuCaP 49 does not express AR, and it is susceptible to the inhibition by E2. Additionally, LuCaP 23.1 (26) and LuCaP 35, which are also inhibited, express wild-type AR. Moreover, LNCaP cells do express an AR, which is mutated in the ligand-binding region and is stimulated by association with E2; yet E2 inhibited growth in vivo with this xenograft, suggesting still more strongly that AR status does not affect the observed inhibition by E2.

In conclusion, we have demonstrated significant inhibition of CaP growth in IF versus OVXF female mice in five of six CaP xenograft lines. We have also shown that E2 is responsible for the growth inhibition observed in IF mice. These models can then be used to delineate pathways whereby estrogen inhibits CaP to identify potential targets for intervention. Moreover, if similar effects are observed in castrated male animals, the possibility exists that estrogen therapy may be of use in androgen-independent CaP, for which there is currently no effective treatment. Our data are intriguing and are likely to stimulate additional research into estrogenic effects on CaP to determine the mechanisms involved and the relevance, if any, to improved treatment of advanced CaP.

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