Received: 8 September 2017 | Accepted: 11 October 2017
DOI: 10.1002/pros.23446

REVIEW ARTICLE

WILEY The Prostate

Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progression

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Background: The androgen receptor (AR) is the classical target for prostate cancer prevention and treatment, but more recently estrogens and their receptors have also been implicated in prostate cancer development and tumor progression.

Methods: Recent experimental and clinical data were reviewed to elucidate pathogenetic mechanisms how estrogens and their receptors may affect prostate carcinogenesis and tumor progression.

Results: The estrogen receptor beta (ERβ) is the most prevalent ER in the human prostate, while the estrogen receptor alpha (ERα) is restricted to basal cells of the prostatic epithelium and stromal cells. In high grade prostatic intraepithelial neoplasia (HGPIN), the ERα is up-regulated and most likely mediates carcinogenic effects of estradiol as demonstrated in animal models. The partial loss of the ERβ in HGPIN indicates that the ERβ acts as a tumor suppressor. The tumor promoting function of the TMPRSS2-ERG fusion, a major driver of prostate carcinogenesis, is triggered by the ERα and repressed by the ERβ. The ERβ is generally retained in hormone naïve and metastatic prostate cancer, but is partially lost in castration resistant disease. The progressive emergence of the ERα and ERα-regulated genes (eg, progesterone receptor (PR), PS2, TMPRSS2-ERG fusion, and NEAT1) during prostate cancer progression and hormone refractory disease suggests that these tumors can bypass the AR by using estrogens and progestins for their growth. In addition, nongenomic estrogen signaling pathways mediated by orphan receptors (eg, GPR30 and ERRα) has also been implicated in prostate cancer progression.

Conclusions: Increasing evidences demonstrate that local estrogen signaling mechanisms are required for prostate carcinogenesis and tumor progression. Despite the recent progress in this research topic, the translation of the current information into potential therapeutic applications remains highly challenging and clearly warrants further investigation.

KEYWORDS
CRPC, ER alpha, ER beta, HGPIN

1 | INTRODUCTION

Estrogens, such as diethylstilbestrol (DES), have been proven effective in the hormonal treatment of metastatic prostate cancer more than 70 years ago and are still used as second-line hormonal therapy. Paradoxically, increasing evidence suggests that estrogens are involved in the development and progression of prostate cancer.[1–5] The therapeutic efficiency of estrogens results from their systemic endocrine effects acting via the pituitary gland to indirectly decrease testicular androgen secretion at castrate levels. The

significance of receptor mediated estrogen action on prostate glandular tissue, however, was recognized only in recent years. The presence of the classical estrogen receptor alpha (ERα) in premalignant changes of the human prostate and in advanced prostate cancer was first described in 1999 by Bonkhoff et al.[6] The second nuclear ER, termed ERβ, which is the most prevalent ER in human prostate tissue, was cloned in 1996 from a rat prostate cDNA library by Gustafsson et al.[7] Since then an increasing amount of evidence has been accumulated demonstrating the impact of estrogen signaling pathways on prostatic carcinogenesis and prostate cancer progression.

The human prostate is equipped with a dual system of estrogen receptors (ERα, ERβ) which undergoes profound remodeling during prostate cancer development and tumor progression.[6,8,9] In the normal prostate, ERα expression is restricted to stromal cells and to the androgen-independent basal cell layer which harbors prostate stem cells and the proliferation compartment of the prostate epithelium.[6,10] The ERβ is predominately expressed in the differentiation compartment consisting of luminal cells which are androgen-dependent, but have a limited proliferation capacity.[9,10] These basic epithelial cell types equipped with different ER also differ in their susceptibility to cytotoxic argents. While luminal cells expressing the ERβ at high levels are particularly vulnerable and undergo programmed cell death after androgen deprivation therapy (ADT), radiation, and chemotherapy, basal cells equipped with the ERα are multidrug resistant and survive such cytotoxic conditions (Figure 1).

2 | THE ROLE OF ESTROGENS AND THEIR RECEPTORS IN PROSTATIC CARCINOGENESIS

Estradiol (the most potent estrogen) exerts carcinogenic effects on the prostatic epithelium. This knowledge firstly derives from experimental data reported in animal models (recently reviewed by Bosland[11]). Briefly, when testosterone is chronically administered to Noble rats at low doses, prostate cancer develops via high grade prostatic intraepithelial neoplasia (HGPIN) in 35-40% of cases. If estradiol is given together with low-dose testosterone, the incidence of prostate cancer increases to nearly 100%. This clearly demonstrates that estrogens are required for a maximal carcinogenic response to androgens, at least in rat prostate. In a novel mouse model, chronic treatment with testosterone + estradiol was unable to induce HGPIN or prostate cancer when the ERα was knocked out (alpha ERKO), indicating that a functional ERα is required for the development of prostate cancer in this mouse model.[12] The most significant precursor of estradiol in men is testosterone. The conversion of testosterone to estradiol is mediated by the P450 aromatase enzyme (CYP19 gene), which is active in adipose tissue, adrenal glands, testes, and even the prostate. Thus, aromatase may be a key regulator of the ratio of androgen to estrogen in the prostate gland.[12] In the mouse model mentioned above, aromatase- knockout (ArKO) mice had reduced prostate cancer incidence, which implicates in situ production of estradiol as an important determinant in prostate cancer development.[12] Another etiological factor involved in prostatic carcinogenesis refers to chronic and recurrent prostate inflammation leading to oxidative DNA damage and to a putative precursor of prostate cancer, termed proliferative inflammatory atrophy (PIA).[13] Administration of estradiol induces chronic inflammation in the mouse prostate, and this inflammatory response is predominately mediated by the ERα.[3]

The question arises whether the oncogenic ERα signaling pathways demonstrated in the rat and murine prostate are equally relevant for the human prostate cancer development. Few studies have addressed this issue in HGPIN, which is considered to be the most likely precursor of prostate cancer in men. During the malignant transformation of the prostatic epithelium (HGPIN), ERα gene expression extends from basal cells to luminal cells, in which dysplastic

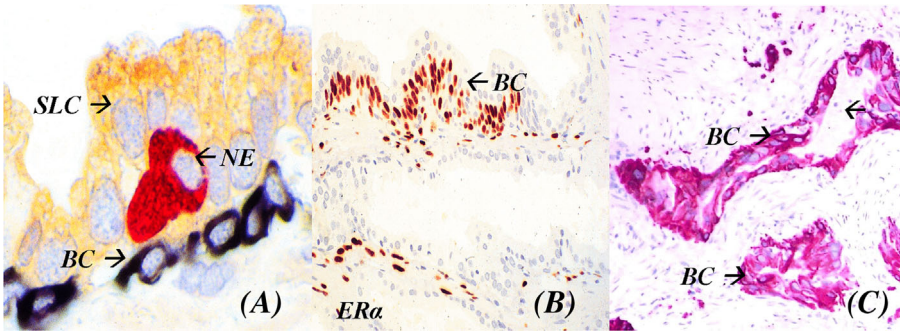

FIGURE 1 Cellular biology of the prostatic epithelium. The normal prostatic epithelium (A) is mainly composed of PSA producing and androgen-dependent secretory luminal cells (SLC) which express the androgen receptor and the estrogen receptor beta (ERβ) at high levels. The basal cell layer (BC), characterized by high molecular weight cytokeratins, harbors the stem cell, and proliferation compartment. Estrogen receptor alpha (ERα) expression is restricted to stromal cells and subsets of basal cells (B). Neuroendocrine cells (NE) expressing chromogranin A lack the androgen receptor and thus are androgen-insensitive. Prostate gland after androgen deprivation and radiation therapy (C). The residual glandular tissue is mainly composed of basal cells (identified by high molecular weight cytokeratins (red)), while luminal cells have undergone apoptosis and are virtually absent. Note apoptotic luminal cells (arrow)

changes occur.[6] In our studies, the ERα was detectable at the mRNA and the protein level in about 30% and 10% of HGPIN, respectively,[6] (Figure 2A). This implicates the ERα as an oncogene, which is overexpressed during the malignant transformation of the human prostatic epithelium. Further evidence of functional ERα signaling pathways involved in prostatic carcinogenesis derives from the differential expression of the ERα-regulated oncogenic TMPRSS2-ERG fusion and the estrogen-inducible PS2 gene in prostate tissue.[14–16] PS2 is undetectable in benign prostate tissue from patients without clinical and histological evidence of cancer, but is expressed at variable degree in benign glandular tissue and HGPIN adjacent to clinically significant prostate cancer in prostatectomy specimens.[14] This indicates that PS2 is involved in early phases of prostatic carcinogenesis, and may be a field effect biomarker for ERα activity in this disease process. TMPRSS2: ERG gene fusions, the most common and prostate cancer specific molecular subtypes of ETS family gene fusions, occur in about 50% of prostate cancer and 10-20% of HGPIN intermingled with adjacent invasive cancer demonstrating identical gene fusions, but not in benign prostate tissue.[15] While TMPRSS2 is an androgen-regulated gene, regulation of the oncogenic TMPRSS2-ERG fusion involves ER signaling pathways.[16] TMPRSS2-ERG expression was found to be increased by ERα agonist (estradiol) and repressed by ERβ agonists.[16] A recent clinical study enrolling patients with biopsy diagnosed HGPIN indicates that TMPRSS2-ERG gene fusion predicts subsequent detection of prostate cancer.[17] It is noteworthy, that ERG expression was detected in 11.1% of patients with isolated HGPIN, which, in turn, is very similar to 10% of HGPIN reported to express the ERα at the protein level[6,17] (Figures 2A and 2B). Taken together, the abnormal expression of ERα and ERα-regulated PS2 and TMPRSS2-ERG fusion in HGPIN documents that functional ERα signaling pathways are implicated in the malignant transformation of the human prostate, and that premalignant lesions equipped with TMPRSS2-ERG fusion (about 10% of biopsy diagnosed HGPIN) are at high-risk to progress to invasive and fusion positive cancer (Figure 2B).

Contrary to the ERα, the ERβ which preferentially binds phytoestrogens is more likely to protect the prostate epithelium from malignant transformation. The initial evidence of anticancer properties of phytoestrogens stemmed from the epidemiological

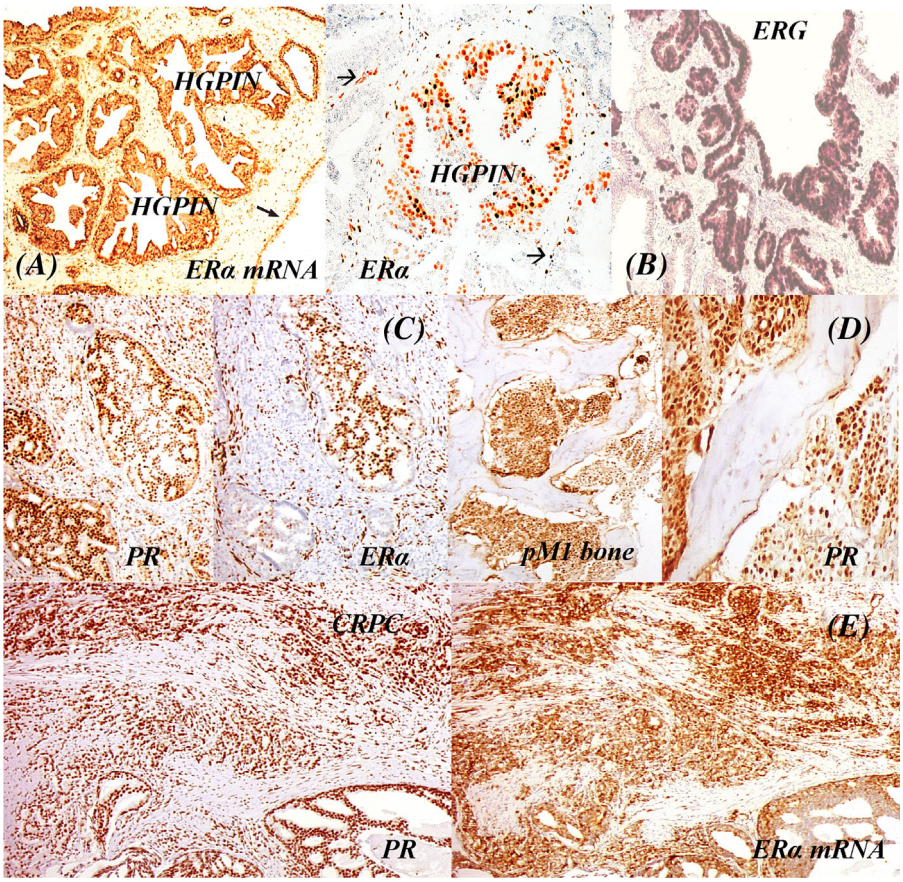

FIGURE 2 Differential expression the of estrogen receptor alpha (ERα) and ERα-regulated genes in prostate carcinogenesis and prostate cancer progression. HGPIN expressing the ERα at the mRNA and protein level in secretory luminal cells in which dysplastic changes occur (A). In the normal prostatic epithelium, ERα expression is restricted to basal cells at mRNA and protein level (arrows). Detection of the ERα-regulated TMPRSS2-ERG fusion by ERG immunohistochemistry in HGPIN progressing to microinvasive prostate cancer (Gleason 3 + 3) (B). Prostate cancer (Gleason 4 + 4) expressing both the ERα and the progesterone receptor (PR) (C). Bone metastasis with extensive and strong expression of the PR (D). Castration resistant prostate cancer (CRPC) with extensive expression of the PR and the ERα at the mRNA level (E)

observation of the low incidence of clinical prostate cancer in Japan with traditionally high dietary intake of phytoestrogens. Moreover, the incidence of clinical cancer among second- and later-generation Japanese populations living in the US has become much closer to that of the general US population, suggesting environmental or dietary factors implicated in prostatic carcinogenesis.[18] Natural phytoestrogens, in particular soy isoflavones such as genistein, and indole-3-carbinol, and resveratrol preferentially bind to the ERβ which exerts protective effects to the prostatic epithelium. The anticancer properties of phytoestrogens have been documented in vitro and in vitro (reviewed by Klein[18] and Bonkhoff & Berges[4]). This includes inhibition of cell proliferation and angiogenesis, and decrease of PSA, 5α-reductase activity, androgen receptor (AR) expression (AR silencing), and tumor volume. ERβ elicits antitumoral activity in prostate cancer cell lines by repressing key oncogenes (PI3K, p45Skp2, c-myc, and cyclin E), increasing expression of antiproliferative genes like PTEN, FOXO3, KLF5, p21WAF1, CDKN1A, and p27Kip1, increasing E-cadherin that maintains epithelial differentiation and opposes dedifferentiation, and last but not least by repressing the oncogenic TMPRSS2-ERG fusion (reviewed by Dey et al[19]). In the mouse ventral prostate, the ERβ opposes AR signaling, inflammation, and proliferation by down-regulating androgen receptor (AR) signaling, inducible nitric oxide synthase, the antioxidant gene glutathione peroxidase 3, and IL-6, and by up-regulating the tumor suppressor PTEN.[20]

In the human prostate, the ERβ is expressed at high levels in luminal cells of the prostatic epithelium, but is partly lost in HGPIN.[9] In our series, the ERβ was markedly decreased or absent in about 40% of HGPIN (Figures 3A and 3B). This implicates the ERβ as a tumor suppressor which is partly lost during prostatic carcinogenesis.[9] Considering that chemopreventive and anticancer properties of phytoestrogens are depending on the presence and activity of the ERβ, it appears that the dietary intake of phytoestrogens is beneficial in terms of chemoprevention for those patients with either no HGPIN or with HGPIN retaining high levels of ERβ expression. A Swedish study has shown that high intake of phytoestrogens substantially reduces prostate cancer risk among men with specific polymorphic variation in the promoter region of the ERβ gene. No association was found between phytoestrogens and prostate cancer among carriers homozygous for the wild-type allele of the ERβ gene.[21] Thus, ERβ-mediated preventive effects of phytoestrogens may depend on specific ERβ gene polymorphisms and the ERβ status in the patient's tissue.

3 | ROLE OF ESTROGENS AND THEIR RECEPTORS IN PROSTATE CANCER PROGRESSION

The ERβ is the most prevalent ER in clinical specimens of prostate cancer. Hormone naïve prostate cancer, unlike HGPIN, generally retains high levels of ERβ expression, even in lymph node and bone metastasis[9] (Figures 3C and 3D). Considering the antitumoral activity elicited by the ERβ in vivo, one would expect ERβ specific agonists to be effective in slowing tumor progression in hormone naïve prostate cancer patients, but this issue has not yet been addressed in clinical studies. A substantial loss of ERβ is encountered only in castration resistant prostate cancer (CRPC) (Figure 3E). Reduced levels of ERβ were found in about 40% of cases. In 10% of these tumors the ERβ was undetectable.[9] The partial loss of the ERβ in CRPC may be related to the markedly decreased levels of bioavailable androgens after androgen deprivation. In fact, androgen-cycling experiments in the ventral rat prostate have shown that androgen deprivation decreases ERβ mRNA expression indicating that the ERβ is an androgen-regulated gene.[1]

The presence of the classical ERα in prostate cancer cells is, in apparent contrast to breast cancer and other estrogen dependent tumors, a late event in disease progression.[6] It is unlikely to find the ERα expressed in low to intermediate grade prostate cancer. In our study, high grade (Gleason grade 4 and 5) tumors revealed ERα protein expression in 43% and 62% of cases, respectively (Figure 2C). The most significant ERα gene expression at mRNA and protein level was observed in metastatic lesions and CRPC with ERα expression in >25% of tumor cells in 45.5% and 55.5% of cases, respectively, indicating that about 50% of these tumors harbors a significant amount of estrogen-responsive tumor cells.[6] If the ERα detectable in these tumors is functionally active, one would expect to find evidence of transcriptional activity of ERα-regulated genes. Among the various ERα-regulated genes, the progesterone receptor (PR) is one of the most important markers for estrogen-regulated growth and responsiveness of ERα antagonists in breast cancer and other estrogen-dependent tumors. In a subsequent study, we were able to detect the PR for the first time not only in the tumor microenvironment, but also in prostate cancer cells, and revealed that the immunoprofile of the PR in prostate cancer runs remarkably parallel with those of the ERα[22] (Figure 2C). In fact, the most consisting and extensive levels of PR expression in prostate cancer cells were detectable in lymph node and bone metastases, and CRPC (Figures 2D and 2E). Significant PR expression (with >20% of PR positive tumor cells) was identified in metastases and CRPC in 60% and 54% of cases, respectively. The progressive emergence of the PR during tumor progression indicates that a substantial amount of metastases and CRPC harbors a functional ERα able to induce PR expression.[22] Beside the PR, other ERα-regulated genes are involved in prostate cancer progression. The estrogen-inducible PS2 is detectable in hormone naïve prostate cancer only in close association with neuroendocrine differentiation, while CRPC expresses PS2 at variable degree even in absence of neuroendocrine differentiation.[14] Conflicting results have been reported on the prognostic significance of the ERα-regulated TMPRSS2-ERG fusion. Its presence in most cases of metastases reported by several studies, the greater predilection for metastasis of fusion positive nodules versus fusion negative nodules in multifocal prostate cancer, and finally, the association with disease-specific death made in clinical observation studies, however, may suggest that TMPRSS2-ERG positive prostate cancer is associated with a more aggressive clinical course than fusion negative cancer.[15] Moreover, the presence of TMPRSS2-ETS fusion with interstitial deletion in all 97 nonosseous metastatic sites of prostate cancer from 30 rapid autopsies of men who

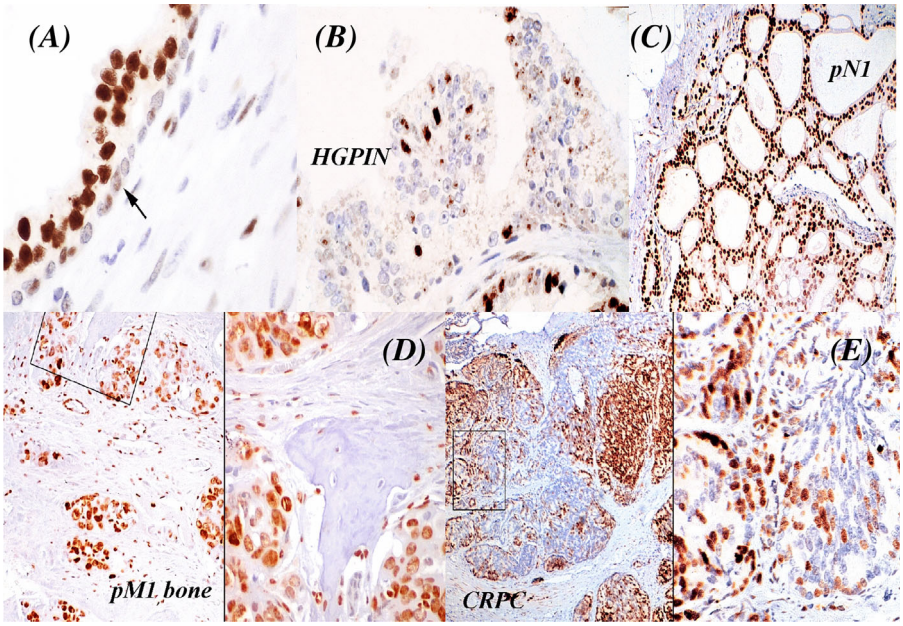

FIGURE 3 Differential expression of the estrogen receptor beta (ERβ) in prostate carcinogenesis and prostate cancer progression. In the normal prostatic epithelium, the ERβ is expressed at high levels in luminal cells and to a lesser degree in basal cells (arrow) (A). HGPIN with severe loss of the ERβ (B). Hormone naïve lymph node (C) and bone metastases (D) with extensive and strong expression of the ERβ (C). Castration resistant prostate cancer (CRPC) with partial loss ERβ expression (E)

died of castration resistant disease documents a functional ERα-signaling pathway involved in lethal prostate cancer.[23] The recent description of an ERα-specific non-coding transcriptome signature in prostate cancer further highlights the impact of ERα signaling pathways on prostate cancer progression.[24] Among ERα-regulated intergenic long non-coding RNAs (lncRNAs), the nuclear enriched abundant transcript 1 (NEAT1) was identified as the most significantly overexpressed lncRNA in prostate cancer. NEAT1 was identified as a new prognostic biomarker for aggressive prostate cancer independent of common clinical and pathologic variables. In prostatectomy specimens, high NEAT1 expression was associated with a significant increase in both biochemical and metastatic recurrence rates compared to those with low NEAT1 expression. Moreover, both ERα and NEAT1 signaling were unaffected by AR antagonists and independent of the AR and ERβ, thus indicating that prostate cancer cells may develop therapeutic resistance through positive selection of an alternate nuclear ERα-NEAT1 signaling pathway during tumor progression.[24]

Taking together, the reappearance of ERα and ERα-regulated gene expression (as exemplified by PR, PS2, TMPRSS2-ERG fusion, and NEAT1) in clinically aggressive tumors documents functional ERα-signaling pathways implicated in prostate cancer progression. This provides a possible mechanism how prostate cancer cells can bypass the AR and ADT by using endogenous or exogenous estrogens for their own growth and raises a cautionary note regarding the use of therapeutic agents with ERα and PR agonist activity, such as estrogens and progestins. It is noteworthy that although the ERα emerges typically in metastatic and castration resistant disease, this receptor can be induced by ADT in a very short period of time. A recent clinical study of 27 patients with high-risk prostate cancer medically castrated with Degarelix 7 days before radical prostatectomy reports up-regulation of ERα expression in prostate cancer cells which, in turn, was associated with differential expression of ERα-regulated genes, and sustained tumor cell proliferation in an androgen deprived milieu.[25] This documents the plasticity of high grade prostate cancer cells to switch from AR to ERα-signaling in response to ADT, and the propensity of ADT to induce pathways implicated in the development of castration resistant disease by bypassing the AR.[25] It is noteworthy that prostate and breast cancer share common steroid receptor signaling pathways, including the ERα, the ERβ, ERα-regulated genes (PR and pS2), and the AR. In fact, about 60-80% of breast cancers are equipped with the AR, and the AR pathway has cross-talk with several other key signaling pathways, including the PI3K/Akt/mTOR, MAPK pathways, ERα, PR, and human epidermal growth factor receptor-2 (HER2- neu).[26] In apparent contrast to prostate cancer, determination of the ERα, PR, and HER2-neu status in breast cancer tissue is a routine procedure to stratify patients according to the likelihood of response to ER-targeted drugs. There is a great need for standardization of detection and reporting these steroid receptors in prostate cancer tissue or liquid biopsy to make these tests available for clinical studies testing the efficiency of ER-targeted drugs in patients with aggressive prostate cancer or castration resistant disease.

4 | ROLE OF ORPHAN RECEPTORS IN PROSTATE CANCER PROGRESSION

Certain orphan receptors together with ERα and/or ERβ may mediate nongenomic estrogen signaling in various cell types. GPR30 is

structurally unrelated to the classical ERs (ERα and ERβ) with high-affinity and low-capacity binding to estrogens, phytoestrogens, antiestrogens, and selective estrogen receptor modulators, but bind G-1, a highly selective non-steroidal GPR30 agonist.[27] Using LNCaP xenograft growing in intact and castrated mice, treatment with the GPR30 agonist G-1 induced massive tumor necrosis in castrated mice bearing CRPC, but not in untreated mice with hormone naïve tumors. It was demonstrated that GPR30 expression is suppressed by androgens via the AR and markedly up-regulated by castration.[27] In clinical specimens, high level of GPR30 expression was detected in 80% of CRPC metastases compared to only 54% of primary, hormone naïve prostate cancer.[27] Clinical studies are warranted to establish GPR30 as a new therapeutic target in CRPC.

Another orphan receptor implicated in prostate cancer progression is the estrogen-related receptor alpha (ERRα) which shares structural similarities with ERα and ERβ, but does not bind estrogen.[28] ERRα is expressed at higher levels in bone metastases from patients with CRPC than in primary hormone naïve prostate cancer. ERRα pathways have been shown to mediate tumor progression in bone by increasing expression of pro-resorption factors (VEGF-A, WNT5A, TGFβ1), by stimulating osteoblasts through increased expression of pro-osteoblastic factors (VEGF-A, WNT5A, TGFβ1), and by increasing the expression of the pro-metastatic factor periostin in tumor infiltrating fibroblasts. These data provide a rationale for the investigation of ERRα as a therapeutic target to treat prostate cancer skeletal-related events.[28]

5 | ESTROGENS AND PROSTATE STEM CELL BIOLOGY

The basal cell layer of the prostatic epithelium harbors a small stem cell population (<1%) which is characterized by a panel of surface stem cell markers (such as CD44, α2ß1 integrin, CD133, CD117, Sca-1, Trop2, and CD49f) and equipped with ERα and ERβ, indicating that human prostate stem cells may be direct estrogen targets.[29] To assess the actions of estrogens on prostate stem and progenitor cells, Prins at al[29] have isolated and enriched adult human prostate stem cells to form prostaspheres (PS). These PS containing AR negative, and ERα and ERβ positive human prostate stem cells were combined with inductive rat urogenital sinus mesenchyme and grafted under the renal capsule of adult male nude mice. After mature glandular prostate (human) tissue was formed, treatment with elevated estradiol levels along with testosterone supplementation induced prostatic carcinogenesis in the human prostatic epithelium via HGPIN in the host mice. To test whether xenoestrogens such as bisphenol (BPA) could influence this process, host mice were orally exposed to low doses of BPA, which, in turn, increased prostate cancer development to about 35% and up to 45% when human stem cell containing prostaspheres were also exposed to BPA (reviewed by Prins et al[29]). These elegant experimental data demonstrate that prostate cancer can be induced from human prostate stem cells exposed to estradiol, and that low doses of BPA increase the susceptibility of the human prostate epithelium to estrogen-driven carcinogenesis.[29] It is noteworthy that BPA-based plastic is found in a wide range of consumer products (such as water bottles, and as coatings on the inside of many food and beverage cans, and on sales receipts) and is one of highest volume of chemicals produced worldwide.

The prostate cancer stem cell hypothesis predicts that cytotoxic agents (such as androgen derivation, radiation, and chemotherapy) may eliminate bulk tumor cells but spare rare cancer stem cells, which may account for the subsequent disease relapse after treatment. Hence, targeting prostate cancer stem cells may provide new treatment strategies. As mentioned above, the basal cell layer harboring the proliferation and stem cell compartment of the prostatic epithelium is particularly resistant to ADT, chemotherapy, and radiation (Figure 1C), and thus shares some important biological features with CRPC.[30–32] In fact, a number of markers restricted to basal cells in normal condition reemerges in high grade prostate cancer and is implicated in the pathogenesis of castration resistant disease, including:

- BCL-2, a major antiapoptotic protein protecting basal cells from programmed cell death, is involved in CRPCa by bypassing the AR and blocking the apoptotic pathway.[32]
- Non-hormonal growth factor receptors (such as erb1/EGFR, erb2/HER2) recruited by CRPC cells to maintain AR signaling pathways in an androgen deprived milieu.[30–32]
- ERα that regulates target genes implicated in tumor progression such as PR, PS2, TMPRSS2-ERG fusion, and NEAT1, and bypasses the AR during ADT.

Although prostate cancer lacks basal cell differentiation, basal cells, and CRPC cells obviously share common multidrug resistance pathways. It is noteworthy that markers restricted to basal cells in normal condition (ie, BCL-2, erb1/EGFR, erb2/HER2, and ERα) extend to luminal cells in HGPIN, but reappear in prostate cancer only in high grade tumors and CRPC.[31] Furthermore, these markers are inducible in high grade prostate cancer by ADT in a relative short period of time.[30,31] The progressive emergence of basal cell related pathways (including BCL-2, erb1/EGFR, erb2/HER2, and the ERα) during progression toward CRPC suggests that these tumor cells recapitulate biological properties of basal cells and stem cells to acquire multidrug resistance. This concept was supported recently by showing that aggressive prostate cancer shares a conserved transcriptional program with normal adult prostate basal stem cells and that this basal cell specific gene signature is differentially enriched in various phenotypes of late-stage metastatic prostate cancer. Targeting normal stem cell transcriptional programs may provide a new strategy for treating advanced prostate cancer.[33] Expressed in human prostate stem cells and implicated in prostate cancer progression, the ERα may be a promising candidate for such targeted therapies.

6 | PRECLINICAL AND CLINICAL STUDIES WITH SELECTIVE ER MODULATORS (SERMS)

A number of selective ER modulators (SERMs) have been tested in preclinical studies (recently reviewed by Bosland,[11] and Bonkhoff &

Berges[4]). Briefly, tamoxifen inhibits proliferation of PC-3 and DU-145 prostate cancer cells and induces apoptosis in LNCaP cells. Tamoxifen also inhibits in vivo growth of the CWR22 prostate cancer xenograft in nude mice. Raloxifene (a mixed estrogen agonist/antagonist) induces apoptosis in LNCaP cells. Both raloxifene and the ERα antagonist trioxifene reduce the development of pulmonary metastasis and extend survival in the PAIII prostatic adenocarcinoma model. The pure antiestrogen ICI 182,780 and the ERα antagonist toremifene inhibit proliferation of PC-3 cells. The human prostate cancer PacMetUT1 cell line equipped with the ERα induces extensive bone formation in vivo and could serve as a useful model for investigating the mechanism of osteoblastic lesion formation. ERα knockdown in PacMetUT1 cells as well as pharmacological inhibition of ERα with ICI 182,780 (fulvestrant) inhibits osteoblastic bone metastasis and lung metastasis in vivo, which documents that fulvestrant is highly efficient to repress this ERα mediated metastatic process in vivo.[34]

Contrary to the rather encouraging results of SERMs in preclinical studies, the few data from clinical trials currently available are rather disappointing. Tamoxifen has been studied in phase II clinical trials with prostate cancer patients, but therapeutic efficacy was uncertain. A major problem with tamoxifen are mixed antagonist and agonist (=estrogenic) effects.[11]

In a recent clinical trial of fulvestrant in CRPC with 500 mg fulvestrant every 14 days for the first month and 250 mg monthly thereafter in seven highly pretreated CRPC patients demonstrated initial reduction of PSA levels in six of these patients even though the levels increased after the dose was reduced to 250 mg.[35] Since this observation clearly implies dose-dependent responses of fulvestrant, future trials on high-dose fulvestrant in CRPC patients are warranted. Another promising ER antagonist in preclinical and clinical studies is toremifene. In the transgenic TRAMP mouse model all animals in the placebo group developed tumors compared with only 35% of animals treated with toremifene. HGPIN was observed in animals in the placebo group, but not in animals treated with toremifene. Moreover, toremifene-treated animals had prolonged survival compared with placebo-treated animals. By 33 weeks of age, 100% of the placebo-treated animals had developed palpable tumors and died; whereas 60% of the toremifene-treated animals were tumor free.[36] Subsequently, the ERα antagonist toremifene was evaluated in a multicenter phase IIb dose-finding study in the treatment/prevention of HGPIN using prostate cancer on follow-up biopsy as a primary end point.[37] A total of 514 men with a history of diagnosed HGPIN were randomized to placebo or one of three escalating doses of toremifene: 20, 40, and 60 mg. Repeat biopsies were carried out at 6 and 12 months using a minimum of eight cores. When comparing the 12-month biopsies only, a 48.2% reduction in cancer incidence was observed in the 20 mg-treated group compared with the placebo group.[37] These encouraging results, however, could not be confirmed by the subsequent randomized phase III, double-blind, placebo-controlled clinical trial of toremifene 20 mg for prevention of prostate cancer in 1,590 men with isolated HGPIN on biopsy.[38] It was argued that the current phase III study includes more stringent baseline sampling criteria, longer treatment period (3 years vs 1 year), and less frequent sampling (yearly vs every 6 months) when compared to the previous phase IIb trail.[38] On the other hand, it should be noted that potential targets of toremifene, such as the ERα and the ERα-regulated TMPRSS2-ERG fusion, are detectable in only about 10% and up to 20% of HGPIN, respectively.[6,15] One would expect toremifene to be effective in reducing cancer detection rate, especially in patients with ERα or TMPRSS2-ERG fusion positive HGPIN, but this issue has not yet been addressed in clinical studies. Nevertheless, toremifene has been reported to elicit some clinically relevant responses in prostate cancer patients receiving ADT. Toremifene significantly increases hip and spine bone mineral density and improves lipid profiles in men receiving ADT.[39,40] The latter includes a significant decrease of total cholesterol, LDL cholesterol, and triglycerides, and increase of HDL cholesterol.[39] Lowering cholesterol therapies may be beneficial for patients with prostate cancer. In fact, prostate cancer uses cholesterol for intratumoral de novo testosterone synthesis which is markedly increased in castration resistant disease.[41] In a double-blind, placebo controlled phase III study with 646 men receiving androgen deprivation therapy for prostate cancer, toremifene significantly decreased the incidence of new vertebral fractures.[40] Preliminary clinical data suggest that toremifene may also delay disease progression. In a randomized controlled phase II a trial enrolling 15 patients, toremifene combined with conventional ADT significantly improved the biochemical recurrence rate in treatment-naïve bone metastatic prostate cancer.[42] Further clinical trials are warranted to confirm the clinical efficacy of toremifene in combination with ADT.

7 | IMMUNOHISTOCHEMICAL DETECTION, RECEPTOR ISOFORMS AND SPLICE VARIANTS

Gene silencing by methylation and inactivation of ERα, ERβ, and PR has been reported in prostate cancer.[43,44] It is conceivable that the detection rate of these steroid receptors by immunohistochemistry is closely related to the methylation status. Conflicting results have been reported on the presence of ERα and PR in human prostate cancer tissue, which obviously reflects differences in the choice of antibodies, immunohistochemical detection tools, and tissue processing.[6,22] The use of fresh tissue immediately fixed in buffered formalin is of paramount importance. Archival paraffin blocks obtained by routine fixation may not be informative. Of equal importance are the use of supersensitive monoclonal antibodies in conjunction with antigen retrieval, and the presence of suitable internal positive controls, (eg, strong nuclear staining of the ERα and PR in stromal and basal cells; strong nuclear staining of the ERβ in luminal cells). These requirements are crucial for reliable immunolocalization of ERα, ERβ, and PR in prostate cancer tissue.[6,9,22]

Another issue refers to the distribution and function of ERβ isoforms and splice variants in prostate tissue. Using an antibody raised against a post-transcriptionally modified short form of the ERβ, Leav et al[8] have immunolocalized the ERβ in basal cells, and reported markedly decreased levels of ERβ in Gleason grade 4/5 tumors and its

absence in transition zone cancer. In our studies, using an antibody raised against the long and short form of the ERβ isoform 1, the ERβ was localized predominantly in luminal cells as described in the rat and murine prostate. In addition, the substantial loss of ERβ in transition zone cancer and in Gleason grade 4/5 tumors reported by Leav et al was not observed.[9]

It is noteworthy that current studies are focused on the ERβ isoform 1, while the localization and function of the ERβ isoforms 2, 3, 4, and 5 are less well established. Recent data indicate that ERβ2 and ERβ5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion.[45] Another recent study performed in radical prostatectomy specimens reported that patients with cytoplasmic ERβ1 and nuclear ERβ2 co-staining had significantly worse 15-year prostate cancer specific mortality than patients with expression of only cytoplasmic ERβ1, only nuclear ERβ2 and neither ER.[46]

Future research in this field is undoubtedly necessary to elucidate the role of the various ERβ isoforms and ERβ splice variants in human prostate tissue.

8 | CONCLUSION

Although the androgen receptor (AR) remains the major target for prostate cancer prevention and treatment, there are multiple lines of evidence to suggest that estrogens and their receptors (ERα, ERβ) are no less involved in prostate cancer development and tumor progression. First of all, it should be noted that human prostate stem cells are equipped with ERα and ERβ, but not with the AR, and that prostatic carcinogenesis can be induced in vivo by exposing these stem cells to estradiol. In the human prostate, the ERα is a functional oncogene overexpressed in HGPIN, while the ERβ acts as tumor suppressor partially lost during the malignant transformation of the prostatic epithelium. The tumor promoting function of the TMPRSS2-ERG fusion, a major driver of prostate carcinogenesis, is triggered by the ERα and repressed by the ERβ. Hormone naïve, primary and metastatic prostate cancers express the ERβ at high levels that offers a promising target for specific ERβ agonists to slow tumor progression. A partial loss of ERβ occurs only after androgen deprivation therapy (ADT). Contrary to the ERβ, the progressive emergence of the ERα in prostate cancer cells is a late event in disease progression. The impact of functional ERα-signaling pathways on prostate cancer progression is further documented by the expression pattern of ERα-regulated genes (ie, PR, PS2, TMPRSS2-ERG fusion, and NEAT1) and their prognostic implication reported in clinical studies. This provides a possible mechanism how prostate cancer cells can bypass ADT by switching from AR to ERα signaling pathways and using endogenous or exogenous estrogens for their own growth.

From a clinical perspective, the translation of the current information into potential therapeutic applications remains highly challenging. There is a significant potential for the use of ERα antagonists and ERβ agonists to prevent prostate cancer and to delay disease progression, especially if the pertinent receptors are present in the patient's tumor tissue. Toremifene is currently the most promising ERα antagonists, although the encouraging results of the phase IIb prevention trial could not be confirmed by the subsequent randomized phase III trial. The preventive efficiency of torimifene in patients with HGPIN equipped with corresponding targets (ERα, TMPRSS2-ERG, pS2), however, is currently unknown. At least, torimifene combined with conventional ADT has shown clinical activity in a randomized controlled phase II trial of treatment-naïve bone metastatic prostate cancer, but further clinical trials are warranted to confirm these preliminary data. Although safe and effective ERβ agonists are currently available, no ERβ agonist has been tested so far in clinical trials to delay prostate cancer progression. The concept has come of age, but much more effort has still to be done to make it run.

ACKNOWLEDGMENT

Dedicated to the memory of Dr. Richard Berges.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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How to cite this article: Bonkhoff H. Estrogen receptor signaling in prostate cancer: Implications for carcinogenesis and tumor progression. *The Prostate*. 2017;1–9. https://doi.org/10.1002/pros.23446