The Evolving Role of Oestrogens and Their Receptors in the Development and Progression of Prostate Cancer

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Abstract

Context: Oestrogens were proven effective in the hormonal treatment of advanced prostate cancer (PCa) >60 yr ago and are still used as second-line hormonal therapy. Paradoxically, oestrogens might also be involved in the development and progression of PCa.

Objective: To examine mechanisms of how oestrogens may affect prostate carcinogenesis and tumour progression.

Evidence acquisition: Recent data obtained from animal, experimental, and clinical studies were reviewed.

Evidence synthesis: The human prostate is equipped with a dual system of oestrogen receptors (oestrogen receptor alpha [ER\alpha], oestrogen receptor beta [ER\beta]) that undergoes profound remodelling during PCa development and tumour progression. In high-grade prostatic intraepithelial neoplasia (HGPIN), the ER\alpha is upregulated and most likely mediates carcinogenic effects of estradiol as demonstrated in animal models. Preliminary clinical studies with the ER\alpha antagonist toremifene have identified the ER\alpha as a promising target for PCa prevention. The partial loss of the ER\beta in HGPIN indicates that the ER\beta acts as a tumour suppressor. The ER\beta is generally retained in hormone-naïve PCa but is partially lost in castration-resistant disease. The progressive emergence of the ER\alpha and the oestrogen-regulated progesterone receptor (PR) during PCa progression and hormone-refractory disease suggests that these tumours can use oestrogens and progestins for their growth. The TMPRSS2-ERG gene fusion recently reported as a potentially aggressive molecular subtype of PCa is regulated by ER\alpha-dependent signalling. TMPRSS2-ERG expression has been found to be increased by ER\alpha agonist (oestrogens) and decreased by ER\beta agonists.

Conclusions: Oestrogens and their receptors are implicated in PCa development and tumour progression. There is significant potential for the use of ER\alpha antagonists and ER\beta agonists to prevent PCa and delay disease progression. Tumours equipped with the pertinent receptors are potential candidates for this new therapeutic approach.

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1. Introduction

The androgen receptor (AR) is the major target for prostate cancer (PCa) prevention and treatment. Nevertheless, there is a growing body of evidence to suggest that oestrogen signalling also plays a significant role in normal and abnormal growth of the prostate gland [1–4]. Oestrogen action in the male must be viewed in at least two different ways: (1) systemic endocrine effects acting through the pituitary gland to indirectly lower androgens, and (2) local effects that directly target prostate tissue by specific oestrogen receptors (ER). The human prostate is equipped with a dual system of ERs (oestrogen receptor alpha [ERα] and oestrogen receptor beta [ERβ]) that undergoes profound remodelling during PCa development and tumour progression [5–7]. In the normal prostate, the ERα is restricted to stromal cells and to the androgen-independent basal cell layer, which harbours prostate stem cells and the proliferation compartment of the prostate epithelium [5,8]. ERβ is predominately expressed in luminal cells, which are androgen dependent but have a limited proliferation capacity [7,8].

2. Evidence acquisition

2.1. Role of oestrogens and their receptors in prostatic carcinogenesis

Oestrogens (estradiol) exert carcinogenic effects on the prostatic epithelium. This knowledge is derived from experimental data reported in animal models (recently reviewed by Bosland [9]). Briefly, when testosterone is chronically administered to Noble rats at low doses, PCa develops through high-grade prostatic intraepithelial neoplasia (HGPIN) in 35–40% of cases. When estradiol is given together with low-dose testosterone, the incidence of prostate carcinomas increases to nearly 100%. This increase clearly demonstrates that oestrogens are required for a maximal carcinogenic response to androgens—at least in rat models. In a novel mouse model, chronic treatment with testosterone plus estradiol was unable to induce HGPIN or PCa when ERα was knocked out (alpha-ERKO), indicating that functional ERα is required for the development of PCa in this mouse model [10]. 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, the testicles, and even the prostate. Therefore, aromatase may be a key regulator of the ratio of androgen to oestrogen in the prostate gland [3]. In the mouse model mentioned above, aromatase-knockout (ArKO) mice had reduced PCa incidence, which implicates in situ production of estradiol as an important determinant in PCa development [10]. Another aetiologic factor involved in prostatic carcinogenesis refers to chronic and recurrent prostate inflammation leading to oxidative DNA damage and to proliferative inflammatory atrophy (PIA), considered a new putative precursor of PCa [11]. Administration of estradiol induces chronic inflammation in the mouse prostate, and this inflammatory response is predominately mediated by ERα [3].

The question arises whether the carcinogenic effects of oestrogens demonstrated in the rat and murine prostate are applicable to the biology of the human prostate. Few studies have addressed this issue in HGPIN, which is the most likely precursor of PCa in men. During the malignant transformation of the prostatic epithelium (HGPIN), ERα gene expression extends from basal cells to luminal cells, in which the dysplastic changes occur [5]. In HGPIN, ERα is detectable at the mRNA and protein level in about 30% and 10% of cases, respectively [5] (Fig. 1a and b). This indicates that the ERα in the human prostate acts as an oncogene, which is overexpressed during the malignant transformation of the prostatic epithelium. The data reported in human tissue are in line with the pivotal oncogenic role of ERα demonstrated in animal models. Further evidence for this concept derives from clinical studies [12,13]. The ERα antagonist toremifene was evaluated in a multicentre phase 2b dose-finding study in the treatment and prevention of HGPIN using PCa on follow-up biopsy as a primary end point. A total of 514 men with a history of diagnosed HGPIN were randomised to placebo or one of three escalating doses of toremifene: 20, 40, and 60 mg. Repeat biopsies were carried out at 6 and 12 mo using a minimum of eight cores. When comparing the 12-mo biopsies only, a 48.2% reduction in cancer incidence was observed in the 20-mg–treated group compared with the placebo group [12]. In apparent contrast to the data reported on finasteride, toremifene does not decrease prostate-specific antigen (PSA) and prostate volume. Interestingly, individuals diagnosed with PCa while receiving toremifene were not more likely to have high-grade disease than those treated with placebo [12,13].

Cumulatively, these data indicate that estradiol potentiates the carcinogenic effects of androgens through ERα, which is a promising new target for chemoprevention with the ERα antagonist toremifene. Considering that both ARs and ERα are
required for prostatic carcinogenesis, it is conceiv-able that the combination of 5α-reductase (5-AR) inhibitors (finasteride, dutasteride) with the ERα antagonist toremifene offers a much more effective protection against the development of PCa in men than 5-AR inhibitors or an ERα antagonist alone. This issue, however, has not yet been addressed by clinical studies.

Another oestrogen receptor involved in prostatic carcinogenesis is ERβ, which had been cloned in 1997 by Gustafsson and colleagues in the rat prostate and ovary and has a high affinity to phytoestrogens [14]. The potential preventive effect of phytoestrogens on PCa stemmed from the epidemiologic observation of the low incidence of clinical PCa among Japanese and Chinese populations with a traditionally high dietary intake of phytoestrogens [15]. Natural phytoestrogens, such as genistein, indole-3-carbinol, and resveratrol preferentially bind to ERβ, which exerts protective

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Fig. 1 – Differential expression of oestrogen receptor alpha (ERα) and progesterone receptor (PR) in (A and B) high-grade intraepithelial neoplasia (HGPIN) and in (C–E) prostate cancer; (A, arrow) ERα at the mRNA level is restricted to basal cells of the normal prostatic epithelium; (A) in HGPIN, ERα gene expression extends to luminal cells; (B) HGPIN with ERα expression at the protein level; (C) prostate cancer with intraductal spread (Gleason 4 + 4) revealing nuclear expression of the ERα and the PR on adjacent sections; (D) bone metastasis with extensive and strong nuclear expression of the PR; (E) castration-resistant prostate cancer with extensive expression of the nuclear PR (left) and high levels of ERα mRNA expression (right) on adjacent sections; in this case, ERα was undetectable by immunohistochemistry. Original magnifications: A, ×200; B, ×40; C, ×50; D (left), ×50; D (right), ×200.
effects on the prostatic epithelium. The anticancer properties of phytoestrogens have been documented in vivo and in vitro (reviewed by Klein [15]), including inhibition of cell proliferation and angiogenesis, a decrease in PSA and 5-AR activity, and a decrease of androgen-receptor expression (AR silencing) (Table 1). In the human prostate, ERβ is expressed at high levels in luminal cells of the prostatic epithelium but is partly lost during prostatic carcinogenesis [7]. In HGPIN, ERβ is markedly decreased or absent in about 40% of cases, which implicates ERβ as a tumour suppressor [7] (Fig. 2a

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SCID = severe combined-immunodeficient; PSA = prostate-specific antigen; TRAMP = transgenic adenocarcinoma mouse prostate; AR = androgen receptor; ER = oestrogen receptor; ERβ = oestrogen receptor beta.

Fig. 2 – Differential expression of the oestrogen receptor beta (ERβ) in (A) the normal prostate, (B) in high-grade intraepithelial neoplasia (HGPIN), and in (C–D) prostate cancer; (A, arrow) ERβ is expressed at high levels in luminal cells of the normal prostatic epithelium and to a lesser degree in basal cells; (B) HGPIN with severe loss of ERβ; (C) bone metastasis with extensive and strong expression of ERβ; (D) castration-resistant prostate cancer with partial loss of ERβ. Original magnifications: A, ×200; B (left), ×20; B (right), ×100; C (left), ×40; C (right), ×100; D (left) ×25; D (right) ×100.
and b). As the chemopreventive and anticancer properties of phytoestrogens depend on the presence and activity of ERβ, one can speculate 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 a high intake of phytoestrogens substantially reduces PCa risk among men with specific polymorphic variation in the promoter region of the ERβ gene. No association was found between phytoestrogens and PCa among carriers homozygous for the wild-type allele of the ERβ gene [25].

2.2 Role of oestrogens and their receptors in prostate cancer progression

Contrary to HGPIN, hormone-naïve PCa generally retains high levels of ERβ expression—even in lymph node and bone metastasis [7] (Fig. 2c). For those patients, treatment with ERβ-specific agonists may slow tumour progression, but this issue has not yet been addressed in clinical studies. A substantial loss of ERβ is encountered in hormone-refractory disease (Fig. 2d). Markedly reduced levels of ERβ are found in about 40% of cases. In 10% of these tumours, ERβ is undetectable [7].

In apparent contrast to breast cancer and other oestrogen-related tumours, the presence of ERα in PCa is a late event in disease progression [5]. One is unlikely to find ERα immunoreactivity in low- to intermediate-grade PCa. High-grade (Gleason grade 4 and 5) tumours reveal ERα protein expression in 43% and 62% of cases, respectively. The most significant ERα gene expression on mRNA and protein levels was observed in metastatic lesions and hormone-refractory tumours [5]. It is quite clear that the mere presence of ERα detected in PCa tissue by immunohistochemistry does not imply that this receptor elicits biologically relevant events. If the ERα present during PCa progression is functionally active, one would expect to find evidence for transcriptional activity of ERα-regulated genes in these tumours. Among the various ERα-regulated genes, the progesterone receptor (PR) is one of the most important markers for oestrogen-regulated growth in oestrogen-dependent tumours. It is not surprising to find that the immunoprofiles of the PR in PCa run remarkably parallel to those of ERα [26] (Fig. 1c). In fact, the most consistent and extensive levels of PR expression in PCa are detectable in hormone-refractory and metastatic lesions, including bone and lymph node metastases (Fig. 1d and 1e). Moderate to strong PR expression is identified in 60% of metastatic lesions and in 54% of recurrent tumours after androgen deprivation therapy (ADT). The progressive emergence of the PR during tumour progression indicates that a substantial number of metastatic and hormone-refractory PCa harbours functional ERα, which can induce PR expression [26]. This provides a possible mechanism for how PCa cells can bypass ADT by using endogenous or exogenous oestrogens for their growth. The current data highlight the need to test ERα-specific antagonists in the treatment of PCa and raise a cautionary flag regarding the use of therapeutic agents with ERα and PR agonist activity, such as oestrogens and progestins.

Another pathway that further underscores the importance of oestrogen signalling in PCa progression has been reported recently [28]. The majority of prostate cancers harbour an acquired chromosomal translocation that results in the fusion of the promoter region of the transmembrane protease serine-2 (TMPRSS2) gene to the coding region of members of the erythroblast transformation-specific (ETS) family of transcription factors, including ERG, ETV1, and ETV4. Prostate cancers with the TMPRSS2-ERG fusion appear to have a more aggressive natural clinical history than other prostate cancers, although this issue remains controversial. One Finish study reported that the TMPRSS2-ERG fusion identifies a subgroup of prostate cancers with a favourable prognosis [27]. Nevertheless, the TMPRSS2-ERG fusion was identified recently in all nonosseous metastasis from 30 rapid autopsies of men who died of androgen-independent disease [29]. In a subsequent study, the authors have identified an 87-gene expression signature for TMPRSS2-ERG tumours that was associated with ER signalling pathways. It was found that TMPRSS2-ERG expression was increased by ERα agonists (oestrogens) and decreased by ERβ agonists [28]. The authors concluded that pharmacologic inhibition of TMPRSS2-ERG expression using drugs that antagonise ERα activity and function as ERβ agonists may have promise as new therapeutic strategy for PCa [28].

2.3 Paracrine actions of oestrogens and tumour microenvironments

The prostatic stroma is equipped with ARs, ERα, PR, and—to a lesser degree—ERβ. Cunha and colleagues have convincingly demonstrated that paracrine oestrogen signalling through stromal-derived growth factors and mesenchymal–epithelial cell interactions is crucial for prostate morphogenesis, epithelial differentiation, and androgen signalling [3,30]. Prostate stromal cells secrete a number of
paracrine growth factors, including the insulin-like growth factor (IGF), fibroblast growth factor (FGF), and transforming growth factor beta (TGFβ) families. Importantly, these potent growth factor signalling pathways have been implicated in PCa and are regulated, in part, through ER signalling [30]. Changes within the stromal steroid receptor system have been documented in several clinical studies. For example, hereditary PCa has been reported to have higher stromal AR and lower stromal ERα levels than sporadic cancer [31]. Increased expression of stromal ERα was observed in pathologic specimens from PCa patients after ADT [32].

In contrast, stromal AR is lost, while AR expression is upregulated in PCa cells during progression. These data are derived from clinical studies relating the AR status in PCa tissue with the Gleason grade, clinical and pathologic stage, and PSA recurrence in patients after radical prostatectomy (RP) [33,34]. PCa cells with high levels of AR expression can use very low levels of androgen for growth and can survive androgen deprivation. This hypersensitive pathway has been recognised as one of the most important mechanisms involved in the development of castration-resistant disease [35]. As the stromal ERα controls AR expression under normal conditions, it is conceivable that increased expression of ERα in the tumour stroma may contribute to the hypersensitive pathway and to tumour progression. It is noteworthy that phytoestrogens acting through the ERβ and the pure anti-oestrogenICI 182 780 (fulvestrant) has been reported to decrease AR expression in PCa cells and inhibit androgen-mediated signalling pathways [20,40]. Thus, ERα antagonists and ERβ agonists may have promise in targeting tumour microenvironment and AR expression in PCa.

3. Evidence synthesis

3.1. Preclinical studies with selective oestrogen receptor modulators

Several selective ER modulators (SERM) have been tested in preclinical studies (recently reviewed by Bosland [9]). Briefly, tamoxifen inhibits proliferation of PC-3 and DU-145 PCa cells and induces apoptosis in LNCaP cells. Tamoxifen also inhibits in vivo growth of the CWR22 PCa xenograft in nude mice. Raloxifene (a mixed oestrogen agonist/antagonist) induces apoptosis in LNCaP cells. Both raloxifene and the ERα antagonist tormifene reduce the development of pulmonary metastasis and extend survival in the PAIII prostatic adenocarcinoma model (Table 2). The pure anti-oestrogenICI 182 780 and the ERα antagonist toremifene inhibit proliferation of PC-3 cells. In the transgenic adenocarcinoma of mouse prostate (TRAMP) model, all

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ADT = androgen deprivation therapy; AR = androgen receptor; CRPC = castration-resistant prostate cancer; HDL = high-density lipoprotein; HGPIN = high-grade prostatic intraepithelial neoplasia; LDL = low-density lipoprotein; PCa = prostate cancer; PSA = prostate-specific antigen; SERM = selective oestrogen receptor modulator; TRAMP = transgenic adenocarcinoma mouse prostate.
animals in the placebo group developed tumours compared with only 35% of the 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 wk of age, 100% of the placebo-treated animals had developed palpable tumours and died, whereas 60% of the toremifene-treated animals were tumour free [42].

3.2. Clinical studies with selective oestrogen receptor modulators

Among the various SERMs, the ER\textsubscript{a} antagonist toremifene is currently the most promising drug for PCa prevention in clinical studies. A phase 2B clinical trial enrolling 514 patients with a history of diagnosed HGPIN revealed a significant (48.2%) reduction in cancer incidence at 12-mo biopsy compared with the placebo group [12]. Contrary to the rather encouraging results of SERMs in preclinical studies, the few data from clinical trials enrolling patients with castration-resistant disease are rather disappointing. Tamoxifen has been studied in phase 2 clinical trials with PCa patients, but therapeutic efficacy was uncertain. A major problem with tamoxifen is the mixed antagonist and agonist (estrogenic) effects [9]. The pure antioestrogen ICI 182,780 (fulvestrant), although effective in preclinical studies, failed to produce clinical or PSA response in a phase 2 clinical trial enrolling 20 patients with castration-resistant prostate cancer (CRPA) [41]. At least toremifene has been reported to elicit some clinically relevant responses in PCa patients receiving ADT. Toremifene significantly increases hip and spinal bone mineral density and improves lipid profiles in men receiving ADT [43,44]. The latter includes a significant decrease in total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides and an increase in high-density lipoprotein (HDL) cholesterol [44]. Cholesterol-lowering therapies may be beneficial for patients with PCa. It has been shown recently that PCa uses cholesterol for intratumoural de novo testosterone synthesis, which is markedly increased in castration-resistant disease [45]. Whether toremifene delays disease progression remains to be established.

3.3. Oestrogens as potential serum markers for prostate cancer staging and progression

Further evidence supporting the role of oestrogens in PCa progression is derived from clinical studies investigating serum oestrogens in patients with PCa [46]. Serum levels of estradiol (E2), oestrone (E1), and oestrone sulphate (E1S) were measured in PCa patients and related to PSA, Gleason score, histologic stage, and surgical margins. Significantly higher E1S levels were found in patients with PSA >10 ng/ml versus PSA \(\leq 10\) ng/ml, stage pT3–T4 versus pT2, and positive versus negative margins. The authors conclude that oestrogens, especially E1S, might represent possible serum markers of PCa staging and progression [46].

3.4. Immunohistochemical detection, gene silencing, and splice variants

Gene silencing by promotor hypermethylation and subsequent inactivation of ER\textsubscript{a} and ER\textsubscript{b} gene expression has been reported in PCa [47,48]. 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 ER\textsubscript{a} and PR in human PCa tissue. These discrepancies obviously reflect differences in the choice of antibodies, immunohistochemical detection tools, and tissue processing [5,26]. The use of supersensitive monoclonal antibodies in conjunction with antigen retrieval and the presence of suitable internal positive controls (eg, strong nuclear staining of ER\textsubscript{a} and PR in stromal and basal cells; strong nuclear staining of ER\textsubscript{b} in luminal cells) are required for reliable immunolocalisation of ER\textsubscript{a}, ER\textsubscript{b}, and PR in PCa tissue [5,7,26]. Of paramount importance for ER\textsubscript{a} immunolocalisation is the use of fresh tissue immediately fixed in buffered formalin. Archival paraffin blocks obtained by routine fixation may not be informative. In this case, negative immunohistochemical results may be obtained even in presence of high ER\textsubscript{a} mRNA levels detected by in situ hybridisation (Fig. 1e). Thus, evaluation of the ER\textsubscript{a} status in human PCa tissue by immunohistochemistry remains difficult and cannot be regarded as a routine procedure as established in breast cancer tissue.

Another issue refers to the expression and function of ER\textsubscript{b} splice variants. Using an antibody raised against a post-transcriptionally modified short form of ER\textsubscript{b}, Leav et al have immunolocalised ER\textsubscript{b} in basal cells and reported markedly decreased levels of ER\textsubscript{b} in Gleason grade 4/5 tumours and its absence in transition-zone cancer [6]. In our studies, using an antibody raised against the long and short form of the ER\textsubscript{b} isoform 1, ER\textsubscript{b} was localised predominantly in the secretory epithelium, as described in the rat and murine prostate. In
addition, the substantial loss of ERβ in transitionzone cancer and in Gleason grade 4/5 tumours reported by Leav et al was not observed [7].

3.5. Importance of receptor isoforms

Studies using semiquantitative reverse transcription polymerase chain reaction (RT-PCR) have shown that ERα and ERβ transcripts are differentially expressed in human PCa cell lines, including the androgen-sensitive LNCaP (ERα+/ERβ+) and the androgen-insensitive PCa cell lines PC-3 (ERα+/ ERβ+), PC3M (ERα+/ERβ+), and DU-145 (ERα−/ERβ+) [38]. Down-regulation of ERα mRNA expression reported in LNCaP, DU-145, and in human PCa tissue has been related to gene silencing through promoter hypermethylation of the ERα gene [48]. This may be true for the ERα isoforms A and B (ERα-A, ERα-B), but not for the ERα isoform C (ERα-C). Sasaki et al have shown that the ERα-C isoform is unmethylated and expressed in various PCa cell lines (ND1, DU-145, PC-3, LNCaP, and DUPro) and in human PCa tissue [47]. It is conceivable that the progressive emergence of ERα during tumour progression reported by immunohistochemistry and in situ hybridisation in human cancer tissue [5] refers to ERα-C but not to ERα isoforms A and B. Little is known about the functional implications of the ERα isoforms A, B, and C for ERα signalling and their localisation in human PCa tissue.

Referring to ERβ isoforms, most of the current studies are confined to the ERβ isoform 1, while the localisation and function of the ERβ isoforms 2, 3, 4, and 5 are less well established. It has been reported that ERβ 1 can form heterodimers with other ERβ isoforms, which may be critical for ERβ signalling and function [49]. Clearly, further studies are required to elucidate the role of the various ERα and ERβ isoforms and ERβ splice variants in human prostate tissue.

4. Conclusions

Although the AR remains the major target for PCa prevention and treatment, there are multiple lines of evidence to suggest that oestrogens and their receptors (ERα, ERβ) are also involved in PCa development and tumour progression. This is particularly evident in prostate carcinogenesis, where ERα signalling potentiates the carcinogenic effects of androgens on the prostatic epithelium. Based on the promising nature of the phase 2b trial outcome with the ERα antagonist toremifene, a phase 3 trial in a cohort of 1500 American men has been recently initiated.

The concept that ERα and ERβ are differentially involved in tumour progression has been strengthened by the recent observation that both receptors regulate a distinct molecular subclass of PCa with potentially aggressive clinical behaviour (ie, the TMPRSS2-ERG fusion).

Nevertheless, the translation of the current information into potential therapeutic applications remains highly challenging. A major problem is still the agonist (estrogenic) effects of ERα antagonists. With the SERMs currently available, it is perhaps unrealistic to expect an objective clinical response in patients with end-stage hormone-refractory disease. The usefulness of SERMs in hormone-naïve PCa in preventing disease progression has not yet been addressed by clinical studies. Little is known about the expression and function of ERβ splice variants, ERα and ERβ isoforms, ligand-dependent and ligand-independent activities, the role of genomic versus nongenomic signalling, and the role of ER coactivators in regulating antagonist/agonist response. Answers to these questions will further our understanding of ER signaling pathways and will open new avenues for drugs designed to antagonize ERα activity and function as ERβ agonists. This approach may provide new preventive and therapeutic strategies for prostate cancer.

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Study concept and design: Bonkhoff, Berges.
Acquisition of data: Bonkhoff, Berges.
Analysis and interpretation of data: Bonkhoff, Berges.
Drafting of the manuscript: Bonkhoff.
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