available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Platinum Priority – Review – Prostate Cancer

Editorial by Alberto Briganti on pp. 543–545 of this issue

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

Helmut Bonkhoff[a,*], *Richard Berges*[b]

[a] *Pathology Laboratories, Tietzenweg 129, 12203 Berlin, Germany*
[b] *Pan-Klinik, Zeppelinstr.1, 50667 Köln, Germany*

Article info

Article history:
Accepted October 27, 2008
Published online ahead of print on November 6, 2008

Keywords:
Oestrogens
Phytoestrogens
Oestrogen receptor alpha and beta
Progesterone receptor
TMPRSS2-ERG
HGPIN
Prostate cancer

EU ★ ACME
www.eu-acme.org/europeanurology

Please visit www.eu-acme.org/europeanurology to read and answer questions on-line. The EU-ACME credits will then be attributed automatically.

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 [ERα], oestrogen receptor beta [ERβ]) that undergoes profound remodelling during PCa development and tumour progression. In high-grade prostatic intraepithelial neoplasia (HGPIN), the ERα is upregulated and most likely mediates carcinogenic effects of estradiol as demonstrated in animal models. Preliminary clinical studies with the ERα antagonist toremifene have identified the ERα as a promising target for PCa prevention. The partial loss of the ERβ in HGPIN indicates that the ERβ acts as a tumour suppressor. The ERβ is generally retained in hormone-naïve PCa but is partially lost in castration-resistant disease. The progressive emergence of the ERα 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-dependent signalling. *TMPRSS2-ERG* expression has been found to be increased by ERα agonist (oestrogens) and decreased by ERβ agonists.

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

* Corresponding author. Pathology Laboratories, Tietzenweg 129, 12203 Berlin, Germany.
Tel. +49 30 84317882; Fax: +49 30 8330335.
E-mail address: info@prostapath.de (H. Bonkhoff).

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

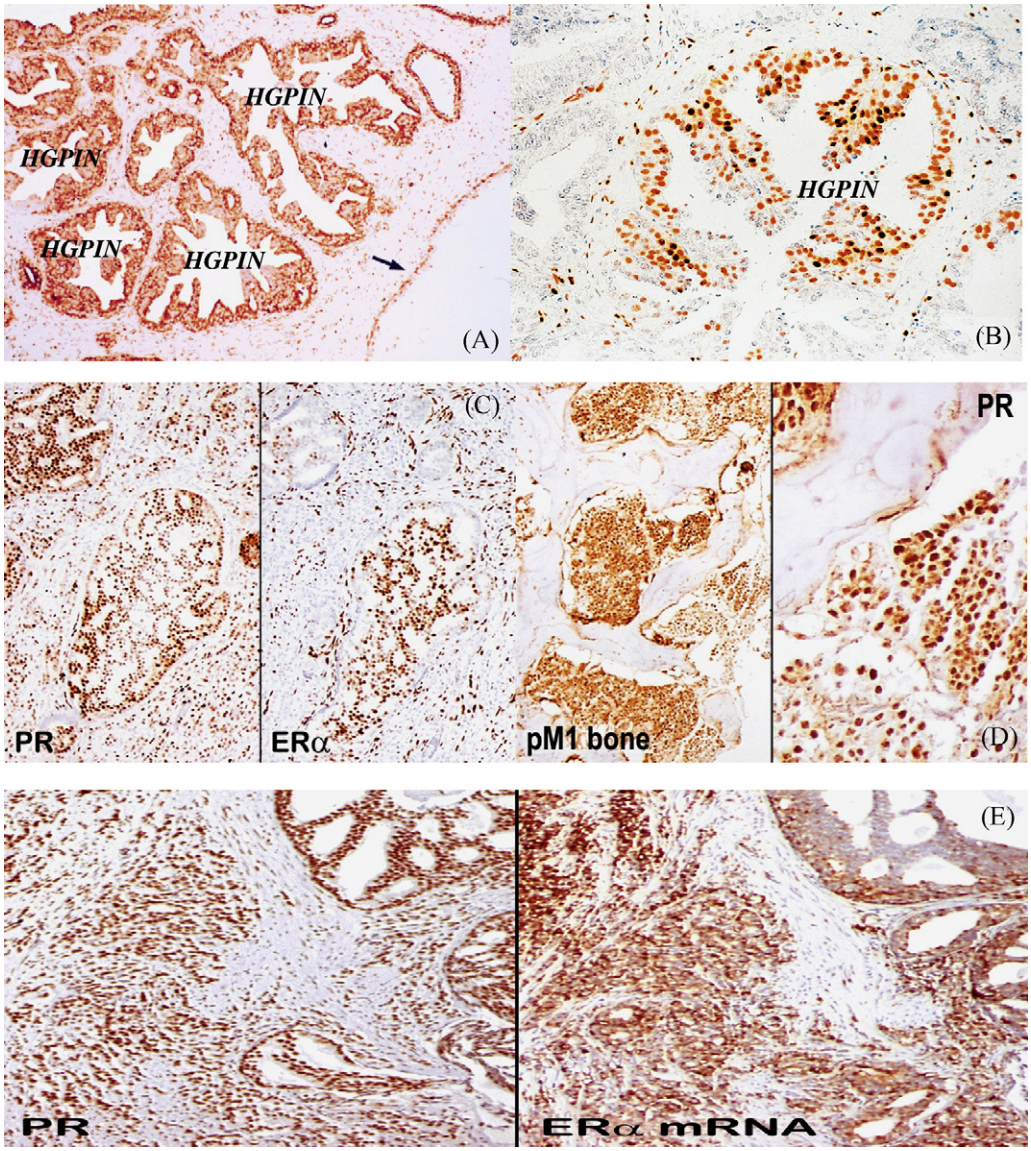

Fig. 1 – Differential expression of oestrogen receptor alpha (ERα) and progesterone receptor (PR) in (A and B) high-grade intraepithelial neoplasia (HGPIN) and in (C–E) prostate cancer; (A, arrow) ERα at the mRNA level is restricted to basal cells of the normal prostatic epithelium; (A) in HGPIN, ERα 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.

required for prostatic carcinogenesis, it is conceivable 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

Table 1 – Anticancer effects of phytoestrogens on prostate cancer documented in animal models and in preclinical studies

Author	Phytoestrogens	Model System	Findings
Zhou et al [16]	Soy protein phytochemical concentrate	LNCaP, SCID mouse xenografts	Reduced tumour volume, Increased apoptotic index Decreased proliferation activity Decreased angiogenesis
Bylund et al [17]	Soy protein	LNCaP nude mouse xenografts	Reduced tumour incidence and volume, lower PSA production
Mentor-Marcel et al [18]	Genistein	TRAMP	Dose-dependent reduction in progression to poorly differentiated tumours
Shen et al [19]	Genistein	LNCaP	Induces G(1) cell-cycle block mediated by p27(KIP 1) and p21(WAF1)
Fritz et al [20]	Genistein	Rat prostate	Downregulates AR and ER
Wang et al [21]	Genistein	TRAMP	50% decrease in poorly differentiated tumours
Stettner et al [22]	Tectorgenin (+ valproic acid)	LNCaP	Upregulation of the ERβ induces antiproliferatve effects
Matsumura et al [23]	Genistein	PC-3	Marked decrease in proliferation activity through the ERβ and p21
Haper et al [24]	Resveratrol	TRAMP	7.7-fold decrease in poorly differentiated tumours, upregulation of the ERβ

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

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

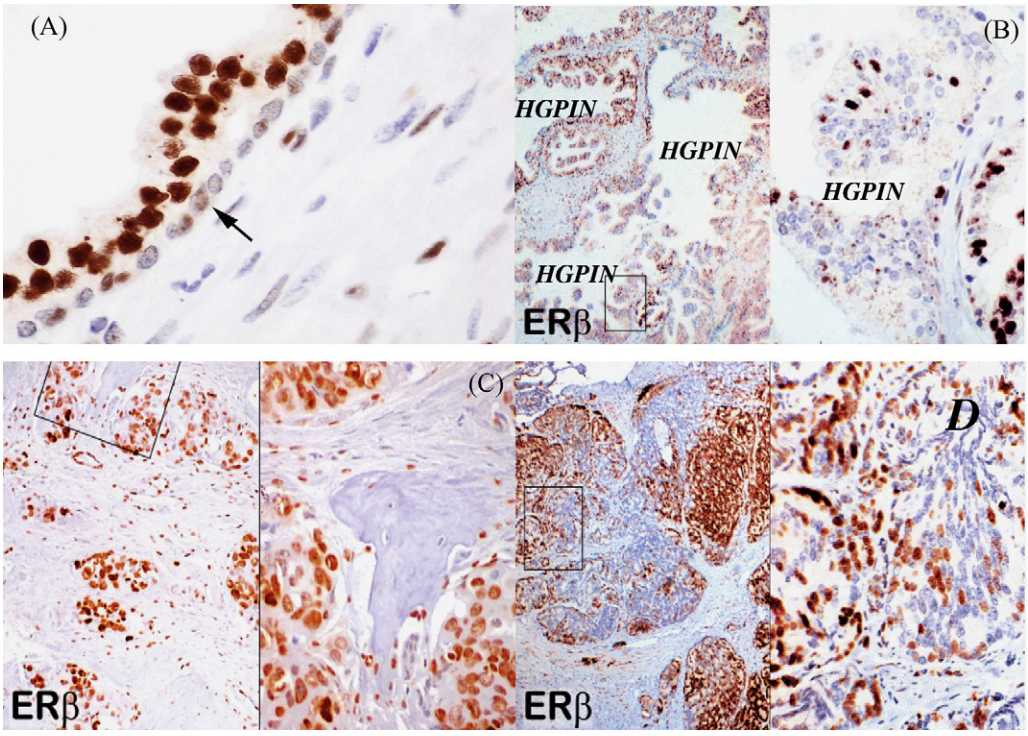

Fig. 2 – Differential expression of the oestrogen receptor beta (ERβ) in (A) the normal prostate, (B) in high-grade intraepithelial neoplasia (HGPIN), and in (C–D) prostate cancer; (A, arrow) ERβ is expressed at high levels in luminal cells of the normal prostatic epithelium and to a lesser degree in 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 mesenchymale–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 antioestrogen ICI 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 trioxifene reduce the development of pulmonary metastasis and extend survival in the PAIII prostatic adenocarcinoma model (Table 2). The pure antioestrogen ICI 182 780 and the ERα antagonist toremifene inhibit proliferation of PC-3 cells. In the transgenic adenocarcinoma of mouse prostate (TRAMP) model, all

Table 2 – Selective oestrogen receptor modulators in preclinical and clinical studies

Authors	SERM	Study design	Findings
Neubauer et al [36]	Raloxifene	PAIII rat prostatic carcinoma model	Marked decrease of metastasis Extended survival
Kim et al [37]	Raloxifene	LNCaP	Induced apoptosis through androgen- independent pathway
Kim et al [38]	Raloxifene	PC-3, PC3M, DU-145	Induced apoptosis through androgen- independent pathway
Neubauer et al [39]	Trioxifene	PAIII rat prostatic carcinoma model	Marked decrease of metastasis Extended survival
Bhattacharyya et al [40]	Fulvestrant (ICI 182 780)	LNCaP	Downregulated AR 70% growth inhibition
Chadha et al [41]	Fulvestrant (ICI 182 780)	20 patients with CRPC Phase 2 study	No PSA or clinical response
Raghow et al [42]	Toremifene	TRAMP	65% decrease in PCa incidence No HGPIN in treated mice Extended survival
Price al [12]	Toremifene	514 patients with HGPIN Phase 2b study—double-blind, placebo-controlled	12-mo incidence of PCa decreased by 48.2%
Smith et al [43]	Toremifene	1392 patients under ADT Phase 3	Increase in bone mineral density
Smith al [44]	Toremifene	1389 patients under ADT Phase 3 study	Decrease in cholesterol, LDL, and triglycerides Increase in HDL

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 ERα 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 ≤ 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 ERα and ERβ 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 ERα 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 ERα and PR in stromal and basal cells; strong nuclear staining of ERβ in luminal cells) are required for reliable immunolocalisation of ERα, ERβ, and PR in PCa tissue [5,7,26]. Of paramount importance for ERα 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 ERα mRNA levels detected by in situ hybridisation (Fig. 1e). Thus, evaluation of the ERα 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 ERβ splice variants. Using an antibody raised against a post-transcriptionally modified short form of ERβ, Leav et al have immunolocalised ERβ in basal cells and reported markedly decreased levels of ERβ 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 ERβ isoform 1, ERβ was localised predominantly in the secretory epithelium, 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 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.

Author contributions: Helmut Bonkhoff had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Bonkhoff, Berges.
Acquisition of data: Bonkhoff, Berges.
Analysis and interpretation of data: Bonkhoff, Berges.
Drafting of the manuscript: Bonkhoff.
Critical revision of the manuscript for important intellectual content: Bonkhoff, Berges.
Statistical analysis: Bonkhoff, Berges.
Obtaining funding: None.
Administrative, technical, or material support: Bonkhoff, Berges.
Supervision: Bonkhoff, Berges.
Other (specify): None.

Financial disclosures: I certify that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

Funding/Support and role of the sponsor: None.

References

[1] Chang WY, Prins GS. Estrogen receptor-beta: implications for the prostate gland. Prostate 1999;40:115–24.

[2] Ho S-M. Estrogens and anti-estrogens: key mediators of prostate carcinogenesis and new therapeutic candidates. J Cell Biochem 2004;91:491–503.

[3] Risbridger GP, Ellem SJ, McPherson SJ. Estrogen action on the prostate gland: a critical mix of endocrine and paracrine signalling. J Mol Endocrinol 2007;39:183–8.

[4] Singh PB, Matanhelia SS, Martin FL. A potential paradox in prostate adenocarcinoma progression: oestrogen as the initiating driver. Eur J Cancer 2008;44:928–36.

[5] Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. Am J Pathol 1999;155:641–7.

[6] 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.

[7] Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ER beta) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. Prostate 2003;54:79–87.

[8] Bonkhoff H, Remberger K. Differentiation pathways and histogenetic aspects of normal and abnormal prostatic growth: a stem cell model. Prostate 1996;28:98–106.

[9] Bosland MC. The role of estrogens in prostate carcinogenesis: a rationale for chemoprevention. Rev Urol 2005; 7(Suppl 3):4–10.

[10] Ricke WA, McPherson SJ, Bianco JJ, Cunha GR, Wang Y, Risbridger GP. Prostatic hormonal carcinogenesis is mediated by in situ estrogen production and estrogen receptor alpha signalling. FASEB J 2008;22:1512–20.

[11] De Marzo AM, Platz EA, Sutcliffe S, et al. Inflammation in prostate carcinogenesis. Nature Reviews Cancer 2007;7: 256–69.

[12] Price D, Stein B, Sieber P, et al. Toremifene for the prevention of prostate cancer in men with high grade prostatic intraepithelial neoplasia: results of a double-blind, placebo controlled, phase IIB clinical trial. J Urol 2006; 176:965–70.

[13] Taneja SS. Drug therapies for eradicating high-grade prostatic intraepithelial neoplasia in the prevention of prostate cancer. Rev Urol 2005;7(Suppl 3):19–29.

[14] Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci USA 1996;93: 5925–30.

[15] Klein EA. Opportunities for prevention of prostate cancer: genetics, chemoprevention, and dietary intervention. Rev Urol 2002;4(Suppl 5):18–28.

[16] Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr 1999;129:1628–35.

[17] Bylund A, Zhang JX, Bergh A, et al. Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. Prostate 2000;42:304–14.

[18] Mentor-Marcel R, Lamartiniere CA, Eltoum IE, Greenberg NM, Elgavish A. Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP). Cancer Res 2001;61: 6777–82.

[19] Shen JC, Klein RD, Wei Q, et al. Low-dose genistein induces cyclin-dependent kinase inhibitors and G(1) cell-cycle arrest in human prostate cancer cells. Mol Carcinog 2000;29:92–102.

[20] Fritz WA, Wang J, Eltoum IE, Lamartiniere CA. Dietary genistein down-regulates androgen and estrogen receptor expression in the rat prostate. Mol Cell Endocrinol 2002;186:89–99.

[21] Wang J, Eltoum IE, Lamartiniere CA. Genistein chemoprevention of prostate cancer in TRAMP mice. J Carcinog 2007;6:3.

[22] Stettner M, Kaulfuss S, Burfeind P, et al. The relevance of estrogen receptor-beta expression to the antiproliferative effects observed with histone deacetylase inhibitors and phytoestrogens in prostate cancer treatment. Mol Cancer Ther 2007;6:2626–33.

[23] Matsumura K, Tanaka T, Kawashima H, Nakatani T. Involvement of the estrogen receptor beta in genistein-induced expression of p21(waf1/cip1) in PC-3 prostate cancer cells. Anticancer Res 2008;28:709–14.

[24] Harper CE, Patel BB, Wang J, Arabshahi A, Eltoum IA, Lamartiniere CA. Resveratrol suppresses prostate cancer progression in transgenic mice. Carcinogenesis 2007; 28:1946–53.

[25] Hedelin M, Bälter KA, Chang ET, et al. Dietary intake of phytoestrogens, estrogen receptor-beta polymorphisms and the risk of prostate cancer. Prostate 2006;66:1512–20.

[26] Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Progesterone receptor expression in human prostate cancer: correlation with tumor progression. Prostate 2001;48: 285–91.

[27] Saramäki OR, Harjula AE, Martikainen PM, Vessella RL, Tammela TL, Visakorpi T. TMPRSS2:ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. Clin Cancer Res 2008;14:3395–400.

[28] Setlur SR, Mertz KD, Hoshida Y, et al. Estrogen-dependent signaling in a molecularly distinct subclass of aggressive prostate cancer. J Natl Cancer Inst 2008;100:815–25.

[29] Mehra R, Tomlines S, Yu J, et al. Characterization of TMPRSS2-ETS gene aberrations in androgen-independent metastatic prostate cancer. Cancer Res 2008;68: 3584–90.

[30] Ricke W, Wang Y, Cunha G. Steroid hormones and carcinogenesis of the prostate: the role of estrogens. Differentiation 2007;75:871–82.

[31] Fromont G, Yacoub M, Valeri A, et al. Differential expression of genes related to androgen and estrogen metabolism in hereditary versus sporadic prostate cancer. Cancer Epidemiol Biomarkers Prev 2008;17: 1505–9.

[32] Kruithof-Dekker IG, Têtu B, Janssen PJ, Van der Kwast TH. Elevated estrogen receptor expression in human prostatic

stromal cells by androgen ablation therapy. J Urol 1996; 156:1194–7.
[33] Henshall SM, Quinn DI, Lee CS, et al. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. Cancer Res 2001;61:423–7.
[34] Li R, Wheeler T, Dai H, Frolov A, Thompson T, Ayala G. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. Am J Surg Pathol 2004;28:928–34.
[35] Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
[36] 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;27:220–9.
[37] Kim IY, Seong DH, 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;62:3649–53.
[38] Kim IY, Kim BC, Seong DH, et al. Raloxifene, a mixed estrogen agonist/antagonist, induces apoptosis in androgen-independent human prostate cancer cell lines. Cancer Res 2002;62:5365–9.
[39] 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;63:6056–62.
[40] Bhattacharyya RS, Krishnan AV, Swami S, Feldman D. Fulvestrant (ICI 182,780) down-regulates androgen receptor expression and diminishes androgenic responses in LNCaP human prostate cancer cells. Mol Cancer Ther 2006;5:1539–49.
[41] Chadha MK, Ashraf U, Lawrence D, et al. Phase II study of fulvestrant (Faslodex) in castration resistant prostate cancer. Prostate 2008;68:1461–6.
[42] Raghow S, Hooshdaran MZ, Katiyar S, Steiner MS. Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. Cancer Res 2002;62:1370–6.
[43] Smith MR, Malkowicz SB, Chu F, et al. Toremifene increases bone mineral density in men receiving androgen deprivation therapy for prostate cancer: interim analysis of a multicenter phase 3 clinical study. J Urol 2008;179:152–5.
[44] Smith MR, Malkowicz SB, Chu F, et al. Toremifene improves lipid profiles in men receiving androgen-deprivation therapy for prostate cancer: interim analysis of a multicenter phase III study. J Clin Oncol 2008;26: 1824–9.
[45] Locke JA, Guns ES, Lubik AA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 2008;68:6407–15.
[46] Giton F, de la Taille A, Allory Y, et al. Estrone sulfate (E1S), a prognosis marker for tumor aggressiveness in prostate cancer (PCa). J Steroid Biochem Mol Biol 2008; 109:158–67.
[47] Sasaki M, Tanaka Y, Perinchery G, et al. Methylation and inactivation of estrogen, progesterone, and androgen receptors in prostate cancer. J Natl Cancer Inst 2002;94: 384–90.
[48] Lau KM, LaSpina M, Long J, Ho SM. Expression of estrogen receptor (ER)-alpha and ER-beta in normal and malignant prostatic epithelial cells: regulation by methylation and involvement in growth regulation. Cancer Res 2000;60: 3175–82.
[49] Leung YK, Mak P, Hassan S, Ho SM. Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signalling. Proc Natl Acad Sci USA 2006;103:13163–7.