

## Review Article

# Estrogen receptors in prostate development and cancer

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**Abstract:** Prostate cancer (PCa) is an androgen-sensitive disease, which can be pharmacologically controlled by androgen blockade. To date, a growing body of evidence showed that estrogen and estrogen receptors (ERs) could regulate prostate development, as well as cancer initiation and progression. This review will address the expression levels and function of ERs in different stages of PCa progression. The functions of ERs in different types of prostate cells, the ligand effect, and the potential applications of selective estrogen modulators (SERMs) will also be discussed. To further dissect ERs' roles in prostate development, cell type specific ER knockout mouse models were generated. Results collected from the prostate cell type-specific ER $\alpha$ KO mouse models provided new insights about the cell type specific ER $\alpha$  roles in prostate development prenatally and postnatally. The results of ERs' roles in mouse PCa mode and the correlation of ERs expression and biomedical outcome will also be discussed.

**Keywords:** Estrogen receptor, environment estrogen, prostate cancer, SERM, tissue specific ER $\alpha$  knockout mouse

### Introduction

Prostate gland growth and development are mainly mediated by androgens, but estrogen has also been suggested to play certain roles in the same processes as well as in prostate carcinogenesis [1-3]. The development of prostate cancer (PCa) commonly occurs at an age when the level of serum testosterone is in decline [4]. In contrast, the levels of estradiol do not reduce and instead remain unchanged or increase with age [5, 6]. Earlier results suggest that the significant decrease in ratio of testosterone to estradiol is related to PCa development. Epidemiological data from adult men further showed an association of elevated plasma estrogens with an increased risk of PCa [7]. Animal studies also demonstrated that 100% of prostatic adenocarcinomas occurred in rats after being treated with 17 $\beta$ -estradiol (E2) plus testosterone (T) for around 44 weeks [8].

PCa is an androgen-sensitive disease, which can be pharmacologically controlled by androgen blockade. To date, a growing body of evidence showed that estrogen and estrogen receptors (ERs) could regulate prostate development, cancer initiation and progression. This review will address the expression levels and function of estrogen receptors in different

stage of PCa progression. The functions of ERs in different types of prostate cells, the ligand effect, the potential applications of selective estrogen modulators (SERMs) will also be discussed.

### *Expression of ER $\alpha$ and ER $\beta$ in prostate and prostate cancer*

Estrogen action is mainly mediated through its specific nuclear receptors that regulate transcription of target genes via binding to estrogen response element (ERE) or non-ERE mediated transactions, as well as non-genomic regulation [9]. There are two major types of ERs including ER $\alpha$  and ER $\beta$  [10-12]. The two ER subtypes are structurally similar, consisting of the six common domains (A-F) but encoded by separate genes (ESR1 and ESR2). To understand ERs' roles in prostate development, hyperplasia and cancer, we will first discuss their expression patterns followed by biochemical functional studies.

In non-malignant human adult prostate tissues, ER $\alpha$  is mainly expressed in the stromal compartment and occasionally found in the basal-epithelial cells [13-15]. ER $\beta$  is mainly detected in basal-epithelial cells [13-15]. As ER $\beta$  was identified in 1996 [11, 12], the studies of ER

expression performed before or close to 1996 may be compounded by the antibody cross reaction of the two subtypes of ERs. To date, the expression of both ER $\alpha$  and ER $\beta$  in PCa have been reported, however, the relative expression levels of ERs in different stages of PCa are still controversial.

Results of Immunostaining of PCa tissues indicated that near 15% of prostate stromal cells were positive for ER $\alpha$  in the normal, BPH, and PCa specimens. In contrast, immunostaining showed little epithelial ER $\alpha$  expression in normal prostates and only 10% BPH epithelial cells showed ER $\alpha$  positive. However, almost 80% of the PCa epithelial cells are ER $\alpha$  positive [15]. In normal prostate, ER $\beta$  was detected in nuclei of basal epithelial cells and in a few stromal cells by immunostaining. However, ER $\beta$  expression level and intensity decline in PCa tissues where only 10% or less PCa cells are ER $\beta$  positive [13].

Bonkhoff H *et al.* [16] used immunohistochemical staining (IHC) and in situ hybridization (ISH) techniques to demonstrate the correlation of protein and mRNA expressions of ER $\alpha$  in pre-malignant lesions and various stages of prostatic adenocarcinoma. High-grade prostatic intraepithelial neoplasia revealed positive ER $\alpha$  mRNA and protein expression at 28% and 11%, respectively. Focal ER $\alpha$  immunoreactivity was detected in a minority of low- to intermediate-grade adenocarcinomas. High-grade (primary Gleason grade 4 and 5) tumors revealed positive ER $\alpha$  protein expression with 43% in grade 4 and 62% in grade 5 cases. The most significant positive ER $\alpha$  expressions at mRNA and protein levels were observed in hormone refractory tumors and metastatic lesions, including lymph nodes and bone metastases [16]. These data suggest that estrogens can affect prostatic tumorigenesis and neoplastic progression through an ER $\alpha$ -mediated process in human prostate tissue. Latil *et al.* [17] studied ERs (ER $\alpha$  and ER $\beta$ ) expression in 12 clinically localized and 11 hormone-refractory sporadic prostate tumors, using real-time quantitative-PCR assays. ER $\beta$  expression level was decreased in both clinically localized and hormone-refractory tumors relative to normal prostate tissues. Surprisingly, this study showed hormone-refractory tumors display a decreased expression of ER $\alpha$ , which is different from the report by Bonkhoff H *et al.* [16] that ER $\alpha$  constantly

increased in high grade PCa and in hormone refractory PCa. However, we should be aware that the tissues used for quantitative-PCR detection of ER $\alpha$  mRNA levels [17] may contain a mixture of cancer epithelial cells and stromal cells.

Another report by Pflueger *et al.* detected ER $\beta$  expression in normal developing human fetal prostate. ER $\beta$  was detected in the nuclei of nearly 100% of epithelial and in the majority of stromal cells with strong intensity in fetal, neonatal, and early prepubertal prostates by immunostaining [18]. Horvath LG *et al.* further focused on determining ER $\beta$  expression patterns in normal, hyperplastic, and malignant human prostate and associations with clinical outcome [19]. In the report, five normal prostates from organ donors and 159 radical prostatectomy specimens from patients with clinically localized PCa were assessed for ER $\beta$  expression using immunohistochemistry. The five normal prostates showed strong ER $\beta$ -nuclear staining in >95% of the epithelial cells and 35% of the stromal cells. The number of ER $\beta$ -positive cases declined to 24.2% (38/157) in hyperplasia areas adjacent to carcinoma and 11.3% (18/159) in PCa cases. ER $\beta$ -positivity was related to relapse-free survival (log-rank P = 0.04).

Although there are some inconsistent results about the ER $\alpha$  and ER $\beta$  expression patterns in different stages of PCa, the above reported data suggest the expression and functions of ER $\alpha$  and ER $\beta$  could modulate the growth and progression of PCa. Overall, loss of ER $\beta$  expression is associated with progression from normal prostate epithelium to PCa, whereas those cancers that retained ER $\beta$  expression were associated with a higher rate of recurrence [19]. The correlation of ER $\alpha$  expression in different type prostate cells with prostate cancer progression remains to be further investigated.

### *Roles of ER $\alpha$ and ER $\beta$ in prostate development*

Prior to addressing the ER functions in PCa, it should be noted that ER $\alpha$  and  $\beta$  are expressed in the developmental stages of prostate glands. Neonatal exposure of estrogenic compounds could lead to abnormal prostate gland development. The long-term exposure of estrogen plus T could lead to neoplasias in murine prostate

[8]. An earlier report showed that estrogen and ER could affect the prostate growth, there was a lack of an *in vivo* model to systematically investigate the impact of ERs in selective prostate cells in the adult stage. Also the conventional ER $\alpha$  knockout mice could generate an alternative splicing ER $\alpha$  chimera protein, which maintains 30% of ER $\alpha$  transactivation capability. Thus, there is a clear need to use additional *in vivo* models to investigate ERs' roles in prostate development and cancer. Chen *et al.* [20] established the Cre-loxP system to knockout the floxed ER $\alpha$  gene in general organs or in a specific cell type. In the initial study, Chen *et al.* produced a total knockout mouse model to demonstrate ER $\alpha$  play important roles in prostate development. The total ER $\alpha$ KO mice were generated via the Cre-loxP system by mating floxed ER $\alpha$  mice with  $\beta$ -actin (ACTB)-Cre mice. Phenotypes from ER $\alpha$ KO male mice suggest that ER $\alpha$  is required for male fertility, acts through a paracrine mechanism to regulate prostatic branching morphogenesis, and is involved in the proliferation and differentiation of the prostatic stromal compartment. Using tissue specific FSP1-cre to breed with floxER $\alpha$  mice, Chen M *et al.* further produced stromal fibroblast specific ER $\alpha$ KO mice [21]. The expression of FSP1-Cre is first detected in the cells of the fibroblastic phenotype after embryonic day 8.5 [22, 23], Chen M *et al.* observed that loss of prostate stromal fibroblast ER $\alpha$ KO can reduce branching morphogenesis and increased stromal cell apoptosis [21]. To further dissect ER $\alpha$  roles in different component cells of stromal in prostate development, TGLN (smooth muscle specific) and FSP/TGLN double (smooth muscle and fibroblast specific) ER $\alpha$ KO mice models were generated. Data indicated smooth muscle ER $\alpha$  regulates cell growth, glandular infolding and ECM deposition and the fibroblast ER $\alpha$  affects branching morphogenesis [24]. Other than prostate stromal ER $\alpha$ KO, the prostate epithelial ER $\alpha$ KO mice were established to study the importance of epithelial ER $\alpha$  roles. Although prostate epithelial specific knockout mice model (pes-ER $\alpha$ KO, probasin-Cre ER $\alpha$ KO mice) indicated epithelial ER $\alpha$  has no function in early stage of prostate branching morphogenesis, epithelial ER $\alpha$  indeed plays important roles in estrogen-mediated cell proliferation postnatally. Squamous metaplasia is a specific phenotype in response to oestrogen in the prostates of postnatal male mice. The SQM proliferation

induced by DES (diethylstilbestrol) treatment was observed in wild type (WT), but not pes-ER $\alpha$ KO mice [25]. The results clearly showed that epithelial ER $\alpha$  plays important roles in estrogen-mediated cell proliferation postnatally.

In summary, the prostate cell type-specific ER $\alpha$ KO mouse models have provided new insights about the cell type specific ER $\alpha$  roles in prostate development prenatally and postnatally. Furthermore, prostate cell type-specific ER $\alpha$ KO mouse models could be applied as a powerful tool to further investigate the prostate cell type specific ER $\alpha$  roles in prostate development as well as PCa progression. Currently, there is no prostate cell type specific ER $\beta$ KO mice reported, it could be due to the unavailability of flox ER $\beta$  mice in the past.

### *Roles of ER $\alpha$ and ER $\beta$ in prostate cancer development*

Earlier reports showed testosterone (T) plus estrogen (E2) co-treatment could induce PCa in rats. A later report further showed ER $\alpha$  is essential in E2 plus T induced PCa, as ER $\alpha$ KO male mice do not develop high-grade PIN or PCa, while ER $\beta$ KO mice are similar to wild-type (WT) mice [26].

The aromatase deficient (ArKO) mice [27] lack the capability to produce estrogen and presumably ER $\beta$  (or ER $\alpha$ ) is not fully activated by estrogen. ArKO male mice showed the phenotypes of prostatic hypertrophy and hyperplasia within 8 weeks of birth. Feeding ER $\beta$  agonist to ArKO male mice results in the selective suppression of prostate hypertrophy and ablation of prostatic hyperplasia [28]. Taken together; these ArKO data suggested that estrogenic action via ER $\beta$  can result in an inhibited cellular proliferation within the prostate and PCa.

### *ER $\alpha$ roles in mouse prostate cancer models*

For PCa research, Hurwitz *et al.* [29] developed the transgenic adenocarcinoma of mouse prostate (TRAMP) mouse model which developed prostatic adenocarcinoma. This mouse model shows pathogenesis progression similar to human PCa progression. Due to amplified neuroendocrine phenotypes, TRAMP mouse PCa model is not perfect, yet is an acceptable and well characterized model in the PCa field. A

study [30] showed PCa cell proliferation signals and ER $\alpha$  levels, but not ER $\beta$  expression levels, are significantly increased in prostates of TRAMP compared to WT littermate mice. Feeding TRAMP mice with SERMs (like Genistein, a component of soy, GTx-006 and arzoxifene) [30, 31] can decrease PCa proliferation in TRAMP mice. The Genistein treatment could down-regulate expression levels of EGFR, IGF-1R, and their downstream mitogen-activated protein kinases, ERK-1 and ERK-2. These data provide a possible mechanism of using SERMs for PCa chemoprevention.

Another Study by Slusarz A *et al.* bred transgenic mice lacking functional ER $\alpha$  or ER $\beta$  with TRAMP mice [32]. The study showed a strong ER $\alpha$  genotypic influence on incidence of poorly-differentiated PCa, and a retarded cancer progression in ER $\alpha$ KO (only 5% developed poorly-differentiated PCa) compared with 19% in the WT littermates. The report also showed an increase incidence of poorly-differentiated PCa in ER $\beta$ KO mice. Interestingly, immunohistochemical analysis showed ER $\alpha$  expression changes from non-nuclear location in well-differentiated PCa to nuclear location in poorly-differentiated PCa, with little change in ER $\beta$  location or expression level. Also in the same study, when they tested the anti-cancer effect of phytoestrogen genistein, Results showed that dietary genistein reduced the incidence of cancer in WT/TRAMP mice but not in ER $\alpha$ KO/TRAMP or ER $\beta$ KO/TRAMP mice [32]. In addition to TRAMP mouse PCa model, the Pten heterozygous deletion mouse is also a widely used mouse PCa model. Pten (phosphatase and tensin homolog deleted from chromosome 10), is a key tumor suppressor. Previous observations showed Pten is frequently inactivated in human cancers [33] and loss of the Pten gene has been linked to many cancers, including PCa [34]. It was observed a significant decrease in ER $\alpha$  nuclear staining in high-grade lesions and carcinoma (Chen and Yeh unpublished data). The data suggest that Pten deficiency allows neoplastic cells to proliferate, yet epithelial ER $\alpha$  expression could be independent of PCa development.

Together, the above mouse PCa study results showed the epithelial ER $\alpha$  role remain controversial, and ER $\beta$  could play a protective roles in

PCa. Both ERs could be important for mediating Genistein inhibited cancer growth. However, the study did not well dissect the stromal vs. epithelial ERs' roles in PCa. Especially, the stromal ER $\alpha$  function remains to be further elucidated.

### *Selective estrogen receptor modulators (SERMs) in PCa therapy*

Selective estrogen receptor modulators (SERMs) have continued to show significant new benefits in management of human diseases in recent years [35]. SERM treatments have been applied to treat ER $\alpha$ -positive breast cancer in postmenopausal women, the results further showed that SERMs treatments could prevent fractures and reduce loss of bone mineral density (BMD) in postmenopausal women [36, 37]. In addition, SERMs were shown to prevent PCa development in men [38, 39]. It is proposed that estrogens are required for prostate carcinogenesis, yet SERMs can compete with E2 and other estrogens for binding to ERs in prostate tissues and inactivate estrogen-regulated genes to decrease cellular proliferation [3]. Several kinds of SERMs, like Tamoxifen, Toremifene, and Raloxifene, have been demonstrated able to suppress PCa growth in *in vitro* assays and in animal PCa models [40-42]. However, clinical trials with a high-dose tamoxifen [43] or toremifene [44, 45] did not show significant effects after treatments. This could be due to some differences in the PCa tumor microenvironment, or the pharmacokinetic difference of those SERMs between human and mouse.

### *ERs' roles in prostate cancer invasion*

Early studies suggested that epithelial to mesenchymal transition (EMT) can be a driving force of cancer invasion [46, 47]. Several reports have shown that ER $\beta$  expression was diminished when prostate cells undergo neoplastic transformation and develop into high Gleason grade prostate carcinomas, suggesting that ER $\beta$  could play a suppressor role in PCa. A study demonstrated that the ER $\beta$  selective ligand, 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3beta-adiol), can suppress EMT that was induced by TGF $\beta$  or hypoxia treatment to maintain an epithelial phenotype, and repress mesenchymal characteristics in prostate carcinoma [48]. Cheng *et al.* introduced ER $\beta$  expression in pros-

tatic carcinoma cells and observed ER $\beta$  plays a tumor-suppressor role by its anti-proliferative, anti-invasive and pro-apoptotic properties [49]. For ER $\alpha$ , there was no study to address the epithelial ER $\alpha$  roles in PCa invasion. Recently, there is a study delineating the role of CAF ER $\alpha$  and found that it could protect against PCa invasion. Immunohistochemistry on prostatectomy specimens showed that PCa patients with ER $\alpha$ -positive stroma had a significantly lower risk for biochemical recurrence. The results from matrigel invasion assay confirmed that the stromal ER $\alpha$  was able to reduce PCa cell invasion. Molecular mechanism dissection revealed that the prostate cancer stromal ER $\alpha$  could function through a CAF-epithelial interaction to inhibit PCa invasion via selectively up-regulating thrombospondin 2 (Thbs2) and downregulating matrix metalloproteinase 3 (MMP3) at the protein and messenger RNA levels. Overall, the study provided multiple evidences with clinical specimen analysis and mouse PCa model studies as well as *in vitro* cell study results demonstrating that stromal ER $\alpha$  could play protective roles for PCa progression [50].

### *ERs' roles in prostate cancer inflammation*

A previous study observed estrogen and ER $\alpha$  played roles in prostatic chronic inflammation. Yatkin *et al.* reported that the reduced androgen to oestrogen ratio may promote inflammation in rat prostate [51]. Their data suggested that higher androgen concentrations are required for preventing proinflammatory and epithelial responses to estrogen in the presence of elevated estrogen concentrations. Harris [52] showed that after 4 days of estrogen treatment, IL-1 $\beta$ , IL-6, MIP-2, and inducible nitric oxide synthase (iNOS) increased in a mouse model. Bianco *et al.* [53] found ArKO mice, which lack the capability of estrogen synthesis, developed epithelial hyperplasia and inflammation following long-term treatment with estrogen as compared to vehicle treatment. Prins *et al.* [54] demonstrated that estrogen-induced inflammation is specifically mediated by ER $\alpha$ . After DES treatment, the epithelial inflammatory cell infiltration was observed with aging in WT and in ER $\beta$ KO mice, but not in ER $\alpha$ KO mice. Ravenna's study analyzed the relationship between the mRNA expression levels of ER $\alpha$ , EGFR, and ER $\beta$  to pro-inflammatory

genes in human clinical samples, and data showed ER $\alpha$  and EGFR, but not ER $\beta$ , mRNA levels correlate with the expression of all pro-inflammatory molecules in all samples [55].

Together, the above reports consistently showed that ER $\alpha$ , but not ER $\beta$ , is involved in the PCa inflammation.

### **Summary**

Increasing evidences demonstrate estrogens are required for prostate development and carcinogenesis. Activated ER $\alpha$  could decrease the androgen production, which in turn contributes significantly to the genesis of benign prostatic hyperplasia and prostate dysplasia. Multiple reports consistently showed that ER $\beta$  could play protective roles in PCa development, yet ER $\alpha$  roles in PCa remain controversial. In addition to epithelial ER roles, a recent study showed that stromal ER $\alpha$  could play protective roles to reduce PCa invasion. SERMs could potentially be applied in combination with current therapies to treat PCa. As the differential roles of ER $\alpha$  and ER $\beta$  are elucidated in prostate progression, the synthetic and natural modulators of ER action may exert a protective activity or become potential therapies against the progression of PCa.

### **Disclosure of conflict of interest**

The authors have nothing to disclose.

### **Abbreviations**

ArKO, aromatase knockout; AR, androgen receptor; E2, 17  $\beta$ -estradiol; ER $\alpha$ , estrogen receptor alpha; ER $\alpha$ KO, ER $\alpha$  knockout mice; ER $\beta$ , estrogen receptor beta; IHC, immunohistochemical staining; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; PCa, prostate cancer; SERM, selective estrogen modulator; T, testosterone; WT, wild type.

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