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Role of estrogens in development of prostate cancer

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Abstract

Estrogens have previously been extensively used in prostate cancer treatment. Serious side effects, primarily in cardiovascular system have, however, limited their use. The therapeutic effect of estrogen in preventing prostate cancer growth was mainly obtained indirectly by feedback inhibition of the hypothalamic release of LRH leading to lowered serum androgen levels and castration like effects.

Prostate tissue is also most probably a target for direct regulation by estrogens. Prostate contains estrogen receptor α (ER α) and β (ER β), which are localized characteristically in stroma and epithelium, respectively. The physiological function of these receptors is not known but there is evidence of the role of estrogens in prostatic carcinogenesis. Developing prostate seems particularly sensitive to increased level of endogenous and/or exogenous estrogens. Perinatal or neonatal exposure of rats and mice to estrogens leads to “imprinting” of prostate associated with increased proliferation, inflammation and dysplastic epithelial changes later in life. Prolonged treatment of adult rodents with estrogens along with androgens also leads to epithelial metaplasia, PIN-like lesions and even adenocarcinoma of prostate speaking for the role of estrogen in prostate cancer development. Recent results concerning antiestrogen inhibition of prostate cancer development beyond PIN-type lesions in transgenic mouse models further suggests a role for estrogens in prostate cancer progression. These results also suggest that direct inhibition of estrogen action at the level of prostate tissue may provide an important novel principle of development of prostate cancer therapies.

1. Introduction

Estrogens regulate the development and function of prostate at several stages of a man's life by indirect and direct mechanisms. Prostate growth, differentiation and function are primarily controlled by androgens but estrogens modulate these effects in several ways. The most important routes of indirect estrogen regulation are interference of androgen production by repression of the hypothalamic-pituitary-gonadal axis and direct effects on testis. Another important, indirect route for estrogen regulation of prostate is via prolactin. Estrogens also clearly have direct effects on prostate, which may be elicited by external hormone or by estradiol produced by local aromatisation of testosterone. It has been very difficult, however, to work out the mechanisms and the physiological importance of these effects.

Estrogen regulation has also been considered as one of hormonal risk factors in association of development of benign prostatic hyperplasia and prostate cancer [1,2,3]. The evidence for the possible role of estrogen in prostate cancer has come from epidemiological and experimental studies. First, the epidemiological evidence includes a strong correlation of prostate cancer incidence with geographical location, which may be associated with dietary factors among other environmental influences and their endocrine consequences. The role of phytoestrogens and other dietary and environmental estrogens have particularly been considered [3]. The correlation of the levels of serum estrogen or estrogenic compounds with the prostate cancer incidence in various population groups has not, however, provided any conclusive evidence of the role of estrogens in explaining the differences in prostate cancer incidence in those groups [4]. Second, the serum estrogens are known to increase and androgens decrease upon aging, which changes precede or coincide with the increasing incidence of prostate cancer. The altered ratio of serum androgens to estrogens and progression of prostate cancer have been suggested to have a causal relationship with each other. However, despite extensive studies, there is no conclusive clinical evidence of a strong correlation between elevated serum estrogen or estrogen/androgen ratio and the increased incidence of prostate cancer [5,6]. Third, there is, however, strong

experimental evidence that shows that excessive or untimely exposure to estrogens can facilitate development of prostatic changes, disorders and even malignancies [7]. These results open up a possibility for direct or indirect effects of locally produced estrogen [8].

The indirect estrogen effects were exploited for a long time as a therapy of prostate cancer [3,9]. Estrogen treatment was based on the primary observations of Huggins and Hodges [10] on estrogen regulation of prostate cancer [10,11]. As suggested in these original papers, the therapeutic effect of estrogen is primarily mediated by suppression of the hypothalamic-pituitary-gonadal axis and by direct effects on the Leydig cells in testis, which together lead to decreased serum levels of testosterone and castration like effects in prostate. In these studies estrogen was also suggested to have direct (then considered “toxic”) effects on prostate [11].

Estrogen treatment was an effective form of therapy of advanced prostate cancer but unfortunately, it was associated with a high risk of serious cardiovascular complications, which eventually strongly limited its clinical use [9].

2. Biological effects of estrogens on prostate

2.1 In vitro effects of estrogen

The evidence for direct prostatic effects of estrogen comes mainly from studies with organ cultures of normal rat or human prostate or samples of human prostate cancer. In those studies estrogen was found to stimulate DNA synthesis and induce metaplastic epithelial morphology both in human and rat prostate [12,13]. Estrogen also regulated expression of prostate specific genes [12,14]. When estrogen was combined with androgen, the effects on DNA synthesis and specific gene expression were mainly synergistic but, interestingly, the induction of metaplastic changes was prevented [12,13] and the organized epithelial morphology largely maintained in the in vitro cultures.

2.2. Neonatal imprinting of prostate by estrogen treatment

Developing prostate seems to be particularly sensitive to the effects of estrogen. Prenatal or neonatal treatment of rodents with high doses of estrogen during the first 5 days after the birth, when tubular branching morphogenesis of prostatic organogenesis occurs leads to marked changes in prostate development and function later in life. This is invariably associated with hyperprolactinemia and hypoandrogenemia, which may explain a major part of the effects. A good example of permanent alterations induced by an early estrogen exposure is the rodent neonatal estrogenization model [15]. Neonatal estrogenization with estradiol or diethylstilbestrol (DES) results in time-of-exposure and dose-dependent inhibition of prostatic growth and function in mice and rats [16-19]. Besides growth inhibition, neonatal estrogenization promotes inflammation, epithelial hyperplasia and development of dysplastic lesions, which histologically resemble PIN-lesions [19,20]. Furthermore, the expression of several important growth-regulatory genes is altered. This includes upregulation of ER α and estrogen-responsive proto-oncogenes *c-myc* and *c-fos* [20-22] and downregulation of AR and ER β [23,24].

Experiments with ER-deficient mouse models indicate that the effects induced by neonatal exposure to high doses of estrogen are largely mediated through stromal ER α . Neonatal exposure to DES fails to induce epithelial dysplasia in ER α -deficient (ERKO) mice, but not in ER β -deficient (BERKO) mice [25]. Tissue recombinant studies further emphasize the central role of stromal microenvironment and, in particular the importance of the expression of stromal ERs and AR in estrogen-androgen-induced prostatic carcinogenesis [26].

Between the years 1940 and 1971, millions of women were prescribed DES during pregnancy. Consequently, there are millions of women and men who were exposed to DES prenatally (DES daughters and DES sons). The long-term adverse consequences in DES daughters have been well documented, but only few studies have addressed the possible long-term health effects in DES sons. With regard to cancer risk, there is no conclusive evidence for elevated risk at any organ site. Most studies have focused on

testicular cancer, which, based on rodent studies would be one of the likely effects, but the association between prenatal DES exposure and testicular cancer in humans remains controversial [27,28]. No studies on the prostate cancer risk in DES sons have been published to date, and it is not known if the effects observed in rodent models are relevant for humans. However, it should be taken into account that prostate cancer is typically diagnosed later in life, and the DES sons are now only approaching this age.

2.3. Estrogen-induced prostate dysplasia and malignancy in rodents

Prolonged treatment of rodents with a high dose of androgen and estrogen together induces growth of prostate, epithelial metaplasia, stromal hypertrophy and a strong inflammatory reaction in stroma [29-32]. These effects may be largely caused by associated hyperprolactinemia since most of them are inhibited with bromocriptine [33-35]. However, high serum levels of estrogen and prolactin associated with very low testosterone levels in aromatase overexpressing transgenic mice (AROM+) [36] or in estrogen-treated hypogonadal (hpg) mice [37] did not induce prostatic hyperplasia emphasising the need for a combined action of androgen and estrogen at least at some stage of prostatic development. In certain rat strains prostatic adenocarcinoma can be induced by a prolonged estrogen-androgen exposure, or with aromatizable androgen alone, but not with non-aromatizable androgen. These results imply that at least some prostatic effects of estrogen can be obtained by targeted aromatization of testosterone to estradiol [8] and further support the synergistic function of estrogens and androgens in prostatic carcinogenesis [38-40].

Lack of production of endogenous estrogen may also lead to development of prostatic enlargement in mice, as has been demonstrated in aromatase knockout (ArKO) mouse model [41,8]. With age, ArKO mice develop hyperplasia of epithelial, interstitial and luminal compartments, as well as up-regulation of androgen receptors. ArKO mice have significantly elevated serum androgen and PRL concentration, which may explain the enhanced prostatic growth. Interestingly, no induction of malignancy has been observed

in ArKO mice, which again speaks for the critical role of estrogens in combination with androgen and possibly prolactin in the development of prostate cancer.

2.4 Role of prolactin in mediation of estrogen effects

Besides androgens, prostatic actions of estrogens are closely associated with those of prolactin [42]. Either pituitary-derived prolactin or prostate epithelium-produced endogenous prolactin [43] regulates prostate by stimulating its secretory activity, proliferation and survival [12,13,23,44-47]. Excessive prolactin stimulation induces hypertrophic changes in prostatic epithelium and stromal compartment in vitro in organ cultures [12,13] and in vivo in prolactin transgenic mice [48]. Similar results were also recently obtained with prostate-targeted prolactin transgenic mice [49]. Furthermore, molecular mimics able to block autocrine functions of prolactin reduced tumor formation and growth in nude mice [50]. These results together demonstrate that local endogenous prolactin is functionally important.

In rodents, estrogen triggers prostatic effects, which are very similar to those of prolactin [29,31]. Neonatal estrogenisation and prolonged treatments with androgen and/or estrogen are associated with periodical or persistent hyperprolactinemia, which clearly mediates some of estrogen effects on prostate [31,33]. Such are, for example development of dysplastic changes and accumulation of inflammatory and immune cells in prostatic stroma. Treatment with bromocriptine, an antagonist of hypothalamic L-dopa inhibiting prolactin release from pituitary, is able to prevent most although not all of estrogen effects [33,51,35].

In prostate epithelial cells estrogen increases the level of prolactin receptors [52]. It is not known whether estrogen regulates endogenous expression of prolactin by prostatic epithelial cells but in prostate-targeted prolactin transgenic mice the proportion of ER α expressing stromal cells is clearly increased and ER α is even expressed in some epithelial cells [49] resembling the situation in estrogen-induced squamous metaplasia

changes of epithelium (Fig. 1). It is thus possible that prolactin mediates estrogen effects in prostate not only at the systemic but also at the cellular level as an important mechanism in estrogen-facilitated development of dysplastic changes in prostate [42].

3. Mechanisms of estrogen effect in prostate

3.1 Estrogen receptors in normal and cancerous human prostate

The presence of estrogen receptors in prostate suggests that estrogens may act directly on multiple sites in prostate. Interestingly, the two ER subtypes have very different expression patterns (Fig. 1). In human and rodent prostate, ER β is the predominant ER subtype, expressed in the majority of the epithelial cells, as well as in some stromal cells (Fig. 1 H-N). ER α is expressed in a more limited manner (Fig. 1A-G), and typically found in stromal cells only (Fig. 1B). However, ER α -positive cells are found in hyperplastic or metaplastic epithelium in the prostatic ducts and posterior periurethral region (Fig. 1 C and J), but the functional relevance of this observation is still unclear. Based on the differential expression patterns of ER α and ER β , it would be tempting to suggest specific roles for the two ER subtypes in the regulation of prostate growth and function in humans. However, at present, no such evidence exists, and the physiological roles of ER subtypes remain to be clarified.

ER β is commonly expressed also in prostate cancer (an example in Fig. 1 M, N and [53-56]) while ER α has been reported only in some studies [57]. Generally, the levels of ER α and ER β in human prostate and prostate cancer seem to be very low at any stage of the disease when compared to breast cancer for example [56]. After the discovery of ER β , there has been, however, considerable interest in its role during prostatic carcinogenesis and also in a possibility of using it as a prognostic marker but the results obtained have been rather conflicting. The studies by Horvath et al. [58] and Leav et al. [59] demonstrated reduction in ER β expression during carcinogenesis, suggesting that ER β might be important for the maintenance of normal prostate epithelium, but it is not

yet known if reduction in ER β expression is causally related to the development of neoplasia. The study by Horwath et al. [58] suggested that cancers retaining ER β expression are associated with a higher rate of recurrence, and Leav et al. [59] reported that ER β is expressed in prostate cancer metastases, which could indicate ER β as a marker of highly malignant phenotype.

It is well known that multiple variant forms of ER β are expressed in normal and cancerous tissues. The variant forms may functionally differ significantly from the “wild type” receptor. One such variant is ER β cx (also called ER β 2), which has structurally different ligand binding domain. It shows very poor ligand binding affinity to known estrogenic ligands, and has been suggested to act as an inhibitor of ER α action [60]. Interestingly, Fujimura et al. [61] reported that expression of wild type (wt) ER β is lower in cancers than in the benign epithelium and inversely correlated with Gleason tumor grade. They also found that low wtER β and high ER β cx expression are associated with poor cancer-specific survival. These results would suggest a shift from wtER β to ER β cx during carcinogenesis, which would be consistent with the results of Horwath et al. [58] and Leav et al. [59] that did not differentiate between wtER β and ER β cx.

Altogether, no clear association between ER β expression and tumor grade or prognosis has been demonstrated so far as also concluded on the basis of recent studies using quantitative real-time RT-PCR [56].

3.2 ER-deficient mouse models

As expected, mice lacking functional ERs develop prostatic phenotypes (recently reviewed by Jarred et al. [62]). Obviously, because mice with prostate-targeted inactivation of ERs are not yet available, the phenotype is not necessarily caused solely by the lack of the receptor in prostatic tissue, but may also be secondary to the altered CNS-gonadal function. An example of prostate phenotype that is likely to be secondary to altered CNS-gonadal function has been observed in ER α KO (ER α -deficient) mouse. Couse and Korach [63] reported that ER α KOs develop enlarged prostates and seminal

vesicles upon aging, without any pathological changes in the prostatic histology [25,64,65]. Serum testosterone levels in ER α KO males are twofold compared to WT littermates [66,67], and this could well explain the prostatic enlargement. Those mice do have, however, also increased serum estrogen levels.

On the other hand, the lack of functional ER α has also direct effects on prostate. It is well known that estrogen treatment induces development of squamous epithelial metaplasia in prostate, in particular at sites where the underlying stroma expresses ER α . This does not occur in ER α KO mouse, indicating that this specific response is ER α -dependent [65,62] and likely to be mediated by prostatic ER α . Squamous epithelial metaplasia has also been observed in human prostate, particularly in the posterior periurethral region (Fig. 1 D), both in fetal prostate [68] (possibly as a response to maternal estrogens), and in aging prostate [69-73]. Interestingly, human prostatic epithelium that is normally devoid of ER α (as in Fig. 1A), may express ER α (as in Fig. 1A), in ducts showing basal cell hyperplasia (as shown in Fig. 1 C), as well as in the metaplastic epithelium in the posterior periurethral region (Fig. 1 D and E) (also demonstrated by Adams et al. [68]). In addition, the epithelium in this site shows high expression of 17 β -HSD type 1, the enzyme responsible for the conversion of estrone to estradiol [74]. Furthermore, a recent report also suggests an altered tissue localization of aromatase in prostate cancer compared to normal prostate [75]. These results further support the idea of regional differences in both estrogen production and responsiveness in prostate. Whether this relates in any way to the differences in the regional differences in the origin of prostate cancer remains to be explored.

Lack of functional ER β may result in a very different prostatic phenotype. ER β is normally expressed in the majority of the epithelial cells in rodent ventral prostate [76] but the specific functions of ER β in prostate epithelium are not yet known. Weihua et al. [77] reported that aged ER β KO mice display hyperplastic foci and increased expression of proliferation marker Ki-67 in the ventral prostate. In contrast, in another ER β -deficient

mouse line, generated by Chambon and coworkers [78], no prostatic abnormalities were observed. At present, there is no explanation to this discrepancy.

4. Exploiting the pathways of estrogenic effects in inhibition of prostate cancer

4.1. Antiestrogen/SERM inhibition of progression of prostate cancer

The results on estrogen stimulation of prostatic proliferation, induction of prostatic dysplastic changes by neonatal estrogenisation or by a long-term treatment with estrogen in combination of androgen have led to several studies on the possibilities of inhibiting development and growth of prostatic malignancies by blocking estrogen action with antiestrogens [7]. Earlier studies with experimental models such as R3327 Dunning rat prostate adenocarcinoma [79] and Nb-2Pr-A tumor transplants in Noble rats [80] demonstrated partial growth regression by tamoxifen treatment. The clinical trials with a high-dose treatment with tamoxifen [81] or toremifene [82] were, however, disappointing. Although occasional positive effects were obtained the overall conclusion was that these antiestrogens used in breast cancer treatment did not produce any objective responses in advanced hormone-refractory prostate cancer.

Recent studies have, however, provided interesting new data that antiestrogens may effectively inhibit development and progression of experimental and even clinical prostate cancer. In a TRAMP transgenic mouse model [83] toremifene decreased development of prostate tumors [84]. Even more interesting was that toremifene treatment also seemed to slow down and inhibit the appearance of high-grade prostatic intraepithelial neoplasia (PIN) lesions, which were found in placebo-treated transgenic mice. The possible mechanism was clearly different from indirect inhibition of androgen pathways. The level of serum estrogen was not essentially changed. These interesting results have led to clinical trials on the efficacy and safety of toremifene treatment of men with high-grade prostatic intraepithelial neoplasia [85,86]. As expected on the basis of past experience of using toremifene in treatment of breast cancer patients [87,88], the

drug is well tolerated also in men [86]. The real clinical significance of these promising proof-of-concept type of observations in prevention of prostate cancer is now being investigated in a placebo-controlled, randomised long-term study [85].

Other antiestrogens such as trioxifene and raloxifene also retard growth or progression of prostate cancer in experimental models. Both decreased the metastatic spreading of PAIII prostatic rat adenocarcinoma from primary tumors in Lobund-Wistar rats and increased the survival of the animals [89,90]. Raloxifene was also shown to induce apoptosis of both androgen dependent and independent prostate cancer cell lines in vitro [91].

The mechanisms of action of the antiestrogens in prostate cancer progression and metastasis remain to be studied. Toremifene is a chlorinated triphenylethylene derivative of tamoxifen, which is used in the treatment of patients with metastatic breast cancer [88]. It is a selective estrogen receptor modulator, which binds both estrogen receptor α and β and elicits antagonist effects at least in mammary gland and breast cancer cells and agonist effects in uterus, bone and liver [92]. Toremifene inhibits DMBA-induced tumorigenesis in mice and rats [87] inhibiting cell proliferation and inducing apoptosis [93]. The profile of prostatic actions of toremifene is not known. Based on the results concerning the requirement of stromal ER α for estrogen treatment-induced development of prostatic dysplasia or cancer one could hypothesise that it is this receptor isoform, which most probably mediates toremifene inhibition of development of high-grade PIN lesions and adenocarcinoma in TRAMP mice. Presently, it is not known, however, which are the gene targets and possible autocrine/paracrine mediators of this SERM action in prostate. Thus, it is not known, either, to which extent the tumor inhibitory effects of toremifene reflect its ability to counteract estrogen effects or its own characteristic SERM effects in prostate [92]. The analysis of the prostatic effects of recently reported ER α and ER β specific ligands [94,95] and estrogen and toremifene target genes in the prostate of mice deficient in ER α or ER β will be of great interest. Those studies will

certainly provide information of the prostatic functions of ER α and ER β and may also help in designing novel, prostate specific SERMs [96,97].

4.2. Role of phytoestrogens in the prevention of prostate cancer

Geographic differences in prostate cancer risk suggest that environmental factors may play a role in prostate carcinogenesis. More specifically, it has been suggested that dietary phytoestrogens, acting as “phytoSERMs” would, at least partly, account for the difference. Epidemiological studies show that phytoestrogen consumption and/or serum phytoestrogen concentrations are inversely correlated with prostate cancer risk [98,99]. Experimental studies support the idea of phytoestrogens as cancer protective agents: genistein (an isoflavonoid phytoestrogen) suppresses chemically induced prostate cancer [100] and reduces the incidence of poorly differentiated adenocarcinomas in TRAMP mouse model [101]. Furthermore, diet rich in isoflavonoid phytoestrogens inhibits or delays the development of DES-induced dysplastic lesions [102]. However, the beneficial effect of phytoestrogens in the prevention or treatment of human prostate cancer remains to be demonstrated. Only a very limited number of clinical trials has been carried out, suggesting a moderate favorable effect, but only in a small number of individuals [103,104].

5. Conclusive remarks

Experimental studies on prostate suggest that although inappropriate exposure to estrogens during the critical phase of differentiation and prostatic organogenesis or later in life may predispose prostate to development of abnormal changes estrogen may also have a role in progression of premalignant lesions to prostate cancer. Other results suggest that inhibition of pathways of estrogen effects may prevent this development, which opens up new possibilities of exploiting estrogen regulatory mechanisms in prostate along with other hormonal effectors, for therapeutic prevention of prostatic carcinogenesis and progression. To this end, information of prostate-specific

mechanisms, signaling mediators and target genes of estrogen receptors would be urgently required.

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7. References

- [1] M.C. Bosland, The role of steroid hormones in prostate carcinogenesis, *J. Natl. Cancer Inst. Monogr.* 27 (2000) 39-66.
- [2] B.E. Henderson, H.S. Feigelson, Hormonal carcinogenesis, *Carcinogenesis* 21 (2000) 427-433.
- [3] K. Griffiths, Estrogens and prostatic cancer. International Prostate Health Council Study Group, *Prostate* 45 (2000) 87-100.
- [4] F.H. de Jong, K. Oishi, R.B. Hayes, J.F. Bogdanowicz, J.W. Raatgever, P.J. van der Maas, O. Yoshida, F.H. Schroeder, Peripheral hormone levels in controls and patients with prostatic cancer or benign prostatic hyperplasia: results from the Dutch-Japanese case-control study, *Cancer Res.* 51 (13) (1991) 3445-3450.
- [5] P.H. Gann, C.H. Hennekens, J. Ma, C. Longcope, M.J. Stampfer, Prospective study of sex hormone levels and risk of prostate cancer, *J. Natl. Cancer Inst.* 88 (1996) 1118-1126.
- [6] N.E. Eaton, G.K. Reeves, P.N. Appleby, T.J. Key, Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies, *Brit. J. Cancer* 80 (1999) 930-934.
- [7] S.M. Ho, Estrogens and anti-estrogens: Key mediators of prostate carcinogenesis and new therapeutic candidates, *J. Cell. Biochem.* 91 (3) (2004) 491-503.
- [8] G.P. Risbridger, J.J. Bianco, S.J. Ellem, S.J. McPherson, Oestrogens and prostate cancer, *Endocr-Relat. Cancer* 10 (2003) 187-191.

- [9] R.L. Cox, E.D. Crawford, Estrogens in the treatment of prostate cancer, *J. Urology* 154 (1995) 1991-1998.
- [10] C. Huggins, C.V. Hodges, Studies on prostate cancer: effect of castration, of estrogen, and of androgen injection on serum phosphatase in metastatic carcinoma of the prostate, *Cancer Res.* 1 (1941) 293-297.
- [11] C. Huggins, W.W. Scott, C.V. Hodges, Studies on prostatic cancer III. The effects of fever, of desoxycorticosterone and of estrogen on clinical patients with metastatic carcinoma of the prostate, *J. Urol.* 46 (1941) 997
- [12] M.T. Nevalainen, E.M. Valve, S.I. Mäkelä, M. Bläuer, P.J. Tuohimaa, P.L. Härkönen, Estrogen and prolactin regulation of rat dorsal and lateral prostate in organ culture, *Endocrinology* 129 (1991) 612-622.
- [13] M.T. Nevalainen, P.L. Härkönen, E.M. Valve, P. Wu, M. Nurmi, P.M. Martikainen, Hormone regulation of human prostate in organ culture, *Cancer Res.* 53 (1993) 5199-5207.
- [14] P. Martikainen, P. Härkönen, T. Vanhala, S. Mäkelä, M. Viljanen, J. Suominen, Multihormonal control of synthesis and secretion of prostatein in cultured rat ventral prostate, *Endocrinology* 121 (1987) 604-611.
- [15] R. Santti, R.R. Newbold, S. Mäkelä, L. Pylkkänen, J.A. McLachlan, Developmental estrogenization and prostatic neoplasia, *Prostate* 24 (2) (1994) 67-78.
- [16] M.J. Naslund, D.S. Coffey, The differential effects of neonatal androgen, estrogen and progesterone on adult rat prostate growth, *J. Urol.* 136 (5) (1986) 1136-40.
- [17] G.S. Prins, Neonatal estrogen exposure induces lobe-specific alterations in adult rat prostate androgen receptors expression, *Endocrinology* 130 (1992) 3703-14.
- [18] G.S. Prins, C. Woodham, M. Lepinske, L. Birch, Effects of neonatal estrogen exposure on prostatic secretory genes and their correlation with androgen receptor expression in the separate prostate lobes of the adult rat, *Endocrinology* 132 (6) (1993) 2387-2398.
- [19] L. Pylkkänen, R. Santti, R. Newbold, J.A. McLachlan, Regional differences in the prostate of the neonatally estrogenized mouse, *Prostate* 18 (2) (1991) 117-129.
- [20] G.S. Prins, L. Birch, Neonatal estrogen exposure up-regulates estrogen receptor expression in the developing and adult rat prostate lobes, *Endocrinology* 138 (5) (1997) 1801-1809.
- [21] L. Pylkkänen, S. Mäkelä, E. Valve, P. Härkönen, S. Toikkanen, R. Santti, Prostatic dysplasia associated with increased expression of c-myc in neonatally estrogenized

mice, *J. Urol.* 149 (6) (1993) 1593-1601.

- [22] L. Salo, S.I. Mäkelä, G.M. Stancel, R.S. Santti, Neonatal exposure to diethylstilbestrol permanently alter the basal and 17 beta-estradiol induced expression of c-fos proto-oncogene in mouse urethrostatic complex, *Mol. Cell. Endocrinol.* 126 (1997) 133-141.
- [23] G.S. Prins, L. Birch, The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen, *Endocrinology* 136 (3) (1995) 1303-1314.
- [24] G.S. Prins, M. Marmer, C. Woodham, W. Chang, G. Kuiper, J.-Å. Gustafsson, L. Birch, Estrogen receptor- beta messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats, *Endocrinology* 139 (3) (1998) 874-883.
- [25] G.S. Prins, L. Birch, J.F. Couse, C. Inho, B. Katzenellenbogen, K.S. Korach, Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor α : Studies with α ERKO and β ERKO Mice, *Cancer Res.* 61 (2001) 6089-6097.
- [26] G.R. Cunha, S.W. Hayward, Y.Z. Wang, W.A. Riche, Role of the stromal microenvironment in carcinogenesis of the prostate, *Int. J. Cancer* 107 (2003) 1-10.
- [27] M. Giusti, M. Giovale, P. Sessarego, A. Carraro, A. Pompei, G. Giordano, Cholinergic modulation of growth hormone, prolactin and thyroid stimulating hormone responses to thyrotropin-releasing hormone in normal aging, *Recent Prog. Med.* 86 (9) (1995) 341-344.
- [28] W.C. Strohsnitter, K.L. Noller, R.N. Hoover, S.J. Robboy, J.R. Palmer, L. Titus-Ernstoff, R.H. Kaufman, E. Adam, A.L. Herbst, E.E. Hatch, Cancer risk in men exposed in utero to diethylstilbestrol, *J. Natl. Cancer Inst.* 93 (7) (2001) 545-551.
- [29] L.E. Tisell, The growth of the ventral prostate, the dorsolateral prostate, the coagulating glands and the seminal vesicles in castrated, adrenalectomized rats injected with oestradiol and/or cortisone. *Acta Endocrinol (Copenh)* 68 (1971) 485-501.
- [30] H. Andersson, L.E. Tisell, Morphology of rat prostatic lobes and seminal vesicles after long-term estrogen treatment, *Acta Pathol. Microbiol. Immunol. Scand. [A]* 90 (6) (1982) 441-8.
- [31] J.A. Belis, L.B. Adlerstein, W.F. Tarry, Influence of estradiol on accessory reproductive organs in the castrated male rats. Effects of bromocriptine and flutamide. *J Androl* 4 (1983) 144-49.

- [32] G. Risbridger, H. Wang, M. Frydenberg, G.R. Cunha, The metaplastic effects of estrogen on prostate epithelium: proliferation of cells with basal cell phenotype, *Endocrinology* 142 (2001) 2443-2450.
- [33] K.E. Lane, I. Leav, J. Ziar, R.S. Bridges, W.M. Rand, S.M. Ho, Suppression of testosterone and estradiol-17 β -induced dysplasia in the dorsolateral prostate of noble rats by bromocriptine, *Carcinogenesis* 18 (1997) 1505-1510.
- [34] C.J. Thompson, N.N. Tam, J.M. Joyce, I. Leav, S.M. Ho, Gene expression profiling of testosterone and estradiol-17 β -induced prostatic dysplasia in Noble rats and response to the antiestrogen ICI 182,780, *Endocrinology* 143 (2002) 2093-2105.
- [35] J.P. Gilleran, O. Putz, M. DeJong, S. DeJong, L. Birch, Y. Pu, L. Huang, G.S. Prins, The role of prolactin in the prostatic inflammatory response to neonatal estrogen, *Endocrinology* 144 (5) (2003) 2046-2054.
- [36] X. Li, E. Nokkala, W. Yan, T. Streng, N. Saarinen, A. Wrri, I. Huhtaniemi, R. Santti, S. Mkel, M. Poutanen, Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase, *Endocrinology* 142 (2001) 2435-2442.
- [37] J.J. Bianco, D.J. Handelsman, J.S. Pedersen, G.P. Risbridger, Direct response of the murine prostate gland and seminal vesicles to estradiol, *Endocrinology* 143 (2002) 4922-33.
- [38] R.L. Noble, L. Hoover, A classification of transplantable tumors in Nb rats controlled by estrogen from dormancy to autonomy, *Cancer Res.* 35 (11 Pt 1) (1975) 2935-2941.
- [39] R.L. Noble, The development of prostatic adenocarcinoma in Nb rats following prolonged sex hormone administration, *Cancer Res.* 37(6) (1977) 1929-1933.
- [40] Leav, F.B. Merk, P.W. Kwan, S.M. Ho, Androgen-supported estrogen-enhanced epithelial proliferation in the prostates of intact Noble rats, *Prostate* 15 (1989) 23-40.
- [41] S.J. McPherson, H. Wang, M.E. Jones, J. Pedersen, T.P. Iismaa, N. Wreford, E.R. Simpson, G.P. Risbridger, Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland, *Endocrinology* 142 (2001) 2458-2467.
- [42] P. Hrknen, Paracrine prolactin may cause prostatic problems, *Endocrinology* 144 (2003) 2266-2268.

- [43] M.T. Nevalainen, E.M. Valve, P.M. Ingleton, M. Nurmi, P.M. Martikainen, P.L. Härkönen, Prolactin and prolactin receptors are expressed and functioning in human prostate, *J. Clin. Invest.* 99 (1997) 618-627.
- [44] J.T. Grayhack, Pituitary factors influencing growth of the prostate, *Natl. Cancer Inst. Monogr.* (1963) 189-199.
- [45] T. Ahonen, P.L. Härkönen, J. Laine, H. Rui, P.M. Martikainen, M.T. Nevalainen, Prolactin is a survival factor for androgen-deprived rat dorsal and lateral prostate epithelium in organ culture, *Endocrinology* 140 (1999) 5412-21.
- [46] E. Reiter, B. Hennuy, M. Bruyninx, A. Cornet, M. Klug, M. McNamara, J. Closset, G. Hennen, Effects of pituitary hormones on the prostate, *Prostate* 38 (1999) 159-165.
- [47] Ruffion, K.A. Al-Sakkaf, B.L. Brown, C.L. Eaton, F.C. Hamdy, P.R. Dobson, The survival effect of prolactin on PC3 prostate cancer cells, *Eur. Urol.* 43 (2003) 301-308.
- [48] H. Wennbo, J. Kindblom, O.G. Isaksson, J. Törnell, Transgenic mice overexpressing the prolactin gene develop dramatic enlargement of the prostate gland, *Endocrinology* 138 (1997) 4410-4415.
- [49] J. Kindblom, K. Dillner, L. Sahlin, F. Robertson, C. Ormandy, J. Tornell, H. Wennbo, Prostate hyperplasia in a transgenic mouse with prostate-specific expression of prolactin, *Endocrinology* 144 (6) (2003) 2269-2278.
- [50] X. Xu, E. Kreye, C.B. Kuo, A.M. Walker, A molecular mimic of phosphorylated prolactin markedly reduced tumor incidence and size when DU145 human prostate cancer cells were grown in nude mice, *Cancer Res.* 61 (2001) 6098-6104.
- [51] T.E. Stoker, C.L. Robinette, B.H. Britt, S.C. Laws, R.L. Cooper, Prepubertal exposure to compounds that increase prolactin secretion in the male rat: effects on the adult prostate, *Biol. Reprod.* 61 (1999) 1636-1643.
- [52] M.T. Nevalainen, E.M. Valve, P.M. Ingleton, P.L. Härkönen, Expression and hormone regulation of prolactin receptors in rat dorsal and lateral prostate, *Endocrinology* 137 (1996) 3078-3088.
- [53] T. Ito, M. Tachibana, S. Yamamoto, J. Nakashima, M. Murai, Expression of estrogen receptor (ER-alpha and ER-beta) mRNA in human prostate cancer, *Eur. Urol.* 40 (5) (2001) 557-563.
- [54] M. Royela, M.P. de Miguel, F.R. Bethencourt, M. Sanchez-Chapado, B. Fraile, M.I. Arenas, R. Paniagua, Estrogen receptors alpha and beta in the normal, hyperplastic and carcinomatous human prostate, *J. Endocrinol.* 168 (2001) 447-

- [55] T. Fixemer, K. Remberger, H. Bonkhoff, Differential expression of the estrogen receptor beta (ER β) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma, *Prostate* 54 (2003) 79-87.
- [56] M.J. Linja, K.J. Savinainen, T.L.J. Tammela, J.J. Isola, T. Visakorpi, Expression of ER α and ER β in prostate cancer, *Prostate* 55 (2003) 180-186.
- [57] H. Bonkhoff, T. Fixemer, I. Hunsicker, K. Remberger, Estrogen receptor expression in prostate cancer and premalignant prostatic lesions, *Am. J. Pathol.* 155 (2) (1999) 641-647.
- [58] L.G. Horvath, S.M. Henshall, C.S. Lee, D.R. Head, D.I. Quinn, S. Mäkelä, W. Delprado, D. Golovsky, P.C. Brenner, G. O'Neill, R. Kooner, P.D. Stricker, J.J. Grygiel, J.-Å. Gustafsson, R.L. Sutherland, Frequent loss of estrogen receptor-beta expression in prostate cancer, *Cancer Res.* 61 (14) (2001) 5331-5335.
- [59] Leav, K.M. Lau, J.Y. Adams, J.E. McNeal, M.E. Taplin, J. Wang, H. Singh, S.M. Ho, 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.* 159 (1) (2001) 79-92.
- [60] S. Ogawa, S. Inoue, T. Watanabe, A. Orimo, T. Hosoi, Y. Ouchi, M. Muramatsu, Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor of estrogen action in human, *Nucleic Acids Res.* 26 (1998) 3505-12.
- [61] T. Fujimura, S. Takahashi, T. Urano, S. Ogawa, Y. Ouchi, T. Kitamura, M. Muramatsu, S. Inoue, Differential expression of estrogen receptor beta (ERbeta) and its C-terminal truncated splice variant ERbetacx as prognostic predictors in human prostatic cancer, *Biochem. Biophys. Res. Commun.* 289 (3) (2001) 692-699.
- [62] R.A. Jarred, B. Cancilla, G.S. Prins, K.A. Thayer, G.R. Cunha, G.P. Risbridger, Evidence that estrogens directly alter androgen-regulated prostate development, *Endocrinology* 141 (9) (2000) 3471-3477.
- [63] J.F. Couse, K.S. Korach, Estrogen receptor null mice: what have we learned and where will they lead us?, *Endocr. Rev.* 20 (3) (1999) 358-417.
- [64] J.F. Couse, S. Curtis Hewitt, K.S. Korach, Receptor null mice reveal contrasting roles for estrogen receptor alpha and beta in reproductive tissues, *J. Steroid Biochem. Mol. Biol.* 74 (5) (2000) 287-96.

- [65] G. Risbridger, H. Wang, P. Young, T. Kurita, Y. Wong, D. Lubahn, J.Å. Gustafsson, G. Cunha, Y.Z. Wong, Evidence that epithelial and mesenchymal estrogen receptor- α mediates effects of estrogen on prostatic epithelium, *Dev. Biol.* 229 (2001) 432-42.
- [66] E.M. Eddy, T.F. Washburn, D.O. Bunch, E.H. Goulding, B.C. Gladen, D.B. Lubahn, K.S. Korach, Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility, *Endocrinology* 137 (11) (1996) 4796-805.
- [67] J. Lindzey, W.C. Wetsel, J.F. Couse, T. Stoker, R. Cooper, K.S. Korach, Effects of castration and chronic steroid treatments on hypothalamic gonadotropin-releasing hormone content and pituitary gonadotropins in male wild-type and estrogen receptor-alpha knockout mice, *Endocrinology* 139 (10) (1998) 4092-101.
- [68] J.Y. Adams, I. Leav, K.M. Lau, S.M. Ho, S.M. Pflueger, Expression of estrogen receptor beta in the fetal, neonatal, and prepubertal human prostate, *Prostate* 52 (1) (2002) 69-81.
- [69] D.K. Das, P.O. Hedlund, T. Lowhagen, P.L. Esposti, Squamous metaplasia in hormonally treated prostatic cancer. Significance during follow-up, *Urology* 38 (1) (1991) 70-75.
- [70] B. Helpap, R. Stiens, The cell proliferation of epithelial metaplasia in the prostate gland. An autoradiographic in vitro study, *Virchows. Arch. B. Cell. Pathol.* 19 (1) (1975) 69-76.
- [71] D.J. Lager, J.A. Goeken, J.D. Kemp, R.A. Robinson, Squamous metaplasia of the prostate, An immunohistochemical study, *Am. J. Clin. Pathol.* 90 (5) (1988) 597-601.
- [72] A.A. Molinolo, R.P. Meiss, P. Leo, A.I. Sens, Demonstration of cytokeratins by immunoperoxidase staining in prostatic tissue, *J. Urol.* 134 (5) (1985) 1037-1040.
- [73] B. Tetu, J.R. Srigley, J.C. Boivin, A. Dupont, G. Monfette, S. Pinault, F. Labrie, Effect of combination endocrine therapy (LHRH agonist and flutamide) on normal prostate and prostatic adenocarcinoma. A histopathologic and immunohistochemical study, *Am. J. Surg. Pathol.* 15 (2) (1991) 111-120.
- [74] L. Pylkkänen, R. Santti, L. Salo, O. Mäentausta, R. Vihko, M. Nurmi, Immunohistochemical localization of estrogen-specific 17 beta-hydroxysteroid oxidoreductase in the human and mouse prostate, *Prostate* 25 (6) (1994) 292-300.
- [75] S.J. Ellem, J.F. Schmitt, J.S. Pedersen, M. Frydenberg, G.P. Risbridger, Local aromatase expression in human prostate is altered in malignancy, *J. Clin. Endocrinol. Metab.* 89 (2004) 2434-41.

- [76] G.G. Kuiper, E. Enmark, M. Peltö-Huikko, S. Nilsson, J.Å. Gustafsson, Cloning of a novel receptor expressed in rat prostate and ovary, *Proc. Natl. Acad. Sci. U S A* 93 (12) (1996) 5925-5930.
- [77] Z. Weihua, R. Lathe, M. Warner, J.Å. Gustafsson, An endocrine pathway in the prostate, ER β , AR, 5 α -androstane, 17 β -diol, and CYP7B1, regulates prostate growth, *Proc. Natl Acad. Sci.* 99 (2002) 13589-13594.
- [78] S. Dupont, A. Krust, A. Gansmuller, A. Dierich, P. Chambon, M. Mark, Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes, *Development*. 127 (19) (2000) 4277-4291.
- [79] M.M. Ip, R.J. Milholland, F. Rosen, Functionality of estrogen receptor and tamoxifen treatment of R3327 Dunning rat prostate adenocarcinoma, *Cancer Res.* 7 (1980) 2188-2193.
- [80] R.L. Noble, Production of Nb rat carcinoma of the dorsal prostate and response of estrogen-dependent transplants to sex hormones and tamoxifen, *Cancer Res.* 40 (1980) 3457-3450.
- [81] R.C. Bergan, E. Reed, C.E. Meyers, D. Headlee, O. Brawley, H.K. Cho, W.D. Figg, A. Tompkins, W.M. Linehan, D. Kohler, S.M. Steinberg, M.V. Blagosklonny, A Phase II study of high-dose tamoxifen in patients with hormone-refractory prostate cancer, *Clin. Cancer Res.* 9 (1999) 2366-2373.
- [82] S. Stein, B. Zoltick, T. Peacock, C. Holroyde, D. Haller, B. Armstaed, S.B. Malcovicz, D.J. Vaughn, Phase II trial of toremifene in androgen-independent prostate cancer: a Penn cancer clinical trials group trial, *Am. J. Clin. Oncol.* 3 (2001) 283-285.
- [83] J.R. Gingrich, R.J. Barrios, R.A. Morton, B.F. Boyce, F.J. DeMayo, M.J. Finegold, R. Angelopoulou, J.M. Rosen, N.M. Greenberg, Metastatic prostate cancer in a transgenic mouse, *Cancer Res.* 56 (18) (1996) 4096-4102.
- [84] S. Raghoe, M.Z. Hooshdaran, S. Katiyar, M.S. Steiner, Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model, *Cancer Res.* 62 (2002) 1370-6.
- [85] M.S. Steiner, S. Raghoe, Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk, *World J. Urol.* 21 (2003a) 31-36.
- [86] M.S. Steiner, S. Raghoe, Phase IIA clinical trial to test the efficacy and safety of toremifene in men with high-grade prostatic intraepithelial neoplasia, *Clin Prostate Cancer* 2 (2003b) 24-31.

- [87] L. Kangas, Review of the pharmacological properties of toremifene, *J. Steroid Biochem.* 36 (1990) 191-195.
- [88] K. Holli, R. Valavaara, G. Blanco, V. Kataja, P. Hietanen, M. Flander, E. Pukkala, H. Joensuu, Safety and efficacy results of a randomized trial comparing adjuvant toremifene and tamoxifen in postmenopausal patients with node-positive breast cancer, Finnish Breast Cancer Group, *J. Clin. Oncol.* 18 (2000) 3487-3494.
- [89] B.L. Neubauer K.L. Best, D.F. Counts, R.L. Goode, D.M. Hoover, C.D. Jones, M.F. Sarosdy, C.J. Shaar, L.R. Tanzer, R.L. Merriman, Raloxifene (LY156758) produces antimetastatic responses and extends survival in the PAll rat prostatic adenocarcinoma model, *Prostate* 27 (1995) 220-9.
- [90] B.L. Neubauer, A.M. McNulty, M. Chedid, K. Chen, R.L. Goode, M.A. Johnson, C.D. Jones, V. Krishnan, R. Lynch, H.E. Osborne, J.R. Graff, The selective estrogen receptor modulator trioxifene (LY133314) inhibits metastasis and extends survival in the PAll rat prostatic carcinoma model, *Cancer Res.* 63 (2003) 6056-6062.
- [91] I.Y. Kim, H. Seong do, B.C. Kim, D.K. Lee, A.T. Ramaley, F. Leach, R.A. Morton, S.J. Kim, Raloxifene, a selective estrogen receptor modulator, induces apoptosis in androgen-irresponsive human prostate cancer cell line LNCaP through an androgen-independent pathway, *Cancer Res.* 13 (2002) 3649-3653.
- [92] B.L. Riggs, L.C. Hartmann, Selective estrogen-receptor modulators – Mechanisms of action and application to clinical practice, *New Engl. J. Med.* 348 (2003) 618-629.
- [93] A.M. Wärri, R.L. Huovinen, A.M. Laine, P.M. Martikainen, P.L. Härkönen, Apoptosis intoremifene-induced growth inhibition of human breast cancer cells in vivo and in vitro, *J. Nat. Cancer Inst.* 85 (1993) 1412-1418.
- [94] H.H. Harris, J.A. Katzenellenbogen, B.S. Katzenellenbogen, Characterization of the biological roles of the estrogen receptors, ER α and ER β , in estrogen target tissues in vivo through the use of an ER α -selective ligand, *Endocrinology* 143 (2002) 4172-4177.
- [95] H.H. Harris, L.M. Albert, Y. Leathurby, M.S. Malamas, R.E. Mewshaw, C.P. Miller, Y.P. Kharode, J. Marzolf, B.R. Komm, R.C. Winneker, D.E. Frail, R.A. Henderson, Y. Shu, J.C. Keith, Evaluation of an estrogen receptor- β agonist in animal models of human disease, *Endocrinology* 144 (2003) 4241-4249.
- [96] D.P. McDonnell, The molecular pharmacology of SERMs, *Trends Endocrinol. Metab.* 10 (1999) 301-311.

- [97] D.P. McDonnell, Mining the complexities of the estrogen signalling pathways for novel therapies, *Endocrinology* 144 (2003) 4237-4240.
- [98] M. Lee, S.L. Gomez, J.S. Chang, M. Wey, R.T. Wang, A.W. Hsing, Soy and isoflavone consumption in relation to prostate cancer risk in China, *Cancer Epidem. Biomarkers Prev.* 12 (7) (2003) 665-668.
- [99] K. Ozasa, M. Nakao, Y. Watanabe, K. Hayashi, T. Miki, K. Mikami, M. Mori, F. Sakauchi, M. Washio, Y. Ito, K. Suzuki, K. Wakai, A. Tamakoshi, Serum phytoestrogens and prostate cancer risk in a nested case-control study among Japanese men, *Cancer Sci.* 95 (1) (2004) 65-71.
- [100] J. Wang, I.E. Eltoum, C.A. Lamartiniere, Dietary genistein suppresses chemically induced prostate cancer in Lobund-Wistar rats, *Cancer Lett.* 186 (1) (2002) 11-18.
- [101] R. Mentor-Marcel, C.A. Lamartiniere, I.E. Eltoum, N.M. Greenberg, A. Elgavish, Genistein in the diet reduces the incidence of poorly differentiated prostatic adenocarcinoma in transgenic mice (TRAMP), *Cancer Res.* 61 (18) (2001) 6777-6782.
- [102] S.I. Mäkelä, L.H. Pylkkänen, R.S. Santti, H. Adlercreutz, Dietary soybean may be antiestrogenic in male mice, *J. Nutr.* 125 (3) (1995) 437-445.
- [103] R.A. Jarred, S.J. McPherson, J.J. Bianco, J.F. Couse, K.S. Korach, G.P. Risbridger, Prostate phenotypes in estrogen-modulated transgenic mice, *Trends Endocrin. Met.* 13 (2002) 163-168.
- [104] R.W. deVere, White, R.M. Hackman, S.E. Soares, L.A. Beckett, Y. Li, B. Sun, Effects of a genistein-rich extract on PSA levels in men with a history of prostate cancer, *Urology* 63 (2) (2004) 259-263.

Legend to Figure 1

EXPRESSION OF ESTROGEN RECEPTORS α AND β (ER α , ER β) IN HUMAN PROSTATE AND IN PROSTATE CANCER

ERs were localized with immunostaining using ER-subtype-specific antibodies. Positive nuclei are indicated by brown color. Immunonegative nuclei show only the blue counterstain. Panel on left (A-G) shows localization of ER α , and panel on right (H-N) that of ER β . In the peripheral zone (A and H, serial sections, K, L), majority of epithelial cells express ER β (H, K, L), while ER α is not detectable (A). Serial sections from the central zone (B and I) demonstrate the presence of ER α -positive but ER β -negative stromal cells. In glands/ducts with basal cell hyperplasia (C and J, serial sections), ER α is expressed in distinct groups of epithelial cells, while only modest ER β expression is seen in basal cell layer. Very intense immunostaining for ER α is seen in the posterior wall of prostatic urethra showing squamous epithelial metaplasia (D), as well as the adjacent urothelium (E). In prostate cancer specimen, ER β is present in some cancer cells, while others are negative (M, N). In the same cancer specimen, ER α was observed only in a small number of stromal cells (F, G).

Fig. 1