

# Commentary

## Estrogen Receptor $\beta$ in Prostate Cancer

### *Brake Pedal or Accelerator?*

Sabina Signoretti and Massimo Loda

*From the Department of Adult Oncology, Dana Farber Cancer Institute, and the Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts*

Estrogens, via the interaction with their receptors, play important roles in the control of cellular growth and differentiation. Specifically, estrogens regulate the growth and development of the mammary gland in embryogenesis as well as in pre- and postpubertal periods, and of ovarian follicles during the reproductive cycle.<sup>1</sup> Importantly, estrogens are known to play a role in the development of the male reproductive system as well.<sup>2</sup> The effects of estrogens on prostate epithelium are still primarily unknown although estrogens have been used in the treatment of prostate cancer because of their growth-inhibitory effects. However, either because of toxicity and/or because of poor response rates, treatment of prostate cancer with estrogen has been discontinued.

Estrogenic activity is mediated by the physical interaction between the estrogen receptors (ERs) and the hormone with subsequent activation of the receptors. These belong to a superfamily of nuclear receptors that are ligand-dependent transactivators and include receptors for retinoic acid, vitamin D, steroid, and thyroid hormones as well as orphan receptors for which ligands are yet to be found.<sup>3</sup>

Although it was initially thought that estrogens mediate their action through a single receptor, the estrogen receptor  $\alpha$  (ER $\alpha$ ), a second ER has been identified relatively recently from a rat prostate library and termed ER $\beta$ .<sup>4</sup> Whereas these two receptors share structural similarities (47% identity) and some functional properties, it is clear that individual characteristics allow them to have distinct biological functions.

Human ERs (hERs) have six functional regions that vary in their degree of conservation. The least conserved region among species, within the receptor superfamily and between ER $\alpha$  and ER $\beta$ , is the hypervariable amino-terminal, important for transactivation. In contrast, the DNA-binding domain is highly conserved (96% identity). Interestingly, there is 59% identity at the amino-acid level

between ER $\alpha$  and ER $\beta$  in the ligand-binding domain. Although both receptors bind the natural ligand 17 $\beta$  estradiol with about equal affinity,<sup>5</sup> phytoestrogens and selective estrogen receptor modulators (SERMs) can bind ER $\alpha$  and ER $\beta$  selectively.<sup>6</sup> However, the putative diverse biological function of the two receptors should not only be ascribed to different ligands but, more importantly, to their different tissue distribution. When inactive, ERs exist as monomers bound to heat shock proteins.<sup>7</sup> On activation, they can form homodimers (ER $\alpha$ /ER $\alpha$ ; ER $\beta$ /ER $\beta$ ) or heterodimers (ER $\alpha$ /ER $\beta$ ). If, for instance, ER $\alpha$  and ER $\beta$  are co-expressed in a given tissue or tumor, the formation of a heterodimer will likely yield a different transcriptional profile from that obtained if homodimers are generated in the presence of natural ligands or SERMs.

Although androgens are required for the normal development and function of the male reproductive system, the role of estrogens is still unclear. ER $\alpha$ , ER $\beta$ , and ER $\alpha$ /ER $\beta$  knock-out mice have been generated to study the biological effects in different tissues including the male reproductive system.<sup>2,8-10</sup> The main findings in these knock-out strains are the requirement of ER $\alpha$  for ovulation and that of ER $\beta$  for granulosa cell proliferation in the female, whereas male infertility was found in the ER $\alpha$  but not in the ER $\beta$  knock-outs ( $\beta$ ERKO). As far as the prostate is concerned, a recent study<sup>10</sup> reports no abnormalities in epithelial proliferation in either ER $\beta$  or double ER $\alpha$  and ER $\beta$  knock-outs (ER $\alpha$  $\beta$ KO) despite previous reports suggesting such abnormalities.<sup>9</sup> The absence of prostatic lesions in the ER $\beta$  knock-outs does not contradict experimental evidence pointing to a role for ER $\beta$  as an inhibitor of prostatic growth, as suggested by Leav and colleagues in this issue of *The American Journal of Pathology*<sup>11</sup> and by the same group of investigators in another recent report.<sup>12</sup> In fact, a case in point is that of the cell-cycle inhibitor p27. p27 has been found to be associated with aggressive behavior in a wide variety of tumors.<sup>13</sup> Its targeted disruption in mice results merely in

---

Accepted for publication May 3, 2001.

Address reprint requests to Massimo Loda, Dana Farber Cancer Institute, D-740, 44 Binney St., Boston, MA 02115. E-mail: massimo\_loda@dfci.harvard.edu.

pituitary tumors.<sup>14–16</sup> However, when these mice are challenged by carcinogens or radiation<sup>17</sup> or are crossed with mouse strains with known susceptibility to prostate cancer such as the PTEN heterozygous-deficient mice,<sup>18</sup> tumors develop at high rates. Studies of this sort will prove useful in both  $\beta$ ERKO and ER $\alpha$  $\beta$ KO genetically engineered strains to determine the putative anti-proliferative function of ER $\beta$  in the prostate.

The discovery of ER  $\beta$  has thus lead to a re-evaluation of the biological functions of estrogens as well as of estrogen antagonists in prostate cancer. It has recently been shown that SERMs function as estrogens in some tissues whereas in others have anti-estrogenic effects.<sup>6</sup> This is thought to result from the induction of distinct conformations in the two ERs, allowing the recruitment of different co-regulators. These, in turn, are thought to determine the transcriptional profile induced by ligands. In fact, opposite transcriptional events occur when the two receptors are exposed to tamoxifen *in vitro*.<sup>19</sup> Thus, by defining the molecular mechanisms of ER signaling in prostate cancer cells it should be theoretically possible to design new SERMs with significant anti-tumoral activity.

Before antibodies became available, localization of the ER subtypes was accomplished either by steroid autoradiography,<sup>20,21</sup> *in situ* hybridization,<sup>22</sup> or, more recently, by reverse transcriptase-polymerase chain reaction.<sup>12,23</sup> Therefore, in most of the studies ER $\beta$  expression in both cell lines and tissues has been investigated at the RNA level. Interestingly, only ER $\beta$ , and not ER $\alpha$  transcripts were previously detected in rodent prostate epithelial cells. ER $\beta$  mRNA appeared to be expressed in both basal and luminal cells.<sup>22</sup> ER $\alpha$  transcripts were also found to be re-expressed after treatment with de-methylation agents in human prostate cell lines suggesting transcriptional regulation of this receptor.<sup>12</sup> Again, at the RNA level, the prostate carcinoma cell lines LNCaP and DU-145 were previously shown to express exclusively ER $\beta$  whereas PC-3 cells expressed transcripts from both receptors.<sup>12</sup> In addition, ER $\beta$  transcripts were found to be decreased in both localized and hormone refractory prostate cancers relative to normal prostate tissue when these were measured by quantitative reverse transcriptase-polymerase chain reaction, suggesting that loss of ER $\beta$  correlated with disease progression.<sup>23</sup>

To date, several antibodies have been generated to the various regions of ER $\beta$  and used in Western blot analysis, immunoprecipitation, and mobility shift assays.<sup>7</sup> Some of these antibodies directed at rat or mouse ER $\beta$ <sup>10,24–27</sup> or human ER $\beta$ <sup>28,29</sup> have also been used in immunohistochemical studies. In this issue, Leav and colleagues<sup>11</sup> describe for the first time the localization of the ER $\beta$  protein in a spectrum of human prostate specimens, ranging from normal, to dysplastic (prostate intraepithelial neoplasia), to invasive and metastatic cancers. To accomplish this, they generated a polyclonal antibody raised against a peptide from the C-terminal region of the receptor. The peptide was chosen on the basis of having no homology to the corresponding region of ER $\alpha$ . As far as normal tissue is concerned, ER $\beta$  was found to be expressed in basal cells of normal prostatic acini as well as in stromal cells. Of note, ER $\beta$  was not expressed in

secretory cells. Importantly, the only previous study in which ER $\beta$  was detected in the human prostate using an antibody raised against the N-terminus region of the receptor, also localized ER $\beta$  in basal acinar cells and in stromal cells.<sup>29</sup>

As expected, ER $\alpha$  was expressed only in stromal cell nuclei. Ho and colleagues<sup>12</sup> have also recently shown that primary, nontransformed, human epithelial prostate cultures express ER $\beta$  but not ER $\alpha$  mRNA. In this article,<sup>11</sup> they confirm ER $\beta$  protein expression in prostate basal cells in culture (PrEC; Clonetics, Inc. San Diego, CA).

Interestingly, Leav and colleagues<sup>11</sup> show that high-grade dysplasia does not express ER $\beta$ . Antiproliferative effects of tamoxifen and the anti-estrogen ICI-182,780 were previously shown to be reversed by ER $\beta$  antisense in prostate cells.<sup>12</sup> Furthermore, increased expression of ER $\beta$  was found to be associated with estrogen-mediated protection from *Apc*-associated tumor formation in *min* mice.<sup>30</sup> Finally, ER $\beta$  protein down-regulation is associated with mitogenic activity in premalignant lesions of the breast.<sup>31</sup> Taken together, these findings raise the attractive possibility that lack of expression of ER $\beta$  may contribute to the initial phases of epithelial tumorigenesis. In terms of the early phases of prostate intraepithelial dysplasia, Leav and colleagues<sup>11</sup> found expression of ER $\beta$  in low-grade prostate intraepithelial neoplasia lesions but lack of expression in cells displaying high-grade dysplasia. Thus, ER $\beta$  may be expressed in the very early phases of dysplasia (low grade), perhaps to counteract proliferative stimuli but lack of ER $\beta$  expression occurs, and may in fact be required, in the lesions with high-grade cytological atypia, known to be associated with invasive carcinomas.

The pattern of expression in the invasive cancers examined was, as expected, more complex. Using the ER $\beta$ -specific antibody they generated, Leav and colleagues<sup>11</sup> showed that the majority of moderately differentiated tumors expressed ER $\beta$  although a difference was observed when ER $\beta$ -negative clear cell carcinomas of the transition zone were compared to ER $\beta$ -positive tumors arising in the peripheral zone. ER $\beta$  was also widely expressed in androgen-independent, metastatic tumors. Although obviously difficult to compare, the immunohistochemical findings seem to contradict the mRNA data previously reported in prostate cancer.<sup>23</sup> However, little is known about posttranscriptional regulation of ER $\beta$  and it may be possible that the ER $\beta$  protein is more stable in androgen-independent, metastatic tumors despite reduced transcriptional activity for this gene. The finding of increased ER $\beta$  in both moderately differentiated cancers and aggressive metastatic tumors is also contradictory. In essence, is ER $\beta$  the good guy or the bad guy? Is its down-regulation associated with tumorigenesis and/or tumor progression or is the expression of ER $\beta$  in prostate tumors of advanced stage transducing signals that favor tumor aggressiveness? Obviously, more detailed studies in human prostate cancer, complete of biochemical correlates, need to be performed to determine the precise role of ER $\beta$  in these tumors.

The finding of positivity of tumor cells for ER $\beta$  in metastatic, androgen-independent lesions, however, could

be of potential therapeutic interest, provided the receptor is functional in this setting. To this end, it is interesting to note that many genes expressed by the basal cells, which do not depend on androgens for survival, such as bcl-2,<sup>32-34</sup> Her-2-neu,<sup>35</sup> and prostate stem cell antigen<sup>36</sup> get re-expressed in advanced, androgen-independent cancers. Intriguingly, ER $\beta$  seems to follow the same path. Except for the keratin profile and the almost universal expression of the androgen receptor in these tumors, metastatic, androgen-independent prostate tumors thus seem to display a basaloid phenotype. This should lend itself to targeted therapeutic manipulations of this late-stage, fatal type of prostate cancer, as Leav and colleagues<sup>11</sup> suggest.

In summary, the different biological activities of ER $\alpha$  and ER $\beta$ , may be ascribed to a variety of factors that will need to be carefully studied in human prostate carcinomas now that tools such as laser capture microdissection, quantitative real-time polymerase chain reaction, and immunohistochemistry are available. These factors include recruitment of different co-activators and co-repressors to modify transcription and homodimerization versus heterodimerization of these receptors. Because heterodimerization can only occur in cells that co-express these receptors, assessment of expression of both ERs as well as that of co-regulators, in normal and tumor tissues, becomes of paramount importance. Leav and colleagues<sup>11</sup> have begun to address this issue in normal and neoplastic human prostate using a novel, anti-hER $\beta$  antibody. They have shown that ER $\beta$  is expressed in basal cells in normal prostatic acini, it is not expressed in high-grade dysplasia, is expressed in the majority of moderately differentiated, invasive cancers whereas it is lost with increasing Gleason score. Interestingly, ER $\beta$  was somewhat surprisingly expressed in androgen-independent metastatic tumors. The data provided generate more questions than they answer. In addition, one must bear in mind that the number of cases assessed is small and that the antibody has not been validated by other groups. Therefore, additional immunohistochemical studies to assess ER $\beta$  expression in prostate cancer are necessary.

In conclusion, ER $\beta$  may play a significant role in prostate cell differentiation and proliferation and may modulate both the initial phases of prostate carcinogenesis and androgen-independent tumor growth. Whether ER $\beta$  enhances or suppresses prostate cancer development and/or progression remains to be established.

## References

- Giguere V, Tremblay A, Tremblay GB: Estrogen receptor  $\beta$ : re-evaluation of estrogen and antiestrogen signaling. *Steroids* 1998, 63:335-339
- Couse JF, Korach KS: Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 1999, 20:358-417
- Kuiper GG, Gustafsson JA: The novel estrogen receptor- $\beta$  subtype: potential role in the cell- and promoter-specific actions of estrogens and anti-estrogens. *FEBS Lett* 1997, 410:87-90
- Kuiper GG, Enmark E, Peltto-Huikko M, Nilsson S, Gustafsson JA: Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996, 93:5925-5930
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, Gustafsson JA: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors  $\alpha$  and  $\beta$ . *Endocrinology* 1997, 138:863-870
- Dutertre M, Smith CL: Molecular mechanisms of selective estrogen receptor modulator (SERM) action. *J Pharmacol Exp Ther* 2000, 295:431-437
- Pavao M, Traish AM: Estrogen receptor antibodies: specificity and utility in detection, localization and analyses of estrogen receptor  $\alpha$  and  $\beta$ . *Steroids* 2001, 66:1-16
- Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O: Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 1993, 90:11162-11166
- Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA, Smithies O: Generation and reproductive phenotypes of mice lacking estrogen receptor  $\beta$ . *Proc Natl Acad Sci USA* 1998, 95:15677-15682
- Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M: Effect of single and compound knockouts of estrogen receptors  $\alpha$  (ER $\alpha$ ) and  $\beta$  (ER $\beta$ ) on mouse reproductive phenotypes. *Development* 2000, 127:4277-4291
- Leav I, Lau K-M, Adams JY, McNeal JE, Taplin M-E, Wang J, Singh H, Ho S-M: Comparative studies of estrogen receptors  $\beta$  and  $\alpha$  and androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am J Pathol* 2001, 159:79-92
- 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, 60:3175-3182
- Slingerland J, Pagano M: Regulation of the cdk inhibitor p27 and its deregulation in cancer. *J Cell Physiol* 2000, 183:10-17
- Fero M, Rivkin M, Tasch M, Porter P, Carow C, Firpo E, Tsai L, Broudy V, Perlmutter R, Kaushansky K, Roberts J: A syndrome of multi-organ hyperplasia with features of gigantism, tumorigenesis and female sterility in p27<sup>Kip1</sup>-deficient mice. *Cell* 1996, 85:733-744
- Nakayama K, Ishida N, Shirane M, Inomata A, Inoue T, Shishido N, Horii I, Loh DY: Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell* 1996, 85:707-720
- Kiyokawa H, Kineman R, Manova-Todorova K, Soares V, Hoffman E, Onoi M, Hayday A, Frohman D, Koff A: Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27<sup>Kip1</sup>. *Cell* 1996, 85:721-732
- Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ: The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature* 1998, 396:177-180
- Di Cristofano A, De Acetis M, Koff A, Cordon-Cardo C, Pandolfi PP: Pten and p27KIP1 cooperate in prostate cancer tumor suppression in the mouse. *Nat Genet* 2001, 27:222-224
- Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ, Scanlan TS: Differential ligand activation of estrogen receptors ER $\alpha$  and ER $\beta$  at AP1 sites. *Science* 1997, 277:1508-1510
- Schleicher G, Drews U, Stumpf WE, Sar M: Differential distribution of dihydrotestosterone and estradiol binding sites in the epididymis of the mouse. An autoradiographic study. *Histochemistry* 1984, 81:139-147
- Cooke PS, Young P, Hess RA, Cunha GR: Estrogen receptor expression in developing epididymis, efferent ductules, and other male reproductive organs. *Endocrinology* 1991, 128:2874-2879
- Shughrue PJ, Lane MV, Scrimo PJ, Merchenthaler I: Comparative distribution of estrogen receptor- $\alpha$  (ER- $\alpha$ ) and  $\beta$  (ER- $\beta$ ) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids* 1998, 63:498-504
- Latil A, Bieche I, Vidaud D, Lidereau R, Berthon P, Cussenot O, Vidaud M: Evaluation of androgen, estrogen (ER  $\alpha$  and ER  $\beta$ ), and progesterone receptor expression in human prostate cancer by real-time quantitative reverse transcription-polymerase chain reaction assays. *Cancer Res* 2001, 61:1919-1926
- Rosenfeld CS, Ganjam VK, Taylor JA, Yuan X, Stiehr JR, Hardy MP, Lubahn DB: Transcription and translation of estrogen receptor- $\beta$  in the male reproductive tract of estrogen receptor- $\alpha$  knock-out and wild-type mice. *Endocrinology* 1998, 139:2982-2987

25. Nilsson LO, Boman A, Savendahl L, Grigelioniene G, Ohlsson C, Ritzen EM, Wroblewski J: Demonstration of estrogen receptor- $\beta$  immunoreactivity in human growth plate cartilage. *J Clin Endocrinol Metab* 1999, 84:370–373
26. Alves SE, Lopez V, McEwen BS, Weiland NG: Differential colocalization of estrogen receptor  $\beta$  (ER $\beta$ ) with oxytocin and vasopressin in the paraventricular and supraoptic nuclei of the female rat brain: an immunocytochemical study. *Proc Natl Acad Sci USA* 1998, 95:3281–3286
27. Sar M, Welsch F: Differential expression of estrogen receptor- $\beta$  and estrogen receptor- $\alpha$  in the rat ovary. *Endocrinology* 1999, 140:963–971
28. Jarvinen TA, Pelto-Huikko M, Holli K, Isola J: Estrogen receptor  $\beta$  is coexpressed with ER $\alpha$  and PR and associated with nodal status, grade, and proliferation rate in breast cancer. *Am J Pathol* 2000, 156:29–35
29. Taylor AH, Al-Azzawi F: Immunolocalisation of oestrogen receptor  $\beta$  in human tissues. *J Mol Endocrinol* 2000, 24:145–155
30. Weyant MJ, Carothers AM, Mahmoud NN, Bradlow HL, Remotti H, Bilinski RT, Bertagnolli MM: Reciprocal expression of ER $\alpha$  and ER $\beta$  is associated with estrogen-mediated modulation of intestinal tumorigenesis. *Cancer Res* 2001, 61:2547–2551
31. Roger P, Sahla ME, Makela S, Gustafsson JA, Baldet P, Rochefort H: Decreased expression of estrogen receptor  $\beta$  protein in proliferative preinvasive mammary tumors. *Cancer Res* 2001, 61:2537–2541
32. McDonnell TJ, Navone NM, Troncoso P, Pisters LL, Conti C, von Eschenbach AC, Brisbay S, Logothetis CJ: Expression of bcl-2 oncoprotein and p53 protein accumulation in bone marrow metastases of androgen independent prostate cancer. *J Urol* 1997, 157:569–574
33. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, Tu SM, Campbell ML: Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res* 1992, 52:6940–6944
34. Colombel M, Symmans F, Gil S, O'Toole KM, Chopin D, Benson M, Olsson CA, Korsmeyer S, Buttyan R: Detection of the apoptosis-suppressing oncoprotein bc1-2 in hormone-refractory human prostate cancers. *Am J Pathol* 1993, 143:390–400
35. Signoretti S, Montironi R, Manola J, Altimari A, Tam C, Bublely G, Balk S, Thomas G, Kaplan I, Hlatky L, Hahnfeldt P, Kantoff P, Loda M: Her-2-neu expression and progression toward androgen independence in human prostate cancer. *J Natl Cancer Inst* 2000, 92:1918–1925
36. Reiter RE, Gu Z, Watabe T, Thomas G, Szigei K, Davis E, Wahl M, Nisitani S, Yamashiro J, Le Beau MM, Loda M, Witte ON: Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci USA* 1998, 95:1735–1740