Insight into the mechanisms of action of estrogen receptor β in the breast, prostate, colon, and CNS

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Abstract

Estrogen and its receptors (ERs) influence many biological processes in physiology and pathology in men and women. ERs are involved in the etiology and/or progression of cancers of the prostate, breast, uterus, ovary, colon, lung, stomach, and malignancies of the immune system. In estrogen-sensitive malignancies, ER β usually is a tumor suppressor and ER α is an oncogene. ER β regulates genes in several key pathways including tumor suppression (p53, PTEN); metabolism (PI3K); survival (Akt); proliferation pathways (p45^{Skp2}, cMyc, and cyclin E); cell-cycle arresting factors (p21^{WAF1}, cyclin-dependent kinase inhibitor 1 (CDKN1A)), p27^{Kip1}, and cyclin-dependent kinases (CDKs); protection from reactive oxygen species, glutathione peroxidase. Because they are activated by small molecules, ERs are excellent targets for pharmaceuticals. ER α antagonists have been used for many years in the treatment of breast cancer and more recently pharmaceutical companies have produced agonists which are very selective for ER α or ER β . ER β agonists are being considered for preventing progression of cancer, treatment of anxiety and depression, as anti-inflammatory agents and as agents, which prevent or reduce the severity of neurodegenerative diseases.

Key Words

- ▶ estrogen receptors
- ▶ anti-proliferative
- ▶ 3 β -Adiol
- ▶ cell cycle

*Journal of Molecular
Endocrinology*
(2013) 51, T61–T74

Introduction

In addition to their well-known role in growth and differentiation of the breast, ovary and uterus in females, and testes and prostate in males, estrogen is also required for proper functioning of the cardiovascular, immune, gastrointestinal, musculoskeletal, and nervous systems as well as skin. The most active estrogen in the body is 17 β -estradiol (E₂), which is synthesized in the granulosa cells of the ovary, Leydig cells of the testis (Stocco 2012), adipose tissue (Rice *et al.* 2012), and brain (Boon *et al.* 2010).

The classical actions of estrogen are mainly mediated via the two nuclear estrogen receptors (ERs), ER α and ER β , both of which bind to E₂ with high affinity in the low nM

range. Two metabolites of E₂, estrone (E₁) and estriol (E₃), circulate at high levels at certain phases in the menstrual cycle and during pregnancy (Gruber *et al.* 2002). E₁ and E₃ have been thought to be the inactive metabolites of E₂, but E₃ has significant effects on the immune system (Zang *et al.* 2002, Zhou *et al.* 2011) and a closer examination of the physiological functions of these two steroids is warranted.

In addition to estrogens, which are synthesized from testosterone and androstenedione through the action of the enzyme aromatase, there is another class of estrogens which do not have an aromatic A-ring. These are 5-androstene-3 β ,17 β -diol (A⁵diol), synthesized from

the adrenal steroid dehydroepiandrosterone, and 5α -androstane- 3β , 17β -diol (3β -Adiol), synthesized from the hormone 5α -dihydrotestosterone (DHT). Estrogenic actions of A^5 diol (Shao *et al.* 1975) and 3β -Adiol (Stewart *et al.* 1977) have been known for many years and their very rapid metabolism in the prostate (Isaacs *et al.* 1980) and pituitary (Guiraud *et al.* 1979) was reported. However, it was not until the discovery of ER β that the estrogenic actions of these steroids were understood (Weihua *et al.* 2002a). Unlike E_2 , A^5 diol and 3β -Adiol do not circulate at high concentrations and serve more paracrine and autocrine functions.

ER α and ER β are transcribed from two genes (*ESR1* and *ESR2* respectively) that are located on different chromosomes (Gosden *et al.* 1986, Enmark *et al.* 1997). ER α was discovered more than 50 years ago by Elwood Jensen and the gene was cloned in 1985 (Walter *et al.* 1985). ER β was discovered in 1996 by cross-hybridization of a consensus DNA-binding domain (DBD) probe in a rat prostate cDNA library (Kuiper *et al.* 1996). ER α protein composed of 595 amino acids (White *et al.* 1987) is a bit longer than the 530 amino acid-long ER β . ER α and ER β are architecturally similar with six regions (A–F) in the primary amino acid sequence, an arrangement found in all members of the nuclear receptor superfamily (Carson-Jurica *et al.* 1990). Overall there are three functional domains: N-terminal domain (NTD), DNA binding domain (DBD), and ligand-binding domain (LBD). The two ERs share ~97% similarity in their DBD and 59% in LBD, whereas the NTD is merely 16% similar (Fig. 1; Pettersson & Gustafsson 2001). Although the differences in the LBD are small, they are significant enough to influence the shape of the ligand-binding pocket and that ligands with unique binding

affinities for the each receptor have been synthesized (for review see Nilsson *et al.* (2011)). These ligands are now referred to as specific ER modulators (SERMs). ER β binds better than ER α to genistein, a phytoestrogen (Kuiper *et al.* 1998), which has traditionally been regarded as a health-promoting and anti-cancer agent. Two years after the discovery of ER β , another splice variant was discovered and named ER β cx/2 (Ogawa *et al.* 1998). Soon after other splice variants – ER β 4, and ER β 5 – were discovered. ER β 4 were identified from a testis library and ER β 5 was identified from the MDA-MB-435 cell line (Moore *et al.* 1998). All the splice variants have a truncated C-terminus with a unique stretch of amino acids fused to the C-terminus. Because of the truncated LBD, these forms have lost their ability to bind estrogens (Leung *et al.* 2006b) and so far no ligands have been found for these variants.

In addition to the classical ER-binding site on DNA known as the estrogen response element (ERE), ERs can also be recruited to the response elements on DNA without themselves binding to DNA. Instead, they are tethered to other transcription factors (activator protein 1 (AP1), Sp1, and nuclear factor κ B (NF κ B); Webb *et al.* 1999) and influence the transcription of genes normally regulated by these rather ubiquitous transcription factors. ER α and ER β can have opposite effects at these sites: ER β -tamoxifen acting through AP1 can activate target genes, whereas ER α -tamoxifen may cause repression of the target genes (Webb *et al.* 2003).

The action of ER β at AP1 sites or EREs is determined by the presence of coactivators. In the absence of E_2 , the co-activator Tip60 enhances ER β transcriptional activity at AP1 sites, but represses it at EREs. ER β -selective ligands abolish the repressive actions of ER β at ERE, while the

Figure 1

Schematic of the structure of the estrogen receptor (ER). The ER is composed of three main domains: the N-terminal domain (NTD), which contains the activation function 1, the DNA-binding domain (DBD), and the

ligand-binding domain (LBD), containing the activation function 2. The DBDs of ER α and ER β are highly conserved and the NTDs are the least conserved. The NTD of ER α is shorter than that of ER β .

antagonist, ICI 182 780, enhances the transcription at AP1 sites. In addition to being a key player in reparation of double stranded breaks in DNA (Sun *et al.* 2010), Tip60 is an interesting coactivator in the relationship between ER β and AR: TIP60 is an AR coactivator (reviewed in Culig & Santer (2012) and a facilitator of AR transport into the nucleus (Shiota *et al.* 2010)). If in the absence of E₂, ER β activation at AP1 sites causes proliferation, then the breasts of post-menopausal women should be proliferating but they are not (Cheng *et al.* 2013), and ER β could increase proliferation in the prostate but it does not (Attia & Ederveen 2012). Clearly a more detailed analysis of the expression of coactivators in the breast and prostate is needed.

Recent genomic landscaping of ER α and ER β binding sites has revealed that while ER α is predominantly found at ERE elements (Carroll *et al.* 2005), whereas ER β is mostly found at AP1 sites (Zhao *et al.* 2010). This difference in DNA interaction has revealed a very important difference between the two ERs: the DBD is less important for transcriptional activity in ER β than it is in ER α . The important consequence of this finding is that although removal of one zinc finger of ER α creates an inactive protein, the same mutation leaves a functional ER β (Price *et al.* 2001). Thus, it is not surprising that a mouse in which the second zinc finger (ER β exon 3) was deleted is normal except for the ability to ovulate (Antal *et al.* 2008).

Mechanisms of ER action

Ligand-dependent activation of ER

ERs primarily reside in an inactive state in the cytoplasm in complex with heat-shock protein (Hsp) 50, 70, and 90, which stabilizes the receptor (reviewed in Sanchez (2012)). In the classical model of estrogen action, Hsp dissociates and the DBD of the receptor becomes unveiled when the ligand-binding pocket is occupied. Upon binding to DNA, the receptor causes recruitment of the p160 family of co-regulators such as steroid receptor coactivators (SRCs) or silencing mediator of retinoid and thyroid receptor (SMRT/NCOR2) (Xu *et al.* 2009). The structure of the ligand that enters the ligand-binding pocket influences the conformation adopted by the receptor and thus either coactivators or corepressors are recruited (Paige *et al.* 1999, Pike *et al.* 1999).

Ligand-independent mechanism of action

ERs can also be activated by ligand-independent mechanisms via cross-talk with other signaling pathways.

Both receptors can be activated via phosphorylation by MAPK and phosphatidylinositol 3-kinase (PI3K; Miller *et al.* 2011, Riggio *et al.* 2012, McGlynn *et al.* 2013). Furthermore, epidermal growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR), upon activation by their cognate peptide ligands, can activate the serine/threonine kinases, ERK and Akt, which in turn catalyze the phosphorylation and activation of ERs. Posttranscriptional modifications of ERs have been nicely reviewed recently (Le Romancer *et al.* 2011). Activation of ER α by kinases is thought to play an important role in breast cancer (reviewed in Thomas & Gustafsson (2011)).

Physiological and pathophysiological actions of ERs

The two ERs have distinct functions and act antagonistically in multiple pathways: ER α plays an important role in growth and proliferation (Maggiolini *et al.* 2001, Ewan *et al.* 2005, Sun *et al.* 2006, Teng *et al.* 2008) while ER β exerts anti-proliferative, differentiative, and apoptotic actions in breast (Lazennec *et al.* 2001, Paruthiyil *et al.* 2004, Strom *et al.* 2004), prostate (Cheng *et al.* 2004, Pravettoni *et al.* 2007, McPherson *et al.* 2010, Dey *et al.* 2012), and colon cells (Hartman *et al.* 2009, Bielecki *et al.* 2011). Both receptors are required for the maintenance of glucose homeostasis with ER β predominating in the skeletal muscle and ER α in the adipose tissue (Barros *et al.* 2009, Barros & Gustafsson 2011).

Breast and ER

In the normal mammary gland, ER is essential for branching morphogenesis and in ER α ^{-/-} mice there is a very rudimentary ductal tree (Couse & Korach 1999). In the adult gland, in both rodents (Saji *et al.* 2000) and women (Cheng *et al.* 2013), ER α is expressed in <10% of mammary epithelial cells. ER β is expressed in 70–80% of the mammary epithelial cells and is also expressed in the stroma and immune cells resident in the breast (Clarke *et al.* 1997, Speirs *et al.* 2002, Li *et al.* 2010). ER β ^{-/-} mice revealed that ER β is not required for ductal growth of the breast but is required for terminal differentiation and for maintaining the organization and differentiation of epithelial tissue (Forster *et al.* 2002).

Breast cancer and ER

According to the statistical evidence (Siegel *et al.* 2013), one in eight women will develop breast cancer and the

5-year survival for metastatic breast cancer is 25%. Although initiation and progression of breast cancer involve a complex interplay of many environmental and genetic factors, estrogen plays a very central role (Hankinson *et al.* 2004). The involvement of estrogen signaling in breast cancer was understood as early as in the 1880s, when surgeons in the USA and Germany were able to treat breast cancer patients by oophorectomy (Love & Philips 2002). Prolonged exposure to estrogen is a well-established risk factor for breast cancer in postmenopausal women (Darbre & Charles 2010, Fernandez & Russo 2010). The combination of multiple gene mutations (*BRCA1*, *TP53*, and *ERBB2*) together with the expression of ER α determines the final course and progression of the tumor (Njiaju & Olopade 2012). About 70% of breast cancers are ER α -positive and respond to anti-estrogen therapies such as tamoxifen, raloxifene, or other SERMs and aromatase inhibitors (Musgrove & Sutherland 2009). However, one-third of the patients treated with tamoxifen become resistant to treatment. The reason for the development of tamoxifen resistance is not yet clear. Loss of ER α expression or mutations in ER α cannot account for all resistance as these occur only in ~15% of patients (Gutierrez *et al.* 2005).

The involvement of ER β in breast cancer is still being investigated. As reviewed by Murphy & Leygue (2012), several studies have demonstrated that ER β expression in breast cancer, irrespective of presence of ER α , is associated with increased response to endocrine therapy, but others have not found this (Murphy & Watson 2006). The functions of ER β splice variants in breast cancer are another unresolved issue. ER β 2 (ER β cx) is a C-terminally spliced form of ER β discovered by Ogawa *et al.* (1998). Some clinical studies have associated the expression of ER β 2 with poor prognosis and decreased survival of breast cancer patients (Saji *et al.* 2002); others have found the opposite (Sugiura *et al.* 2007). Some found that ER β 2 is not indicative of tamoxifen sensitivity (Esslimani-Sahla *et al.* 2004) while others have found that it is (Vinayagam *et al.* 2007). Clearly, more work needs to be done in order to clarify these discrepancies. One suggestion which might be helpful is the use of samples bigger than the traditional biopsy. This is because there is heterogeneity in any breast cancer, with many stages of the disease in each sample and this can only be seen in samples of larger size. Thus some regions of the cancer express ER β and some express ER β cx and these foci of expression can be missed if small samples are used.

Breast cancer cell lines MCF7, T47D, and MDA231 have been used extensively to study the functions of ER α

and ER β . One of the curious results of these studies is that when ER-negative breast cancer cells, MDA-MB-231, are engineered to express ER α or ER β , both receptors can inhibit proliferation and invasion. The difference is that ER α requires the presence of ligand for its actions but ER β does not (Lazennec *et al.* 2001). Studies in the T47D breast cancer cell line revealed that in the presence of E₂, ER α regulates expression of cell cycle genes such as *c-MYC* (*MYC*), cyclin E, cyclin D, and p45^{Skp2} (Strom *et al.* 2004). ER α interacts with a distal half-ERE and an AP1 site in the enhancer of *c-myc* and transcriptionally upregulates *c-myc* expression (Dubik & Shiu 1992, Wang *et al.* 2011). Inhibition of ER α with tamoxifen causes a decrease in cyclin D1 and *c-myc* expression, which represses downstream targets such as Bcl2 and increases cell death (Butt *et al.* 2005, Nehra *et al.* 2010). Very recently, it has been shown that in breast cancer cell lines, ER β augmented the antiproliferative effects of tamoxifen (Lattrich *et al.* 2013).

Many studies show that loss of ER β expression occurs frequently in ductal breast, partly because of promoter hypermethylation. Esslimani-Shaha *et al.* ascribe the progression of the malignancy and tamoxifen resistance to the decreased expression of ER β (Shaaban *et al.* 2003, Esslimani-Sahla *et al.* 2004, Hopp *et al.* 2004). However, there are also studies which show high ER β expression in ductal cancer and in these studies, ER β expression is associated with poor prognosis (Markey *et al.* 2009, Kim *et al.* 2012). The reasons for the reported differences in the contribution of ER β to breast cancer progression will no doubt be explained as more studies are done and the antibodies used become more standardized.

Despite studies showing beneficial effects of ER β in breast cancer, a novel mechanism through which ER β might contribute to aggressiveness in Her2-positive breast cancer has been described by Chen *et al.* According to this study conducted in several breast cancer cell lines, ER β expression is associated with high expression of interleukin 8, a cytokine which increases tumor angiogenesis and metastasis (Chen *et al.* 2011). If it is the relative expression of ERs (ER β 1/ER β 2/ER α) which determines the overall effect of ER β in breast cancer, expression of all three receptors and their localization must be measured in order to assess the role of ERs in the prognosis of breast cancer.

Prostate and ER

E₂ via ER α is required for the development and branching morphogenesis of the prostate (Bosland 2000, Taplin & Ho 2001, Omoto *et al.* 2005). However, in the adult mouse prostate there is very little ER α expression and most of it is

in the stromal compartment. ER β , on the other hand, is abundantly expressed in the epithelium of the adult mouse and human prostate. It is also expressed in the stroma and the infiltrating immune cells (Schulze & Barrack 1987, Brenner *et al.* 1990, Schulze & Claus 1990, Prins & Birch 1997, Tsurusaki *et al.* 2003). The circulating level of E₂ in males is similar to that in postmenopausal women, i.e. 30 pg/ml (Taylor *et al.* 2012). However, in the prostate, the most abundant estrogen is not E₂ but 3 β -Adiol (Weihua *et al.* 2002b).

Prostate cancer and ER

Studies on ER $\beta^{-/-}$ mice showed prostatic hyperplasia in 5-month-old mice and PIN lesions in mice older than 1 year (Weihua *et al.* 2002b). This was the first in a series of observations made in mouse models and cancer cell lines, demonstrating an anti-proliferative function of ER β . *In vitro* models for studying the role of ER β in prostate cancer are very artificial because prostate cancer cell lines do not express or express very low levels of ER β . It is always questionable as to whether results obtained by overexpression of ER β in a cancer cell line can be extrapolated to clinical situations. Nevertheless, interesting information about the antiproliferative effects of ER β has been obtained when ER β was introduced into prostate cancer cell lines. In 2008, Hurtado *et al.* (2008) found that overexpression of ER β in LNCaP cells caused G1 cell cycle arrest. More recently we found that when ER β is expressed in PC3 and 22RV1 cells, there is a decrease in the expression of proliferative and oncogenic factors (p45^{Skp2}, c-myc, and cyclin E) and an increase in cell-cycle arresting factors (p21^{WAF1}, cyclin-dependent kinase inhibitor 1 (CDKN1A), p27^{Kip1}, and cyclin-dependent kinase (Dey *et al.* 2012)). Thus ER β can inhibit proliferation in both androgen-dependent (LNCaP and 22RV1) and androgen-independent (PC3) prostate cancer cell lines.

In DU145 cells, ER β is activated by ICI 182 780, an antiestrogen. Treatment of DU145 cells with ICI 182 780 stabilized the tumor suppressor, Krüppel-like zinc finger transcription factor 5 (KLF5), thereby increasing the expression of the antiproliferative transcription factor, forkhead box protein O1 (FOXO1). The increase in transcription of the *FOXO1* gene involves the recruitment of KLF5-ER β -CBP (CREB-binding protein) complex to the promoter of *FOXO1*. The increase in transcription can be opposed by E₂, which promotes proteasome-dependent degradation of KLF5 (Nakajima *et al.* 2011). This is one possible mechanism through which ER β can oppose E₂-induced proliferation. Another anti-proliferative route

used by ER β was shown by Leung *et al.* (2006a) in DU145 cells. In these cells, ICI 182 780 in the presence of ER β opposed the action of NF κ B which led to decreased cell proliferation. In a recent study we have found that ER β promotes apoptosis in prostate by the upregulation of p53-upregulated modulator of apoptosis (PUMA) via transcriptional activation of FOXO3a (Dey *et al.* 2013).

Results obtained from prostate cancer cell lines point to an important role of ER β in repressing key oncogenes (PI3K, p45^{Skp2}, c-myc, and cyclin E; Dey *et al.* 2012) and increasing expression of antiproliferative genes like *PTEN* (Lindberg *et al.* 2011), *FOXO3*, *KLF5*, p21^{WAF1}, *CDKN1A*, and p27^{Kip1}; why has this not translated to a more enthusiastic embrace of ER β as a target in prostate cancer by urologists? The problem probably lies in the confusion over the phenotype of ER $\beta^{-/-}$ mice and the questions raised in laboratories where no prostatic phenotype was observed in ER $\beta^{-/-}$ mice (Antal *et al.* 2008).

As discussed above, exon 3 deletion does not create an ER $\beta^{-/-}$ mouse, so some of the controversies are related to inappropriate strategy for creating ER $\beta^{-/-}$ mice. A key clinical study published in 2008 should have dispelled all doubts about the beneficial role of ER β in protecting against prostate cancer. This study showed that expression of the fusion gene *TMPRSS2:ERG*, which is thought to be responsible for up to 80% of prostate cancers, is repressed by ER β . The study was a meta-analysis of biopsy samples collected from 455 prostate cancer patients in the Swedish Watchful Waiting cohort (1987–1999) and the USA-based Physicians Health Study cohort (1983–2003) and the effect of ER β was confirmed in NCI-H660 cancer cells, where the inhibition of *TMPRSS2:ERG* fusion is prevented by ER β but not ER α agonists (Setlur *et al.* 2008). Transmembrane protease, serine 2 (*TMPRSS2*) is an AR-regulated gene (Paoloni-Giacobino *et al.* 1997). In certain forms of prostate cancer, there is a chromosomal translocation followed by a gene fusion which results in *TMPRSS2* insertion into the promoter region of the erythroblast transformation-specific (ETS) family of transcription factors, *ERG* and *ETV1*. Expression of this fusion gene leads to castration resistant prostate cancer (Tomlins *et al.* 2005, Yu *et al.* 2010). To date although there are safe and effective ER β agonists (Malamas *et al.* 2004), no ER β agonist has been tested in prostate cancer patients. A similar remarkable effect of ER β agonists has been observed in the TRAMP mouse (transgenic mouse model for prostate cancer; Gingrich *et al.* 1996). In this mouse model of prostate cancer, dietary genistein, is an ER β -dependent pathway, reduced the incidence of prostate

cancer and the severity of the invasive cancer was increased in ER $\beta^{-/-}$ mice (Slusarz *et al.* 2012).

One other confounding factor occurring in prostate cancer is the expression of the ER β splice variant ER β 2. As rodents do not express this splice variant, conclusions about its role in prostate cancer have come from its measurement in prostate cancers (Fujimura *et al.* 2001, Lee *et al.* 2013) and from overexpression in cell lines (Dey *et al.* 2012). So far, there is agreement that the expression of ER β 2 in prostate cancer is associated with a poor prognosis (Leung *et al.* 2010). The reason for the negative effects of ER β 2 is not clear but if it has actions, which are opposite to those of ER β (Dey *et al.* 2012), then it might promote survival under the conditions of hypoxia. One of the mechanisms through which ER β exerts antiproliferative effects is its facilitation of HIF1 α degradation (Mak *et al.* 2013). The effects of ER β 2 on the stability of HIF1 α remain to be determined.

Understanding androgen metabolism in prostate cancer

As is the case with metabolites of estrogen, metabolites of DHT may play important physiological roles. DHT is more a potent androgen than testosterone, so inhibition of the enzyme which catalyzes the conversion of testosterone to DHT should reduce AR activity. However, the use of 5 α -reductase inhibitors showed an important negative consequence of inhibition of this enzyme. In the Prostate Cancer Prevention Trial (PCPT), patients treated with the 5 α -reductase inhibitor, finasteride, showed a decrease of 25% in prostate cancer prevalence, but a higher rate of Gleason grade (7–10) (37%) over the placebo-treated group (22.2%). A possible explanation for this aberration is that the inhibition of synthesis of DHT also results in the loss of the DHT metabolite, 3 β -Adiol, a prostatic ligand of ER β (Imamov *et al.* 2004, Briganti 2009). More recently, the long-term treatment of prostate cancer with finasteride has revealed that this drug has no effect on prostate cancer (McCarthy 2013). Treatment of mice with 3 β -Adiol resulted in a decreased cell proliferation in the ventral prostate of wild type but not in ER β knockout animals (Weihua *et al.* 2002a).

Additional beneficial effects of ER β in prostate cancer

In addition to its antiproliferative actions, ER β is also anti-migratory and is an inhibitor of epithelial to mesenchymal transition (EMT). In one of the first cell-based studies demonstrating an anti-migratory effect of ER β , adenovirus-mediated delivery of ER β led to a strong decrease in

the invasiveness of DU145 cells compared with the control transfected cells (Cheng *et al.* 2004). Metastasis of ER β -expressing xenografts of DU145 was blocked by treatment with 3 β -Adiol (Guerini *et al.* 2005). Seven years later, Grubisha *et al.* (2012) investigated the mechanism through which ER β , activated by 3 β -Adiol, opposes cell migration and motility of DU145 cells. They found that this anti-migratory effect of ER β is opposed by transforming growth factor β (TGF β) signal-derived reactive oxygen species (ROS) such as H₂O₂. Cyclooxygenase 2 (Cox2) in the stromal cells is responsible for the production of H₂O₂, which, in turn, prevents binding of ER β to the promoter of E-cadherin. Agents neutralizing H₂O₂ and Cox2 could reverse the inhibition of ER β by ROS (Fig. 2; Grubisha *et al.* 2012).

Mak *et al.* (2010) showed that ER β promotes an epithelial phenotype and opposes EMT in both androgen-dependent and androgen-independent cell lines. The authors suggested that TGF β and hypoxia may cause a decrease in ER β expression and promote migration and invasion, features of EMT. The paper highlights the point that the Hif1 α /VEGF-A/Snail pathway, required for the genesis of EMT, is opposed by ER β (Mak *et al.* 2010). Reduced EMT and migration occurs in xenographs of PC3 and 22Rv1 cells when they are induced to overexpress ER β . We found that ER β represses the metastasis factor Runx2, the EMT factor Slug and β -catenin (Dey *et al.* 2012). ER β is expressed in BPH and low Gleason grade prostate cancer, but is reduced above Gleason grade 3 (Yang *et al.* 2007, Mak *et al.* 2010). However, there is a report that ER β expression re-emerges in metastatic prostate cancer (Lai *et al.* 2004).

Colon

The Women's Health Initiative Study in 2002 not only showed an increased risk of stroke and heart disease in women on hormone replacement therapy, but it also showed a reduced risk of colon cancer (Rossouw *et al.* 2002). In agreement with this finding, a study by Cleveland *et al.* showed that disruption of estrogen signaling increased intestinal neoplasia in Apc (Min/+) mice. Furthermore, it appears that ER β is important for the tumor suppressive effect, while disruption of ER α signaling has no effect (Cleveland *et al.* 2009). Several other studies support the tumor suppressive effect of ER β in colon. One study showed increased induction of mucin-depleted foci in ER $\beta^{-/-}$ mice (Saleiro *et al.* 2010), while another study showed that ER $\beta^{-/-}$ mice developed more colitis-associated neoplasia (Saleiro *et al.* 2012). In agreement

Figure 2

Pathways regulated by ER β . ER β upon binding to its ligand 3 β -Adiol/8 β -VE2/DPN gets activated and transcriptionally upregulates downstream target genes such as FOXO3a and PHD2. The target genes carry ER β binding elements such as ERE or AP1-ERE-half-site upstream or downstream of the transcriptional start site. The activated FOXO3a in turn transcriptionally upregulates PUMA, p21, and p27. PHD2 inhibits HIF1 α by prolyl

hydroxylation of the oxygen-dependent degradation domain (ODDD), which targets HIF1 α proteins for proteasomal degradation by promoting their interaction with von Hippel–Lindau (VHL). ER β also inhibits proliferation of genes such as c-MYC and p45^{Skp2} by an as yet unknown mechanism. Moreover, some of the EMT and bone metastasis genes, such as β -catenin, SLUG/SNAIL and TWIST, are opposed by ER β .

with this finding ER β has been shown to stimulate anti-inflammatory networks in colon cancer cell lines (Edvardsson *et al.* 2011). Expression of ER β in colon cancer is correlated to prognosis where a decreased expression is found in higher grade and larger tumors (Konstantinopoulos *et al.* 2003, Rudolph *et al.* 2012), and the level is inversely correlated with more advanced Dukes' staging (Jassam *et al.* 2005). A role of ER α in colon cancer has not been found so far. A hint that it may be involved in progression comes from a study by Armstrong *et al.* using azoxymethane to induce colon cancer. The study showed that ER β expression decreases in colonic epithelial cells as the cancer progresses, while expression of ER α increases, suggesting a possible role of ER α in colorectal cancer progression (Armstrong *et al.* 2013). From the phenotype of ER $\beta^{-/-}$ mice, ER β in the colon appears to decrease proliferation and increase apoptosis, suggesting a tumor suppressive and tumor preventive effect (Wada-Hiraike *et al.* 2006). ER β has also been shown to change the micro RNA pool in human

colorectal cancer cells (Edvardsson *et al.* 2013) and to regulate miR-135b and mismatch repair gene expressions in colorectal cells (He *et al.* 2012).

The incidence of colorectal cancer is much lower in Asian countries than in the Western world, an effect that possibly can be explained by a high dietary intake of phytoestrogens in the form of soy (Lechner *et al.* 2005). The protective effects of soy are thought to be mediated by binding of genistein, the main phytoestrogen in soy, to ER β . Genistein binds to ER β with 20-fold higher affinity than ER α (Kuiper *et al.* 1997). Genistein is also a tyrosine kinase inhibitor and some of its actions in the colon may be elicited through its inhibition of tyrosine kinase (Wang *et al.* 2012). Using the Apc (Min/+) mouse model of colorectal cancer, Javid *et al.* (2005) showed that coumestrol, another phytoestrogen with no tyrosine kinase inhibitory activity, as well as E₂ inhibit intestinal tumorigenesis in ovariectomized mice. Both clinical studies and animal experiments suggest that phytoestrogens and E₂ have a preventive effect on colorectal cancer by acting through ER β .

CNS

ER β and neuro-protection ERs have been shown to affect the brain in several ways. ER α is responsible for imprinting of the male brain during embryonic development (Naftolin 2008), while ER β is involved in cortical layering and migration of interneurons in the fetal period and neonatal period (Wang *et al.* 2003, Fan *et al.* 2006).

ER β agonists can modulate tryptophan hydroxylase 1 mRNA expression in the raphe nucleus and cause antidepressant-like effects (Clark *et al.* 2012, Suzuki *et al.* 2013). The mechanism involves increase of serotonergic activity by regulating synthesis of serotonin. In addition, serotonin receptors are regulated by estrogens in an ER β -dependent way (Osterlund 2010). Both ER α and ER β appear to have protective roles in the brain, where E₂ attenuates secondary injury via ER α in the rat brain following subarachnoid hemorrhage (Raval *et al.* 2013). In the paraventricular nucleus (PVN) and rostroventrolateral medulla, ER β protects against aldosterone/salt-induced hypertension in female rats by inhibiting mineralocorticoid receptor induced increases in ROS (Xue *et al.* 2013). Ma *et al.* (2013) have shown that dietary soy and isoflavones by reducing VEGF are neuroprotective in cerebral ischemia. Not much is known about the ER β splice variants in the brain with the exception of ER β 5, which is increased in glioma cells (Li *et al.* 2013) where its expression is induced by hypoxia and HIF1 α . Furthermore, in these cells, ER β 5 reduced cell proliferation and AKT signaling and increased PTEN expression and may play a role in repression of tumor growth.

ER β has profound effects on neuroinflammation and ER β -selective ligands are very effective in the treatment of neuroinflammation in mouse models (Kumar *et al.* 2013, Wu *et al.* 2013). In addition to multiple sclerosis, neuroinflammation plays a role in the etiology and/or progression also of other CNS diseases. These include Parkinson's disease (Koutsilieri *et al.* 2013), Alzheimer's disease (Fuster-Matanzo *et al.* 2013), bipolar disorder (Stertz *et al.* 2013), and autism (Theoharides *et al.* 2013). In neurodegenerative diseases, one of the mechanisms of progression and damage to healthy neurons in the vicinity of lesions is overactive microglia. Microglia are the policeman of the brain sensing and kill diseased cells and foreign invaders by secreting powerful cytokines (Benjamins 2013). In this process, neighboring normal cells can be damaged. ER β but not ER α is expressed in microglia and ER β -selective ligands can prevent microglial activation and secretion of cytokines (Wu *et al.* 2013). The anti-inflammatory effects of ER β are not confined to microglia.

ER β is also expressed in T cells, in particular in Th1 cells, where it regulates indolamine-2,3-dioxygenase (IOD), the rate-limiting step in tryptophan degradation (Harden & Egilmez 2012). This enzyme causes cell death by depleting tryptophan and by cytotoxicity of the tryptophan metabolite, kynurenine. IOD is an important mediator of immune tolerance via inhibition of Th1 responses and its loss is associated with autoimmune disease (McGaha *et al.* 2012). The immunosuppressive property of IOD is essential for immune tolerance and in inhibition of Th1 cells that are responsible for experimental autoimmune encephalitis, a mouse model of multiple sclerosis.

ERs and feeding behavior E₂ has long been recognized as an anorexigenic factor, but the mechanisms behind this effect are still being investigated (Barros & Gustafsson 2011, Eckel 2011). ER α and/or ER β are expressed in the regions of the hypothalamus that regulate feeding and satiety (Roepke 2009; Table 1).

In the arcuate nucleus, ER α is expressed in the pro-opiomelanocortin neurons which secrete two anorexigenic peptides, α -melanocyte-stimulating hormone, and cocaine- and amphetamine-regulated

Table 1 Feeding peptides regulated by estrogen receptors (ERs) in the hypothalamus

Nucleus of the hypothalamus	ER expressed	Peptide expressed	Function
Arcuate nucleus	ER α	Cocaine- and amphetamine-regulated transcripts	Anorexigenic
		α -Melanocyte-stimulating hormone	Anorexigenic
Arcuate nucleus	ER α and ER β	Neuropeptide Y Agouti gene-related protein	Orexigenic Orexigenic
Lateral	None	Melanin-concentrating hormone (MCH)	Orexigenic
Dorsomedial	ER α and ER β	Orexin neurons	Orexigenic
		Neuropeptide Y Cocaine- and amphetamine-regulated transcripts	Orexigenic Anorexigenic
Paraventricular nucleus	ER β	Urocortin	Anorexigenic (repressed by ER β)
Ventromedial	ER α	Brain-derived neurotrophic factor	Anorexigenic
		Urocortin	Anorexigenic

transcripts (CART). Both ER α and ER β are expressed in the neuropeptide Y (NPY) neurons (Roepke 2009, de Souza *et al.* 2011) which secrete NPY and agouti gene-related protein, two orexigenic peptides (Pillot *et al.* 2011).

In the lateral hypothalamus, there are two types of orexigenic neurons, the melanin-concentrating hormone neurons and the orexin neurons (Horvath 2006, Nahon 2006). Neither of the ERs have been identified in either of these neurons, and it is thought that the regulation of feeding and satiety by ERs in the lateral hypothalamus is indirect (Muschamp & Hull 2007). The dorsomedial hypothalamus expresses both ER α and ER β and secretes both the orexigenic peptide, NPY, and the anorexigenic peptide CART. In the PVN, which responds to glucose and lipids, ER β is abundant and decreases the expression of urocortin, a potent anorexigenic peptide (Haeger *et al.* 2006). Stimulation of this nucleus increases food intake (Horvath 2006, Roepke 2009). The ventromedial hypothalamus is mainly regulated by ER α and is an important inhibitor of satiety and food intake (Xu *et al.* 2003). Inhibition of ER α signaling in this nucleus is associated with hyperphagia, obesity, decreased glucose tolerance, and reduced energy expenditure (Musatov *et al.* 2007). Brain-derived neurotrophic factor (Gotoh *et al.* 2013) and urocortin (Chen *et al.* 2012) are the anorexigenic peptides secreted by neurons of the ventromedial hypothalamus.

Although the precise role of each ER in the regulation of feeding and satiety is still under investigation, it seems that ER α plays a dominant role in anorexia and ER β in the stimulation of feeding.

Conclusions

This review covers recent advances in estrogen signaling with a focus on ER β . Since its discovery in 1996, ER β has changed our understanding of the mechanisms of estrogen action. It appears that the two ERs often have an antagonistic or yin/yang relationship, e.g. in control of cell proliferation where ER α is pro-proliferative whereas ER β is anti-proliferative. Accordingly, ER β is a promising potential target for anti-cancer drugs. Furthermore, ER β is an important mediator of estrogen action in the CNS, again offering promise as a drug target in the treatment of, e.g. multiple sclerosis, Parkinson's disease, and eating disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

This work was supported by The Cancer Prevention and Research Institute of Texas (CPRIT) grant numbers HIRP100680 and RP110444, the Texas Emerging Technology Fund under Agreement no. 300-9-1958, the Robert A Welch Foundation (E-0004), and the Swedish Cancer Fund.

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Received in final form 4 September 2013

Accepted 9 September 2013

Accepted Preprint published online 12 September 2013