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, ERβ usually is a tumor suppressor and ERα is an oncogene. ERβ regulates genes in several key pathways including tumor suppression (p53, PTEN); metabolism (PI3K); survival (Akt); proliferation pathways (p45Skp2, cMyc, and cyclin E); cell-cycle arresting factors (p21WAF1, cyclin-dependent kinase inhibitor 1 (CDKN1A)), p27Kip1, 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. ERα 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 ERα or ERβ. ERβ 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
- 3β-Adiol
- cell cycle

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 17β-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), ERα and ERβ, 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-3β,17β-diol (Aβ-diol), synthesized from
the adrenal steroid dehydroepiandrosterone, and 5α-androstane-3β,17β-diol (3β-Adiol), synthesized from the hormone 5α-dihydrotestosterone (DHT). Estrogenic actions of 3β-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 ERβ that the estrogenic actions of these steroids were understood (Weihua et al. 2002a). Unlike E2, 3β-diol and 3β-Adiol do not circulate at high concentrations and serve more paracrine and autocrine functions.

ERα and ERβ are transcribed from two genes (ESR1 and ESR2 respectively) that are located on different chromosomes (Gosden et al. 1986, Enmark et al. 1997). ERα was discovered more than 50 years ago by Elwood Jensen and the gene was cloned in 1985 (Walter et al. 1985). ERβ 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). ERα protein composed of 595 amino acids (White et al. 1987) is a bit longer than the 530 amino acid-long ERβ. ERα and ERβ 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). ERβ binds better than ERα 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 ERβ, another splice variant was discovered and named ERβcx/2 (Ogawa et al. 1998). Soon after other splice variants – ERβ4, and ERβ5 – were discovered. ERβ4 were identified from a testis library and ERβ5 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 kB (NFkB); Webb et al. 1999) and influence the transcription of genes normally regulated by these rather ubiquitous transcription factors. ERα and ERβ can have opposite effects at these sites: ERβ-tamoxifen acting through AP1 can activate target genes, whereas ERα-tamoxifen may cause repression of the target genes (Webb et al. 2003).

The action of ERβ at AP1 sites or EREs is determined by the presence of coactivators. In the absence of E2, the co-activator Tip60 enhances ERβ transcriptional activity at AP1 sites, but represses it at EREs. ERβ-selective ligands abolish the repressive actions of ERβ at AP1, while the

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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 ERα and ERβ are highly conserved and the NTDs are the least conserved. The NTD of ERα is shorter than that of ERβ.
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 ERβ 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, ERβ 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 ERβ 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 landscape of ERα and ERβ binding sites has revealed that while ERα is predominantly found at ERE elements (Carroll et al. 2005), whereas ERβ 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 ERβ than it is in ERα. The important consequence of this finding is that although removal of one zinc finger of ERα creates an inactive protein, the same mutation leaves a functional ERβ (Price et al. 2001). Thus, it is not surprising that a mouse in which the second zinc finger (ERβ 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 ERs by kinases is thought to play an important role in breast cancer (reviewed in Thomas & Gustafsson (2011)).

Physiological and pathophysiological actions of ERs


Breast and ER

In the normal mammary gland, ER is essential for branching morphogenesis and in ERα−/− 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), ERα is expressed in <10% of mammary epithelial cells. ERβ 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). ERβ−/− mice revealed that ERβ is not required for ducital 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 ERα determines the final course and progression of the tumor (Njiaju & Olopade 2012). About 70% of breast cancers are ERα-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 ERα expression or mutations in ERα cannot account for all resistance as these occur only in ~15% of patients (Gutierrez et al. 2005).

The involvement of ERβ in breast cancer is still being investigated. As reviewed by Murphy & Leygue (2012), several studies have demonstrated that ERβ expression in breast cancer, irrespective of presence of ERα, is associated with increased response to endocrine therapy, but others have not found this (Murphy & Watson 2006). The functions of ERβ splice variants in breast cancer are another unresolved issue. ERβ2 (ERβcx) is a C-terminally spliced form of ERβ discovered by Ogawa et al. (1998). Some clinical studies have associated the expression of ERβ2 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 ERβ2 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 ERβ and some express ERβcx 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 ERα and ERβ. One of the curious results of these studies is that when ER-negative breast cancer cells, MDA-MB-231, are engineered to express ERα or ERβ, both receptors can inhibit proliferation and invasion. The difference is that ERα requires the presence of ligand for its actions but ERβ does not (Lazennc et al. 2001). Studies in the T47D breast cancer cell line revealed that in the presence of E2, ERα regulates expression of cell cycle genes such as c-MYC (MYC), cyclin E, cyclin D, and p45Skp2 (Strom et al. 2004). ERα 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 ERα 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, ERβ augmented the antiproliferative effects of tamoxifen (Lattrich et al. 2013).

Many studies show that loss of ERβ 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 ERβ (Shaaban et al. 2003, Esslimani-Sahla et al. 2004, Hopp et al. 2004). However, there are also studies which show high ERβ expression in ductal cancer and in these studies, ERβ expression is associated with poor prognosis (Markey et al. 2009, Kim et al. 2012). The reasons for the reported differences in the contribution of ERβ 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 ERβ in breast cancer, a novel mechanism through which ERβ 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, ERβ 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 (ERβ1/ERβ2/ERα) which determines the overall effect of ERβ 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 ERα 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 ERα expression and most of it is
in the stromal compartment. ERβ, 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 Δ3-Adiol (Weihua et al. 2002b).

**Prostate cancer and ER**

Studies on ERβ−/− 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 ERβ. *In vitro* models for studying the role of ERβ in prostate cancer are very artificial because prostate cancer cell lines do not express or express very low levels of ERβ. It is always questionable as to whether results obtained by overexpression of ERβ in a cancer cell line can be extrapolated to clinical situations. Nevertheless, interesting information about the antiproliferative effects of ERβ has been obtained when ERβ was introduced into prostate cancer cell lines. In 2008, Hurtado et al. (2008) found that overexpression of ERβ in LNCaP cells caused G1 cell cycle arrest. More recently we found that when ERβ is expressed in PC3 and 22RV1 cells, there is a decrease in the expression of proliferative and oncogenic factors (p45Skp2, c-myc, and cyclin E) and an increase in cell-cycle arresting factors (p21WAF1, cyclin-dependent kinase inhibitor 1 (CDKN1A), p27Kip1, and cyclin-dependent kinase (Dey et al. 2012)). Thus ERβ can inhibit proliferation in both androgen-dependent (LNCaP and 22RV1) and androgen-independent (PC3) prostate cancer cell lines.

In DU145 cells, ERβ 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-ERβ-CBP (CREB-binding protein) complex to the promoter of FOXO1. The increase in transcription can be opposed by E2, which promotes prostatesome-dependent degradation of KLF5 (Nakajima et al. 2011). This is one possible mechanism through which ERβ can oppose E2-induced proliferation. Another anti-proliferative route used by ERβ was shown by Leung et al. (2006a) in DU145 cells. In these cells, ICI 182 780 in the presence of ERβ opposed the action of NFkβ which led to decreased cell proliferation. In a recent study we have found that ERβ 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 ERβ in repressing key oncogenes (PI3K, p45Skp2, c-myc, and cyclin E; Dey et al. 2012) and increasing expression of antiproliferative genes like PTEN (Lindberg et al. 2011), FOXO3, KLF5, p21WAF1, CDKN1A, and p27Kip1; why has this not translated to a more enthusiastic embrace of ERβ as a target in prostate cancer by urologists? The problem probably lies in the confusion over the phenotype of ERβ−/− mice and the questions raised in laboratories where no prostatic phenotype was observed in ERβ−/− mice (Antal et al. 2008).

As discussed above, exon 3 deletion does not create an ERβ−/− mouse, so some of the controversies are related to inappropriate strategy for creating ERβ−/− mice. A key clinical study published in 2008 should have dispelled all doubts about the beneficial role of ERβ 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 ERβ. 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 ERβ was confirmed in NCI-H660 cancer cells, where the inhibition of TMPRSS2:ERG fusion is prevented by ERβ but not E2 agonists (Setlur et al. 2008). Transmembrane protease, serine 2 (TMPRSS2) is an AR-regulated gene (Paolini-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 ERβ agonists (Malamas et al. 2004), no ERβ agonist has been tested in prostate cancer patients. A similar remarkable effect of ERβ 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 ERβ-dependent pathway, reduced the incidence of prostate
cancer and the severity of the invasive cancer was increased in ER\(^{-/-}\) mice (Slusarz et al. 2012).

One other confounding factor occurring in prostate cancer is the expression of the ER\(^{-}\) splice variant ER\(^{b}\). 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\(^{b}\) in prostate cancer is associated with a poor prognosis (Leung et al. 2010). The reason for the negative effects of ER\(^{b}\) is not clear but if it has actions, which are opposite to those of ER\(^{a}\) (Dey et al. 2012), then it might promote survival under the conditions of hypoxia. One of the mechanisms through which ER\(^{b}\) exerts antiproliferative effects is its facilitation of HIF1\(\alpha\) degradation (Mak et al. 2013). The effects of ER\(^{b}\) on the stability of HIF1\(\alpha\) 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 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 5α-reductase inhibitors showed an important negative consequence of inhibition of this enzyme. In the Prostate Cancer Prevention Trial (PCPT), patients treated with the 5α-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, 3β-Adiol, a prostatic ligand of ER\(^{b}\) (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 3β-Adiol resulted in a decreased cell proliferation in the ventral prostate of wild type but not in ER\(^{b}\) knockout animals (Weihua et al. 2002a).

Additional beneficial effects of ER\(^{b}\) in prostate cancer

In addition to its antiproliferative actions, ER\(^{b}\) 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\(^{b}\), adenovirus-mediated delivery of ER\(^{b}\) led to a strong decrease in the invasiveness of DU145 cells compared with the control transfected cells (Cheng et al. 2004). Metastasis of ER\(^{b}\)-expressing xenografts of DU145 was blocked by treatment with 3β-Adiol (Guerni et al. 2005). Seven years later, Grubisha et al. (2012) investigated the mechanism through which ER\(^{b}\), activated by 3β-Adiol, opposes cell migration and motility of DU145 cells. They found that this anti-migratory effect of ER\(^{b}\) is opposed by transforming growth factor β (TGF\(\beta\)) signal-derived reactive oxygen species (ROS) such as H\(_2\)O\(_2\). Cyclooxygenase 2 (Cox2) in the stromal cells is responsible for the production of H\(_2\)O\(_2\), which, in turn, prevents binding of ER\(^{b}\) to the promoter of E-cadherin. Agents neutralizing H\(_2\)O\(_2\) and Cox2 could reverse the inhibition of ER\(^{b}\) by ROS (Fig. 2; Grubisha et al. 2012).

Mak et al. (2010) showed that ER\(^{b}\) promotes an epithelial phenotype and opposes EMT in both androgen-dependent and androgen-independent cell lines. The authors suggested that TGF\(\beta\) and hypoxia may cause a decrease in ER\(^{b}\) expression and promote migration and invasion, features of EMT. The paper highlights the point that the Hif1α/VEGF-A/Snail pathway, required for the genesis of EMT, is opposed by ER\(^{b}\) (Mak et al. 2010). Reduced EMT and migration occurs in xenographs of PC3 and 22Rv1 cells when they are induced to overexpress ER\(^{b}\). We found that ER\(^{b}\) represses the metastasis factor Runx2, the EMT factor Slug and β-catenin (Dey et al. 2012). ER\(^{b}\) 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\(^{b}\) 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\(^{a}\) is important for the tumor suppressive effect, while disruption of ER\(^{b}\) signaling has no effect (Cleveland et al. 2009). Several other studies support the tumor suppressive effect of ER\(^{a}\) in colon. One study showed increased induction of mucin-depleted foci in ER\(^{a/-}\) mice (Saleiro et al. 2010), while another study showed that ER\(^{a/-}\) mice developed more colitis-associated neoplasia (Saleiro et al. 2012). In agreement
with this finding ERβ has been shown to stimulate anti-inflammatory networks in colon cancer cell lines (Edvardsson et al. 2011). Expression of ERβ 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 ERα 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 ERβ expression decreases in colonic epithelial cells as the cancer progresses, while expression of ERα increases, suggesting a possible role of ERα in colorectal cancer progression (Armstrong et al. 2013). From the phenotype of ERβ−/− mice, ERβ in the colon appears to decrease proliferation and increase apoptosis, suggesting a tumor suppressive and tumor preventive effect (Wada-Hiraike et al. 2006). ERβ 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 ERβ. Genistein binds to ERβ with 20-fold higher affinity than ERα (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 ERβ.

Figure 2
Pathways regulated by ERβ. ERβ upon binding to its ligand 3β-Adiol/8β-VE2/DPN gets activated and transcriptionally upregulates downstream target genes such as FOXO3a and PHD2. The target genes carry ERβ 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 HIF1α by prolyl hydroxylation of the oxygen-dependent degradation domain (ODDD), which targets HIF1α proteins for proteasomal degradation by promoting their interaction with von Hippel–Lindau (VHL). ERβ also inhibits proliferation of genes such as c-MYC and p45Skp2 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 ERβ.

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CNS

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

ERβ 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 ERβ-dependent way (Osterlund 2010). Both ERα and ERβ appear to have protective roles in the brain, where E₂ attenuates secondary injury via ERα in the rat brain following subarachnoid hemorrhage (Raval et al. 2013). In the paraventricular nucleus (PVN) and rostroventrolateral medulla, ERβ 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 ERβ splice variants in the brain with the exception of ERβ5, which is increased in glia cells (Li et al. 2013) where its expression is induced by hypoxia and HIF1α. Furthermore, in these cells, ERβ5 reduced cell proliferation and AKT signaling and increased PTEN expression and may play a role in repression of tumor growth.

ERβ has profound effects on neuroinflammation and ERβ-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. ERβ but not ERα is expressed in microglia and ERβ-selective ligands can prevent microglial activation and secretion of cytokines (Wu et al. 2013). The anti-inflammatory effects of ERβ are not confined to microglia.

ERβ 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.

**ERα 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). ERα and/or ERβ are expressed in the regions of the hypothalamus that regulate feeding and satiety (Roepke 2009; Table 1).

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

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

<table>
<thead>
<tr>
<th>Nucleus of the hypothalamus</th>
<th>ERα expressed</th>
<th>Peptide expressed</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcuate nucleus</td>
<td>ERα and ERβ</td>
<td>Cocaine- and amphetamine-regulated transcripts</td>
<td>Anorexigenic</td>
</tr>
<tr>
<td>Lateral</td>
<td>None</td>
<td>α-Melanocyte-stimulating hormone</td>
<td>Orexigenic</td>
</tr>
<tr>
<td>Dorsomedial</td>
<td>ERα and ERβ</td>
<td>Neuropeptide Y Agouti gene-related protein melanin-concentrating hormone (MCH)</td>
<td>Orexigenic</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td>ERβ</td>
<td>Neuropeptide Y Cocaine- and amphetamine-regulated transcripts</td>
<td>Orexigenic</td>
</tr>
<tr>
<td>Ventromedial</td>
<td>ERα</td>
<td>Urocortin</td>
<td>Orexigenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain-derived neurotrophic factor Urocortin</td>
<td>Anorexigenic (repressed by ERβ)</td>
</tr>
</tbody>
</table>

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transcripts (CART). Both ERα and ERβ 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 ERα and ERβ and secretes both the orexigenic peptide, NPY, and the anorexigenic peptide CART. In the PVN, which responds to glucose and lipids, ERβ 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 ERα and is an important inhibitor of satiety and food intake (Xu et al. 2003). Inhibition of ERα 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 ERα plays a dominant role in anorexia and ERβ in the stimulation of feeding.

Conclusions

This review covers recent advances in estrogen signaling with a focus on ERβ. Since its discovery in 1996, ERβ 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 ERα is pro-proliferative whereas ERβ is anti-proliferative. Accordingly, ERβ is a promising potential target for anti-cancer drugs. Furthermore, ERβ 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.

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