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1. Introduction

ERs are members of the nuclear receptor superfamily and have a broad range of biological roles, such as growth, differentiation and physiology of the reproductive system (Pearce & Jordan, 2004). These enzymes also have roles in non-reproductive tissues such as bone, cardiovascular system, brain and liver (Heldring et al., 2007). Until 1996, only one human estrogen receptor (ER) was known. That year Kuiper et al. discovered a novel nuclear estrogen receptor cloned from rat prostate. The known ER was renamed and called ERα to differentiate it from the novel ER, ERβ (Kuiper et al., 1996). The complete human ERβ cDNA sequence was published in 1998 by Ogawa et al (Ogawa et al., 1998a).

1.1 Estrogen receptors and signalling function

Estrogen receptors are products of distinct genes localized on different chromosomes; human ERα is encoded on chromosome 6q24-q27 (Gosden et al., 1986), while the gene encoding human ERβ is localized on chromosome 14q22-q24 (Enmark et al., 1997). Despite their distinct localization, the gene organization of the two receptors is well conserved. ESR1 (ERα) and ESR2 (ERβ) genes contain eight exons, separated by seven long intronic sequences. As members of the nuclear receptor superfamily, ERs contain 6 regions in their protein structure common for all nuclear receptors, namely: A, B, C, D, E and F which form functionally different but interacting domains (figure 1). Exon 1 encodes the A/B region in ERα and ERβ, exons 2 and 3 encode part of the C region. Exon 4 encodes the remaining part of region C, the whole of region D and part of region E. Exons 5 to 8 contain the rest of region E and region F is encoded by part of exon 8 [reviewed in (Ascenzi et al., 2006)].
Although ERα and ERβ are encoded separately they share a high degree of homology. The most conserved domain among ERs is the DNA binding domain (DBD) corresponding to the C region, with 96% homology between α and β ER subtypes. The DBD is responsible for binding to specific DNA sequences (Estrogen Responsive Elements or EREs) in target gene promoter regions. High structure similarity in this region suggests similar target promoter sites for both receptors. The A/B region located in the N-terminus of the protein encompasses the AF-1 domain responsible for ligand independent transactivation. The AF-1 domain is the least conserved part among the two ERs with only 30% homology and it is functional only in the ERα subtype (Hall & McDonnell, 1999). The C-terminus of the protein contains the ligand dependent transactivation domain AF-2, the ligand binding domain (LBD) and the homo-/heterodimerization site. Homology between the E/F regions of both proteins is only 53%, explaining differences in ligand binding affinities between the two receptors. The hinge region localized in the D domain contains the nuclear localization signal of the ERs as well as post translational modification sites (Sentis et al., 2005). Information on structure/function relationship of this region is very limited and it appears to be a variable and not well conserved part of the ERs (only 30% homology).

Estrogen (E2) binding to the receptor induces the LBD to undergo a conformational change, upon which the receptor dimerizes, binds to DNA, and stimulates gene expression (Cowley et al., 1997; Katzenellenbogen & Katzenellenbogen, 2000).

1.2 Estrogen receptor distribution

The distribution of ERs varies both between and within human tissues (see Table 1). The cardiovascular system, brain, and bones express both receptors. ERβ is predominant in the male reproductive system. Expression of both ERα and ERβ has been found in all major human uterine cell types at every menstrual stage. However, expression varies from cell-type to cell-type with expression of ERα mRNA generally being higher than that of ERβ (Matsuzaki et al., 1999). Changes in expression of estrogen receptors has been found in certain tumour types. Normal mammary tissue in man predominantly expresses ERβ mRNA, whereas most ER-positive breast tumours appear to exhibit increased ratios of ERα/ERβ (Leygue et al., 1998).
Likewise, an increased ratio of ERα/ERβ mRNA has been demonstrated in ovarian carcinoma compared with normal tissue or cysts (Bardin et al., 2004). High concentrations of ERβ have also been found within the human gut (Enmark et al., 1997).

Therefore, the ultimate estrogenic effect of a certain compound on cells or tissues will be dependent on the receptor phenotype of these cells or tissues.

### Table 1. Tissue distribution of ER subtypes in humans.

<table>
<thead>
<tr>
<th>Organ/Tissue</th>
<th>Human ER subtype</th>
<th>Organ/Tissue</th>
<th>Human ER subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>ERα ✓</td>
<td>ERβ ✓</td>
<td>Adrenal</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>✓</td>
<td>Kidney</td>
</tr>
<tr>
<td>Vascular</td>
<td>✓</td>
<td>-</td>
<td>Prostate</td>
</tr>
<tr>
<td>Bladder</td>
<td>-</td>
<td>✓</td>
<td>Testes</td>
</tr>
<tr>
<td>Epididymus</td>
<td>-</td>
<td>✓</td>
<td>Brain</td>
</tr>
<tr>
<td>Pituitary</td>
<td>-</td>
<td>✓</td>
<td>Thymus</td>
</tr>
<tr>
<td>Liver</td>
<td>✓</td>
<td>-</td>
<td>Breast</td>
</tr>
<tr>
<td>Muscle</td>
<td>-</td>
<td>-</td>
<td>Uterus</td>
</tr>
<tr>
<td>Fat</td>
<td>-</td>
<td>-</td>
<td>Endometrium</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>-</td>
<td>✓</td>
<td>Vagina</td>
</tr>
<tr>
<td>Colon</td>
<td>-</td>
<td>✓</td>
<td>Fallopian tube</td>
</tr>
<tr>
<td>Small intestine</td>
<td>-</td>
<td>✓</td>
<td>Ovary</td>
</tr>
<tr>
<td>Bone</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

**1.3 Mechanism of estrogen action**

Estrogens act on target tissues by binding to ERs. These proteins function as transcription factors when they are activated by a ligand. Biological action of ERs involves complex and broad mechanisms. For the ERs two main mechanisms of action have been described, including a genomic and a non-genomic pathway (Figure 2).

The *genomic action* of ERs occurs in the nucleus of the cell, when the receptor binds specific DNA sequences directly ("direct activation" or classical pathway) or indirectly ("indirect activation" or non-classical pathway). In the absence of ligand, ERs are associated with heat-shock proteins. The Hsp90 and Hsp70 associated chaperone machinery stabilizes the ligand binding domain (LBD) and makes it accessible to the ligand. Liganded ER dissociates from the heat-shock proteins, changes its conformation, dimerizes, and binds to specific DNA sequences called estrogen responsive elements (EREs) in order to regulate transcription (Nilsson et al., 2001). In the presence of the natural ligand E2, ER induces chromatin remodelling and increases transcription of estrogen regulated genes (Berno et al., 2008).
Fig. 2. Mechanisms of estrogen receptor (ER) action. In the direct activation, ERs dimerize after ligand binding and attach to the ERE in the promoter of target genes. In the indirect activation manner, ligand-bound ER dimers might activate transcription of non-ERE containing genes, by binding to other transcription factors (e.g. AP1 or SP1). In the non-genomic pathway, ligand-bound ERs interact directly with and change the function of proteins some of which function as ‘second messengers’ (SM). ERs can also be activated by phosphorylation in the absence of ER ligands (ligand-independent activation). Based on Morani et al. 2008 (Morani et al., 2008).

In the non-classical pathway, AP-1 (DeNardo et al., 2005) and SP-1 (Kim et al., 2003) are alternative regulatory DNA sequences used by both isoforms of the receptor, ERα and ERβ, to modulate gene expression. In this case, ER does not interact directly with DNA but interacts with other DNA-bound transcription factors such as c-Jun or c-Fos, or with other proteins (Kushner et al., 2003). Both AF-1 and AF-2 domains of ER are required for the interaction with Fos/Jun complex and both receptors differentially affect AP-1 dependent genes. In the presence of ERα, E2 works as AP-1 agonist by enhancing activity of the proteins at AP-1 sites (Brzozowski et al., 1997), while in the presence of ERβ it antagonizes...
AP-1 activity (Nilsson et al., 2001). When both receptors are present, ERβ inhibits the action of ERα on AP-1 promoters (Matthews et al., 2006). Interactions of ERs with other transcription factors might be also selectively modulated by different ligands, such as genistein and quercetin, which are not able to stimulate AP-1 dependent transcription (Figtree et al., 2003; Schreihof, 2005).

Even though ERs are considered transcription factors they can act through non-genomic mechanisms. Rapid ER effects were first observed in 1960s when administration of a physiological dose of E2 was reported to increase uterine cAMP levels in ovariectomized rats within 15 seconds (Szego and Davis, 1967), a time scale that is considered too fast for a genomic action. There is still no agreement if receptors responsible for rapid actions of estrogens are the same nuclear ERs or distinct G-protein coupled steroid receptors (Funakoshi et al., 2006; Maggiolini et al., 2004; Pedram & Levin, 2006; Warner & Gustafsson, 2006). However, a broad range of other rapid pathways induced by E2 has been identified so far. Some of these pathways include MAPK/ERK pathway, activation of endothelial nitric oxide synthase (eNOS), PLC stimulated IP3 production, calcium influx and PI3K/Akt pathway activation (Stirone et al., 2005; Virgili F, 2004; Ascenzi et al., 2006).

Similarly to non-classic mechanisms of activation, phytoestrogens might affect rapid pathways in a different way than E2. Quercetin for example has been shown to fail to phosphorylate ERK-2 kinase (opposite to E2) nor did it stimulate transcription of Cyclin D1, the transcription of which sometimes depends on rapid ER pathways (Virgili F, 2004). The stimulation of eNOS, which plays a role in cardiovascular health effects induced by E2 also seems to be regulated differently by phytoestrogens. Rapid activation of eNOS in the presence of E2 is dependent on ERα (Simoncini et al., 2005), while both receptors are required for prolonged effects. However phytoestrogens do not activate eNOS in a rapid manner but seem to activate it through a prolonged, ERβ dependent transcriptional mechanism (Simoncini et al., 2005).

In addition to ligand dependent mechanisms, ERα has ligand independent activity mediated through AF-1, which has been shown to be associated with stimulation of MAPK through growth factors such as Insulin like Growth Factor – 1 (IGF-1) and Epidermal Growth Factor (EGF). Activity of AF-1 is dependent on phosphorylation of Ser 118. A good example of the cross-talk between ER and growth factor signalling is phosphorylation of Ser 118 by MAPK in response to growth factors, such as IGF-1 and EGF (Kato et al., 1995). The importance of growth factors in ER signalling is well illustrated by the fact that EGF can mimic effects of E2 in the mouse reproductive tract (Nilsson et al., 2001).

1.4 Ligand dependent effects and cofactors

The overall biological effects of E2 and other estrogenic compounds are the result of complex interplay between various mechanisms, which largely depend on cellular context, ratio between ER subtypes, expression of coactivators in the cell, sequences of target EREs but also cross-talk with growth factor pathways and activity of kinases and phosphatases. All these factors together enable a precise and targeted response to the natural hormone. However a broad range of pathways involved in ER signaling provides many points of possible signal modulation by estrogens and estrogen-like compounds and small structural changes between different ligands might result in significantly different responses.
Structural differences in the LBD underlie differences in affinity and transcriptional activity of certain ER ligands and provide one of the mechanisms for selective modulation of ER responses. ERβ has an impaired AF-1 domain compared with ERα and the necessary synergy with AF-2 is dramatically reduced (Cowley & Parker, 1999). These differences suggest that it is possible to develop ligands with different affinities, potencies, and agonist vs antagonist behavior for the two ER subtypes.

It has been demonstrated that E2 has higher affinity towards ERα than to ERβ (Bovee et al., 2004; Veld et al., 2006), and certain selective estrogen receptor modulators (SERMs) might exhibit a preference towards one of the receptors (Escande et al., 2006). Plant derived phytoestrogens, which are structurally similar to E2 (Figure 3) provide a good example of ligand selectivity (Kuiper et al., 1998). Genistein is the major isoflavone present in soy and fava beans whereas quercetin is present in red onions, apples, cappers or red grapes among others (Kuiper et al., 1998). In vitro studies with reporter gene assays proved that phytoestrogens are able to stimulate ERE-dependent genes at high concentrations. Therefore they are considered weak ER agonists with the majority of them preferentially binding to ERβ (Chrzan & Bradford, 2007; Harris et al., 2005). The main hypothesis on the positive role of phytoestrogens in modulation of ER signaling is their higher affinity towards the ERβ subtype, which can silence ERα dependent signaling and decrease overall cell sensitivity to E2 (Hall & McDonnell, 1999), which is thought to be significant in cancer prevention.

![Fig. 3. Chemical structure of estradiol, genistein and quercetin.](image)

ERs can associate with distinct subsets of coactivators and corepressors depending on binding affinities and relative abundance of these factors (Chen & Evans, 1995; Halachmi et al., 1994). Several ER coactivators and corepressors have been described (Nilsson et al., 2001). Differences between ERα and ERβ in coactivator and corepressor recruitment have also been reported (Cowley & Parker, 1999; Suen et al., 1998), and therefore this preferential binding of certain coactivators and corepressors to one of the ERs may have consequences for specific ligand signalling and the ultimate biological effect elicited by ligand binding.

NCoR and SMRT corepressors and the p160 family coactivators are widely expressed (Horlein et al., 1995; Misiti et al., 1998; Ohate et al., 1995). Low levels of SRC-3 have been demonstrated for human proliferating endometrium with increased expression in the late secretory phase (Gregory et al., 2002) while overexpression of SRC-3 is frequently observed in breast, ovarian, and prostate cancers (Anzick et al., 1997; Gnanapragasam et al., 2001; McKenna et al., 1999). Similar expression levels of CBP, p300, AIB1, GRIP1, p300, NCoR, and SMRT have been measured for Ishikawa uterine and MCF-7 breast cancer cells (Shang and Brown, 2002). High levels of SRC-1 expression are found in Ishikawa cells, and this might
correlate with the agonist activity of tamoxifen in this cell line (Shang and Brown, 2002). We have seen in our studies (Sotoca et al., 2011), that the T47D breast cancer cells express the ER coactivator PRMT1. Recruitment of this coactivator is accompanied by histone methylation (Huang et al., 2005; Klinge et al., 2004). Recently, PRMT1 gene expression has been used as a marker of unfavourable prognosis for colon cancer patients (Mathioudaki et al., 2008).

Thus, other signalling events within the cell may affect nuclear receptor transcriptional responses via alteration in the expression of certain coregulators, and therefore it is predicted that significant differences in coactivator and corepressor expression found in various cell and tissue types would be important determinants of specific receptor modulator activity.

In addition, distribution of particular splicing variants of both ERs should be taken into account when considering tissue response to estrogens and cofactor recruitment as they have differential and sometimes antagonistic properties and their relative abundance might significantly influence biological responses to hormones. The main physiological role of ER splice variants in breast cancer development is however far from clear and might be a crucial determinant for clinical parameters.

2. ER Isoforms: ERα and ERβ

Full length ERα and ERβ proteins are approximately 66 and 59 kDa respectively (Ascenzi et al., 2006; Fuqua et al., 1999), although as a result of alternative splicing both receptors can form different isoforms. ERα has been shown to form over 20 alternative splice variants in breast cancer and other tumors (Poola et al., 2000), three of them with proven functionality, while at least five ERβ variants have been reported in human (Lewandowski et al., 2002).

The function and physiological significance of all isoforms have not been described so far, but some of them are powerful modulators of ER signaling pathways in normal tissues.

2.1 ERα splice variants

The two most referenced ERα isoforms that seem to be of particular significance are ERα46 and ERα36 as they were reported to oppose genomic actions of full length ERα66 (figure 4).

The ERα46 isoform has been identified in the MCF7 breast cancer cell line (Penot et al., 2005) in which it is coexpressed with full length ERα66. The presence of ERα46 has also been confirmed in osteoblasts (Wang et al., 2005) and endothelial cells (Figtree et al., 2003). This isoform is formed by skipping exon 1 encoding the N-terminus (A/B) and it is devoid of AF-1 activity. In contrast with full length ERα66, the truncated isoform ERα46 does not mediate E2 dependent cell proliferation and high levels of this isoform have been shown to be associated with cell cycle arrest in the G0/G1 phase and a state of refractoriness to E2 stimulated growth, which is normally reached at hyperconfluency of the cells (Penot et al., 2005). Similarly to ERβ, ERα46 is a potent ligand-dependent transcription factor containing AF-2 and a powerful inhibitor of ERα AF-1 dependent transcription (Figtree et al., 2003). By inhibition of ERα66 dependent gene transcription, ERα46 isoform inhibits estrogenic induction of c-Fos and Cycclin D1 promoters, which are involved in cell cycle control. Coexpression of ERα46 with ERα66 in an SaOs osteoblast cell line results in concentration dependent inhibition of E2 stimulated cell
proliferation (Ogawa et al., 1998b), an effect similar to the consequence observed with coexpression of ERα with ERβ (Sotoca et al., 2008; Ström et al., 2004).

The second truncated ERα isoform ERα36 was first described recently (Wang et al., 2005), and it has been shown to lack both the AF-1 and AF-2 transactivation functions of full length ERα. However it has functional DBD, partial dimerization and LBD domains. ERα36 contains an exon coding for myristoylation sites, hence predicting an interaction with the plasma membrane. Transcription of this ERα36 is initiated from a previously unidentified promoter in the first intron of the ERα gene and the unique 27 amino acid C-terminal sequence is encoded by a novel ERα exon, localized downstream of exon 8 to replace the last 138 amino acids encoded by exon 7-8 (Wang et al., 2005).

This novel isoform has been cloned from a human placenta cDNA library, which indicates that it is a naturally occurring isoform of ERα. With no functional AF-1 and AF-2 ERα36 does not have any direct transcriptional activity. However, it is a robust inhibitor of full length ERα and ERβ dependent transactivation (ZhaoYi Wang et al., 2006). It is mainly localized in the plasma membrane and works in a different way than full length protein. Even though it lacks transcriptional activity it can activate non genomic ER pathways such as MAPK/ERK signaling in response to E2 which is of particular significance in response to antiestrogens such as tamoxifen, 4OH-tamoxifen and ICI-182,780 (ZhaoYi Wang et al., 2006). As a result of MAPK/ERK pathway activation by E2 and these antiestrogens a signal is transduced to the nucleus and consequently Elk1 transcription factor is activated. The effect of MAPK/ERK activation mediated by ERα36 is increased cell proliferation in response to E2 as well as antiestrogens in doses that shut down transcriptional activity of full length ERα and ERβ proteins (ZhaoYi Wang et al., 2006).

The ERα80 isoform was detected in the MCF7:2A cell line, which is a subclone MCF7 cell line derived from long term growth in the absence of E2. This ERα80 isoform was produced by duplication of exons 6 and 7 (Pink et al., 1996). No evident function has been described so far.

![Fig. 4. Schematic comparison between full length ERα and its most referenced truncated isoforms.](image-url)
Several other multiple splice variants (ERαΔE2, ERαΔE3, ERαΔE4, ERαΔE5, ERαΔE6, ERαΔE5,7, ERαΔE7…) as a result of exon splicing deletions have been confirmed in human (Poola et al., 2000; Zhang et al., 1996) showing a dominant inhibitory effect in normal ER function. A list of selected ERα splice variants and their expression in various breast tissues (normal and tumor) and breast cancer cell lines is given in Table 2.

<table>
<thead>
<tr>
<th>Splice variant</th>
<th>Breast</th>
<th>MCF7</th>
<th>T47D</th>
<th>MDA-MB-231</th>
<th>MDA-MB-435</th>
<th>BT-474</th>
<th>BT-20</th>
<th>ZR-75</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>ERα36</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Shi et al., 2009; Lee et al., 2008; Zhao Yi Wang et al., 2006)</td>
</tr>
<tr>
<td>ERα46 (or ERαΔ1)</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Penot et al., 2005)</td>
</tr>
<tr>
<td>ERαΔ2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Wang and Miksicek, 1991; Zhang et al., 1996; Bollig and Miksicek, 2000; Poola and Speirs, 2001; Miksicek et al., 1993; Poola et al., 2000)</td>
</tr>
<tr>
<td>ERαΔ3</td>
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<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Wang and Miksicek, 1991; Poola and Speirs, 2001; Bollig and Miksicek, 2000; Zhang et al., 1996; Koduri et al., 2006; Erenburg et al., 1997; Miksicek et al., 1993; Fuqua et al., 1993)</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Pfeffer et al., 1993; Zhang et al., 1996; Bollig and Miksicek, 2000; Poola et al., 2000; Poola and Speirs, 2001)</td>
</tr>
<tr>
<td>ERαΔ5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>(Zhang et al., 1993; Zhang et al., 1996; Bollig and Miksicek, 2000; Poola and Speirs, 2001; Zhang et al., 1996; Fuqua et al., 1991; Daffada et al., 1994)</td>
</tr>
<tr>
<td>ERαΔ6</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Poola and Speirs, 2001; Bollig and Miksicek, 2000)</td>
</tr>
<tr>
<td>ERαΔ7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Wang and Miksicek, 1991; Fuqua et al., 1992; Poola and Speirs, 2001; Bollig and Miksicek, 2000; Fuqua et al., 1992; Miksicek et al., 1993)</td>
</tr>
<tr>
<td>ERαΔ5,7</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Zhang et al., 1996)</td>
</tr>
</tbody>
</table>

Table 2. List of selected ERα splice variants and their expression in various breast tissues (normal and tumour) and breast cancer cell lines.
2.2 ERβ splice variants

The presence of ERβ isoforms has been confirmed in various human cell lines as well as in a broad range of tissues at different levels (Leung et al., 2006; Moore et al., 1998), which provides another possible mechanism of tissue-dependent modulation of the ER response. Therefore distribution of particular isoforms of both ERs should be taken into account when considering tissue response to estrogens as they have differential and sometimes antagonistic properties and their differential distribution might significantly influence biological response to hormone.

Different isoforms of ERβ have been described (figure 5) with a variable C-terminus, and which were cloned from a testis cDNA library (Moore et al., 1998). At present their functional significance is poorly understood. The ERβ isoform whose function has been described in most detail of all ERβ isoforms studied is ERβ1, which is a full length protein with LBD and active AF-2 domain. ERβ2, 4 and 5 have a shortened Helix 11 and a full length Helix 12 is present only in ERβ1 and β2. In ERβ2, Helix 12 has a different orientation than in ERβ1 due to the shorter Helix 11. It has been reported that the displaced Helix 12 in ERβ2 limits ligand access to the binding pocket. As a consequence of their altered structure, ERβ2, 4 and 5 cannot form homodimers and have no transcriptional activity on their own, although they have been shown to heterodimerize with ERβ1 upon E2 treatment and enhance its AF-2 mediated transcriptional activity (Leung et al., 2006). Studies of interactions between different ERβ isoforms with ERα are very limited. However ERβ2 (also named ERβcx) was shown to limit DNA binding of ERα66 and inhibit its transcriptional activity in similar manner to ERβ1 (Ogawa et al., 1998b).

![Fig. 5. Comparison between full length ERβ and it is most referenced truncated isoforms.](image)

Two new exon-deleted variants were detected in the cancer cell line MDA-MB-231, ERβΔ1,2,5 and ERβΔ1,2,5,6 of approximately 35 and 28 kDa, respectively (Treeck et al., 2008). Both proteins are predicted not to contain AF-1, and to have deletions in the DBD and LBD. Therefore, these two variants are expected to be devoid of or have significantly reduced ligand-dependent and ligand independent activities, and their expression did not affect growth of cancer cell lines tested. A list of selected ERβ splice variants and their
expression in various breast tissues (normal and tumor) and breast cancer cell lines is given in Table 3.

Various studies reveal that physiological levels of ERα and ERβ may vary depending on the cell or tissue type (Ennmark et al., 1997; Bonkhoff et al., 1999; Makinen et al., 2001; Pearce et al., 2004) and as a consequence the biological response to endogenous or exogenous ligands can differ significantly.

<table>
<thead>
<tr>
<th>Splice variant</th>
<th>Breast</th>
<th>MCF7</th>
<th>T47D</th>
<th>MDA-MB-231</th>
<th>MDA-MB-453</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERβ2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(Davies et al., 2004; Zhao et al., 2007; Girault et al., 2004; Saji et al., 2005; Cappelletti et al., 2006; Leung et al., 2006)</td>
</tr>
<tr>
<td>ERβ3</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Girault et al., 2004)</td>
</tr>
<tr>
<td>ERβ4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>(Moore et al., 1998; Girault et al., 2004; Poola et al., 2005)</td>
</tr>
<tr>
<td>ERβ5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(Davies et al., 2004; Girault et al., 2004; Moore et al., 1998; Fuqua et al., 1999; Leung et al., 2006; Cappelletti et al., 2006)</td>
</tr>
<tr>
<td>ERβΔ2</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Poola et al., 2002a)</td>
</tr>
<tr>
<td>ERβΔ3</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Poola et al., 2002a; Poola et al., 2002b)</td>
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<tr>
<td>ERβΔ4</td>
<td>+</td>
<td></td>
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<td></td>
<td>(Poola et al., 2002a; Poola et al., 2002b)</td>
</tr>
<tr>
<td>ERβΔ5</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>(Poola et al., 2002a; Speirs et al., 2000; Leygue et al., 1998)</td>
</tr>
<tr>
<td>ERβΔ6</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Poola et al., 2002a; Leygue et al., 1998)</td>
</tr>
<tr>
<td>ERβΔ1,2,5</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Treeck et al., 2008)</td>
</tr>
<tr>
<td>ERβΔ1,2,5,6</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Treeck et al., 2008)</td>
</tr>
</tbody>
</table>

Table 3. List of selected ERβ splice variants and their expression in various breast tissues (normal and tumour) and breast cancer cell lines.
3. Conclusion

Cell proliferation in normal developing breast tissue is stimulated by estrogens and estrogens may prevent osteoporosis by increasing bone mineral density (Douchi et al., 2007). However, as cells can have their own set of ER splice variants that varies in time and abundance the estrogen receptor proteins can be expected to have a role in developmental regulation depending on splice variant and ligand present. ER splice variants are widely expressed in normal, premalignant and cancerous tissues and cell lines [reviewed in (Taylor et al. 2010)]. Co-expression of splice variants remains under investigation to understand its biological implications. Here, we briefly summarize ER expression and its role in positive or negative transcriptional activation in breast cancer.

Several studies have demonstrated that estrogens stimulate the growth of a large proportion of ERα positive breast cancers (Lazennec, 2006; Monroe et al., 2005; Pedram et al., 2006; Weitzmann & Pacifici, 2006). Furthermore, a decreased ERβ expression in cancer tissues as compared to benign tumours or normal tissues has been reported, whereas ERα expression seems to persist (Lazennec et al., 2001, Bardin et al., 2004). Recent progress in cellular experiments confirmed that ERβ opposes ERα actions in breast cancer cell lines (Sotoca et al., 2011; Sotoca et al., 2008; Ström et al., 2004).

The main roles of ER splice variants in breast cancer development are, however, far from clear (Davies et al., 2004; Saji et al., 2005). ERα positivity in breast cancer in vivo is strongly associated with more favourable clinicopathological parameters. ERβ positive patients have been shown to have favourable prognosis and better survival due to better endocrine-treatment response compared with ERβ negative breast tumor patients (Davies et al., 2004; Saji et al., 2005).

When bound to estrogens as homodimers, each receptor activates transcription of certain target genes bearing a classical ERE in their promoter region. However, estrogen binding to ERβ can also inhibit gene transcription via AP-1 sites, while binding to ERα leads to activation. Furthermore, when heterodimers are formed, when the two receptors are co-expressed, ERβ can inhibit ERα function. Given that ER regulates cell proliferation by different mechanisms, we summarize (Table 4 and 5) by which molecular characteristics of ER this proliferation is driven.

Full activation of AF-1 in ERα induces cell proliferation in breast cancer cells (Fujita et al., 2003). AF-1 activity of estrogen-ERβ is weaker compared with that of estrogen-ERα on ERE, whereas their AF-2 activities are similar (Cowley & Parker, 1999). In general ERβ has antiproliferative effects in breast cancer cells. All ERβ variants have negative effect on ERα by heterodimerization and reduce or abrogate both ligand-dependent and ligand-independent activities. Especially the ERβ2 isoform inhibits ERα-mediated estrogen action. In addition, several short ERα isoforms are able to oppose genomics actions of ERβ.

The most important point is that ERα expression induces significant cell proliferation in the absence of ERβ but not the other way around. Cell proliferation is triggered by classical genomic and non-genomic pathways. Only the wild type ERα isoform is able to induce hormone-dependent proliferation. It has been shown that most of the ER variants do not mediate ligand-dependent proliferation.
In conclusion, the overall biological effects of E2 and other estrogenic compounds in breast cancer cells are the result of complex interplay between various mechanisms, which depend on cellular context, balance between ER subtypes, coactivators and corepressors, sequences of target EREs but also cross-talk with growth factor pathways and activity of certain kinases and phosphatases. All these factors taken together enable response to estrogens or antiestrogens.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Feature</th>
<th>AF-1</th>
<th>DBD</th>
<th>LBD</th>
<th>AF-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα66</td>
<td>Wild type form Induces cell proliferation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ERα46</td>
<td>Does not mediate E2-dependent proliferation Opposes genomic action of ERα66 and ERβ Inhibitor of AF-1 dependent transactivation Potent AF-2 ligand dependent transcription activity</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ERα36</td>
<td>Opposes genomic actions of ERα66 Can activate non-genomic ER pathways via MAPK/ERK No direct transcriptional activity Inhibitor of ERα and β dependent transactivation</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ERα80</td>
<td>Not described</td>
<td>+</td>
<td>+</td>
<td>-*</td>
<td>-*</td>
</tr>
<tr>
<td>ERαΔ2</td>
<td>No transcriptional regulation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ERαΔ3</td>
<td>Binds ligand Dominant negative at ERE Interacts with AP-1 sites Suppresses E2-stimulated gene expression</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ERαΔ4</td>
<td>Dominant negative transcriptional effect</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ERαΔ5</td>
<td>Dominant positive transcriptional effect Dominant negative at ERE Coexpresses with ERα and enhances ERE-Luc</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ERαΔ7</td>
<td>Dimerizes with ERα and hERβ Binds to ERE Dominant negative transcriptional effect</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
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Table 4. Summary of ERα mechanism.
<table>
<thead>
<tr>
<th>Isoform</th>
<th>Features</th>
<th>AF-1</th>
<th>DBD</th>
<th>LBD</th>
<th>AF-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERβ1</td>
<td>Wild type form</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ERβ2</td>
<td>Dimerizes with ERβ1 and ERα Does not bind ligand</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ERβ3</td>
<td>Dimerizes with ERβ1 Does not bind ligand</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ERβ4</td>
<td>Dimerizes with ERβ1 and ERα Does not bind ligand</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ERβ5</td>
<td>Dimerizes with ERβ1 and ERα Does not bind ligand</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ERβΔ5</td>
<td>Negative effect on ERβ1 and ERα</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERβΔ1,2,5</td>
<td>Reduced both ligand-dependent and ligand independent activities</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ERβΔ1,2,5,6</td>
<td>Reduced both ligand-dependent and ligand independent activities</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 5. Summary of ERβ mechanism.

4. Acknowledgements

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5. References


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Human ERα and ERβ Splice Variants: Understanding Their Domain Structure in Relation to Their Biological Roles in Breast Cancer Cell Proliferation


Over the recent years, biochemistry has become responsible for explaining living processes such that many scientists in the life sciences from agronomy to medicine are engaged in biochemical research. This book contains an overview focusing on the research area of proteins, enzymes, cellular mechanisms and chemical compounds used in relevant approaches. The book deals with basic issues and some of the recent developments in biochemistry. Particular emphasis is devoted to both theoretical and experimental aspect of modern biochemistry. The primary target audience for the book includes students, researchers, biologists, chemists, chemical engineers and professionals who are interested in biochemistry, molecular biology and associated areas. The book is written by international scientists with expertise in protein biochemistry, enzymology, molecular biology and genetics many of which are active in biochemical and biomedical research. We hope that the book will enhance the knowledge of scientists in the complexities of some biochemical approaches; it will stimulate both professionals and students to dedicate part of their future research in understanding relevant mechanisms and applications of biochemistry.

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