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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

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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 ER $\alpha$  to differentiate it from the novel ER, ER $\beta$  (Kuiper *et al.*, 1996). The complete human ER $\beta$  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 ER $\alpha$  is encoded on chromosome 6q24-q27 (Gosden *et al.*, 1986), while the gene encoding human ER $\beta$  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 (ER $\alpha$ ) and ESR2 (ER $\beta$ ) 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 ER $\alpha$  and ER $\beta$ , 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 ERa and ER $\beta$  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  $\alpha$  and  $\beta$  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 ERa 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).



Fig. 1. Proteomic format, domain structure of human ER $\alpha$  (A) and ER $\beta$  (B). Based on Matthews and Gustafsson (Matthews & Gustafsson, 2003).

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. ER $\beta$  is predominant in the male reproductive system. Expression of both ER $\alpha$  and ER $\beta$  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 ER $\alpha$  mRNA generally being higher than that of ER $\beta$  (Matsuzaki *et al.*, 1999). Changes in expression of estrogen receptors has been found in certain tumour types. Normal mammary tissue in man predominantly expresses ER $\beta$  mRNA, whereas most ER-positive breast tumours appear to exhibit increased ratios of ER $\alpha$ /ER $\beta$  (Leygue *et al.*, 1998).

Likewise, an increased ratio of ER $\alpha$ /ER $\beta$  mRNA has been demonstrated in ovarian carcinoma compared with normal tissue or cysts (Bardin *et al.*, 2004). High concentrations of ER $\beta$  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.

Organ/Tissue	Human ER subtype		Organ/Tissue	Human ER subtype	
	ERa	ERβ		ERa	ERβ
Heart	$\checkmark$	$\checkmark$	Adrenal	$\checkmark$	-
Lung	-	$\checkmark$	Kidney	$\checkmark$	$\checkmark$
Vascular	$\checkmark$	$\checkmark$	Prostate	-	$\checkmark$
Bladder	-	$\checkmark$	Testes	-	$\checkmark$
Epididymus	-	$\checkmark$	Brain	$\checkmark$	$\checkmark$
Pituitary	-	$\checkmark$	Thymus	-	$\checkmark$
Liver	$\checkmark$	-	Breast	$\checkmark$	$\checkmark$
Muscle	-	-	Uterus	$\checkmark$	$\checkmark$
Fat	-	-	Endometrium	$\checkmark$	$\checkmark$
Gastrointestinal tract	-	$\checkmark$	Vagina	$\checkmark$	-
Colon	-	$\checkmark$	Fallopian tube	-	$\checkmark$
Small intestine	-	$\checkmark$	Ovary	$\checkmark$	$\checkmark$
Bone	$\checkmark$	$\checkmark$			

Table 1. Tissue distribution of ER subtypes in humans.

#### 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, ERa and ER $\beta$ , 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 ERa, 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 ER $\beta$  it antagonizes

AP-1 activity (Nilsson *et al.*, 2001). When both receptors are present, ER $\beta$  inhibits the action of ER $\alpha$  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; Schreihofer, 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 proteins as 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 IP<sub>3</sub> 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 ERa (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,  $ER\beta$  dependent transcriptional mechanism (Simoncini et al., 2005).

In addition to ligand dependent mechanisms, ERa 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. ER $\beta$  has an impaired AF-1 domain compared with ER $\alpha$  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 ER $\alpha$  than to ER $\beta$  (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 ER $\beta$  (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 ER $\beta$  subtype, which can silence ER $\alpha$  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.

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 ERa and ER $\beta$  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; Oñate *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 ER $\alpha$  and ER $\beta$  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. ER $\alpha$  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 ER $\beta$  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 ERa isoforms that seem to be of particular significance are **ERa46** and **ERa36** as they were reported to oppose genomic actions of full length **ERa66** (figure 4).

The **ERa46** isoform has been identified in the MCF7 breast cancer cell line (Penot *et al.*, 2005) in which it is coexpressed with full length ERa66. The presence of ERa46 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 ERa66, the truncated isoform ERa46 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 refraction to E2 stimulated growth, which is normally reached at hyperconfluency of the cells (Penot *et al.*, 2005). Similarly to ER $\beta$ , ERa46 is a potent ligand-dependent transcription factor containing AF-2 and a powerful inhibitor of ERa AF-1 dependent transcription (Figtree *et al.*, 2003). By inhibition of ERa66 dependent gene transcription, ERa46 isoform inhibits estrogenic induction of c-Fos and Cyclin D1 promoters, which are involved in cell cycle control. Coexpression of ERa46 with ERa66 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 ER $\alpha$  with ER $\beta$  (Sotoca *et al.*, 2008; Ström *et al.*, 2004).

The second truncated ER $\alpha$  isoform ER $\alpha$ 36 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 ER $\alpha$ . However it has functional DBD, partial dimerization and LBD domains. ER $\alpha$ 36 contains an exon coding for myristoylation sites, hence predicting an interaction with the plasma membrane. Transcription of this ER $\alpha$ 36 isoform is initiated from a previously unidentified promoter in the first intron of the ER $\alpha$  gene and the unique 27 amino acid C-terminal sequence is encoded by a novel ER $\alpha$  exon, localized downstream of exon 8 to replace the last 138 amino acids encoded by exon 7-8 (Wang *et al.*, 2005).



Fig. 4. Schematic comparison between full length ERa and its most referenced truncated isoforms.

This novel isoform has been cloned from a human placenta cDNA library, which indicates that it is a naturally occurring isoform of ER $\alpha$ . With no functional AF-1 and AF-2 ER $\alpha$ 36 does not have any direct transcriptional activity. However, it is a robust inhibitor of full length ER $\alpha$  and ER $\beta$  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 ER $\alpha$ 36 is increased cell proliferation in response to E2 as well as antiestrogens in doses that shut down transcriptional activity of full length ER $\alpha$  and ER $\beta$  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.

Several other multiple splice variants (ER $\alpha\Delta$ E2, ER $\alpha\Delta$ E3, ER $\alpha\Delta$ E4, ER $\alpha\Delta$ E5, ER $\alpha\Delta$ E6, ER $\alpha$ E $\Delta$ 5,7, ER $\alpha$ E $\Delta$ 7,...) 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 ER $\alpha$  splice variants and their expression in various breast tissues (normal and tumor) and breast cancer cell lines is given in Table 2.



Table 2. List of selected ERa 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 ER $\beta$  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 ER $\beta$  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 ER $\beta$  isoform whose function has been described in most detail of all ER $\beta$  isoforms studied is **ER\beta1**, which is a full length protein with LBD and active AF-2 domain. **ER\beta2**, **4 and 5** have a shortened Helix 11 and a full length Helix 12 is present only in ER $\beta$ 1 and  $\beta$ 2. In ER $\beta$ 2, Helix 12 has a different orientation than in ER $\beta$ 1 due to the shorter Helix 11. It has been reported that the displaced Helix 12 in ER $\beta$ 2 limits ligand access to the binding pocket. As a consequence of their altered structure, ER $\beta$ 2, 4 and 5 cannot form homodimers and have no transcriptional activity on their own, although they have been shown to heterodimerize with ER $\beta$ 1 upon E2 treatment and enhance its AF-2 mediated transcriptional activity (Leung *et al.*, 2006). Studies of interactions between different ER $\beta$  isoforms with ER $\alpha$  are very limited. However **ER\beta2** (also named **ER\betacx**) was shown to limit DNA binding of ER $\alpha$ 66 and inhibit its transcriptional activity in similar manner to ER $\beta$ 1 (Ogawa *et al.*, 1998b).



Fig. 5. Comparison between full length  $ER\beta$  and it is most referenced truncated isoforms.

Two new exon-deleted variants were detected in the cancer cell line MDA-MB-231, **ER** $\beta$ **Δ1**,2,5 and **ER** $\beta$ **Δ1**,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 ER $\beta$  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 ER $\alpha$  and ER $\beta$  may vary depending on the cell or tissue type (Enmmark *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.

Splice variant	Breast	MCF7	T47D	MDA-MB-231	MDA-MB-435	BT20	References
ΕRβ2	+	+	+	+		+	(Davies <i>et al.</i> , 2004; Zhao <i>et al.</i> , 2007; Girault <i>et al.</i> , 2004; Saji <i>et al.</i> , 2005; Cappelletti <i>et al.</i> , 2006; Leung <i>et al.</i> , 2006)
ERβ3	+						(Girault <i>et al.</i> , 2004)
ERβ4	+		+		+		(Moore <i>et al.,</i> 1998; Girault <i>et al.,</i> 2004; Poola <i>et al.,</i> 2005)
ΕRβ5	+	+	+	+	+	+	(Davies <i>et al.,</i> 2004; Girault <i>et al.,</i> 2004; Moore <i>et al.,</i> 1998; Fuqua <i>et al.,</i> 1999; Leung <i>et al.,</i> 2006; Cappelletti <i>et al.,</i> 2006)
Ε <b>R</b> βΔ2	+			+			(Poola <i>et al.,</i> 2002a)
ΕRβΔ3	+						(Poola <i>et al.,</i> 2002a; Poola <i>et al.,</i> 2002b)
ΕRβΔ4	+						(Poola <i>et al.,</i> 2002a; Poola <i>et al.,</i> 2002b)
ΕRβΔ5	$\mathcal{L}$	+		+			(Poola <i>et al.,</i> 2002a; Speirs <i>et al.,</i> 2000; Leygue <i>et al.,</i> 1998)
ΕRβΔ6	+						(Poola <i>et al.,</i> 2002a; Leygue <i>et al.,</i> 1998)
ERβΔ1,2,5		+		+			(Treeck <i>et al.,</i> 2008)
ERβΔ1,2,5,6		+		+			(Treeck <i>et al.,</i> 2008)

Table 3. List of selected  $ER\beta$  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 ER $\alpha$  positive breast cancers (Lazennec, 2006; Monroe *et al.*, 2005; Pedram *et al.*, 2006; Weitzmann & Pacifici, 2006). Furthermore, a decreased ER $\beta$  expression in cancer tissues as compared to benign tumours or normal tissues has been reported, whereas ER $\alpha$  expression seems to persist (Lazennec *et al.*, 2001, Bardin *et al.*, 2004). Recent progress in cellular experiments confirmed that ER $\beta$  opposes ER $\alpha$  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). ER $\alpha$  positivity in breast cancer in vivo is strongly associated with more favourable clinicopathological parameters. ER $\beta$  positive patients have been shown to have favourable prognosis and better survival due to better endocrine-treatment response compared with ER $\beta$  negative breast tumor patients (Davies *et al.*, 2004; Saji *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 ER $\beta$  can also inhibit gene transcription via AP-1 sites, while binding to ER $\alpha$  leads to activation. Furthermore, when heterodimers are formed, when the two receptors are coexpressed, ER $\beta$  can inhibit ER $\alpha$  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 ER $\alpha$  induces cell proliferation in breast cancer cells (Fujita *et al.*, 2003). AF-1 activity of estrogen-ER $\beta$  is weaker compared with that of estrogen-ER $\alpha$  on ERE, whereas their AF-2 activities are similar (Cowley & Parker, 1999). In general ER $\beta$  has antiproliferative effects in breast cancer cells. All ER $\beta$  variants have negative effect on ER $\alpha$  by heterodimerization and reduce or abrogate both ligand-dependent and ligand-independent activities. Especially the ER $\beta$ 2 isoform inhibits ER $\alpha$ -mediated estrogen action. In addition, several short ER $\alpha$  isoforms are able to oppose genomics actions of ER $\beta$ .

The most important point is that ER $\alpha$  expression induces significant cell proliferation in the absence of ER $\beta$  but not the other way around. Cell proliferation is triggered by classical genomic and non-genomic pathways. Only the wild type ER $\alpha$  isoform is able to induce hormone-dependent proliferation. It has been shown that most of the ER variants do not mediate ligand-dependent proliferation.

Human ER $\alpha$  and ER $\beta$  Splice Variants: Understanding Their Domain Structure in Relation to Their Biological Roles in Breast Cancer Cell 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.

Isoform	Feature	AF-1	DBD	LBD	AF-2
ERa66	Wild type form Induces cell proliferation	+	+	+	+
ERa46	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	-	+	+	+
ERa36	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	-	+	+	-
ERa80	Not described	+	+	+*	+*
ΕRαΔ2	No transcriptional regulation	-	-	-	-
ERαΔ3	Binds ligand Dominant negative at ERE Interacts with AP-1 sites Suppresses E2-stimulated gene expression	+		+	+
ERa∆4	Dominant negative transcriptional effect	+			+
ERαΔ5	Dominant positive transcriptional effect Dominant negative at ERE Coexpresses with ERα and enhances ERE-Luc	+	+	-	-
ERαΔ7	Dimerizes with ERα and hERβ Binds to ERE Dominant negative transcriptional effect	+	+	+	-

Table 4. Summary of ERa mechanism.

Isoform	Features	AF-1	DBD	LBD	AF-2
ΕRβ1	Wild type form	+	+	+	+
ERβ2	Dimerizes with ERβ1 and ERα Does not bind ligand	+	+	+	- ()
ΕRβ3	Dimerizes with ERβ1 Does not bind ligand	+	+)(		-
ERβ4	Dimerizes with ERβ1 and ERα Does not bind ligand	+	+	+	-
ERβ5	Dimerizes with ERβ1 and ERα Does not bind ligand	+	+	+	-
ΕRβΔ5	Negative effect on ER $\beta$ 1 and ER $\alpha$	+	+		
ERβΔ1,2,5	Reduced both ligand-dependent and ligand independent activities	-	+	-	+
ERβΔ1,2,5,6	Reduced both ligand-dependent and ligand independent activities	-	+	-	+

Table 5. Summary of ER $\beta$  mechanism.

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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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