We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

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

Ana M. Sotoca[1,2], Jacques Vervoort[2],
Ivonne M.C.M. Rietjens[1] and Jan-Åke Gustafsson[3,4]

[1]*Toxicology section, Wageningen University, Wageningen*
[2]*Laboratory of Biochemistry, Wageningen University, Wageningen*
[3]*Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signaling, University of Houston, Science & Engineering Research Center, Houston*
[4]*Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Huddinge*
[1,2]*The Netherlands*
[3]*USA*
[4]*Sweden*

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).
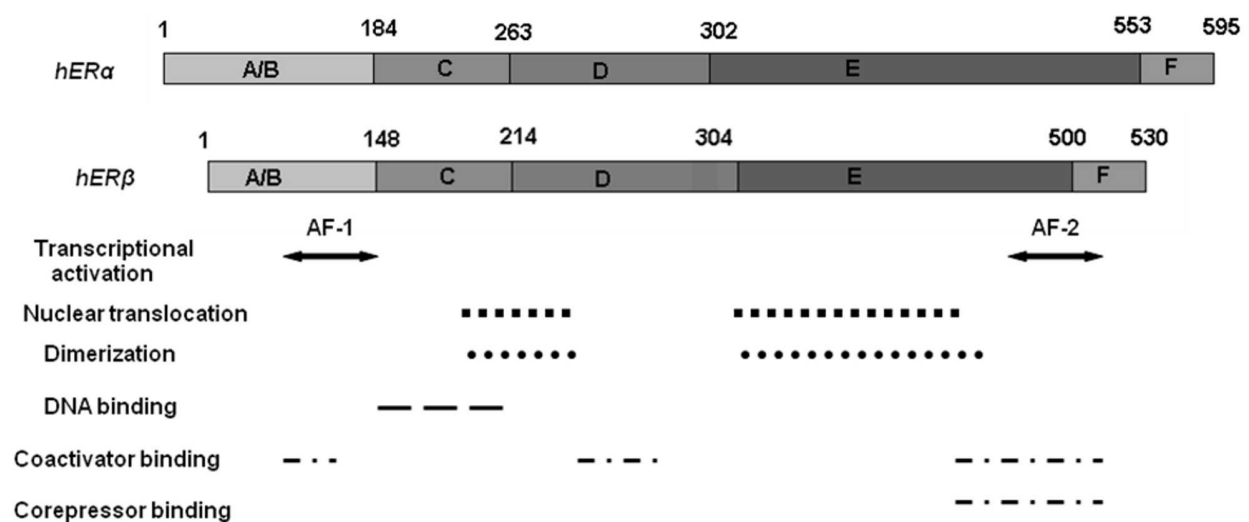

Fig. 1. Proteomic format, domain structure of human ERα (A) and ERβ (B). Based on Matthews and Gustafsson (Matthews & Gustafsson, 2003).

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. 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).

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

143

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.

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

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).
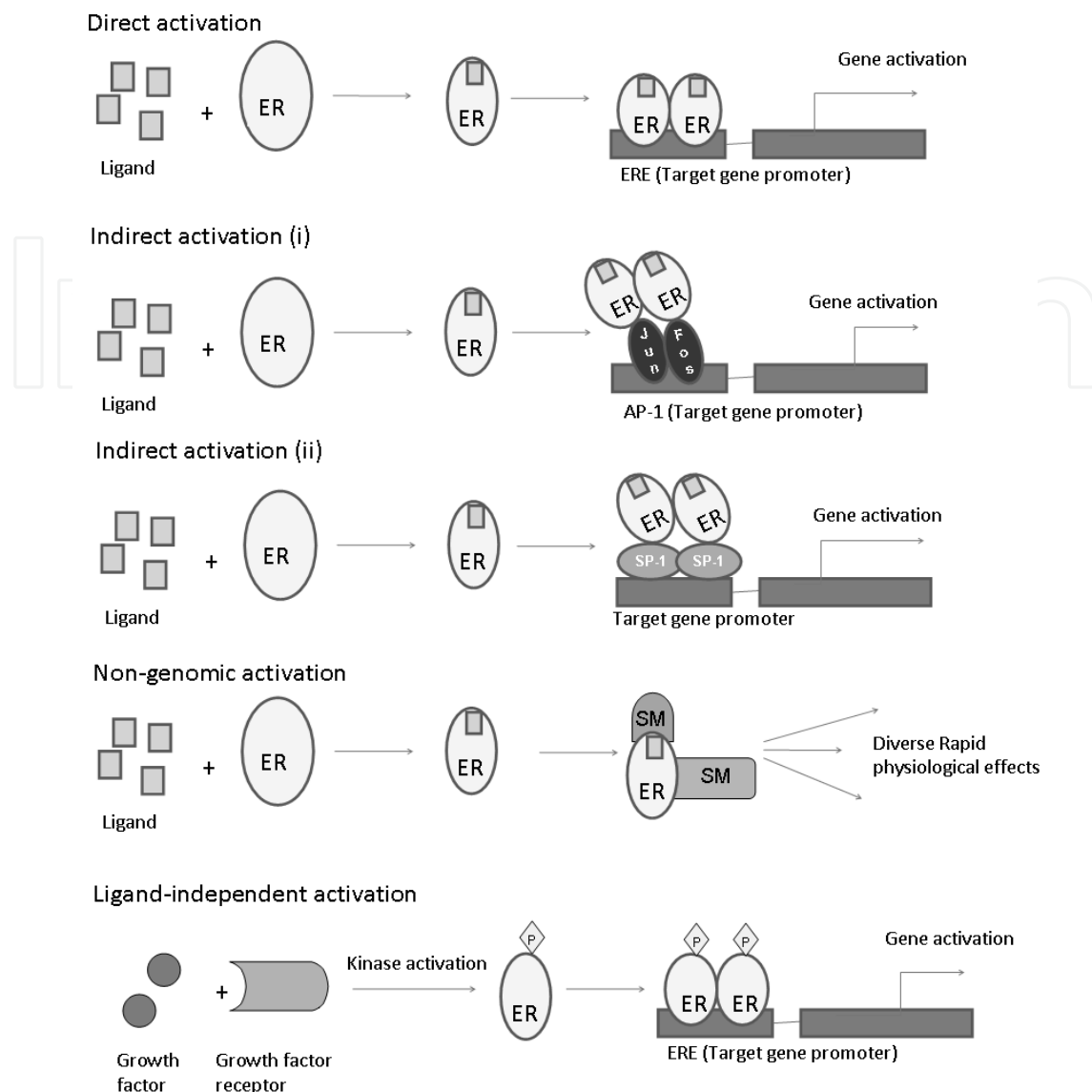
Fig. 2. Mechanisms of estrogen receptor (ER) action. In the *direct activation*, ERs dimerize after ligand binding and attach to the ERE in the promoter of target genes. In the *indirect activation* manner, ligand-bound ER dimers might activate transcription of non-ERE containing genes, by binding to 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, 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; 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_3 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.
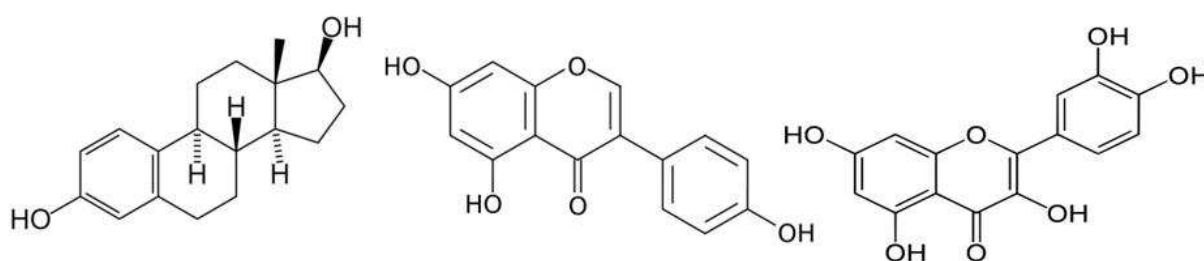

Fig. 3. Chemical structure of estradiol, genistein and quercetin.

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; 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 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 refraction 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 Cyclin 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 isoform 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).
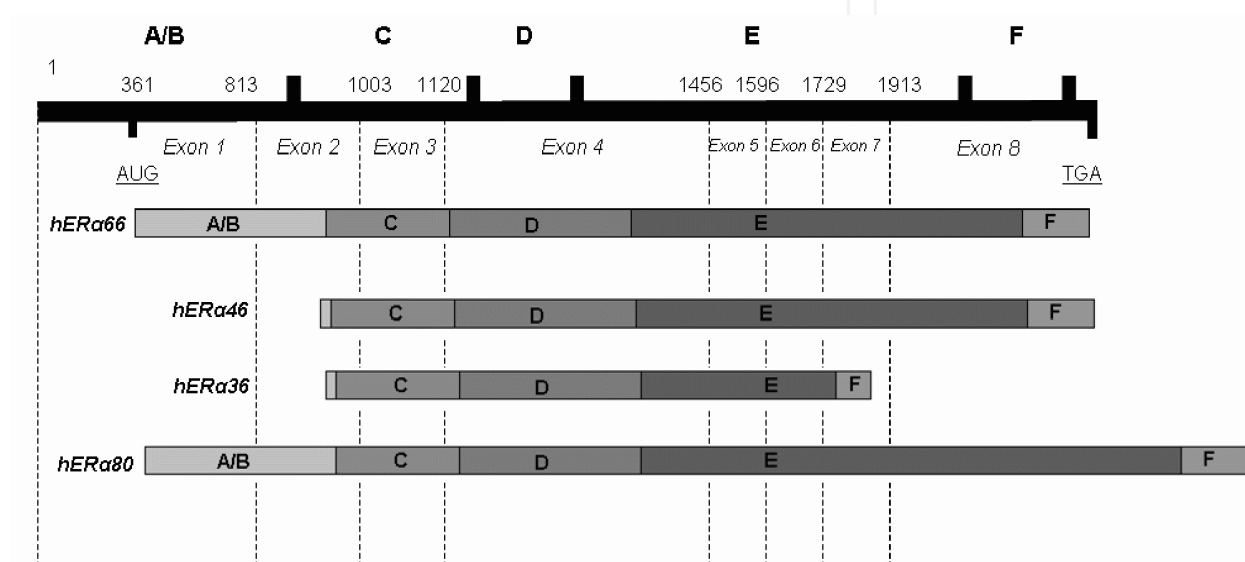

Fig. 4. Schematic comparison between full length ERα and its most referenced truncated isoforms.

This novel isoform has been cloned from a human placenta cDNA library, which indicates that it is a naturally occurring isoform of 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.

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

149

Several other multiple splice variants (**ERαΔE2, ERαΔE3, ERαΔE4, ERαΔE5, ERαΔE6, ERαEΔ5,7, ERαEΔ7**…) 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.

Splice variant	Breast	MCF7	T47D	MDA-MB-231	MDA-MB-435	BT-474	BT20	ZR-75	References
ERα36	+			+					(Shi *et al.*, 2009; Lee *et al.*, 2008; ZhaoYi Wang *et al.*, 2006)
ERα46 (or ERαΔ1)		+							(Penot *et al.*, 2005)
ERαΔ2	+	+	+		+				(Wang and Miksicek, 1991; Zhang *et al.*, 1996; Bollig and Miksicek, 2000; Poola and Speirs, 2001; Miksicek *et al.*, 1993; Poola *et al.*, 2000)
ERαΔ3	+	+	+	+					(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)
ERαΔ4		+			+			+	(Pfeffer *et al.*, 1993; Zhang *et al.*, 1996; Bollig and Miksicek, 2000; Poola *et al.*, 2000; Poola and Speirs, 2001)
ERαΔ5	+	+	+	+	+	+	+	+	(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)
ERαΔ6	+								(Poola and Speirs, 2001; Bollig and Miksicek, 2000)
ERαΔ7	+	+	+	+					(Wang and Miksicek, 1991; Fuqua *et al.*, 1992; Poola and Speirs, 2001; Bollig and Miksicek, 2000; Fuqua *et al.*, 1992; Miksicek *et al.*, 1993)
ERαΔ5,7	+								(Zhang *et al.*, 1996)

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).
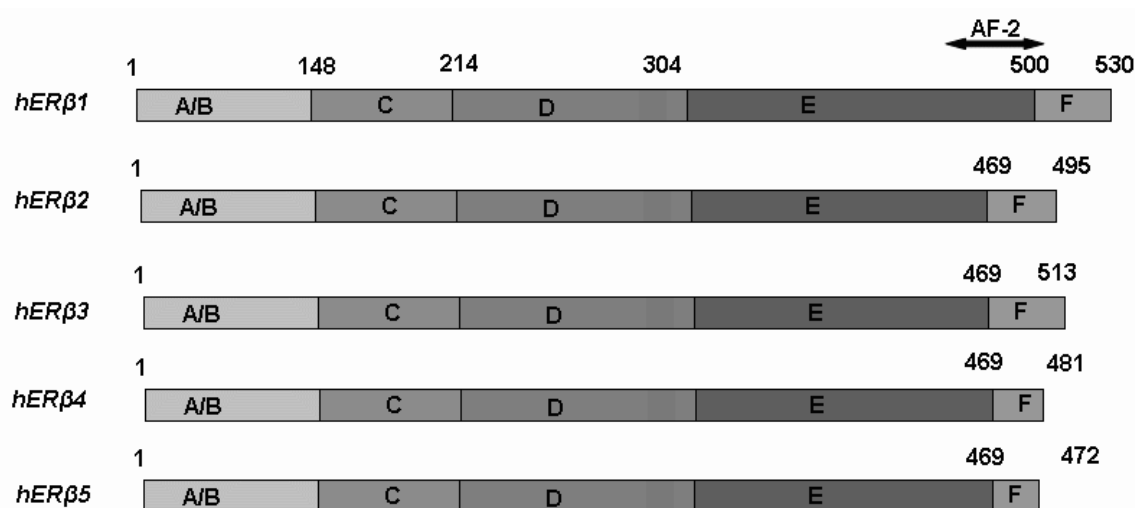

Fig. 5. Comparison between full length ERβ and it is most referenced truncated isoforms.

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 (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
ERβ2	+	+	+	+		+	(Davies *et al.*, 2004; Zhao *et al.*, 2007; Girault *et al.*, 2004; Saji *et al.*, 2005; Cappelletti *et al.*, 2006; Leung *et al.*, 2006)
ERβ3	+						(Girault *et al.*, 2004)
ERβ4	+		+		+		(Moore *et al.*, 1998; Girault *et al.*, 2004; Poola *et al.*, 2005)
ERβ5	+	+	+	+	+	+	(Davies *et al.*, 2004; Girault *et al.*, 2004; Moore *et al.*, 1998; Fuqua *et al.*, 1999; Leung *et al.*, 2006; Cappelletti *et al.*, 2006)
ERβΔ2	+			+			(Poola *et al.*, 2002a)
ERβΔ3	+						(Poola *et al.*, 2002a; Poola *et al.*, 2002b)
ERβΔ4	+						(Poola *et al.*, 2002a; Poola *et al.*, 2002b)
ERβΔ5	+	+		+			(Poola *et al.*, 2002a; Speirs *et al.*, 2000; Leygue *et al.*, 1998)
ERβΔ6	+						(Poola *et al.*, 2002a; Leygue *et al.*, 1998)
ERβΔ1,2,5		+		+			(Treeck *et al.*, 2008)
ERβΔ1,2,5,6		+		+			(Treeck *et al.*, 2008)

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.

Isoform	Feature	AF-1	DBD	LBD	AF-2
ERα66	Wild type form Induces cell proliferation	+	+	+	+
ERα46	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	-	+	+	+
ERα36	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	-	+	+	-
ERα80	Not described	+	+	+*	+*
ERαΔ2	No transcriptional regulation	-	-	-	-
ERαΔ3	Binds ligand Dominant negative at ERE Interacts with AP-1 sites Suppresses E2-stimulated gene expression	+	-	+	+
ERαΔ4	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 ERα mechanism.

Isoform	Features	AF-1	DBD	LBD	AF-2
ERβ1	Wild type form	+	+	+	+
ERβ2	Dimerizes with ERβ1 and ERα Does not bind ligand	+	+	+	-
ERβ3	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	+	+	+	-
ERβΔ5	Negative effect on ERβ1 and ERα	+	+		
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 ERβ mechanism.

4. Acknowledgements

5. References

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan X-Y *et al* (1997). AIB1, a Steroid Receptor Coactivator Amplified in Breast and Ovarian Cancer. *Science* 277: 965-968.

Ascenzi P, Bocedi A, Marino M (2006). Structure-function relationship of estrogen receptor [alpha] and [beta]: Impact on human health. *Molecular Aspects of Medicine* 27: 299.

Bardin A, Boulle N, Lazennec G, Vignon F, Pujol P (2004). Loss of ER{beta} expression as a common step in estrogen-dependent tumor progression. *Endocrine-Related Cancer* 11: 537-551.

Berno V, Amazit L, Hinojos C, Zhong J, Mancini MG, Dave Sharp Z *et al* (2008). Activation of Estrogen Receptor-alpha by E2 or EGF Induces Temporally Distinct Patterns of Large-Scale Chromatin Modification and mRNA Transcription. *PLoS ONE* 3: e2286.

Bollig A, Miksicek RJ (2000). An Estrogen Receptor-{alpha} Splicing Variant Mediates Both Positive and Negative Effects on Gene Transcription. *Mol Endocrinol* 14: 634-649.

Bonkhoff H, Fixemer T, Hunsicker I, Remberger K (1999). Estrogen Receptor Expression in Prostate Cancer and Premalignant Prostatic Lesions. *Am J Pathol* 155:641-647.

Bovee TFH, Helsdingen RJR, Rietjens IMCM, Keijer J, Hoogenboom RLAP (2004). Rapid yeast estrogen bioassays stably expressing human estrogen receptors [alpha] and [beta], and green fluorescent protein: a comparison of different compounds with both receptor types. *The Journal of Steroid Biochemistry and Molecular Biology* 91: 99.

Brzozowski AM, Pike ACW, Dauter Z, Hubbard RE, Bonn T, Engstrom O *et al* (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* 389: 753-758.

Cappelletti V, Miodini P, Fronzo GD, Daidone MG (2006). Modulation of estrogen receptor-beta isoforms by phytoestrogens in breast cancer cells. *International journal of Oncology* 28: 1185-91.

Chen JD, Evans RM (1995). A transcriptional co-repressor that interacts with nuclear hormone receptors. *Nature* 377: 454-457.

Chrzan BG, Bradford PG (2007). Phytoestrogens activate estrogen receptor beta1 and estrogenic responses in human breast and bone cancer cell lines. *Molecular Nutrition & Food Research* 51: 171-177.

Cowley SM, Hoare S, Mosselman S, Parker MG (1997). Estrogen Receptors alpha and beta Form Heterodimers on DNA. *Journal of Biological Chemistry* 272: 19858-19862.

Cowley SM, Parker MG (1999). A comparison of transcriptional activation by ER alpha and ER beta. *Journal of Steroid Biochemistry and Molecular Biology* 69: 165-175.

Daffada AAI, Johnston SRD, Nicholls J, Dowsett M (1994). Detection of wild type and exon 5-deleted splice variant oestrogen receptor (ER) mRNA in ER-positive and - negative breast cancer cell lines by reverse transcription/polymerase chain reaction. *Molecular Endocrinology* 13: 265-273.

Davies MPA, O'Neill PA, Innes H, Sibson DR, Prime W, Holcombe C *et al* (2004). Correlation of mRNA for oestrogen receptor beta splice variants ER{beta}1, ER{beta}2/ER{beta}cx and ER{beta}5 with outcome in endocrine-treated breast cancer. *J Mol Endocrinol* 33: 773-782.

DeNardo DG, Kim H-T, Hilsenbeck S, Cuba V, Tsimelzon A, Brown aPH (2005). Global Gene Expression Analysis of Estrogen Receptor Transcription Factor Cross Talk in Breast Cancer: Identification of Estrogen-Induced/Activator Protein-1-Dependent Genes. *Molecular Endocrinology* 19: 362-378.

Douchi T, Yonehara Y, Kosha S, Iwamoto I, Rai Y, Sagara Y *et al* (2007). Bone mineral density in breast cancer patients with positive estrogen receptor tumor status. *Maturitas* 57: 221-225.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G *et al* (1997). Human Estrogen Receptor {beta}-Gene Structure, Chromosomal Localization, and Expression Pattern. *J Clin Endocrinol Metab* 82: 4258-4265.

Erenburg I, Schachter B, Lopez RMy, Ossowski L (1997). Loss of an Estrogen Receptor Isoform (ER{alpha}{Delta}3) in Breast Cancer and the Consequences of Its Reexpression: Interference with Estrogen-Stimulated Properties of Malignant Transformation. *Mol Endocrinol* 11: 2004-2015.

Escande A, Pillon A, Servant N, Cravedi J-P, Larrea F, Muhnd P *et al* (2006). Evaluation of ligand selectivity using reporter cell lines stably expressing estrogen receptor alpha or beta. *Biochemical Pharmacology* 71: 1459-1469.

Figtree GA, McDonald D, Watkins H, Channon KM (2003). Truncated Estrogen Receptor {alpha} 46-kDa Isoform in Human Endothelial Cells: Relationship to Acute Activation of Nitric Oxide Synthase. *Circulation* 107: 120-126.

Fujita T, Kobayashi Y, Wada O, Tateishi Y, Kitada L, Yamamoto Y *et al* (2003). Full activation of estrogen receptor alpha (ER alpha) activation function-1 (AF-1) induces proliferation of breast cancer cells. *J. Biol. Chem.*: M301031200.

Funakoshi T, Yanai A, Shinoda K, Kawano MM, Mizukami Y (2006). G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochemical and Biophysical Research Communications* 346: 904.

Fuqua SAW, Allred DC, Elledge RM, Krieg SL, Benedix MG, Nawaz Z *et al* (1993). The ER-positive / PgR-negative breast cancer phenotype is not associated with mutations within the DNA binding domain. *Breast Cancer Research and Treatment* 26: 191-202.

Fuqua SAW, Fitzgerald SD, Allred DC, Elledge RM, Nawaz Z, McDonnell DP *et al* (1992). Inhibition of Estrogen Receptor Action by a Naturally Occurring Variant in Human Breast Tumors. *Cancer Research* 52: 483-486.

Fuqua SAW, Fitzgerald SD, Chamness GC, Tandon AK, McDonnell DP, Nawaz Z *et al* (1991). Variant Human Breast Tumor Estrogen Receptor with Constitutive Transcriptional Activity. *Cancer Research* 51: 105-109.

Fuqua SAW, Schiff R, Parra I, Friedrichs WE, Su J-L, McKee DD *et al* (1999). Expression of Wild-Type Estrogen Receptor {{beta}} and Variant Isoforms in Human Breast Cancer. *Cancer Res* 59: 5425-5428.

Girault I, Andrieu C, Tozlu S, Spyratos F, Bièche I, Lidereau R (2004). Altered expression pattern of alternatively spliced estrogen receptor [beta] transcripts in breast carcinoma. *Cancer Letters* 215: 101-112.

Gnanapragasam VJ, Leung HY, Pulimood AS, Neal DE, Robson CN (2001). Expression of RAC 3, a steroid hormone receptor co-activator in prostate cancer. *Br J Cancer* 85: 1928-1936.

Gosden J, Middleton P, Rout D (1986). Localization of the human oestrogen receptor gene to chromosome 6q24----q27 by in situ hybridization. *Cytogenetics and cell genetics* 43: 218-20.

Gregory CW, Wilson EM, Apparao KBC, Lininger RA, Meyer WR, Kowalik A *et al* (2002). Steroid Receptor Coactivator Expression throughout the Menstrual Cycle in Normal and Abnormal Endometrium. *J Clin Endocrinol Metab* 87: 2960-2966.

Halachmi S, Marden E, Martin G, MacKay H, Abbondanza C, Brown M (1994). Estrogen receptor-associated proteins: possible mediators of hormone-induced transcription. *Science* 264: 1455-1458.

Hall JM, McDonnell DP (1999). The Estrogen Receptor {beta}-Isoform (ER{beta}) of the Human Estrogen Receptor Modulates ER{alpha} Transcriptional Activity and Is a Key Regulator of the Cellular Response to Estrogens and Antiestrogens. *Endocrinology* 140: 5566-5578.

Harris DM, Besselink E, Henning SM, Go VLW, Heber aD (2005). Phytoestrogens induce differential estrogen receptor alpha- or Beta-mediated responses in transfected breast cancer cells. *Experimental Biology and Medicine* 230: 558-568.

Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J *et al* (2007). Estrogen Receptors: How Do They Signal and What Are Their Targets. *Physiological Reviews* 87: 905-931.

Horlein AJ, Naar AM, Heinzel T, Torchia J, Gloss B, Kurokawa R *et al* (1995). Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. *Nature* 377: 397-404.

Huang S, Litt M, Felsenfeld G (2005). Methylation of histone H4 by arginine methyltransferase PRMT1 is essential in vivo for many subsequent histone modifications. *Genes & Development* 19: 1885-1893.

Kato S, Endoh H, Masuhiro Y, Kitamoto T, Uchiyama S, Sasaki H *et al* (1995). Activation of the Estrogen Receptor Through Phosphorylation by Mitogen-Activated Protein Kinase. *Science* 270: 1491-1494.

Katzenellenbogen BS, Katzenellenbogen JA (2000). Estrogen receptor transcription and transactivation: Estrogen receptor alpha and estrogen receptor beta - regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res* 2: 335 - 344.

Kim K, Thu N, Saville B, Safe aS (2003). Domains of Estrogen Receptor alpha (ERalpha) Required for ERalpha/Sp1-Mediated Activation of GC-Rich Promoters by Estrogens and Antiestrogens in Breast Cancer Cells *Molecular endocrinology* 17: 804-817.

Klinge CM, Jernigan SC, Mattingly KA, Risinger KE, Zhang aJ (2004). Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors alpha and beta by coactivators and corepressors. *Journal of Molecular Endocrinology* 33: 387-410.

Koduri S, Goldhar A, Vonderhaar B (2006). Activation of vascular endothelial growth factor (VEGF) by the ER-α variant, ERΔ3. *Breast Cancer Research and Treatment* 95: 37-43.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-A (1996). Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *PNAS* 93: 5925-5930.

Kuiper GGJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT *et al* (1998). Interaction of Estrogenic Chemicals and Phytoestrogens with Estrogen Receptor {beta}. *Endocrinology* 139: 4252-4263.

Kushner PJ, Webb P, Uht RM, Liu M-M, and Richard H. Price J (2003). Estrogen receptor action through target genes with classical and alternative response elements. *Pure Applied Chemistry* 75, Nos: 1757-1769.

Lazennec G (2006). Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis. *Cancer Letters* 231: 151-7.

Lazennec G, Bresson D, Lucas A, Chauveau C, Vignon F (2001). ER beta inhibits proliferation and invasion of breast cancer cells. *Endocrinology* 142: 4120-30.

Lee LMJ, Cao J, Deng H, Chen P, Gatalica Z, Wang Z-Y (2008). ER-α36, a Novel Variant of ER-α, is Expressed in ER-positive and -negative Human Breast Carcinomas. *Anticancer research* 28: 479-483.

Leung Y-K, Mak P, Hassan S, Ho S-M (2006). Estrogen receptor (ER)-beta isoforms: A key to understanding ER-beta signaling. *PNAS* 103: 13162-13167.

Lewandowski S, Kalita K, Kaczmarek L (2002). Estrogen receptor [beta]: Potential functional significance of a variety of mRNA isoforms. *FEBS Letters* 524: 1-5.

Leygue E, Dotzlaw H, Watson PH, Murphy LC (1998). Altered Estrogen Receptor {alpha} and {beta} Messenger RNA Expression during Human Breast Tumorigenesis. *Cancer Res* 58: 3197-3201.

Maggiolini M, Vivacqua A, Fasanella G, Recchia AG, Sisci D, Pezzi V *et al* (2004). The G Protein-coupled Receptor GPR30 Mediates c-fos Up-regulation by 17{beta}-Estradiol and Phytoestrogens in Breast Cancer Cells. *J. Biol. Chem.* 279: 27008-27016.

Makinen S, Makela S, Weihua Z, Warner M, Rosenlund B, Salmi S, Hovatta O, Gustafsson J-A (2001). Localization of oestrogen receptors alpha and beta in human testis. *Mol Hum Reprod* 7:497-503

Mathioudaki K, Papadokostopoulou A, Scorilas A, Xynopoulos D, Agnanti N, Talieri M (2008). The PRMT1 gene expression pattern in colon cancer. *Br J Cancer* 99: 2094-2099.

Matsuzaki S, Fukaya T, Suzuki T, Murakami T, Sasano H, Yajima A (1999). Oestrogen receptor {alpha} and ß mRNA expression in human endometrium throughout the menstrual cycle. *Mol. Hum. Reprod.* 5: 559-564.

Matthews J, Gustafsson J-A (2003). Estrogen Signaling: A Subtle Balance Between ER{alpha} and ER{beta}. *Mol. Interv.* 3: 281-292.

Matthews J, Wihlen B, Tujague M, Wan J, Strom A, Gustafsson J-A (2006). Estrogen Receptor (ER) {beta} Modulates ER{alpha}-Mediated Transcriptional Activation by Altering the Recruitment of c-Fos and c-Jun to Estrogen-Responsive Promoters. *Mol Endocrinol* 20: 534-543.

McKenna NJ, Lanz RB, O'Malley BW (1999). Nuclear Receptor Coregulators: Cellular and Molecular Biology. *Endocr Rev* 20: 321-344.

Miksicek RJ, Lei Y, Wang Y (1993). Exon skipping gives rise to alternatively spliced forms of the estrogen receptor in breast tumor cells. *Breast Cancer Research and Treatment* 26: 163-174.

Misiti S, Schomburg L, M. Yen P, Chin WW (1998). Expression and Hormonal Regulation of Coactivator and Corepressor Genes. *Endocrinology* 139: 2493-2500.

Monroe DG, Secreto FJ, Subramaniam M, Getz BJ, Khosla S, Spelsberg TC (2005). Estrogen Receptor {alpha} and {beta} Heterodimers Exert Unique Effects on Estrogen- and Tamoxifen-Dependent Gene Expression in Human U2OS Osteosarcoma Cells. *Mol Endocrinol* 19: 1555-1568.

Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL *et al* (1998). Cloning and Characterization of Human Estrogen Receptor [beta] Isoforms. *Biochemical and Biophysical Research Communications* 247: 75-78.

Morani A, Warner M, Gustafsson JÅ (2008). Biological functions and clinical implications of oestrogen receptors alfa and beta in epithelial tissues. *Journal of Internal Medicine* 264: 128-142.

Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G *et al* (2001). Mechanisms of Estrogen Action. *Physiol. Rev.* 81: 1535-1565.

Ogawa S, Inoue S, Watanabe T, Hiroi H, Orimo A, Hosoi T *et al* (1998a). The Complete Primary Structure of Human Estrogen Receptor [beta] (hER[beta]) and Its Heterodimerization with ER [alpha]in Vivoandin Vitro. *Biochemical and Biophysical Research Communications* 243: 122-126.

Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y *et al* (1998b). Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor ofestrogen action in human. *Nucl. Acids Res.* 26: 3505-3512.

Oñate SA, Tsai SY, Tsai M-J, O'Malley BW (1995). Sequence and Characterization of a Coactivator for the Steroid Hormone Receptor Superfamily. *Science* 270: 1354-1357.

Pearce ST, Jordan VC (2004). The biological role of estrogen receptors alpha and beta in cancer. *Critical Reviews in Oncology/Hematology* 50: 3-22.

Pedram A, Levin MRaER (2006). Nature of Functional Estrogen Receptors at the Plasma Membrane. *Molecular Endocrinology* 20: 1996-2009.

Pedram A, Razandi M, Wallace DC, Levin ER (2006). Functional Estrogen Receptors in the Mitochondria of Breast Cancer Cells. *Mol. Biol. Cell* 17: 2125-2137.

Penot G, Le Peron C, Merot Y, Grimaud-Fanouillere E, Ferriere F, Boujrad N *et al* (2005). The Human Estrogen Receptor-{alpha} Isoform hER{alpha}46 Antagonizes the Proliferative Influence of hER{alpha}66 in MCF7 Breast Cancer Cells. *Endocrinology* 146: 5474-5484.

Pfeffer U, Fecarotta E, Castagnetta L, Vidali G (1993). Estrogen Receptor Variant Messenger RNA Lacking Exon 4 in Estrogen-responsive Human Breast Cancer Cell Lines. *Cancer Research* 53: 741-743.

Pink JJ, Wu SQ, Wolf DM, Bilimoria MM, Jordan VC (1996). A novel 80 kDa human estrogen receptor containing a duplication of exons 6 and 7. *Nucl. Acids Res.* 24: 962-969.

Poola I, Abraham J, Baldwin K (2002a). Identification of ten exon deleted ER[beta] mRNAs in human ovary, breast, uterus and bone tissues: alternate splicing pattern of estrogen receptor [beta] mRNA is distinct from that of estrogen receptor [alpha]. *FEBS Letters* 516: 133-138.

Poola I, Abraham J, Baldwin K, Saunders A, Bhatnagar R (2005). Estrogen receptors beta4 and beta5 are full length functionally distinct ERβ isoforms. *Endocrine* 27: 227-238.

Poola I, Abraham J, Liu A (2002b). Estrogen receptor beta splice variant mRNAs are differentially altered during breast carcinogenesis. *The Journal of Steroid Biochemistry and Molecular Biology* 82: 169-179.

Poola I, Koduri S, Chatra S, Clarke R (2000). Identification of twenty alternatively spliced estrogen receptor alpha mRNAs in breast cancer cell lines and tumors using splice targeted primer approach. *The Journal of Steroid Biochemistry and Molecular Biology* 72: 249-258.

Poola I, Speirs V (2001). Expression of alternatively spliced estrogen receptor alpha mRNAs is increased in breast cancer tissues. *The Journal of Steroid Biochemistry and Molecular Biology* 78: 459-469.

Saji S, Hirose M, Toi M (2005). Clinical significance of estrogen receptor β in breast cancer. *Cancer Chemotherapy and Pharmacology* 56: 21-26.

Schreihofer DA (2005). Transcriptional regulation by phytoestrogens in neuronal cell lines. *Molecular and Cellular Endocrinology* 231: 13-22.

Sentis S, Le Romancer M, Bianchin C, Rostan M-C, Corbo L (2005). Sumoylation of the Estrogen Receptor {alpha} Hinge Region Regulates Its Transcriptional Activity. *Mol Endocrinol* 19: 2671-2684.

Shang Y, Brown M (2002). Molecular Determinants for the Tissue Specificity of SERMs. *Science* 295: 2465-2468.

Shi L, Dong B, Li Z, Lu Y, Ouyang T, Li J *et al* (2009). Expression of ER-{alpha}36, a Novel Variant of Estrogen Receptor {alpha}, and Resistance to Tamoxifen Treatment in Breast Cancer. *J Clin Oncol* 27: 3423-3429.

Simoncini T, Fornari L, Mannella P, Caruso A, Garibaldi S, Baldacci C *et al* (2005). Activation of nitric oxide synthesis in human endothelial cells by red clover extracts. *Menopause:The Journal of The North American Menopause Society* 12: 69-77.

Sotoca AM, Sollewijn Gelpke MD, Boeren S, Stöm A, Gustafsson J-Å, Murk AJ *et al* (2011). Quantitative proteomics and transcriptomics addressing the estrogen receptor subtype-mediated effects in T47D breast cancer cells exposed to the phytoestrogen genistein. *Molecular and cellular proteomics* 10: M110.002170.

Sotoca AM, van den Berg H, Vervoort J, van der Saag P, Ström A, Gustafsson J-Å *et al* (2008). Influence of Cellular ER{alpha}/ER{beta} Ratio on the ER{alpha}-Agonist Induced Proliferation of Human T47D Breast Cancer Cells. *Toxicological sciences* 105: 303-311.

Speirs V, Adams IP, Walton DS, Atkin SL (2000). Identification of Wild-Type and Exon 5 Deletion Variants of Estrogen Receptor {beta} in Normal Human Mammary Gland. *J Clin Endocrinol Metab* 85: 1601-1605.

Stirone C, Duckles SP, Krause DN, Procaccio V (2005). Estrogen Increases Mitochondrial Efficiency and Reduces Oxidative Stress in Cerebral Blood Vessels. *Mol Pharmacol* 68: 959-965.

Ström A, Hartman J, Foster JS, Kietz S, Wimalasena J, Gustafsson aJ-Å (2004). Estrogen receptor beta inhibits 17beta-estradiol-stimulated proliferation of the breast cancer cell line T47D. *Proc Natl Acad Sci U S A* 101: 1566-71.

Suen C-S, Berrodin TJ, Mastroeni R, Cheskis BJ, Lyttle CR, Frail DE (1998). A Transcriptional Coactivator, Steroid Receptor Coactivator-3, Selectively Augments Steroid Receptor Transcriptional Activity. *Journal of Biological Chemistry* 273: 27645-27653.

Szego CM, Davis JS (1967). Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen. *Proc Natl Acad Sci USA* 58: 1711–1718.

Taylor SE, Martin-Hirsch PL, Martin FL (2010). Oestrogen receptor splice variants in the pathogenesis of disease. *Cancer Letters* 288: 133-148

Treeck O, Juhasz-Boess I, Lattrich C, Horn F, Ortmann RGaO (2008). Effects of exon-deleted estrogen receptor b transcript variants on growth, apoptosis and gene expression of human breast cancer cell lines. *Breast Cancer Reserach Treatment* 110: 507–520.

Veld MGRt, Schouten B, Louisse J, Es DSv, Saag PTvd, Rietjens IMCM *et al* (2006). Estrogenic Potency of Food-Packaging-Associated Plasticizers and Antioxidants As Detected in ER and ER Reporter Gene Cell Lines. *Journal or Agriculture and Food Chemistry* 54 4407 -4416.

Virgili F AF, Ambra R, Rinna A, Totta P, Marino M. (2004). Nutritional flavonoids modulate estrogen receptor alpha signaling. *IUBMB Life* 56: 145-151.

Wang Y, Miksicek RJ (1991). Identification of a Dominant Negative Form of the Human Estrogen Receptor. *Molecular Endocrinology* 5: 1707-1715.

Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF (2005). Identification, cloning, and expression of human estrogen receptor-[alpha]36, a novel variant of human estrogen receptor-[alpha]66. *Biochemical and Biophysical Research Communications* 336: 1023-1027.

Warner M, Gustafsson J-A (2006). Nongenomic effects of estrogen: Why all the uncertainty? *Steroids* 71: 91-95.

Weitzmann MN, Pacifici R (2006). Estrogen deficiency and bone loss: an inflammatory tale. *J. Clin. Invest.* 116: 1186-1194.

Zhang Q-X, Borg A, Fuqua SAW (1993). An Exon 5 Deletion Variant of the Estrogen Receptor Frequently Coexpressed with Wild-Type Estrogen Receptor in Human Breast Cancer. *Cancer Research* 53: 5882-5884.

Zhang Q-X, Hilsenbeck SG, Fuqua SAW, Borg Å (1996). Multiple splicing variants of the estrogen receptor are present in individual human breast tumors. *The Journal of Steroid Biochemistry and Molecular Biology* 59: 251-260.

Zhao C, Matthews J, Tujague M, Wan J, Strom A, Toresson G *et al* (2007). Estrogen Receptor {beta}2 Negatively Regulates the Transactivation of Estrogen Receptor {alpha} in Human Breast Cancer Cells. *Cancer Res* 67: 3955-3962.

ZhaoYi Wang, XinTian Zhang, Peng Shen, Brian W Loggie, YunChao Chang, Deuel. TF (2006). A variant of estrogen receptor-{alpha}, hER-{alpha}36: Transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. *PNAS* 103: 9063-9068.
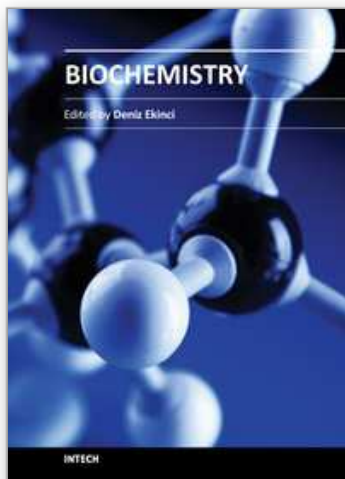
Biochemistry

Edited by Prof. Deniz Ekinci

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.

INTECH
open science | open minds