Loss of ERβ expression as a common step in estrogen-dependent tumor progression

A Bardin[1], N Boulle[1,2], G Lazennec[1], F Vignon[1] and P Pujol[1,2]

[1]Unité INSERM 540, 60 rue de Navacelles, 34090 Montpellier, France.

[2]Laboratoire de Biologie Cellulaire, Centre Hospitalier Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, 271, Av G. Giraud, 34295 Montpellier, France.

(Requests for offprints should be addressed to Dr P Pujol, Service de Biologie Cellulaire, Hôpital Arnaud de Villeneuve, CHU, 271, Av G. Giraud, 34295 Montpellier, France; Tel: 33 (0) 467 33 58 77; Fax: 33 (0) 467 33 95 90; E-mail: p-pujol@chu-montpellier.fr)

Running title: ERβ and estrogen-dependent cancers

Abbreviations: hERα or β, human estrogen receptor α or β; E2, 17β-estradiol; IHC, immunohistochemistry; ISH, *in situ* hybridization; Apc, adenomatosis polyposis coli; ERE, estrogen response element; SFRE, steroid factor response element ; AP-1, activator protein-1; wt, wild-type; SAOS-2, human osteoblast-like cells; HOSE, Human Ovarian Surface Epithelium; WHI, Women's Health Initiative; RT-PCR, reverse transcription polymerase chain reaction; mRNA, messenger RNA; PARP, Poly (ADP-ribose) polymerase.

Abstract

The characterization of estrogen receptor beta (ERβ) brought new insight into the mechanisms underlying estrogen signaling. Estrogen induction of cell proliferation is a crucial step in carcinogenesis of gynecologic target tissues and the mitogenic effects of estrogen in these tissues (e.g. breast, endometrium and ovary) are well documented both *in vitro* and *in vivo*. There is also an emerging body of evidence that colon and prostate cancer growth is influenced by estrogens. In all of these tissues, most studies have shown decreased ERβ expression in cancer as compared to benign tumors or normal tissues, whereas ERα expression persists. The loss of ERβ expression in cancer cells could reflect tumor cell dedifferentiation but may also represent a critical stage in estrogen-dependent tumor progression. Modulation of the expression of ERα target genes by ERβ, or ERβ specific gene induction could indicate that ERβ has a differential effect on proliferation as compared to ERα. ERβ may exert a protective effect and thus constitute a new target for hormone therapy, e.g. via ligand specific activation. The potential distinct roles of ERα and ERβ expression in carcinogenesis, as suggested by experimental and clinical data, are discussed in this review.

Introduction

It is well documented that the mitogenic actions of estrogens are critical in the etiology and progression of human breast and gynecological cancers (Henderson *et al.* 1988, Pike *et al.* 1993). The promoting effect of estrogens was recently highlighted by the results of large prospective studies, showing that estradiol intake during menopause increased the risk of breast cancer (Nelson *et al.* 2002, Rossouw *et al.* 2002, Chlebowski *et al.* 2003, Beral *et al.* 2003). In ovarian cancer, although the question is still debated (Coughlin *et al.* 2000), several recent prospective studies have indicated a risk of ovarian cancer for women undergoing long-term estrogen replacement therapy (Rodriguez *et al.* 2001, Lacey *et al*. 2002, Anderson *et al*. 2003, Folsom *et al*. 2004). In contrast, estrogens appear to exert a protective effect on the risk of colon cancer (Rossouw *et al.* 2002).

The effects of estrogens are mediated by ERα and ERβ receptors, which are members of the nuclear steroid receptor superfamily. ERα and ERβ classically mediate their action by ligand-dependent binding to the estrogen response element (ERE) of target genes, leading to their transcription regulation (Green *et al.* 1986, Mosselman *et al.*1996, Kuiper *et al*. 1996, Tremblay *et al*. 1997). Both of these proteins have a high degree of homology in the DNA binding domain (Mosselman *et al.* 1996), but differ considerably in the N-terminal domain and to a lesser extent in the ligand-binding domain (E domain) (Mosselman *et al.* 1996, Kuiper *et al.*

1996). These differences suggest that the two receptors could have distinct functions in terms of gene regulation and biological responses and may contribute to the selective actions of E2 in different target tissues (Gustafsson *et al.* 2000).

Recently, various studies have shown decreased expression of ERβ mRNA and protein (or an increased ERα/ERβ mRNA ratio) in tumor versus normal tissues in many cancers, including breast, ovary, colon, and prostate (Brandenberger *et al.* 1998, Campbell-Thompson *et al.* 2001, Fixemer *et al.* 2003, Foley *et al.* 2000, Pujol *et al.*1998, Roger *et al.* 2001, Rutherford *et al*. 2000). The ERα/ERβ gene expression ratio thus appears to increase during carcinogenesis, suggesting that ERα and ERβ specific pathways may have distinct roles in this process (Leygue *et al.* 1998). The differential expression of ERα and ERβ in cancer cells and experimental data on their respective roles on proliferation are reviewed in this report.

Differential ERβ expression as a common feature of estrogen-dependent tumor progression in clinical studies

Analysis of ERα and ERβ expression in estrogen sensitive cancers (Tables 1 to 4)

In breast tissues, several studies have indicated an increase in ERα/ERβ mRNA and protein ratios in cancer as compared to benign tumors and normal tissues. In immunochemical analyses, Roger *et al.* (2001) found a higher percentage of ERβ-positive cells in normal mammary glands as compared to non-proliferative benign breast disease (BBD) (85%), proliferative BBD without atypia (18.5%) and carcinoma *in situ* (33.8%). In contrast, an increase in ERα protein expression was noted during progression. Moreover, ERβ was inversely correlated with Ki67, a marker of cell proliferation. The authors thus suggest that ERβ has a protective role against the mitogenic activity of estrogens in mammary premalignant lesions. This conclusion is also supported by the results of another study (Shaw et *al.* 2002), which revealed lower ERβ protein expression in carcinomas and demonstrated that ERα but not ERβ protein expression was correlated with tumor grade. Similar findings were obtained at the mRNA level using the RT-PCR method in study of Iwao *et al*. (2000), who also showed that ERα mRNA is increased and ERβ mRNA decreased during breast carcinogenesis. Recently, Park *et al.* (2003) compared ERβ mRNA levels in various breast tissues using mRNA *in situ* hybridization. ERβ expression was decreased in breast cancer (BC) and metastatic lymph node tissues as compared with normal mammary and

benign breast tumor (BBT) tissues. The intensity and extent of ERβ expression were significantly higher in normal and BBT tissues than in BC or metastatic lymph node tissues (Park *et al.* 2003).

In invasive breast cancer, other studies using IHC and ISH methods revealed that ERβ expression was associated with indicators of low biological aggressiveness (low tumor grade, low S-fraction, and negative lymph node status), suggesting that ERβ might be a good prognostic indicator (Jarvinen *et al.* 2000). In a study of Omoto *et al*. (2001), a survival analysis showed that patients with ERβ-positive tumors had increased disease-free survival at 5 years as compared with those with ERβ-negative tumors. Fuqua *et al.* (2003) studied ERβ expression using IHC in a pilot series of 242 breast cancer patients and showed that ERβ expression is not associated with clinical and biological parameters, including PR expression, tumor grade and S-phase fraction. ERβ was only found to be correlated with aneuploidy. The findings of this study suggested that ERβ could be a useful biomarker on its own in clinical breast tumors. To gain insight into the possible role of ERβ in breast carcinogenesis, Skliris *et al.* (2003) performed an IHC analysis of ERβ on 512 breast specimens. Moreover, real time PCR was used to investigate the ERβ gene methylation status in the ERβ-negative breast cancer cell lines SK-BR-3 and MDA-MB-435. The results suggested that the loss of ERβ expression is one of the hallmarks of breast carcinogenesis and that it may be a reversible process involving methylation. Zhao *et al.* (2003) also concluded that decreased ERβ mRNA expression may be associated with breast tumorigenesis and that DNA methylation is an important mechanism for ERβ gene silencing in breast cancer (Table 1). Collectively, ERβ expression decreases in the process of breast cancer development.

The ovary (Table 2) contains both ER isoforms but ERβ seems to be the predominant species expressed in normal ovary in rats (Byers *et al.* 1997) and humans (Kuiper *et al.* 1996, Enmark *et al*. 1997). Our laboratory (Pujol *et al.* 1998) documented an increase in the ERα/ERβ mRNA ratio in ovarian carcinomas as compared with normal ovaries and cysts and our findings suggested that overexpression of ERα relative to ERβ mRNA may be a marker of ovarian carcinogenesis. This conclusion was further supported by Brandenberger *et al.* (1998) and Rutherford *et al.* (2000). The latter study revealed that the balance between ERα and ERβ receptors might be essential for maintaining normal cellular function, suggesting that, as ERβ decreases, uncontrolled cellular proliferation leads to a metastatic state. In a study of Lau *et al.* (1999) no differences in ERβ mRNA expression were found between normal and cancer epithelial cells, but these authors only analyzed a few HOSE cell primary cultures (n=4) and ovarian cancer cell lines (n=3) using a non-quantitative PCR method. Decreasing levels of ERβ expression seems to be a common denominator between breast and ovarian carcinogenesis.

In prostate, it has been suggested that estrogens and their receptors may be involved in cancer development and progression (Santti *et al.* 1994, Farnsworth *et al.* 1999, Jarred *et al.* 2000). Estrogen exposure during prostate development may initiate cellular processes resulting in future neoplasia (Santti *et al.* 1994). In a study of Latil *et al*. (2001), ERα and ERβ mRNA expression were quantified by real-time RT-PCR in both benign and malignant prostate. ERβ mRNA level was decreased in most of the tumor samples as compared to normal prostate, suggesting that ERα and ERβ expression status could be used to identify advanced prostate tumor patients. This result is in agreement with those obtained at the protein level. Pasquali *et al.* (2001a) investigated ERβ expression in benign and malignant prostate tissue specimens using a polyclonal antibody directed against the C-terminal domain of the ERβ protein. In contrast to normal tissues, ERβ nuclear immunostaining was undetectable in all cancer sections, showing that malignancy seems to be associated with the disappearance of ERβ expression in prostate tissue. Horvath *et al.* (2001), using IHC, also found that the ERβ protein was progressively lost in hyperplasia and neoplastic lesions. This is in agreement with the results of Fixemer *et al.* (2003) in a study in which a new monoclonal antibody revealed the differential expression of ERβ in tissue sections from 132 patients with prostate cancer. Moreover, these authors showed partial loss of ERβ in high grade prostatic intraepithelial neoplasia (HGPIN) (Table 3). Once more the change in ERα/ERβ ratio seems to be correlated with malignancy.

In colon cancer**,** the protective effect of estrogen replacement therapy is supported by a number of clinical observations (Calle *et al.* 1995, Newcomb *et al.* 1995, Persson *et al*. 1996, Kampman *et al*. 1997), including the results of recent randomized studies named "WHI" (Nelson *et al.* 2002, Rossouw *et al.* 2002). These studies demonstrated that women with a history of current or past hormone replacement therapy had a significantly decreased risk of colon cancer. These findings have led many investigators to search for the biological mechanisms by which estrogen may influence the pathogenesis of colorectal cancer. Since ERα is reported to be minimally expressed in normal colon mucosa and colon cancer cells (Waliszewski *et al.* 1997, Campbell-Thompson *et al*. 2001), the effects of estrogen on colon cancer susceptibility may be mediated by ERβ. Campbell-Thompson *et al.* (2001) showed, using semi-quantitative RT-PCR, that ERβ is the predominant ER subtype in the human colon and that decreased ERβ1 (ERβwt) and ERβ2 (ERβcx) mRNA levels were associated with colonic tumorigenesis in women. In a recent study using IHC analysis (Konstantinopoulos *et al.* 2003), it was shown that ERβ expression was significantly lower in colon cancer cells than in normal colonic epithelium and that there was a progressive decline in ERβ expression, which paralleled the loss of malignant

colon cell dedifferentiation. These findings are in accordance with a previous study of Foley *et al.* (2000) who also detected a selective loss of ERβ protein in malignant human colon by Western immunoblotting. Weyant *et al.* (2001) worked with a model of mice bearing germline mutations in murine Apc. These mice develop multiple intestinal tumors that show loss of wild-type Apc protein. In this model, E2-induced prevention of Apc-associated tumor formation was correlated with an increase in ERβ protein and a decrease in ERα in target tissues. Altogether, these results strongly suggest that ERβ provides protection against colon carcinogenesis (Table 4).

ERβ as a predictive factor for antiestrogen therapy?

Although many reports suggest the protective role of ERβ against tumor progression, controversies have arisen regarding the clinical value of ERβ expression in terms of predicting the adjuvant hormonal therapy response in breast cancer. Some studies suggest that the ERβ status in breast cancer is a predictor of the response to tamoxifen (Leygue *et al*. 1998, Jarvinen *et al.* 2000, Mann *et al.* 2001) whereas others suggest that ERβ is significantly upregulated in tamoxifen resistant breast cells and could be involved in tamoxifen resistance (Speirs *et al.* 1999).

The type of analysis, patient selection criteria, the type of splicing variants detected in RNA analyses or the small number of patients analysed to date could ultimately explain these controversial results. The first findings were obtained in studies involving RT-PCR based techniques, but the quantification of gene expression at the mRNA level may not be directly linked qualitatively or quantitatively to the protein expression. There have been very few studies in which ERs were measured by Western immunoblotting or IHC because of the lack of reliable antibodies. Finally, the choice of statistical analysis and different parameters selected for analysis could also influence the results.

ERβ as a potential tumor suppressor gene?

The results of these different studies, showing a loss of ERβ expression in cancer as compared to normal cells, are in line with the hypothesis that the ERβ gene may act as a tumor suppressor (Iwao *et al.* 2000). This concept needs to be confirmed but could make sense in view of the location of ERβ on chromosome 14q (Enmark *et al.* 1997). A loss of 14q has been detected by comparative genomic hybridization in some breast cancers (Loveday *et al.* 2000, Burki *et al.* 2000). Interestingly, in ovarian cancer, two potential tumor suppressor

gene loci have been mapped to 14q (Bandera *et al.* 1997). 14q deletions are also observed in colon carcinoma (Young *et al.* 1993) and prostate cancer (Kasahara *et al.* 2002). These overall findings suggest a potential tumor suppressive function for ERβ. However, further studies are required before definitive conclusions on the tumor suppressive function of ERβ can be drawn.

What are the potential molecular mechanisms underlying ERα and ERβ differential actions?

Several *in vitro* studies have focused on the molecular mechanisms underlying the differential roles of ERα and ERβ. Differences in ligand affinity, transcriptional activation, interactions with cofactors or putative heterodimerisation have been proposed.

Structural properties of ERα and ERβ and consequences on their transcriptional activities

Estrogen receptors α and β belong to the large nuclear steroid/thyroid hormone receptor family. Like most other members of the family, ERs have a modular architecture of four interacting domains: the N-terminal A/B domain, the C or DNA binding domain (DBD), the D or hinge domain and the C-terminal E/F or ligand binding domain (LBD) (Fig. 1). There is only 56% amino-acid identity between the two receptors in the LBD whereas the homology in the DBD is 97%. This suggests that ERβ would recognize and bind to the same EREs as ERα but that each receptor might have a distinct spectrum of ligands (Kuiper *et al*. 1997a). A number of novel selective ER subtype ligands have now been developed. The propyl pyrazole triol (PPT) compound was found to be an ERα-specific agonist, activating gene transcription only through ERα (Sun *et al*. 1999, Stauffer *et al.* 2000). A number of other known ligands are also somewhat ERβ selective. Some phytoestrogens, such as genistein and coumestrol, show a higher affinity toward ERβ than ERα (Kuiper *et al.* 1997b). The diaryl-propionitrile (DPN) compound is a potency-selective agonist for ERβ with a more than 70-fold higher binding affinity for ERβ than ERα (Meyers *et al.* 2001). Recently, Ghosh *et al.* (2003) have investigated a novel series of heterocycle ligands for the ERs based on a diazene core motif. In this process, they have found diazenes that have high binding affinity for the ERs and some of these show preferential affinity for ERα or for ERβ.

The N-terminal domain of nuclear receptors encodes a ligand-independent activation function (AF-1) (Berry *et al.* 1990, McInerney *et al*. 1996a, Tora *et al.* 1989), a region of the receptor involved in protein-protein interactions (Onate *et al.* 1998), and transcriptional stimulation of target gene expression. The activation function-2 (AF-2) domain, located in the ligand-binding domain (Tora *et al.* 1989), is responsible for hormone-dependent activation through recruitment of coactivator proteins (Tremblay et *a*l. 1997, White et *al.* 1997). There is very little conservation in the N-terminal AF-1 domain, which could explain why different sets of proteins in the transcription complexes may interact with ERα and ERβ and direct them to specific targets. Dissimilarity in the NH_2-terminal extremity of ERα and ERβ is one possible explanation for the difference in the response of the two receptors to various ligands. In fact, the two receptors are distinct in their responses to the synthetic antiestrogens tamoxifen, raloxifen and ICI-164, 384. On an ERE-based reporter gene assay, tamoxifen, 4-OH-tamoxifen, raloxifen, and ICI-164, 384 have an ERα-selective partial agonist/antagonist function but pure E2 antagonist effect through ERβ (Barkem *et al.* 1998, McDonnell *et al.* 1995, McInerney *et al.* 1998). Watanabe *et al*. (1997) showed that the agonistic effect of tamoxifen depends on the cell type, ERE-promoter context, and ER subtypes, and that this action is ERα specific. Tamoxifen is an ERα antagonist in breast (Jordan *et al.* 1992) but an agonist in bone (Love *et al.* 1992) and uterine tissues (Kedar *et al*. 1994). Raloxifene is also an ERα antagonist in breast tissue, but it exerts agonistic activity in bone but not in uterine tissue (Black *et al.* 1994).

ERα and ERβ are capable of regulating gene transcription through a classical mechanism involving the consensus estrogen response element (ERE) but ERβ seems to be a weaker transactivator (Cowley *et al.* 1999). Cowley and Parker (1999) have shown that the AF-1 activity of ERβ is weak compared with that of ERα on estrogen-responsive reporters, whereas their AF-2 activities are similar. In turn, when both AF-1 and AF-2 functions are active in a particular cell and/or on a particular promoter, the activity of ERα greatly exceeds that of ERβ, whereas ERα and ERβ activities are similar when only AF-2 is required (Cowley *et al.* 1999, McInerney *et al.* 1998). ERα and ERβ have similar but also different effects on gene transcription mediated via the ERE. To date only a limited number of genes have been shown to be regulated by one of the two E2-liganded ER subtypes in this classical mode of action. In this way, gene encoding the catalytic subunit of human telomerase hTERT is regulated by ERα and not by ERβ in human ovary epithelium cells (Misiti *et al,* 2000) and in human prostate cancer (Nanni *et al,* 2002). In the same way, Lazennec *et al.* (2001) reported that, ERα, but not ERβ was able to regulate c-myc proto-oncogene expression. The metallothionein gene is known to be specifically upregulated by E2 via ERβ in SAOS-2 cells (Harris *et al.* 2001). However, recently, Stossi *et al*.

(2004) have compared the gene regulatory activities of ERα and ERβ in bone and showed a highly similarity but also significant differences in gene targets for these two ERs. Thus, genes encoding for cystatin D, autotaxin or stromal antigen 2 appear to be E2-regulated specifically by ERβ in human osteosarcoma cells.

Estrogens (and antiestrogens) also transcriptionally regulate target genes via ERs though a non-ERE mode of action. These effects are mediated through promoter elements that bind various transcription factors, including AP-1-binding sites (Webb *et al.* 1995), Sp1 binding sites (Porter *et al.* 1997), the SF1 response element (SFRE) (Vanacker *et al.* 1999), electrophophilic/antioxidant response element (EpRE/ARE) (Montano *et al.* 1997) and cyclic AMP response element (CRE) (Sabbah *et al.* 1999). At AP-1 sites, ERα and ERβ could have opposite transcriptional effects in some circumstances (Paech *et al.* 1997). In fact, ERβ is able to potentiate an AP-1 containing reporter in the presence of the anti-estrogen tamoxifen but not in the presence of estrogens in a tissue-specific manner. ERα stimulates AP-1 activity in the presence of antiestrogens in endometrial cells (Webb *et al.* 1995, Paech *et al.* 1997), but antiestrogens decrease or have no effect on AP-1 activity in breast cancer cells (Philips *et al.* 1993, Webb *et al.* 1995). Of particular note, ERβ is more potent overall than ERα on AP-1 sites, whereas the contrary occurs on EREs (Paech *et al.* 1997, Cowley *et al.* 1999, Hall *et al.* 1999). Similar to AP-1, E2 binding to ERα induces transcriptional activation when associated with SP1 in GC-rich regions. However, E2 interaction with ERβ does not result in the formation of a transcriptionally active complex at a promoter containing Sp1 elements (Saville *et al.* 2000). Vanacker *et al.* (1999) described that the osteopontin gene promoter is stimulated through SFRE sequences by ERα but not by ERβ.

Consequently, these differences in ligand interaction or transcriptional activity between the two ER subtypes may account for the major differences in their tissue-specific biological actions. This complexity is further enhanced by ERβ isoforms, the ability of ERs to form homodimers and heterodimers, and their capacity to interact with various coregulators.

ER isoforms

Several groups have reported and cloned different ERβ isoforms with exon deletions (Lu *et al.* 1998), insertions (Hanstein *et al.* 1999), or C-terminal splice variants (Ogawa *et al.* 1998, Moore *et al.* 1998). These isoforms can also bind ligands, mediate estrogen signalling (Kuiper *et al.* 1997b, Paech *et al.* 1997, Cowley *et al.* 1999, Bollig *et al.* 2000) and exhibit different properties, thus further enhancing the complexity in the spectrum of potential cellular responses to estrogen. The key element lies perhaps in the balance between the expression of

these different variants and their relative quantities. It has been shown that ERβ splice variants have dramatically different localization patterns in living cells and this localization can be altered by estrogen agonists and antagonists (Price *et al.* 2000). Interestingly, Poola *et al.* (2002) recently showed that estrogen receptor β splice variant mRNAs were differentially altered during breast carcinogenensis. ERβcx, which utilizes an alternative exon 8, is the most extensively studied splice variant. Ogawa *et al.* (1998) showed that this isoform may act as a potential inhibitor of ERα transactivation, possibly due to ERα/ERβcx heterodimer formation. Using IHC, it has been shown that differential expression of ERβwt and ERβcx may be used as a prognostic marker in human prostate (Fujirama *et al.* 2001). Peng *et al*. (2003) showed that all ERβ isoforms inhibited ERα transcriptional activity on an ERE, while only ERβwt had transcriptional activity of its own. It has been shown, using cDNA microarrays in MCF-7 cells stably transfected with ERβwt and ERβcx MCF-7, that these two isoforms inhibit ERα function differently (Omoto *et al*. 2003). Consequently, it can be hypothesized that the differential expression of ERβ isoforms may have a role in the modulation of estrogen action.

ER homo- and hetero-dimers

The functional formation of ERα and ERβ heterodimers has been demonstrated (Pettersson *et al.* 1997, Cowley *et al*. 1997). They are able to bind to DNA with an affinity similar to that of ERα and greater than that of ERβ homodimers, to interact with coactivators, and to stimulate the transcription of reporter gene in transfected cells (Pettersson *et al.* 1997, Cowley *et al*. 1997). The possible involvement of ERα and ERβ dimerization would increase the complexity of transcription activation in response to E2 and suggests the existence of two previously unrecognized estrogen signaling pathways, i.e. via ERβ homodimers and ERα/ERβ heterodimers. Moreover, it has been reported that various ERα and ERβ ratios in different cells, resulting in different homodimer and heterodimer compositions, may constitute a key for gaining insight into the tissue-specific effects of estrogen and antiestrogens (Kuiper *et al.* 1997a). Homodimers and heterodimers could bind to distinct response elements and consequently activate specific gene expression patterns in given target tissues. For such interactions, ERα and ERβ must be coexpressed in cells, as noted in breast, ovarian and endometrium tissues. However, future studies will be required to determine the physiological roles of ERα and ERβ homo- and heterodimers *in vivo*.

Interactions with coactivators and corepressors

There is one further confounding factor in the ER-mediated estrogen action equation. The ER-mediated transcriptional activity of estrogen is influenced by several regulatory factors, known as coactivators and corepressors, which activate or repress the transcription of ER-responsive genes (Klinge *et al.* 2000). The p160/SRC (steroid receptor coactivator) family is one of the most studied classes of coactivators, and includes SRC1, SRC2 (GRIP1/TIF-2) (McKenna *et al.* 1999) and other more recently described coactivators such as ACTR (Chen *et al*. 1997), RAC3 (Li *et al.* 1997), AIB1 (Anzick *et al.* 1997) and TRAM-1 (Takeshita *et al*. 1997). Most of interactions of these coregulators with the ER is ligand-dependent but some coactivators have also been shown to be recruited in a ligand-independent manner by the AF-1 domain of ERs (Mc Inerney *et al*. 1996b; Tremblay *et al.* 1999). SRC-1 activated ERβ AF-1 upon MAPK-induced phosphorylation of serine residues (Tremblay *et al.* 1999). Deblois *et al.* (2003) studied the steroid receptor RNA activator (SRA) and showed that SRA potentiated the estrogen-induced transcriptional activity of both ERα and ERβ. They demonstrated that the transcriptional activity of ERα can be enhanced by SRA in a ligand-independent manner through the AF-1 domain. However, this AF-1 dependent effect of SRA is not observed on ERβ. Very few receptor-specific ERβ cofactors have been identified so far. Warnmark *et al.* (2001) showed that TRAP220 displays a preference for ERβ and suggested that the coregulator selectivity of ER subtypes is an additional layer of specificity that influences the transcriptional response in estrogen target cells. Kurebayashi *et al.* (2000) also showed, using multiplex RT-PCR, that ERβ expression levels were correlated with some activators such as AIB1, CBP, P/CAF, and a corepressor, N-CoR, but the significance of this correlation is unclear. Nuclear receptors usually bind the corepressors N-CoR and SMRT in the absence of ligand or in the presence of antagonists. Agonist binding leads to corepressor release and coactivator recruitment. A recent study (Webb *et al*. 2003) demonstrated that, *in vitro* and *in vivo*, ERβ binds to N-CoR and SMRT in the presence of ER agonists such as estradiol and phytoestrogens like genistein, but not in the presence of antagonists. ERα and ERβ present completely distinct modes of action with coregulators, which could be of major importance in terms of potential effects on physiological behaviour (Webb *et al*. 2003).

What do we know about the role of ERβ in cell proliferation and death?

ERβ and cell proliferation

Although the specific functions of ERβ in cancer are not known, there is some evidence that ERβ could have inhibitory effects on cellular proliferation. First, as indicated previously, the levels of ERβ are highest in normal tissue (breast, ovary, prostate) as well as in benign disease and it decreases during carcinogenesis (Table 1-3). Our laboratory obtained the first evidence that ERβ is an important modulator of proliferation and invasion of breast cancer cells, thus supporting the hypothesis that the loss of ERβ expression could be one of the events leading to breast cancer development (Lazennec *et al.* 2001). Whereas ERα was able to regulate reporter genes and endogenous genes in a ligand-dependent manner, ERβ inhibited MDA-MB231 cell proliferation in a ligand-independent manner. This suggests that the two ERs inhibit cancer cell proliferation via different mechanisms (Lazennec *et al.* 2001).

Omoto *et al.* (2003) recently developed cell lines expressing ERβwt and ERβcx by stable transfection of each expression plasmid in MCF7 cells and demonstrated that this constitutive expression significantly reduced the percentage of cell population in S-phase and the number of colonies in an anchorage-independent assay. Recently, two studies showed that the induced expression of ERβ in ERα-positive breast cancer cells inhibits their growth (Paruthiyil *et al.* 2004, Strom *et al.* 2004). These reports also suggest that ERβ might reduce cell proliferation by inhibiting cyclin D1gene, a key factor controlling the G1-S transition of the cell cycle, and thus cell proliferation. Strom *et al.* (2004) also indicated that numerous other components of the cell cycle associated with proliferation such as cyclin E or Cdc25A were decreased. These results are in accordance with the study of Bièche *et al.* (2001), showing a negative correlation between ERβ and CCND1 (cyclin D1) expression. *In vitro* studies are in agreement with the hypothesis of Liu *et al.* (2002) who showed that E2 activates cyclin D1 gene transcription through ERα, but inhibits cyclin D1 gene transcription through ERβ in HeLa cells.

The contrasting phenotypes observed in individual lines of ER knockout mice, i.e. ERαKO and ERβKO, which exhibit phenotypes that generally mirror the respective ER expression patterns, provides further evidence that the two ERs have distinct biological functions. Weihua *et al.* (2000) observed that, in immature uterus, ERα and ERβ are expressed at comparable levels in the epithelium and stroma, and E2 treatment decreases ERβ in the stroma. Increased cell proliferation and the exaggerated response to E2 in ERβKO mice suggested that ERβ plays a role in the modulation of the effects of ERα and also (or consequently) has an

antiproliferative function in immature uterus. A second study in ERβ-/- mice showed that ERβ is implicated in the regulation of epithelial growth, and its absence results in hyperplasia of the prostatic epithelium (Weihua *et al.* 2001). The inhibition of ERα transcriptional activity could be a molecular mechanism by which ERβ has antiproliferative effects. Previous *in vitro* data indicate that ERβ could act as a dominant negative regulator of ERα activity. Hall *et al.* (1999) have provided direct proof that ERβ modulates/represses ERα transcriptional activity in transient transfection cells. In bone, it has been shown that ERβ inactivation by gene targeting results in increased cortical bone formation. Windalh *et al.* (2001) showed that, when present, ERβ acts in a repressive manner on trabecular bone, possibly by inhibiting the stimulatory action of ERα. Finally, Lindberg *et al*. (2003) showed that in some mouse tissues, ERβ reduces ERα-regulated gene transcription, thus indicating that there is a balanced relationship between ERα and ERβ (Fig. 2).

ERβ and apoptotic pathways?

A decrease in the human cancer cell population *in vitro* or tumor regression *in vivo* reflects a change in the balance of cellular growth events and could involve arrested cell proliferation or an enhanced cell death or both. Several studies have suggested that estrogen may regulate apoptotic pathways in cancers (Kyprianou *et al*. 1991, Perillo *et al.* 2000, Choi *et al.* 2001). We could assume that E2 effects involve both proliferation induction and apoptosis inhibition. Choi *et al*. (2001) showed that E2 may be associated with upregulation of the anti-apoptotic bcl-2 gene at the mRNA level. It has been suggested that ERα may play a role in ovarian tumorigenesis by preventing apoptosis whereas the ERβ-induced inhibition of proliferation could be explained by the inhibition of the bcl-2 gene, as supported by a recent report of Nilsen *et al.* (2000). They showed that estradiol can function as a neuroprotective agent or an inducer of apoptosis, depending on the estrogen receptor-subtype present in the cell. ERα is thus associated with a neuroprotective effect, while ERβ mediates the induction of apoptosis in neuronal cells. Similarly, Sapi *et al.* (2002) demonstrated estrogen-induced upregulation of FasL, an apoptotic protein ligand, in ovary. This could seem paradoxical since estrogen is known to be anti-apoptotic in different cells. The authors proposed that in normal ovary the apoptotic protein ligand FasL is probably upregulated by ERβ, the predominant form of ER in this tissue (Fig. 3). Recently, we have demonstrated (Cheng *et al.* in press) that the expression of ERβ in prostate carcinoma cells triggers apoptosis, notably by increasing baxα levels as well as cleavage of PARP and caspase-3 expression.

Conclusions

Numerous clinical and *in vitro* studies suggest that imbalanced ERα/ERβ expression is a common feature and could be a critical step of estrogen dependent tumor progression. ERβ seems to play a key role in the mitogenic action of estrogen by providing protection against ERα-induced hyperproliferation. A role in apoptosis might also be possible.

ERα and ERβ have some overlapping tissue distribution but also display high relative tissue-specific expression. Moreover, a number of molecular mechanisms could be proposed to explain the differential roles of ERα and ERβ, including differences in ligand affinity and transactivation, distinct cofactor interactions and putative heterodimerisation. Splicing variant ERs isoforms may also be important in modulating the cellular response.

In conclusion, the imbalance in ERα/ERβ expression in estrogen dependent cancer opens a new field in hormone therapy of cancer. Targeted ERβ therapies, including the development of ERβ specific ligands, may constitute a new therapeutic approach particularly for pre-invasive or proliferative lesions. The clinical value of ERβ in cancer prognosis and its possible usefulness for prediction of the hormone response should be assessed in large-scale and prospective clinical studies.

Acknowledgments

This work was supported by the "Ligue Nationale Contre le Cancer", the "Centre Hospitalier Universitaire de Montpellier" (Grant # AOI7619), the "Association pour la Recherche contre le Cancer" (Grant n° 4302) and the "Institut National de la Santé et de la Recherche Médicale".

References

Anderson GL, Judd HL, Kaunitz AM, Barad DH, Beresford SA, Pettinger M, Liu J, McNeeley SG & Lopez AM; Women's Health Institute Investigators 2003 Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial. *The Journal of the American Medical Association* **290** 1739-1748.

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM & Meltzer PS 1997 AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* **277** 965-968.

Bandera CA, Takahashi H, Behbakht K, Liu PC, LiVolsi VA, Benjamin I, Morgan MA, King SA, Rubin SC & Boyd J 1997 Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma. *Cancer Research* **57** 513-515.

Barkhem T, Carlsson B, Nilsson Y, Enmark E, Gustafsson J & Nilsson S 1998 Differential response of estrogen receptor alpha and estrogen receptor beta to partial estrogen agonists/antagonists. *Molecular Pharmacology* **54** 105-112.

Beral V & Million Women Study Collaborators 2003 Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* **362** 419-27.

Berry M, Metzger D & Chambon P 1990 Role of the two activating domains of oestrogen receptors in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO Journal* **9** 2811-2818.

Bieche I, Parfait B, Laurendeau I, Girault I, Vidaud M & Lidereau R 2001 Quantification of estrogen receptor alpha and beta expression in sporadic breast cancer. *Oncogene* **20** 8109-8115.

Black LJ, Sato M, Rowley ER, Magee DE, Bekele A, Williams DC, Cullinan GJ, Bendele R, Kauffman RF, Bensch WR, *et al.* 1994 Raloxifene (LY139481 HCI) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *The Journal of Clinical Investigation* **93** 63-69.

Bollig A & Miksicek JR 2000 An estrogen receptor-alpha splicing variant mediates both positive and negative effects on gene transcription. *Molecular Endocrinology* **14** 634-649.

Brandenberger AW, Tee MK & Jaffe RB 1998 Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER-beta in neoplastic tissues. *The Journal of Clinical Endocrinology & Metabolism* **83** 1025-1028.

Burki NG, Caduff R, Walt H, Moll C, Pejovic T, Haller U & Ward DC 2000 Comparative genomic hybridisation of fine needle aspirates from breast carcinomas. *International Journal of Cancer* **15** 607-613.

Byers M, Kuiper GJ, Gustafsson JA & Park-Sarge OK 1997 Estrogen receptor-β mRNA expression in rat ovary: down-regulation by gonadotropins. *Molecular Endocrinology* **11** 172-182.

Calle EE, Miracle-Mahill HL, Thun MJ & Health CWJr 1995 Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. *Journal of the National Cancer Institute* **87** 517-523.

Campbell–Thompson M, Lynch J & Bhardwaj B 2001 Expression of estrogen receptor (RE) subtypes ER beta isoforms in colon cancer. *Cancer Research* **61** 632-640.

Chen H, Lin RJ, Schiltz RL, Chakravarti D, Nash A, Nagy L, Privalsky ML, Nakatani Y & Evans RM 1997 Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90** 569-580.

Cheng J, Lee EJ, Madison LD & Lazennec G 2004 Expression of Estrogen Receptor Beta in Prostate Carcinoma Cells Inhibits Invasion and Proliferation and Triggers Apoptosis. *FEBS Letters* (in press).

Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, Rodabough RJ, Gilligan MA, Cyr MG, Thomson CA, Khandekar J, Petrovitch & McTiernan; WHI Investigators 2003 Influence of estrogens plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *The Journal of the American Medical Association* **289** 3243-3253.

Choi KC, Kang SK, Tai CJ, Auersperg N & Leung PC 2001 Estradiol up-regulates antiapoptotic Bcl-2 messenger ribonucleic acid and protein in tumorigenic ovarian surface epithelium cells. *Endocrinology* **142** 2351-2360.

Coughlin SS, Giustozzi A, Smith SJ & Lee NC 2000 A meta analysis of estrogen replacement therapy and risk of epithelial ovarian cancer. *Journal of Clinical Epidemiology* **53** 367-375.

Cowley SM, Hoare S, Mosselman S & Parker MG 1997 Estrogen Receptors α and β Form heterodimers on DNA. *The Journal of Biological Chemistry* **272** 19858-19862.

Cowley SM & Parker MG 1999 A comparison of transcriptional activation by REα and REβ. *Journal of Steroid Biochemistry and Molecular Biology* **69** 165-175.

Deblois G & Giguère V 2003 Ligand-independent coactivation of ERα AF-1 by steroid receptor RNA activator (SRA) via MAPK activation. *Journal of Steroid Biochemistry and Molecular Biology* **85** 123-131.

Enmark E, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjdd M & Gustafsson JA 1997 Human estrogen receptor β - gene structure, chromosome localization, expression pattern. *The Journal of Clinical Endocrinology & Metabolism* **82** 4258-4265.

Farnsworth WE 1999 Estrogen in the etiopathogenesis of BPH. *Prostate* **41** 263-274.

Fixemer T, Remberger K & Bonkhoff H 2003 Differential expression of the estrogen receptor beta in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. *Prostate* **54** 79-87.

Foley EF, Jazaeri AA, Shupnik MA, Jazaeri O & Rice LW 2000 Selective loss of estrogen receptor beta in malignant human colon. *Cancer Research* **60** 245 -248.

Folsom AR, Anderson JP & Ross JA 2004 Estrogen replacement therapy and ovarian cancer. *Epidemiology* **15** 100-104.

Fujimura T, Takahashi S, Urano T, Ogawa S, Ouchi Y, Kitamura T, Muramatsu M & Inoue S 2001 Differential expression of Estrogen Receptor β (ERβ) and its C-terminal Truncated Splice variant ERβcx as Prognostic Predictors in Human Prostatic Cancer. *Biochemical and Biophysical Research Communications* **289** 692-699.

Fuqua SA, Shiff R, Parra I, Moore JT, Moshin SK, Osborne CK, Clark GM & Allred C 2003 Estrogen Receptor β Protein in Human Breast Cancer: Correlation with Clinical Tumor Parameters. *Cancer Research* **63** 2434-2439.

Gosh U, Ganessunker D, Sattigeri VJ, Carlson KE, Mortensen DJ, Katzenellenbogen BS & Katzenellenbogen JA 2003 Estrogenic Diazenes: Heterocyclic Non-steroidal Estrogens of Unusual Structure with Selectivity for Estrogen Receptor Subtypes. *Bioorganic & Medicinal Chemistry* **11** 629-657.

Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P & Chambon P 1986 Human estrogen receptor cDNA: sequence, expression and homology to v-erbA. *Nature* **320** 134 –139.

Gustafsson JA & Warner M 2000 Estrogen Receptor β in the breast: role in estrogen responsiveness and development of breast cancer. *Journal of Steroid Biochemistry and Molecular Biology* **74** 245-248.

Hall JM & McDonnell DP 1999 The estrogen receptor β-isoform (ERβ) of the human estrogen receptor modulates ERα transcriptional activity and is a key regulator of the cellular response to estrogens and antiestrogens. *Endocrinology* **140** 5566-5578.

Hanstein B, Liu H, Yancisin MC & Brown M 1999 Functional Analysis of a Novel Estrogen Receptor-β Isoform. *Molecular Endocrinology* **13** 129-137.

Harris HA, Henderson RA, Bhat RA & Komm BS 2001 Regulation of metallothionein II messenger ribonucleic acid measures exogenous estrogen receptor-β activity in SAOS-2 and LNCaPLN3 cells. *Endocrinology* **142** 645-652.

Henderson BE, Ross R & Bernstein L 1988 Estrogens as a cause of human cancer. The Richard and Linda Rosenthal Foundation award lecture. *Cancer Research* **48** 246-253.

Horvath LG, Henshall SM, Lee CS, Head DR, Quinn DI, Makela S, Delprado W, Golovsky D, Brenner PC, O'Neill G, Kooner R, Stricker PD, Grygiel JJ, Gustafsson JA & Sutherland RL 2001 Frequent loss of estrogen receptor-beta expression in prostate cancer. *Cancer Research* **61** 5331-5335.

Iwao K, Miyoshi Y, Egawa C, Ikeda N & Noguchi S 2000 Quantitative analysis of estrogen receptor-β mRNA and its variants in human breast cancers. *International Journal of Cancer* **88** 733-736.

Jarred RA, Cancilla B, Prins GL, Thayer KA, Cunha GR & Risbridger GP 2000 Evidence That Estrogens Directly Alter Androgen-Regulated Prostate Development. *Endocrinology* **141** 3471-3477.

Jarvinen TA, Pelto-Huikko M, Holli K & Isola J 2000 Estrogen receptor beta is coexpressed with ERalpha and PR and associated with nodal status, grade, and proliferation rate in breast cancer. *The American Journal of Pathology* **156** 29-35.

Jordan VC 1992 The role of tamoxifen in the treatment and prevention of breast cancer. *Current Problems in Cancer* **16** 129-76.

Kampman E, Potter JD, Slattery ML, Caan BJ & Edwards S 1997 Hormone replacement therapy, reproductive history, and colon cancer: a multi-center, case-control study in the United States. *Cancer Causes and Control* **8** 146-158.

Kasahara K, Taguchi T, Yamasaki I, Kamada M, Yuri K & Shuin T 2002 Detection of genetic alterations in advanced prostate cancer by comparative genomic hybridisation. *Cancer Genetics and Cytogenetics* **137** 59-63.

Kedar RP, Bourne TH, Powles TJ, Collins WP, Ashley SE, Cosgrove DO & Campbell S 1994 Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. *Lancet* **343** 1318-1321.

Klinge CM 2000 Estrogen receptor interaction with co-activators and co-repressors. *Steroids* **65** 227-251.

Konstantinopoulos PA, Kominea A, Vandoros G, Sykiotis GP, Andricopoulos P, Varakis I, Sotiropoulou-Bonikou G & Papavassiliou AG 2003 Œstrogen receptor beta (ERβ) is abundantly expressed in normal colonic mucosa, but declines in colon adenocarcinoma paralleling the tumor's dedifferentiation. *European Journal of Cancer* **39** 1251-1258.

Kuiper G, Enmark E, Pelto-Huikko M, Nilsson S & Gustafsson JA 1996 Cloning of a novel estrogen receptor expressed in rat prostate and ovary. *PNAS* **93** 5925-5930.

Kuiper G, Carlsson B, Grandien K, Enmark E, MS Nilsson & Gustafsson JA 1997a Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptor α and β. *Endocrinology* **138** 863-870.

Kuiper GG & Gustafsson JA 1997b The novel estrogen receptor-beta subtype: potential role in the cell- and promoter-specific actions of estrogens and antiestrogens. *FEBS Letters* **410** 87-90.

Kurebayashi J, Sonoo H, Inaji H, Nishimura R, Iino Y, Toi M, Kobayashi S & Saeki T 2000 Endocrine therapies for patients with recurrent breast cancer: predictive factors for responses to first- and second-line endocrine therapies. *Oncology* **59** 31-37.

Kyprianou N, English HF, Davidson NE & Isaacs JT 1991 Programmed cell death during regression of the MCF-7 human breast cancer following estrogen ablation. *Cancer Research* **51** 162-166.

Lacey JVJr, Mink PJ, Lubin JH, Sherman ME, Troisi R, Hartge P, Schatzkin A & Schairer C 2002 Menopausal hormone replacement therapy and risk of ovarian cancer. *The Journal of the American Medical Association* **288** 334-341.

Latil A, Bieche I, Vidaud D, Liderau R, Berthon P, Cussenot O & Vidaud M 2001 Evaluation of Androgen, Estrogen (ERα and ERβ), and Progesterone Receptor Expression in Human Prostate Cancer by Real-Time Quantitative reverse Transcription-Polymerase Chain Reaction assays. *Cancer Research* **61** 1919-1926.

Lau KM, Mok SC & Ho SM 1999 Expression of human estrogen receptor-alpha and -beta, progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells. *PNAS* **96** 5722-5727.

Lazennec G, Bresson D, Lucas A, Chauveau C & Vignon F 2001 ERβ inhibits proliferation and invasion Breast Cancer Cells. *Endocrinology* **142** 4120-4130.

Leav I, Lau KM, Adams JY, McNeal JE, Taplin ME, Wang J, Singh H & Ho SM 2001 Comparative studies of the estrogen receptors beta and alpha and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *The American Journal of Pathology* **159** 13-16.

Leygue EH, Dotzlaw H, Watson PH & Murphy LC 1998 Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. *Cancer Research* **58** 3197-3201.

Li H, Gomes PJ & Chen JD 1997 RAC3, a steroid/nuclear receptor-associated coactivator that is related to SRC-1 and TIF2. *PNAS* **94** 8479-8484.

Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA & Ohlsson C 2003 Estrogen Receptor (ER)-beta Reduces ERalpha-Regulated Gene Transcription, Supporting a "Ying Yang" Relationship between ERalpha and ERbeta in Mice. *Molecular Endocrinology* **17** 203-208.

Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, Price RH Jr, Prestell RG & Kushner PJ 2002 Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. *The Journal of Biological Chemistry* **277** 24353-24360.

Love RR, Mazess RB, Barden HS, Epstein S, Newcomb PA, Jordan VC, Carbone PP & DeMets DL 1992 Effects of tamoxifen on bone mineral density in postmenopausal women with breast cancer. *New England Journal of Medecine* **326** 852-856.

Loveday RL, Greenman J, Simcox DL, Speirs V, Drew PJ, Monson JR & Kerin MJ 2000 Genetic changes in breast cancer detected by comparative genomic hybridisation. *International Journal of Cancer* **15** 494-500.

Lu B, Leygue E, Dotzlaw H, Murphy LJ, Murphy LC & Watson PH 1998 Estrogen receptor-beta mRNA variants in human and murine tissues. *Molecular Cell Endocrinology* **138** 199-203.

Mann S, Laucirica R, Carlson N, Younes PS, Ali N, Younes A, Li Y & Younes M 2001 Estrogen receptor beta expression in invasive breast cancer. *Human Pathology* **32** 113-118.

McDonnell DP, Clemm DL, Hermann T, Goldman ME & Pike JW 1995 Analysis of estrogen receptor function in vitro reveals three distinct classes of antiestrogens. *Molecular Endocrinology* **9** 659-669.

McInerney EM & Katzenellenbogen BS 1996a Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. *The Journal of Biological Chemistry* **271** 24172-24178.

McInerney EM, Tsai MJ, O'Malley BW & Katzenellenbogen BS 1996b Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *PNAS* **93** 10069-10073.

McInerney EM, Weis KE, Sun J, Mosselman S & Katzenellenbogen BS 1998 Transcription Activation by the Human Estrogen Receptor Subtype β (ERβ) Studied with ERβ and ERα receptor Chimeras. *Endocrinology* **139** 4513-4521.

McKenna NJ, Lanz RB, O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. *Endocrine Reviews* **20** 321-344.

Meyers MJ, Sun J, Carlson KE, Marириner GA, Katzenellenbogen BS & Katzenellenbogen JA 2001 Estrogen receptor-β potency selective ligands: structure-activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *Journal of Medecine and Chemistry* **44** 4230-4251.

Misiti S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirte HW, Piaggio G, Sacchi A, Pontecorvi A, Bachetti S & Farsetti A 2000 Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. *Molecular and Cellular Biology* **20** 3764-3771.

Montano MM & Katzenellenbogen BS 1997 The quinone reductase gene: a unique estrogen-regulated gene that is activated by antiestrogens. *PNAS* **94** 2581-2586.

Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Home EL, Su JL, Kliewer SA, Lehmann JM & Willson TM 1998 Cloning and characterization of human estrogen receptor beta isoforms. *Biochemical and Biophysical Research Communications* **9** 75-78.

Mosselman S, Polman J & Dijkema R 1996 RE-beta - identification and characterization of a novel human estrogen receptor. *FEBS Letters* **392** 49-53.

Nanni S, Narducci M, Della Pietra L, Moretti F, Grasseli A, De Carli P, Sacchi A, Pontecorvi A & Farsetti A 2002 Signaling through estrogen receptors modulates telomerase activity in human prostate cancer. *The Journal of Clinical Investigation* **110** 219-227.

Nelson HD, Humphrey LL, Nygren P, Teutsch SM & Allan JD 2002 Postmenopausal hormone replacement therapy: scientific review. *The Journal of the American Medical Association* **288** 872- 881.

Newcomb PA & Storer BE 1995 Postmenopausal hormone use and risk of large-bowel cancer. *Journal of the National Cancer Institute* **87** 1067-1071.

Nilsen J, Mor G & Naftolin F 2000 Estrogen-Regualted developmental Neuronal Apoptosis Is determined by estrogen receptor Subtype and the Fas/Fas Ligand System. *Journal of Neurobiology* **43** 64-78.

Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y & Muramatsu M 1998 Molecular cloning and characterization of human estrogen receptor βcx: a potential inhibitor of estrogen action in human. *Nucleic Acids Research* **26** 3505-3512.

Omoto Y, Inoue S, Ogawa S, Toyama T, Yamashita H, Muramatsu M, Kobayashi S & Iwase H 2001 Clinical Value of the wild-type estrogen receptor β expression in breast cancer. *Cancer Letters* **163** 207-212.

Omoto Y, Eguchi H, Yamamoto-Yamaguchi Y & Hayashi S 2003 Estrogen receptor (ER) β1 and ERβcx/β2 inhibit ERα function differently in breast cancer cell line MCF7. *Oncogene* **22** 5011-5020.

Onate SA, Boonyaratanakornkit V, Spencer TE, Tsai SY, Tsai MJ, Edwards DP & O'Malley BW 1998 The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF-1) and AF-2 domains of steroid receptors. *The Journal of Biological Chemistry* **15** 12101-12108.

Paech K. Webb P, Kuiper G, Nilsson S, Gustafsson JA, Kushner PJ & Scalan TS 1997 Differential Ligand Activation of Estrogen Receptors REα and REβ at AP-1 sites. *Science* **277** 1508-1510.

Park BW, Kim KS, Heo MK, KO SS, Hong SW, Yang WI, Kim JH, Kim GE & Lee KS 2003 Expression of estrogen receptor-β in normal mammry and tumor tissues : is it protective in breast carcinogenesis ? *Breast Cancer Research and Treatment* **80** 79-85.

Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL & Leitman DC 2004 Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Research* **64** 423-428.

Pasquali D, Staibano S, Prezioso D, Franco R, Esposito D, Notaro A, De Rosa G, Bellastella A & Sinisi AA 2001a Estrogen receptor β expression in human prostate tissue. *Molecular and Cellular Endocrinology* **178** 47-50.

Pasquali D, Rossi V, Esposito D, Abbondanza C, Puca GA, Bellastella A & Sinisi AA 2001b Loss of Estrogen Receptor β Expression in Malignant Human prostate Cells in Primary Cultures and in prostate Cancer Tissues. *The journal of Clinical Endocrinology & Metabolism* **86** 2051-2055.

Peng B, Lu B, Leygue E & Murphy LC 2003 Putative functional characteristics of human estrogen receptor-beta isoforms. *Journal of Molecular Endocrinology* **30** 13-29.

Perillo B, Sasso A, Abbondanza C & Palumbo G 2000 17β-Estradiol Inhibits Apoptosis in MCF-7 Cells, Inducing bcl-2 Expression via two Estrogen-responsive Elements Present in the coding sequence. *Molecular and Cellular Biology* **20** 2890-2901.

Persson I, Yuen J, Bergkvist L & Schairer C 1996 Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy: long term follow-up of a Swedish cohort. *International Journal of Cancer* **67** 327-332.

Pettersson K, Grandien K, Kuiper G & Gustafsson JA 1997 Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Molecular Endocrinology* **11** 1486-1496.

Philips A, Chalbos D & Rochefort H 1993 Estradiol Increases and Antiestrogens Antagonize the Growth Factor-induced Activator Protein-1 Activity in MCF7 Breast Cancer cells without Affecting *c-fos* and *c-jun* Synthesis. *The Journal of Biological Chemistry* **268** 14103-14108.

Pike MC, Spicer DV, Dahmoush L & Press MF 1993 Estrogens, progestagens, normal breast cancer proliferation, and breast cancer risk. *Epidemiology Reviews* **15** 17-35.

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

Porter W, Saville B, Hoivik D & Safe S 1997 Functional synergy between the transcription factor Sp1 and the estrogen receptor. *Molecular Endocrinology* **11** 1569-1580.

Price RH, Butler CA, Webb P, Uht R, Kushner P & Handa RJ 2001 A Splice Variant of Estrogen Receptor β Missing Exon 3 Displays Altered Subnuclear Localization and Capacity for Transcriptional Activation. *Endocrinology* **142** 2039-2049.

Pujol P, Rey JM, Nirde P, Roger P, Gastaldi M, Laffargues F, Rochefort H & Maudelonde T 1998 Differential expression of estrogen receptor-alpha and -beta messenger RNAs as a potential marker of ovarian carcinogenesis. *Cancer Research* **58** 5367-5373.

Rodriguez C, Patel AV, Calle EE, Jacob EJ & Thun MJ 2001 Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women. *The Journal of the American Medical Association* **285** 1460-1465.

Roger P, Sahla ME, Makela S, Gustafsson JA, Baldet P & Rochefort H 2001 Decreased expression of receptor beta protein in proliferative preinvasive mammary tumors. *Cancer Research* **61** 2537-2541.

Rossouw JE, Anderson GL, Prentrice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM & Ockene J; Writing Group for Women's Health Institute Investigators 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. *The Journal of the American Medical Association* **288** 321-333.

Rutherford T, Brown WD, Sapi E, Aschkenazi S, Munoz A & Mor G 2000 Absence of estrogen receptor-beta expression in metastatic ovarian cancer. *Obstetrics and Gynecology* **96** 417-21.

Sabbah M, Courilleau D, Mester J & Redeuilh G 1999 Estrogen induction of the cyclin D1 promoter : Involvement of a cAMP response-like element. *PNAS* **96** 11217-11222.

Santti R, Newbold RR, Makela S, Pylkkanen L & McLachlan JA 1994 Developmental estrogenization and prostatic neoplasia. *Prostate* **24** 67-78.

Sapi E, Brown WD, Aschkenazi S, Lim C, Munoz A, Kacinski BM, Rutherford T & Mor G 2002 Regulation of Fas ligand expression by estrogen in normal ovary. *Journal of the Society for Gynecologic Investigation* **9** 243-250.

Saville B, Wormke M, Wang F, Nguyen T, Enmark E, Kuiper G, Gustafsson JA & Safe S 2000 Ligand-, Cell-, and estrogen-receptor subtype (alpha/beta)-dependent activation at GC-rich (Sp1) promoter elements. *The Journal of Biological Chemistry* **275** 5379-5387.

Shaw JA, Udokang K, Mosquera JM, Chauhan H, Jones JL & Walker RA 2002 Œstrogen receptors alpha and beta differ in normal human breast and breast carcinomas. *The Journal of Pathology* **198** 450-457.

Skliris GP, Munot K, Bell SM, Carder PJ, Lane S, Hoirgan K, Lansdown MRJ, Parkes A, Hanby AM, Markham AF & Speirs V 2003 Reduced expression of œstrogen receptor β in invasive breast cancer and its re-expression using DNA methyl transferase inhibitors in a cell line model. *The Journal of Pathology* **201** 213-220.

Speirs V, Malone C, Walton DS, Kerin MJ & Atkin SL 1999 Increased expression of Estrogen Receptor β mRNA in Tamoxifen-resistant Breast Cancer Patients. *Cancer Research* **59** 5421-5424.

Stauffer SR, Coletta CJ, Tedesco R, Nishiguchi G, Carlson K, Sun J, Katzenellenbogen BS & Katzenellenbogen JA 2000 Pyrazole ligands: structure-affinity/activity relationships and estrogen receptor-α-selective agonists. *Journal of Medicinale Chemistry* **43** 4934-4947.

Stossi F, Barnett DH, Frasor J, Komm B, Lyttle CR & Katzenellenbogen BS 2004 Transcriptional Profiling of Estrogen-Regulated Gene Expression via Estrogen receptor α or Estrogen receptor β in Human Osteosarcoma Cells: Distinct and Common Target Genes for these Receptors. *Endocrinology* (ahead of print).

Strom A, Hartman J, Foster JS, Kietz S, Wimalasena J & Gustafsson JA 2004 Estrogen receptor {beta} inhibits17{beta}-estradiol-stimulated proliferation of the breast cancer cell line T47D. *PNAS* **101** 1566-1571.

Sun J, Meyers MJ, Fink BE, Rajendra R, Katzenellenbogen JA & Katzenellenbogen BS 1999 Novel ligands that function as selective estrogens or antiestrogens for estrogen receptor-α or estrogen receptor-β. *Endocrinology* **140** 800-804

Takeshita A, Cardona GR, Koibuchi N, Suen CS & Chin WW 1997 TRAM-1, A novel 160-kDa thyroid hormone receptor activator molecule, exhibits distinct properties from steroid receptor coactivator-1. *The Journal of Biological Chemistry* **272** 27629-27634.

Tora L, White J, Brou C, Tasset D, Webster N, Scheer E & Chambon P 1989 The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell* **59** 477-487.

Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F & Giguere V 1997 Cloning chromosomal localization, and functional analysis of the murine estrogen receptor beta. *Molecular Endocrinology* **11** 353-365.

Tremblay GB, Tremblay A, Labrie F & Giguere V 1999 Ligand-independent recruitement of SRC-1 by estrogen receptor β through phosphorylation of activation function AF-1. *Molecular Cell* **3** 513-519.

Vanacker JM, Pettersson K, Gustafsson JA & Laudet V 1999 Transcriptional targets shared by estrogen receptor- related receptors (ERRs) and estrogen receptor (ER) alpha, but not by ERbeta. *EMBO Journal* **18** 4270-4279.

Waliszewski P, Blaszcyk M, Wolinska-Witort E, Drews M, Snochowski M & Hurst RE 1997 Molecular study of sex steroid receptor gene expression in human colon and in colorectal carcinomas. *Journal of Surgery Oncology* **64** 3-11.

Watanabe T, Inoue S, Ogawa S, Ishii Y, Hiroi H, Ikeda K, Orimo A & Muramatsu M 1997 Agonistic effect of tamoxifen is dependent on cell type, ERE-promoter context, and estrogen receptor subtype: functional difference between estrogen receptors alpha and beta. *Biochemical and Biophysical Research Communications* **236** 140-145.

Warnmark A, Almlof T, Leers J, Gustafsson JA & Treuter E 2001 Differential recruitment of the mammalian mediator subunit TRAP220 by estrogen receptors ERalpha and ERbeta. *The Journal of Biological Chemistry* **29** 23397-23404.

Webb P, Lopez GN, Uht RM & Kushner PJ 1995 Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. *Molecular Endocrinology* **9** 443-456.

Webb P, Valentine C, Nguyen P, Price RHJr, Marimuthu A, West BL, Baxter JD & Kushner PJ 2003 ERbeta Binds N-CoR in the Presence of Estrogens via an LXXLL-like Motif in the N-CoR C-terminus. *Nuclear Receptor* **28** 4.

Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV, Warner M & Gustafsson JA 2000 Estrogen receptor (ER) beta, a modulator of ERalpha in the uterus. *PNAS* **23** 5936-5941.

Weihua Z, Makela S, Andersson LC, Salmi S, Saji S, Webster I, Jensen EV, Nilsson S, Warner M & Gustafsson JA 2001 A role for estrogen receptor β in the regulation of growth of the ventral prostate. *PNAS* **98** 6330-6335.

Weyant MJ, Carothers AM, Mahmoud NN, Bradlow L, Remotti H, Bilinski RT & Bertagnolli MM 2001 Reciprocal Expression of ERα and ERβ is associated with Estrogen-mediated Modulation of Intestinal Tumorigenesis. *Cancer Research* **61** 2547-2551.

White R, Sjoberg M, Kalkhoven E & Parker MG 1997 Ligand-independent activation of the œstrogen receptor by mutation of a conserved tyrosine. *EMBO Journal* **16** 1427-1435.

Windahl SH, Hollberg K, Vidal O, Gustafsson JA, Ohlsson C & Andersson G 2001 Female estrogen receptor beta-/- mice are partially protected against age-related trabecular bone loss. *Journal of Bone and Mineral Research* **16** 1388-1398.

Young J, Legget B, Gustafson C, Ward M, Searle J, Thomas L, Buttenshaw R & Chenevix-Trench G 1993 Genomic instability occurs in colorectal carcinomas but not in adenomas. *Human Mutation* **2** 351-354.

Zhao C, Lam WFE, Sunters A, Enmark E, Tamburo De Bella M, Coombes RC, Gustafsson JA & Dahlman-Wright K 2003 Expression of estrogen receptor β isoforms in normal breast epithelial cells and breast cancer: regulation by methylation. *Oncogene* **22** 7600-7606.

Table 1: Relative expression of ERα and ERβ in breast tumor progression.

References	Tissues	Number	Methods	ERα	ERβ	Comments
				SQ Ov.	SQ Ov.	
Roger *et al.* (2001)	Normal	118	IHC	+	+++	ERβ + cells decrease during pre-invasive tumor progression
	NP-BBD	18		+	++	
	P-BBD	37		++	++	
	P-BBDWA	13		++	+	
	CIS	25		++	+	
	High grade CIS	35		++	-	
Iwao *et al.* (2000)	Normal	11	Real Time-PCR	++	+++	Changes in ERβ1 and ERβ2 mRNA levels in breast cancer.
	Cancer	112		+++	++	
Park *et al.* (2003)	Normal	89	ISH	/	+++	ERβ mRNA level decreases during tumor progression. High ERβ level associated with poor differentiation.
	BBT	11			+++	
	Breast Cancer	85			+	
	Met. lymph node	10			+	
Skliris *et al.* (2003)	Normal	138	IHC	/	++++	Reduced expression of ERβ in invasive breast cancer. Loss of ERβ may be a reversible process involving methylation.
	PDCIS	16			+++	
	Invasive cancers	319			++	
	Met. lymph node	31			+	
	Recurrences	8			+	
Speirs *et al.* (1999)	Normal	23	RT-PCR	+	+++	22% of normal breast expressing exclusively ERβ mRNA. 50% of breast tumors coexpressing ERα and ERβ.
	Cancer	60		+++	+	
Leygue *et al.* (1998)	Normal (adjacent tissues)	18	Multiplex RT-PCR	+	++	Increase in ERα and decrease in ERβ during tumor progression.
	Cancer	18		++/+++	+	

Gustafsson *et al.* (2000)	Normal BBD Cancer	Total of 30 samples	RT-PCR Western-Blot, IHC	↓	ERβ is the predominant form in normal mammary gland.

The number of + indicates the ERs relative expression. The arrows indicate a decrease (↓), an increase (↑) or no variations in expression (↔) between normal and cancer tissues. SQ= semiquantitative, Ov= overall trends, BBD= Benign Breast Disease, NP-BBD= Non Proliferative BBD, P-BBD= Proliferative BBD, P-BBDWA= Proliferative BBD with atypia, BBT= Benign Breast Tumors, CIS= carcinoma *in situ*, HGPIN= High grade prostatic intraepithelial neoplasia, IHS= *in situ* hybridization, IHC= immunohistochemistry, Met= metastatic, RT-PCR= reverse transcription polymerase chain reaction

Table 2: Relative expression of ERα and ERβ in ovarian tumor progression

References	Tissues	Number	Methods	ERα SQ Ov.	ERβ SQ Ov.	Comments
Pujol *et al.* (1998)	Normal	6	Competitive RT-PCR	+	+++	ERα/ERβ mRNA ratio increases during tumor progression
	Cysts	24		+	+++	
	Borderline tumors	3		++	++	
	Cancers	10		++	+	
Brandenberger *et al.* (1998)	Normal	10	Northern Blot RT-PCR	++	++	ERβ mRNA level decreases in cancer
	Cancer	10		+++	+	
Rutherford *et al.* (2000)	Normal	9	RT-PCR Western Blot	++	++	ERβ mRNA and protein levels decrease in ovarian cancer and metastases
	Primary cancer	8		++	+	
	Met cancer	8		+++	-	

Table 3: Relative expression of ERα and ERβ in prostate tumor progression

References	Tissues	Number	Methods	ERα SQ Ov.	ERβ SQ Ov.	Comments
Latil *et al.* (2001)	Normal	4	Real-time PCR	++	+++	Decreased expression of ERβ mRNA in the hormone-resistant group
	Cancer	23		+ to ++	+	
Pasquali *et al.* (2001a)	Normal	5	IHC	/	+++	ERβ protein expression decreases in cancer
	Cancer	10			+	
Pasquali *et al.* (2001b)	Normal	6	RT-PCR Western blot	++	++	ERβ mRNA expression decreases in cancer
	Cancer	5		++	+	

Horvath *et al*. (2001)	Normal Hyperplasia Cancer	5 157 159	IHC	/	+++ - or + - or +	Loss of ERβ protein expression during tumor progression
Leav *et al*. (2001)	Dysplasia - moderate grade - high grade Carcinoma - grade III - grade IV/V Metastasis	Total of 50 samples	IHC RT-PCR	- - -/+ -/+ -	+ - + -/+ +	Decrease in ERβ protein and mRNA expression in high grade dysplasia and carcinoma.
Fixemer *et al*. (2003)	HGPIN Adenocarcinoma Gleason grade: III IV V Metastatic	47 17 29 14 12	IHC mono- clonal antibody	/	+++ + ++ + +	ERβ protein expression decreases during tumor progression. ERβ expression higher in Gleason grade IV than in in grade III and V

Table 4: Relative expression of ERα and ERβ in colon tumor progression

References	Tissues	Number	Methods	ERα SQ	ERα Ov.	ERβ SQ	ERβ Ov.	Comments
Campbell-Thompson *et al*. (2001)	Normal cancer	26 26	RT-PCR Southern	+ +		+++ +		ERβ1 and ERβ2 mRNA expressions decrease in cancer
Foley *et al*. (2000)	Normal Cancer	11 11	RT-PCR Western	+ +		+++ +		Decrease ERβ protein but not mRNA expression in cancer. Post-transcriptional mechanism?

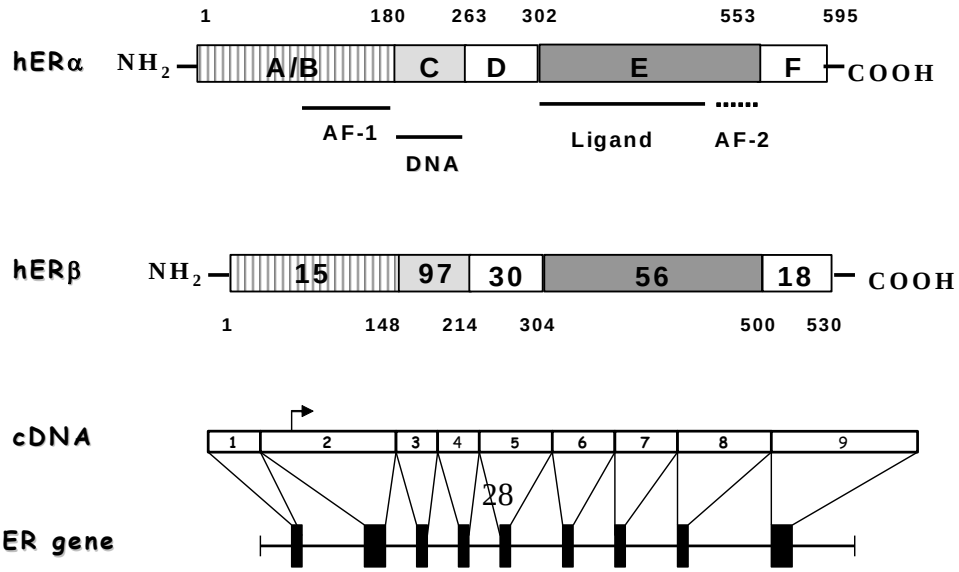

Figure 1 Schematic representation of the structure of human ERα and ERβ nuclear receptors. The A/B domain at the NH-2 terminal contains the ligand independent transcriptional-activation function AF-1, the C domain represents the DNA-binding-domain, D corresponds to the hinge region, E domain contains the hormone binding domain and the hormone-dependent transcriptional-activation function AF-2. Numbers outside each box refer to amino acid number whereas the number inside each box of ERβ refers to the percentage of amino acid identity. The arrow indicates the translation starting site in ER cDNA.

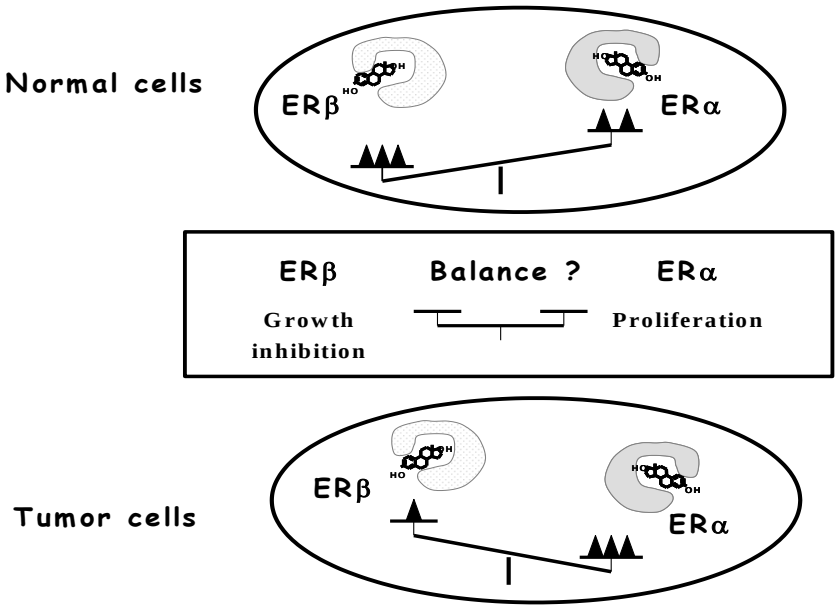

Figure 2 Schematic representation of ERα and ERβ imbalance in estrogen-dependent tumor progression

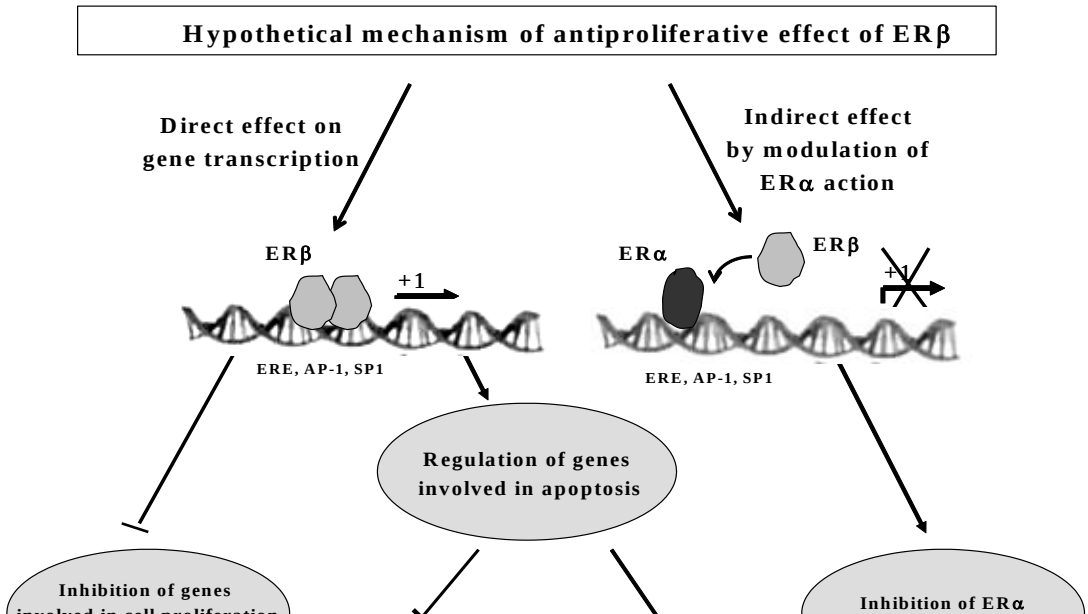

Figure 3 Hypothetical mode of ERβ action on cell proliferation pathways.