The different roles of ER subtypes in cancer biology and therapy

Christoforos Thomas and Jan-Åke Gustafsson*‡*

Abstract | By eliciting distinct transcriptional responses, the oestrogen receptors (ERs) ERα and ERβ exert opposite effects on cellular processes that include proliferation, apoptosis and migration and that differentially influence the development and the progression of cancer. Perturbation of ER subtype-specific expression has been detected in various types of cancer, and the differences in the expression of ERs are correlated with the clinical outcome. The changes in the bioavailability of ERs in tumours, together with their specific biological functions, promote the selective restoration of their activity as one of the major therapeutic approaches for hormone-dependent cancers.

Co-activators
Proteins that increase gene expression by binding to a transcription factor that binds DNA through its DNA-binding domain.

Co-repressors
Proteins that decrease gene expression by binding to a transcription factor that contains a DNA-binding domain.

**Center for Nuclear Receptors and Cell Signalling, Department of Biology and Biochemistry, University of Houston, Houston 77204, Texas, USA.*
‡Department of Biosciences and Nutrition, Novum, Karolinska Institute, Huddinge S-14183, Sweden.
Correspondence to J.-A.G.
e-mail:
jgustafs@Central.UH.EDU
doi:10.1038/nrc3093

By regulating cell growth and differentiation, steroid hormones influence normal physiology, reproduction and behaviour. Oestrogens mediate their effects in target tissues through two members of the nuclear receptor superfamily, oestrogen receptor-α (ERα) and ERβ[1]. ERα and ERβ from various species have been cloned, and several ERα and ERβ variants have been identified since the discovery of ERα in the late 1950s and ERβ in 1996 (REFS 2–5). ERs and their variants exert distinct cellular functions following activation in response to ligand binding or in a ligand-independent manner. The ligand-binding domains of ERα and ERβ share a medium degree of homology (59%), and this accounts for their specificity in binding endogenous ligands, dietary oestrogens and anti-oestrogens[1]. As transcription factors, active ERs can differentially control gene expression by recruiting different co-activators and co-repressors at specific DNA sequences. They can also interact and modulate the activity of one another, alter the activity of other transcription factors and participate in separate membrane or cytoplasmic signalling cascades[6].

Many lines of evidence suggest a relationship between the perturbation of oestrogen signalling and cancer initiation, progression and response to treatment[7,8]. The diverse actions of oestrogens and competitive inhibitors of oestrogen–ER binding in breast, endometrial and prostate cancer in combination with the variation of ERα/ERβ ratio in these tissues indicate that the ER subtypes have different functions in cancer biology and therapy[9,10]. This notion has been further supported by numerous animal and cell model studies in which the use of specific ligands and other methods to disrupt receptor subtype-specific expression has shown that ERα and ERβ have opposite effects on cell proliferation and apoptosis[11]. Furthermore, clinical studies revealed that there are ER subtype-specific expression changes in cancer that vary depending on the tumour type and the disease stage[10,12–16]. These findings placed the idea of improving the outcome of patients after selectively targeting or restoring ER levels and activity in cancer tissues at the centre of the current therapeutic strategies for hormone-dependent cancers. Modulations in the levels of endogenous ligands and co-regulators, transcriptional misregulation, including hypermethylation of the ER promoters, growth factor-mediated post-transcriptional modification and proteasome-mediated degradation of ERs, are emerging as candidate causes for the altered activity of the receptors in cancer tissues[17–20]. However, the exact mechanisms through which these factors regulate the levels and the activity of ERs, as well as their contribution in different stages and types of cancer, are not well understood. In addition to the variability of wild-type ERα and ERβ expression, the levels and function of the receptor splice variants seem to contribute to the complexity of ER action. On the basis of the diverse actions of ERs in cancer tissues, current research is focusing on developing and evaluating the efficacy of ERα- and ERβ-selective ligands that either activate or block the receptors depending on their status in different stages of the disease. Alternative strategies that target ER signalling beyond ligand–ER interaction include components of growth factor signalling, methylases, ubiquitin ligases and chaperones. Finally, cell-based and animal model studies are aiming to identify novel ER-associated pathways that are relevant to cancer biology.

At a glance

- Oestrogen receptor (ER) subtypes (ERα and ERβ) influence the development and progression of hormone-related cancers by exerting distinct biological functions. ERα is associated with aberrant proliferation, inflammation and the development of malignancy. ERβ seems to oppose ERα actions on cell proliferation by modulating the expression of many ERα-regulated genes and exhibits antimigratory and anti-invasive properties in cancer cells.
- Multiple factors affect the ER-mediated regulation of gene expression and may account for the adverse and beneficial effects of oestrogens and anti-oestrogens. Both ER genomic and non-genomic actions often converge at certain regulatory sites of the adjacent ER-responsive genes. The final gene and the subsequent cancer biological responses may vary depending on the combination of transcription factors; the ratio and the cellular localization of ERα and ERβ; the expression levels of various co-regulators and signal transduction components; and the nature of extracellular stimuli. These variables are altered during cancer transformation and are divergent in different cancer cells.
- Owing to the practical limitations in detection, only a few truncated ERα and ERβ variant isoforms have been examined in tumour samples and correlated with clinical outcome. Some of these variants are localized in the cytoplasm and plasma membrane, show variable expression in cancer tissues and influence cancer progression and response to therapy either through genomic pathways by modulating the activity of wild-type ERs or by interacting with the membrane and cytoplasmic signalling cascade.
- Perturbation of ER subtype-specific expression has been detected in different stages of various types of cancer, with the levels of ERα and ERβ declining in most cancers as the disease develops. The hypermethylation of the ER promoters, microRNAs that target the ER mRNAs and increased proteasomal degradation are among the factors that are responsible for the reduced levels of ERs in cancer tissues.
- ERα is the principal biomarker for the response of breast cancers to endocrine therapy, and its truncated isoform ERα-36 seems to confer resistance to tamoxifen. On-going research is trying to fully clarify the prognostic and predictive role of ERβ. So far, it seems that the nuclear wild-type ERβ complements ERα in predicting response to endocrine therapy and is associated with better overall outcome and the metastatic potential of breast and prostate cancer. The cytoplasmic ERβ2 (also known as ERβcx) isoform correlates with worse survival and metastatic phenotype.
- Insights into the mechanisms of ER action and regulation have suggested possible therapeutic approaches for hormone-related cancers. The development of selective ERα and ERβ agonists and antagonists, and alternative strategies that target the ER signalling beyond the ligand-binding activity, including as targets components of growth factor signalling, methylases, ubiquitin ligases, and chaperones are under investigation.

In this Review, we discuss how ERs differentially control cellular processes that are relevant to cancer biology. In addition, we provide new insights into the distinct prognostic role of ERs in cancer, the regulation of ER signalling in normal cells and how the deregulation of this signalling is associated with cancer initiation, progression and response to treatment. Finally, we discuss how this information could open new avenues in the development of novel therapeutic strategies.

Mechanism of action and regulation of ERs

ERα and ERβ are the products of individual genes (*ESR1* and *ESR2*, respectively) that are located on different chromosomes[21,22]. As members of the nuclear receptor superfamily, both ER subtypes have a six region structure and contain defined functional domains that have considerable homology[5] (FIG. 1). The classical mechanism of ER action involves ligand binding to the ligand-binding domain of the receptor, which induces ligand-specific conformational changes of the protein. The ligand-bound receptors dimerize and bind to DNA through their zinc finger-containing DNA-binding domains at sequence-specific response elements known as oestrogen response elements (EREs). Once bound to EREs the receptors recruit co-activator or co-repressor multiprotein complexes on the basis of the shape of the ligand–receptor complex. These complexes influence the activity of the receptors, which activate or repress gene transcription. The multiprotein co-activator complex is formed after the active co-activator (for example, the steroid receptor co-activators SRC1 or SRC3) recruits co-regulatory proteins, such as histone acetyltransferases, which modify the chromatin structure, ubiquitin ligases and protein remodellers (including, protein isomerases, heat-shock proteins (HSPs) and proteasome ATPases). After the initiation of transcription, post-translational modifications, such as methylation and acetylation, promote the dissociation of the complex, and the simultaneous ubiquitylation of ERs either results in further activation of the receptors or induces their degradation[1].

Binding to other transcription factors. On ligand binding, ERα and ERβ can also regulate gene transcription by interacting with and activating other direct DNA-binding transcription factors, such as activating protein 1 (AP1), specificity protein 1 (SP1), cAMP response element-binding protein (CREB), runt-related transcription factor 1 (RUNX1), nuclear factor-κB (NF-κB), p53 and signal transduction and activator of transcription 5 (STAT5)[6,23,24]. During this interaction, ERs can bind to full or imperfect ERE motifs, such as ERE half-sites, or can indirectly interact with chromatin through a tethering mechanism that involves a partner transcription factor[25,26]. Interestingly, although full ERE and ERE half-sites can be located in the proximal promoter region, within 5 kb upstream of the transcription start site or at distal enhancer regulatory sites, recent studies suggest that most of the oestrogen-responsive genes could be regulated by distal regulatory regions[25].

ER-mediated transcription in the absence of ligand. ERs can elicit transcriptional responses in the absence of ligand. Hyperactive growth factor receptors, such as epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGFIR), can stimulate protein kinase cascades that phosphorylate and activate the ERs in the absence of ligand[17]. There is also accumulating evidence that membrane and cytoplasmic populations of ER subtypes can mediate the rapid (also known as non-genomic) effects of oestrogens. MAPK, PI3K, endothelial nitrogen oxide synthase (eNOS; also known as NOS3), ERBB2 (also known as HER2 and neu), caveolin 1, EGFR, IGFIR, SRC and G proteins are among the components that are activated by ERs within 3–15 minutes after oestrogen treatment and that can signal to regulate gene expression through the activation of other transcription factors[6,27–34]. Oestrogen can also induce the transient methylation of ERα by protein arginine *N*-methyltransferase 1 (PRMT1). This methylation event, which is frequent in breast cancer, results

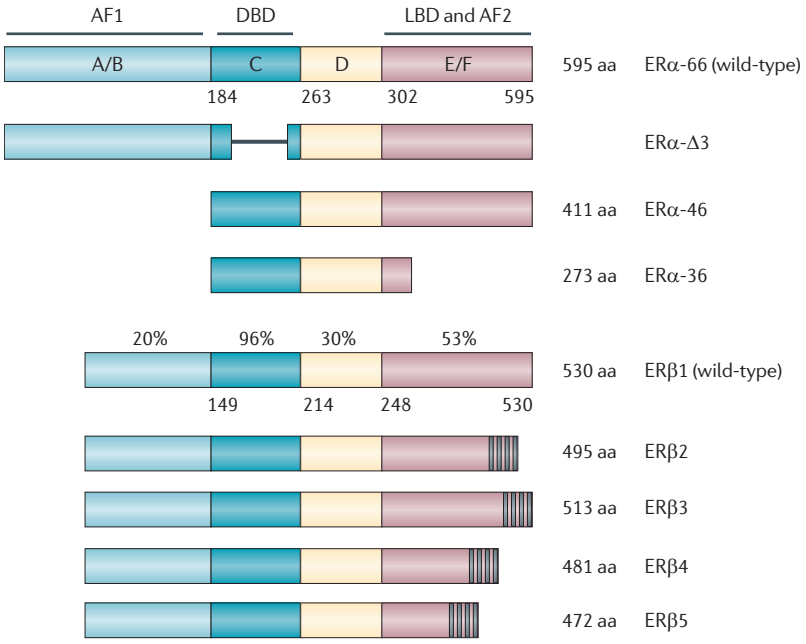

Figure 1 | **Schematic representation of structural and functional domains of the ERs.** The structural domains are labelled A–F with the amino acid numbers indicated below. Relative positions of some of the known functional domains are represented by solid bars. The percentage amino acid homologies between wild-type oestrogen receptor-α (ERα) and ERβ are also shown. The amino-terminal A/B regions contain a transactivation domain (AF1) with ligand-independent function and a co-regulatory domain that is responsible for the recruitment of co-activators and co-repressors. The C region corresponds to the DNA-binding domain (DBD), which is required for binding to specific oestrogen response elements (EREs) in the proximal promoter region or at distal regulatory elements of oestrogen-responsive genes. The carboxy-terminal regions E and F contain the ligand-binding domain (LBD) and have a ligand-dependent transactivation function. This region is also responsible for the binding to co-regulatory and chaperone proteins, as well as for receptor dimerization and nuclear translocation. Finally, the D region contains several functional domains, including the hinge domain, part of the ligand-dependent activating domain and the nuclear localization signal. Human ERα and ERβ variant isoforms are presented below the wild-type forms. Most of these variants are expressed in malignant tissues and influence cancer biology. ERβ variants are formed from alternative splicing of the last coding exon (shown by the striped bars).

in the formation of cytoplasmic complexes that contain ERα, PI3K, SRC and focal adhesion kinase (FAK), which influence gene transcription through the activation of AKT[13].

Different transcriptional responses. Although there are general similarities in the mechanism of ERα and ERβ action, ERs can elicit different transcriptional responses. Microarray analysis of endogenous genes in mouse tissues and breast cancer cells revealed that ERs regulate common and different genes[11]. These studies also showed that ERs can differentially regulate the expression of the same genes. The divergent transcriptional responses of ERα and ERβ can be seen in their different affinity to binding ligands, their different response following binding to the same ligand, and the separate membrane and cytoplasmic signalling cascades that activate the receptors. The activating function 1 (AF1) domain that is responsible for the recruitment of co-regulatory proteins is poorly conserved (only 20% amino acid homology) between ERα and ERβ, and this difference can partly account for the functional differences of the ER subtypes. Agonists, such as 17β-oestradiol, induce conformational changes in the receptor that favour the binding of co-activators to ERα and co-activators or co-repressors to ERβ. By contrast, the antagonist tamoxifen recruits co-repressors when bound to ERα but not to ERβ[35,36]. This also indicates that the relative balance of co-activator and co-repressor expression within cells can affect the relative agonist and antagonist activity of ligands. Increased expression of SRC1 and SRC3, which induce cell proliferation and migration, was found in breast and ovarian cancers[20,37], and reduced expression of nuclear receptor co-repressor (NCOR) and silencing mediator of retinoid and thyroid receptor (SMRT; also known as NCOR2) has been correlated with tamoxifen resistance in breast cancer[5,38]. In addition, ERs have been shown to regulate the activity of one another by forming heterodimers that influence the receptor–DNA binding or by altering the amounts of the receptor. The expression of ERβ in ERα-positive cells has been shown to repress the transcriptional activity of ERα by inhibiting the recruitment of ERα at EREs or the ERα-mediated binding of other transcription factors at their cognate motifs. ERα and ERβ differentially regulate the nuclear transcription factor MYC, which displays mitogenic effects by inducing cell cycle progression[39,40]. They also exert opposite actions, through regulatory regions that include CREB and AP1 motifs, on the transcription of *CCND1* (which encodes cyclin D1), which is overexpressed in various cancers and can affect the response of these cancers to systemic therapy[41].

It is evident that multiple factors affect the ER-mediated regulation of gene expression. Both ER nuclear and rapid cytoplasmic actions often converge at certain response elements. The final gene expression pattern and the subsequent biological responses can vary depending on the combination of transcription factors bound to the chromatin regulatory sites of a gene; the ratio and the cellular localization of ERα and ERβ; the expression levels of various co-regulatory proteins and signal transduction components; and the nature of extracellular stimuli. These variables are altered during cancer transformation and they are divergent in different cancer cells. It seems that ER subtypes can elicit distinct gene expression and biological functions in normal and cancer cells by signalling through different pathways depending on the cellular context (FIG. 2).

Changes in ER expression levels. The alteration of ER expression is an important step in the development and progression of hormone-related cancers, and it influences cancer response to systemic therapy[13,42–45] (TABLE 1). The intracellular concentration of ERs results from a dynamic balance between ER synthesis and ER breakdown[18]. The transcription of ERs is regulated by several factors, which, in the case of ERα, include GATA-binding factor 3 (GATA3), forkhead box protein O3A (FOXO3A), forkhead box protein M1 (FOXM1) and ERα, which can regulate its own expression[25,46–48]. ER

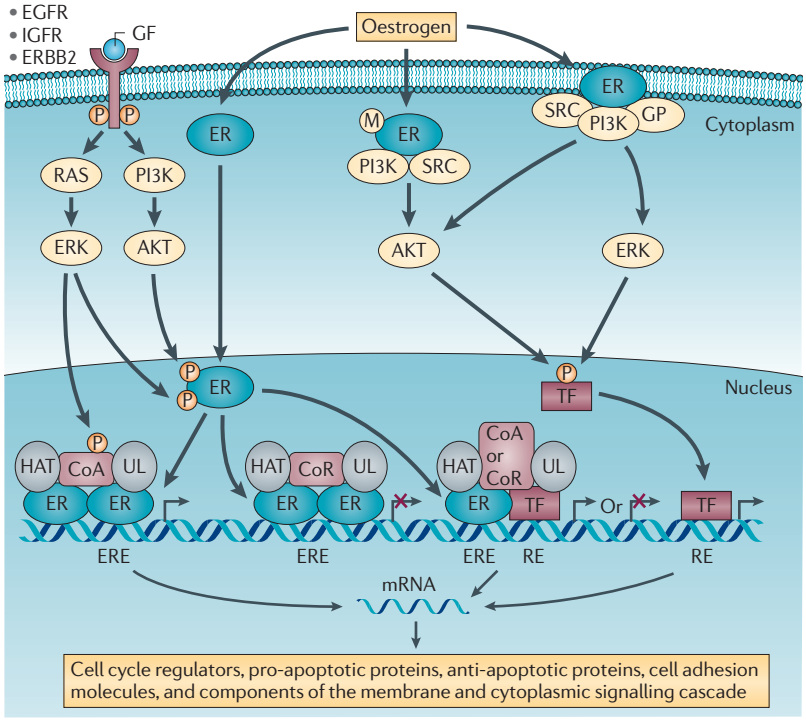

Figure 2 | **Molecular mechanism of ER action.** In the classical mechanism of oestrogen receptor (ER) action, ligands such as oestrogen bind to ERs and the ligand–ER complexes dimerize and bind to DNA at sequence-specific response elements that are known as oestrogen response elements (EREs). At these sites, the ERs interact with co-activator (CoA) or co-repressor (CoR) multiprotein complexes to activate or to repress gene transcription, respectively. The core of these multiprotein complexes is the active (phosphorylated (P)) co-activator or co-repressor that recruits co-regulatory proteins such as a histone acetyltransferase (HAT) and an ubiquitin ligase (UL). ERs can alternatively regulate gene expression by interacting with other direct DNA-binding transcription factors (TFs). According to this model of action, ERs can bind to ERE motifs that are near the response element (RE) of the interacting transcription factor or can indirectly interact with chromatin through tethering to the partner transcription factor. Oestrogen-bound ERs localized in the cytoplasm (ERα can be methylated (M)) or at the cell membrane can interact with SRC, PI3K and G proteins (GPs) and mediate non-genomic signalling. This signalling through the activation of protein kinase cascades results in the phosphorylation and activation of target TFs. TFs can regulate transcription through their cognate sites (RE sites). Growth factor receptors, such as epidermal growth factor receptor (EGFR), including ERBB2 (also known as HER2 and neu) and insulin-like growth factor receptor (IGFR) in response to growth factors (GFs) can activate ERK and AKT serine/threonine kinases, which can phosphorylate and activate ERs in a ligand-independent manner.

synthesis is repressed by the methylation of ER promoters. Promoter hypermethylation has been significantly associated with the loss of ERα and ERβ in the majority of cancers and cancer cell lines. DNA demethylating agents (such as, 5-AZAC (5-aza-2D-deoxycytidine)) were reported to restore ERβ expression in cancer cells[19,43]. A number of ER subtype-specific microRNAs (miRNAs) can affect ER expression, and an inverse correlation between specific miRNAs and ER levels has been detected in various cancers[49,50–53]. Following translation, the native, unbound ER subtypes are stable as they form a complex with the HSPs[54]. Ligand binding dissociates the receptors from these chaperones and increases their proteasome-mediated degradation. This process requires alteration in the phosphorylation status of ERs and the interaction of the receptors with several proteins, including ubiquitin ligases and ubiquitin-binding proteins. Modulations in the levels of endogenous ligands, alterations in the tumour microenvironment (such as, hypoxia), downregulation of chaperones and increased expression of ubiquitin ligases are implicated in the proteasome-mediated degradation of the ERs (FIG. 3). HSP90 inhibitors, including geldanamycin, radicicol, physiological oestrogens and 'pure' anti-oestrogens (such as, fulvestrant), but not 'partial' anti-oestrogens (such as, tamoxifen), downregulate ERα by increasing its rate of degradation[18]. Ubiquitin-protein ligase E3A (UBE3A; also known as E6AP) and MDM2 are among the ubiquitin ligases that have been shown to promote the degradation of ERα[55,56] (FIG. 3). Two recent studies have suggested that CUE domain-containing protein 2 (CUEDC2), a ubiquitin-binding motif-containing protein, and lemur tyrosine kinase 3 (LMTK3) regulate the stability of ERα and affect the endocrine resistance of breast cancer[57,58]. It remains for future studies to identify whether the upregulation of novel ubiquitin ligases is a crucial step in the downregulation of the ERs in cancer. This post-transcriptional regulation of the ERs questions the validity of studies that based their clinical assessment of ERs on the detection of *ER* mRNA and may account for the discrepancy between *ER* mRNA and protein levels.

ERs and cell proliferation

ERα. Evidence from cell-based and animal model studies has established the idea that the ER subtypes influence cancer biology and therapy. Analysis of *Esr1*-knockout mice showed phenotypic changes that are associated with the gonads, skeletal system and reproductive tract and revealed that ERα is required for the normal development of the mammary gland[59,60]. Similar effects of ERα in the breast have been observed in mice with CRE-mediated deletion of *Esr1* in the epithelium of the mammary gland[61]. When *Esr1*-knockout mice were crossed with mice that develop tumours owing to the expression of the mouse mammary tumour virus (MMTV)–*Wnt1* transgene or a mammary-specific mutant of *Erbb2*, the onset of tumour development in the offspring was delayed[62,63]. Similarly, the incidence of oestrogen- and DMBA-induced mouse mammary tumours was reduced by the loss of ERα, suggesting that ERα can influence mammary carcinogenesis[64,65]. ERα has additionally been implicated in prostate tumorigenesis[10]. Oestrogens were able to stimulate proliferation and the appearance of multilayered prostatic epithelial cells, a phenotype known as squamous metaplasia (SQM), in wild-type, but not in *Esr1*-knockout, mice, indicating the involvement of ERα in the induction of this pathology[66]. This ERα-mediated increase in proliferation has been linked to inflammation and tumour development. Treatment with the synthetic oestrogen, diethylstilbestrol (DES), during neonatal life stimulated inflammatory cell infiltration in the prostate of wild-type, but not of *Esr1*-knockout, mice[67–69]. In addition, the ERα-selective antagonist toremifene decreased the incidence of high-grade prostatic intraepithelial neoplasia (PIN) and prostate cancer in transgenic adenocarcinoma

'Pure' anti-oestrogens
Drugs that bind the oestrogen receptor, thereby blocking the effect of oestrogen, but that have no detectable oestrogen-like effects. Most have a steroidal structure.

Table 1 | **ER subtypes in cancer biology**

Cancer type	Role of ERs	Refs
Breast	• Wild-type ERα stimulates cell proliferation by inducing MYC and cyclin D1 expression • ERα-36 mediates E2- and tamoxifen-stimulated cell proliferation by activating MAPK–ERK signalling • Lack of ERα delays the onset of WNT1- and ERBB2-induced mouse mammary tumours • Wild-type ERα correlates with better response to tamoxifen treatment. ERα-36 is associated with tamoxifen resistance • ERβ1 inhibits the expression of ERα target genes that regulate cell proliferation • ERβ1 expression in ERα-positive cells inhibits cell proliferation *in vitro*, as well as cell growth and angiogenesis in xenografts, by repressing cyclin D1, cyclin A, MYC, VEGF and PDGFβ and by inducing p21 and p27 expression • ERβ1 is associated with better survival in triple-negative breast cancers and better response to tamoxifen monotherapy. Nuclear ERβ2 correlates negatively with metastasis and vascular invasion. By contrast, cytoplasmic ERβ2 correlates with worse outcome and poorer response to chemotherapy	39,74, 84–88,98, 106,166, 167
Prostate	• ERα expression is required for the proliferation and multi-layering of the prostatic epithelial cells. ERα mediates oestrogen-induced inflammatory response in the mouse prostate • Wild-type ERα expression is inversely correlated with histological grade and pathological stage • ERβ1 impedes EMT in prostate cancer cells by upregulating E-cadherin, inhibits proliferation and induces apoptosis; ERβ1 decreases in highly aggressive Gleason grades • ERβ2 and ERβ5 enhance the invasiveness of prostate cancer cells. Both nuclear ERβ2 and cytoplasmic ERβ5 are associated with a more metastatic phenotype	10,16, 45,112
Colon	• Expression of ERβ1 induces cell cycle arrest by downregulating MYC, cyclin E and by inducing p21 and p27 expression. Lack of ERβ enhances small intestinal tumorigenesis in $Apc^{Min/+}$ mice • Low ERβ1 expression was associated with poor differentiation • ERβ2 is associated with lymph-node metastasis	12,40, 183–186
Lung	• ERα expression is associated with a poor prognosis among patients with NSCLC and correlates with EGFR mutations • ERβ1 is associated with better survival in men. A strong nuclear expression of ERβ1 is associated with EGFR mutations, and ERβ1 is a favourable predictor of the response of patients with lung adenocarcinoma to an EGFR tyrosine kinase inhibitor	15, 169–174
Ovarian	• ERα and ERβ have opposite actions on the transcription of cyclin D1, and ERβ1 can inhibit cell growth and induce apoptosis • ERβ2 induces EMT by downregulating E-cadherin in ovarian cancer cells • Increased ERα mRNA and protein levels are associated with a better outcome in patients with ovarian cancer • Reduced ERβ1 levels correlates with the occurrence of lymph-node metastasis	120,134, 135, 179,180, 187,188
Endometrial	• Wild-type ERα expression increases cell proliferation • ERα-36 mediates tamoxifen-stimulated cell proliferation through MAPK–ERK and PI3K–AKT pathways • ERα-46 mediates the E2-mediated activation of PKC in endometrial cancer cells	9,41, 95,181

APC, adenomatous polyposis coli; E2, 17β-oestradiol; EGFR, epidermal growth factor receptor; EMT, epithelial–mesenchymal transition; ER, oestrogen receptor; NSCLC, non-small-cell lung cancer; PDGF, platelet-derived growth factor; PKC, protein kinase C; VEGF, vascular endothelial growth factor.

of the mouse prostate (TRAMP) mice[70,71]. Overall, ERα seems to contribute to tumorigenesis primarily by stimulating cell proliferation.

It has been suggested that by increasing the rate of cell division ERα can result in the accumulation of random DNA mutations, some of which may be carcinogenic. A plethora of studies has confirmed the mitogenic effects of ERα in cells and animal tissues. The increased expression of MYC, which promotes cell cycle progression, and cyclin D1, which induces the G1/S cell cycle transition, are among the events that connect ERα activity with the induction of cell proliferation. ERα has been shown to increase the transcription of *CCND1* following ligand binding through the interaction between SRC3 and ERα, increasing the proliferation of breast cancer cells both *in vivo* and *in vitro*[39,72–74]. In the absence of ligand, treatment with EGF or overexpression of the EGFR family member ERBB2 enhances ERα transcriptional activity through MAPK- and PI3K-mediated phosphorylation and induces the proliferation of breast cancer cells[75,76].

ERβ. Although several different mouse models have confirmed the involvement of ERα in tumorigenesis, phenotypic differences among *Esr2*-knockout mouse models that were developed and analysed in different laboratories resulted in a controversy regarding the role of ERβ in cancer biology. One group reported decreased terminal differentiation and increased proliferation in the alveoli of the lactating *Esr2*-knockout mice. These mice develop age-related cystic breast disease and hyperplasia of the prostatic epithelium[77–80]. These phenotypes were not observed in other *Esr2*-knockout mutant lines

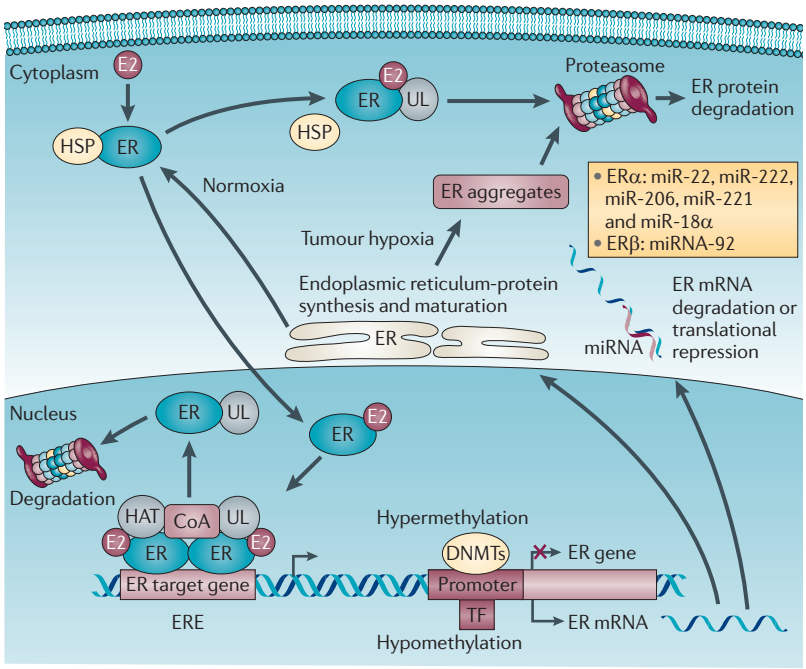

Figure 3 | **Regulation of the cellular levels of ERs.** Transcription factors (TFs) regulate the expression of oestrogen receptors (ERs). GATA-binding protein 3 (GATA3), forkhead box protein O3A (FOXO3A), forkhead box protein M1 (FOXM1) and ERα regulate ERα transcription. DNA methylation occurs in CG sites (CpGs) in the ER promoters and it is catalysed by DNA methyltransferases (DNMTs). Hypermethylation of the ER promoters correlates with decreased ER expression in a variety of cancers. The expression of ERs is regulated by microRNAs (miRNAs). miR-22, miR-222, miR-221, miR-206 and miR-18α repress ERα expression, and miR-92 downregulates ERβ by targeting the mRNA 3′ untranslated region. Aberrant miRNA expression has been associated with the alteration of ER levels in cancers. The ubiquitin–proteasome pathway is the major mechanism for targeted protein degradation in eukaryotic cells. In the absence of ligands (E2 (17β-oestradiol)), ERs are stable as complexes with chaperones. On ligand binding, the receptor dissociates from heat-shock proteins (HSPs), is ubiquitylated by ubiquitin ligases (ULs) and is targeted for degradation. After the transcription initiation, the ER co-activator (CoA)–histone acetyltransferase (HAT) transcription complex disassembles and the ERs are targeted for degradation. The active transcription depends on continuous reloading and degradation of the ER transcription complex. Finally, the tumour microenvironment affects the synthesis of functional ERs. Oxygen as the ultimate source of oxidizing power for disulphide bond formation is important for the proper folding of endoplasmic reticulum client proteins. Tumour hypoxia can induce the accumulation of ER aggregates that represent non-functional proteins that are targeted for degradation. ERE, oestrogen response element.

that were developed by a different group[81,82]. It has been suggested that the expression of splice variants (discussed below) lacking the targeted exons may account for the discrepancy in the results of the different laboratories. In addition, a number of additional somatic mutations may be required before a malignant phenotype arises in the *Esr2*-knockout mice. Further insight into this controversy might be provided by the development of multigenic models of *Esr2*. In support of the ERβ tumour suppressor properties that were observed in one *Esr2*-knockout mouse model, most *in vitro* studies that have analysed the biological effects of ERβ expression in cancer cells have shown that ERβ has antiproliferative effects. However, the mechanisms that govern the cancer biological responses of ERβ have not been fully elucidated. ERβ expression was found to inhibit cell growth and to induce G1 cell cycle arrest in various types of cancer cells by regulating the expression of cyclin D1, CDC25A, p21, MYC, FOXO1, p53 and ARF (also known as p14), which act on the same cell cycle checkpoint[40,83–87]. The ERβ-mediated alteration in the expression of cell cycle regulatory genes has been correlated with the inhibition of cell proliferation *in vitro* and *in vivo* and the cytotoxicity of DNA-damaging chemotherapy[40,83,88,89]. To date, a few *in vitro* studies have proposed a proliferative and anti-apoptotic role of ERβ in cancer cells. This occurred in one study when ERβ was introduced into ERα-negative metastatic breast cancer cells, and it was additionally observed with the endogenous cytoplasmic ERβ following treatment of lung cancer cells with selective ERβ agonists[90–92].

Isoforms of ERα and ERβ

Each ER subtype exists as several isoforms that are derived from alternative splicing and promoter usage[93]. ERα and ERβ splice variants that differ from wild-type receptors in structure and function have been detected in normal and cancer tissues. PCR and sequencing enabled the detection of a large number of ERα mRNA splice variants in various cancer cell lines and samples from breast, endometrial and ovarian cancer, with wild-type ERα being the predominant variant[5,94–97]. Owing to the lack of specific antibodies, only a few truncated ERα variant isoforms have been examined in tumour samples and correlated with the clinical outcome[5,98–100] (FIG. 1a). The truncated ERα variant ERα-36 is localized in the plasma membrane and the cytoplasm and mediates membrane-initiative effects of oestrogen signalling. Its expression in the presence of wild-type ERα has been reported to confer endocrine resistance in breast cancer. This ERα variant promotes tamoxifen agonist action in endometrial cancer cells through MAPK–ERK and PI3K–AKT-mediated upregulation of MYC and this may explain the association of anti-oestrogens with increased risk of endometrial cancer[95,98]. Following expression in cancer cell lines, ERα isoforms modified the transcriptional activity of the wild-type receptor and influenced cell growth through genomic and non-genomic pathways[101]. Many fewer ERβ variants and naturally occurring point mutants have been identified compared with ERα, owing to the later discovery of ERβ. Five full-length splice variants of ERβ exist as a result of alternative splicing of the last exon, most of which have been detected in cancer tissues. ERβ2 (also known as ERβcx) and ERβ5 have been reported to antagonize wild-type ERα and to modulate wild-type ERβ (ERβ1) transcriptional activity through heterodimerization and are associated with clinical outcome[102–108]. The antiproliferative effects of wild-type ERβ and its variant isoform ERβ2 have been associated with their ability to prevent the ERα transcriptional complexes from activating genes that induce cell cycle progression. The induction of ERβ1 and ERβ2 expression in ERα-positive breast cancer cells and ERβ1 in ERα-transfected cervical adenocarcinoma cells inhibited ERα transcriptional activity and modulated the expression of many ERα-regulated genes, including *CCND1* and *CDKN1A* (which encodes p21)[85–87,103,109].

Gleason grade
The assignment of a number between 1 and 5 to indicate the degree of differentiation of the cells in the cancer specimen. It is used to establish the Gleason score. Cancers with a higher Gleason score are more aggressive and have a worse prognosis.

Hormone replacement therapy
(HRT). The administration of hormones to correct a deficiency, such as postmenopausal oestrogen replacement therapy.

SERMs
Drugs that bind the oestrogen receptor and thereby block the effects of oestrogen on tissues such as the breast but that function similarly to oestrogen in other tissues, such as the endometrium. These drugs are not steroidal in structure.

Aromatase inhibitors
Drugs that block aromatase, the enzyme that converts androgens to oestrogens in tissues including the breast and adipose tissue.

ERs and invasive behaviour

Although a decline of ERα levels is detected in invasive breast cancers, a few studies have reported the regulation of cell migration and invasion by ERα. According to these studies, ERα controls epithelial–mesenchymal transition (EMT) by repressing transcription from NF-κB and AP1 sites and increases E-cadherin expression by dowregulating its transcriptional repressors SNAI1 and SLUG (also known as SNAI2)[110,111]. Recent data have also associated ERβ with the regulation of cell migration and invasion. Downregulation of wild-type ERβ was found to induce EMT in prostate cancer cells, and this was correlated with the loss of ERβ in high Gleason grade prostate carcinoma. This effect was specific to ERβ, as the ERβ-specific ligand 3β-adiol — but not 17β-oestradiol, which activates both ERs — was able to sustain an epithelial phenotype and repress invasion by inducing the expression of E-cadherin[112]. Downregulation of E-cadherin was additionally observed in mouse mammary epithelial cells following knockdown of ERβ1 (REF. 113). Similar to E-cadherin, ERβ1 was found to induce the expression of integrins α1 and β1 and to enhance the adhesion of breast cancer cells[114]. The decline of ERβ1 expression in primary invasive breast and prostate cancers supports the idea that ERβ1, through the inhibition of EMT, functions to maintain a differentiated and epithelial phenotype[43,45,115,116]. The positive correlation between ERβ1 and epithelial markers may also account for the re-expression of the receptor in bone metastasis where metastatic prostate cells undergo mesenchymal–epithelial transition (MET) to form tumours with similar histological characteristics to those in the primary site. Although wild-type ERβ has been shown to inhibit EMT in breast and prostate cancer cell lines, and its increased nuclear expression correlates with better survival in breast cancer, ERβ2 and ERβ5, which are localized in both the cytoplasm and the nucleus, have recently been found to increase the invasiveness of prostate cancer cells and are associated with poor outcome[16]. ERβ1, ERβ2 and ERβ5 have a unique AF2 domain that controls the ligand-dependent transcriptional activity of the receptor and that regulates dimerization, subcellular localization and the stability of the protein. This might explain their different responses in the presence of ligands and also account for their distinct subcellular localization and variable expression in benign versus malignant tissues. ERβ2 can heterodimerize with wild-type ERβ and ERα and can alter their transcriptional activity[117]. In addition, cytoplasmic populations of ERβ2 and ERβ5, as well as the truncated ERα-36, which lacks the AF2 domain, could interact with the membrane and cytoplasmic signalling cascades and oppose the genomic actions of ligand-activated nuclear wild-type ERα and ERβ. On-going and future laboratory and clinical studies will confirm whether the anti-migratory and anti-invasive responses represent an ERβ-specific action and whether wild-type ERβ and its variant isoforms have pivotal roles in early steps of invasion, as well as after extravasation of the metastatic cells at distant sites. This will also establish the idea that oestrogenic signalling is involved not only in tumorigenesis but also in cancer progression.

ERs in cancer prognosis and targeted therapies

The first evidence for the involvement of oestrogenic signalling in the development of breast cancer was provided as early as the 1880s when it was shown that oophorectomy in postmenopausal women caused tumour regression[118]. To date, sustained exposure to exogenous and endogenous oestrogens is considered a well-established cause of breast cancer[8]. Epidemiological, clinical and animal studies additionally implicate oestrogens in the aetiology of prostate, ovarian, lung and endometrial cancer[119–123]. However, the reduced incidence of colon cancer in postmenopausal women receiving combined hormone replacement therapy (HRT) — oestrogen plus progesterone — and the beneficial effects of dietary oestrogens in prostate cancer[124,125] manifest the complexity of oestrogen action in cancer tissues. Similarly, anti-oestrogens, such as selective oestrogen receptor modulators (SERMs), which act as competitive blockers of oestrogen–ER binding, and aromatase inhibitors, which target oestrogen synthesis, have been successfully used for the treatment and the prevention of breast cancer. Adjuvant therapy with tamoxifen treatment reduces the rate of disease recurrence and has led to a significant reduction in breast cancer mortality in the past few decades. Importantly, aromatase inhibitors have greater efficacy than tamoxifen in late-stage disease[126,127]. In addition, the SERM toremifene, which is used for the treatment of breast cancer, has been shown to prevent the progression of high-grade prostatic intraepithelial neoplasia (PIN) to prostate cancer[10]. However, both SERMs and aromatase inhibitors have limitations. Tamoxifen functions as an agonist in endometrial epithelial and stromal cells and stimulates cell proliferation, increasing the risk of endometrial cancer in women taking tamoxifen by 2.5-fold[9]. In addition, aromatase inhibitors were unsuccessful for the treatment of prostate cancer, indicating that the complete ablation of oestrogens is not a valuable therapeutic option for this type of cancer[128].

The different expression patterns of ERα and ERβ can partly account for their distinct activation in cancer tissues. ERα expression increases in early breast cancers from the low-grade ductal carcinoma *in situ* (DCIS) to high-grade DCIS and then declines in more invasive cancers[5,129,130]. Conversely, a gradual reduction of ERβ1 has been observed from normal to pre-invasive lesions and invasive carcinomas[115,116]. Owing to limitations in detection, the few studies that examined the levels of ERβ isoforms in breast cancer showed variable expression, with ERβ1 and ERβ2 the most commonly expressed[105]. Increased expression of ERβ2 was found in DCIS compared with normal epithelium, and higher ERβ5 mRNA was detected in cancer compared with normal tissue[131,132]. Contradictory data were produced regarding the expression of ERα in primary and metastatic prostate cancers[10,45,133], whereas dynamic changes characterize the expression of ERβ in the same tissue. Interestingly, the levels of ERβ decline as prostate cancer develops in the gland but reappear in lymph node and bone metastases[43,45,112]. Changes in the methylation of the *ESR2* promoter are involved in the fluctuation of the receptor expression in different stages of prostate cancer,

and treatment of prostate cell lines with demethylating agents increases the levels of ERβ[43]. Both ER subtypes are expressed in lung and gynaecological cancers. Although high-grade ovarian and endometrial cancers have lower wild-type ERα levels than low-grade tumours[134–138], the ERα-36 variant, which is constitutively active in the absence of ligand, is expressed at higher levels in endometrial carcinoma compared with endometrial hyperplasia[5]. Owing to promoter hypermethylation, the mRNA levels of ERβ1, ERβ2 and ERβ4 are significantly lower in ovarian cancer tissues than in their corresponding normal counterparts, and ERβ2 is expressed in both normal and malignant endometrium[139–141]. Finally, the loss of ERβ1 and ERβ2 but not of ERβ5 was found in different stages of colorectal carcinomas[12,42,142].

Can these differences in the bioavailability of ERs alone account for their distinct activation and function and explain the subsequent adverse and beneficial effects of ligands in cancer? As discussed above, ERs elicit their biological functions by interacting with several other proteins that either stimulate their activity or are regulated by the receptors. Although the predominance of the anti-proliferative and pro-apoptotic ERβ in colon cancer can explain the beneficial effects of HRT in postmenopausal women with the disease, the relative balance of co-activator and co-repressor concentration within the endometrial cells may account for the tamoxifen agonist action in this tissue. The equilibrium of co-activators and co-repressors in endometrial cells is probably shifted towards co-activator function by antagonists. Overexpression of SRC1 has been shown to enhance tamoxifen-stimulated ERα activity in endometrial cells[143], and SRC1 and ERα mRNA levels correlate in endometrial carcinoma tissue, suggesting that the SRC1–ERα protein interaction may account for the adverse effects of tamoxifen during endometrial carcinogenesis[143,144]. Cellular signalling can also influence the subcellular localization and activity of ER, transcription factors and their co-regulators. The phosphorylation of ERα and ERβ on serine and tyrosine residues has been observed in both the presence and the absence of ligands[145–147]. Increased phosphorylation of ERα at S167 correlates with better survival and can predict responses to endocrine therapy. According to some studies, ERα-S118 phosphorylation predicts good outcome in non-selected cohorts and in patients treated with tamoxifen, whereas other studies associate this phosphorylation, as well as S305 phosphorylation, with the development of endocrine resistance[148–154]. Similarly, phosphorylation of ERβ at S105 was associated with better survival, even in tamoxifen-resistant cases[155]. It is evident that constitutively active growth factor receptors and kinases in cancer cells in the presence or absence of growth stimuli, depending on the ERα/ERβ ratio, determine the relative beneficial versus adverse actions of ERs in cancer tissues.

Despite the complexity of ER actions in cancer tissues, these receptors are used as independent prognostic factors in cancer. In breast cancer, ERα protein expression correlates with low tumour grade and negative lymph node status, and ER-positive tumours are usually less invasive and have a more favourable prognosis[156]. ERα is the principal biomarker for the response of breast cancers to tamoxifen treatment. This is associated with the ability of tamoxifen to inhibit the expression of ERα target genes that regulate cell cycle and apoptosis[13]. Repression of cyclin D1 and MYC expression, reduced activity of the transcription factors SP1 and NF-κB and downregulation of the NF-κB target gene *BCL2* are among the events that account for the increased cell death that is observed in tamoxifen-treated ERα-positive breast cancers[13,157–159]. One-third of the women treated with tamoxifen for 5 years will have recurrence of the disease within 15 years, and among the mechanisms of *de novo* or intrinsic endocrine resistance is the lack of ERα expression[13]. Alteration in the levels of CUEDC2 and LMTK3 has recently been reported to influence the tamoxifen resistance of breast cancer by modulating the stability of ERα[57,58]. In addition, overexpression of ERBB2 is a well-known mechanism of endocrine resistance, and ERα has recently been shown to regulate the expression of ERBB2 by interacting with the paired box 2 gene product (PAX2) and the co-activator SRC3. PAX2 competes with SRC3 for binding to ERα and represses *ERBB2* transcription. Increased amounts of PAX2 and decreased expression of SRC3 are associated with low ERBB2 levels and a better clinical outcome in tamoxifen-treated ERα-positive breast cancers[160]. The cytoplasmic localization of proline, glutamate and leucine-rich protein 1 (PELP1) and the expression of the truncated ERα variant ERα-36 have been reported to promote constitutive ERα-mediated transcription, which also decreases sensitivity *in vitro* and is associated with reduced responsiveness to tamoxifen treatment[98,161–163]. In addition to well-defined pathways, the splicing of the X-box-binding protein 1 (XBP1) and the unfolded protein response have recently emerged as ER-regulated processes that increase endocrine resistance in breast cancer[164,165].

Currently, the prognostic and predictive value of ERβ remains unclear because previous clinical studies have produced contradictory data. Most retrospective studies used poorly validated antibodies, analysed a small number of cases and failed to account for the contribution of the ERβ isoforms. More recent studies have examined a larger number of samples with well-validated antibodies and have shown an association between the expression of ERβ isoforms and clinical outcome[14]. With the exception of one study, which suggested that wild-type ERβ predicts a high risk of relapse in a lymph node-positive group and is related to a more aggressive phenotype[106,166,167], positivity for wild-type ERβ has been associated with a better survival in patients with ERBB2-positive and ERα-, PR- and ERBB2-negative (triple-negative or basal) breast cancers and a better response to tamoxifen monotherapy[166]. This is consistent with the increased tamoxifen sensitivity of ERα-positive breast cancer cells following the expression of wild-type ERβ[11]. Nuclear ERβ2 was negatively associated with metastasis and vascular invasion, and cytoplasmic ERβ2 was associated with a worse outcome, although nuclear

Unfolded protein response
A cellular response to stress that senses misfolded proteins in the endoplasmic reticulum. It activates pathways that help cells to survive the toxicity that is caused by unfolded proteins or to activate mechanisms of cell death.

ERβ5 was associated with better survival[106]. In prostate cancer, although *in vitro* studies strongly suggested that ERβ1 inhibits EMT and its expression diminishes in high Gleason grades, a few clinical studies showed an association between ERβ immunopositivity in high-grade prostate carcinomas and poor relapse-free survival time[112,168]. This can be explained by the different function of ERβ in prostate carcinogenesis and prostate cancer progression, as well as by the variable expression of the different ERβ isoforms during different stages of the disease. Recent studies correlated ERβ5 with a worse clinical outcome in prostate cancer, and ERβ2 was associated with a more metastatic phenotype in prostate carcinoma. Such findings are consistent with data from *in vitro* studies that link the ERβ variants with the induction of migration and invasion in prostate cancer cells[12,16]. A similar picture exists regarding the prognostic role of ERβ in lung cancer, with most of the studies proposing an association between ERβ1 positivity and better survival in men and worse outcome in women. A few studies have reported a correlation between cytoplasmic ERβ1 and worse clinical course[15,169–175]. Increased *ESR1* mRNA and protein expression pointed to a favourable outcome in patients with ovarian cancer and showed a significant association with tumour grading[176–179], whereas ERβ levels correlated with the occurrence of lymph node metastasis[180]. Similarly to ovarian cancer, ERα positivity correlated with better survival in patients with endometrial carcinoma, and ERβ expression did not demonstrate any significant correlations with clinicopathological characteristics[181,182].

On the basis of these observations, it can be postulated that ERα, ERβ and their variants can influence cancer biology and therapy by exerting distinct biological functions. Future studies should elucidate which clinical responses are either ER subtype-specific or result from the interaction of both receptors.

Future directions and challenges

The variable expression of ER subtypes and their variant isoforms in cancer tissues, as well as alterations in the levels of endogenous ligands, co-regulators, transcription factors, components of the membrane and cytoplasmic signalling cascade, all compose a complex environment that influences ER action and that affects cancer biology and therapy. This also proposes a variety of different approaches to target ER signalling and to improve the outcome of patients with hormone-related cancers. Recent laboratory and clinical research has been focusing on developing and evaluating ERα- and ERβ-selective agonists and antagonists. It seems that when ERα is expressed it mediates the adverse effects of oestrogens in most types of cancers. This supports the rationale for the use of anti-oestrogenic compounds that specifically target and block ERα signalling for the prevention and treatment of cancer. By contrast, the activation of wild-type ERβ has been associated with the beneficial effects of oestrogens, suggesting the application of an ERβ agonist for the treatment of this disease. However, the application and use of agonists, SERMs and aromatase inhibitors for the treatment of cancer is not as simple as it would seem. The variability of ERα and ERβ expression in cancer tissues complicates the issue and dictates the necessity to elucidate the mechanisms that regulate ER expression. This would lead to the identification of novel biomarkers that can complement ERs in the prognosis and prediction of therapeutic response. In addition, targeting pathways that control the receptor turnover and that result in the restoration of ER levels in cancer tissues may have therapeutic potential in the treatment of cancer. Finally, exploration of genomic and non-genomic pathways that interact with and influence ER signalling may help to overcome the problem of tumour heterogeneity by identifying biomarkers for molecular profiling and tracking therapeutic treatment and outcome.

1. Heldring, N. *et al.* Estrogen receptors: how do they signal and what are their targets. *Physiol. Rev.* **87**, 905–931 (2007).
This review discusses new insights into the mechanism of ER action and the molecular mechanism of anti-oestrogen signalling.
2. Toft, D. & Gorski, J. A receptor molecule for estrogens: isolation from the rat uterus and preliminary characterization. *Proc. Natl Acad. Sci. USA* **55**, 1574–1581 (1966).
3. Walter, P. *et al.* Cloning of the human estrogen receptor cDNA. *Proc. Natl Acad. Sci. USA* **82**, 7889–7893 (1985).
4. Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S. & Gustafsson, J. A. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl Acad. Sci. USA* **93**, 5925–5930 (1996).
5. Herynk, M. H. & Fuqua, S. A. Estrogen receptor mutations in human disease. *Endocr. Rev.* **25**, 869–898 (2004).
This reference extensively describes the splice variants and mutations of the human ER subtypes and illustrates their role in disease.
6. Bjornstrom, L. & Sjoberg, M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol. Endocrinol.* **19**, 833–842 (2005).
7. Colditz, G. A. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J. Natl Cancer Inst.* **90**, 814–823 (1998).
8. Hankinson, S. E., Colditz, G. A. & Willett, W. C. Towards an integrated model for breast cancer etiology: the lifelong interplay of genes, lifestyle, and hormones. *Breast Cancer Res.* **6**, 213–218 (2004).
9. Shang, Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. *Nature Rev. Cancer* **6**, 360–368 (2006).
10. Ellem, S. J. & Risbridger, G. P. Treating prostate cancer: a rationale for targeting local oestrogens. *Nature Rev. Cancer* **7**, 621–627 (2007).
This article discusses the complexity of oestrogen action in the prostate and suggests the selective targeting of ER subtypes as a potential new and more effective therapeutic approach.
11. Fox, E. M., Davis, R. J. & Shupnik, M. A. ERβ in breast cancer—onlooker, passive player, or active protector? *Steroids* **73**, 1039–1051 (2008).
12. Wong, N. A. *et al.* ERβ isoform expression in colorectal carcinoma: an *in vivo* and *in vitro* study of clinicopathological and molecular correlates. *J. Pathol.* **207**, 53–60 (2005).
13. Musgrove, E. A. & Sutherland, R. L. Biological determinants of endocrine resistance in breast cancer. *Nature Rev. Cancer* **9**, 631–643 (2009).
14. Speirs, V. *et al.* Clinical importance of estrogen receptor β isoforms in breast cancer. *J. Clin. Oncol.* **26**, 5825–5826 (2008).
15. Stabile, L. P. *et al.* Combined analysis of estrogen receptor β-1 and progesterone receptor expression identifies lung cancer patients with poor outcome. *Clin. Cancer Res.* **17**, 154–164 (2011).
This article summarizes new clinical findings regarding the prognostic role of ERβ in lung cancer.
16. Leung, Y. K. *et al.* Estrogen receptor β2 and β5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion. *Endocr. Relat. Cancer* **17**, 675–689 (2010).
17. Britton, D. J. *et al.* Bidirectional cross talk between ERα and EGFR signalling pathways regulates tamoxifen-resistant growth. *Breast Cancer Res. Treat* **96**, 131–146 (2006).
18. Nonclercq, D., Journe, F., Body, J. J., Leclercq, G. & Laurent, G. Ligand-independent and agonist-mediated degradation of estrogen receptor-α in breast carcinoma cells: evidence for distinct degradative pathways. *Mol. Cell Endocrinol.* **227**, 53–65 (2004).
19. Swedenborg, E., Power, K. A., Cai, W., Pongratz, I. & Ruegg, J. Regulation of estrogen receptor β activity and implications in health and disease. *Cell. Mol. Life Sci.* **66**, 3873–3894 (2009).
20. Xu, J., Wu, R. C. & O'Malley, B. W. Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. *Nature Rev. Cancer* **9**, 615–630 (2009).
21. Enmark, E. *et al.* Human estrogen receptor β-gene structure, chromosomal localization, and expression pattern. *J. Clin. Endocrinol. Metab.* **82**, 4258–4265 (1997).
22. Gosden, J. R., Middleton, P. G. & Rout, D. Localization of the human oestrogen receptor gene to chromosome 6q24----q27 by *in situ* hybridization. *Cytogenet. Cell Genet.* **43**, 218–220 (1986).

23. Biswas, D. K., Singh, S., Shi, Q., Pardee, A. B. & Iglehart, J. D. Crossroads of estrogen receptor and NF-κB signaling. *Sci. STKE* **2005**, pe27 (2005).
24. Fox, E. M., Andrade, J. & Shupnik, M. A. Novel actions of estrogen to promote proliferation: integration of cytoplasmic and nuclear pathways. *Steroids* **74**, 622–627 (2009).
25. Carroll, J. S. *et al.* Genome-wide analysis of estrogen receptor binding sites. *Nature Genet.* **38**, 1289–1297 (2006).
This reference describes distinct mechanisms of ER-mediated gene regulation.
26. Menendez, D., Inga, A. & Resnick, M. A. Estrogen receptor acting in *cis* enhances WT and mutant p53 transactivation at canonical and noncanonical p53 target sequences. *Proc. Natl Acad. Sci. USA* **107**, 1500–1505 (2010).
27. Sotgia, F. *et al.* Caveolin-1, mammary stem cells, and estrogen-dependent breast cancers. *Cancer Res.* **66**, 10647–10651 (2006).
28. Chambliss, K. L. *et al.* Estrogen receptor α and endothelial nitric oxide synthase are organized into a functional signaling module in caveolae. *Circ. Res.* **87**, e44–e52 (2000).
29. Kousteni, S. *et al.* Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. *Cell* **104**, 719–730 (2001).
30. Massarweh, S. & Schiff, R. Unraveling the mechanisms of endocrine resistance in breast cancer: new therapeutic opportunities. *Clin. Cancer Res.* **13**, 1950–1954 (2007).
References 13 and 30 summarize and evaluate new insights into the mechanism of endocrine resistance based on global gene expression profiling approaches and functional genetic screens and suggest new therapeutic opportunities to target resistance and improve breast cancer disease outcomes.
31. Razandi, M., Pedram, A., Merchenthaler, I., Greene, G. L. & Levin, E. R. Plasma membrane estrogen receptors exist and functions as dimers. *Mol. Endocrinol.* **18**, 2854–2865 (2004).
32. Kahlert, S. *et al.* Estrogen receptor α rapidly activates the IGF-1 receptor pathway. *J. Biol. Chem.* **275**, 18447–18453 (2000).
33. Shupnik, M. A. Crosstalk between steroid receptors and the c-Src-receptor tyrosine kinase pathways: implications for cell proliferation. *Oncogene* **23**, 7979–7989 (2004).
34. Song, R. X. *et al.* Linkage of rapid estrogen action to MAPK activation by ERα-Shc association and Shc pathway activation. *Mol. Endocrinol.* **16**, 116–127 (2002).
35. Smith, C. L., Nawaz, Z. & O'Malley, B. W. Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Mol. Endocrinol.* **11**, 657–666 (1997).
36. Webb, P. *et al.* ERβ binds N-CoR in the presence of estrogens via an LXXLL-like motif in the N-CoR C-terminus. *Nucl. Recept.* **1**, 4 (2003).
37. Bouras, T., Southey, M. C. & Venter, D. J. Overexpression of the steroid receptor coactivator AIB1 in breast cancer correlates with the absence of estrogen and progesterone receptors and positivity for p53 and HER2/neu. *Cancer Res.* **61**, 903–907 (2001).
38. Klinge, C. M., Jernigan, S. C., Mattingly, K. A., Risinger, K. E. & Zhang, J. Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors α and β by coactivators and corepressors. *J. Mol. Endocrinol.* **33**, 387–410 (2004).
39. Dubik, D. & Shiu, R. P. Mechanism of estrogen activation of c-myc oncogene expression. *Oncogene* **7**, 1587–1594 (1992).
40. Hartman, J. *et al.* Tumor repressive functions of estrogen receptor β in SW480 colon cancer cells. *Cancer Res.* **69**, 6100–6106 (2009).
41. Liu, M. M. *et al.* Opposing action of estrogen receptors α and β on cyclin D1 gene expression. *J. Biol. Chem.* **277**, 24353–24360 (2002).
42. Foley, E. F., Jazaeri, A. A., Shupnik, M. A., Jazaeri, O. & Rice, L. W. Selective loss of estrogen receptor β in malignant human colon. *Cancer Res.* **60**, 245–248 (2000).
43. Zhu, X. *et al.* Dynamic regulation of estrogen receptor-β expression by DNA methylation during prostate cancer development and metastasis. *Am. J. Pathol.* **164**, 2003–2012 (2004).
44. Rutherford, T. *et al.* Absence of estrogen receptor-β expression in metastatic ovarian cancer. *Obstet. Gynecol.* **96**, 417–421 (2000).
45. Leav, I. *et al.* Comparative studies of the estrogen receptors β and α and the androgen receptor in normal human prostate glands, dysplasia, and in primary and metastatic carcinoma. *Am. J. Pathol.* **159**, 79–92 (2001).
46. Eeckhoute, J. *et al.* Positive cross-regulatory loop ties GATA-3 to estrogen receptor α expression in breast cancer. *Cancer Res.* **67**, 6477–6483 (2007).
47. Guo, S. & Sonenshein, G. E. Forkhead box transcription factor FOXO3a regulates estrogen receptor α expression and is repressed by the Her-2/neu/phosphatidylinositol 3-kinase/Akt signaling pathway. *Mol. Cell Biol.* **24**, 8681–8690 (2004).
48. Madureira, P. A. *et al.* The Forkhead box M1 protein regulates the transcription of the estrogen receptor α in breast cancer cells. *J. Biol. Chem.* **281**, 25167–25176 (2006).
49. Adams, B. D., Furneaux, H. & White, B. A. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-α (ERα) and represses ERα messenger RNA and protein expression in breast cancer cell lines. *Mol. Endocrinol.* **21**, 1132–1147 (2007).
50. Liu, W. H. *et al.* MicroRNA-18a prevents estrogen receptor-α expression, promoting proliferation of hepatocellular carcinoma cells. *Gastroenterology* **136**, 683–693 (2009).
51. Zhao, J. J. *et al.* MicroRNA-221/222 negatively regulates estrogen receptor α and is associated with tamoxifen resistance in breast cancer. *J. Biol. Chem.* **283**, 31079–31086 (2008).
52. Al-Nakhle, H. *et al.* Estrogen receptor β1 expression is regulated by miR-92 in breast cancer. *Cancer Res.* **70**, 4778–4784 (2010).
53. Pandey, D. P. & Picard, D. miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor α mRNA. *Mol. Cell Biol.* **29**, 3783–3790 (2009).
References 49–53 illustrate the role of miRNAs in the regulation of the expression of ER subtypes in cancer cells and tissues.
54. Richter, K. & Buchner, J. Hsp90: chaperoning signal transduction. *J. Cell Physiol.* **188**, 281–290 (2001).
55. Duong, V. *et al.* Differential regulation of estrogen receptor α turnover and transactivation by Mdm2 and stress-inducing agents. *Cancer Res.* **67**, 5513–5521 (2007).
56. Li, L., Li, Z., Howley, P. M. & Sacks, D. B. E6AP and calmodulin reciprocally regulate estrogen receptor stability. *J. Biol. Chem.* **281**, 1978–1985 (2006).
57. Giamas, G. *et al.* Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. *Nature Med.* **17**, 715–719 (2011).
58. Pan, X. *et al.* Elevated expression of CUEDC2 protein confers endocrine resistance in breast cancer. *Nature Med.* **17**, 708–714 (2011).
References 57 and 58 demonstrate how changes in ER protein stability alter cancer response to therapy.
59. Bocchinfuso, W. P. & Korach, K. S. Mammary gland development and tumorigenesis in estrogen receptor knockout mice. *J. Mammary Gland Biol. Neoplasia* **2**, 323–334 (1997).
60. Korach, K. S. Insights from the study of animals lacking functional estrogen receptor. *Science* **266**, 1524–1527 (1994).
61. Feng, Y., Manka, D., Wagner, K. U. & Khan, S. A. Estrogen receptor-α expression in the mammary epithelium is required for ductal and alveolar morphogenesis in mice. *Proc. Natl Acad. Sci. USA* **104**, 14718–14723 (2007).
62. Bocchinfuso, W. P., Hively, W. P., Couse, J. F., Varmus, H. E. & Korach, K. S. A mouse mammary tumor virus-Wnt-1 transgene induces mammary gland hyperplasia and tumorigenesis in mice lacking estrogen receptor-α. *Cancer Res.* **59**, 1869–1876 (1999).
63. Hewitt, S. C. *et al.* Lack of ductal development in the absence of functional estrogen receptor α delays mammary tumor formation induced by transgenic expression of ErbB2/neu. *Cancer Res.* **62**, 2798–2805 (2002).
64. Miermont, A. M., Parrish, A. R. & Furth, P. A. Role of ERα in the differential response of Stat5a loss in susceptibility to mammary preneoplasia and DMBA-induced carcinogenesis. *Carcinogenesis* **31**, 1124–1131 (2010).
65. Yoshidome, K., Shibata, M. A., Couldrey, C., Korach, K. S. & Green, J. E. Estrogen promotes mammary tumor development in C3(1)/SV40 large T-antigen transgenic mice: paradoxical loss of estrogen receptorα expression during tumor progression. *Cancer Res.* **60**, 6901–6910 (2000).
66. Risbridger, G. *et al.* Evidence that epithelial and mesenchymal estrogen receptor-α mediates effects of estrogen on prostatic epithelium. *Dev. Biol.* **229**, 432–442 (2001).
67. Bianco, J. J., Handelsman, D. J., Pedersen, J. S. & Risbridger, G. P. Direct response of the murine prostate gland and seminal vesicles to estradiol. *Endocrinology* **143**, 4922–4933 (2002).
68. Bianco, J. J., McPherson, S. J., Wang, H., Prins, G. S. & Risbridger, G. P. Transient neonatal estrogen exposure to estrogen-deficient mice (aromatase knockout) reduces prostate weight and induces inflammation in late life. *Am. J. Pathol.* **168**, 1869–1878 (2006).
69. Prins, G. S. *et al.* Estrogen imprinting of the developing prostate gland is mediated through stromal estrogen receptor α: studies with αERKO and βERKO mice. *Cancer Res.* **61**, 6089–6097 (2001).
70. Gingrich, J. R. *et al.* Metastatic prostate cancer in a transgenic mouse. *Cancer Res.* **56**, 4096–4102 (1996).
71. Raghow, S., Hooshdaran, M. Z., Katiyar, S. & Steiner, M. S. Toremifene prevents prostate cancer in the transgenic adenocarcinoma of mouse prostate model. *Cancer Res.* **62**, 1370–1376 (2002).
72. Castro-Rivera, E., Samudio, I. & Safe, S. Estrogen regulation of cyclin D1 gene expression in ZR-75 breast cancer cells involves multiple enhancer elements. *J. Biol. Chem.* **276**, 30853–30861 (2001).
73. List, H. J. *et al.* Ribozyme targeting demonstrates that the nuclear receptor coactivator AIB1 is a rate-limiting factor for estrogen-dependent growth of human MCF-7 breast cancer cells. *J. Biol. Chem.* **276**, 23763–23768 (2001).
74. Planas-Silva, M. D., Shang, Y., Donaher, J. L., Brown, M. & Weinberg, R. A. AIB1 enhances estrogen-dependent induction of cyclin D1 expression. *Cancer Res.* **61**, 3858–3862 (2001).
75. Levin, E. R. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Mol. Endocrinol.* **17**, 309–317 (2003).
76. Driggers, P. H. & Segars, J. H. Estrogen action and cytoplasmic signaling pathways. Part II: the role of growth factors and phosphorylation in estrogen signaling. *Trends Endocrinol. Metab.* **13**, 422–427 (2002).
77. Forster, C. *et al.* Involvement of estrogen receptor β in terminal differentiation of mammary gland epithelium. *Proc. Natl Acad. Sci. USA* **99**, 15578–15583 (2002).
78. Imamov, O., Lopatkin, N. A. & Gustafsson, J. A. Estrogen receptor β in prostate cancer. *N. Engl. J. Med.* **351**, 2773–2774 (2004).
79. Imamov, O. *et al.* Estrogen receptor β regulates epithelial cellular differentiation in the mouse ventral prostate. *Proc. Natl Acad. Sci. USA* **101**, 9375–9380 (2004).
80. Weihua, Z. *et al.* A role for estrogen receptor β in the regulation of growth of the ventral prostate. *Proc. Natl Acad. Sci. USA* **98**, 6330–6335 (2001).
81. Antal, M. C., Krust, A., Chambon, P. & Mark, M. Sterility and absence of histopathological defects in nonreproductive organs of a mouse ERβ-null mutant. *Proc. Natl Acad. Sci. USA* **105**, 2433–2438 (2008).
82. Dupont, S. *et al.* Effect of single and compound knockouts of estrogen receptors α (ERα) and β (ERβ) on mouse reproductive phenotypes. *Development* **127**, 4277–4291 (2000).
83. Nakajima, Y. *et al.* Estrogen regulates tumor growth through a nonclassical pathway that includes the transcription factors ERβ and KLF5. *Sci. Signal.* **4**, ra22 (2011).
This article demonstrates the tumour suppressive properties of ERβ and describes one of the mechanisms through which ERβ regulates tumour growth.
84. Strom, A. *et al.* Estrogen receptor β inhibits 17β-estradiol-stimulated proliferation of the breast cancer cell line T47D. *Proc. Natl Acad. Sci. USA* **101**, 1566–1571 (2004).
85. Lin, C. Y. *et al.* Inhibitory effects of estrogen receptor β on specific hormone-responsive gene expression and association with disease outcome in primary breast cancer. *Breast Cancer Res.* **9**, R25 (2007).

86. Lindberg, M. K. *et al.* Estrogen receptor (ER)-β reduces ERα-regulated gene transcription, supporting a "ying yang" relationship between ERα and ERβ in mice. *Mol. Endocrinol.* **17**, 203–208 (2003).
87. Williams, C., Edvardsson, K., Lewandowski, S. A., Strom, A. & Gustafsson, J. A. A genome-wide study of the repressive effects of estrogen receptor β on estrogen receptor α signaling in breast cancer cells. *Oncogene* **27**, 1019–1032 (2008).
88. Hartman, J. *et al.* Estrogen receptor β inhibits angiogenesis and growth of T47D breast cancer xenografts. *Cancer Res.* **66**, 11207–11213 (2006).
89. Thomas, C. G., Strom, A., Lindberg, K. & Gustafsson, J. A. Estrogen receptor β decreases survival of p53-defective cancer cells after DNA damage by impairing G_2/M checkpoint signaling. *Breast Cancer Res. Treat.* **127**, 417–427 (2011).
90. Hershberger, P. A. *et al.* Estrogen receptor β (ERβ) subtype-specific ligands increase transcription, p44/p42 mitogen activated protein kinase (MAPK) activation and growth in human non-small cell lung cancer cells. *J. Steroid Biochem. Mol. Biol.* **116**, 102–109 (2009).
91. Hou, Y. F. *et al.* ERβ exerts multiple stimulative effects on human breast carcinoma cells. *Oncogene* **23**, 5799–5806 (2004).
92. Zhang, G. *et al.* Estrogen receptor β functions through nongenomic mechanisms in lung cancer cells. *Mol. Endocrinol.* **23**, 146–156 (2009).
93. Mangelsdorf, D. J. *et al.* The nuclear receptor superfamily: the second decade. *Cell* **83**, 835–839 (1995).
94. Barone, I., Brusco, L. & Fuqua, S. A. Estrogen receptor mutations and changes in downstream gene expression and signaling. *Clin. Cancer Res.* **16**, 2702–2708 (2010).
95. Lin, S. L. *et al.* ER-α36, a variant of ER-α, promotes tamoxifen agonist action in endometrial cancer cells via the MAPK/ERK and PI3K/Akt pathways. *PLoS ONE* **5**, e9013 (2010).
96. Poola, I. & Speirs, V. Expression of alternatively spliced estrogen receptor α mRNAs is increased in breast cancer tissues. *J. Steroid Biochem. Mol. Biol.* **78**, 459–469 (2001).
97. Poola, I., Koduri, S., Chatra, S. & Clarke, R. Identification of twenty alternatively spliced estrogen receptor α mRNAs in breast cancer cell lines and tumors using splice targeted primer approach. *J. Steroid Biochem. Mol. Biol.* **72**, 249–258 (2000).
98. Shi, L. *et al.* Expression of ER-α36, a novel variant of estrogen receptor α, and resistance to tamoxifen treatment in breast cancer. *J. Clin. Oncol.* **27**, 3423–3429 (2009).
99. Castles, C. G., Fuqua, S. A., Klotz, D. M. & Hill, S. M. Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line. *Cancer Res.* **53**, 5934–5939 (1993).
100. Desai, A. J. *et al.* Presence of exon 5-deleted oestrogen receptor in human breast cancer: functional analysis and clinical significance. *Br. J. Cancer* **75**, 1173–1184 (1997).
101. Erenburg, I., Schachter, B., Mira y Lopez, R. & Ossowski, L. Loss of an estrogen receptor isoform (ER α δ 3) in breast cancer and the consequences of its reexpression: interference with estrogen-stimulated properties of malignant transformation. *Mol. Endocrinol.* **11**, 2004–2015 (1997).
102. Moore, J. T. *et al.* Cloning and characterization of human estrogen receptor β isoforms. *Biochem. Biophys. Res. Commun.* **247**, 75–78 (1998).
103. Omoto, Y., Eguchi, H., Yamamoto-Yamaguchi, Y. & Hayashi, S. Estrogen receptor (ER) β1 and ERβcx/β2 inhibit ERα function differently in breast cancer cell line MCF7. *Oncogene* **22**, 5011–5020 (2003).
104. Peng, B., Lu, B., Leygue, E. & Murphy, L. C. Putative functional characteristics of human estrogen receptor-β isoforms. *J. Mol. Endocrinol.* **30**, 13–29 (2003).
105. Green, C. A., Peter, M. B., Speirs, V. & Shaaban, A. M. The potential role of ER β isoforms in the clinical management of breast cancer. *Histopathology* **53**, 374–380 (2008).
106. Shaaban, A. M. *et al.* Nuclear and cytoplasmic expression of ERβ1, ERβ2, and ERβ5 identifies distinct prognostic outcome for breast cancer patients. *Clin. Cancer Res.* **14**, 5228–5235 (2008).
107. Saji, S. *et al.* Expression of estrogen receptor (ER) (β) cx protein in ER(α)-positive breast cancer: specific correlation with progesterone receptor. *Cancer Res.* **62**, 4849–4853 (2002).
108. Yan, M., Rayoo, M., Takano, E. A. & Fox, S. B. Nuclear and cytoplasmic expressions of ERβ1 and ERβ2 are predictive of response to therapy and alters prognosis in familial breast cancers. *Breast Cancer Res. Treat* **126**, 395–405 (2010).
109. Pettersson, K., Delaunay, F. & Gustafsson, J. A. Estrogen receptor β acts as a dominant regulator of estrogen signaling. *Oncogene* **19**, 4970–4978 (2000).
110. Wang, X. *et al.* Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nature Cell Biol.* **9**, 470–478 (2007).
111. Ye, Y. *et al.* ERα signaling through slug regulates E-cadherin and EMT. *Oncogene* **29**, 1451–1462 (2010).
112. Mak, P. *et al.* ERβ impedes prostate cancer EMT by destabilizing HIF-1α and inhibiting VEGF-mediated snail nuclear localization: implications for Gleason grading. *Cancer Cell* **17**, 319–332 (2010).
An elegant proof of the anti-migratory and anti-invasive properties of ERβ, and a demonstration of its pivotal role in the early steps of the invasion and metastasis process.
113. Helguero, L. A. *et al.* Different roles of estrogen receptors α and β in the regulation of E-cadherin protein levels in a mouse mammary epithelial cell line. *Cancer Res.* **68**, 8695–8704 (2008).
114. Lindberg, K. *et al.* Expression of estrogen receptor β increases integrin α1 and integrin β1 levels and enhances adhesion of breast cancer cells. *J. Cell Physiol.* **222**, 156–167 (2010).
115. Shaaban, A. M. *et al.* Declining estrogen receptor-β expression defines malignant progression of human breast neoplasia. *Am. J. Surg. Pathol.* **27**, 1502–1512 (2003).
116. Skliris, G. P. *et al.* Reduced expression of oestrogen receptor β in invasive breast cancer and its re-expression using DNA methyl transferase inhibitors in a cell line model. *J. Pathol.* **201**, 213–220 (2003).
117. Leung, Y. K., Mak, P., Hassan, S. & Ho, S. M. Estrogen receptor (ER)-β isoforms: a key to understanding ER-β signaling. *Proc. Natl Acad. Sci. USA* **103**, 13162–13167 (2006).
118. Boyd, S. Remarks on oophorectomy in the treatment of cancer of the breast. *Br. Med. J.* **1**, 257–262 (1899).
119. Bosland, M. C. *et al.* Chemoprevention strategies for prostate cancer. *Eur. J. Cancer Prev.* **11**, S18–S27 (2002).
120. Couse, J. F. & Korach, K. S. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr. Rev.* **20**, 358–417 (1999).
121. Deroo, B. J. & Korach, K. S. Estrogen receptors and human disease. *J. Clin. Invest.* **116**, 561–570 (2006).
122. Mah, V. *et al.* Aromatase expression predicts survival in women with early-stage non small cell lung cancer. *Cancer Res.* **67**, 10484–10490 (2007).
123. Henderson, B. E. & Feigelson, H. S. Hormonal carcinogenesis. *Carcinogenesis* **21**, 427–433 (2000).
124. Jarred, R. A. *et al.* Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones. *Cancer Epidemiol. Biomarkers Prev.* **11**, 1689–1696 (2002).
125. Rossouw, J. E. *et al.* Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* **288**, 321–333 (2002).
126. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* **365**, 1687–1717 (2005).
127. Johnston, S. R. & Dowsett, M. Aromatase inhibitors for breast cancer: lessons from the laboratory. *Nature Rev. Cancer* **3**, 821–831 (2003).
128. Smith, M. R. *et al.* Selective aromatase inhibition for patients with androgen-independent prostate carcinoma. *Cancer* **95**, 1864–1868 (2002).
129. Leygue, E., Dotzlaw, H., Watson, P. H. & Murphy, L. C. Altered estrogen receptor α and β messenger RNA expression during human breast tumorigenesis. *Cancer Res.* **58**, 3197–3201 (1998).
130. Roger, P. *et al.* Dissociated overexpression of cathepsin D and estrogen receptor α in preinvasive mammary tumors. *Hum. Pathol.* **31**, 593–600 (2000).
131. Chi, A., Chen, X., Chirala, M. & Younes, M. Differential expression of estrogen receptor β isoforms in human breast cancer tissue. *Anticancer Res.* **23**, 211–216 (2003).
132. Esslimani-Sahla, M. *et al.* Increased estrogen receptor βcx expression during mammary carcinogenesis. *Clin. Cancer Res.* **11**, 3170–3174 (2005).
133. Bonkhoff, H., Fixemer, T., Hunsicker, I. & Remberger, K. Progesterone receptor expression in human prostate cancer: correlation with tumor progression. *Prostate* **48**, 285–291 (2001).
134. Hogdall, E. V. *et al.* Prognostic value of estrogen receptor and progesterone receptor tumor expression in Danish ovarian cancer patients: from the 'MALOVA' ovarian cancer study. *Oncol. Rep.* **18**, 1051–1059 (2007).
135. Issa, R. M. *et al.* Estrogen receptor gene amplification occurs rarely in ovarian cancer. *Mod. Pathol.* **22**, 191–196 (2009).
136. Jazaeri, A. A. *et al.* Well-differentiated endometrial adenocarcinomas and poorly differentiated mixed mullerian tumors have altered ER and PR isoform expression. *Oncogene* **20**, 6965–6969 (2001).
137. Saegusa, M. & Okayasu, I. Changes in expression of estrogen receptors α and β in relation to progesterone receptor and pS2 status in normal and malignant endometrium. *Jpn. J. Cancer Res.* **91**, 510–518 (2000).
138. Tan, D. S., Lambros, M. B., Marchio, C. & Reis-Filho, J. S. ESR1 amplification in endometrial carcinomas: hope or hyperbole? *J. Pathol.* **216**, 271–274 (2008).
139. Critchley, H. O. *et al.* Wild-type estrogen receptor (ERβ1) and the splice variant (ERβcx/β2) are both expressed within the human endometrium throughout the normal menstrual cycle. *J. Clin. Endocrinol. Metab.* **87**, 5265–5273 (2002).
140. Skrzypczak, M. *et al.* Evaluation of mRNA expression of estrogen receptor β and its isoforms in human normal and neoplastic endometrium. *Int. J. Cancer* **110**, 783–787 (2004).
141. Suzuki, F. *et al.* Loss of estrogen receptor β isoform expression and its correlation with aberrant DNA methylation of the 5′-untranslated region in human epithelial ovarian carcinoma. *Cancer Sci.* **99**, 2365–2372 (2008).
142. Jassam, N., Bell, S. M., Speirs, V. & Quirke, P. Loss of expression of oestrogen receptor β in colon cancer and its association with Dukes' staging. *Oncol. Rep.* **14**, 17–21 (2005).
143. Shah, Y. M. & Rowan, B. G. The Src kinase pathway promotes tamoxifen agonist action in Ishikawa endometrial cells through phosphorylation-dependent stabilization of estrogen receptor (α) promoter interaction and elevated steroid receptor coactivator 1 activity. *Mol. Endocrinol.* **19**, 732–748 (2005).
144. Smith, C. L. & O'Malley, B. W. Coregulator function: a key to understanding tissue specificity of selective receptor modulators. *Endocr. Rev.* **25**, 45–71 (2004).
145. Picard, N. *et al.* Phosphorylation of activation function-1 regulates proteasome-dependent nuclear mobility and E6-associated protein ubiquitin ligase recruitment to the estrogen receptor β. *Mol. Endocrinol.* **22**, 317–330 (2008).
146. Sauve, K., Lepage, J., Sanchez, M., Heveker, N. & Tremblay, A. Positive feedback activation of estrogen receptors by the CXCL12-CXCR4 pathway. *Cancer Res.* **69**, 5793–5800 (2009).
147. Skliris, G. P. *et al.* Immunohistochemical validation of multiple phospho-specific epitopes for estrogen receptor α (ERα) in tissue microarrays of ERα positive human breast carcinomas. *Breast Cancer Res. Treat* **118**, 443–453 (2009).
148. Holm, C. *et al.* Phosphorylation of the oestrogen receptor α at serine 305 and prediction of tamoxifen resistance in breast cancer. *J. Pathol.* **217**, 372–379 (2009).
149. Kok, M. *et al.* Estrogen receptor-α phosphorylation at serine-118 and tamoxifen response in breast cancer. *J. Natl Cancer Inst.* **101**, 1725–1729 (2009).
150. Murphy, L. *et al.* Phospho-serine-118 estrogen receptor-α detection in human breast tumors *in vivo*. *Clin. Cancer Res.* **10**, 1354–1359 (2004).
151. Murphy, L. C., Niu, Y., Snell, L. & Watson, P. Phospho-serine-118 estrogen receptor-α expression is associated with better disease outcome in women treated with tamoxifen. *Clin. Cancer Res.* **10**, 5902–5906 (2004).
152. Sarwar, N. *et al.* Phosphorylation of ERα at serine 118 in primary breast cancer and in tamoxifen-resistant tumours is indicative of a complex role for ERα phosphorylation in breast cancer progression. *Endocr. Relat. Cancer* **13**, 851–861 (2006).

153. Yamashita, H. *et al.* Phosphorylation of estrogen receptor α serine 167 is predictive of response to endocrine therapy and increases postrelapse survival in metastatic breast cancer. *Breast Cancer Res.* **7**, R753–R764 (2005).
154. Yamashita, H. *et al.* Low phosphorylation of estrogen receptor α (ERα) serine 118 and high phosphorylation of ERα serine 167 improve survival in ER-positive breast cancer. *Endocr. Relat. Cancer* **15**, 755–763 (2008).
155. Hamilton-Burke, W. *et al.* Phosphorylation of estrogen receptor β at serine 105 is associated with good prognosis in breast cancer. *Am. J. Pathol.* **177**, 1079–1086 (2010).
References 147–155 provide clinical evidence that the phosphorylation status of ER subtypes can be used in prognosis of breast cancer.
156. Platet, N., Cathiard, A. M., Gleizes, M. & Garcia, M. Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Crit. Rev. Oncol. Hematol.* **51**, 55–67 (2004).
157. Butt, A. J., McNeil, C. M., Musgrove, E. A. & Sutherland, R. L. Downstream targets of growth factor and oestrogen signalling and endocrine resistance: the potential roles of c-Myc, cyclin D1 and cyclin E. *Endocr. Relat. Cancer* **12**, S47–S59 (2005).
158. Musgrove, E. A. *et al.* Identification of functional networks of estrogen- and c-Myc-responsive genes and their relationship to response to tamoxifen therapy in breast cancer. *PLoS ONE* **3**, e2987 (2008).
159. Nehra, R. *et al.* BCL2 and CASP8 regulation by NF-κB differentially affect mitochondrial function and cell fate in antiestrogen-sensitive and -resistant breast cancer cells. *FASEB J.* **24**, 2040–2055 (2010).
160. Hurtado, A. *et al.* Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. *Nature* **456**, U663–U693 (2008).
This reference indicates the crucial role of co-regulatory proteins in determining ER-mediated transcriptional responses.
161. Ali, S. & Coombes, R. C. Endocrine-responsive breast cancer and strategies for combating resistance. *Nature Rev. Cancer* **2**, 101–112 (2002).
162. Osborne, C. K. *et al.* Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J. Natl Cancer Inst.* **95**, 353–361 (2003).
163. Ring, A. & Dowsett, M. Mechanisms of tamoxifen resistance. *Endocr. Relat. Cancer* **11**, 643–658 (2004).
164. Ishii, Y. *et al.* Bortezomib enhances the efficacy of fulvestrant by amplifying the aggregation of the estrogen receptor, which leads to a pro-apoptotic unfolded protein response. *Clin. Cancer Res.* **17**, 2292 (2011).
165. Scriven, P. *et al.* Activation and clinical significance of the unfolded protein response in breast cancer. *Br. J. Cancer* **101**, 1692–1698 (2009).
166. Honma, N. *et al.* Clinical importance of estrogen receptor-β evaluation in breast cancer patients treated with adjuvant tamoxifen therapy. *J. Clin. Oncol.* **26**, 3727–3734 (2008).
167. Novelli, F. *et al.* A divergent role for estrogen receptor-β in node-positive and node-negative breast cancer classified according to molecular subtypes: an observational prospective study. *Breast Cancer Res.* **10**, R74 (2008).
References 106, 166 and 167 represent three recent studies which, by analysing large number of samples using well-validated antibodies, provide clinical evidence for the prognostic and predictive role of ERβ in breast cancer.
168. Horvath, G., Leser, G., Hahlin, M. & Henriksson, M. Exon deletions and variants of human estrogen receptor mRNA in endometrial hyperplasia and adenocarcinoma. *Int. J. Gynecol. Cancer* **10**, 128–136 (2000).
169. Kawai, H. *et al.* Estrogen receptor α and β are prognostic factors in non-small cell lung cancer. *Clin. Cancer Res.* **11**, 5084–5089 (2005).
170. Nose, N. *et al.* Association between estrogen receptor-β expression and epidermal growth factor receptor mutation in the postoperative prognosis of adenocarcinoma of the lung. *J. Clin. Oncol.* **27**, 411–417 (2009).
171. Nose, N., Uramoto, H., Iwata, T., Hanagiri, T. & Yasumoto, K. Expression of estrogen receptor β predicts a clinical response and longer progression-free survival after treatment with EGFR-TKI for adenocarcinoma of the lung. *Lung Cancer* **71**, 350–355 (2010).
172. Raso, M. G. *et al.* Immunohistochemical expression of estrogen and progesterone receptors identifies a subset of NSCLCs and correlates with EGFR mutation. *Clin. Cancer Res.* **15**, 5359–5368 (2009).
173. Schwartz, A. G. *et al.* Nuclear estrogen receptor β in lung cancer: expression and survival differences by sex. *Clin. Cancer Res.* **11**, 7280–7287 (2005).
174. Skov, B. G., Fischer, B. M. & Pappot, H. Oestrogen receptor β over expression in males with non-small cell lung cancer is associated with better survival. *Lung Cancer* **59**, 88–94 (2008).
175. Wu, C. T., Chang, Y. L., Shih, J. Y. & Lee, Y. C. The significance of estrogen receptor β in 301 surgically treated non-small cell lung cancers. *J. Thorac. Cardiovasc. Surg.* **130**, 979–986 (2005).
176. Alonso, L. *et al.* Gonadotropin and steroid receptors as prognostic factors in advanced ovarian cancer: a retrospective study. *Clin. Transl. Oncol.* **11**, 748–752 (2009).
177. Darb-Esfahani, S. *et al.* Estrogen receptor 1 mRNA is a prognostic factor in ovarian carcinoma: determination by kinetic PCR in formalin-fixed paraffin-embedded tissue. *Endocr. Relat. Cancer* **16**, 1229–1239 (2009).
178. Faggad, A. *et al.* Prognostic significance of Dicer expression in ovarian cancer-link to global microRNA changes and oestrogen receptor expression. *J. Pathol.* **220**, 382–391 (2010).
179. Zamagni, C. *et al.* Oestrogen receptor 1 mRNA is a prognostic factor in ovarian cancer patients treated with neo-adjuvant chemotherapy: determination by array and kinetic PCR in fresh tissue biopsies. *Endocr. Relat. Cancer* **16**, 1241–1249 (2009).
180. Burges, A. *et al.* Prognostic significance of estrogen receptor α and β expression in human serous carcinomas of the ovary. *Arch. Gynecol. Obstet.* **281**, 511–517 (2010).
181. Shabani, N. *et al.* Prognostic significance of oestrogen receptor α (ERα) and β (ERβ), progesterone receptor A (PR-A) and B (PR-B) in endometrial carcinomas. *Eur. J. Cancer* **43**, 2434–2444 (2007).
182. Suthipintawong, C., Wejaranayang, C. & Vipupinyo, C. Prognostic significance of ER, PR, Ki67, c-erbB-2, and p53 in endometrial carcinoma. *J. Med. Assoc. Thai* **91**, 1779–1784 (2008).
183. Barone, M. *et al.* Dietary-induced ERβ upregulation counteracts intestinal neoplasia development in intact male ApcMin/+ mice. *Carcinogenesis* **31**, 269–274 (2010).
184. Giroux, V., Lemay, F., Bernatchez, G., Robitaille, Y. & Carrier, J. C. Estrogen receptor β deficiency enhances small intestinal tumorigenesis in ApcMin/+ mice. *Int. J. Cancer* **123**, 303–311 (2008).
185. Martineti, V. *et al.* ERβ is a potent inhibitor of cell proliferation in the HCT8 human colon cancer cell line through regulation of cell cycle components. *Endocr. Relat. Cancer* **12**, 455–469 (2005).
186. Wilkins, H. R., Doucet, K., Duke, V., Morra, A. & Johnson, N. Estrogen prevents sustained COLO-205 human colon cancer cell growth by inducing apoptosis, decreasing c-myb protein, and decreasing transcription of the anti-apoptotic protein bcl-2. *Tumour Biol.* **31**, 16–22 (2010).
187. Bardin, A. *et al.* Involvement of estrogen receptor β in ovarian carcinogenesis. *Cancer Res.* **64**, 5861–5869 (2004).
188. Fan, X. *et al.* Gonadotropin-positive pituitary tumors accompanied by ovarian tumors in aging female ERβ-/- mice. *Proc. Natl Acad. Sci. USA* **107**, 6453–6458 (2010).

Acknowledgments
Research in the authors' laboratory is supported by the Welch Foundation and the Texas Emergency Technology Fund, under Agreement No 300-9-1958.

Competing interests statement
The authors declare competing financial interests. See Web version for details.

DATABASES
National Cancer Institute Drug Dictionary:
http://www.cancer.gov/drugdictionary
fulvestrant | tamoxifen | toremifene

FURTHER INFORMATION
Jan-Åke Gustafsson's homepage: http://cnrcs.uh.edu/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF