The different roles of ER subtypes in cancer biology and therapy

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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.

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. 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. 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.

Many lines of evidence suggest a relationship between the perturbation of oestrogen signalling and cancer initiation, progression and response to treatment. 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. 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. 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. 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. However, the exact mechanisms through which these factors regulate the levels and 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, methyleases, 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

1. 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 antiinflammatory and anti-invasive properties in cancer cells.

2. 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.

3. 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.

4. 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 ERβcc) isoform correlates with worse survival and metastatic phenotype.

5. 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. As members of the nuclear receptor superfamily, both ER subtypes have a six region structure and contain defined functional domains that have considerable homology. 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 remodelers (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.

**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). 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. 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.

**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. 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 nitric 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. Oestrogen can also induce the transient methylation of ERs by protein arginine N-methyltransferase 1 (PRMT1). This methylation event, which is frequent in breast cancer, results...
Although there are formed from alternative splicing of the last coding exon (shown by the striped bars). Variants are expressed in malignant tissues and influence cancer biology. ER D region contains several functional domains, including the hinge domain, part of proteins, as well as for receptor dimerization and nuclear translocation. Finally, the function. This region is also responsible for the binding to co-regulatory and chaperone regulatory elements of oestrogen-responsive genes. The carboxy-terminal regions E and oestrogen response elements (EREs) in the proximal promoter region or at distal regulatory elements. 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).

**Different transcriptional responses.** Although there are general similarities in the mechanism of ERα and ERβ action, ERs elicit different transcriptional responses. Microarray analysis of endogenous genes in mouse tissues and breast cancer cells revealed that ERs regulate common and different genes. 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 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.

**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. The intracellular concentration of ERs results from a dynamic balance between ER synthesis and ER degradation. This also indicates changes in the localization of ERα and ERβ, which display mitogenic effects by inducing cell cycle progression and reduced expression of nuclear receptor co-repressor (SMRT; also known as NCOR2) has been correlated with tamoxifen resistance in breast cancer.

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 and 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. 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.

**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. 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).
REVIEW

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 (ERs 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.

‘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.

with several proteins, including ubiquitin ligases and ubiquitin-binding proteins. Modulations in the levels of endogenous ligands, alterations in the tumour micro-environment (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 degradation14. 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 ERα5,6 (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 cancer7,8,9. 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 gland10,11. Similar effects of ERα in the breast have been observed in mice with CRE-mediated deletion of Esr1 in the epithelium of the mammary gland12. 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 delayed13–15. Similarly, the incidence of oestrogen- and DMBA-induced mouse mammary tumours was reduced by the loss of ERα, suggesting that ERα can influence mammary carcinogenesis16–20. ERα has additionally been implicated in prostate tumorigenesis21,22. 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 pathology23. 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, mice24–26. In addition, the ERα-selective antagonist toremifene decreased the incidence of high-grade prostatic intraepithelial neoplasia (PIN) and prostate cancer in transgenic adenocarcinoma
of the mouse prostate (TRAMP) mice. 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. 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.

**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 ERβ-2 knockout mice. These mice develop age-related cystic breast disease and hyperplasia of the prostatic epithelium.

### Table 1 | ER subtypes in cancer biology

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Role of ERs</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Breast</td>
<td>Wild-type ERα stimulates cell proliferation by inducing MYC and cyclin D1 expression</td>
<td>39,74, 84–88, 98, 106, 166, 167</td>
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<td></td>
<td>ERα-36 mediates E2- and tamoxifen-stimulated cell proliferation by activating MAPK–ERK signaling</td>
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<td>Lack of ERα delays the onset of WNT1- and ERBB2-induced mouse mammary tumours</td>
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<td>Wild-type ERα correlates with better response to tamoxifen treatment. ERα-36 is associated with tamoxifen resistance</td>
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<td></td>
<td>ERβ1 induces cell cycle arrest by downregulating MYC, cyclin E and by inducing p21 and p27 expression</td>
<td>10,16, 45, 112</td>
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<td></td>
<td>ERα expression is required for the proliferation and multi-layering of the prosthetic epithelial cells. ERα mediates oestrogen-induced inflammatory response in the mouse prostate</td>
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<td>Prostate</td>
<td>ERα expression is inversely correlated with histological grade and pathological stage</td>
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<td></td>
<td>ERβ1 impedes EMT in prostate cancer cells by upregulating E-cadherin, inhibits proliferation and induces apoptosis; ERβ1 decreases in highly aggressive Gleason grades</td>
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<td>ERβ2 and ERβ5 enhance the invasiveness of prostate cancer cells. Both nuclear ERβ2 and cytoplasmic ERβ5 are associated with a more metastatic phenotype</td>
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<td>Colon</td>
<td>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 ApcΔmin mice</td>
<td>12, 40, 183–186</td>
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<td></td>
<td>Low ERβ1 expression was associated with poor differentiation</td>
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<td></td>
<td>ERβ2 is associated with lymph-node metastasis</td>
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<tr>
<td>Lung</td>
<td>ERα expression is associated with a poor prognosis among patients with NSCLC and correlates with EGFR mutations</td>
<td>15, 169–174</td>
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<td></td>
<td>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</td>
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<td>Ovarian</td>
<td>ERα and ERβ have opposite actions on the transcription of cyclin D1, and ERβ1 can inhibit cell growth and induce apoptosis</td>
<td>120, 134, 135, 179, 180, 187, 188</td>
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<td></td>
<td>ERβ2 induces EMT by downregulating E-cadherin in ovarian cancer cells</td>
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<td></td>
<td>Increased ERα mRNA and protein levels are associated with a better outcome in patients with ovarian cancer</td>
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<td>Reduced ERα levels correlates with the occurrence of lymph-node metastasis</td>
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<td>Endometrial</td>
<td>Wild-type ERα expression increases cell proliferation</td>
<td>9, 41, 95, 181</td>
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<td></td>
<td>ERα-36 mediates tamoxifen-stimulated cell proliferation through MAPK–ERK and PI3K–AKT pathways</td>
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<td></td>
<td>ERα-46 mediates the E2-mediated activation of PKC in endometrial cancer cells</td>
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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.
that were developed by a different group. 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. 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. 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.

**Isoforms of ERα and ERβ**

Each ER subtype exists as several isoforms that are derived from alternative splicing and promoter usage. 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 mRNAs splice variants in various cancer cell lines and samples from breast, endometrial and ovarian cancer, with wild-type ERα being the predominant variant. 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. (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. 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. 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. The antiproliferative effects of wild-type ERβ and its variant isof orm 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).© 2011 Macmillan Publishers Limited. All rights reserved

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-18a 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.
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 downregulating its transcriptional repressors SNAI1 and SLUG (also known as SNAI2). 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. 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. 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. 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 tumors 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. 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. 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. To date, sustained exposure to exogenous and endogenous oestrogens is considered a well-established cause of breast cancer. Epidemiological, clinical and animal studies additionally implicate oestrogens in the aetiology of prostate, ovarian, lung and endometrial cancer. 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 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. 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. 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. 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.

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. Conversely, a gradual reduction of ERβ1 has been observed from normal to pre-invasive lesions and invasive carcinomas. 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. Increased expression of ERβ2 was found in DCIS compared with normal epithelium, and higher ERβ5 mRNA was detected in cancer compared with normal tissue. Contradictory data were produced regarding the expression of ERα in primary and metastatic prostate cancers, 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. 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
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, 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. 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. Finally, the loss of ERβ1 and ERβ2 but not of ERβ5 was found in different stages of colorectal carcinomas.

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, 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. 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. 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. Similarly, phosphorylation of ERβ at S105 was associated with better survival, even in tamoxifen-resistant cases. 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. 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. Repression of cyclin D1 and MYC expression, reduced activity of the transcription factors SP1 and NF-xB and downregulation of the NF-xB target gene BCL2 are among the events that account for the increased cell death that is observed in tamoxifen-treated ERα-positive breast cancers. 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. The role of ERβ in breast cancer 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. With the exception of one study, which suggested that wild-type ERβ predicts a high risk of relapse in a lymph node-negative group and is related to a more aggressive phenotype, 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. This is consistent with the increased tamoxifen sensitivity of ERα-positive breast cancer cells following the expression of wild-type ERβ. Nuclear ERβ2 was negatively associated with metastasis and vascular invasion, and cytoplasmic ERβ2 was associated with a worse outcome, although nuclear
ERβ5 was associated with better survival. 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. 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. 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 cytoplasmatic ERβ1 and worse clinical course. Increased ESR1 mRNA and protein expression pointed to a favourable outcome in patients with ovarian cancer and showed a significant association with tumour grading, whereas ERβ levels correlated with the occurrence of lymph node metastasis. 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.

On the basis of these observations, it can be postulated that ERαs, ERβs 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 ERαs 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 ERαs 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.

REVIEWS


This reference describes distinct mechanisms of estrogen-mediated gene regulation.


35. References 68–85 illustrate the role of miRNAs in the regulation of the expression of ER subtypes in cancer cells and tissues.


41. References 68–85 demonstrate how changes in ER protein stability alter cancer response to therapy.


This article demonstrates the tumor suppressive properties of ERα and describes one of the mechanisms through which ERα regulates tumour growth.


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230. Critchley, H. O. et al. Wild-type estrogen receptor (ER1) and the splice variant (ERαx2) both are expressed within the human endometrium throughout the normal menstrual cycle. J. Clin. Endocrinol. Metab. 87, 5265–5272 (2003).


REVIEWS


References 167–155 provide clinical evidence that the phosphorylation status of ER subtypes can be used in prognosis of breast cancer.


This reference indicates the crucial role of co-regulatory proteins in determining ER-mediated transcriptional responses.


References 106, 166 and 157 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.


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Competing interests statement

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

DATABASES


FURTHER INFORMATION

Jan-Åke Gustafsson’s homepage: http://ccresc.uh.edu/

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