Abstract

Estrogens are implicated in a diverse range of functions varying from reproduction, circulation, skeletal health to neuroprotection. Estrogens are also being increasingly recognized for their pathological contribution to cancers of various organs. This has spurred several investigations on estrogen-initiated signaling mechanisms in various cell types in physiological and pathological conditions. Estrogens exert their biological actions through a class of conventional nuclear receptors known as estrogen receptors (ERs), majorly of two subtypes – ERα and ERβ, both encoded by different genes, and each has multiple isoforms. It is reported that different ER subtypes and their specific isoforms have overlapping and nonoverlapping functions. Moreover, ER functions are highly cell-context specific. Thus, it is difficult to propose a unified scheme for estrogen signaling. Another layer of complexity is added by diverse subcellular localization, i.e., nucleus, plasma membrane, and cytosol, of ERs in estrogen-responsive tissues. Size as well as site dictates the sequence of cellular events triggered by estrogen signaling. This review compiles the existing information on different subtypes, different isoforms, and different sites of subcellular localization of ERs.

Keywords: Estrogen, estrogen receptors, isoforms, signaling, subtype

Introduction

The human body is a complex enclosed system with multiple functions occurring simultaneously. Peptide, protein, and steroid hormones play critical roles in ensuring synchrony between different functions and physiological homeostasis. Like peptide and protein hormones, steroids act on a wide range of cells, tissues, and organs. Chemically steroids are lipophilic in nature and influence physiological processes such as growth, metabolism, sexual development, and differentiation through specific receptors.[1]

It was previously believed that steroids which are small molecules and products of metabolic pathways have not undergone molecular evolution. However, now, there exists evidence to demonstrate that metabolic pathways have also been modified during evolutionary descent and steroids have also evolved. Markov et al. demonstrated that in vertebrates, steroids are synthesized through side-chain cleavage and estrogen synthesis invariably involves aromatization, a more ancient estrogenic pathway.[2]

Estrogen synthesis initiates with side-chain cleavage of cholesterol to pregnenolone by the CYP11a enzyme and ends with aromatization of testosterone by CYP19A after a series of biochemical steps. However, pregnenolone synthesis from cholesterol is believed to be a vertebrate-specific phenomenon since CYP11a expression is restricted to vertebrates. Intriguingly, aromatization has been detected in mollusks and cnidarians which lack the CYP19A aromatase. To explain this, it was proposed that not only androgens but other steroids also undergo aromatization via a paralog CYP19 and form paraesters. Paraesters comprise of cholesterol side chain and its synthesis seems to be independent of the presence of progesterone, testosterone, or any side-chain intermediate. Thus, paraesters appear to be the most primitive estrogen-like compound.[2]

Interestingly, mollusks which show aromatization despite lack of CYP19 display specific estrogenic actions, as revealed with aromatization of testosterone by CYP19A after a series of biochemical steps. However, pregnenolone synthesis from cholesterol is believed to be a vertebrate-specific phenomenon since CYP11a expression is restricted to vertebrates. Intriguingly, aromatization has been detected in mollusks and cnidarians which lack the CYP19A aromatase. To explain this, it was proposed that not only androgens but other steroids also undergo aromatization via a paralog CYP19 and form paraesters. Paraesters comprise of cholesterol side chain and its synthesis seems to be independent of the presence of progesterone, testosterone, or any side-chain intermediate. Thus, paraesters appear to be the most primitive estrogen-like compound.[2]

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How to cite this article: Desouza J, Gadkar S, Jagtap D, Sachdeva G. Size, site, and signaling: Three attributes of estrogen receptors. Biomed Res J 2019;6:37-48.

Received: 28 September, 2019, Revised: 15 October, 2019,
Accepted: 06 November, 2019, Published: 22 November, 2019.
by estrogen binding to specific proteins or receptor-like sequences in their genome. The ancient steroid receptor discovered in mollusks was found to have 22 out of 26 sites in the ligand-binding pocket similar to that of estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Sequence analysis of steroid receptors through the assessment of their evolutionary relationship has predicted ER to belong to the one clade and 3-ketosteroid receptor to the other clade. The 3-ketosteroid receptor further evolved to form progesterone receptor (PR), corticoid receptor, and androgen receptor. This diversification of steroid receptors resulted due to genome duplication and ligand exploitation.\(^\text{[9]}\) Thus, the origin of estrogens and ERs date back to more than 500 million years ago in terms of evolutionary scale and this dyad of steroid-steroid receptor continues to be of extreme relevance in human physiology and pathology.

Estrogens, classically known as the female hormone, is synthesized mainly in the ovary in females and testes in males. Estrogens regulate multiple physiological functions by acting on gonads, brain, heart, liver, and bone. Estrogenic functions are not restricted to pubertal and adult lives but extend to embryonic development or life before birth.\(^\text{[4]}\) Imbalance in the levels or functions of estrogens is associated with etiology of various cancers, metabolic diseases, and neurological diseases.\(^\text{[5]}\) Estrogen-dependent cancers include cancers of reproductive organs, i.e., endometrium, breast, and ovary, wherein estrogens influence initiation, progression, and metastasis.\(^\text{[5,6]}\) Estrogens mediate their function through ER signaling, which activates expression of various oncogenes and cell cycle genes.\(^\text{[7]}\)

Conventional ERs act as transcription factors and there exist two major subtypes, i.e., ERα and ERβ. ERα is believed to execute oncogenic functions, whereas ERβ exerts protective functions.\(^\text{[8]}\) However, both subtypes are known to have different isoforms and these isoforms exert regulatory functions by homo- or hetero-dimerization between themselves. It is very likely that the presence of more than one subtype and more than one isoform of each subtype allows precision in the regulation of estrogen signaling, intrinsically linked with cell proliferation. Nonetheless, co-expression of more than one subtype and more than one isoform of each subtype renders the mechanism of estrogen signaling more intricate. This also makes therapeutic targeting of estrogen signaling extremely difficult.

**ESTROGEN RECEPTOR ARCHITECTURE AND SIGNALING**

Estrogens mediate their pleiotropic effects via two major classical receptors – ERα and ERβ. These receptors belong to type I class of nuclear receptors and on ligand binding act as transcription factors. Both the major classical ERs – ERα and ERβ – are evolutionarily conserved, coded by genes located on the chromosome 6 and 14, respectively, in humans. ERs have molecular structure of six domains encoded by eight exons namely A–F which differ in their functions. A/B domain codes for activation function 1 (AF1) which regulates transcription in a ligand-independent cell-specific manner.\(^\text{[9]}\) The C domain encodes for DNA-binding domain (DBD).

Within this domain exists a pair of C4-type zinc fingers which mediate binding of ERs to response elements called estrogen response elements (EREs) and coactivators. The first zinc finger within the DNA-binding monomer identifies the sequence 5’-AGGTCAG-3’ of the ERE duplex within the major groove while the second zinc finger is involved in the homodimerization of DBD.\(^\text{[10]}\)\(^\text{[11,12]}\) Domain D comprises the hinge region that interconnects DBD and LBD and codes for the nuclear localization signal.\(^\text{[13]}\) Domain E/F encodes for the ligand-binding domain (LBD), the heart of the structure, and AF2, which brings about transcription of downstream genes in ligand-dependent fashion.\(^\text{[14]}\) The E domain structurally consists of 11 α-helices arranged in an antiparallel sandwich fold such that helices 4, 5, 6, 8, and 9 are surrounded on the one side by helices 1 and 3 while on the other side with helices 7, 10, and 11.\(^\text{[14]}\) The conformation of the E domain does not differ much when compared in its ligand bound and unbound state, except for the structural orientation of the alpha 12 helix. Originally, the alpha 12 is oriented outward to make way for the ligand which on ligand-binding repositions itself to form a hydrophobic pocket exposing a coregulatory protein-binding surface.\(^\text{[9,10]}\)\(^\text{[14,17]}\) Sequence alignment of both the receptors demonstrates a homology of 97% in their DBD, 60% in LBD, and 18% in the AF-2 domain.\(^\text{[18]}\)

For ERα, majorly, three isoforms [Figure 2] have been reported. The ERα66, the full-length receptor having molecular weight (M.W.) of 66 kDa, has been extensively studied. The other isoforms include ERα46 (M.W. 46 kDa)
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which lacks the first 173 amino acids mapping the AF1 domain and the ERα36 (M.W. 36 kDa) transcribed through an alternative transcription start site lacks a part of AF2 in addition to AF1 domain. ERα36 also has unique C terminal amino acid. Functionally, ERα46 represses ERα66 when co-expressed. ERα36, on the other hand, inhibits signaling by ERα66 and ERβ, independent of its ligand status. To carry out transcriptional activities, ERα forms homodimers as well as heterodimers with ERβ. In tissues with higher expression of ERα compared to ERβ, there occurs preferential homodimerization of ERα and heterodimerization of α/β than ERβ homodimerization.

ERβ, on the other hand, has five major isoforms resulting from exon exclusion or skipping [Figure 3]. These isoforms are truncated in the F and E domains. ERβ1 (59 kDa), the full length and most studied ERβ, is fully functional and can induce transactivation on binding to the estrogen. ERβ2 (56 kDa) isoform has altered structure, with altered ligand-binding ability and also impaired ligand-induced transcriptional activity. ERβ2 has been found to negatively regulate ERα. Expression of ERβ3 is reported to be restricted to the testis. ERβ4 (54 kDa) and ERβ5 (53 kDa) transcripts contain a part of exon 7 and different exon 8 sequence that creates 3D structures without 12α helix and F domain. This leads to formation of an open conformation of the LBD pocket affecting the ligand-binding affinity. Due to structural alterations, the ERβ2, ERβ4, and ERβ5 isoforms do not have transcriptional activity in their homodimer forms. However, these isoforms can heterodimerize with ERβ1. These heterodimers have higher transactivation activity than ERβ1 homodimers.

ERs predominantly localize in the nucleus which on activation by estradiol (E2) or estrogen-like molecule initiate genomic signaling pathway. ERs undergo a conformational change on binding with E2 wherein they homo-or hetero-dimerize as ERα/ERβ and then translocate to the nucleus. In the nucleus, dimers bind to EREs, a 15 bp palindromic inverted repeats in or near the promoter regions, introns, or 3′untranslated regions of target genes, and recruit coregulators to initiate transcription. ERs can also regulate transcription of their target genes lacking ERE through their interaction with DNA-bound transcription factors such as SP1 or AP1. ERs modulate transcriptional machinery by interacting with coactivators which in turn recruit chromatin remodeling complexes. This leads to modification of nucleosomal structures that enable recruitment of general transcription factors and other components of transcription machinery. ER functions are also regulated by posttranslational modifications. Phosphorylation can modify the ability of ERα to bind to the

**Figure 2:** Diagrammatic representation of estrogen receptor alpha isoforms (Adapted from Jia et al., 2015)

**Figure 3:** Human estrogen receptor beta isoforms: Estrogen receptor beta 1 is the full length receptor while the other isoforms (β2-5) are generated through alternative splicing. These isoforms differ in the number of amino acids at the C-terminal (Adapted from Lipovka et al., 2016)
DNA and thus affect transcription. In addition to genomic signaling, estrogenic effects are mediated through nongenomic signaling pathway by a pool of ERs present on the plasma membrane and other extranuclear sites, such as endoplasmic reticulum and mitochondria. The translocation of ERs on to the membrane occurs through posttranslational modifications, such as acylation, palmitoylation, and myristylation brought out by lipids,[28,29] Activation of these membrane receptors by estrogen leads to interaction with factors such as Src, Ras, and Raf, resulting in rapid downstream effects which involve mobilization of intracellular calcium, stimulation of adenylate cyclase activity, cAMP production, as well as MAPK signaling pathway[30] [Figure 4].

**Physiological Roles of Estrogen Receptor Alpha and Estrogen Receptor Beta**

Full-length ERα and ERβ are reported to be expressed in various tissues [Table 1]. ERα appears to be of predominance in uterus, pituitary glands, skeletal muscle, adipose tissue, and bone. In contrast, ERβ is known as the more predominant receptor in ovary, prostate, lung, central nervous system, and cardiovascular system.[31] Even within the same tissue, distribution of ER subtypes varies. For example, in the ovary, ERα is more abundant in theca cells and ERβ in the granulosa cells, while in the prostate, ERα is predominantly expressed in the stroma and ERβ in both cell compartments, i.e., stromal and epithelial cells.[32] Differential expression of ERα and ERβ probably allows estrogenic effects to be tightly regulated in a spatial manner.

Knockout studies in mice have demonstrated the role of ERα in the development of reproductive organs, such as uterus, mammary gland, and ovarian follicle maturation in females. In males, ERα is expressed in the prostate during embryonic development, and loss of ERα affects testes development and sperm production. Thus, ERα knockout (ERαKO) mice are infertile. In nonreproductive organs such as bone, ERα is positively associated with bone mineral density despite being expressed in low levels.[33] ERα also regulates glucose metabolism by modulating genes involved in gluconeogenesis, such as Pck-1 and G6Pase.[34] In endothelial cells, E2 activation of ERα is found to mediate remodeling of mesenteric arteries and in reendothelization.[35,36] ERα is also expressed in neuronal cells as well as nonneuronal cells.[37,39] ERα in the hypothalamus is found to be expressed in the arcuate nucleus, the ventromedial nucleus of the hypothalamus, pro-opiomelanocortin, and SF1 neurons wherein it regulates energy homeostasis as they are the key centers for controlling food intake.[40,41] This was further confirmed through hypothalamus-specific ERαKO mouse model, which tends to develop metabolic syndrome.[42] ERα also regulates body weight through the neurons of the medial amygdala known as single-minded–1.[43] ERβ in reproductive organs such as ovary has a role in preovulatory follicle maturation and ovulation.[44] In males, ERβ is expressed in prostate and testis but is not indispensable for fertility.[33,45] In the brain, ERβ is expressed in the hypothalamus in paraventricular nucleus and regulates the hypothalamus–pituitary axis function in response to stress.[46]

Figure 4: Estrogen signaling pathway: 1: Estrogen receptor on ligand activation initiates transcription by binding to DNA. 2: Stimulating protein 1 is involved in indirect association of estrogen receptors to the DNA leading to transcription of genes. 3: Estrogen on binding to estrogen receptors on the membrane activates different downstream molecules through interaction with adaptor proteins such as Shc and sos leading to modulation in cell growth and differentiation. 4: Membrane-initiated signal transduction pathways can influence genomic signaling through phosphorylation of estrogen receptors and coactivators.
Furthermore, in the hippocampus, ERβ is found to mediate an antistress and antianxiety effect on stimulation with E2 and along with ERα exerts neuroprotective effects through upregulation of Beclin in the hippocampal neuron.[73]

Among immune cells, ERs are expressed in myeloid as well as lymphoid cells. On comparing the relative levels of the two ER subtypes, CD4 T-cells and macrophages showed higher levels of ERα while monocytes and B-cells predominantly express ERβ.[74] Human primary monocytes have also been shown to express ERα46.[75] Activation of these receptors through estrogen stimulation regulates differentiation, maturation, and activation of the immune cells.[76] Stimulation of T-cells with increasing doses of estrogen has shown to influence the T-helper 1 response via ERα activation.[76] Estrogen stimulation of CD4+CD25+ cells that are found to express ERα transform into regulatory T-cells.[77] ERα is reported to be involved in differentiation of dendritic cells from bone marrow cells.[78] ERα and ERβ also influence the release of pro-inflammatory cytokines such as tumor necrosis factor α and IL1β by mononuclear cells via reduction in the expression of CD16.[79]

**Pathological Role of Estrogen Receptor Alpha and Estrogen Receptor Beta**

**Breast cancer**

Breast cancer therapeutics is mainly directed to curb the estrogen action mediated through ERs. Based on the expression of ERs, breast tumors are classified as ERα-positive and ERα-negative tumors. The receptor status in breast tumors acts as a determinant for prognosis and choosing appropriate therapeutic options.[80]

In normal mammary glands and tumors, ERα66 is localized in the nuclei of epithelial cells that line the breast ducts and lobules.[45,81] It is found to promote carcinogenesis by inhibiting apoptosis and regulating survival and multidrug resistance gene.[62] ERα function is also modulated through phosphorylation of its serine residues 118 and 167. Clinical studies have associated these phosphorylation at two potential sites with better prognosis and high survival rates.[83] *In vitro* attenuation of phosphorylation of ERα at these two sites in MCF7 breast cancer cell lines leads to increase in proliferation and metastasis potential and also alters expression of ERα.[84] In addition to ERα66, ERα46 is expressed in breast tumors and a higher expression is observed in lower grades and smaller size tumors. ERα46 is known to abrogate the proliferative effects of ERα66. These two isoforms elicit different signaling through differential recruitment of cofactors and coactivators.[85] ERα36 is expressed in ER-positive and ER-negative cancer cell lines and localized in the cytoplasm and plasma membrane of breast tumors.[86,87] The mechanism behind this translocation of ERα36 to the membrane is not known but has been assumed to involve potential posttranslational modifications of amino acids 25–30, 76–81, and 171–176 in the A/B domain by myristoylation. ERα36 membrane localization was found positively associated with patient survival in triple-negative tumors.[88]

### Table 1: Localization of estrogen receptor α and estrogen receptor β in different tissues under nonpathological conditions

| Serial number | Site of localization                  | Subtypes of isoform | Type of signaling reported | References |
|---------------|--------------------------------------|---------------------|---------------------------|------------|
| 1 A           | Pituitary gland, lung, liver, prostate, epididymis, testis, smooth muscle cells of coronary, artery, osteoblasts, liver, adipose tissue, cardiomyocytes, ovary (theca interna, germinal epithelium, interstitial glands) gastrointestinal tract, endometrium, testis (Leydig cells), brain (hypothalamus, hippocampus, amygdala), immune cells (T-cells, macrophages) | ERα66               | Genomic                   | [37,40,47-59] |
| 1 B           | Myometrium                           | ERαA7               | Genomic                   | [60]       |
| 1 C           | Adrenal gland, pituitary gland, gastrointestinal tract (colon, rectum), kidney, urinary bladder, skeletal muscle, prostate, ovary (granulosa cells, germinal epithelium, breast, thymus, spleen, pancreas, endometrium, testis (Leydig cells, Sertoli cells), immune cells (dendritic cells, B-cells, monocytes), brain (hippocampus, hypothalamus), endothelial cells, vascular smooth muscles | ERβ1                | Genomic                   | [48,49,51-56,58,61-65] |
| 2 A           | Pituitary (lactotropes and somatotropes) | ERα66               | Nongenomic                | [66]       |
| 2 B           | Endothelial cells, osteoblasts, immune cells (monocytes) | ERα46               | Nongenomic                | [47,67]    |
| 2 C           | Osteoblasts and osteocytes           | ERα36               | Nongenomic                | [68]       |
| 2 D           | Duodenal epithelial cells            | ERα and ERβ1        | -                         | [69]       |
| 3 A           | Uterine smooth muscle                | ERα36               | Nongenomic signaling (proposed) | [70]       |
| 3 B           | Hippocampal neurons, cardiomyocytes, heart | ERβ1                | -                         | [71]       |

ER: Estrogen receptor
breast cancer tumors.\[88\] However, another study reported association of ERα36 expression with poor prognosis in breast cancer patients.\[89\] In \textit{vitro} experiments have shown ERα36 has procancerous effects in the presence of E2. This occurs via protein kinase C-induced ERK1/2 (MAPK) activation and PLD-LPAP13K signaling, leading to proliferation, metastasis, and antiapoptosis. Activation of signaling cascade leads to increased expression of downstream genes such as SNAIL 1 and RankL and reduced expression of E-cadherin.\[89,90\] In addition to this, ERα36-mediated nongenomic signaling occurs through EGFR/Src/shc complex. This implies that during estrogen signaling, ERα36 can dynamically change its interacting partners and involves crosstalk among multiple pathways.\[86,91\]

ERα-30 is a novel splice variant of ERα identified in breast cancer tissue [Figure 2]. This variant lacks amino acids from the hinge region, the LBD and AF2 with an additional 10 unique amino acids at the C terminal end. This implies different transcriptional activities as compared to ERα-66. Further, expression levels of ERα-30 at transcript level correlate with absence of ERα66 and progesterone receptor in breast tumors. In MDA-MB-231 cell line, ERα30 overexpression led to higher migration, invasion, and proliferation. Although further investigations need to be carried out, the results demonstrate that ERα-30 could possibly act as a promising biomarker for metastasis and recurrence.\[92\]

Several other splice variants of ERα lacking certain regions of the exons such as A3, A4, A6, and A7 have been reported in certain breast cancer cell lines. However, their functional relevance is yet to be known.\[93,94\] Wild-type ERα expression was found to be lower, while ERα variants with mutation in DBD were higher at in breast tumors compared to normal breast tissue at transcript level.\[95\] Variants in breast tumors mostly included those containing 1, 2, or 3 exon deletions.\[95\] Thus, there exist multiple variants of ERα66, suggesting that the gene is under selective pressure during tumorigenesis. It will be of interest to investigate whether deletion, mutation and alternate splicing allow ER to exercise its function, irrespective of the presence of ligand, coactivators, and cofactors. On the other hand, it is also possible that such modifications relieve the ERs from some inhibitory influences.

ERβ is localized in the nuclei of ductal and luminal epithelial cells as well as stroma and myoepithelial cells of the breast tissue.\[95\] At the transcript level, ERβ receptor isoforms 1–5 are expressed in major breast cancer cell lines as well as primary breast cancer tissue.\[96\] At the protein level, till date, only ERβ1, ERβ2, and ERβ5 have been detected. These isoforms were found to be localized in the nuclei as well as cytoplasmic compartments.\[97\] The compartment-specific distribution pattern (cytosolic or nuclear) of the isoforms has been reported to correlate with overall survival (OS), disease-free survival (DFS), and treatment outcome. However, there is a lack of consensus with regard to isoform-specific outcome. Shaaban \textit{et al.} reported no correlation of ERβ1 expression with OS while nuclear expression of ERβ2 was found correlated with better OS, DFS, and treatment response.\[97\] On the other hand, cytoplasmic ERβ2 (cERβ2) expression was found to correlate with poor outcomes and is often expressed in high-grade tumors and metastasis. In patients undergoing tamoxifen treatment, ERβ2 is associated with poor OS and DFS.\[98\] A positive correlation of ERβ1 with DFS and OS is reported in triple-negative breast cancer.\[99\] ERβ1 is proposed as a potential prognostic marker in patients treated with chemotherapy.\[100\]

ERβ seems to have a dual role in breast cancer cells. In MCF7 cells, ERβ induces antiproliferative effects and increases apoptosis via abrogation of ERα repression of p53. This activation of ERα repressed gene is under epigenetic regulation, wherein ERβ increases H3K4me3 mark, an indicator of gene activation.\[101\] However, in MDA-MB-231 cells, knockdown of ERβ leads to a gain in epithelial-like phenotype and decreased migration. The migratory potential of MDA-MB231 is regulated via interactions between ER/EGFR/IGF-IR pathways.\[102\]

ERα and ERβ have also been localized in the mitochondria in MCF-7 breast cancer cells, wherein they are reported to bind to the D loop of mitochondrial DNA that contains sequences which are homologous to EREs.\[103\] However, a direct or indirect role of ERs in regulating mitochondrial DNA transcription or transcription of nuclear-encoded mitochondrial respiratory complex genes remains to be deciphered.

**Ovarian cancer**

Clinical studies have not only correlated the expression levels of ERs with pathological grade but have also shown that differential compartmental expression of ERs can predict survival and aggressiveness of ovarian cancer.

ERα expression is found to increase from normal to benign and is highest in malignant cases while its loss is observed in clear cell carcinoma and mucinous carcinomas of the ovary.\[104,105\] Further, Chan \textit{et al.} have shown that the localization of ERα in different cellular compartments can predict disease progression, OS, and DFS.\[106\] Nuclear ERα (nERα) expression was found to be associated with more aggressive type of cancer while cytoplasmic ERα (cERα) may have a positive correlation with OS and DFS.\[105\] ERβ1, ERβ2, and ERβ5 isoforms were found to be differentially expressed in nuclear and cytoplasmic compartments of all different grades of ovarian cancer.\[105,107\] In addition to these conventional isoforms, two other splice variants of ERβ generated by exon skipping were found to be expressed at the transcript level in normal ovary and ovarian cancer tissue. These two variants were first identified in MDA-MD-231 breast cancer cell lines. The proteins coded by these isoforms are expected to have different functions in comparison to the conventional forms since they lack AF1 and have deletions in the LBD and DBD.\[108,109\]

nERβ1 expression is highest at transcript as well as protein level in normal ovarian tissue while it progressively decreases in high-grade, poorly differentiated, and metastatic foci.
Expression of ERβ2 and β5 has also been assessed in ovarian cancer tissues. nERβ5 is expressed in high levels in advanced carcinoma stages. Overexpression of ERβ5 in OVCAR420 and ES-2 cell lines led to increased migration and invasion which was found to be mediated through FAK/Src activation. ERβ2 is expressed in both the cellular compartments, and high-positive cytoplasmic reactivity has been linked with aggressive disease, chemoresistance, and decreased survival. The cause of this compartmental shift has been attributed to estrogen and could be demonstrated in ovarian cancer xenograft mice. These mice when ovarectomized express high levels of cERβ2 than their intact counterparts. This has led to speculations that the drop in the levels of estrogen during menopause can lead to the development of a more aggressive ovarian cancer phenotype through ERβ2 regulation.

**Prostate cancer**

Clinical studies undertaken to understand the role of estrogens in prostate carcinogenesis have largely focused on the expression of ERs in different stages of disease progression such as prostatic epithelial neoplasia (PIN), high-grade PIN, and metastatic cancer. Although expression levels of ERs vary with stage and grade of disease, ERs are found to be localized in both nuclear and cytoplasmic compartments of prostate cells. When compared across different grades, ERα expression increases with increased severity of the disease. ERαΔ5, a splice variant, has been reported in benign prostate hyperplasia (BPH) and tissue associated with tumor (TA). Its expression was found to be six times more in TA compared to BPH. ERαΔ5 variant lacks the LBD and has only 5% of the constitutive activity of the full-length receptor. Another isoform ERα36 was also found to be present in the androgen-independent PC3 cells at transcript and protein levels. Activation of this receptor initiates rapid responses and involves ERK-1 phosphorylation. In normal prostate tissues, wild-type ERβ (ERβ1) and its isoforms are reported to be expressed in basal as well as luminal epithelial cells. However, in prostate cancer (PCa) tissues, expression of ERβ1 is reduced whereas that of its isoform ERβ2 and β5 increases. This implies that the expression of ERβ1 and its isoform is regulated by independent mechanisms. Zhang et al. demonstrated that the ERβ transcription is driven by two promoters 0K and 0N-upstream to 5’ most-untranslated exons, preceding exon 1. Both promoters were found to be active in normal and cancerous prostate tissues. Promoter 0N with higher transcriptional activity has AP-2 site, whereas promoter 0K has CpG-rich region with lower transcriptional activity than that triggered by promoter 0N. Zhang et al. demonstrated preferential use of promoter 0N for ERβ1 transcription. Lee et al. extended that these observations to demonstrate that ERβ1 and ERβ2 are transcribed from both promoters 0N and 0K whereas ERβ5 transcription is predominantly initiated from promoter 0K. It was demonstrated that the transcripts transcribed from promoter 0K contain different combinations of untranslated exons 0Xs (0X1–8) which do not contribute to protein expression. Instead, the presence of 0Xs is known to inhibit protein translation through ribosome stalling, premature release of ribosome, and increased mRNA instability. It is likely that cis-regulating elements (modified due to epigenetic modification) and trans-acting factors eventually dictate the type of promoter usage. Nonetheless, these observations suggest that different isoforms of ERβ are generated not only by alternative splicing but also through posttranscriptional modifications.

Although most of the reports support the oncogenic role of ERα, contrasting inference has been reported in aggressive PCa cell line ARCaP which expresses low levels of ERα and androgen receptor. ERα ectopically expressed in ARCaP human prostate cells have shown to mediate antiproliferation via growth arrest in G1 cell cycle phase in E2-independent fashion. ERβ1 acts as a tumor suppressor and multiple signaling cascades have been reported that help ERβ1 achieve its function. ERβ1-mediated apoptosis is induced by FOXO3a through downstream targeting of p53 upregulated modulator of apoptosis and activation of intrinsic apoptotic pathway by caspase 9. ERβ1 is also found to have profound effects on epithelial–mesenchymal transition (EMT). PC3 cells treated with TGFβ or subjected to hypoxic microenvironment were found to be more invasive and migratory, and this was accompanied by a loss in ERβ1. ERβ1 loss leads to decrease or loss of E-cadherin promoter activity. The mechanism involved is ERβ1-mediated regulation of EMT through repression of VEGF (Vascular Endothelial Growth Factor)-A. VEGF-A also regulates localization of Snail-1 from the cytoplasm to the nucleus, a phenomenon observed in hypoxia. The repression of VEGF-A is mediated by ERβ1 which involves two different mechanisms. The first involves destabilization of HIF-1 (Hypoxia Inducible Factor) which regulates VEGF-A. The second is through repression of VEGF transcription via ERE. Ectopic expression of ERβ2 or 5 in PC3 cells led to an increase in their invasiveness whereas in vitro migration was found to be increased only in ERβ5-overexpressing PC3 cells. Transcriptome analysis of PC3 cells transfected with a construct encoding ERβ1 showed a downregulation of cell-cycle regulating genes – C-myc and upregulation of Rb either at transcript or protein level. ERβ1 also negatively regulates RUNX2 and its downstream target SLUG. RUNX2 is an osseous master transcription factor that enhances metastatic ability of PC3 cells. Thus, ERβ1 may have a potential inhibitory role in PCa metastasis to the bone. ERβ2-expressing cells show higher expression of genes involved in proliferation, migration, and invasive behavior. In addition to this, ERβ2 was found to
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positively regulate MGAT5 which activates matriptase, known to influence the invasive potential of PCa cells.\textsuperscript{[130,131]} These results strongly support conclusions drawn by Leung et al., 2010,\textsuperscript{[121]} supporting an antiproliferative role for ERβ1 and poor prognosis associated with higher expression of ERβ2.

Our group has been pursuing studies on detection and functional characterization of ERs on the plasma membrane of PCa cells. We demonstrated the presence of membrane-bound ERα and ERβ in normal as well as tumorigenic prostate epithelial cells.\textsuperscript{[132]} Surface plasmon resonance experiments revealed binding of antibodies to conventional ERs to the plasma membrane extracts of LNCaP cells [Figure 5a and c] and this binding was displaced by addition of E2 [Figure 5b and d]. Interestingly, ERα showed remarkable co-localization with caveolin-1, an integral membrane protein and an important component of caveolae [Figure 6]. Thus, there exists a possibility of ERα protein transported from or to the plasma membrane via caveolae-derived vesicles in PCa cells. Further, estrogen binding to the plasma membrane of androgen-dependent as well as androgen-independent PCa cells was observed. Overall, these observations suggest the presence of both ERα and ERβ on the plasma membrane of PCa cells. However, it remains to be investigated whether plasma membrane localization of ERs is a constitutive process or a regulated event.

**Conclusion**

ERs, a class of nuclear receptors, play crucial roles in various physiological functions. Their aberrant expression is not limited to cancer and extends to metabolic and aging-related diseases. It has emerged that while wild-type full-length transcripts of ERα and ERβ have contrasting functions, proteins encoded by the truncated forms of ERα and ERβ have functions, often opposing to the functions executed by the wild-type isoforms. Further, in clinical settings, the expression of ER isoforms in various tumor types is reported to be associated with survival and therapy outcomes. Presence of various isoforms explains variable effect of therapy seen in

![Figure 5: SPR analysis showing the real-time binding of cell surface protein fraction (CSP) of LNCaP cells containing estrogen receptors to immobilized polyclonal ERα and ERβ antibodies. Sensogram showing the binding of CSP (500ng-8μg), flowed over a surface of sensor chip immobilized with antibody specific against estrogen receptor alpha and estrogen receptor beta respectively (a and c). Sensograms showing the binding of CSP (1μg) to estrogen receptor alpha and estrogen receptor beta antibodies immobilized over a sensor chip in absence and presence of different concentrations of estradiol (1μM and 10μM) (b and d).](image1)

![Figure 6: Colocalization of caveolin-1 with estrogen receptor alpha in LNCaP cells. (a) caveolin-1 localization, (b) estrogen receptor alpha localization, (c) DAPI staining, (d) Merged picture showing colocalization of caveolin-1 with estrogen receptor alpha](image2)
some patients. Detailed investigations need to be undertaken to elucidate the mechanisms by which different isoforms of ERs and ERβ are generated and transported to different subcellular compartments. In addition, it will be worthwhile to decode the functions of each isoform in different compartments. Furthermore, extensive efforts are required to test whether it is feasible to develop ER isoform-specific agonists and antagonists.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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