CMLS, Cell. Mol. Life Sci. 61 (2004) 281–291
1420-682X/04/030281-11
DOI 10.1007/s00018-003-3222-5

CMLS Cellular and Molecular Life Sciences

Review

Novel estrogen receptor coregulators and signaling molecules in human diseases

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Received 12 June 2003; received after revision 21 July 2003; accepted 29 July 2003

Abstract. The steroid hormone estrogen and signaling from its receptors are increasingly recognized as critical mediators of a variety of organ-specific biological processes. Recent advances in the identification and functional characterization of novel estrogen receptor interacting proteins clearly show the complexity of hormonal signaling regulation, but may also contribute to our understanding of the roles of estrogen signaling in normal physiology and the pathobiology of human disease.

Key words. Estrogen receptor; coregulators; breast cancer; signaling pathways; chromatin remodeling.

Introduction

The steroid hormone 17β-estradiol (estrogen) plays an important role in controlling the expression of genes involved in a wide variety of biological processes, including development, differentiation, and homeostasis in a wide variety of tissues, including bone, brain, breast, uterus, testis and cardiovascular systems [1–3]. In addition to the normal functions of estrogen, existing evidence also suggest a role of estrogen signaling in human breast cancer progression. There are numerous organ- and cell-type-specific responses to estrogen, suggesting a complexity of signaling pathways activated by this hormone. The biological effects of estrogen are mediated by its binding to the structurally and functionally distinct estrogen receptors (ERs) ERα and ERβ [4]. Along with the two ERs, a number of coregulator proteins have been identified which influence the function of ERs. These new findings are beginning to unravel the complexity of estrogen-ER signaling. In this review we will summarize recent developments in tissue-specific responses to estrogen and the involvement of ER coregulators in estrogen signaling in both physiological and pathophysiological conditions.

ER (α and β)

ERs are hormone-responsive transcription factors, comprised of several conserved domains: a N-terminal ligand independent activation function (AF1) domain, a DNA-binding domain and a C-terminal ligand-binding region which contains an activation function (AF2) domain [5] (Fig. 1). The ligand-dependent activation function AF2 of the ER is located in the ligand binding domain, while the N-terminal activation function domain (AF1) functions both in a ligand-dependent (through allosteric interaction of ligands with aspartic acid 351 [6, 7]) and ligand-independent manner. Both AF1 and AF2 exhibit cell type and promoter context specificity [5, 8]. The activity of AF2 is regulated by binding of ligands, while the activity of AF1 is regulated by phosphorylation via activation of signaling kinases [9]. Upon binding of E2 to ERα, the ligand-activated ER binds to the estrogen-response-enhancer (ERE) element in the target genes [10]. Ligand-depen-

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dent conformational changes in the ligand binding domain (LBD) are thought to recruit coactivators to DNA-bound ER [11]. However, the outcome of estrogen-ER signaling is also determined by the regulatory proteins present in a given cell/tissue. The transcription functions of ER are shown to be influenced by several coactivators, including SRC1, GRIP1, AIB1, CBP, p300, PGC1, E6AP, PCAF and SNF2 [11]. In addition to activation through the ligand-dependent AF2 domain of ERα, some coactivators such as SRC1 and GRIP1 also interact with the ligand-independent AF1 domain of ERα [12, 13]. It is generally accepted that some of the diverse functions of estrogens depend on differential recruitment of coregulators to the E2-ER complex.

ERα and ERβ exhibit similar domain structure. The highest similarity between the receptors was observed in the DNA binding domain (95%) and AF2 domain (58%) and therefore was expected to bind identical DNA response elements. The least similarity between the ER receptors was localized in the N-terminal AF1 domain (16%). Evidence suggests that ERα AF1 has stronger transcriptional activity compared with ERβ [4]. In addition to structural differences, a number of isoforms of both ERα and ERβ have been reported, adding to the complexity of ER signaling [3].

ERα and ERβ are differentially expressed in various tissues and may play a role in differential responses to estrogen ligands observed in different tissues. ERα is predominantly expressed in the mammary gland and uterus, while ERβ is highly expressed in the ovary, hypothalamus, thymus, skeletal, male reproductive system and the cardiovascular system [14]. Some tissues are known to express both ERα and ERβ, such as thyroid, epididymus, bone and brain [2, 4].

Mechanisms of estrogen action

Emerging evidence suggests that there are at least four different means by which estrogen and ER can regulate biological processes [15].

(1) Classical pathway. This is the most studied ER signaling pathway and is dependent upon ligand binding. In this pathway, ligand-activated ER in the nucleus binds to the ERE in the target genes and stimulates gene transcription [16].

(2) Non-classical pathway. In this pathway, ligand-activated ER complexes with DNA-bound transcription factors such as promoter-specific cellular transcription factor (SP)1 and activating protein-1 (AP-1) [17, 18] and activates transcription from a number of genes that do not contain classical ERE elements. As examples, ER can activate transcription of collagenase and insulin-like growth factor (IGF)1 via AP1 and SP1 sites, respectively. Both the classical and nonclassical ER signaling pathways typically function through transcriptional control and involve activation of regulatory (early) genes (i.e. c-*myc*, c-*fos*, c-*jun*) and also a number of late-response genes [19]. These pathways can thus elicit both quick responses as well as the slower responses of longer-term protein expression.

(3) Nongenomic pathway. This pathway is mainly implicated in the generation of rapid estrogen-mediated cytoplasmic signaling, and it has been observed in a number of tissues. Nongenomic functions are mediated by membrane-associated ER in conjunction with activation of intracellular signal transduction pathways involving kinases such as Src, mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) [20, 21]. Some evidence also suggests that the existence of a novel G-protein-coupled membrane-associated ER that is distinct from the classical ERs and is involved in the rapid nongenomic effects of estrogen [22].

(4) Ligand-independent pathway. Growth factor signaling or activation of a number of signaling pathways leads to activation of signaling kinases which can phosphorylate and thus activate ER in the absence of ligand. This activated pathway is suspected of contributing significantly to the hormonal independence observed in some tumors, as discussed in greater detail below [2, 9, 20, 23].

Both ligand-dependent and -independent ER actions have been implicated in a number of membrane-associated cytoplasmic signaling pathways independent of direct ER transcriptional regulation. These nongenomic effects include the rapid regulation of membrane-based ion fluxes, activation of second-messenger signaling cascades and activation of receptor tyrosine kinases. Through these multiple mechanisms, ER signaling can also indirectly affect transcription at non-ERE-containing promoters via the activation of transcription modulators downstream of these other activated signaling pathways.

ER coregulators

The transcriptional activity of ERs is regulated not only by hormones but also by several regulatory proteins called coactivators and corepressors [24, 25]. Coactivators usually do not bind to DNA but are recruited to the target gene promoters through protein-protein interactions with the ERs and function as linker molecules between DNA binding proteins and DNA and protein-modifying enzymes, which facilitate local structural alterations [26]. Evidence suggests that multiprotein complexes containing coactivators, ER and other transcriptional regulators assemble in response to estrogen binding to ER and subsequently activate transcription [2, 9, 20, 24]. Structural analysis of coactivators has identified a motif consisting of five amino acids – LXXLL, where X is any amino acid – that is sufficient to mediate

coactivator binding to the ligand-bound nuclear hormone receptors (NRs) [27]. In addition to the LXXLL motif, amino acids surrounding the core LXXLL motif also play a role in binding specificity [28]. LXXLL motifs are present in several ER coactivators. Similarly, corepressors utilize a variation of sequence I/L-x-x-I/V-I (CoRNR box) to interact with NRs [29, 30]. A multistep model has been proposed for transcriptional activation by NRs. According to this model, binding of NRs to the enhancer region directs modification of local chromatin structure into a transcriptionally permissive state through the activity of peripheral bound enzymes. These structural changes are followed by the recruitment of general transcription factors to form a preinitiation complex [24]. Histone acetylation and deacetylation have been suggested as mechanisms by which NRs modify chromatin structure [25].

Histone modification and ER coactivators

The p160/SRC family is a well-studied group of NR coactivators. Its members are steroid receptor coactivator (SRC1), glucocorticoid receptor interacting protein (GRIP1 also known as SRC2, TIF2) and P/CIP (also known as SRC3, AIB1, TRAM1 and RAC3) [31]. The P160/SRC family members share a common structural frame that includes an N-terminal basic helix-loop-helix domain, a PAS (PER, ARNT and SIM) homology domain, a C-terminal transcriptional activation domain and a central region containing three NR-interacting LXXLL motifs [24]. SRC1 and SRC3 are to shown to have histone acetyltransferase activity, which is necessary for the formation of an open chromatin structure [32]. In addition, SRC coactivators also interact with general coactivators such as the CREB binding protein (CBP) and p300 [33], and thus may help ERs to recruit more HAT enzymes upon ligand binding to the vicinity of target sites. In addition to HAT activity, coactivator-mediated methylation of proteins in the transcription machinery may also contribute to transcriptional regulation by NRs [34]. For example, coactivator-associated arginine methyltransferase 1 (CARM1) binds to the carboxyl-terminal region of p160/SRC coactivators, methylates histone H3 and enhances transcriptional activation by nuclear receptors [35].

Chromatin-remodeling functions and ER coactivators

Accumulating evidence suggests that ER recruits multiprotein complexes that regulate higher-order chromatin domains into which nucleosomes are organized [16, 36]. SW1/SNF, a complex that possesses ATPase activity, alters nucleosomal structure and is shown to be involved in the transcriptional regulation of NR [37]. Factors involved in the structural remodeling of chromatin have also been shown to mediate hormone-dependent transcriptional activation by ERs. One example is the coactivator brahma-related gene 1 (BRG-1), which is recruited by ERs in response to estrogen. Estrogen-mediated stimulation of ER-BRG-1 association may couple BRG-1 to specific regions of chromatin, including estrogen-responsive promoters, and facilitate the activity of other recruited factors that alter the acetylation state of chromatin [38].

Emerging novel ER coactivators

In addition to the above well-studied family of coactivators, emerging evidence suggests that the ER coactivator repertoire is much larger than originally anticipated. The TRAP/DRIP complex, a large multiprotein complex, interacts with ERs and is thought to connect ERs with the basal transcription machinery and thus influence ER-mediated transcription [39, 40]. Another ER-binding protein, the steroid receptor activator (SRA), is unique among coactivators in that it functions as an RNA transcript rather than as a protein [41]. In addition, the E6-associated protein E6-AP, a ubiquitin ligase, has been identified as a coactivator of ER, and expression of E6-AP was shown to be deregulated in breast cancers [42, 43].

A previously uncharacterized molecule, the MTA1-interacting coactivator (MICoA), was recently identified in a screen of proteins which bind the corepressor metastasis-associated protein 1 (MTA1) and was shown to be an ER coactivator. MICoA cooperates with other ER coactivators, stimulates ER-transactivation functions, and associates with the endogenous ER bound to promoter sequences of target genes [44].

Another novel ER coactivator, the aryl hydrocarbon receptor nuclear translocator (ARNT), is an obligatory heterodimerization partner for the aryl hydrocarbon receptor (AhR) and hypoxia-inducible factor 1α, and also functions as a potent coactivator of ERα- and ERβ-dependent transcription [45]. Indeed, the AhR/ARNT heterodimer was recently shown to bind directly to ERα and ERβ and recruit unliganded ER and the coactivator p300 directly to estrogen-responsive gene promoters, while the functions of liganded ERs were attenuated [46]. These data provide a mechanistic explanation for the documented but poorly understood link between exposure to dioxin-type environmental contaminants and adverse estrogen-related actions.

Proline-, glutamic acid-, leucine-rich protein 1 (PELP1), a novel ER coregulator, contains 10 NR interacting LXXLL motifs for NR interaction and plays an important role in estrogen-mediated transcriptional functions [47].

Modulator of nongenomic action of ER (MNAR) is another novel coregulator of ER which is important in the nongenomic actions of ER via activation of Src/MAPK pathways [21]. Subsequent studies showed that PELP1 and MNAR are identical proteins and suggested that PELP1/MNAR is a novel coactivator of ER that regulates both the genomic and nongenomic functions of ER [48]. Overexpression of PELP1 sensitizes cells to estrogen-mediated cell cycle progression, PELP1 interaction with the retinoblastoma protein, and upregulates cyclin D1 expression [48]. These data suggest that PELP1 also links cell cycle regulators to ER.

The p21-activated kinase 1 (Pak1), a signaling kinase with diverse functions that was originally linked with cytoskeletal changes and cell motility, also interacts with ER and functions as a coactivator of ER transcriptional activity. Recent kinase-active T423E Pak1 transgenic mouse studies have established a role of Pak1 signaling in the development of hyperplasia in mammary epithelium, with an underlying mechanism which involves direct phosphorylation of ER at Ser305 and subsequent transactivation independent of estrogen [49]. Another Pak family member, Pak6, also interacts with ERα, and Pak6-ERα binding was enhanced by 4-hydroxytamoxifen [50]. The ability of Pak members to interact with steroid hormonal receptors suggests that Pak kinases may play an important role in the cross-talk between steroid hormone receptors and Rho GTPase-mediated signal transduction pathways, which could influence the hormonal independence which is seen in tumorigenesis [50].

ER corepressors

Corepressors have been shown to preferentially associate with antagonist-occupied NRs [51]. Among the corepressors, the nuclear receptor corepressor (NCoR) and silencing mediator for retinoic and thyroid receptor (SMRT) have been widely characterized and have been implicated in the transcriptional silencing of ER [24, 52]. A yeast two-hybrid screen using SMRT as a bait led to the isolation of a novel human protein termed SHARP (SMRT/HDAC1 associated repressor protein). SHARP is a potent transcriptional repressor of NRs. In addition, SHARP also binds SRA and suppresses SRA-potentiated steroid receptor transcription activity. Interestingly, the expression of SHARP itself is steroid inducible, suggesting a simple feedback mechanism for attenuation of hormonal response [53].

Some of the known corepressors appear to be selective for the ER pathway. One such protein, repressor of estrogen receptor activity (REA), is an ER-selective coregulator, identified using a yeast two-hybrid screen. REA potentiates the inhibitory activities of both dominant-negative ERs and anti-estrogen-liganded ER, and competitively inhibits SRC-mediated ERα coactivation functions [54]. The orphan receptor DAX-1 interacts with the estrogen receptors ERα and ERβ, and functions as an inhibitor of ER activation in mammalian cells. The mechanism of DAX-1-mediated inhibition involves occupation of the ligand-induced coactivator-binding surface and subsequent recruitment of corepressors [55].

A novel DEAD box RNA helicase (97 kDa, DP97) interacts with the hormone binding/activation function-2 region of ERs, as well as several other nuclear receptors. The knockdown of endogenous cellular DP97 by antisense DP97 or RNA interference (siRNA for DP97) results in significant enhancement of the expression of estrogen-ER-stimulated genes and attenuation of the repression of genes inhibited by the estrogen-ER. These findings suggest that RNA helicases can associate with nuclear receptors and function as coregulators to modulate receptor transcriptional activity [56]. Recent data also implicates histone H1 as a potent repressor of ERα-mediated transcription by selectively inhibiting ER-mediated transcriptional initiation [57].

The MTA1 gene product, a target of growth factor signal transudation, was recently identified as a corepressor of ER and might play a role in the growth factor-mediated hormonal independence frequently seen in cancer cells via recruitment of histone deacetylases (HDACs) to ERs [58]. Subsequent studies identified an isoform of MTA1 that is overexpressed in ER-negative tumors (MTA1s). MTA1s was found to inhibit ER transactivation functions by sequestering ERs in the cytoplasm and to promote tumorigenesis [21, 59].

A yeast two-hybrid screen with the MTA1 C-terminal domain as bait identified MAT1 (ménage à trois 1) as an MTA1-binding protein. MAT1 is an assembly/targeting factor for cyclin-dependent kinase-activating kinase (CAK), which functionally interacts with the general transcriptional factor TFIIH, a known inducer of ER transactivation. MTA1 inhibited CAK stimulation of ER transactivation, which was partially relieved by HDAC inhibitor trichostatin A [60]. These data suggest that MTA1 might inhibit the CAK-induced transactivation function of ER by recruiting HDAC to ER transcriptional complexes and altering chromatin structure. Furthermore, MTA1 overexpression inhibited the ability of CAK complex to phosphorylate ER, suggesting that the transactivation functions of ER might be influenced by the regulatory interactions between CAK and MTA1 in breast cancer cells [21, 60].

A few bifunctional coregulators that can act as both coactivators and corepressors of NRs have also been reported. Examples include a mouse zinc-finger protein, a regulator of apoptosis and cell cycle arrest [61], an NR-binding set domain-containing protein [62], a coactivator-independent activator of AF2 function (CIA) [63], and RIP 140 [64]. Nuclear matrix protein/scaffold attachment fac-

tor HET/SAF-B is also an ER-interacting protein which associates with ER independent of estrogen, but its binding is increased by the antiestrogen tamoxifen [65]. HET/SAF-B functions as a corepressor of ER and also enhances tamoxifen antagonism of estrogen-induced ER-mediated transactivation, and may be important for the antagonist effect of tamoxifen.

ER coregulators in cancer

Antiestrogens and selective estrogen receptor modulators (SERMs) have been shown to be effective in controlling the progression of hormone-dependent human cancers, including breast and endometrium. However, many patients that initially respond to SERMs eventually develop resistance to hormonal treatment [66, 67]. The causes of resistance to hormonal therapy remain elusive. Previous studies suggest that mechanisms for resistance and progression of breast cancer from hormone dependence to hormone independence are multifactoral and include levels of ERα and ERβ, expression of variant or mutant ER, ligand-independent activation of ER, adaptation of tumors to lower concentrations of estrogen and pharmacological alterations [68, 69]. In breast cancer, ERα rather than ERβ correlated with most of the prognostic factors [70]. The function of ERβ in breast pathobiology and tamoxifen therapy is unclear, partly because most studies have focused on its messenger RNA (mRNA) rather than the protein [71]. Some correlation was reported with high ERβ expression and tamoxifen sensitivity [72]. Differential SERM effects on corepressor binding were proposed as a mechanism to explain the differential effects of SERMs on ERα [30].

Coregulators of ER are thought to play a role in tumor progression. Relative amounts of expression of coactivators and corepressors are shown to play a role in modulating differential response from ER by agents such as tamoxifen [73]. In breast cancer, amplification of the steroid receptor coactivator gene AIB1 is correlated with ER and PR positivity [74–76]. Another study found a significant correlation between ERα expression and levels of the coregulators TIF2, AIB1, PCAF and NCoR in tissue samples [77]. A recent study found NCoR expression as a promising independent predictor of tamoxifen resistance in patients with ERα-positive breast tumors [21, 78]. Emerging evidence suggests that cross-talk of growth factor and other oncogene signaling with E2-ER pathways may lead to hormonal independence or may lead to resistance to SERMs [9, 20, 21, 23]. Altered growth factor signaling seen in cancers may deregulate corepressors such as MTA1, which can then promote hormonal independence [5, 58]. Alternatively, deregulation of growth factor-mediated signaling pathways may lead to aberrant activation of kinases such as PAK1 and thus lead to activation of ER in the absence of ligand [49]. Even though few studies to date have examined the role of coactivators in cancer, existing data suggest the potential role of ER coregulators in cancer progression and point towards the potential use of coregulator expression patterns in conjunction with the expression levels of ERs as prognostic markers for human cancers.

The molecular configuration of the cell dictates the response to a particular estrogen or antiestrogen. A recent example of this principle comes from the study of tamoxifen resistance in breast cancer patients, where a subset of breast tumors overexpressing the receptor tyrosine kinase (RTK) HER2 also showed high levels of the ER coactivator SRC3 (AIB1) [79]. Coordinate overexpression of HER2 and SRC3 led to an estrogenic rather than anti-estrogen response in this subset of patients. Indeed, cell surface signaling can enhance SRC3 phosphorylation and ER activation [80]. Furthermore, estrogen causes a reduction in SRC3 mRNA, but antiestrogens increase SRC3 mRNA levels [81]. Along these lines, a recent study of endometrial cancer cells showed that levels of the related activating transcription factor SRC1 dictated gene activation in response to tamoxifen [82]. Thus an increase in coactivator concentration and activity, through increased receptor tyrosine kinase activity, may enhance signal transduction of an antiestrogen-ER complex. Complicating this scheme is the established enhancement by cytoplasmic ER activity of signaling via the RTKs IGF1R and EGFR, opening the possibility of cytoplasmic ER enhancement of HER2 signaling.

These data also raise the question of the role of corepressors of ER transcriptional activity in modulating the effects of estrogen and/or SERMs on ER transcriptional regulation. SERMs promote the interaction of ERα with repressor complexes as a mechanism of suppressing ER-dependent transcriptional activity [82]. Indeed, a recent report suggests that differential SERM repression of ERα AF1 transcriptional activity depends upon functional corepressor histone deacetylase activity in breast cancer cells, and that SERM-mediated inhibitory effects depend upon functional HDAC-containing repressor complexes [83]. This finding raises the additional possibility that corepressor protein levels and/or activity might be advantageously modulated by RTK and/or ER cytoplasmic signaling as a means of enhancing nuclear ER functions. A greater understanding of the interplay between RTK and ER signaling will enhance our ability to coordinate targeted ER therapies.

ER and the neuroprotective effects of estrogen

Estrogen has numerous direct effects on the nervous system, including the brain, including, influences in development, differentiation, maturation, function to protection from injury. Indeed, administration of estrogen be-

fore brain injury is directly neuroprotective (reviewed in [84]). This effect seems to be quite sweeping, as estrogen is protective for a range of insults, from neurotransmitter excitotoxicity to acute oxidative stress and ischemic events. Estrogen therapy has also been one of the most compelling possible preventive and therapeutic strategies for age-related neurodegenerative ailments such as Alzheimer's disease. Prospective and case-control studies showed a 50% lower risk of developing Alzheimer's disease in women who took estrogen [85– 88]. These multiple protective effects and possible mechanisms of estrogen action were the subject of a recent comprehensive review [84] and will not be reviewed here.

Despite preclinical work and observational studies which supported the hypothesis that hormone therapy should decrease the risk of dementia in women, recently published results from the Women's Health Initiative (WHI) hormone replacement trial actually show the opposite effect. The WHI randomized prospective trial investigated the effect of treatment with 0.625 mg of conjugated estrogen and 2.5 mg of medroxyprogesterone acetate on multiple endpoints, including central nervous system outcomes. The data presented in two reports [89, 90] suggest an increased risk for all types of dementia and a more rapid decline in cognitive function in the hormone treatment group as compared with placebo. These surprising results raise the possibility that progestin may protect women from the increased risk of endometrial cancer which occurs with estrogen treatment alone, but at the same time eliminate the cognitive benefits of estrogen alone [91]. Moreover, rationale design of novel SERMs with selected tissue-specific actions may circumvent some of the current issues surrounding the preventive strategies of hormone replacement therapy [92, 93].

ER in bone

Numerous lines of evidence indicate that estrogen is a key regulator of bone homeostasis in both sexes, with ERα as the dominant mediator. As this area has been extensively reviewed, we will just briefly discuss recent reports relevant to our evolving understanding of the role that immune cells and inflammatory cytokines play in regulatory balance between bone-forming osteoblasts and bone-resorbing osteoclasts. Postmenopausal women secrete high levels of interleukin 1 (IL1) and tumor necrosis factor α (TNFα), for which there are receptors on both precursor cells and mature osteoclasts. Initial studies documenting increased IL1 and TNFα after estrogen loss presumed monocytes to be the main source of these cytokines, but recent reports show that mesenchymal cells and activated T cells are also major sources [94, 95]. Furthermore, the cytokine IL7 is now implicated as a major regulator of bone mass. IL7 is unique in that it performs the dual functions of suppressing osteoblasts while stimulating osteoclast formation and activity. Recent studies established T cells as critical mediators of these IL7 effects [96] and showed that estrogen deficiency significantly increases bone marrow IL7 mRNA and protein while estrogen restoration reverses these changes [97]. Thus evidence is mounting for T cell function as a critical element in bone resorption following estrogen decline, and IL7 appears to be a major effector of these changes. Progress in this area may aid in the development of future targeted clinical interventions for the prevention of osteoporosis.

ER and the cardiovascular system

The onset of menopause and increased risk of negative cardiovascular events has long been recognized, but the underlying mechanism for these effects and the potential use of SERMs to counteract these detrimental effects are only recently emerging. A number of beneficial cardiovascular effects have been attributed to estrogen, including vasodilation, inhibition of response to blood vessel injury, limiting myocardial injury and cell death after infarction, and reducing cardiac hypertrophy [98]. In general, cardiovascular risk factors impair endothelium-de-

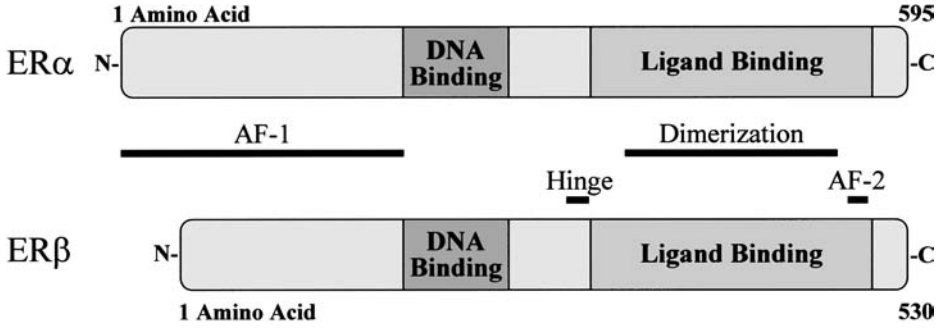

Figure 1. Structural comparison of ERα and ERβ. Both ERs possess nuclear receptor functional domains, including agonist-independent (AF-1) and agonist-dependent (AF-2) functional domains, a conserved DNA binding domain, a ligand binding domain and multiple protein interaction motifs, including a dimerization region.

pendent vasodilation and the ability of the vasculature to respond to insult, and thus predispose to vascular injury and subsequent negative outcomes.

A loss of circulating estrogen rapidly precipitates impaired endothelial function and decreased vascular response [99], but can be reversed by administration of estrogen. Much of the restored vasodilatory function is thought to be mediated through the regulation of endothelial nitric oxide synthase (eNOS) activity and release of the vasodilatory nitric oxide (NO) by both genomic and nongenomic ER mechanisms. Activated ER in the cytoplasm of endothelial cells rapidly interacts with mitogen-activated protein kinase (MAPK), which directly phosphorylates and activates eNOS, and also with PI3K, which initiates a signaling cascade whereby AKT/PKB (protein kinase B) can activate eNOS [100, 101]. In addition to these mechanisms of ER-mediated stimulation of eNOS activity, mounting evidence suggests that ER may release eNOS from the inactivating protein caveolin within caveolae at the cell membrane and thus free the enzyme for NO production [102].

Circulating estrogen may also trigger dynamic alterations in the expression of ERα and ERβ [103]. In isolated endothelial cells, short-term (2 h) estrogen treatment downregulates ERα and ERβ expression, while longer (> 6 h) exposure upregulates ERα expression while downregulating ERβ. These dynamic alterations may provide a feedback loop whereby estrogen fluctuations can modulate the responsiveness of the vasculature to circulating hormone levels. Coronary artery smooth muscle cell proliferation, which is also associated with vascular disease, is inhibited by estradiol metabolites, demonstrating another recently identified protective effect of estrogen upon the vasculature [104].

It is important to note that in the WHI trial (see above), the combination of estrogen plus progestin resulted in a twofold higher risk of stroke compared with placebo [105, 106]. As was noted in reports of cognitive function in WHI trial participants [89, 90], the unexpected negative outcome may implicate progestin in counteracting the beneficial cardiovascular effects of estrogen alone. It is also possible that the effects of the combination of estrogen plus progestin on brain vasculature and negative cognitive outcomes are causally linked [91, 107]. However, this possibility remains to be investigated. Despite these recent negative implications, the use of estrogen alone or in combination with novel SERMs [92, 93] may have protective cardiovascular effects.

With increased use of SERMs for protection against breast cancer risk and age-associated bone and neurodegenerative diseases has come a need to understand the impact on the cardiovascular system and potential benefits of SERM use. Tamoxifen appears to exert agonistic effects on vascular endothelial cells and improves vascular tone in postmenopausal women [108]. Raloxifene use has also been associated with cardiovascular benefit and is currently the subject of a multinational study [109], although not all studies of raloxifene vascular effects have shown a benefit [110]. Some of the positive effects of estrogen may come from its structure, as estrogen and other phenolic antioxidants including resveratol [111] have been shown to upregulate eNOS activity and to be car-

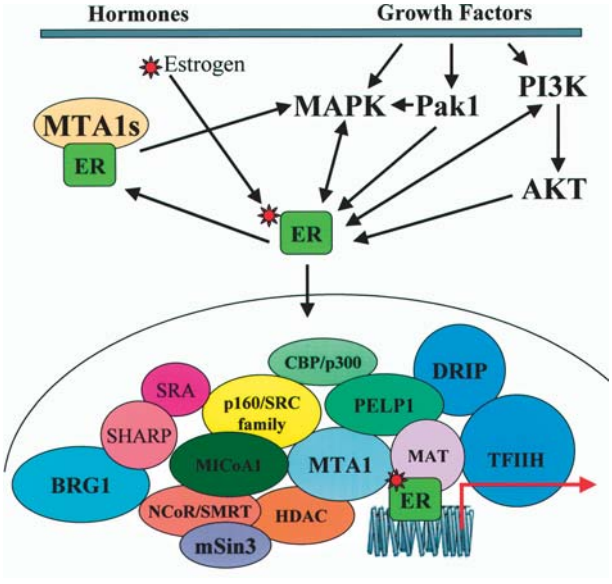

Figure 2. New ER cytoplasmic signaling cascades and ER interacting coregulators. Emerging evidence of the interaction between hormonal and growth factor signaling pathways is depicted. These multiple signaling pathways coordinately regulate the dynamic regulation of ER-mediated transcriptional regulation. A number of recently reported regulatory molecules and their interacting proteins are shown, along with elements of the constitutive transcriptional complex in blue. Proteins were placed in an arbitrary location relative to ER.

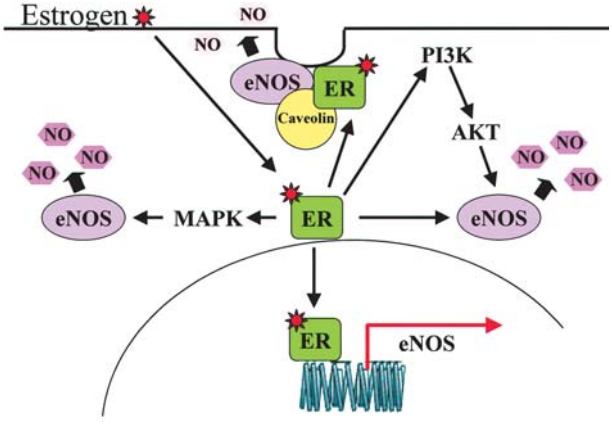

Figure 3. Genomic and nongenomic mechanisms of ER regulation of eNOS. NO produced by eNOS is a critical mediator of vasodilatory function. Emerging evidence indicates that there are multiple mechanisms by which circulating estrogen and the ER may regulate eNOS function and thus endothelial vasodilatory response.

dioprotective [112]. Although promising data exist in this beneficial application of estrogen and selective SERMs, much work remains to be done before these compounds could be recommended for routine cardioprotective use.

Conclusions

Recent progress in the study of ER interactions, regulation and function has expanded the complexity of estrogen signaling. Control of the hierarchy of possible ER interactions and functions remains largely unknown. However, investigations of the tissue-specific regulatory control of ERα and ERβ function in normal homeostasis and pathophysiology holds great promise for future modulation of ER signaling as a means of disease prevention and treatment.

Acknowledgements. Research in the authors' laboratory is supported by National Institutes of Health grants CA90970, CA98823 and CA84456 (R. K.).

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