Estrogen Receptor Function and Molecular Mechanisms

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Abstract: Estrogen receptors (ERs), ERα and ERβ, are ligand-dependent transcriptional factors that mediate various estrogen actions. The ERs belong to the superfamily of nuclear receptors and are composed of six modular domains, A to F. Estrogen binds to ERs via their E-F domains (ligand-binding domain, LBD) and ERs bind to DNA as dimers through their C domain (DNA-binding domain, DBD) at specific estrogen responsive elements (EREs) on the genome, leading to activate transcription of their target genes. Using genomic binding-site cloning technique, our group newly identified several primary estrogen-responsive genes including NR2D and Efp. NR2D is a subunit of N-methyl-D-aspartate receptor complex expressed in the brain and may function in sexual behavior. Efp has been revealed as a RING finger-type ubiquitin ligase that targets the proteolysis of a cell-cycle checkpoint 14-3-3α. Efp promotes proliferation of breast cancer through breakdown 14-3-3α. Analysis of each estrogen-responsive gene may elucidate the diverse physiological roles of estrogen functions.

Key words: Estrogen; Nuclear receptors; Breast cancer; Efp; Ubiquitin Ligase

Introduction

Estrogen is a sex steroid hormone that is essential for the manifestation of female secondary sexual characteristics and reproductive functions. It has diverse physiological effects on the central nervous system, immune system, and cardiovascular system as well as lipid and bone metabolism. The hormone plays a crucial role in the growth and development of women, while the postmenopausal decline of estrogen secretion is closely associated with the onset as well as treatment of various menopausal symptoms including hot flushes, osteoporosis, and dementia. Recent studies have shown that estrogen is important for men as well, since

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Mechanisms of Estrogen Receptor Function

The ERs are members of the nuclear receptor superfamily including receptors for steroids, retinoic acid, thyroid hormone, and vitamin D, functioning as ligand-dependent transcription factors.1–3) In the nucleus, estrogen binds to ERs via their C-terminal ligand-binding domain (LBD). The estrogen-bound ERs in turn bind to genomic EREs in dimerized forms via a central DNA-binding domain (DBD), and thus control the transcription of target genes to exert specific physiological actions (Fig. 1A).

Since 1996, a novel subtype of ER designated as ERβ has been identified in rodents and humans. Consequently, the classical ER has been renamed ERα to distinguish it from ERβ. A comparison between the amino acid sequence of the full-length human ERβ isolated by our group5 and ERα showed that the A/B domain of ERβ is shorter than that of ERα. The amino acid sequence of ERβ is closely related to that of ERα, as the C domain (DBD) of ERβ is 96% identical to that of ERα, while the E domain (LBD) of ERβ shows 53% similarity to that of ERα (Fig. 1B). ERβ has a similar affinity and specificity to 17β-estradiol compared with ERα and its transactivation through EREs is also enhanced in the presence of estrogen.

Fig. 1A  Mechanism of estrogen action
Estrogen-bound ERα and ERβ bind to estrogen-responsive elements (EREs) in forms of homodimers or heterodimers, leading to activate the transcription of target genes.

Structure and Function of the ER

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there are reports that estrogen action is involved in spermatogenesis and estrogen receptors (ERs) are expressed in the testis and prostate. It has been also revealed that estrogen is a requisite in male bone homeostasis based on the clinical data of the first male patient with ERα deficiency. Furthermore, estrogen is profoundly related to the etiology, diagnosis, treatment, and prognosis of hormone-dependent cancers such as breast cancer, endometrial cancer, and prostate cancer.

The various functions of estrogen are mediated through its cognate nuclear receptors, ERs. The estrogen-bound ERs form dimers by themselves and bind to specific estrogen response elements (EREs) on genomic DNA, leading to activate transcription of various target genes. Thus, ERs act as ligand-dependent transcription factors. The first ER was cloned in late 1980s and its structure and functions have been extensively studied. Recently, another subtype of ER (ERβ) has been identified in the prostate, and there have been efforts to reevaluate estrogen signaling.

This article focuses on the mechanism of estrogen action mediated through ERs, the comparisons between ERα and ERβ, and the physiological functions of estrogen-targeted genes.
of 17β-estradiol and reduced by tamoxifen. On the other hand, the ligand specificities of ERα and ERβ are not identical regarding various estrogen-like compounds. It has been shown, for example, phytoestrogen exerts functions on ERβ at a lower concentration compared with ERα.

Regarding tissue distribution of ERβ, it has been shown that the receptor is abundantly expressed in prostates and ovaries in rats. The ERβ expression has also been observed in bladder, lungs, uterus, testes, brain, and arteries. It is notable that ERβ expression is not so strong in organs that highly express ERα, such as uterus, hypothalamus, and pituitary gland, while ERβ is abundantly expressed in the prostate.

There is an overall similarity of physiological functions between ERβ and ERα, yet there might be functional differences between two ERs based on their structural variances. Multiple functional domains are usually involved in the transcription activation of nuclear receptors. The A/B and E domains are particularly important in the process and they are also known as two transcription activation domains AF-1 (activation function-1) and AF-2, respectively. In regard to these transcription activation domains, ERβ has a shorter AF-1 compared to ERα, and the amino acid identities of AF-1 and AF-2 are not so high between the two ERs. Thus, the functional analysis of AF-1 and AF-2 may be useful for understanding the differences of transactivation machinery and specific actions between ERα and ERβ.

**Estrogen Receptor Downstream Genes and Their Physiological Functions**

The estrogen-dependent transcription factor ER elicits diverse estrogen functions by modulating the transcription of various downstream genes that locate in the vicinity of EREs. The known estrogen-inducible genes can be classified into three groups. One is a group of primary estrogen-responsive genes that can be directly activated by ERs, the other two are groups of secondary and tertiary estrogen-responsive genes that are downstream of primary estrogen-responsive genes (Fig. 2).

Vitellogenin is well studied as a prototypic primary estrogen-responsive gene. There is a functional ERE that locates in the vicinity of vitellogenin gene, and it has been revealed that transcription of vitellogenin gene can be induced promptly by estrogen stimulation. It has been also shown that the estrogen-induced transcriptional activation of vitellogenin is not affected by protein synthesis inhibitors such as cyclohexamide. Prolactin, pS2, and c-fos are thought to be other examples of primary estrogen-responsive genes, yet apparent EREs have not been identified in the vicinity of those genes. In such cases, the estrogen-induced transactivations are mediated through alternative pathways such as activating protein-1 (AP-1). BRCA1 has been reported as a secondary or tertiary estrogen-responsive gene. Much attention has been given to this gene in association with estrogen and estrogen-dependent proliferation of its target tissues including mammary epithelial cells, breast cancers, and the endometrium.

We are searching for novel primary estrogen-
responsive genes using a method designated as “genomic binding-site (GBS) cloning”, which has been developed by us to identify EREs on the genome (Fig. 3). Among the estrogen-responsive genes that we have recently identified, NR2D is a subunit involved in the N-methyl-d-aspartate (NMDA) receptor complex, a receptor for excitatory neurotransmitter glutamate. Because the presence of ERα or ERβ has been shown in the nuclei of specific neurons, it is likely that the estrogen action on sexual behavior, memory, and emotion are mediated through
the ERs expressed in the central nervous system and subsequently through the ER downstream factors such as the NMDA receptor.

In regard to the estrogen-inducible RING finger protein Efp\textsuperscript{5} that has been also identified by GBS cloning, the tissue distribution of the gene in reproductive tissues including endometrial cells, mammary epithelial cells, and ovarian granulose cells\textsuperscript{6} was overlapped with that of ER\textalpha. Our experimental data showed that the levels of Efp mRNA in the uterus were rapidly elevated and reached a peak 2 hours after injection of 17\textbeta-estradiol into mice,\textsuperscript{9} suggesting that the gene mediates a specific estrogen action as a primary estrogen-responsive gene. In addition, homozygous mice lacking Efp could grow normally, but female mice showed underdevelopment of the uterus and reduced estrogen responsiveness.\textsuperscript{9} These findings suggest that Efp positively regulates the proliferation of endometrium in an estrogen-dependent manner. Moreover, we have recently shown that Efp is a RING finger-type ubiquitin ligase that targets the ubiquitination and the proteolysis of 14-3-3\textsigma, a cell cycle check point that arrests cell cycle at G\textsubscript{2} phase\textsuperscript{10,11} (Fig. 4). The Efp-mediated breakdown of 14-3-3\textsigma provides a new mechanism for the proliferation of breast cancer.

**Conclusion**

Here we have briefly reviewed recent progress in the research addressing the structure, the functions, and the target genes of ERs. The discovery of a novel ER subtype ER\beta, cofactors recruited by ERs, and ER downstream genes have provided an insight into the
molecular mechanisms and pathophysiological implications of estrogen actions. Although not much described in this article, it has been revealed that estrogen exerts its effects on diverse physiological functions including the homeostasis in postmenopausal women, spermatogenesis and sexual behaviors in men, cardiovascular system, and bone metabolism as well as female reproduction and sexuality, based on new knowledge provided by the generation and analysis of ER-deficient mice along with the discovery and the clinical manifestation of a male patient with ERα null mutation.

Among the multiple aspects of estrogen action, the effect on the cardiovascular system has lately become the focus of attention. The recent randomized clinical trials performed by the Women’s Health Initiative (WHI) in the United States\(^2\) indicate that the hormone replacement therapy using estrogen plus progestin in postmenopausal women increases the risk of thrombosis and heart attack. Further studies are required in terms of the risks and the benefits of estrogen use on the vasculature. Moreover, progress in the study addressing the oncogenic role of estrogen in hormone-dependent cancers has been also achieved.

Future advances in various areas of estrogen research may elucidate the precise molecular mechanisms and the diverse physiological roles of estrogen functions, leading to the clinical application of novel diagnostic procedures and therapeutic strategies.

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