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Review

Genomic and non-genomic actions of estrogen: recent developments

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Abstract

Estrogen affects transcriptional status by activating its corresponding nuclear receptor, the estrogen receptor (ER). It can also induce rapid cellular reactions within a few minutes, and this feature cannot be explained by the transcription-mediated effects of estrogen. The latter mechanisms are called 'non-genomic actions' of estrogen. In contrast, the former classic modes of action came to be called 'genomic actions'. One of the recent developments of research on estrogen was the substantiation of the non-genomic actions of estrogen; these were initially observed and reported as intriguing phenomena more than 40 years ago. The interacting molecules as well as the biological significance of non-genomic actions have now been shown. In the field of genomic actions, invention and spread of new technologies, including high-throughput sequencers, promoted a comprehensive view of estrogen-mediated transcriptional regulation.

Keywords: estrogen; estrogen receptor; genomic action; non-genomic action.

Introduction

Estrogen plays an important role in biological and pathological processes, such as female reproduction, circulation, bone metabolism, and development of breast cancer. The action of estrogen is mediated by its corresponding nuclear receptor, the estrogen receptor (ER). ER has two subtypes, namely, ERα and ERβ. On the stimulation of agonistic ligands, ER forms complexes with coactivators and histone acetyltransferases, resulting in conformational changes of chromatin and activation of transcription pathways. In contrast, antagonistic ligands induce complexes of ER, corepressors, and histone deacetylases (HDACs), which alter the chromatin conformation into a transcriptionally inactive form.

Estrogen can cause rapid changes in signal-transducing molecules within a few minutes. This phenomenon cannot be explained by transcriptional regulation, which requires time for transcription and translation, and came to be known as 'non-genomic estrogen action', whereas the classic function mediated by transcriptional regulation is termed 'genomic estrogen action' (Figure 1).

In this review, we discuss the physiological and pathological significance of newly found 'non-genomic estrogen actions', and recent developments in the analysis of 'genomic estrogen action' promoted by technological advancement.

Membrane ERs

The existence of rapid estrogen reactions was reported as early as the 1960s. In 1967, Szego and Davis (1) described rapid increase in cyclic AMP (cAMP) in the rat uterus after estrogen stimulation. In 1975, it was observed that estrogen stimulus caused a rapid increase in calcium uptake in rat endometrial cells (2). In 1977, estrogen-specific binding sites were predicted on the outer membrane of endometrial and hepatic cells (3). However, after the cloning of ERα was successful in the middle of 1980s (4), researchers tended to focus on the analysis of transcriptional regulation. In the 1990s, phenomena that could not be explained by genomic actions began to draw attention again. In 1992, Morley and colleagues (5) reported rapid calcium release in chicken granulosa cells induced by estrogen stimulus. In 1994, it was discovered that estrogen stimulus caused elevated cAMP in breast cancer cells and uterine cells, which was not blocked by inhibitors of RNA and protein synthesis (6). At this time, the receptor mediating these rapid effects was not clarified.

In 1996, Migliaccio and coworkers (7) demonstrated rapid transient activation of mitogen-activated kinase (MAPK) by estrogen in breast cancer cells. They showed the interaction of ER and c-Src in this process by immunoprecipitation assay. In 2002, estrogen-induced membrane translocation of the ER was shown in breast cancer cells by immunostaining using an ERα antibody (8). Thus, non-genomic action mediated by ER, the same receptor mediating genomic action,

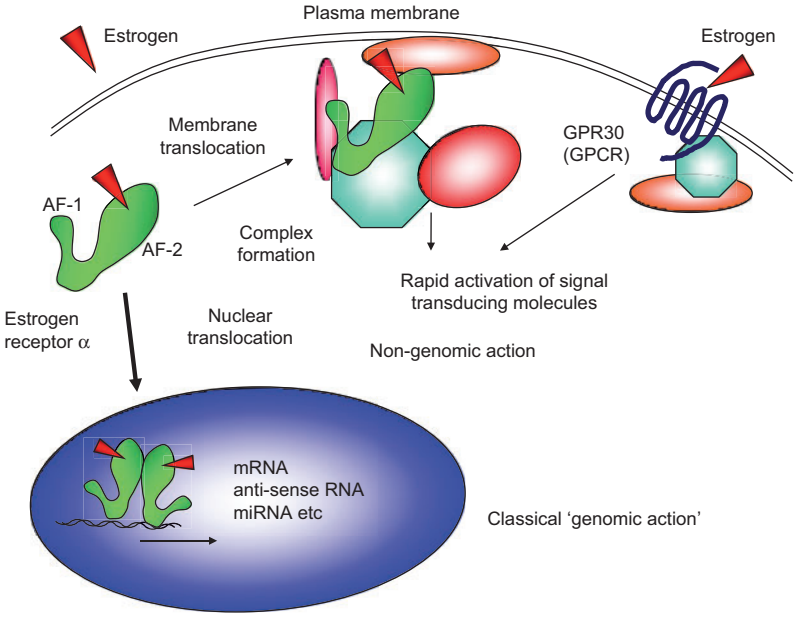

Figure 1 Mechanisms of estrogen action.
The ER functions as a ligand-dependent transcription factor, and this classic function is called 'genomic action'. Messenger RNA, anti-sense RNA, and small RNA are transcriptionally regulated by estrogen. However, estrogen also alters signal transduction molecules within a few minutes. This action is called 'non-genomic action'. Membrane-localized ERs and GPR30 are reported to mediate this function.

came to be accepted, and the non-genomic actions of estrogen were attributed to ERα functioning. Recently, other than full-length 66-kDa ERα, shorter variants of ER – ERα36 (36 kDa) and ER46 (46 kDa) – were shown to mainly localize at the plasma membrane and mediate non-genomic actions of estrogen (9, 10). Some lines of evidence indicated that fatty acid modification of the ER might be important for the mechanism of membrane translocation of ER. Acconcia et al. (11) demonstrated that S-palmitoylation of the Cys447 ERα residue is responsible for membrane localization by a mutation that changes Cys477 to Ala. Another post-transcriptional modification of ERα, methylation of Arg260 in the DNA binding domain, may also be involved in the non-genomic action of estrogen (12). This modification occurs rapidly after estrogen stimulus and promotes the interaction of signal-transducing molecules, such as phosphatidylinositol-3-OH kinase (PI3K) and c-Src.

Some researchers assumed that other estrogen membrane receptors mediate the non-genomic actions of estrogen. In 2000, Filardo et al. (13) showed MAPK was activated by estrogen, even in breast cancer cells without ER expression. They demonstrated that GPR30, a member of the seven-transmembrane-spanning G-protein coupled receptors, can act as an ER at the membrane (13). Expression of GPR30 was reported in several cancer cell lines, including breast (13), endometrial (14), and ovarian (15) cancer cells. Expression of GPR30 was also detected in several tissues, such as the reproductive tissues (16), pancreas (17), bone tissue (18), blood vessels (19), and brain (20, 21). The wide range of GPR30 expression suggested that the non-genomic actions of estrogen mediated by GPR30 can affect physiological and pathological processes in various organs.

Non-genomic actions of estrogen involved in physiological processes

The reproductive tissues are important targets of estrogen action. Non-genomic actions of estrogen in the endometrium were discovered long before the ER was isolated. Rapid increases of cAMP in rat uterus (1) and calcium uptake in rat endometrial cells (2) were shown following an estrogen stimulus. Interestingly, Rambo and Szego (22) reported rapid morphological changes in the endometrial cell microvilli. Presently, GPR30 expressed in the endometrium (16) as well as ERα could also mediate the non-genomic actions of estrogen in the uterus.

The discovery of rapid calcium release from the granulosa cells of chicken preovulatory follicles after estrogen stimulus was one of the early findings of the non-genomic actions of estrogen (5). Tesarik and colleagues (23) found that rapid calcium influx is induced by estrogen in human oocytes during the germinal vesicle stage. Recent studies have suggested that GPR30 is expressed in neonatal hamster ovary and is involved in primordial follicle formation (24).

As for the effects of estrogen on male reproductive tissues, it was reported that effects on the spermatozoa in which estrogen stimulus improved ova penetration (25). As spermatozoa contain densely packaged DNA, genomic action is supposed to be impossible. Therefore, the effects of estrogen on the spermatozoa are likely mediated by non-genomic actions. Aquila et al. (26) demonstrated ERα (and ERβ) expression in the spermatozoa, and interactions between ERα and the p55 regulatory subunit of PI3K, which could explain the mechanisms of the non-genomic actions of estrogen in the spermatozoa.

Estrogen is assumed to have a protective effect on the cardiovascular system, although this effect is still controversial in clinical studies. Simoncini et al. (27) reported evidence that non-genomic actions are involved in these effects. In vascular endothelial cells, estrogen rapidly induces nitric oxide production by activating the PI3K pathway. During this action, membrane-localizing ERα associates with the p85α regulatory subunit of PI3K, leading to the activation of Akt and endothelial nitric oxide synthase. Recently, Haas et al. (19) reported GPR30 also mediates a rapid vasodilating effect.

Estrogen also plays a beneficial role in the bone tissue. In osteoblasts, the membrane-localizing ERα mediates anti-apoptotic effects by activating the Src/Shc/extracellular signal-regulated kinase (ERK) pathway (28). During this action, the membrane-localizing ERα associates with c-Src. In osteoblast progenitor cells, GPR30 is also expressed, and GPR30-mediated signaling promotes the proliferation of these cells (18).

Some studies show the involvement of estrogen-mediated non-genomic actions in the central nervous system. In 1991, Hayden-Hixon and Ferris (29) reported that estrogen injections into the anterior hypothalamus of male hamsters caused rapid behavioral changes, i.e., increased flank marking. Similar rapid behavioral changes were also found in experiments involving rats, in which estrogen administration facilitated anogenital investigation and mounting behavior within 35 min (30). Recently, Dewing and coworkers (31) revealed part of the molecular mechanism of behavioral changes in female rats. They demonstrated that ERα and the metabotropic glutamate receptor 1a directly interact to mediate a rapid estradiol-induced activation of the μ-opioid receptor in the medial preoptic nucleus, leading to female sexual receptivity, such as lordosis. In the central nervous system, estrogen can also influence memory and hippocampal function. Fernandez et al. (32) demonstrated that these effects were mediated by the non-genomic actions of estrogen accompanied with MAPK activation in the dorsal hippocampus. They showed that cerebroventricular infusion of membrane-impermeable bovine serum albumin-conjugated estrogen caused enhanced object recognition and hippocampal extracellular signal-regulated kinase activation. Neuronal cells also contain GPR30. Regulation of energy homeostasis in hypothalamic neurons (20), and prolactin secretion in the hypothalamic-pituitary axis (21) are reported to be mediated by GPR30 signaling.

Non-genomic actions of estrogen involved in pathological processes

The non-genomic actions of estrogen are also analyzed under pathological conditions, such as breast cancer. Ligand-dependent rapid phosphorylation of MAPKs caused by estrogen was revealed in ERα-positive breast cancer MCF-7 cells (7). As MCF-7 cells also were later shown to be GPR30 positive, this phenomenon could be attributed to both membrane-localized ER and GPR30.

Further studies on membrane-localizing ER led to the discovery of several associated molecules at the plasma membrane. One of these proteins is MNAR [modulator of non-genomic action of estrogen, also known as PELP1 (proline-, glutamic acid-, and leucine-rich protein-1)], which functions as a scaffold protein binding with ERα and c-Src at the same time, leading to c-Src activation (33). Recently, MNAR was found to interact with integrin-linked kinase 1, and this complex mediates estrogen-induced cytoskeletal reorganization and enhanced motility of breast cancer cells (34). Enhancement of motility was also explained by rapid tubulin deacetylation by estrogen stimulus (35). We revealed that the membrane-localizing ER associates with tubulin and HDAC6 in the cytoplasm. In this case, HDAC6 deacetylates tubulin instead of histone. Interestingly, tamoxifen, which functions as an antagonist to the genomic actions of ER, behaves as an agonist for the non-genomic actions of ER. It was also reported that another tubulin-binding protein, the hematopoietic PBX-interaction protein, associates with ERα (36) and functions as a scaffold protein, associating with c-Src, the p85 subunit of PI3K, and tubulin. Formation of this complex leads to activation of Akt and MAPK, and depolymerization of microtubule, which causes enhanced cell motility. Another scaffold protein, p130Cas, was also shown to be associated with ERα and c-Src (37). p130Cas could mediate non-genomic actions, such as MAPK activation in ER-positive breast cancer cells (37).

GPR30-mediated enhanced proliferation and motility in ER-negative/GPR30-positive SKBr3 breast cancer cells was also shown (38). GPR30-induced transcription of connective tissue growth factor was necessary for these observed effects. Hydroxytamoxifen was an agonist in this action.

Advancement of genomic action analysis

Since the discovery of ERα, various studies have been performed to clarify genomic actions of estrogen, including identification of estrogen target genes and the cofactors involved in estrogen-induced transcriptional regulation. After completion of the human genome project, and with the advent of newly developed sequencing technology, a more comprehensive vision of the estrogen transcriptome was elucidated.

The chromatin immunoprecipitation (ChIP) method helps in understanding temporal regulation and special occupancy of the promoter/enhancer regions by ERα and its cofactors. Using ChIP experimentation, Reid et al. (39) revealed that ERα and associated cofactors were recruited in a cyclic pattern. This cyclic recruitment depended on nuclear proteasome activity, suggesting a rapid mechanism involving on/off regulation in the presence and absence of ligands. By combining ChIP and microarray analysis, Carrol et al. performed chromosome-wide (40), and later, genome-wide (41) analysis of the ER-binding sites. These revealed many potential ERα-regulated genes, and possible transcriptional regulation from a remote enhancer at as far as 500 kb from the target genes. In the late 2000s, high-throughput sequencing technology became widely available. This technology is potentially a valuable tool for transcriptional analysis. In 2009, Welboren et al. (42) combined ChIP and high-throughput sequencing (ChIP-seq) methods to comprehensively analyze

the genomic binding sites of ERα in response to different ligands. Recently, investigations have focused on the three-dimensional structure of chromatin, which could explain the mechanism of ERα-mediated transcriptional regulation from the remote enhancer sites. Fullwood et al. (43) undertook a comprehensive analysis of the ERα-bound chromatin interactome by using a strategy called chromatin interaction analysis by paired-end tag sequencing (ChIA-PET). In this strategy, ChIP and high-throughput sequencing methods were used after cross-linking of chromatin and proximal ligation of DNA. High-throughput sequencing can also be applied for the analysis of estrogen-induced transcription. Hah et al. (44) focused on newly synthesized RNA using global nuclear run-on and sequencing (GRO-seq), and demonstrated estrogen-mediated induction of mRNA, antisense RNA, divergent RNA, and small RNAs, which were overlooked in steady-state RNA sequencing. Thus, comprehensive studies of the genomic actions of estrogen promote functional understanding of the roles of estrogen in cells, tissues, and organs.

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