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ER-independent actions of estrogen and estrogen metabolites in bone cells

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Introduction

The physiological actions of mammalian estrogens are mediated through two known estrogen receptors (ERα and ERβ). This process takes place through the regulation of transcription, involving nuclear translocation and binding of ligand-activated receptor to DNA elements leading to regulation of gene expression (genomic effects). Many investigations in the past few years have identified estrogen-mediated rapid responses that involve non-transcriptional mechanisms (non-genomic effects). These involve binding of the ligand-activated receptors to other transcription factors or kinases. Recent reports also demonstrate that some of the actions of estrogens are taking place independent of ERs through the activation of several signal transduction pathways. These non-genomic effects of estrogens are not sensitive to the inhibitory effects of the estrogen receptor antagonists. We would like to present two of our recent studies in bone cells where we have demonstrated ER-independent effects of estrogens.

ER-independent actions of 2-Methoxyestradiol in osteosarcoma cells

The mammalian estrogen is metabolized into several compounds that have unique biological effects. Estrogen and estrogen metabolites have been associated with several types of human and animal cancers. 2-Methoxyestradiol (2-ME), a metabolite of 17β-estradiol, is produced by sequential 2-hydroxylation and O-methylation[1]. Unlike the parent compound, 17β-estradiol, that stimulates proliferation of estrogen-receptor dependent cancer cells, 2-ME has been reported to exhibit anti-cancer properties in a variety of models and inhibit cell proliferation and angiogenesis[2-7].

The molecular mechanisms of 2-ME mediated anti-tumor effects are under intensive investigation. Two general mechanisms of action for the anti-angiogenic activity of 2-ME have been proposed: 1) disruption of the cytoskeleton during cell division and 2) induction of apoptosis. 2-ME inhibits tubulin formation, causes disturbances in mitosis and produces abnormal metaphase in some cell types[8]. In other cell types, 2-ME stimulates the expression of p53 gene, which leads to the induction of apoptosis[9]. Recently it has been suggested that 2-ME can induce apoptosis in the absence of p53[4-6]. 2-ME activates several caspases through the upregulation of Death Receptor 5 protein, which leads to the induction of apoptosis. This implies that 2-ME induced effects are cell type specific and in addition to interacting with tubulin and regulating p53, other mechanisms must exist for 2-ME mediated induction of cell killing.

Osteosarcoma is a bone tumor that occurs most frequently during adolescence. Although a combination of surgery and chemotherapy has led to improved survival rate, a specific therapy is not yet available for this devastating disease and the mortality rate is still quite high. We have recently shown that 2-ME induces cell death in osteosarcoma cells and not in normal osteoblasts[10]. This effect is accompanied by an increase in the interferon-α mRNA expression. E2, which has a 2,000-fold higher binding affinity to the estrogen receptor than 2-ME, has much less effect than its metabolite on osteosarcoma cell survival. This finding suggests that the toxic effects of 2-ME might not depend on estrogen receptors, a conclusion that is supported by two additional independent lines of evidence. First, 2-ME was equally effective in killing cells expressing both low and high levels of endogenous estrogen receptors. Second, the cytotoxic effects of 2-ME in osteosarcoma cells were neither antagonized nor potentiated by the high affinity estrogen receptor ligand ICI 182,780[10].

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The direct effects of 2-ME on normal osteoblasts have not been studied in detail. Recent studies from our group indicate that the administration of high doses of 2-ME to growing female rats did not disturb normal bone turnover[11]. Also, it has been demonstrated that 2-ME prevents bone loss in ovariectomized rats resulting from estrogen deficiency at doses that are not uterotrophic and that are well below those required for tumor suppression[12]. These *in vivo* actions are not ER dependent and occur in the presence of ICI 182,780. These findings imply that 2-ME actions are independent of estrogen receptors in bone cells. Taken together, these observations suggest that 2-ME which does not affect normal osteoblasts could be a useful therapeutic agent in bone cancer and should be further studied in this regard.

ER-independent effects of estrogen in human fetal osteoblasts

Several investigations demonstrate that estrogen actions involve non-genomic signaling. Although possible mechanisms for this non-genomic effect have been investigated, the nature of these mechanisms is still far from being completely known. Non-transcriptional/non-genomic estrogen actions involve putative membrane ERs[13-16]. Some investigations show that ER can localize to cell membrane, but a structurally distinct ER isoform with membrane localization sequences has not yet been identified. G-protein coupled receptors (GPCRs) have been implicated as a mediator in some non-genomic actions of estrogens in osteoblast cells[17]. ERα interacts with G-proteins and couples to phospholipase C that results in a rapid increase in intracellular Ca2+ concentration. Also, estrogen has been shown to regulate transmembrane ion fluxes in vascular smooth muscle cells. However, the involvement of ERs in this action has not yet been demonstrated.

Several signal transduction pathways are involved in the non-genomic actions of estrogen. These include mitogen-activated protein kinase (MAPK), several tyrosine kinases and lipid kinases[16,18,19]. It has been demonstrated that phosphorylation of ER leads to ligand-independent activation. Also, there is evidence in the literature that estrogen treatment leads to rapid activation of extracellular-signal-regulated kinase (ERK) 1/2 module in various cell types. Estrogen treatment results in the activation of tyrosine kinases, which seems to play a role in the recruitment of other non-genomic pathways like MAPK cascades. For example, estrogen signaling to ERK kinases can occur through activation of src-kinase and tyrosine phosphorylation of Shc adaptor protein[16,19]. Non-transcriptional actions of sex steroid hormone can be mediated by the recruitment of lipid kinases. After binding to estrogen, ERα can physically couple with the regulatory subunit of lipid kinase, phosphatidylinositol 3-kinase (PI3K) and can trigger the activation of catalytic subunit resulting in an increase in intracellular production of phosphoinositides[20]. This, in turn, could activate the downstream effects involving Akt and endothelial isoform of nitric oxide (NO) synthase (eNOS). PI3 kinase activation by estrogen triggers association of ERα with Src and p85[21].

Signal transducers and activators of transcription (Stats) mediate signals from a variety of cytokines and growth factors[22-24]. Recently it has been shown in endothelial cells that estrogen treatment leads to the activation of Stat3 and Stat5 proteins by non-genomic mechanisms through ER-dependent regulation of MAP kinase, PI3 kinase and Src kinase pathways[25]. We have found that 17β-estradiol treatment in human fetal osteoblast (hFOB) cells leads to the activation of Stat1 protein and provide the first direct evidence that Stat1 is a transducer for estrogen signaling in human osteoblasts. This finding in hFOB cells that do not have detectable levels of ER demonstrates a previously unrecognized ER-independent non-genomic mechanism for estrogen signaling in bone cells.

Summary

The non-transcriptional and non-genomic responses to steroid hormone treatment are not rare. The majority of them involve binding of ER to functional proteins while some of them could be ER-independent. Our investigations demonstrate that estrogens can also exert physiological effects (apoptosis, regulation of gene expression and activation of signal transduction pathways) in the absence of classical ER in cultured osteoblasts. The nature of the receptor that mediates these effects of estrogens is unknown. In general, insight is also lacking regarding the specific signaling pathways in ER-dependent non-genomic actions. Thus, a full understanding of the molecular mechanisms associated with these novel effects of estrogen is yet to be accomplished in the skeletal system.

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