Insulin-Like Growth Factor-I and Estrogen Interactions in Breast Cancer

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ABSTRACT There is increasing evidence that estradiol and insulin-like growth factor-I (IGF-I) act through a complex cross-talk mechanism to stimulate the proliferation of normal mammary epithelium to increase the risk of breast cancer. The emerging model of cross-talk suggests that estradiol regulates the expression of IGF-I and the IGF receptor I. The subsequent binding of IGF-I to its receptor initiates an intracellular signal transduction pathway that activates transcription factors, including the estrogen receptor. Recent studies show that the effects of IGF-I on estrogen receptor activity are mediated in part by the protein kinase A and phosphatidylinositol-3-kinase/Akt pathways. J. Nutr. 132: 3799S–3801S, 2002.

KEY WORDS: • insulin-like growth factor-I • estradiol • estrogen receptor

There is increasing evidence for a complex mechanism of cross-talk between peptide and steroid pathways. The emerging model of cross-talk between insulin-like growth factor-I (IGF-I) and estrogens suggests that estrogens, acting through the estrogen receptor (ER), induce the expression of IGF-I. IGF-I, in turn exerts its actions through binding to the IGF receptor I, a transmembrane protein with tyrosine kinase activity. The binding of IGF-I to its receptor activates the tyrosine kinase and initiates a cascade of phosphorylations that activate intracellular kinases and nuclear transcription factors, including the ER.

IGF-I is an endocrine factor that belongs to a family of growth factors involved in the regulation of normal and malignant cell growth, differentiation and development. It is a 70-residue, single-chain polypeptide synthesized in the liver in response to growth hormone (1). Besides its endocrine effects, IGF-I is produced in most organs and tissues where it can function in both an autocrine and a paracrine manner to stimulate cell growth (2). The binding of IGF-I to the IGF receptor is modulated by a group of soluble proteins, called IGF binding proteins, that are found in all extracellular fluids (2).

The IGF-I receptor appears to play a critical role in the regulation of breast cancer cell growth (2,3). The amount of the IGF-I receptor is significantly higher in breast cancer than in normal breast tissue or benign tumors. In primary breast cancer, there is a correlation between tumor size, the amount of insulin receptor substrate 1 (IRS-1), an intracellular substrate of the IGF-I receptor, and recurrence of the disease (4). Inhibition of IGF receptor I signaling with anti-IGF receptor antibodies, antisense RNA to the receptor or antisense oligonucleotides to IRS-1 restricts breast cancer cell growth in vitro and in vivo (5). A similar effect is also observed in breast tumor xenografts in vivo. In the MCF-7 breast cancer cell line, the coexpression of IRS-1 (6), IGF receptor I (7) or IGF-II (8) reduces the dependence of cell growth on estrogens (5), whereas IGFs dramatically increase cell proliferation in the presence of estrogen (9). Based on these observations, it has been suggested that estradiol and IGF-I may act together to stimulate proliferation in normal mammary epithelium and increase the risk of breast cancer. In fact estrogens regulate the expression of IGF-I and IGF receptor I in ER-positive breast cancer cell lines as well as in neuroblastoma cells (10), in uterine tissue (11,12) and in osteoblasts (11). Antiestrogens, on the other hand, inhibit IGF receptor I dependent growth by down-regulating the IGF autocrine pathway and modulating the expression of IGF binding protein (13). In addition, antiestrogens decrease the expression of IGF-I binding sites and suppress the activation of IRS-1 associated phosphatidylinositol-3-kinase.

In contrast to the effects of estrogens on the IGF-I pathway, regulation of estrogen signaling by IGF is less well defined. The effects of estrogen are mediated by the ER, which belongs to a superfamily of ligand-inducible transcription factors (14–19). Two distinct regions within the ER contribute to its transcriptional activity: the AF-1 domain, located in the amino terminus, and the AF-2 domain, located in the carboxyl-terminal hormone binding domain. The AF-1 and AF-2 domains regulate transcription both independently and synergistically depending on the promoter and cell type (20). In the absence of hormone, the inactive receptor is complexes with a host of proteins, including heat shock proteins, which prevent it from interacting with the cellular transcription apparatus. On binding estradiol, the receptor undergoes a conformational change that permits it to bind to coactivators and initiate the tran-
scription of target genes. Activation of the ER is also associated with an increase in phosphorylation (21) on serines S104, S106, S118 and S167 (22) located in the amino-terminal AF-1 domain (23) and on tyrosine T537, located in the ligand-binding domain (24).

Although estradiol is necessary for ER activation, a great body of evidence has accumulated demonstrating that IGF-I activates the ER in the absence of estradiol. Early studies demonstrated that antiestrogens inhibited IGF-mediated proliferation (25). It was suggested that inhibition was the result of regulation of receptor binding sites and tyrosine kinase activity by the antiestrogen. However a number of later studies demonstrated that IGF, as well as other growth factors, directly increased the transcriptional activity of ER and that activation was specifically inhibited by antiestrogens (3,10,11,26–28). In vivo studies in ER-α knockout mice provide further support that IGF-I induces a hormone-independent transactivation of the ER (29). Although the mechanism by which growth factors activate the ER remains unclear, receptor phosphorylation is thought to play a role (12). However phosphorylation of the receptor does not always correlate with transcriptional activity (3). The goal of our work is to understand the mechanism by which IGF-I regulates both the expression and activity of ER-α in human breast cancer cells. To begin these studies, the role of IGF-I in the regulation of ER-α was investigated in MCF-7 cells (30). A study of the effects of IGF-I on ER-α expression showed that the growth factor decreased expression of the receptor by blocking transcription from the ER-α promoter. When cells were treated with IGF-I, there was a corresponding decrease in the concentration of ER-α protein and in the number of estradiol-binding sites and there was no effect on the binding affinity of the receptor for estradiol. The decrease in ER-α protein concentration was found to parallel a decrease in the steady-state amount of ER-α mRNA. When a reporter gene under the control of the ER-α promoter was transfected into MCF-7 cells, treatment with IGF-I resulted in a decrease in CAT activity, suggesting that the IGF-I-induced decrease in ER-α mRNA was the result of the inhibition of ER-α gene transcription. We then examined the effects of IGF-I on ER-α activity. In contrast to the effects on ER-α expression, treatment with IGF-I induced two endogenous estrogen-regulated genes: progesterone receptor and pS2. The antiestrogenICI-164,384 blocked this induction, suggesting that ER-α mediates the effects of IGF-I on the expression of progesterone receptor and pS2. Transient co-transfections of ER-α and a reporter gene controlled by an estrogen response element demonstrated that IGF-I increased reporter gene activity. This effect was also blocked by the antiestrogen, further supporting the ability of IGF-I to activate ER-α.

To define the pathway used by IGF-I to activate the ER, kinase inhibitors were used. In these studies, inhibitors that blocked protein kinase A and phosphatidylinositol-3-kinase blocked the effects of IGF-I on ER-α expression and activity, suggesting that protein kinase and phosphatidylinositol-3-kinase may be involved in the cross-talk between the IGF-I and ER-α pathways. Because the serine/threonine protein kinase Akt, also known as protein kinase B, is downstream of phosphatidylinositol-3-kinase, experiments were conducted to determine whether Akt is involved in the cross-talk between IGF-I receptors and ER-α. When MCF-7 cells were treated with IGF-I, there was a rapid phosphorylation and activation of Akt that were blocked by inhibitors of phosphatidylinositol-3-kinase. Stable transfection of cells with a dominant negative Akt mutant also blocked the effects of IGF-I on ER-α expression and activity, whereas stable transfection of cells with a constitutively active Akt mutant mimicked the effects of IGF-I. In the latter cells, there was a decrease in the amount of ER-α and an increase in the amount of progesterone receptor protein and pS2. Coexpression of ER-α and the dominant negative Akt mutant also blocked IGF-I stimulated activation of ER-α, whereas coexpression of the ER with the constitutively active Akt mutant increased ER-α activity, providing additional evidence that the effects of IGF-I are mediated by Akt. Studies using mutants of ER-α demonstrated that Akt increases ER activity through serines in the amino-terminal AF-1 domain, most likely as a result of serine phosphorylation. It is not clear at the present time whether Akt phosphorylates ER-α directly or indirectly through a downstream kinase or cross-talk with other signaling pathways. However serine S167 is a good candidate for phosphorylation by Akt; it is located within the sequence RERAS, which conforms to the consensus Akt phosphorylation site RXRXXS (31), suggesting a direct interaction of Akt with ER-α and phosphorylation of at least one of the serines in AF-1 domain. The activity of Akt to directly phosphorylate ER-α on serine S167, as well as on other serines, remains to be tested. Although the IGF-I pathway(s) that mediate the effects on ER-α activity remain to be defined, the emerging model of cross-talk between IGF-I and ER-α in breast cancer cells suggests that IGF-I regulates both the expression and the activity of ER-α in part through the phosphatidylinositol-3-kinase/Akt pathway.

LITERATURE CITED


