Review

Tyrosine kinase signalling in breast cancer
Insulin-like growth factors and their receptors in breast cancer

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Abstract

The insulin-like growth factor (IGF) system exerts pleiotropic effects on mammalian cells. This review focuses on type I IGF receptor (IGF1R)-mediated signal transduction and its relevance in breast cancer. Upon activation by the IGFs, IGF1R, a transmembrane tyrosine kinase receptor, undergoes autophosphorylation, and then binds and phosphorylates additional signaling molecules. These intermediates initiate a series of downstream signaling events that are involved in multiple physiologic processes for cells. Recent data demonstrate that the IGF receptor system actively interacts with the estrogen receptor and integrin receptor systems. Cross-talk among these pathways regulates breast cancer proliferation, protection from cell death, and metastasis. Better understanding of IGF biochemical signaling pathways is of utmost importance for developing therapies for breast cancer.

Keywords: breast cancer, insulin-like growth factor, insulin-like growth factor receptor, signal transduction

Introduction

The IGF system is composed of IGF ligands, receptors, and binding proteins. These system components form a highly regulated network of interactions both among themselves and between other biologic signaling pathways.

The two well-characterized ligands, IGF-I and IGF-II, are mitogenic peptides that are highly homologous to each other and to insulin [1]. Whereas insulin is composed of two chains (A and B) of 21 and 30 amino acids, respectively, the IGFs are single-chain molecules that retain the equivalent of the connecting (C)-peptide of proinsulin between the A and B domains. IGF-I and IGF-II are thought to have autocrine, endocrine, and paracrine roles in normal mammary development and in the etiology of breast cancer [2–5].

Unlike insulin, circulating IGFs are found complexed to high-affinity binding proteins known as IGF-binding proteins (IGFBPs). Six distinct species have been cloned. An additional family of structurally homologous proteins has been identified and named IGFBP-related proteins, because their affinity for the IGFs is significantly lower than that of the IGFBPs [6,7]. Cleavage of IGFBPs by specific proteases modulates levels of free IGFs and IGFBPs, and thereby their actions. In addition, IGFBPs may also have effects that are completely independent of their role in modulating the action of IGF [8].

CSK = carboxyl-terminal src kinase; ER = estrogen receptor; FAK = focal adhesion kinase; IGF = insulin-like growth factor; IGF1R = type I IGF receptor; IGF2R = type II IGF receptor; IGFBP = insulin-like growth factor-binding protein; IRS = insulin receptor substrate; MAPK = mitogen-activated protein kinase; PI-3K = phosphatidyl inositol-3 kinase.

The cellular actions of IGFs are mediated by type I and type II receptors, insulin receptor, and insulin receptor–IGF1R hybrids. The type II IGF receptor (IGF2R) is a multifunctional nontyrosine kinase receptor [9–11] that is also known as the cation-independent mannose-6-phosphate receptor, and its function in regulating the action of IGF-II has been controversial. IGF1R is a glycosylated heterotetramer that is composed of two extracellular α-subunits and two transmembrane β-subunits that have intrinsic tyrosine kinase activity [12,13]. This review focuses on IGF1R-mediated signaling and its relevance in breast cancer.

Type I insulin-like growth factor receptor signaling

Activation of the IGF1R by IGFs results in its oligomerization, autophosphorylation, and activation of the intrinsic tyrosine kinase [12–15]. The IGF1R tyrosine kinase further directly phosphorylates various intracellular substrates. Several substrates of the IGF1R have been identified, including insulin receptor substrates (IRSs) 1, 2, and 4 [16–19], src-homology 2/collagen-α proteins (Shc) [20,21], phosphatidyl inositol-3 kinase (PI-3K) [22], growth factor receptor-binding protein 10 [23], focal adhesion kinase (FAK) [24•], and carboxyl-terminal src kinase (CSK) [25•].

IRS-1 is a well-characterized IGF1R-signaling molecule that has multiple sites for tyrosine phosphorylation and acts as a 'docking protein' for other signaling molecules [26,27]. Upon activation of IGF1R, IRS-1 binds and becomes rapidly tyrosine phosphorylated, allowing docking sites for SH2 domain-containing proteins. IRS-1 phosphorylation results in the activation of many downstream signaling pathways; many of these pathways are implicated in mitogenesis and protection from apoptosis. For instance, the following are all known to be stimulated through IRS-1: PI-3K pathway through the association with the p85 regulatory subunit of PI-3K [28]; Ras–mitogen-activated protein kinase (MAPK) cascade through Grb-2/Sos [29]; Syp phosphatase [30]; as well as other pathways involving adapters Nck and Crk [31,32]. Upon tyrosine phosphorylation by the activated IGF1R, Shc (a common substrate of most tyrosine kinase receptors) also recruits Grb-2–Sos complexes and activates the Ras–MAPK pathway.

Additional pathways may be affected by IGF1R activation. For example, the cytoplasmic tyrosine kinase c-Src can phosphorylate IGF1R on the same sites as the IGF-induced autophosphorylation sites [33]. CSK, a negative regulator of Src activity, associates with activated IGF1R, and therefore may play a role in the decrease in Src activity after IGF-I stimulation. Other substrates of Src are almost exclusively proteins that regulate actin cytoskeleton dynamics, such as FAK, p130 Crk-associated substrate, cortactin, and p190RhoGAP. IGF-I through its receptors has been shown to positively or negatively modulate tyrosine phosphorylation of focal adhesion proteins such as FAK, p130 Crk-associated substrate, and paxillin [24•,34,35]. Thus, activation of IGF1R, via its interaction with Src, could influence aspects of cytoskeletal organization and cell adhesion.

Insulin-like growth factors and insulin-like growth factor receptor signaling in breast cancer

Expression of IGF-I and IGF-II has been measured in normal and breast tumor tissues by *in situ* hybridization and immunohistochemistry. IGF-I is found mainly in stromal cells that are adjacent to normal breast cells [2]. IGF-II is also mainly expressed in the stroma [5], but may occasionally be found in malignant epithelial cells [4]. Increased IGF-II expression is seen in stromal cells that are adjacent to malignant epithelial cells, whereas levels are lower in stroma that are adjacent to benign and normal breast epithelium [36,37]. Furthermore, malignant breast epithelial cells can induce IGF-II expression in breast stroma *in vitro* [38]. High IGF-II expression is reported to be associated with poor prognostic features in breast cancer [39].

Endocrine levels of IGF-I have been implicated in breast cancer. Breast cancer patients have higher serum IGF-I levels than do matched control individuals [40]. Higher IGF-I levels have also been associated with an increased risk of developing breast cancer [41••].

IGF1R has been found to be both significantly overexpressed [42–44] and highly activated [45••] in cancer cells with respect to its status in normal or benign breast tissues. Recent reports [46•] have suggested that insulin receptor may mediate the IGF-II response in breast cancer cells. In addition, insulin receptor–IGF1R hybrids are overexpressed in breast cancer, and these receptors can also mediate IGF responsiveness [47•]. Although the IGF2R does not appear to function in a signaling pathway, there is significant loss of heterozygosity at the IGF2R locus in breast cancer, suggesting that IGF2R may represent a breast tumor suppressor gene [48]. Mutation in the IGF-II binding domain of the remaining IGF2R allele has been identified in cancer cells [49•]. These observations suggest that IGF2R loses the ability to bind IGF-II in some cancer cells. This would allow enhanced interaction of IGF-II with the tyrosine kinase receptors and, perhaps, tumor promotion.

Expression of the IGF downstream signaling molecule IRS-1 is also detected in breast cancer. Increased levels of expression were found to correlate with estrogen receptor (ER) status in 200 node-negative patients, and identified patients with a decreased disease-free survival in a subset of patients with small tumors [50•]. Taken together,

these studies show that the IGFs are freely available to the malignant epithelial cells from endocrine or paracrine sources. Furthermore, IGF receptors are present on breast cancer cells to mediate the biologic effects of the IGFs.

Consequence of insulin-like growth factor activation in breast cancer

Activation of the IGF system is known to have substantial pleiotropic effects on mammalian cells. Mitogenesis, transformation, and antiapoptosis induced by IGF1R stimulation could account for many aspects of the malignant phenotype. Both IGF-I and IGF-II stimulate ER-positive breast cancer cell proliferation at picomolar to nanomolar concentrations [51]. Once IGFs interact with receptors, we found that IRS-1 is the predominant signaling molecule activated in ER-positive human breast cancer cells [52•].

There is also accumulating evidence that IGF action influences breast cancer cell responsiveness to estrogen. It is well established that estrogens stimulate the growth of ER-positive breast cancer cells. ER acts as a ligand-activated transcription factor. Two forms of ER have been cloned, ERα and ERβ [53–55]. Erα contains a hormone-binding domain, a DNA-binding domain, and two transcriptional activation domains (AF-1 and AF-2). Estradiol binding to ERα results in dimerization and subsequent binding of the hormone–receptor complex to specific DNA palindromic sequences (estrogen response elements) to initiate gene transcription, and therefore induce the expression of growth promoting genes. To date, a similar role for ERβ has not been found. Antiestrogens, such as tamoxifen, influence ERα function by blocking the initiation of transcription from estrogen response elements without interfering with the binding of ligand–receptor complex to DNA.

Estrogen induces the expression of several members of the IGF family, including IGF-II [3,56,57], IGF1R [58], IGF2R [59], IGFBPs [60], and IRS-1 and IRS-2 [61•]. The increased expression of IGF1R and IRS-1 results in an enhanced response to IGF-I that is manifested in greater downstream signaling through MAPK. Removal of estrogen results in a dramatic decrease in IRS-1 expression and MAPK activity. Antiestrogens may inhibit IGF action by increasing IGFBP-3 [62,63], affecting phosphorylation of IGF1R or IRS-1 [64•,65,66], downregulating expression of IGF1R and IRS-1 [58,67], and inhibiting ligand-independent activation of the ER by IGF-I [68–71]. Thus, several members of the IGF family could be the growth-promoting genes that are regulated by estrogen.

On the other hand, IGF-I also directly increases the transcriptional activity of the ER and increases expression of estrogen-inducible genes, such as the progesterone receptor gene [71]. Furthermore, IGFBP-1, an inhibitor of IGF-1 action, not only inhibited IGF-mediated activation of the ER, but also had a significant inhibitory effect upon estrogen-mediated activation of the ER. Although the mechanisms that account for this cross-talk are not clear, it is obvious that both signaling pathways can positively influence each other, resulting in reinforcement of biologic effects for both estrogen and IGFs.

Many model systems have shown that IGF1R activation protects cells from programmed cell death. The PI-3K pathway and its substrate AKT1 probably mediate this effect. It has been reported [72] that AKT1 is highly expressed in several human breast carcinoma cell lines, and its activity in MCF-7 cells is modulated by estradiol and IGF-I. Overexpression and activation of AKT1 produces estrogen and IGF-I independent proliferation and controls an antiapoptotic pathway. IGF-I reduces apoptosis in doxorubicin-treated and paclitaxel-treated MCF-7 cells [73]. Detailed studies indicate that IGF-I rescue of MCF-7 cells from chemotherapy-induced cell death involves at least two mechanisms: inhibition of apoptosis through PI-3K and induction of proliferation through both PI-3K and MAPK cascades. In clinical specimens, high levels of IGF1R may protect cells from radiation therapy-induced apoptosis [74•].

Several reports have documented the interaction of IGF and integrin signaling pathways. The direct interaction of the two pathways was demonstrated through a physical association between $\alpha_v\beta_3$ integrin and IRS-1 [75••]. Later reports [24•] also showed that FAK, a downstream signaling molecule of integrins, is a substrate for the insulin receptor and IGF1R. In vascular smooth muscle cells, ligand occupancy of $\alpha_v\beta_3$ integrin is required for full activation of the IGF1R β-subunit and IRS-1 by IGF-I stimulation [76,77•]. IGFs are chemoattractants for breast cancer cells, perhaps due to the ability of IGF to affect the integrins [78]. Activation of integrin signaling pathways have been reported [79] to inhibit the mitogenic effect of IGF-I in human breast cancer cell lines. Recently, IGF1R activation was shown to induce rapid and transient tyrosine dephosphorylation of FAK, p130 Crk-associated substrate, and paxillin in MCF-7 breast cancer epithelial cells [80]. Finally, IGFs may be involved in cell migration and invasion, because dominant-negative IGF1R constructs inhibit invasion and metastasis of MDA-435 breast cancer cells *in vitro* and *in vivo* [81•].

Conclusion

Breast cancer is a lethal disease because the transformed epithelial cells proliferate, metastasize, and are protected from programmed cell death. The pathways responsible for each of these phenotypes are only now becoming understood. Despite the multiple accumulated genetic abnormalities that cause malignant transformation, however, it is evident that some of the transformed cells can still respond to signals from their external environment. Notably, the inhibition of ER function has proven to be a powerful weapon in breast cancer treatment.

There now is a large body of evidence showing that IGF activation is involved in these malignant processes; clearly some fully transformed cells can still respond to these cues. It is also evident that the signaling pathways that are activated by the IGFs are not simple or linear. Multiple divergent and convergent biochemical signaling pathways are stimulated after receptor activation, which then impinge upon multiple other pathways that are known to be important in breast cancer biology. We are now just beginning to understand how the IGFs affect breast cancer biology. The next challenge will be to untangle the web of signal cascades initiated by these factors. By doing so, we will be better positioned to develop therapies based on interruption of the key signaling pathways that are responsible for the malignant phenotype.

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