The Role of the Insulin Receptor Isoforms in the Insulin-Like Growth Factor Signaling Axis in Cancer

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Abstract

The Insulin-like Growth Factor (IGF) signaling system is known for regulating critical cellular processes related to growth and apoptosis. The IGF-axis is activated upon IGF stimulation of the IGF-1 receptor (IGF-1R), and it has been well demonstrated that increased expression of IGF-1R and the IGF-2 ligand is implicated in cell transformation and tumor propagation. For several decades, efforts have been focused on developing treatment strategies aimed at inhibiting IGF-1R action but produced suboptimal results. Recent research has confirmed the presence of an autocrine signaling loop involving IGF-2 and an insulin receptor isoform A (IR-A), which is produced as a result of alternative splicing of the IR. Stimulation of full-length IR (IR-B) by insulin typically activates glucose regulating pathways, whereas IR-A activation by IGF-2 leads to mitogenic signaling. IR-A is a naturally occurring isoform, prevalent during fetal development and in certain tissues. It is also shown to be highly upregulated in many cancer types, increasing the cancer cell's responsiveness to IGF-2 thus providing the tumor cell a growth advantage. In this mini review, we discuss the mechanism of IR alternative splicing and its role in cancer and treatment resistance.

Keywords: Cancer; IGF-2; Insulin receptor; Insulin-like growth factor-1 receptor; Splicing

Introduction

The Insulin-like Growth Factor (IGF) system plays an important role in the regulation of cellular growth and development. Many cancers overexpress IGF hormones and receptors leading to enhanced autocrine and paracrine signaling to promote growth and inhibit apoptosis, presenting the tumor cell with a growth advantage [1-6]. The IGF system is therefore, an attractive target for cancer therapeutic development. Recent therapeutic development has targeted the IGF-axis using various antibody and small molecule approaches against the IGF-1 receptor (IGF-1R) and the related Insulin Receptor (IR), as well as the ligands that activate these receptors. This approach to slow tumor growth by inhibiting the IGF signaling system showed promise in preclinical development but clinical trial results were disappointing [7]. Recently evaluated therapeutic strategies focused on the IGF-signaling pathway have been reviewed previously [8].

It is well known that intracellular signaling pathways are very complex and involve a high level of interconnectivity [9]. Crosstalk between the IGF-1R and IR signaling pathways is likely a key factor in the failure of these clinical trials [10-13]. Insulin Receptor isoform A (IR-A) is stimulated by IGF-2, which leads to activation of mitogenic signaling, bypassing the IGF-1R inhibitors. This IR isoform is often upregulated in many cancers and has been the subject of recent interest since it is becoming clear that therapeutic strategies should consider the IR signaling family. This review summarizes the prevalence and mechanism of IR-A in cancer and its role in IGF-1R-targeted therapies.

The IGF and insulin signaling family

The IGF and insulin signaling pathways are activated by IGF-1, IGF-2 or the homologous hormone insulin. These factors activate at least six receptors: IGF-1R, two forms of the IR produced from alternative splicing (IR-A and IR-B), and various hybrid receptors (Figure 1). Both IGF-1R and IR are transmembrane tyrosine kinase receptors that function as a dimer. Each monomer consists of an extracellular α subunit and a membrane-spanning β subunit, both synthesized from a single mRNA. The protein is cleaved by furin into the two subunits linked by disulfide bonds to form the αβ chain, which dimerizes to form the functional receptor [14].

In addition to the homodimer receptors, αβ chains from IGF-1R and IR can dimerize with each other to make heterodimers receptors referred to as hybrid receptors. To add another layer

Figure 1: The insulin and IGF-1 receptor family.
The insulin and IGF-1 receptors exist as homodimers or heterodimers, each with distinct ligand binding preferences and signaling outcomes. Each "half" of the dimer is comprised of an αβ chain synthesized from one pre-mRNA transcript that is proteolytically cleaved and joined by disulfide linkages (black bars). IR (green receptor) is expressed as two isoforms, differing by the presence or absence of exon 11 (pink box). Both the full-length IR-B and exon 11 deficient IR-A isoforms dimerize to form functional receptors or hybrid receptors as indicated below each receptor. IGF-1R (purple receptor) can also form functional receptors with both IR isoforms. The IR-B has strong affinity for insulin, which results in signaling of metabolic pathways. IGF-1R activation by either IGF-1 or IGF-2 leads to processes promoting cell proliferation and motility and antiapoptotic signaling. Hybrid receptors with IR and IGF-1R as well as the homodimer IR-A activate mainly mitogenic pathways when stimulated by IGF-2 [25,30].

of complexity to the IGF and insulin signaling family, there are two isoforms of the IR that result from alternative splicing of exon 11 during maturation of the pre-mRNA. The full-length receptor is known as IR-B and includes exon 11, which resides at the C-terminus of the α chain and is predicted to influence ligand binding. On the other hand, IR-A lacks exon 11, which allows increased affinity for signaling ligands in addition to insulin. This alternatively spliced exon is only 36 nucleotides in length and encodes for 12 amino acids but the receptor lacking exon 11 is able to bind IGF-2 with high affinity, unlike the full-length IR-B receptor [15,16].

Splicing is influenced by proteins that bind specific sequences of the pre-mRNA. These splicing factors can either recruit or block the spliceosomal snRNPs, leading to either the recognition of specific exons and removal of the intervening introns, or the silencing of splicing signals at exon boundaries, resulting in the removal of one or a series of exons and introns. The regulation of IR exon 11 splicing is controlled by repressor and enhancer sequences in exon 11 and the surrounding introns [17]. Positive regulators of splicing that promote exon 11 inclusion include muscle blind-like splicing regulator 1 [18-20] and serine/arginine-rich splicing factors 1 and 3 [21]. Alternatively, splicing factors that cause skipping of exon 11 include heterogeneous nuclear riboprotein A1 [22] and CUG-binding protein 1 [21]. Expression of the IR isoforms is developmentally regulated. Fetal tissue including brain, muscle, liver, kidney, and fibroblasts were assayed for IR-A expression and compared to adult tissues. In all fetal tissues except brain, there was higher expression of IR-A than the respective adult tissues [15]. Expression of the two IR isoforms is also tissue specific. The full-length form can be mostly found in insulin-sensitive tissues such as liver, muscle, adipocytes and kidney whereas the IR-A form is widely expressed [23]. Both forms of the insulin receptor can produce homo- and heterodimers as well as hybrid receptors with IGF-1R allowing for crosstalk between these two receptor families and for a complex signaling axis [24].

Binding affinity and receptor activation for IGF-1R and both forms of IR receptors has been characterized [15,25]. IGF-1R is activated

by IGF-1 and IGF-2, which bind to the extracellular α-subunit of the receptor and cause a conformational change in the β-subunit. This leads to autophosphorylation of the β-subunit and recruitment of adapter proteins and subsequent activation of mitogenic signaling cascades including Mitogen Activated Protein Kinases (MAPK) and phosphatidylinositol 3-kinase (PI3K)-Akt pathways to promote cell growth and motility and antiapoptotic signaling [26-28]. Both forms of the IR have high affinity for insulin, which leads to primarily metabolic effects through PI3K-Akt signaling. It is also known that IGF-2 can stimulate the IR to activate the mitogenic pathways. Previous studies using mouse fibroblasts deficient for IGF-1R and expressing low levels of IR failed to proliferate in serum-free conditions when stimulated with growth factors. When IR is expressed, these cells are able to grow when stimulated with IGF-2 [29]. Subsequent studies demonstrated that IR-A is the isoform that is stimulated by IGF-2, activating mitogenic pathways and allowing for proliferation of these receptor-deficient fibroblast cell lines [15].

IR-A binds IGF-2 and is auto-phosphorylated with relatively high affinity, whereas IR-B only has strong affinity for insulin. It has also been shown that hybrid receptors with IGF-1R and IR-A can also bind IGF-2 along with IGF-1 and insulin (Figure 1) [30]. Activation by these two types of ligands leads to activation of distinct cellular processes. When the receptors bind IGFs or insulin, this induces structural changes and subsequent auto-phosphorylated of tyrosine residues like that of IGF-1R.While IR-B signaling promotes mainly metabolic processes related to glucose homeostasis, IR-A also activates mitogenic signaling cascades when stimulated by IGF-2.

IR-A and cancer

It is becoming increasingly evident in recent decades the prevalence of IR-A in a variety of cancer cell types. A number of labs have reported increased IR-A:IR-B ratios in a number of neoplasms including breast, colon, lung, thyroid, liver, and bone cancers [15,31-34]. It was reported by Sciacca and colleagues [15] that breast cancer tissue samples had increased IR-A expression (40-80% IR-A) as compared to normal breast tissue (30-50% IR-A). They also determined IGF-2 stimulated breast cancer cell growth. Moreover, the potency of IGF-2 was correlated to IR-A expression, indicating the presence of autocrine and paracrine signaling via the IGF-2/IR-A interaction. Similar overexpression of IR-A in thyroid cancer cells and tissue specimens was reported [32]. Interestingly, poorly differentiated thyroid cells produced IGF-2 and overexpressed IR-A, again indicating the presence of an autocrine loop promoting cancer cell growth. Similar lines of investigation confirmed over expression of IR-A in prostate, lung, leiomyosarcoma, osteosarcoma, and colon cancer cells and tissue samples, further emphasizing the pervasiveness of IGF-2/IR-A signaling in cancer cells [12,15,31,34-36].

IR-A resistance in IGF-1R therapies

Therapeutic strategies have focused on inhibiting tumor growth through the IGF-1R signaling axis due to the frequent over expression of this receptor in cancer cells and its key role in regulating cell proliferation and apoptosis. The three most investigated strategies include: receptor-targeting antibodies, tyrosine kinase inhibition, and ligand-targeting neutralization antibodies [8]. Therapies targeted to IGF-1R have been promising but there is evidence to suggest that the IR compensates for IGF-1R inhibition as this single line of therapy is not sufficient to inhibit tumor growth. A recent study of Ewing's sarcoma investigated the effect of several anti-IGF-1R therapies, and the researchers found that cells not responding to these drugs

had higher expression of IR-A. They report that tumors with a low ratio of IGF-1R:IR are unlikely to benefit from anti-IGF-1R therapies [37]. They also conclude Ewing's sarcoma cells may adapt to anti-IGF-1R therapies through activation of an IR-A-dependent pathway. Increased IGF-2 expression was also noted in resistant cells indicating a switch from IGF-1/IGF-1R to IGF-2/IR-A dependency to maintain mitogenic signaling pathways.

A recent study by Forest and colleagues investigated the correlation of resistance of cixutumumab, an anti-IGF-1R and IR and IGF-1R expression [12]. By analyzing transcript levels from tissue samples in their tumor models (Rhabdoid, Ewing's sarcoma, rhabdomyosarcoma, glioblastoma, neuroblastoma and osteosarcoma), they discovered that high IR expression levels indeed correlated to poor antitumor efficacy of cixutumumab. It is interesting to note that IR-A was the predominant form present in the tissue samples and IR-B expression was rather weak but IR-A expression alone failed to correlate cixutumumab efficacy, suggesting that both forms of IR may contribute to anti-IGF-1R resistance. In experiments using stably induced breast cancer cells over expressing IR-B, treated with cixutumumab or an anti-IGF-2 neutralizing drug, there was only partial inhibition of colony formation. However, when cells were treated with both therapies, the drug resistant phenotype was reversed, suggesting that IGF-2 is implicated in the resistance of tumor phenotypes by IR-B along with previously reported IR-A resistance to anti-IGF-1R therapies [13]. It is also possible that resistance is conferred through IR and IGF-1R hybrid receptors but it has been shown that cixutumumab and other anti-IGF-1R therapies are very effective at receptor internalization and subsequent degradation, effectively neutralizing hybrid receptors [38].

Conclusion

IGF-1R is expressed at high levels in several tumor types inclusive of breast, ovarian, prostate, head and neck, and squamous lung cancer and tumor types [12]. The IGF-signaling axis plays a key role in promoting cellular proliferation and inhibiting apoptosis and represents an attractive and heavily developed avenue for therapeutic development. Another key player in this complex signaling pathway is the IR, which exists in two isoforms that result from alternative splicing and give rise to receptors with different ligand affinities and signaling outcomes. It is becoming apparent that the unexpected poor performance of therapies targeting the IGF-signaling axis through IGF-1R inhibition and ligand neutralization can be attributed to IGF-2 stimulation of IR-A, activating mitogenic pathways and circumventing this therapeutic strategy. A recent study highlighted the prevalence of IR-A expression with an RNA-seq analysis that evaluated RNA expression in 6,943 samples representing 21 tumor types and found IR-A to be present in all tumor types. This same study also provides evidence for IGF-2 stimulation through IR-B to promote cellular growth, further complicating this signaling cascade [12]. It is clear that co-targeting of IGF-1R and IR is necessary for an effective therapeutic strategy. This will represent a delicate task since IR expression is required for glucose metabolism, a critical cellular process. Therefore, it is critical to fully understand the action of the two IR isoforms and their role in mitogenic and metabolic signaling pathways to effectively target tumor cells and to develop ways to overcome tumor resistance associated with IR isoforms.

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