IGF system in cancer: from bench to clinic

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Insulin-like growth factors (IGFs) are important mediators of growth, development, and survival, and have been implicated in the pathogenesis of malignancies. The IGF system is a complex system comprising two growth factors (IGF-I and IGF-II), cell surface receptors (IGF-IR and IGF-IIR), six specific high-affinity binding proteins (IGFBP-1 to IGFBP-6), IGFBP proteases, and several other IGFBP-interacting molecules that regulate and propagate IGF actions in several tissues. IGFs are produced by almost any cell in the body; circulate in more than 1000-fold higher concentrations than most other peptide hormones, such as insulin, and their action is modulated by several binding proteins. Studies have revealed that IGFs may promote cell cycle progression and inhibition of apoptosis either by directly associating with other growth factors or indirectly by interacting with other molecular systems that have an established role in carcinogenesis and cancer promotion, such as steroid hormones and integrins. In addition, studies also suggest that increased serum levels of IGFs and/or altered levels of their binding proteins are associated with increased risk of developing cancers. These data underline the significance of IGFs system in the development of cancer risk, and a potential target for novel anticancer treatments and/or preventative strategies in high-risk groups. The researchers review the IGFs pathway and its implications in cancer development and treatment. *Anti-Cancer Drugs* **22:206–212 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Insulin-like growth factor system background information

The insulin-like growth factor (IGF) system has been extensively studied and has been shown to have an integral role in normal growth and development and in the pathophysiology of various malignancies. Research detailing the different aspects of the IGF system has been ongoing since the 1970s. The IGF system has been shown to consist of a series of circulating ligands, transmembrane receptor tyrosine kinases, circulating hormones, and ligand-binding proteins that work in synchrony to regulate cell growth. Dysregulation of the IGF system has been implicated in carcinogenesis and as early as the 1980s, upregulation of IGF receptors was identified in resected surgical specimens from various solid tumors [1].

The IGF system has been shown to have two key circulating ligands, IGF-I and IGF-II, which share an approximately 50% homology to insulin [2]. IGF-I is produced primarily in the liver as a response to the circulating levels of the growth hormone [3]. IGF-I and IGF-II target specific cell surface receptors are designated as IGF-IR and IGF-IIR. The IGF-IR and the insulin receptor share approximately 60% homology and as such can form hybrid receptors [4]. On account of the homology shared between IGF-I, IGF-II, and insulin, IGF-IR may also be activated by IGF-II and insulin. However, the affinity of IGF-IR to IGF-II and insulin is approximately 10-fold and 1000-fold lower than for IGF-I, respectively [5].

The IGF system is regulated by a family of receptors that exist as hybrid heterotramers and homotetramers that regulate cell metabolism and growth. Two distinct insulin receptor isoforms have been identified and are known to hybridize to IGF-IR. The insulin receptor isoform A (IR-A) is generated through the deletion of exon 11 of the insulin receptor gene whereas the insulin receptor isoform B (IR-B) retains the exon 11 [6]. In normal vertebrate development, IR-A is the predominant isoform in fetal tissues whereas IR-B appears in postnatal life within insulin-target tissues [7,8]. IR-B signaling seems to stimulate differentiation and maturation whereas increased IR-A signaling seems to favor undifferentiated proliferation [9–13]. This is supported by various studies that have identified a substantially increased IR-A:IR-B ratio in various poorly differentiated malignancies including breast cancer, poorly differentiated thyroid carcinoma, colon cancer, and lung cancer [13–15].

Knockout studies undertaken in the 1990s helped to delineate the role of the IGF system in normal embryonic and postnatal development [16]. These studies showed that mice lacking IGF-I, IGF-II, and IGF-IR, displayed a substantial reduction in the rate of mitosis during

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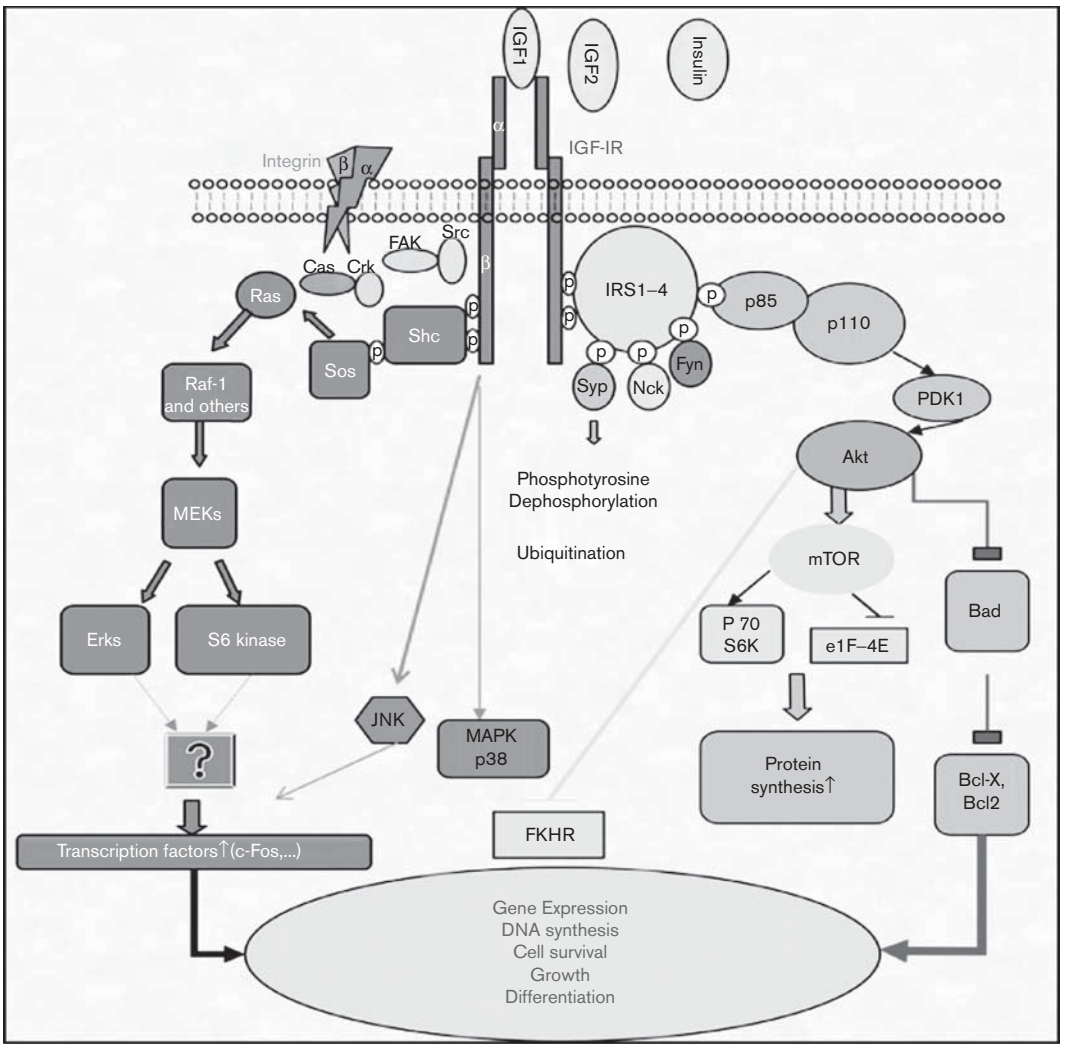
embryogenesis and postnatal development. This resulted in a decreased overall size of the mice and significant organ hypoplasia. IGF-IR null mice, invariably, die shortly after birth.

IGF-IR is a heterotetrameric transmembrane receptor with a structure consisting of two extracellular α subunits and two intracellular β subunits linked by disulfide bonds [17]. The intracellular component of IGF-IR has intrinsic tyrosine kinase activity that requires ligand binding for activation [18]. Overexpression of IGF-IR alone is therefore thought not to be sufficient for receptor activation. The downstream signals of IGF-IR are primarily mediated by the insulin receptor substrate (IRS) family of adaptor proteins [19]. IGF-IR-mediated phosphorylation of IRS-1 activates the Ras/Raf pathway leading to mitogen-activated protein kinase (MAPK)-induced cell growth and proliferation (Fig. 1) [20]. IRS-1 phosphorylation by IGF-IR also activates PI3K and Akt leading to inhibition of proapoptotic signals [21].

IGF-IR is also thought to increase cell motility through two mechanisms. First, IRS-1 phosphorylation can influence the interaction between E-cadherin and β-catenin, leading to decreased cell–cell contact [22]. Second, increased cell motility has been shown through IGF-IR phosphorylation of IRS-2 and subsequent changes in integrin expression [23,24]. Conversely, IGF-IIR lacks an

Fig. 1



Insulin-like growth factor (IGF)-I signaling pathway. Activation of the IGF I surface receptor (IGF-IR) by IGF ligands results in enhanced proliferation and strong survival signals in tumor cells. Although complex, the signaling pathways involved are primarily the Ras-Raf-mitogen-activated protein kinase (MAPK)-Erk proliferation pathway and the PI3-PKB-Akt survival pathway. Exposure of MCF-7 breast cancer cells to either the IGF-I or IGF-II ligand results in increased phosphorylation of key signaling proteins in these two pathways. Adapted from Samani *et al. Endocr Rev* 2007; 28:20–47. FKHR, forkhead transcription factor; MEK, methyl ethyl ketone; mTOR, mammalian target of rapamycin.

intracellular tyrosine kinase domain and its primary function is believed to be to decrease the effects of circulating IGF-II [25].

The IGF system is also regulated by a group of at least six high-affinity ligand-binding proteins (IGFBPs). IGF-I circulates almost entirely bound to IGFBPs and the acid labile subunit in a large ternary complex [26,27]. Interestingly, these IGFBPs serve not only to increase the half-life of the IGF-I (when bound to acid labile subunit), but, in addition, they have been shown to have biological activity that is independent of IGF-I/IGF-IR. The IGF-I/IGF-IR-independent actions of IGFBPs seem to be quite diverse and are not well understood. Experiments have shown these proteins to increase both proliferative and apoptotic signaling depending on the cell line they are exposed to *in vitro*. IGFBP-3, for example, has been shown to augment cellular proliferation of human breast cancer cells, but when exposed to chick embryo fibroblasts and mouse embryo fibroblasts, IGFBP-3 has been shown to inhibit growth and proliferation [28–31].

Insulin-like growth factor system in cancer

The IGF system has been shown to play an important role in controlling the rate of cell proliferation and apoptosis. These discoveries have led to intense research in describing the role of the IGF system in carcinogenesis. Resected tumor specimens have been shown to contain high concentrations of IGF-IR [1]. Furthermore, population studies have also shown that high levels of circulating IGF-I and IGFBP-3 may be independent risk factors to developing several different cancers including colorectal cancer, prostate cancer, breast cancer, and lung cancer [32–34].

Early knockout studies have shown that the IGF system plays a key role in controlling the rate of mitosis of cells during embryogenesis and postnatal development [6]. Therefore, individuals with higher levels of circulating IGF-I have a higher baseline rate of mitosis occurring concomitant with increased activation of the Ras/Raf and PI3K pathways and increased cell motility signaling. This increased rate of mitosis occurring in the backdrop of a microenvironment favoring survival and motility is believed to increase the probability of developing somatic mutations sufficient for carcinogenesis.

Dietary factors and lifestyle have also been shown to have a significant effect on the IGF system activation [35]. In animal models a starvation diet significantly reduces the activation of the IGF system [36]. This may, in part, account for why animals subjected to a starvation diet live significantly longer and have decreased the rates of carcinogenesis when compared with their obese and non-diet-restricted counterparts. As expected, experiments have shown that the protective effects of starvation can be reversed through the infusion of IGF-I [37].

Strategies to target the insulin-like growth factor system

As a result of the compelling evidence implicating the IGF system in carcinogenesis, agents that target different components of the IGF system are currently being developed. Strategies to interrupt the IGF system include the use of monoclonal antibodies (moAbs) targeting IGF-IR, small molecule tyrosine kinase inhibitors (TKIs) of IGF-IR, reducing IGF-IR gene expression by antisense RNA and oligonucleotide technologies, decreasing IGF-I levels through growth hormone antagonists or growth hormone-releasing hormone antagonists, and developing agents to modulate IGFBPs. Preclinical data suggest that agents used to target the IGF system may be more effective when used in combination with chemotherapy versus when used as monotherapy [38,39].

Immunotherapy/monoclonal antibodies

MoAbs targeted against IGF-IR are the furthest in development and are currently being studied in multiple trials in various malignancies. Designing antibodies with a high degree of affinity to IGF-IR remains an attractive treatment option because, in theory, they could inhibit the IGF system with minimal cross-inhibition of the insulin receptor and glucose metabolism. Preclinical and early clinical data indicate that moAbs targeted against IGF-IR may be more effective when used in combination with traditional chemotherapy versus being used as monotherapy [40]. This correlates with earlier clinical trials using other moAbs; trastuzumab and bevacizumab, although active as single agents, have been shown to have increased efficacy when used in combination with chemotherapy.

Hyperglycemia has been a common toxicity encountered in clinical trials using moAbs targeting IGF-IR. The exact mechanism of action for why these agents lead to hyperglycemia remains unclear. A potential drawback to the use of moAbs is that although they may be highly specific toward IGF-IR, they may also bind to hybrid insulin–IGF-I receptors. The interaction with hybrid receptors could, in part, account for why hyperglycemia has been a toxicity observed with the use of various moAbs targeting IGF-IR. Another potential explanation for why highly specific IGF-IR moAbs induce hyperglycemia involves the disruption of the hypothalamic–pituitary axis. MoAbs systemically decrease IGF-IR signaling, which may interrupt the negative feedback signal that IGF-I exerts on the hypothalamus and the pituitary gland. This disruption could induce increased circulating growth hormone levels leading to increased liver gluconeogenesis and systemic insulin resistance [41,42].

Another potential problem with the strategies that selectively target IGF-IR is that they may be overcome by the proliferative and antiapoptotic signaling of IR-A.

Various tumor subtypes have been shown to have markedly increased levels of IR-A, which is known to hybridize with IGF-IR. The relative abundance of IR-A and IGF-IR/IR-A hybrids in poorly differentiated cancer cells may confer resistance to strategies that are highly selective for the IGF-IR.

Small molecule inhibitors of insulin-like growth factor I surface receptor: tyrosine kinase inhibitors and inhibition of insulin-like growth factor I/insulin-like growth factor I surface receptor binding

The intracellular effects of the IGF-IR are initiated through a tyrosine kinase domain that is activated after the binding of the circulating ligand. The binding of IGF-I to the extracellular IGF-IR α-domain triggers a conformational change in the intracellular β-domain, which, in turn, initiates tyrosine kinase activity [43]. Small molecules that bind and inhibit the tyrosine kinase domain of IGF-IR are attractive potential therapeutic agents. The tyrosine kinase domain of IGF-IR shares substantial homology with the insulin receptor; however, and like moAbs, TKIs must achieve a high degree of specificity to minimize potential toxicity. There are increasing emerging data describing the selectivity and efficacy of TKIs in preclinical models. NVP-AEW541, NVP-ADW742, and cyclolignan picropodophyllin (PPP, AXL 1717) are examples of compounds that have been shown to selectively inhibit the IGF-IR tyrosine kinase in preclinical models [44–49]. Other small molecules such as AG 538 have been reported to selectively bind IGF-IR and prevent ligand-dependent activation [50].

In an effort to minimize hyperglycemic toxicities inherent in insulin receptor manipulation, drug development has focused on finding highly selective inhibitors of IGF-IR. It remains to be seen in large randomized clinical trials whether the strategies using moAbs and TKIs will be able to deliver the therapeutic antineoplastic benefits seen in the laboratory without significant insulin-related toxicities. Furthermore, as the insulin receptor has also been implicated in cancer proliferation, it is possible that strategies that completely avoid the insulin receptor will be less effective than those that have dual receptor activity.

Decreased insulin-like growth factor I surface receptor expression

Another potential therapeutic strategy targeting the IGF system involves the inhibition of IGF-IR expression. One method being developed to decrease the expression of IGF-IR involves engineering antisense oligonucleotide sequences designed to be complimentary to the IGF-IR transcript. Preclinical experiments and early clinical trials indicate that antisense oligonucleotide sequences may inhibit the IGF-IR system by two mechanisms. First, by directly forming duplexes with the IGF-IR transcript, translation is inhibited [51–53]. Second, the formation of these duplexes is hypothesized to elicit a targeted immune response against the affected cells [53–56].

An additional approach being studied to decrease the IGF-IR expression involves the use of small interfering RNAs or short hairpin RNAs. Small interfering RNAs and short hairpin RNAs can be delivered through a virus or a plasmid vector and have been shown *in vitro* to effectively inhibit IGF-IR synthesis and expression [57–59]. When compared with TKIs and moAbs targeted against IGF-IR, these treatment strategies have the potential advantage of being highly specific to the IGF-IR transcript and can disrupt the IGF system without interfering with the insulin receptor and glucose metabolism. This treatment strategy is early in the process of development and although phase I and preclinical data are encouraging, larger randomized clinical trials evaluating the efficacy and safety of these novel agents are needed. Furthermore, their use in combination with traditional chemotherapy or radiotherapy, either concurrently or sequentially, remains of great interest.

Targeting the insulin-like growth factors binding proteins

IGFBPs have been shown to be essential components that help to regulate the IGF system. They have also been shown to have diverse biological effects that are independent of IGF-I/IGF-IR signaling and may be of potential therapeutic significance. More research is needed to better understand exactly how these proteins exert their effects on cellular function. In the future, proteases targeting a specific IGFBP may prove significant in targeting certain malignancies. Alternatively, increasing the serum concentration of a specific IGFBP may be warranted in tumors with a specific genetic signature (Table 1).

Translational correlates

On the basis of the preponderance of evidence implicating the IGF system in carcinogenesis, studies have attempted to determine whether IGF-IR expression is of prognostic importance. Cappuzzo *et al.* [76] published a study in 2006 that looked at IGF-IR expression in 77 patients with metastatic nonsmall cell lung cancer treated with gefitinib. Although no differences were identified in response rates, a statistically significant difference of 10.3 months (17.8 vs. 7.3) in median survival favoring IGF-IR expression was identified ($P < 0.013$). Several other studies have confirmed that IGF-IR expression portends improved survival in patients treated with antiepidermal growth factor receptor (EGFR) agents [77,78]. Interestingly, a retrospective study of 369 patients who underwent surgical resection of nonsmall cell lung cancer did not find IGF-IR expression to independently correlate with survival. In this retrospective analysis, none of the patients were treated with anti-EGFR agents [79]. It is not clear why patients who

Table 1 Drugs targeting the insulin-like growth factor system in development

Compound	Phase of development	Manufacturer	References
IGF-IR monoclonal antibodies			
Figitumumab/CP 751 871	Phase II/III	Pfizer	[40,60]
Cixutumumab/IMC-A12	Phase II	Imclone	[61]
Ganitumab/AMG 479	Phase II	Amgen	[62]
Dalotuzumab/MK0646	Phase I/II/III	Merk	[63]
AVE 1642	Phase II	Immunogen and Sanofi Aventis	[64]
BIIB022	Phase I/II	Biogen	[65]
SCH 717454	Phase II	Schering-Plough	[66]
R1507	Phase I	Roche	[67]
h7c10	Preclinical	Merck	[68]
19D12	Preclinical	Scheirng-Plough	[69]
Small molecule tyrosine kinase inhibitors			
OSI-906	Phase III	OSI pharmaceuticals	[70]
AXL1717	Phase I	Axelar	[48,49]
BMS-754807	Phase I/II	Bristol-Myers Squibb	[71]
BMS-554417	Preclinical	Bristol-Myers Squibb	[72]
BMS-536924	Preclinical	Bristol-Myers Squibb	[73]
NVP-ADW742	Preclinical	Novartis	[44]
NVP-AEW541	Preclinical	Novartis	[45]
Dual inhibitors			
INSM-18	Phase I/II	Insmed	[74]
EXEL-228: IGF-IR & Src	Phase I/II	Exelixis	[75]
Other agents			
LR4437-001A	Preclinical	Lynx therapeutic	[53]

IGF-IR, insulin-like growth factor I surface receptor.

overexpress IGF-IR tend to do better when treated with anti-EGFR therapies. One possible explanation is that the tumors coexpressing IGF-IR and EGFR are more driven by the Ras-Raf-MAPK pathway. Downstream signals triggered by the activation of the EGFR and IGF-IR converge on the Ras-Raf-MAPK pathway leading to increased survival. IGF-IR over-expression may be a surrogate marker for increased MAPK activation. This may, in part, account for the observation that patients who coexpress EGFR and IGF-IR have improved outcomes when treated with anti-EGFR agents. Prospective trials looking at the coinhibition of IGF-IR and EGFR are needed to better understand the biology of these tumors. Further study of the mechanism of improved clinical benefit of anti-EGFR therapy in patients with high IGF-IR expression is warranted.

The role of circulating tumor cells (CTCs) as prognostic markers is also being studied. CTCs have been shown to express IGF-IR, but it is not clear whether this is of prognostic or therapeutic significance [80]. Determining the ratio of IGF-IR + CTCs to IGF-IR – CTCs before and after treatment with systemic therapy could provide important prognostic information. Furthermore, the degree to which IGF-IR + CTCs decrease after treatment may also confer important treatment information. Identifying the ratio of IGF-IR + CTCs to IGF-IR – TCs may prove to be an important surrogate marker of IGF system activation and prognosis.

In analyzing the results of the ongoing trials using strategies that target the IGF system it will also be important to identify mechanisms of resistance. Preclinical data suggest that signaling from the IR-A isoform and IGF-IR/IR-A hybrids may overcome targeted IGF-IR inhibition. Analyzing the overall expression of IR-A and the IR-A:IR-B ratio in cancer cells of patients who do not respond to IGF-IR targeted therapies could provide valuable information. Moreover, strategies that target both IR-A and IGF-IR may prove superior to targets that are highly selective to IGF-IR. Further studies are needed to fully understand the role of increased IR-A expression in cancer.

Conclusion

Preclinical data and early clinical trials using agents targeting IGF-IR suggest that disrupting the IGF system may be a clinically significant therapeutic strategy with applications in various malignancies. The data thus far indicate that these agents are generally well tolerated and have a relatively favorable toxicity profile. However, large randomized clinical trials using these agents as monotherapy and in combination with traditional chemotherapy or radiotherapy are needed to evaluate their efficacy and long-term safety profile. Developers of the agents targeting the IGF system are faced with the challenge of devising an assay that can identify those patients that are likely to benefit from the inhibition of the IGF system. More than 30 years of research defining the different components of the IGF system has culminated in the development of targeted agents that may be of clinical benefit in a wide range of malignancies.

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