

Drug Development Series: Review

Disrupting insulin-like growth factor signaling as a potential cancer therapy

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Abstract

The type I insulin-like growth factor receptor (IGF-IR) plays multiple roles in several cancers and increased circulating levels of insulin-like growth factor-I (IGF-I) are associated with increased risk of breast, colon, and prostate cancers. Because IGF-II and insulin signal via the insulin receptor (IR) to stimulate the growth of cancer cells, inhibition of IR might be necessary to totally disrupt the action of IGFs and their receptors. This review describes the well-recognized roles of IGF-IR in driving the malignant phenotype, examines the evidence that perhaps IR should also be targeted to inhibit the effects of the IGF ligands and insulin in cancer, describes the strategies to disrupt IGF signaling in cancer, and highlights some key issues that need to be considered as clinical trials targeting IGF-IR proceed. [Mol Cancer Ther 2007;6(1):1–12]

Introduction

In the 20 years since the purification and cloning of the first receptor tyrosine kinase (RTK; ref. 1), much progress has been made in understanding their structure, the mechanisms RTKs use to signal to intracellular messengers (2, 3), and the roles they play in oncogenesis and maintenance of the malignant phenotype (4, 5). The success of drugs such as trastuzumab (Herceptin), a humanized antibody against HER2, in the treatment of early and advanced breast cancer (6–8), and cetuximab (Erbix), an antibody against epidermal growth factor receptor (EGFR) in colorectal

cancer (9), has proved that targeting growth factor receptors is beneficial to patients. Furthermore, targeting the tyrosine kinase activity of RTKs has been successful in cancer therapies, as exemplified by the use of erlotinib (Tarceva), which targets the activity of EGFR in non-small-cell lung cancer (10). This suggests that both the expression and activity of RTKs play important roles in the acquisition or maintenance of the transformed phenotype.

The insulin-like growth factor (IGF) system is composed of the circulating ligands, insulin-like growth factor-I (IGF-I), IGF-II, and insulin; multiple receptors; and binding proteins. The type I IGF receptor (IGF-IR) is a RTK closely related to the insulin receptor (IR). In normal physiology, ligand activation of IGF-IR plays a role in fetal growth and linear growth of the skeleton and other organs, whereas insulin acts via IR to regulate glucose homeostasis.

Multiple lines of evidence have shown that IGF-IR plays a role in maintaining the malignant phenotype (11), and disruption of IGF-IR activation has been shown to inhibit growth and motility of a wide range of cancer cells *in vitro* and in mouse models (12, 13). It is also becoming increasingly evident that stimulation of IR by insulin or IGF-II (14) enhances mitogenesis in cancer cells, suggesting that perhaps both IGF-IR and IR might be targets for cancer therapy. Thus, inhibition of multiple RTKs may be necessary to maximally inhibit tumor growth. This must be balanced by the fact that inhibition of IR leads to type II diabetes, which is a major health concern.

IGF System Components

The IGF system is composed of multiple receptors and ligands as shown in Fig. 1.

Type I IGF Receptor

IGF-IR is composed of two covalently linked polypeptide chains, each with an extracellular α -subunit and a transmembrane β -subunit, which possesses tyrosine kinase activity (15), as shown in Fig. 1. IGF-IR is transported to the membrane fully assembled in the dimeric form, and ligand binding of IGF-I or IGF-II to IGF-IR results in a conformational change leading to transphosphorylation of one β -subunit by the other. Activated IGF-IR recruits and phosphorylates adaptor proteins belonging to the insulin receptor substrate (IRS) family or SHC. The phosphorylated adaptor proteins then serve as docking sites for other signaling molecules, resulting in the activation of the downstream pathways such as phosphatidylinositol 3'-kinase and extracellular signal-regulated kinase 1/2 of the mitogen-activated protein kinase (MAPK) pathways, as shown in Fig. 1.

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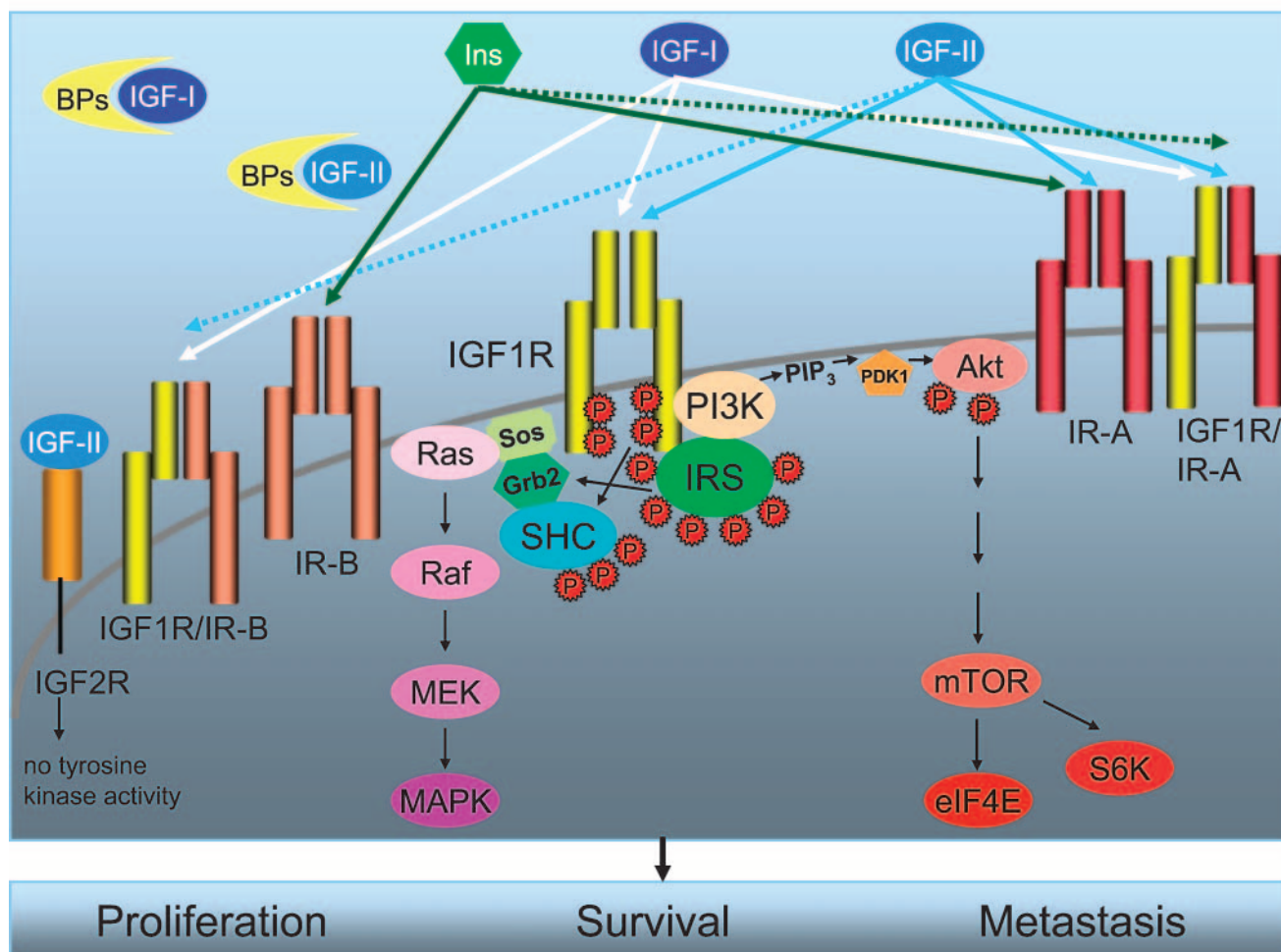


Figure 1. The IGF system components. The IGF system is composed of three ligands: IGF-I, IGF-II, and insulin. IGF-I and IGF-II are found in the circulation complexed to a family of binding proteins, called IGF binding proteins (BPs), which serve to regulate bioavailability of these ligands in the tissues. There are multiple receptor conformations. IGF-IR is a transmembrane tyrosine kinase and consists of two $\alpha\beta$ chains and has high affinity for both IGF-I and IGF-II. The insulin receptor (IR) mediates the effects of insulin. Two isoforms of IR, IR-A and IR-B, are found in breast cancer cells, with IR-A being overexpressed in many cancers. In addition, hybrid receptors assembled with one $\alpha\beta$ chain of IGF-IR (*IGF1R*) and one $\alpha\beta$ chain of IR-A or IR-B also exist. Arrows, ligand specificity of the various receptors; solid arrows, high-affinity binding; dotted arrows, low-affinity binding. Thus, IGF-I binds to IGF-IR and IGF-IR/IR-B with high affinity (solid white arrows) and to IGF-IR/IR-A hybrid receptor with much reduced affinity (white dotted arrow). IGF-II binds to IGF-IR, IR-A, and hybrid IGF-IR/IR-B and IGF-IR/IR-A receptors. For simplicity, only signaling initiated by the activated IGF-IR is shown. Activation of IGF-IR results in phosphorylation of adaptor proteins belonging to the IRS family or SHC. Activation of IRS and SHC leads to activation of extracellular signal-regulated kinase (*ERK*) 1/2 of the MAPK cascade via the growth factor receptor binding protein 2 (*Grb2*)/*Sos*/Ras/Raf/MAPK extracellular signal-regulated kinase (*MEK*) pathway. IRS proteins also bind to the p110 subunit of phosphatidylinositol 3'-kinase (*PI3K*), leading to the generation of phosphatidylinositol 3,4,5-triphosphate (*PIP*₃) and phosphorylation of Akt by phosphoinositide dependent kinase (*PDK1*). Phosphorylation of Akt leads to subsequent activation of mammalian target of rapamycin (*mTOR*), eukaryotic translation initiation factor 4E (*eIF4E*), and p70S6 kinase (*S6K*). Activation of these downstream signaling pathways leads to enhanced proliferation, survival, and metastasis in cancer cells. Similar signaling pathways are activated by IR and the various hybrid receptors shown.

Insulin Receptor

IGF-IR shares a high degree of homology to IR with their kinase domains exhibiting 84% homology (16). Two isoforms of IR are generated by alternative splicing of exon 11, giving rise to the exon 11+ or B-isoform (IR-B) and exon 11- or A-isoform (IR-A), which lacks the 12 amino acids due to exclusion of exon 11. The two isoforms are expressed in a developmentally specific manner with IR-A more highly expressed in fetal tissue and IR-B found in

adult tissues. IGF-II binds IR-A with high affinity whereas IGF-I does not (17). Activation of IR also leads to phosphorylation of IRS adaptor proteins and activation of downstream phosphatidylinositol 3'-kinase and MAPK pathways. Evidence from gene deletion studies suggests that the functions of IR and IGF-IR, although physiologically distinct, are still partially overlapping, with IR capable of stimulating growth (18) and IGF-IR able to regulate a metabolic response (19). It is believed that, in addition to

intrinsic signaling differences, tissue distribution, and relative number of cell-surface receptors, developmental regulation could determine the specificity of signaling via IR compared with IGF-IR (20).

Hybrid Receptors

Many cells and tissues have hybrid receptors assembled with one chain of the IGF-IR and one of the IR (21, 22). Both IGF-IR/IR-A and IGF-IR/IR-B hybrids have been described. IGF-IR/IR-B hybrids have higher affinity for IGF-I whereas IGF-IR/IR-A hybrids have equal affinity for IGF-II and insulin (23). The existence of these hybrid receptors adds another level of complexity to the insulin/IGF system.

Type II IGF Receptor

The type II IGF receptor (IGF-IIR) or the cation-independent mannose-6-phosphate receptor (24) binds IGF-II but lacks tyrosine kinase activity and does not transduce signals (24). It binds IGF-II, among other proteins, and seems to serve as a sink for IGF-II. Loss of heterozygosity of IGF-IIR has been shown in breast cancer. It has been hypothesized that loss of functional IGF-IIR allows for enhanced interaction of IGF-II with IGF-IR.

IGF-IR Is a Target for Cancer Therapy

Multiple lines of evidence implicate IGF-IR, IGF-I, and IGF-II in various aspects of the malignant phenotype, suggesting that IGF-IR is a target for cancer therapy.

IGFs, IGF-IR, and Cancer

The IGF system has been implicated in several different cancers including breast cancer, prostate cancer, colon cancer, liver cancer, pancreatic cancer, melanoma, multiple myeloma, mesothelioma, glioblastoma, and childhood malignancies. Abundant data from cell culture, animal, and human epidemiologic studies have suggested that IGFs and IGF-IR regulate all aspects of the malignant phenotype. IGF-IR is not activated by overexpression as seen with the EGFR family of receptors and can only be activated by ligands (25). Thus, activation of IGF-IR is ligand dependent; endocrine as well as autocrine/paracrine sources of IGFs have been implicated. Liver-specific IGF-I-deficient mice that were created by the Cre/loxP recombination system and have a 75% reduction in circulating levels of IGF-I compared with control mice (26) showed decreased growth of both the primary colon tumors and hepatic metastases (27). Furthermore, these mice also showed decreased incidence of mammary tumors (28). Epidemiologic studies have shown that high circulating levels of IGF-I are associated with increased risk of developing breast, prostate, colon, and lung cancer (29, 30). High circulating IGF-I levels have also been reported in breast cancer patients. Transgenic mice overexpressing IGF-I or IGF-II showed increased incidence of mammary tumors (31, 32). There is increased expression of IGF-I and IGF-II in many cancers including breast cancer and multiple myelomas (33).

IGF-IR stimulates proliferation, survival, and motility of various cancer cells. Overexpression of IGF-IR leads to

ligand-dependent transformation of fibroblasts (25). Fibroblasts derived from mice lacking IGF-IR cannot be transformed by some (34), but not all (35), oncogenes. In several cancers, overexpression and increased tyrosine kinase activity of IGF-IR have been reported (36).

IGF-IR and Metastasis

The role of IGF-IR in regulating tumor growth is well understood. However, it is becoming increasingly clear that IGF-IR can also regulate other phenotypes associated with malignancy. Activation of IGF-IR results in recruitment of multiple adaptor proteins and IGF-stimulated phenotypes may be dependent on activation of specific adaptor proteins species. We have shown that IRS-1 activation is associated with proliferation of cancer cells (37) whereas IRS-2 is associated with motility of breast cancer cells (38). These observations have also been confirmed *in vivo* by studies showing that IRS-2 null animals had significantly decreased incidence of metastasis compared with wild-type mice when mated with transgenic mice that express the polyoma virus middle T antigen in the mammary gland (39). Several studies have reported that inhibition of IGF-IR inhibits metastasis of various cancer cells (40–42).

Dunn et al. (41) have shown that a soluble truncated IGF-IR inhibited metastasis of MDA-435 breast cancer cells. Using a COOH-terminally truncated dominant negative IGF-IR construct that was overexpressed in a metastatic variant of MDA-435 cells, LCC6 cells, we have shown that cells with both wild-type and dominant negative IGF-IR formed tumors in mice (42). However, only mice harboring parent LCC6 cells with wild-type IGF-IR formed lung metastases. These results indicate that IGF-IR can regulate metastasis independently from tumor growth. Using a similar dominant negative IGF-IR approach, it has also been shown that IGF-IR regulates liver metastasis of colon cancer cells as colon cancer cells with dominant negative IGF-IR failed to form liver metastases after splenic injection or direct injection into the liver of mice (43).

Furthermore, in a transgenic mouse model of pancreatic islet cell tumorigenesis, RIP1-Tag2 mice, in which SV40 T antigen (Tag) oncoproteins are expressed in the β cells of the pancreatic islets under the control of the rat insulin promoter (RIP), high levels of IGF-IR increased invasive carcinomas and lymph node metastases (44). These suggest that IGF-IR plays an important role in the metastasis of cancer cells.

Thus, the preclinical data suggest that IGF-IR is an important target for cancer therapy. To validate this potential, drugs directed against this receptor target need to be tested in clinical trials. Several approaches have been used to inhibit signaling via IGF-IR as described later in this review.

Is IR Also a Target for Cancer Therapy?

Whereas the metabolic effects of insulin and IR on glucose metabolism have been widely recognized and studied, it

has also been recognized for decades that insulin can stimulate proliferation of breast cancer cells (45). Several lines of evidence show that, along with both IGFs, insulin, acting via IR, also regulates breast cancer biology (45, 46). Increased levels of insulin have been related to increased risk of breast cancer and mortality from it (47) and colon cancer (48). It has been shown that human breast cancer specimens have higher IR content than normal breast tissue and fibroadenoma specimens (49). Increasing evidence also suggests that, in breast cancers, there is higher expression of IR-A (14, 17). As IGF-IR expression levels are also elevated in primary breast cancer tissues, this could result in the formation of hybrid IGF-IR/IR-A receptors. Because IGF-II also binds with high affinity to IR-A and IGF-IR/IR-A hybrid receptors (22), targeting of both IR and IGF-IR may be necessary to inhibit IGF action. Thus, a strategy that targets only IGF-IR may not be sufficient to block all of the receptors important in regulating IGF action as IR may also need to be targeted. However, due to the importance of IR in glucose homeostasis, an ideal anti-IGF strategy would target both IGF-IR and IR only in tumor cells and leave host IR in insulin target tissues (liver, muscle, and fat) unaffected.

Methods of Disrupting IGF Signaling in Cancer

In this section, the various strategies that can be used to disrupt IGF signaling in cancer will be reviewed with a focus on small-molecule tyrosine kinase inhibitors and antibodies.

Reduction of IGF-I Levels

Since increased levels of IGF-I have been associated with increased risk of breast, prostate, colon, and lung cancers (29) and mice with low circulating IGF levels have decreased tumor incidence and growth (27, 28, 50), lowering circulating serum IGF-I levels could affect established cancers.

Growth Hormone-Releasing Hormone Antagonists.

During puberty, growth hormone-releasing hormone (GHRH) is secreted by the hypothalamus to stimulate release of pituitary growth hormone. Growth hormone, acting via the growth hormone receptor, then regulates the hepatic synthesis and secretion of IGF-I. Thus, endocrine IGF-I levels could be affected at many levels. One strategy for decreasing levels of IGF-I is to inhibit the action of GHRH. GHRH antagonists inhibit release of growth hormone, which controls synthesis of IGF-I, and function either indirectly to block the endocrine growth hormone-IGF axis or directly on tumor cells to suppress autocrine/paracrine IGF-I, IGF-II, or GHRH. Some antagonists of GHRH have shown promise in inhibiting tumor growth in animal models. Thus, JV-1-38, a GHRH antagonist, inhibited growth of non-small-cell lung cancer cells *in vitro* and in athymic mice (51). JV-1-36, another GHRH antagonist, also inhibited the growth and metastasis of MDA-MB-435 breast cancer cells (52).

Growth Hormone Antagonists. Because IGF-I is synthesized in the liver under the control of growth hormone

signals, a second method to reduce IGF-I levels is to block growth hormone action. Pegvisomant is a polyethylene glycol derivative of growth hormone that acts as a selective growth hormone antagonist and is used for the treatment of acromegaly (53). Pegvisomant has been shown to successfully reduce IGF-I levels in patients with acromegaly and in normal volunteers (54, 55). Therefore, pegvisomant was tested in models of human cancer. Pegvisomant has shown efficacy against meningiomas *in vitro* and *in vivo* (56) and against colon and breast cancer cell lines *in vivo* (57). Furthermore, transgenic mice expressing a mutant growth hormone that behaves as an antagonist also have decreased IGF-I levels and reduced incidence of mammary tumors (58). Thus, disruption of growth hormone receptor function could be used to inhibit cancer growth.

Neutralization of IGF-I/IGF-II

Because IGF-IR is activated predominantly by binding of the natural ligands, neutralization of ligands can inhibit activation of the receptors.

Binding Proteins. IGF binding proteins (IGFBP) are a family of proteins that function to regulate bioavailability of IGF-I and IGF-II to interact with the receptors. The binding affinity of IGFBPs for the ligands is higher than that of IGF-IR for the ligands, and one approach that is being investigated is the use of binding proteins to neutralize IGF-I and IGF-II. In addition, many of them have IGF-independent effects, such as inducing apoptosis, which can be useful in cancer therapy. We have been interested in using IGFBP-1 to inhibit the effects of IGFs in breast cancer. We have shown that IGFBP-1 inhibited activation of IGF-IR by IGF-I and that it inhibited IGF-I-, estrogen-, and serum-induced proliferation (59) and anchorage-independent growth of breast cancer cells (60). Furthermore, we have also shown that IGFBP-1 inhibited the motility of MDA-231BO cells (61), which are a bone-seeking metastatic variant of the MDA-MB-231 cells.

To test the ability of IGFBP-1 to inhibit breast cancer growth *in vivo*, we administered recombinant human IGFBP-1 by osmotic pumps over a 2-week period to mice bearing MCF-7 xenografts. As shown in Fig. 2, infusion of IGFBP-1 inhibited xenograft tumor growth of MCF-7 cells in athymic mice compared with mice infused with albumin. These results suggest that neutralization of IGF-I by pharmacologic excess of binding protein can be useful. In this experiment, IGFBP-1 had to be administered via osmotic pumps because of its very short half-life (62) and it was ineffective on a twice-daily schedule *in vivo* (data not shown). Thus, strategies that increase the half-life of IGFBP-1 would greatly increase its attraction as an agent for therapeutic intervention. Several approaches can be used to increase the half-life of IGFBP-1, including conjugation to polyethylene glycol, which we have shown to be capable of increasing its half-life (60), and fusing it to the Fc domain of human immunoglobulin G. This approach of using a circulating protein in the treatment of a disease has a successful precedent in the treatment of rheumatoid arthritis

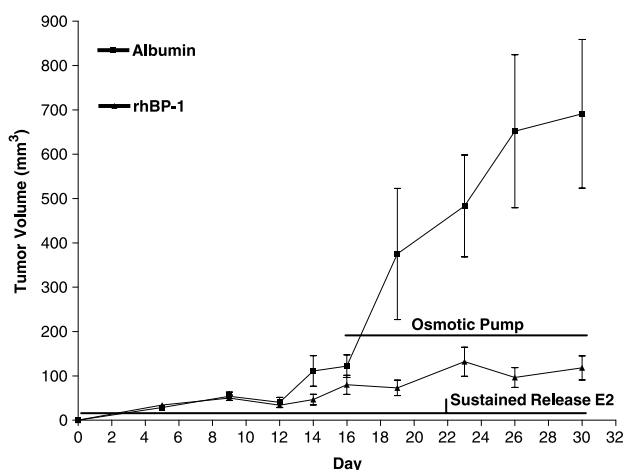


Figure 2. Recombinant human IGFBP-1 inhibited xenograft growth of MCF-7 breast cancer cells. MCF-7 cells (5×10^6) were injected into the mammary fat pad of each female athymic mouse supplemented with a 60-d-release pellet of 0.5 mg 17β -estradiol. Sustained-release osmotic pumps (Alzet) loaded with either bovine serum albumin or *E. coli*-expressed, purified recombinant human IGFBP-1, which released 480 μ g of bovine serum albumin or IGFBP-1/d, were implanted s.c. in the mice. Tumors were measured bidirectionally and tumor volumes calculated using the following formula: length \times breadth² / 2. Mice treated with IGFBP-1 showed significant inhibition of xenograft growth compared with mice that received bovine serum albumin.

with etanercept (63), which is a soluble fusion protein with the ligand binding domain of the tumor necrosis factor- α receptor fused to Fc of human immunoglobulin G, which binds and neutralizes tumor necrosis factor- α .

Soluble Receptor. A second method to neutralize IGF action is to bind circulating ligand with soluble IGF-IR. This approach has been tested by creating a mutant of IGF-IR with a stop codon at base 486 (known as 486 stop). This 486 stop mutant was soluble and functioned as a decoy receptor to bind circulating IGF-I (41). This protein has been shown to inhibit the motility and metastasis of several types of cancer cells including breast cancer cells (41), lung cancer cells (64), and colon cancer and pancreatic cancer cells (65). Like all of the IGF neutralization strategies, it is not clear if tumor or host IGF-IR is the target for these therapies.

Antibodies against IGF-I and IGF-II. A third way to neutralize the ligand that was recently described is the use of antibodies against IGF-I and IGF-II (66). A rat monoclonal antibody, KM1468, which neutralized both IGF-I and IGF-II, inhibited bone metastases of MDA PCa 2b prostate cancer cells (66), indicating that such an approach could have clinical value.

Decreased Expression of IGF-IR

Several approaches have been used to decrease IGF-IR expression.

Antisense Strategies. Antisense RNA or oligodeoxynucleotides form heteroduplexes with the target mRNA thereby inhibiting translation of the mRNA or causing mRNA degradation by RNase H. Antisense strategies to

IGF-IR that result in decreased IGF-IR levels have been shown to inhibit multiple cancer types (67). These data suggest that inhibition of IGF-IR expression by antisense strategies could be clinically useful. In fact, an antisense oligonucleotide to IGF-IR has been tested in a clinical trial for malignant astrocytomas (68). Although safe and efficient delivery of antisense reagents still needs to be optimized, direct intratumoral injection of IGF-IR antisense oligodeoxynucleotides was reported to inhibit the growth of C4HD mammary adenocarcinomas in mice (69). As newer viral vectors designed to deliver the antisense RNA to tumors with higher efficiencies are developed (70), the attractiveness of antisense approaches in IGF-IR-targeted cancer therapy will increase.

RNA Interference. Small interfering RNAs against IGF-IR have recently been described and these have been shown to decrease IGF-IR levels and IGF-IR signaling in cancer cells (71). Similar to antisense strategies, the delivery of these reagents is still inefficient and the *in vivo* specificity of these reagents needs to be optimized.

Inhibition of IGF-IR Activation

These strategies perhaps hold the most promise in translating an anti-IGF-IR reagent from the bench to the clinic and are the furthest along in terms of development for clinical purposes (72). The development and undertaking of a clinical trial with an anti-IGF-IR reagent will provide the ultimate validation of the importance of this system in cancer. Currently, the antibodies CP-751,871, A12, and h7C10 are in phase I clinical trials in hematologic malignancy and solid tumors.

Small-Molecule Inhibitors. These compounds inhibit the tyrosine kinase activity by binding to the ATP binding site or substrate binding site in the kinase domain of IGF-IR or by blocking substrate binding to the activated receptor. Several small-molecule inhibitors have been described over the years. However, the development of an inhibitor specific for IGF-IR has been hampered by the high degree of similarity of the active sites of IGF-IR and IR. Several of the earlier described inhibitors were the tyrphostins, such as AG1024 and AG1034, which are synthetic protein tyrosine kinase inhibitors and were not very specific for IGF-IR (73). Blum et al. (74, 75) studied several other tyrphostins such as AG538 and developed bioisosteres of AG538 but all inhibited IR kinase activity with similar efficacy to IGF-IR. Another class of compounds, called cycloolignans, in particular picropodophyllin, selectively inhibited basal IGF-IR tyrosine phosphorylation in melanoma cells and inhibited growth of these cells *in vivo* in severe combined immunodeficient mice (76).

In 2004, two small-molecule inhibitors specific for IGF-IR were described. NVP-AEW541 (77) and NVP-ADW742 (78) are pyrrolopyrimidines. NVP-AEW541 was reported to be specific for IGF-IR (27-fold more potent towards IGF-IR compared with IR) based on cellular kinase assay using NIH 3T3 cells expressing either IGF-IR or IR (77); however, in whole-cell-based assays using cancer cells, which express both IGF-IR and IR, NVP-AEW541 inhibited both

IGF-IR and IR activation.¹ This may not be detrimental if either hybrid, IGF-IR/IR or holo-IR, also needs to be targeted to inhibit the effects of IGFs. However, it will be desirable to target IR only in tumor cells and not in other tissues where IR plays important roles in glucose metabolism. Nevertheless, both NVP-AEW541 and NVP-ADW742 inhibited growth of a wide variety of cancer cells (77, 78). Two dual-specificity tyrosine kinase inhibitors that inhibited both IGF-IR and IR tyrosine kinase activity, BMS-536924 (79) and BMS-554417 (80), were recently developed and shown to inhibit tumor growth.

Antibodies against IGF-IR. Several antibodies against IGF-IR have been developed over the years. One of the first antibodies developed against IGF-IR was α IR3 (81), which inhibited *in vitro* growth of several breast cancer cell lines including MCF-7 (82). However, it did not inhibit xenograft growth of MCF-7 cells but inhibited the xenograft growth of many types of cancer cells including MDA-231 breast cancer cells, rhabdomyosarcoma (83), Ewing's sarcoma (84), and non-small-cell lung cancer cells (85). Although the description of α IR3 was simultaneous or preceded the generation of the parent antibodies from which trastuzumab (86) and cetuximab (87) were derived, it was never further developed for clinical use.

However, in the past 5 years, several additional antibodies against IGF-IR have been described and development of IGF-IR inhibitors has finally come of age. One of the initial antibodies was a single-chain humanized antibody against IGF-IR, called scFv-Fc, which partially inhibited xenograft growth of MCF-7 breast and PC-3 prostate cancer cells (88). We have shown that scFv-Fc bound and activated IGF-IR, resulting in receptor phosphorylation. However, it efficiently down-regulated IGF-IR via the endocytic pathway, which led to diminished levels of IGF-IR, rendering cells refractory to ligand stimulation (89). scFv-Fc also down-regulated IGF-IR *in vivo* in the tumors, suggesting that it functions directly to block tumoral IGF-IR (89). EM164, a second antibody against IGF-IR, has been shown to inhibit *in vitro* growth of MCF-7 cells (90). In contrast to scFv-Fc, EM164 did not stimulate IGF-IR autophosphorylation but still down-regulated IGF-IR *in vitro* (ref. 90; Fig. 3) and *in vivo* (91). It also inhibited xenograft growth of BxPC3 human pancreatic cancer cells (90). More recently, A12, a fully human antibody against IGF-IR, was reported to inhibit *in vivo* growth of MCF-7 breast cancer, BxPC3 pancreatic cancer, and Colo-205 colorectal cancer cells and also down-regulated IGF-IR (92). Finally, in 2005, another fully human antibody, CP-751,871, was described which inhibited xenograft tumor growth of multiple cancer types, including breast cancer, lung cancer, and colorectal cancer, and down-regulated IGF-IR (93). Two other fully human antibodies against IGF-IR, h7C10 (94) and 19D12 (95), have also been described and inhibited growth of multiple cancer cells.

¹ D. Sachdev and D. Yee, unpublished observation.

Figure 3 shows the effect of these antibodies on receptor level (89, 90, 92). In this experiment, MCF-7 cells were treated with scFv-Fc, α IR3 (top), EM164, or A12 (bottom) over time and IGF-IR levels were examined by immunoblot. All four antibodies down-regulated IGF-IR over a similar time course. Thus, receptor down-regulation is a common mechanism of action of these antibodies and the initial biochemical agonist or antagonist effect does not affect this down-regulation. Whether the agonist properties have any clinical relevance may be discovered in clinical trials using these drugs. Furthermore, we have also recently reported that antibodies directed specifically against IGF-IR down-regulated IR in breast cancer cells coexpressing IGF-IR and IR (91). This down-regulation of IR was not seen in cells expressing low or no IGF-IR levels. Figure 4A shows the effect of scFv-Fc and EM164 on IR levels in T47D-C42W breast cancer cells that express very low levels of IGF-IR (top). T47D-C42W cells were treated with scFv-Fc or EM164 for 15 min or 24 h and IGF-IR (top) and IR (bottom) levels were examined by immunoblot. Neither scFv-Fc nor EM164 down-regulated IR in these cells after a 24-h treatment (bottom, lanes 7 and 8). Moreover, EM164 and scFv-Fc also did not down-regulate IR in HepG2 cells (Fig. 4B), which have very low levels of IGF-IR. EM164 also did not inhibit insulin-stimulated activation of IR as assayed by phosphorylation of IRS-1 in HepG2 cells after a 15-min or 24-h pretreatment (Fig. 4C, lanes 3 and 4 compared with lane 2). These data suggest that coexpression of both receptors is required for down-regulation of IR, and in insulin-responsive tissues, such as the liver, which do not express

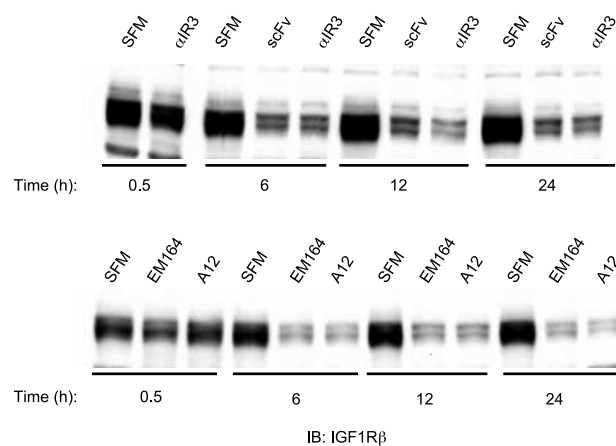


Figure 3. Anti-IGF-IR antibodies down-regulated IGF-IR. MCF-7 cells were plated in 60-mm dishes in full-growth media. Seventy-percent confluent cells were serum starved overnight in serum-free media (SFM). MCF-7 cells were untreated (SFM), treated with 60 nmol/L α IR3, 250 nmol/L scFv-Fc (top), 120 nmol/L EM164, or 100 nmol/L A12 (bottom) for various times as indicated. Cellular proteins were subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted for IGF-IR levels using an antibody against the β -subunit of IGF-IR (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). All anti-IGF-IR antibodies down-regulated IGF-IR. The down-regulation of IGF-IR by different antibodies shown here was previously reported by several groups [scFv-Fc by Sachdev et al. (89); EM164 by Maloney et al. (90); and A12 by Burtrum et al. (92)] as indicated in the text.

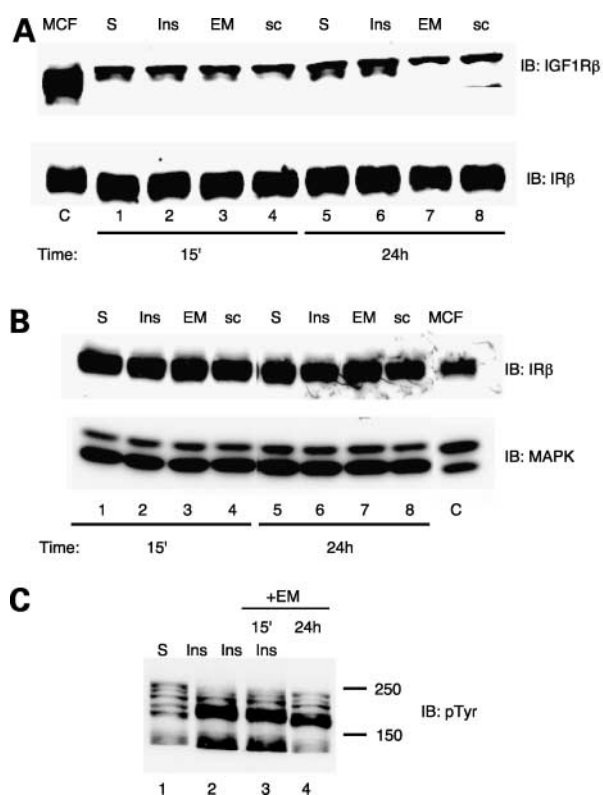


Figure 4. Anti-IGF-IR antibodies did not down-regulate IR or inhibit IR activation in cells with low levels of IGF-IR. **A**, scFv-Fc and EM164 did not down-regulate IR in cells with low IGF-IR levels. T47D-C42W cells (a variant of T47D breast cancer cells) were untreated (S), treated with 10 nmol/L insulin (Ins), 120 nmol/L EM164 (EM), or 250 nmol/L scFv-Fc (sc) for 15 min (lanes 1–4) or 24 h (lanes 5–8). MCF-7 cells were used as a control (lane C). Cellular proteins were subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted for IGF-IR (top) or IR levels (bottom). scFv-Fc and EM164 did not down-regulate IR in T47D-C42W cells, which have very low IGF-IR levels even after a 24-h treatment. **B**, scFv-Fc and EM164 did not down-regulate IR in hepatoma cells with very low levels of IGF-IR. HepG2 cells were untreated, treated with 10 nmol/L insulin, 120 nmol/L EM164, or 250 nmol/L scFv-Fc for 15 min (lanes 1–4) or 24 h (lanes 5–8). MCF-7 cells were used as a control (lane C). Cellular proteins were subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted for IR levels (top) or total MAPK as loading control (bottom). Anti-IGF-IR antibodies did not down-regulate IR in liver cells. **C**, EM164 did not inhibit insulin-stimulated activation of IR in liver cells. HepG2 cells were untreated (lane 1) or treated with 10 nmol/L insulin (lanes 2–4) in the absence of EM164 (lane 2), after a 15-min pretreatment with EM164 (lane 3), or after 24-h pretreatment with EM164 (lane 4). Cellular proteins were subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted with an antiphosphotyrosine antibody. Insulin treatment resulted in activation of IR as assayed by phosphorylation of IRS-1 (lane 2 compared with lane 1). Neither a 15-min nor a 24-h treatment with EM164 inhibited activation of IR by insulin (lanes 3 and 4 compared with lane 2). Thus, antibodies against IGF-IR do not inhibit IR activation in cells with very low IGF-IR levels.

IGF-IR, antibodies directed only against IGF-IR will not inhibit IR function.

Thus, in the past several years, many antibodies against IGF-IR have been developed or modified for clinical purposes for use in patients. In fact, currently, CP-751,871 is being tested in a phase I clinical trial in multiple

myeloma patients,² A12 in solid tumors,³ and h7C10 in solid tumors.⁴

Because antibodies bearing a human immunoglobulin G domain could direct antibody-dependent cellular cytotoxicity, this could have important considerations for human clinical trials given the expression of IGF-IR in normal tissues. We have shown that scFv-Fc does not enhance lysis of tumor cells by antibody-dependent cellular cytotoxicity *in vitro* (89), and recently, it has been reported that CP-751,871 also does not enhance antibody-dependent cellular cytotoxicity (93). This may be a desirable property in an antibody when targeting IGF-IR for cancer therapy due to the ubiquitous expression of IGF-IR.

Conduct of Clinical Trials with Anti-IGF-IR Reagents

Potential Toxicities from Inhibiting IGF-IR

In this section, some of the anticipated toxicities as a result of inhibiting IGF-IR are discussed.

Metabolic Effects. Whereas the systemic effects and toxicities of inhibiting IGF-IR in the adult are not known, the existence of human populations with low levels of IGF-I (Laron dwarfs; ref. 96) or no IGF-I (97) indicates that anti-IGF-IR therapy might be tolerated reasonably well. There is also a reported case of a human with a homozygous deletion of *igf1* gene (98). This human displayed intrauterine growth failure and has extreme short stature, deafness, and mild retardation but leads an otherwise healthy life, suggesting that loss of total IGF-I gene expression is not lethal. Furthermore, children with dysfunctional IGF-IR mutations have been described (99). These individuals have severe growth deficits, and in one case also has developmental abnormalities. Taken together, these children demonstrate that complete loss of IGF-I gene expression or partial suppression of IGF-IR function is still compatible with life. Whether or not suppression of this signaling pathway in adults with normal IGF-I/IGF-IR function will have substantial toxicity awaits the conduct of phase I clinical trials. All of the *in vivo* studies with anti-IGF-IR reagents have been done in adult mice, which have no circulating IGF-II levels, so the effect of inhibiting IGF-II in adults remains unknown. Furthermore, humans deficient in IGF-II expression have not been described and thus toxicities associated with inhibition of IGF-II are difficult to appreciate without data from phase I clinical trials. However, initial reports of preliminary results of phase I trials with CP-751,871 in multiple myeloma patients indicated no overt toxicity.

Furthermore, if anti-IGF-IR reagents affect IR, then some toxicity associated with glucose homeostasis will likely be

² <http://www.mayoclinic.org/multiple-myeloma/clinicaltrials.html> or http://www.moffitt.usf.edu/about_moffitt/publications/clinical_trials_update/nbcmonths/2004s4.pdf

³ http://www.medical.washington.edu/studies/study_details.asp?study=20051313

⁴ <http://www.clinicaltrials.gov/ct/show/NCT00282737?order=1>

seen. Whereas it is true that inhibition of host IR would not be desirable for chronic therapy, it may be tolerated for short-term treatment. Hopefully, the vast knowledge of abnormal glucose homeostasis and its management in patients with diabetes will be useful in dealing with this toxicity. Obviously, if IR also has to be targeted in tumor cells, a therapy that blocks host IR and IGF-IR may not be tolerated over prolonged periods.

Immunologic Effects. Given the abundant expression of IGF-IR on many normal cells, antibodies that do not enhance antibody-dependent cellular cytotoxicity may be desirable so as not to cause autoimmune disease. While tumoral IGF-IR is down-regulated by the antibodies, making antibody-dependent cellular cytotoxicity a likely minor mechanism for the tumor response, the regulation of IGF-IR on normal tissue has not been studied. Because all of the available antibodies, with the exception of A12 (92), do not affect mouse IGF-IR, it has not been possible to determine the effect of these reagents on host IGF-IR. However, mice treated with A12 did not experience weight loss (100) or have abnormal glucose levels,⁵ although antibody-dependent cellular cytotoxicity has not been evaluated in these mouse model systems. Two of the antibodies against IGF-IR, h7C10 and 19D12, have been reported to enhance antibody-dependent cellular cytotoxicity *in vitro* (94, 95), and as they are in clinical development, the contribution of antibody-dependent cellular cytotoxicity in cancer therapy targeting IGF-IR will be better understood.

Effects on Specific Tissues. Due to the role of IGF-IR in the pancreatic β cells, heart, and bone, inhibition of IGF-IR may have some toxicity in these tissues. Of special concern is central nervous system toxicity. IGF-I has been shown to be a survival factor in neurons, and the therapeutic benefit of IGF-I in the treatment of amyotrophic lateral sclerosis is currently under way. In this regard, monoclonal antibodies could be less toxic than small-molecule inhibitors in the central nervous system by their inability to cross the blood-brain barrier.

Designing Clinical Trials to Test IGF-IR – Targeted Therapy

As we approach the conduct of clinical trials with anti-IGF-IR drugs in solid tumors, we need to incorporate the lessons learned from the trials involving anti-EGFR agents. Gefitinib (Iressa), a small-molecule tyrosine kinase inhibitor of EGFR, was granted accelerated Food and Drug Administration approval for locally advanced and metastatic non-small cell lung cancer (101). However, in large confirmatory trials mandated by the Food and Drug Administration, gefitinib failed to show prolongation of overall survival (102).

In 2004, it was reported that two different EGFR-activating mutations were associated with clinical response to gefitinib (103, 104). In the large clinical trials, there were no selection criteria for enrollment (105). Certainly, if there

were a better understanding of the role of EGFR-activating mutations in lung cancer, the clinical trials of gefitinib might have been done more efficiently and shown a survival benefit for this subset of patients. In contrast, another EGFR tyrosine kinase inhibitor, erlotinib, which also received accelerated Food and Drug Administration approval for advanced non-small-cell lung cancer, showed an overall survival advantage of 2 months compared with placebo. In this smaller phase III randomized trial of 731 patients, 55% of the patients that were enrolled had high EGFR levels. Furthermore, in this study with erlotinib, overall survival benefit was higher in patients with high levels of EGFR compared with patients with low EGFR levels (106). Thus, appropriate design and selection of key biomarkers for growth factor-targeted therapies might reveal the clear benefit of such therapy.

The first priority for undertaking clinical trials with anti-IGF-IR reagents will be to identify patients with IGF-driven tumors. The mere presence and activation of IGF-IR may not be sufficient to indicate potential response. For example, we have shown that inhibition of IGF-IR function in the LCC6 cell line does not affect tumor growth; rather, metastatic function is blocked (42). Because phase II clinical trials only detect tumor growth inhibition, regulation of this phenotype by IGF-IR blockade would not be observed. The activated IGF-IR must also phosphorylate appropriate adaptor proteins to enhance growth regulation. In breast cancer cell lines, IRS-2 activation is associated with IGF-mediated cell migration, whereas IRS-1 activation is associated with growth responses. Thus, developing appropriate biomarker studies, beyond simple measurement of IGF-IR and its phosphorylation status, must be considered.

Second, constitutive activation of downstream signaling pathways, such as the phosphatidylinositol 3'-kinase and MAPK, may render IGF-IR irrelevant as a target because breast cancer cells with constitutive activation of Akt are refractory to anti-IGF-IR antibodies and the small-molecule inhibitor NVP-AEW541.⁶ Similarly, limited response to EGFR inhibitors was observed in cases of persistent MAPK or Akt activity (107). Thus, patients with constitutive activation of this signaling pathway, such as may be seen in phosphatase and tensin homologue mutant tumors, may not respond to anti-IGF-IR therapy.

Third, because multiple phenotypes may be regulated independently by IGF-IR, the conduct of phase II trials should consider other methods to measure response beyond tumor growth. At a minimum, inhibition of IGF-IR should disrupt biochemical signaling pathways within tumor cells. Ideally, noninvasive measurement of the biochemical activation of IGF-IR would be the best way to examine efficacy. The development of noninvasive diagnostic methods such as magnetic resonance spectroscopy to measure intratumoral choline levels might be one such technique. Recent studies have linked total choline

⁵S. Plymate, personal communication.

⁶In preparation.

content with aggressive behavior and activation of MAPK (108). We have evaluated changes in total choline content in primary breast cancers after presurgical systemic chemotherapy (neoadjuvant chemotherapy) by magnetic resonance spectroscopy. We found that a decrease in total choline concentration within 24 h of cytotoxic chemotherapy was associated with clinical response measured at the end of four cycles of treatment (109). Thus, functional imaging technology might provide insight into early responses induced by anti-IGF-IR therapy. Alternate imaging techniques such as dynamic-contrast enhanced-MRI and positron emission tomography could also identify changes in tumor vasculature and glucose uptake mediated by anti-IGF-IR therapy.

Anti-IGF-IR Reagents in Combination Therapy

Whereas anti-IGF-IR therapy may have activity alone, it is also highly likely that disruption of IGF-IR signaling would enhance the cytotoxic effects of conventional therapy. Rochester et al. (71) recently showed that small interfering RNA to IGF-IR increased sensitivity of prostate cancer cells to various DNA-damaging agents such as etoposide, mitoxantrone, and ionizing radiation. In this study, sensitization to paclitaxel or 5-fluorouracil, which do not damage DNA, was not seen, suggesting that chemosensitization results from impairment of the DNA damage response. There is also abundant evidence of crosstalk between IGF-IR and ER (110). Thus, it is clear that IGF-IR-targeted therapy might be most effective in combination with chemotherapy or, in breast cancer, with agents targeting the estrogen receptor, such as tamoxifen, a selective estrogen receptor modulator, or aromatase inhibitors.

Recent preclinical studies have provided additional rationale for combination therapy. CP-751,871, a monoclonal antibody directed against IGF-IR, given in combination with 5-fluorouracil, caused significantly greater inhibition of xenograft growth of Colo-205 colon cancer cells compared with 5-fluorouracil alone (93). Furthermore, CP-751,871 in combination with tamoxifen was better at inhibiting MCF-7 xenograft growth (93). Treatment of nude mice bearing A549 non-small-cell lung cancer tumors with anti-IGF-IR antibody h7C10 combined with either a chemotherapeutic agent (vinorelbine) or an anti-EGFR antibody (C225) was superior to either agent alone (94). These results support the conclusion that anti-IGF-IR antibodies have tremendous potential for cancer therapy when combined with either a chemotherapeutic agent or an antibody that targets other growth factor receptors, such as EGFR. In the near future, development of bispecific or multispecific antibodies targeting multiple tyrosine kinases will no doubt be invaluable in combination cancer therapy. There are already reports of bispecific antibodies targeting IGF-IR and EGFR (111).

Conclusion

Multiple lines of evidence implicate IGF-I, IGF-II, and insulin acting via IGF-IR and IR in regulating various

aspects of the malignant phenotype, suggesting that both IGF-IR and IR are targets for cancer therapy. However, it is still not completely clear if IR needs to be inhibited and, thus, the role of IR in cancer needs to be more completely identified. While inhibition of IR function in the patient over a sustained period of time is not desirable, short-term inhibition of IR along with IGF-IR may be tolerated, especially in combination with other proapoptotic stimuli. Identification of all the key targets in the IGF system is necessary to ensure success of trials with anti-IGF-IR reagents in solid tumors. Preclinical studies examining rational combinations of therapy may also provide information for future clinical trial designs.

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