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EDITORIAL

A candidate targeting molecule of insulin-like growth factor-Ⅰ receptor for gastrointestinal cancers

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Abstract

Advances in molecular research in cancer have brought new therapeutic strategies into clinical usage. One new group of targets is tyrosine kinase receptors, which can be treated by several strategies, including small molecule tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs). Aberrant activation of growth factors/receptors and their signal pathways are required for malignant transformation and progression in gastrointestinal (GI) carcinomas. The concept of targeting specific carcinogenic receptors has been validated by successful clinical application of many new drugs. Type I insulin-like growth factor (IGF) receptor (IGF-IR) signaling potently stimulates tumor progression and cellular differentiation, and is a promising new molecular target in human malignancies. In this review, we focus on this promising therapeutic target, IGF-IR. The IGF/IGF-IR axis is an important modifier of tumor cell proliferation, survival, growth, and treatment sensitivity in many malignant diseases, including human GI cancers. Preclinical studies demonstrated that downregulation of IGF-IR signals reversed the neoplastic phenotype and sensitized cells to anticancer treatments. These results were mainly obtained through our strategy of adenoviruses expressing dominant negative IGF-IR (IGF-IR/dn) against gastrointestinal cancers, including esophagus, stomach, colon, and pancreas. We also summarize a variety of strategies to interrupt the IGFs/IGF-IR axis and their preclinical experiences. Several mAbs and TKIs targeting IGF-IR have entered clinical trials, and early results have suggested that these agents have generally acceptable safety profiles as single agents. We summarize the advantages and disadvantages of each strategy and discuss the merits/demerits of dual targeting of IGF-IR and other growth factor receptors, including Her2 and the insulin receptor, as well as other alternatives and possible drug combinations. Thus, IGF-IR might be a candidate for a molecular therapeutic target in human GI carcinomas.

Key words: Dominant negative; Gastrointestinal cancer; Insulin like growth factor-I receptor; Monoclonal antibody; Tyrosine kinase inhibitor

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INTRODUCTION

Signals from a variety of growth factors and their receptors are required for tumorigenesis, cancer development, and maintenance of the malignant phenotype[1]. Those signals alter regulation of the cell cycle, induction of apoptosis, and interactions of tumor cells with their environment, which affect the continuous growth potential of gastrointestinal (GI) cancer cells[1].

Recently, advances in molecular cancer research have brought new therapeutic arms from the bench into clinical usage. One group of new targets is the receptor tyrosine kinases (RTKs), including epidermal growth factor receptor (EGFR, erbB1), Her2/neu (c-erbB2), c-Kit (stem cell factor receptor), and vascular endothelial growth factor receptor (VEGFR). RTKs can be blocked by small molecule tyrosine kinase inhibitors (TKIs), for example gefitinib[2] and imatinib[3], targeting EGFR and c-kit, respectively. Multikinase inhibitors are also available for several tumors, including sorafenib (targeting Raf, VEGFR, PDGFR, c-kit, Flt-3, and RET)[4] and sunitinib (targeting for Flt-3, c-kit, VEGFR, and PDGFR)[5]. RTK signals can be inhibited by human or humanized monoclonal antibodies (mAb), e.g. trastuzumab[6] and cetuximab[7], targeting Her2 and EGFR, respectively. Bevacizumab is a mAb against VEGF-A, which is a ligand for VEGFRs, and is also in clinical use for patients with colorectal cancer[8]. Insulin-like growth factor (IGF) receptor-I (IGF-IR) could be the next molecular target in RTKs of human neoplasms[9].

INSULIN-LIKE GROWTH FACTOR/IGF-I RECEPTOR AXIS

IGF-IR is synthesized as a single precursor peptide of 1367 amino acid residues, which is then cleaved at residue 706, into the α-chain (containing the extracellular domain) and the β-chain (having the transmembrane and tyrosine kinase domains) (Figure 1)[10]. IGF-IR is transported to the membrane fully assembled in the dimeric form with two α-chains and two β-subunits. IGF-I and IGF-II are the ligands of IGF-IR and are produced by the liver and by many extrahepatic sites, including tumor cells and stromal fibroblasts. After the ligands bind to IGF-IR, which is autophosphorylated to stimulate tyrosine kinase activity, IGF-IR subsequently phosphorylates intracellular substrates, including insulin receptor substrates-1 to -4 (IRS-1~4) and Shc. These early events activate multiple signaling pathways, including the mitogen-activated protein kinase [MAPK, extracellular signal-regulated kinase (ERK)] and phosphatidylinositide 3-kinase (PI3-K)/Akt-1 (protein kinase B) pathways[11,12]. Those pathways then switch on several cellular functions, including anti-apoptosis, transcription, metabolism, proliferation, growth, and translation.

In normal cells, the IGF/IGF-IR system is controlled by multiple steps (Figure 2)[13]. Growth hormone-releasing hormone (GHRH) stimulates the expression of growth hormone (GH), which is produced in the pituitary gland. GH then stimulates the secretion of IGFs and IGF binding proteins (IGFBPs) from hepatocytes. Activation of IGF-IR is tightly regulated by the amount of the free forms of the ligands, which is controlled by the action of IGFBP and the non-stimulatory receptor type 2 IGF receptor (IGF-IIR, also known as mannose 6-phosphate receptor)[14,15]. IGFBP-1 to -6 circulate and modulate IGF activity by reducing IGFs bioavailability to bind to the IGF-IR. The complex balance between IGFs and IGFBPs is modulated by specific IGFBP proteases, such as matrix metalloproteinase (MMP)[16]. IGFBPs have IGF-independent actions, but their role in cancer is not yet clear. IGF-IIR is also a negative regulator of IGF signaling, and works by as a decoy by binding IGFs.

THE ROLES OF IGF-IR SIGNALS IN HUMAN NEOPLASMS, ESPECIALLY GASTOROINTESTINAL CANCERS

Dysregulation of the IGFs/IGF-IR system has been implicated in the proliferation of numerous tumors[17]. IGF-IR appears to be essential for malignant transformation in certain systems, for example, fetal fibroblasts with a disruption of the IGF-IR gene, while viable, cannot be transformed by the potent oncogene, SV40 T antigen[11,18]. Elevation of serum IGF-I increases the risk of developing several cancers, e.g. colon, prostate, and breast[14,19,20]. In addition, low serum concentration of IGFBP3 increases the risk of cancer[14]. Increased IGF-II expression has been implicated as a biomarker of colorectal cancer risk[21]. Overexpression of IGFs and the receptor, either by gene amplification, loss of imprinting, or overexpression of convertases or transcription factors, have been observed, as well as posttranslational modifications of the IGF-IR by glycosylation. IGF-IR is also important for the maintenance, as well as the initiation, of malignancy[11]. Moreover, reduction of IGF-IR has been shown to induce apoptosis in tumor cells, but produces only growth arrest in untransformed cells[1], implying that receptor blockade might have a greater therapeutic index than strategies targeting fundamental cell mechanisms, such as DNA synthesis or the cell cycle. In support of this, IGF-IR knockout mice are viable (though physically smaller than normal and ultimately die of respiratory failure), indicating that relatively normal tissue development and differentiation can occur in its absence[22].

Exogenous IGFs stimulate the proliferation of colon, gastric, esophageal, hepatocellular, and pancreatic cancer cells, whereas blocking IGF-IR inhibits tumor progression[23-29]. Intestinal fibroblast-derived IGF-II has been shown to stimulate proliferation of intestinal epithelial cells in a paracrine manner[30]. Both IGF-II and IGF-IR expressions are increased in gastrointestinal cancers[23,28,29,31-33]. Soluble IGF-IIR rescues Apc(Min/+) intestinal adenoma progression induced by loss of IGF-II imprinting[15]. Previously, we reported that detection of IGF-II/IGF-IR might be useful for the prediction of recurrence and poor prognosis of ESCC and for selecting patients for IGF-IR targeting therapy[33]. IGF-I has also

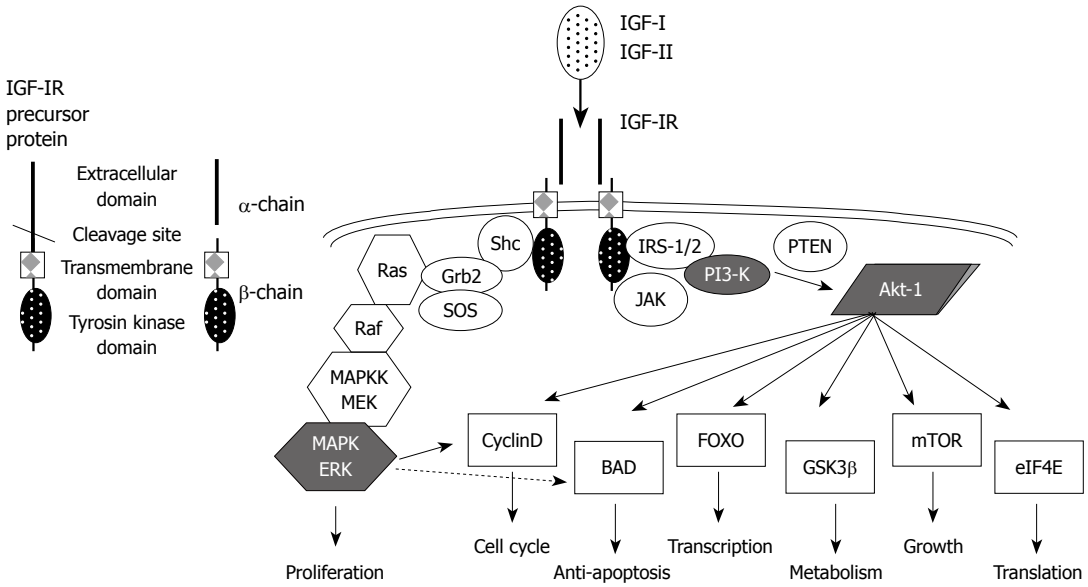

Figure 1 The structure, signal transductions, and effects of the type I insulin-like growth factor receptor system. Type I insulin-like growth factor receptor (IGF-IR) is synthesized as a single precursor peptide and then is cleaved into the α-subunit (extracellular domain) and the β-subunit (transmembrane and tyrosine kinase domains). After binding to the ligands (IGF-I and IGF-II), IGF-IR, which is constructed with two α- and two β-chains, turns on its signal transductions *via* two major pathways, such as mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3-K)/Akt, results in survival and mitogenesis. IRS: Insulin receptor substrate; Shc: Src homology and collagen-containing protein; Grb2: Growth factor receptor-bound protein 2; PTEN: Phosphatase and tensin homolog; JAK: Janus kinase; MAPKK: MAPK kinase; MEK: MAPK/ERK kinase; ERK: Extracellular signal-regulated kinase; BAD: Bcl-2-associated death promoter; FOXO: Forkhead box O; GSK3β: Glycogen synthase kinase 3 beta; eIF4E: Eukaryotic translation initiation factor 4E.

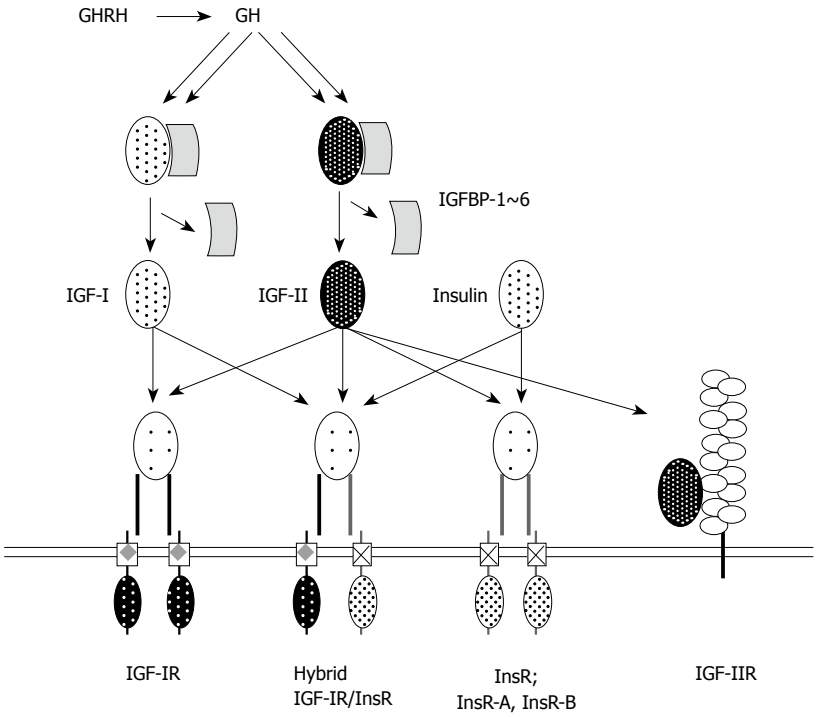

Figure 2 Insulin-like growth factor/type I insulin-like growth factor receptor and insulin/insulin receptor systems. Growth hormone-releasing hormone (GHRH) can stimulate secretion of growth hormone (GH), which upregulates insulin-like growth factors (IGFs) expression. IGF-I and IGF-II, which have about 40% sequence similarity to pro-insulin, predominantly activate type I IGF receptor (IGF-IR), which is a similar structure to insulin receptor (InsR) (59% sequence similarity). IGF-II is able to bind IR and both IGFs can bind hybrid IGF-IR/IR receptors. Ligand supply of both IGFs is regulated by two components. One is IGF binding proteins, which comprise at least six proteins [IGF binding protein (IGFBP)-1~6]. Another is IGF-IIR (lacks tyrosine kinase activity), which internalizes IGF-II for degradation in the pre-lysosomal compartment. Insulin can activate both IR and hybrid IGF-IR/InsR. Two isoforms of InsR exist, the A-isoform (InsR-A) and the B-isoform (InsR-B).

been shown to antagonize the antiproliferative effects of cyclooxygenase-2 inhibitors on pancreatic cancer cells[34]. Thus, overexpressed IGF-IR signals are also important in tumor dissemination through the control of adhesion, migration, and metastasis.

IGF-II, in conjunction with IGF-IR, IGF-I, COX-2, and MMP-7, seems to play a key role in the early stage of colorectal carcinogenesis[35,36]. Matrilysin (MMP-7) can cleave all six IGFBPs and can thus cause increased IGF-mediated IGF-IR phosphorylation[37]. Moreover, matrilysin is also able to generate bioactive IGF-II by degrading the IGF-II/IGFBP-2 complex binding to heparan sulfate proteoglycan in the ECM of HT29[16]. We have previously reported a positive feedback loop between the IGF/IGF-IR axis and matrilysin in the progression and invasiveness of GI cancers[38] (Figure 3).

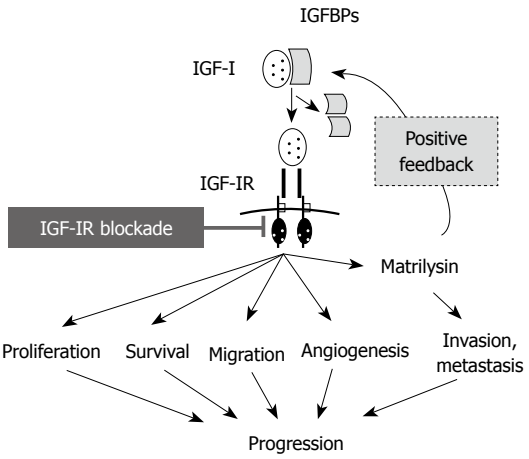

Figure 3 Blockades of type I insulin-like growth factor receptor reduce tumor progression through several interruptions of type I insulin-like growth factor receptor-mediated functions, including type I insulin-like growth factor receptor/matrilysin positive feedback system. IGF-IR: Type I insulin-like growth factor receptor; IGFBPs: IGF binding proteins.

These findings suggest a potential basis for tumor selectivity in therapeutic applications in GI cancers.

INSULIN AND INSULIN RECEPTOR AXIS

The insulin receptor (InsR) is also a key component of the IGF signaling pathway (Figure 2). IGF-IR shares a high degree of sequence similarity to InsR. The ATP binding sites of these two receptors display 100% sequence identity, whereas the entire kinase domains share 84% sequence identity, both with each other and across species[39]. InsR activation leads to cell proliferation in addition to glucose metabolism. In addition to insulin, InsR can also bind IGF-II and initiate mitogenic signaling[40]. In cells that express both receptors, IGF-IR/InsR hybrids form by random association. The hybrid receptors bind both IGF-I and IGF-II at physiological concentrations.

Epidemiological studies linked long standing type 2 diabetes, obesity, and metabolic syndrome with increased risk for developing cancer, including pancreatic and colon cancer[41]. High levels of both insulin and IGF-I are risks for breast cancer in postmenopausal women[12,42]. Phosphorylated IGF-IR/InsR is present in all breast cancer subtypes, and is related to poor survival[43]. InsR and IGF-IR/InsR hybrid receptors might also be involved in cancer biology, as both insulin and IGF-I contribute to the development and progression of adenomatous polyps[44].

Two isoforms of InsR are generated by alternative splicing of exon 11[45]. The A-isoform (InsR-A) is a fetal type and does not contain exon 11, and the B-isoform (InsR-B) is a classic form and contains exon 11[45]. InsR-A can bind IGF-II in addition to insulin and initiates mitogenic signaling[40]. InsR-B is able to bind IGF-I in addition to insulin. Cancers are now known to express InsRs, particularly the fetal variant InsR-A that mediates proliferation and apoptosis protection in response to IGF-II.

THE EFFECTS OF DOMINANT NEGATIVE IGF-IR IN COLORECTAL, GASTRIC, PANCREATIC, AND ESOPHAGEAL CANCER CELLS

Of the many potent strategies targeting the IGF/IGF-IR axis in GI cancer, we will first discuss data generate by our own group[33,38,46-48]. We constructed dominant negative (dn) versions IGF-IR, which can inhibit the function rather than the expression of the naturally expressed receptor[46,49]. We generated two different truncated IGF-IR constructs (950 and 482 amino acid residue IGF-IRs, IGF-IR/950st and IGF-IR/482st, respectively). The former lacks the tyrosine kinase domain and is thought to reside in the membrane of the transduced cells. The latter produces a defective α-chain of IGF-IR and should thus be a secreted form that may affect signal transduction in adjacent cells in addition to the transduced cells. We then constructed adenoviruses expressing two IGF-IR/dns, Ad-IGF-IR/dns (Ad-IGF-IR/482st and Ad-IGF-IR/950st).

In vitro effects and signal transduction of IGF-IR/dn

The Ad-IGF-IR/dns effectively reduced ligand dependent DNA synthesis, an index of mitogenesis, and colony formation, an index of *in vitro* tumorigenicity. IGF-IR/dns induced apoptosis and upregulated stressor (serum starvation, heat, and ethanol)-induced apoptosis.

IGF-IR/482st is a secreted protein and has a bystander effect, which suggest that IGF-IR/482st might enhance antitumor effects.

The IGF-IR/dns reduced ligands-induced phosphorylated Akt-1, but did not influence those of ERKs significantly. IGF-IR/dn can block not only IGF-I but also IGF-II stimulation, broadening the potential activity of IGF-IR/dn as an antitumor therapeutic. Although insulin induced Akt-phosphorylation, IGF-IR/482st did not block this phosphorylation, indicating that Ad-IGF-IR/dn has a high degree of receptor selectivity.

In vivo effects of IGF-IR/dn in GI tumor cells

When the GI cancer cells expressed IGF-IR/dn, the subcutaneous (SC) tumor formation was diminished significantly. Moreover, tumors derived from IGF-IR/dn expressing cells showed limited invasion into the underlying muscle. These results indicate that IGF-IR/dn effectively downregulates *in vivo* tumorigenicity and invasiveness.

Intratumoral (it) injection of Ad-IGF-IR/dn resulted in growth retardation or shrinkage of established GI tumors. The anti-tumor effect of IGF-IR/482st was stronger than that of IGF-IR/950st, undoubtedly due to the bystander effect of IGF-IR/482st. Moreover, IGF-IR/dn suppressed the invasiveness of SC tumors via downregulation of matrilysin expression and increased the number of apoptotic cells in the tumors.

In addition, GI cancer cells form peritoneal tumor

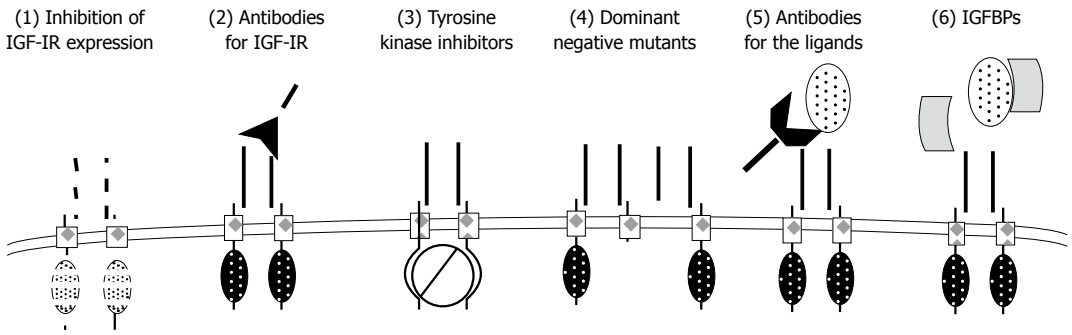

Figure 4 The summary of strategies to inactivate type I insulin-like growth factor receptor system. (1) Inhibition of type I insulin-like growth factor receptor (IGF-IR) protein expression by blocking its translation [with antisense oligodeoxynucleotides and short interfering RNA (siRNA)]; (2) IGF-IR function can be blocked with inactivating monoclonal antibodies (mAb); (3) The tyrosine kinase activity of IGF-IR can be abolished with small-molecule inhibitors (TKI); (4) IGF-IR mutants lacking β-subunits can act as dominant-negative (dn) receptors; (5) Ligand availability can be reduced by mAb for IGFs; and (6) IGF binding proteins (IGFBPs) can reduce active forms of IGFs.

nodules after intraperitoneal (ip) transplantation. Tumor bearing mice were treated by administration (ip) of Ad-IGF-IR/482st. IGF-IR/dn reduced the number of masses and resulted in a significant prolongation of survival in these mice, indicating that IGF-IR/dn can prevent and treat peritoneal cancer dissemination.

Combination effects with chemotherapy or radiotherapy

IGF-IR/dn enhanced chemotherapy (5FU and cisplatin)-induced apoptosis in GI cancer cells. The effects of the combinations were greater than addition of the effects of each monotherapy. IGF-IR/dn also upregulated radiation induced apoptosis. The combination of IGF-IR/482st (it) and 5FU (ip) for established SC tumors on mice was more effective than each single compound, and one-third of masses on the mice treated with this combo were cured; neither monotherapy cured any masses. This indicates that IGF-IR/dn has the potential to enhance the effectiveness of standard cancer therapies. Primary resistance to cytotoxic drugs is a serious problem in GI carcinomas and this approach has the potential to overcome this lack of responsiveness.

METHODS FOR THE BLOCKADE OF IGF/IGF-IR AXIS

Figure 4 shows six methods of disrupting IGF/IGF-IR signaling in cancer: (1) Blocking IGF-IR translation [with antisense (AS) oligodeoxynucleotides, AS RNA constructs, and RNA interference (RNAi)] or transcription (with triple helices) can bring about reduction or elimination of IGF-IR protein expression; (2) Binding of mAbs to IGF-IR can abolish its function; (3) Small-molecule TKIs can reduce the activity of IGF-IR; (4) Defective IGF-IRs, either mutated or lacking the tyrosine kinase domain, can act as dn receptors; (5) mAbs for the ligands can reduce their binding to the receptor; (6) Excess IGF binding proteins or inhibition of ligand expression (at the transcriptional or post-transcriptional level) can reduce the active ligands. There are several other ways to inactivate IGF-IR signals[50]; (7) IGF mimetic peptides can compete with the natural ligands; (8) Expression of a myristoylated IGF-IR C-terminus, which is a domain with intrinsic pro-apoptotic activity, can downregulate signals; (9) GHRH antagonists could diminish IGF-I levels[51]; and (10) Adnectins™ (Bristol-Myers Squibb), a novel class of targeted biologics, are proteins designed to either block or stimulate therapeutic targets of interest[52]. Recently, an optimized Adnectins specific that specifically blocks IGF-IR has been developed.

Agents useful for blocking IGF/IGF-IR signaling in cancer are listed in Table 1. Two of the ways to inhibit IGF-IR expression are RNAi technology and the AS technique[53,54]. We constructed a recombinant adenovirus expressing an AS to IGF-IR that decreases receptor numbers and inhibits soft agar colony forming efficiency, and treatment with this virus can significantly prolong the survival of nude mice bearing human lung tumor xenografts[55]. ATL-1101 (Antisense therapeutics) is an AS oligodeoxynucleotide and was developed for the treatment of psoriasis (stopped after a phase I study)[56]. We have also reported that adenoviral vectors expressing this short-hairpin RNA from IGF-IR induced effective IGF-IR silencing in lung and five GI cancers, as manifested by effective blocking of the downstream pathway of IGF-IR and by antitumor effects[57,58]. Although an adenoviral vector has several advantages, certain side effects have been reported for gene therapy using adenovirus vectors. Thus, there are some unsolved hurdles in practical application.

Many mAbs for IGF-IR had been developed over the years. Although αIR3[59] is a famous mAb for IGF-IR and inhibited cancer cell growth *in vitro*; however, it did not inhibit xenograft growth of breast cancer cell, MCF-7[60]. Thus, none of the first generation mAbs had the precise characteristics for clinical use. Recently, great advances have been in the cloning and production of mAbs by several pharmaceutical companies, e.g. figitumumab (CP-751,871)[61] by Pfizer, SCH 717454[62] by Schering-Plough, IMC-A12[63] by imClone systems, R1507[64] by Roche, AMG 479[65] by AMG, BIIB022[13] by Biogen Idec, MK-0646[66] by Merck, and AVE1642[67] by Sanofi-Aventis. The first six are whole human type mAbs and the latter two are humanized mAbs. These mAbs may have the qualities necessary for clinical usage and currently under phase study. Current IGF-IR targeting mAbs seem to

Table 1 Type I insulin-like growth factor receptor targeting agents

Class	Name	Company	Other targets than IGF-IR	Clinical study	Target organs of GI	Target organs other than GI
Inhibition of IGF-IR expression	Antisense oligonucleotide Antisense RNA siRNA					
Antibodies for IGF-IR	Figitumumab (CP-751,871)[1]	Pfizer		Phase III	Colon	Lung, head and neck, breast, prostate, sarcoma, advanced solid tumor
	SCH 717454 (19D12)[1]	Schering-Plough		Phase II	Colon	sarcoma, advanced solid tumor
	IMC-A12[1]	ImClone Systems		Phase II	Colon, HCC, pancreas, islet cell cancer	Lung, head and neck, breast, prostate, kidney, thymic, adrenocortical, sarcoma, advanced solid tumor, CMPD, leukemia, lymphoma
	R1507 (RG1507)[1]	Roche		Phase II		Lung, breast, sarcoma, advanced solid tumor
	AMG 479[1]	Amgen		Phase II	Colon, pancreas, carcinoid, neuroendocrine cell	Lung, ovarian, prostate, sarcoma, advanced solid tumor
	BIIB022[1]	Biogen Idec		Phase I	Liver	Lung, solid tumor
	MK-0646 (h7C10)[2]	Merck		Phase II	Colon, pancreas, neuroendocrine cell	Lung, breast, myeloma, advanced solid tumor
	AVE 1642[2]	Sanofi-Aventis		Phase II	Liver	Breast
Tyrosine kinase inhibitors	NVP-AEW541[3]	Novartis				
	NVP-ADW742[3]	Novartis				
	NVP-TAE226[3]	Novartis	FAK			
	BMS-536924[3]	Bristol Myers Squibb				
	BMS-554417[3]	Bristol Myers Squibb	IR			
	BMS-754807[3]	Bristol Myers Squibb	IR	Phase I/II	Colon	Breast, head and neck, advanced solid tumor
	EGCG (tea polyphenol)[3]			Phase II	Esophagus	Lung, breast, prostate, bladder, leukemia
	OSI-906 (PQIP)[3]	OSI pharma		Phase III	Colon, liver	Adrenocortical, ovarian, breast, advanced solid tumor
	A-928605[3]	Abbott				
	XL-228	Exelixis	Abl, SFK, Src, Aurora kinase A	Phase I		CML, lymphoma, cancer
	BVP-51004 (cyclolignan PPP)[4]	Biovitrum				
	INSM-18[4] [Nordihydroguaiaretic acid (NDGA)]	INSMED	Her2	Phase II		Prostate, brain, advanced solid tumor, leukemia, MDS, lymphoma
Dominant negative mutants	IGF-IR/482st IGF-IR/486STOP IGF-IR/950st IGF-IR/952STOP					
Antibodies for the ligands	KM1468	Kyowa Hakko				
	KM3168	Kyowa Hakko				
	KM3002	Kyowa Hakko				
IGFBPs	Recombinant human IGFBP3 protein					

[1]Fully human antibody; [2]Humanized antibody; [3]Adenosine triphosphate (ATP) antagonists; [4]Non-ATP antagonists. IGF-IR: Type I insulin-like growth factor receptor; GI: Gastrointestinal; HCC: Hepatocellular carcinoma; CMPD: Chronic myeloproliferative disorder; FAK: Focal adhesion kinase; CML: Chronic myeloid leukaemia; MDS: Myelodysplastic syndromes; IGFBP: IGF binding protein.

share a common mechanism of drug action, namely to blocking ligand binding, decreasing cell surface receptor expression through receptor internalization, and blocking intracellular signaling, particularly through the PI3K/Akt pathway[63,68]. Most mAb are IgG1 class, humanized or fully human, to reduce immunogenicity. IgG1 and IgG3 classes can mediate antibody-dependent cellular cytotoxicity[63,68], which might strengthen anticancer activity and lymphocytic toxicity through recruitment of immune effector cells to antibody-antigen complexes. However, IGF-IR-mAb-directed cellular cytotoxicity could also enhance toxicity to normal IGF-IR-bearing tissues. As

CP-751,871 is an IgG2 subtype, which are usually poor activators of cellular immune response, and BIIB022 is a nonglycosylated IgG4 antibody, ongoing clinical studies should clarify whether these agents have significantly different properties from IgG1 class.

Small molecular TKIs for IGF-IR are synthesized by several companies. Novartis pharma produced three agents, NVP-ADW742[69], NVP-AEW541[70], and NVP-TAE226[71], which has dual targets on IGF-IR and focal adhesion kinase (FAK). Bristol-Myers-Squibb constructed three materials, a specific inhibitor of IGF-IR, BMS-536924[72], and dual inhibitors for InsR and IGF-IR, BMS-554417[73] and BMS-754807[74]. OSI-906[75] is made by OSI pharma and A-928605[76] by Abbott. Tea polyphenol (-)-epigallocatechin-3-gallate (EGCG) is also identified as a TKI for IGF-IR[77]. These nine medicines inhibit IGF-IR kinase activity by an ATP-competitive mechanism. In contrast, there are two compounds that are IGF-IR TKI non-ATP antagonists, cyclolignan picropodophyllin (PPP)[78] and INSM-18[13]. The latter is a dual TKI for IGF-IR and Her2. XL-228 is a multi-target TKI for IGF-IR, Bcl-Abl, SFK, Src, and Aurora kinase A[13]. At least five TKIs are currently in clinical studies.

We used two dn inhibitors of IGF-IR, as detailed above. Several groups have used IGF-IR/486STOP[79,80] and IGF-IR/952STOP[81,82], the former resembles our IGF-IR/482st and the latter is similar to our IGF-IR/950st.

mAbs for IGF-I and IGF-II, KM1468[83,84], KM3168[85], and KM3002, are made by Kyowa Hakko. KM1468 neutralized both ligands and inhibited bone metastasis in an animal model.

The last approach is a recombinant human IGFBP-3 protein, which is available for intravenous injection[86] and is beginning clinical testing.

TOXICITY AND COMBINATION OF IGF-IR TARGETING STRATEGIES

The two major potential toxicities of IGF-IR blockade are based on the IGF-IR expression in normal tissues and homology between IGF-IR and InsR. Long-term IGF-IR blockade might cause growth retardation during childhood and might influence the function of IGF-dependent tissues, including the myocardium and brain at any age[87,88]. IGF-IR-inhibitory drugs are predicted to influence glucose tolerance. TKIs might directly inhibit the InsR kinase in some degree, because of binding to a well-conserved ATP binding pocket. In fact, some TKIs can inhibit both receptors, e.g. NVP-TAE226, BMS-554417, and INSM-18. Several anti-IGF-IR mAbs, such as scFv-Fc and EM164, might induce downregulation of InsR *via* endocytosis of hybrid receptors or InsR, which was observed in cancer cells expressing both receptors, but not in cells expressing InsR only[89]. This suggests that anti-IGF-IR mAbs will not inhibit InsR function in insulin-responsible tissues, e.g. hepatocytes, which do not express IGF-IR. In addition, both IGF-IR mAbs and TKIs might result in loss of the hypoglycemic effects of IGF-I, and blockade of pituitary IGF-IRs might result in a compensatory increase in serum concentration of GH, which could contribute to insulin resistance[90].

Although IGF-IR mAbs are exquisitely specific inhibitors of receptor function (by inducing rapid internalization and down-regulation of the receptor), TKIs suffer from a lack of selectivity. TKIs, in general, do not lead to internalization or downregulation of IGF-IR, and will probably represent a broad spectrum of specificity against IGF-IR and InsR and a unique profile of toxicity. Possible toxicity of the central nervous system deserves particular attention during treatment with TKIs, because other molecules in this class have been shown to infiltrate the blood-brain barrier in central nervous system tumors[91]. Nevertheless, IGF-IR TKIs offer several advantages, such as oral administration and of the ability to control the duration of drug exposure, in contrast to long-acting mAbs.

Recently, it has been revealed that the insulin/InsR axis has certain roles in carcinogenicity and tumor development. Chronic hyperinsulinaemia might be a cause of colon and pancreas cancers[92]. IGFs have some potential for binding to and activating InsR. In addition, hybrid receptors of IGF-IR and InsR exist on malignant cells. Thus, blockade of InsR is another matter of concern to eliminate cancer cells. Thus, dual targeting TKIs for IGF-IR and InsR have merits for terminating tumor cells; however, they would, again, have adverse effects of glucose homeostasis.

According to several clinical studies, it has been reported that the adverse effects of IGF-IR mAb are hyperglycemia, mild skin toxicities (rash, flushing, pruritus, and acne), and fatigue as common toxicities of these antibodies[64,93,94]. Other observed toxicities, such as CD4+ lymphocytopenea, thrombocytopenia, and transaminitis, do not seem to be related to the mechanism of their specific action. An IGF-IR mAb caused hyperglycemia in around 20% of patients, but was tolerable, mild to moderate (grades 1 and 2), reversible, and manageable with oral hypoglycemic drugs. Patients with previous glucose intolerance or with steroids usage were at risk of hyperglycemia.

IGF-IR is a mediator of resistance to therapy. IGF-IR activation is known to protect tumor cells against apoptosis induced by cytotoxic drugs, and might also influence the repair of DNA damage[95,96]. There is considerable preclinical data to support the view that IGF-IR inhibition can enhance sensitivity to chemotherapy and radiotherapy. In addition, blockade of IGF-IR might have combination effects with other molecular targeting therapies, especially for RTKs.

Recently, a new role for IGF-IR has been proposed in that its signals might be an escape pathway in cancer cells for drug resistance. Many patients who achieve an initial response to trastuzumab acquire resistance within one year of treatment initiation. Two mechanisms for this trastuzumab resistance have been reported; one is overexpression of IGF-IR[97] and the other is the formation of a IGF-IR/Her2 heterodimer[98]. In addition, IGF-induced PI3-K/Akt activation mediates resistance to EGFR blockade in glioblastomas[99].

Thus, there is a hypothesis for horizontal blockade of two different growth factor receptors, such as Her/EGFR and IGF-IR. Several groups have tried these dual targeting therapies. Recently, a candidate combination treatment with an IGF-IR TKI, BMS-536924, and EGFR/Her2 inhibitors was reported[100].

On the other hand, nonselective inhibitors might have a different profile and alternative benefits. Some TKIs inhibit other kinases, such as Src (XL-228) or Her2 (INSM-18), and these multi-kinase inhibitors can expand the activity of the agent. It could also add toxicity mediated by target and off-target effects, complicating the combination with other agents.

Treatment with CP-751,871 decreased both total circulating tumor cell count and IGF-IR-positive circulating tumor cell count, suggesting that circulating tumor cells could be used as a biomarker of drug effect[93]. High concentrations of serum free IGF-I might be a marker of high responder of patients with non small cell lung carcinoma treated with figitumumab.

Apart from mAbs and TKIs, there are individual approaches using short interfering RNA (siRNA), peptides, proteins, or antisense oligonucleotides to antagonize IGF-IR. As mentioned above, several group, including ours, have revealed that both dn and siRNA for IGF-IR show powerful anti-tumor effects. However, the delivery systems of these approaches represent a significant hurdle for clinical use. Given a suitable delivery tool for humans, we would want to start using both dn and siRNA for IGF-IR in the patients with GI cancer.

CONCLUSION

The IGF/IGF-IR axis plays pivotal roles in the carcinogenicity and progression of GI cancers. We have presented the efficacy of IGF-IR targeting strategies using our data of IGF-IR/dn against GI cancers. Blockade of IGF-IR suppresses carcinogenicity, and upregulates apoptosis-induction and the effects of chemotherapy, both *in vitro* and *in vivo*. We summarized several approaches to blocking IGF-IR signals and discussed the merits and demerits of each strategy. In addition to combination with classical chemotherapy, several attempts at dual targeting for IGF-IR and other growth factor receptors have been made. Many drugs blocking IGF-IR function are now entering clinical trials. Thus, IGF-IR might be a candidate therapeutic molecular target in gastrointestinal malignancies.

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