# Targeting the type 1 insulin-like growth factor receptor as anti-cancer treatment

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**The type 1 insulin-like growth factor receptor (IGF1R) is overexpressed by many tumors, and mediates growth, motility and protection from apoptosis. Inhibition of IGF1R expression or function has been shown to block tumor growth and metastasis, and enhance sensitivity to cytotoxic drugs and irradiation. Thus the IGF1R is a highly promising anti-cancer treatment target. This review describes approaches to target the IGF1R using antibodies, small molecule inhibitors of the IGF1R tyrosine kinase, and molecular agents such as antisense and small interfering RNAs. Problems for the clinical introduction of this approach may include toxicity due to normal tissue IGF1R expression and cross-reactivity with the insulin receptor. The next few years will see clinical trials of IGF1R targeting, which offers genuine potential to inhibit tumor growth and chemoresistance in patients with cancer. *Anti-Cancer Drugs* 14:669–682 © 2003 Lippincott Williams & Wilkins.**

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## Introduction

The insulin-like growth factor (IGF) axis plays a key role in normal growth and development. Altered expression of components of the IGF system has been implicated in the development and maintenance of the malignant phenotype in many tumor types, suggesting that therapeutic agents targeting this system may have potential as anti-cancer therapy. This review will describe the ligands, binding proteins and receptors that comprise the IGF system, the rationale for IGF targeting, the strategies available to achieve this, and potential problems associated with this approach.

## Components of the IGF axis

### Ligands

IGF-I and -II have 60% homology with proinsulin. They differ from insulin in that they are synthesized widely by many cell types and are secreted immediately rather than stored intracellularly. Circulating IGF-I derives principally from the liver in response to growth hormone [1]. In most tissues, IGF-II expression is subject to imprinting: only the paternal allele is expressed [2].

### Receptors

The IGF receptor family includes the type 1 IGF receptor (IGF1R), the type 2 IGF receptor (IGF2R) and the insulin receptor (IR). The IGF1R and the IR share an $\alpha_2\beta_2$ tetrameric structure including extracellular ligand-binding $\alpha$ and $\beta$ subunits with extracellular, transmembrane and intracellular domains. The IGF1R is 70% homologous at the amino acid level to the IR, with 84% similarity in the tyrosine kinase domain and only 44% in the C-terminus, reflecting differences in signaling and function [3]. Binding of IGF-I, IGF-II and insulin at supraphysiological concentrations induces a conformational change leading to autophosphorylation of tyrosines 1131, 1135 and 1136 in the kinase domain, juxtamembrane tyrosines and C-terminal serines [4,5]. These phosphorylation events induce recruitment of signaling intermediates including insulin-receptor substrate (IRS)-1 and -2, Shc, Grb10, and 14-3-3ε [6–9]. This in turn leads to activation of distinct signaling pathways including the ras–raf–mitogen-activated protein kinase (MAPK) cascade and the phosphatidylinositol 3-kinase (PI3K)–Akt pathway [10–12]. The IGF1R also activates phospholipase Cγ and protein kinase C (PKC), the signal transducers and activators of transcription (STATs), and their negative regulators the suppressors of cytokine signaling (SOCS) [13–15].

In contrast to the widespread expression of the IGF1R, IR expression is restricted to the liver, adipose tissue and muscle. Insulin binding induces IR activation leading to glucose uptake and inhibition of gluconeogenesis in the liver [9,16,17]. Many factors appear to contribute to differences in IGF1R and IR signaling, including the different patterns of receptor expression, kinetics of ligand binding, recruitment of signaling intermediates and effects on gene expression [18–20].

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The IGF2R gene encodes a monomeric transmembrane protein that lacks a tyrosine kinase domain and shows no evidence of signaling capability [21]. It mediates endocytosis and degradation of IGF-II, thereby acting as a negative regulator of IGF activity [22]. The IGF2R is identical to the mannose-6-phosphate (M6P) receptor, which binds lysosomal enzymes and other M6P-containing proteins for transfer to the lysosome [23,24].

#### Binding proteins

There are at least six IGF binding proteins (IGFBP1–6) and several other related proteins. IGFBPs are produced by the liver and range in size from 22 to 31 kDa. More than 95% of circulating IGF-I is bound to BP3 in a 150-kDa complex with an acid-labile subunit [25]. IGFBP actions vary with cell type, but in general they inhibit interaction of IGFs with the IGF1R. IGFBP1, IGFBP3 and IGFBP5 have been shown to have ligand-independent actions: IGFBP3 promotes IGF-independent apoptosis and has been detected in the nucleus where it interacts with the retinoid X receptor-α to influence gene transcription [26–30]. Various protease enzymes including prostate-specific antigen (PSA) can act as IGF binding protein proteases, which by cleaving IGFBPs reduce their affinity for IGFs, favoring ligand binding to the IGF1R [31,32].

## Rationale for targeting the IGF axis

Two factors underpin the concept of the IGF axis as an anti-cancer treatment target: the IGF1R mediates many characteristics of the transformed phenotype and expression of IGF axis components is perturbed in many cancers.

#### IGF1R signaling

IGF-induced activation of downstream signaling promotes cell growth and proliferation, principally (but not exclusively) via the MAPK and PI3K pathways [33–37]. Depending on the cellular context, however, IGFs can use this same pathway to induce differentiation of, for example, myoblasts, adipocytes and neurones. This endpoint appears to follow from Shc-mediated MAPK activation and is thought to be favored in cells where Shc signaling predominates over IRS-1 [38–40].

IGF1R overexpression is able to transform NIH-3T3 fibroblasts [41], while $IGF1R^{-/-}$ fibroblasts (R cells) are refractory to transformation by oncogenes, with the exception of v-*src* [42–45]. The transforming function has been linked to the IGF1R C-terminus, specifically between residues 1245 and 1310 [46,47]. Within this region at least two domains are involved, including tyrosine 1251 and the quartet of serine residues at 1280–1283, which when phosphorylated create a binding site for 14-3-3 proteins [7,48]. Both the PI3K and MAPK pathways have also been shown to play a role in transformation [12].

IGF-induced apoptosis protection occurs principally via the PI3K pathway, activating Akt to stimulate inhibitory phosphorylation of BAD, a member of the bcl-2 family of proteins. BAD phosphorylation can also result from IGF-induced activation of MAPK and 14-3-3-mediated mitochondrial translocation of Raf-1 [49]. The IGF axis plays a key role in protection from apoptosis induced by agents including osmotic stress, loss of matrix adhesion, Fas, hypoxia, low pH and low glucose, and by a range of anti-cancer drugs [50–62]. In glioblastoma and neuroblastoma cells, apoptosis susceptibility is regulated by the number of functional IGF1R sites per cell [53,63]. Recent *in vivo* studies reveal an interesting paradox: while short-term IGF treatment undoubtedly protects from killing induced by hypoxia and other microenvironmental stresses [61,64], heterozygous IGF1R knockout mice display enhanced resistance to oxidative stress and prolongation of life-span [65]. Similarly, life-span is extended in mice lacking the insulin receptor in adipose tissue and despite a normal food intake these animals are protected from age-related obesity [66]. This tallies with the observation that only calorie restriction is known to prolong life-span in humans, perhaps mediated, at least in part, by suppression of plasma IGF-I [67,68].

IGF1R activation confers additional properties that contribute to the malignant phenotype. IGF1R activation is required for hypoxia signaling and expression of vascular endothelial growth factor (VEGF) [69–71], and local IGF-I expression is associated with high microvessel density in colorectal cancer [72]. IGF-I is also known to stimulate cell motility [73–76] through IRS-1 and PI3K-mediated crosstalk between IGF and actin polymerization/integrin clustering pathways [77–79]. IGFs can influence cell–cell adhesion via the influence of IRS-1 on E-cadherin function [74,80,81]. *In vitro* tumor cell invasion is enhanced by IGFIR activation in many cell types [82–84]. Furthermore the exon 11 isoform of the IR (IR-A), which is expressed on fetal and tumor cells, can be activated by IGF-II leading to proliferation, apoptosis protection and invasion [85,86]. These data suggest that IGFs may enhance the propensity for metastasis *in vivo*. Supporting this concept are studies on Rip1-Tag2 mice, which express SV40 large T antigen from the rat insulin receptor and develop hyperproliferative islets progressing to pancreatic tumors [87]. Rip1-Tag2 mice transgenic for pancreatic IGF1R overexpression have been shown to develop invasive pancreatic cancers that spontaneously metastasize [88]. Furthermore, IGF1R overexpression in Lewis lung carcinoma cells induces VEGF expression and lymph node metastasis [89]. In contrast, however, antisense-mediated IGF1R downregulation in MCF-7 cells appears to confer a more motile phenotype with

reduction in cellular adhesion [90]. A possible reason for this discrepancy may lie in the observation that, in MCF-7 cells that overexpress the IGF1R, IGF-I leads to stabilization of the E-cadherin–catenin complex [74]. In contrast, as we previously reported, this complex is disrupted by IGF-I treatment in colorectal cancer cells, consistent with IGF-induced enhancement of cell detachment and metastasis [80].

#### Altered expression of IGF axis components

IGF-II overexpression occurs in several tumors including pancreatic and colorectal cancer, and may be associated with loss of imprinting for IGF-II expression. This can occur not only in the tumor, but also in the surrounding normal colonic mucosa and in peripheral blood mononuclear cells of patients with colorectal cancer [91–94]. Local IGF-II supply may also be increased as a consequence of mutation and loss of heterozygosity at the IGF2R locus, described in a number of primary tumors including breast, lung and hepatocellular carcinoma [95–98]. Indeed forced overexpression of IGF2R in colorectal cancer cells is known to inhibit cell growth [99].

Although the IGF1R is present on the surface of most normal cells, it is overexpressed relative to levels in the equivalent normal tissue by tumors including melanoma, prostate, colon and pancreatic cancers [93,100–103]. This may relate to the ability of oncogenes and tumor suppressor genes to influence IGF1R promoter activity, which is enhanced by n-*myc* and c-*myb*, and suppressed by wild-type p53, BRCA1 and WT1, the Wilm's tumor suppressor gene [104–109]. Overexpression of the IGF1R is associated with poor prognosis in renal cancer and uveal melanoma [110,111]. Conflicting data come from the study of breast cancer, where IGF1R overexpression has been reported to confer favorable prognosis [112], but in a later report has been associated with clinical radioresistance [113]. In the transgenic adenocarcinoma of mouse prostate (TRAMP) model of prostate cancer, in which prostatic expression of SV40 large T antigen leads to the development of metastatic prostate cancer, IGF1R levels are dramatically downregulated during progression to advanced metastatic disease [114]. Similarly, non-metastatic SV40-immortalized human prostate epithelial cells have been shown to express higher IGF1R levels than a metastatic subline, possibly due to high WT1 expression [115].

Several large epidemiological studies have shown that high normal plasma IGF-I levels confer a significantly increased risk of development of cancers, including prostate, colorectal, bladder, ovarian and premenopausal breast cancer [116–122]. However, not all reports have confirmed the association between high IGF-I levels and increased cancer risk, and there are conflicting data regarding a possible link between BP3 levels and cancer risk [123–126]. There is no evidence that IGFs themselves can initiate carcinogenesis, but it is plausible that IGFs could promote the survival of cells harboring mutations, that would otherwise have undergone apoptosis. Indeed patients with acromegaly are at increased risk of colon cancer [127]. In mice, transgenic overexpression of IGF-II induces spontaneous lung tumors [128]. Alterations in IGF supply can influence the growth of intestinal polyps in the min mouse model of adenomatous polyposis coli [129] and of human sarcoma xenografts in IGF-I-deficient hosts homozygous for the *lit* mutation [130].

## Methods of targeting the IGF axis

Many strategies have been developed to block the IGF axis. Early attempts to suppress plasma IGF-I levels were not generally successful in influencing tumor growth, perhaps because circulating levels may poorly reflect IGF bioavailability at the tissue level (reviewed in [131], [132]). Most recent studies have been designed to block either the expression or the function of the IGF1R. The following sections will review the results obtained with each approach, and the potential advantages and disadvantages of each.

## Strategies for inhibiting protein function

#### Small molecule inhibitors

Chemical inhibitors have many advantages as drugs: they can be designed for target specificity and for favorable pharmacokinetic properties including solubility and stability, and they can often be delivered orally with high bioavailability. Small molecule inhibitors of tyrosine kinase activity have been successfully developed to specifically target the epithelial growth factor (EGF) receptor and Bcr–Abl [133,134]. The most successful design strategy thus far has been the creation of small molecules that mimic ATP and compete for binding in the kinase active site [133]. Specificity is a major design hurdle, as there are numerous enzymes that catalyze reactions using ATP and the majority of tyrosine kinase ATP-binding domains are highly conserved. This is a particular problem for design of IGF1R inhibitors, given the high degree of homology with the IR [3]. However, recent structural studies have revealed regions of dissimilarity within the IGF1R and IR kinase domains, suggesting that it may be possible to design specific inhibitors of the IGF1R [135–137].

#### Blocking antibodies

Antibodies that block receptor function by interfering with ligand binding and/or initiating receptor internalization are attractive agents for use against circulating or transmembrane oncogenes. A monoclonal antibody to the IGF1R, αIR3, competes with IGF-1 (but not IGF-2) for binding to the receptor and blocks receptor activation

**Table 1 Effects of antibody-mediated blockade of IGF signaling in cancer cell lines**

| Antibody | Cell type(s) | Comments | Reference |
|---|---|---|---|
| αIR3 | WM 373, WM 852 human melanoma | inhibits growth of xenografts in athymic mice | [214] |
| αIR3 | SK-mel-5, -21, -28, -31 human melanoma | inhibits growth and/or induces apoptosis | [149] |
| αIR3 | human colorectal cancer | inhibits *in vitro* growth of Caco-2, HT-29, LS411N, LS513, LS1034, WiDr and SW620 | [215] |
| αIR3 | MCF-7 and MDA-MB-231 human breast cancer | inhibits growth *in vitro* and MDA-MB-213 tumor formation *in vivo* | [216–218] |
| αIR3 | Ewing's sarcoma | inhibits tumorigenesis and metastasis in athymic mice, increases sensitivity to doxorubicin and vincristine | [219,220] |
| αIR3 and MAB391 | human MCF-7 breast, HT29 colon cancer, DU145 prostate cancer | inhibit IGF-induced IGF1R autophosphorylation and Akt phosphorylation; block growth of MCF-7 in soft agar | [140] |
| ScFv-Fc | human MCF-7 breast | *in vitro* activates IGF1R, enhances monolayer growth; *in vivo*, IGF1R downregulation, partial inhibition of xenograft growth | [141] |

[138]. However, αIR3 can act as an IGF-I mimetic in cells overexpressing the IGF1R [139]. New monoclonal antibodies against the IGF1R are currently in development [140,141]. Table 1 summarizes the evidence that these antibodies can inhibit tumor cell growth *in vitro* and *in vivo*. However, the efficacy of monoclonal antibodies in solid tumors is often limited. The large size of the therapeutic molecule restricts its access to tumor cells, particularly in central regions of solid tumors [142]. Smaller fragments are being studied as a substitute for whole antibodies in an effort to improve access and uptake [141,143,144].

### Dominant-negative receptors

Dominant-negative proteins are designed to interfere with the function of wild-type protein, either by direct binding, in the case of proteins that function as oligomers, or by competing for binding partners. In the case of the IGF1R, dominant-negative receptors have been constructed as proteins truncated within the β subunit, resulting in the formation of inactive heterodimers of mutant and wild-type receptors unable to transduce downstream signals [145]. Other IGF1R dominant-negatives lack the transmembrane region and so are secreted from the cell to compete with wild-type receptors for ligand binding [146]. In many cases, this strategy has been shown to successfully suppress IGF1R function, resulting in reduced growth and/or tumorigenicity (Table 2). This approach has yet to be tested in the clinic; potential problems will include the difficulties of gene transduction *in vivo*, although viral vectors may be more efficient than plasmid-based systems in this context [147,148].

Other agents that have been used to block IGF1R function include tunicamycin, which prevents glycosylation and translocation of the IGF1R to the cell surface, and *N*-acetyl-cysteine, which leads to downregulation of cell surface IGF1R [149,150].

## Strategies for blocking gene expression

Several approaches utilize sequence homology to inhibit translation of target mRNA. The most well-characterized is the antisense approach, but this is now being superseded by the recent demonstration that profound gene silencing can be induced in mammalian cells by small interfering RNAs (siRNAs) [151].

### Antisense

Antisense agents can be generated by expression within cells of antisense RNA or by chemical synthesis of short antisense oligonucleotides (ASOs). These agents are designed to be complementary to the target mRNA. Protein production is prevented, either by directly blocking the translation machinery or by digestion of the mRNA by RNase H, which is activated by the formation of duplexes between mRNA and ASOs (Fig. 1) [152]. The antisense strategy is attractive because of the sequence-specificity imposed by virtue of the mechanism requiring Watson–Crick base-pairing with complementary mRNA. However, only 4–6 bases of homology are required to induce RNase H activity, and furthermore ASOs have well-recognized sequence-related and -unrelated effects including protein binding. Thus ASOs may induce downregulation of proteins in addition to the intended target [153].

There are many reports detailing antisense-mediated downregulation of the IGF1R in tumor cell lines using antisense RNA or ASOs (Table 3). Several groups have constructed inducible or constitutive mammalian vectors expressing antisense RNA to the 5′ 300–700 bp of the IGF1R cDNA. Those employing ASOs have used chemically synthesized 18–20mer phosphorothioate oligonucleotides targeted to the translational start site [154]. This is the conventional target region for ASO design because it is assumed that this region of the transcript lacks extensive secondary structure in order to facilitate translation initiation and therefore is accessible to ASO binding [155]. Upon stable or transient antisense transfection, antisense-mediated downregulation of the IGF1R has been shown to inhibit survival *in vitro* and tumorigenicity *in vivo* of a wide range of tumor types. In addition to blocking tumor growth, this strategy also inhibits metastasis [156,157]. Furthermore these approaches also enhance sensitivity to cytotoxic drugs, both

**Table 2 Effects of dominant-negative IGF1R expression in cancer models**

| Dominant-negative | Cell type(s) | Comments | Reference |
|---|---|---|---|
| Amino acids 1–952 (952/STOP) | transformed Rat-1 fibroblasts | inhibits anchorage-independent growth and tumor formation | [221] |
| Several DN designs | C6 rat glioma | inhibits clonogenic survival in monolayer and soft agar | [222] |
| Amino acids 1–486 (486/STOP) | C6 rat glioma | inhibits growth (monolayer and soft agar) and tumorigenesis and induces apoptosis *in vivo* | [146] |
| Amino acids 1–486 (486/STOP) | five different tumor cell lines | inhibits growth in soft agar and/or tumor formation in nude mice | [223] |
| Amino acids 1–486 (486/STOP) | MDA 435 and 231 human breast cancer | suppresses adhesion, invasion, growth and metastasis | [224,225] |
| Amino acids 1–486 (486/STOP) | A549 human lung carcinoma | suppresses tumorigenicity and increases sensitivity to UV irradiation and proteosome inhibitors | [226] |
| Amino acids 1282–1298 (C-terminus of the β subunit) | human prostate cancer | inhibits growth in soft agar and tumor formation in nude mice, induces apoptosis | [227] |
| IGF1R with mutation in ATP-binding domain | Ewing's sarcoma (TC-71) | induces apoptosis, inhibits tumorigenesis and enhances chemosensitivity | [228] |
| Amino acids 1–952 | human KM12L4 colon cancer | inhibits VEGF expression, vessel count, xenograft growth, liver metastasis | [229] |
| Adenoviral vector, amino acids 1–482, 1–950 | colorectal cancer | blocks IGF-induced Akt activation, enhances chemosensitivity *in vitro*, *in vivo* | [230] |
| Adenovirus expressing amino acids 1–950 or 1–498 | A549 and NCI H460 lung carcinoma | blocks growth and Akt activation *in vitro* and suppressed growth in xenografts | [231] |

in tumors that are chemosensitive, such as sarcoma and bladder cancer [157,158], and also in tumors such as prostate cancer, that are intrinsically chemoresistant [159].

Following the demonstration that IGF1R overexpression is associated with clinical radioresistance in breast cancer [113], we have used antisense IGF1R to explore the role of the IGF axis in the DNA damage response. Murine melanoma cells stably expressing antisense IGF1R transcripts show increased sensitivity to γ-irradiation compared with sense-transfected controls [160]. Furthermore, antisense IGF1R transfectants display radioresistant DNA synthesis and attenuated post-irradiation p53 response. These features are reminiscent of cells bearing a mutation in the *ATM* (*A*taxia-*T*elangiectasia *M*utated) gene, which encodes a large protein with a key role in the initiation of cell cycle checkpoints and DNA repair pathways after DNA damage [161]. We found that antisense IGF1R B16 transfectants show reduced levels of Atm protein and impaired activation of the Atm kinase after irradiation [160]. These findings suggest that the IGF system plays a key role in the cellular response to DNA damage and also suggest a specific mechanism for antisense IGF1R-induced chemosensitization, in addition to the simple removal of apoptosis protection. Indeed in murine fibroblasts it appears that anti-apoptotic signaling via the PI3K pathway is dispensable for radioresistance mediated by the IGF1R [162,163].

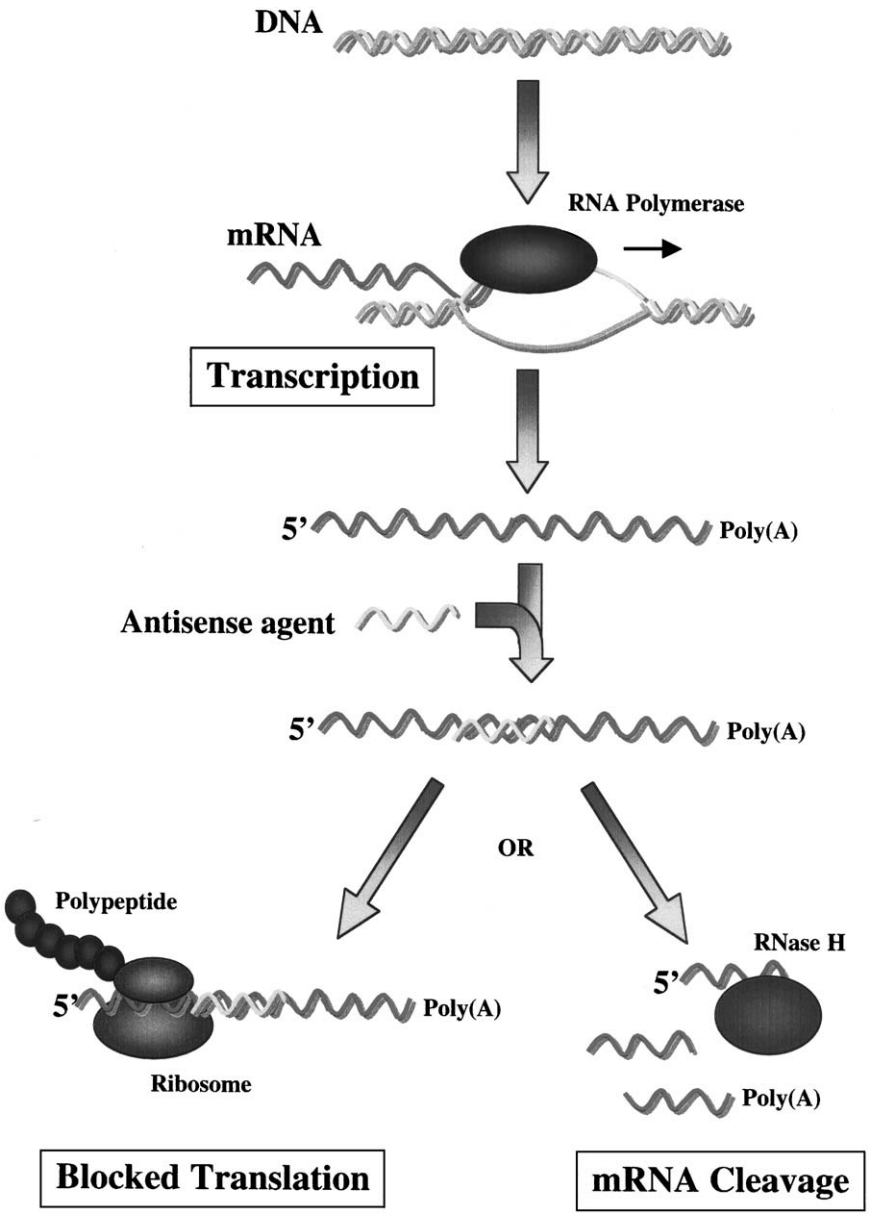
In addition to inhibiting tumor growth, the use of antisense agents has shown that tumor cells killed *in vivo* following IGF-I or IGF1R downregulation may induce a systemic immune response capable of protecting the host from tumor cell rechallenge [164,165]. The protective effect has been shown to operate following rechallenge with glioblastoma cells injected into the subcutaneous tissues and also into the brain [165]. In support of an immune effect, direct injection of an IGF1R antisense plasmid into established neuroblastomas induces much more profound inhibition of tumor growth in syngeneic (i.e. immunocompetent) animals than in nude (i.e. immunodeficient) mice [166]. The mechanism of this effect may involve upregulation of class I and co-stimulatory B-7 molecules in tumor cells expressing antisense IGFI [167]. In a pilot clinical study, surgically removed malignant astrocytoma tumor cells have been treated *ex vivo* with IGF1R ASO and reimplanted for 24 h in a diffusion chamber in the patients' rectus sheath. Eight of 12 patients have shown clinical improvement, including three cases of intracranial recurrence with unexpected spontaneous or postsurgical regression [168]. These remarkable results provide a rationale for further development of clinical applications of IGF1R downregulation in the treatment of established tumors.

In a clinical setting, ASOs may prove to be more effective than antisense expression vectors, because their small size favors efficient uptake into cells [169]. In addition, stabilizing modifications to the backbone (e.g. phosphorothioate) or sugar ring (e.g. 2′-*O*-methyl) confer enhanced resistance to nucleases [170,171]. Encouragingly, phosphorothioate ASO-based treatments have entered the clinic, and some are showing objective anti-cancer activity [172,173].

### RNA interference (RNAi)

RNAi has recently emerged as a potent method of gene silencing that can be applied to mammalian cells. It was first recognized during the course of antisense experiments in *Caenorhabditis elegans*, where profound gene

**Fig. 1**




Mechanism of antisense action. Phosphorothioate ASOs induce RNase H activity, leading to a decrease in steady-state mRNA levels and thus reduction in protein expression. Antisense RNA appears to function by blocking translation, with no detectable change in mRNA levels.

silencing has been shown to be due to contaminating double-stranded RNA (dsRNA) in the single-stranded RNA preparations [174,175]. In differentiated mammalian cells, introduction of long dsRNA (above 50 bp) activates the interferon response, resulting in generalized suppression of protein synthesis [176,177]. In *C. elegans* and *Drosophila* it appears that RNAi occurs in a two-step process (Fig. 2), involving cleavage of dsRNA and incorporation of the resulting short duplexes into a nuclease complex that destroys homologous mRNA [178–182].

The most potent RNAi effectors in *Drosophila* are RNA duplexes with 19 bp of homology to the target gene and two nucleotide 3′ overhangs (Fig. 2) [183]. These 21–23 nucleotide small interfering RNAs (siRNAs) have been shown to effect potent and sequence-specific silencing of exogenous and endogenous genes in mammalian cells [151]. In many cases, the gene silencing effect is more robust and less variable than that induced by antisense or ribozyme techniques [184–186]. However, only about 50% of siRNAs are effective and the determinants of activity are unclear. We have used scanning oligonucleo-

**Table 3 Effects of IGF1R antisense RNA or phosphorothioate ASO on tumor growth in cancer models**

| Design of antisense RNA and/or phosphorothioate ASO | Cell type(s) | Comments | References |
|---|---|---|---|
| Inducible antisense RNA expression plasmid (1–309 bp) and translation start site phosphorothioate ASO | FO-1 human melanoma | inhibits tumor formation in nude mice | [232] |
| Inducible antisense RNA expression plasmid (1–309 bp) and/or phosphorothioate ASO to translation start site | C6 rat glioma | inhibits clonogenic survival and tumor formation and induces regression of established tumors | [165,233,234] |
| Constitutive antisense RNA expression plasmid (1–291 bp) | MCF-7 human breast cancer | inhibits growth *in vitro* | [235] |
| Adenoviral antisense RNA expression (1–300 bp) | NCI H460 and SCC5 human lung cancer | causes regression of established tumors upon viral infection | [236] |
| Inducible antisense RNA expression plasmid (1–738 bp) | PA-III rat prostate cancer | suppresses tumor formation | [237] |
| Constitutive antisense RNA expression plasmid (1–1581 bp) | N2A murine neuroblastoma | induces regression of established tumor | [166] |
| Constitutive antisense RNA expression plasmid (1–697 bp) | MDA 435 human breast cancer | inhibits cell growth and clonogenic survival *in vitro* and tumor growth and metastasis *in vivo* | [156] |
| Inducible antisense RNA expression plasmid (1–309 bp) | human cervical cancer | Inhibits clonogenic survival in soft agar, slows tumor formation in nude mice. | [238] |
| Constitutive antisense RNA expression plasmid (1–309 bp) | B16.F1 murine melanoma | inhibits growth, survival and tumorigenicity; enhances radiosensitivity, impairs Atm function | [160] |
| Antisense 1–309 bp in retroviral vector | H9 metastatic Lewis lung | inhibits invasion *in vitro*, metastasis *in vivo* | [239] |
| Inducible antisense RNA expression plasmid (1–309 bp) | Ewing's sarcoma cells (TC-71) | decreases cell growth, motility, tumorigenesis and metastasis and increases sensitivity to doxorubicin | [157] |

tide arrays to identify regions within *IGF1R* mRNA that are accessible to bind antisense oligonucleotides [187]. We synthesized siRNAs homologous to accessible or inaccessible regions of the transcript and have been able to show that secondary structure in the IGF1R transcript has a major effect on the efficacy, not only of ASOs, but also of siRNAs that mediate *IGF1R* gene silencing [188]. The requirement for access comparable to that required for ASO binding supports the concept, as originally proposed when RNAi was first recognized [175,182], of direct interaction by base-pairing between the transcript and component(s) of the duplex. This is consistent with the recent demonstration that antisense strands can mediate RNAi in mammalian cytoplasmic lysate [189]. Encouragingly, chemically synthesized and plasmid-based siRNAs are now being used *in vivo* [190–195]. It seems clear that RNA interference is not only a powerful research tool for studying gene function, but also shows genuine therapeutic potential.

### Other molecular approaches

Ribozymes are catalytic RNA molecules that cleave RNA substrates. Two of the common types, hairpin and hammerhead ribozymes, contain an auto-catalytic motif flanked by antisense guide sequences that bind to target mRNA [196]. Ribozymes have been used to induce downregulation of the IGF2R, reducing target protein levels and enhancing growth of human breast cancer cells [197]. Prostate cancer cell growth can be inhibited using IGF-II ribozymes, but the effects are similar using a mutant ribozyme, suggesting that inhibition of IGF-II expression and growth is attributable to the antisense effect of the complementary flanking sequences [198]. Oligonucleotides have also been designed to induce triplex formation in the IGF1R promoter, to block IGF1R gene replication and transcription [199,200]. The triplex approach has the theoretical advantage that there are only two target molecules per cell (if diploid), compared with the thousands of copies of mRNA that are the target of antisense. However, unlike antisense, which can be targeted to any sequence, the triplex approach is limited to targets with certain sequence characteristics. The interaction is weak at physiological pH and temperature, but can be stabilized by incorporation of reactive groups [201].

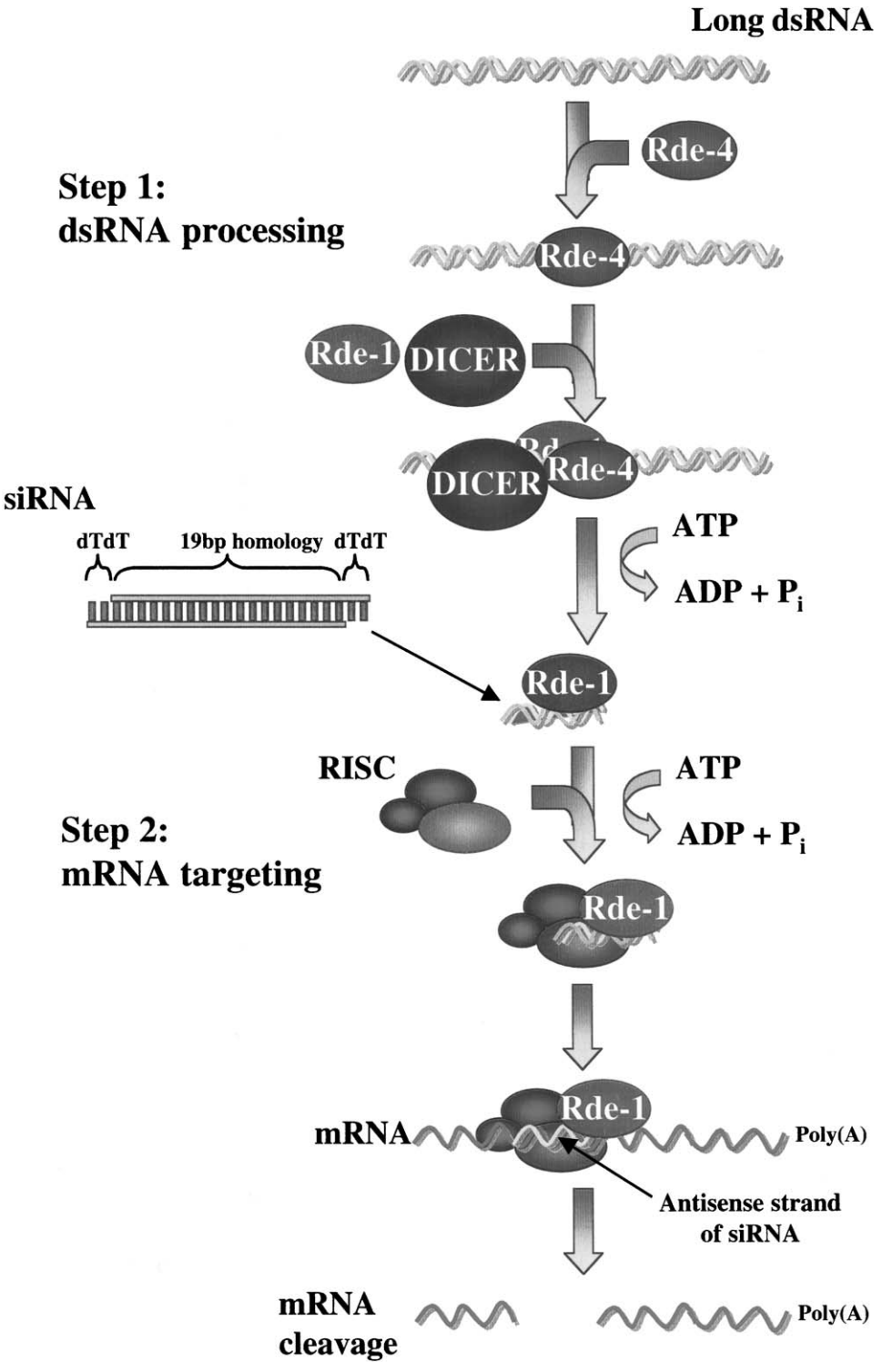
## Potential problems

### Normal tissue toxicity

The IGF1R is essentially ubiquitous [132]. Although receptor density may be higher in some malignant tumors than in benign tissues, normal tissue toxicity is a major consideration for this approach. A measure of selectivity may be provided by the fact that normal tissue growth is anchorage-dependent, conforming to tissue planes, and this mode of growth is influenced only to a minor extent by IGF signaling. In contrast neoplastic growth is anchorage-independent, a parameter that is influenced to a much greater extent by the IGF1R [202].

Although the IGF1R is clearly important in early development [34,35], it is unclear to what extent IGF1R expression and function are required in the mature adult. It is possible that IGF1R inhibitors could cause toxicity to tissues that are rapidly proliferating, such as the bone

**Fig. 2**




RNA interference. In *C. elegans*, the initiation step involves binding of dsRNA to proteins of the Rde family for delivery to the nuclease Dicer, a member of the RNase III family. Dicer cleaves dsRNA into siRNAs, which are bound by Rde proteins and loaded into the RNA-induced silencing complex (RISC). Here the antisense strand of the siRNA serves as a guide to direct cleavage of the target mRNA by nucleases contained within the effector complex. In mammalian cells long dsRNAs activate an antiviral response leading to generalized inhibition of translation. Chemically synthesized or transcribed 21- to 23-bp siRNAs are too small to activate this response, yet by mimicking Dicer products they can enter the RNAi pathway at the effector step.

marrow and epithelial lining of the gastrointestinal tract. IGF1R levels are relatively low in some hemopoietic subsets, e.g. T cells [203], but this may not protect from toxicity. Thus it is possible that the side-effects of IGF1R targeting could resemble the toxicity of conventional chemotherapy. One specific concern that would be a major barrier to this approach is the possibility of impairment of neurological function, given that IGFs play an important role in neuronal survival [204,205]. Systemic administration of a small molecule therapeutic, such as a chemical inhibitor or oligonucleotide, could thus have potentially severe effects on peripheral or central nervous system function that might be irreversible. Large molecules such as antibodies may be less likely to cross the blood–brain barrier, potentially protecting from this problem. However, certain antibody isotypes can cause toxicity by antibody-dependent cellular cytotoxicity (ADCC) [206,207], which could damage even those tissues with low-level IGF1R expression. For molecular approaches the problem of normal tissue toxicity could be ameliorated by the use of tissue-specific promoters [208] to direct expression to a specific population of cells.

Toxicity may also arise from agents that cross-react with the insulin receptor, blocking its expression or function. This is likely to be a significant consideration for small molecule IGF1R kinase inhibitors, given the similarity between the IGF1R and insulin receptor kinase domains [137]. Therefore it will be important to monitor glucose tolerance during clinical trials of these agents.

### Magnitude of clinical activity

Efficacy is the key consideration that will determine whether this approach will be successful in the clinic. Preclinical studies have suggested that IGF1R targeting may be effective in a range of tumor types, but clinical activity may be more limited. For example, trastuzumab (Herceptin) is effective only in patients with tumors that are strongly $HER2^+$ [209]. It is unclear at present whether IGF1R inhibitors will block growth of only those tumors with high IGF1R overexpression. In the first instance it would be reasonable to extend clinical trials of IGF1R targeting to patients with a wide spectrum of tumor types. It will be important, however, to measure IGF1R levels and activity in all cases, to allow correlation with clinical response and, hopefully, identification of tumor types/subgroups where this approach is effective.

At present it is not clear whether the IGF axis is essential for the maintenance of the malignant, metastatic phenotype *in vivo* nor to what extent another growth factor pathway could compensate for loss of IGF signaling. It is notable that the most successful biological agent currently in use, STI571 (Gleevec; Novartis), inhibits the kinase activity of the Bcr–Abl fusion protein, which appears to be absolutely required for the growth of the malignant cells in chronic myeloid leukemia [210]. IGF1R upregulation has been shown to mediate tumor cell resistance to inhibitors of the EGF receptor and HER2, via continued activation of PI3K signaling [211,212]. It is possible that reciprocal upregulation of EGF receptor family members could negate effects of IGF1R targeting *in vivo*. As a logical extension of this concern, biological agents may be used in combination, such as the recently reported use of Herceptin together with expression of dominant-negative IGF1R to target HER2-overexpressing human breast cancer cells [213].

## Conclusion

The next few years will see the introduction of IGF1R targeting into the clinic. As with all new therapies, the extent of its success will depend upon the balance between anti-tumor activity and toxicity. The key issue that will determine utility is whether the undoubted preclinical activity of IGF1R targeting, documented in a plethora of studies, will translate to clinical efficacy in patients with metastatic cancer.

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