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IGF-IR blockade strategies in human cancers

Review Article

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Abbreviations: Antisense Oligonucleotides, (AS ODNs); chronic myelocytic leukemia, (CML); dominant negative IGF-IR, (dn IGF-IR); Ewing's sarcoma, (ES); insulin receptor substrate-1, (IRS-1); insulin-like growth factor receptor, (IGF-IR); insulin-like growth factor, (IGF); mitogen-activated protein kinase, (MAPK); phosphatidylinositide 3-kinase, (PI3-K); RNA interference, (RNAi)

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Summary

Growth factor receptor signals, like those from insulin-like growth factor (IGF)-I receptor (IGF-IR), are required for carcinogenesis and tumor progression in many human malignancies. The concept of targeting specific tumorigenic receptors has been validated by the successful clinical application of multiple new drugs, such as trastuzumab and gefitinib. Genetic blockade of IGF-IR has been accomplished by antisense, dominant negative inhibition, siRNA, and triplex formation mediated by plasmid vector transfection, oligonucleotide, or viral transduction, whereas non-genetic blockade of IGF-IR has been accomplished using soluble IGF-IR, monoclonal antibodies, and IGF-IR tyrosine kinase inhibitor. IGF-IR blockade induces apoptosis of cancer cells by blocking antiapoptotic signaling pathways resulting in the regression of established tumors. However, the lack of a suitable means for inducing an effective IGF-IR blockade remains an obstacle to the clinical application of IGF-IR blockade strategies. Furthermore, the structural similarity between IGF-IR and insulin receptor increases the importance that any inhibitor used in the clinic be both highly specific as well as effective. Here we review the current status of IGF-IR blockade strategies for cancer treatment and suggest possible future development directions.

I. Introduction

A. Growth receptors: Emerging new therapeutic targets for cancer

A variety of growth factor signals are required for carcinogenesis and tumor progression in human malignancies (Baserga, 1994). Signals from these receptors alter cell cycle regulation, induce apoptosis, and induce interactions between tumor cells and their environment which affect the continuous growth potential of tumor cells (Baserga, 1995).

Recently, advances in the molecular biology of cancer have resulted in the introduction of several new drugs that target growth factor receptors. Trastuzuamb (Herceptin) is a human antibody against HER2 (EGFR 2; type 1 transmembrane tyrosine kinase), whereas erlotinib (Tarceva) competes for the ATP binding site of the EGFR tyrosine kinase domain. Imatinib (Gleevec: STI571), another successful drug, is a small molecule that acts by targeting Bcr-Abl cytoplasmic tyrosine kinase, which is constitutively active in chronic myelocytic leukemia (CML). Advances evidenced by the introduction of these drugs have encouraged scientists involved in anticancer drug development to investigate the likely potential of targeting growth factor receptors. Insulin-like growth factor (IGF)-I receptor (IGF-IR) has been a major target of these investigations for some time.

B. Insulin-like growth factor I receptor in human tumors

Most cancer cells of epithelial origin, including those of lung, colon, and pancreas show IGF-mediated growth responsiveness (Nakanishi et al 1988; Bergmann et al 1995; Freier et al 1999). Recent studies have shown that elevated levels of IGF-I in serum increase the risk of cancer development [e.g. colon, prostate, and breast (Chan et al, 1998; Hankinson et al, 1998; Ma et al, 1999)]. And, many have found that IGFs/IGF-IR signals affect tumor development other than by their mitogenic or anti-apoptotic effects. Thus it appears that overactive IGF-IR signaling is important for tumor dissemination through effects on adhesion, migration, and metastasis.

IGFs and their receptors are important during lung development and respiratory system cell growth (Stiles et al, 1990), and many human lung cancer cell lines produce both IGF ligand and receptor. It has been suggested that they mediate autocrine proliferation (Nakanishi et al, 1988; Ankrapp et al, 1993). In human colorectal carcinomas, exogenous IGFs stimulated cancer cell proliferation, and conversely blocking IGF-IR inhibited tumor growth (Remacle-Bonnet et al 1992; Lahm et al, 1994). Moreover, intestinal fibroblast-derived IGF-II has been shown to stimulate the proliferation of intestinal epithelial cells in a paracrine manner (Simmons et al, 1999). In pancreatic cancer it has been suggested that paracrine and autocrine mechanisms aberrantly activate IGF-IR (Bergmann et al, 1995). The IGF/IGF-IR system has also been reported to be important in hematologic tumors, e.g. multiple myeloma (Tai et al, 2003). Thus, the blockade of IGF-IR might be relevant in a wide range of malignancies.

IGF-IR is synthesized as a single precursor peptide of 1367 amino acid residues, which is subsequently cleaved at residue 706, into an α subunit, which contains an extracellular domain, and a β subunit, which possesses a transmembrane and tyrosine kinase domains (Ullrich et al, 1986). The binding of IGF-I or IGF-II, to IGF-IR (a heterotetramer composed of two α and two β chains), causes receptor autophosphorylation and tyrosine kinase activation, and this activated tyrosine kinase subsequently phosphorylates a host of intracellular substrates, including insulin receptor substrate-1 (IRS-1) and Shc. Moreover, these early events activate multiple signaling pathways, which include the mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3-K)/Akt-1 (protein kinase B) pathways (Baserga, 1995; Yu et al, 2000).

IGF-IR signaling can potently stimulate cellular proliferation and induce cellular differentiation (Sara et al, 1990). And, in certain systems, IGF-IR appears to be essential for malignant transformation (Sell et al, 1993; Baserga, 1995). IGF-IR was also found to be important for the maintenance and the initiation of malignancy (Baserga, 1995).

The activation of IGF-IR by IGF-I has a strong antiapoptotic effect on cancer cells, which involves multiple pathways. The main signaling pathway is mediated through the activation of IRS, which is followed by the activations of PI3-K and Akt/protein kinase B (Kulik et al, 1997) and by the phosphorylation of BAD (Datta et al, 1997). Alternative pathways include those involving the activation of MAPK and the mitochondrial translocation of Raf (Peruzzi et al, 1999); both of these two latter pathways also result in BAD phosphorylation.

Reductions in the levels of IGF-IR have been shown to induce apoptosis in tumors, but only growth arrest in untransformed cells (Baserga, 1994), which implies that an IGF-IR blockade based strategy has greater therapeutic potential than strategies that target more fundamental cell processes such as DNA synthesis or the cell cycle. This notion is supported by the finding that IGF-IR knockout mice remain viable (though physically smaller than the wild type), thus indicating that relatively normal tissue development and differentiation can occur in the absence of IGF-IR (Liu et al, 1993).

II. Strategies for blocking IGF-IR signaling

Summarizing the above studies, it is therefore clear that IGF-IR meets several requirements that present it as an attractive target for cancer therapy because: (1) IGF-I is strongly implicated in malignant transformation and in the maintenance of the malignant phenotype (2) IGF-IR and its ligands are found abundantly in clinically important human tumors (3) Blocking the IGF-I pathway induces tumor growth suppression, apoptosis, and loss of tumorigenicity (Surmacz, 2003). To date, several means of blocking IGF-IR signaling have been reported (**Figure 1**). As mentioned above, the development of a highly sensitive and specific molecule that targets IGF-IR, but not the insulin receptor, is fundamental to these approaches. Here, we discuss and review current strategies targeting IGF-IR in terms of the mechanisms and methods involved.

A. Antisense strategy

1. Plasmid vectors expressing antisense IGF-IR

A commonly used strategy for blocking IGF-IR in experimental systems involves directly reducing receptor expression using antisense cDNA vectors. The stable transfection of antisense plasmids expressing the first 300 bp of IGF-IR was found to reduce the tumorigenicity of a variety of tumor cell lines, and has been reported to induce systemic antitumor effects against established tumors in animal models (Resnicoff et al, 1994, Long et al, 1995).

This antitumor effect of blocking IGF-IR in these studies was mediated at least in part by inducing a systemic immune response. Liu X et al (1998) found that direct injections of antisense expression vector into established tumors (neuroblastoma) induced tumor regression in syngeneic mice but not in SCID mice. However, the mechanism of immune response induction by blocking IGF-IR was not well investigated. Nevertheless, two studies on immune induction by antisense IGF-I (not IGF-IR) suggest a mechanism. The observed increased expressions of MHC class I and costimulatory B7 in IGF-I antisense transfected brain tumor cells might contribute the immune recognition (Trojan et al, 1996). Furthermore, cotransfection of

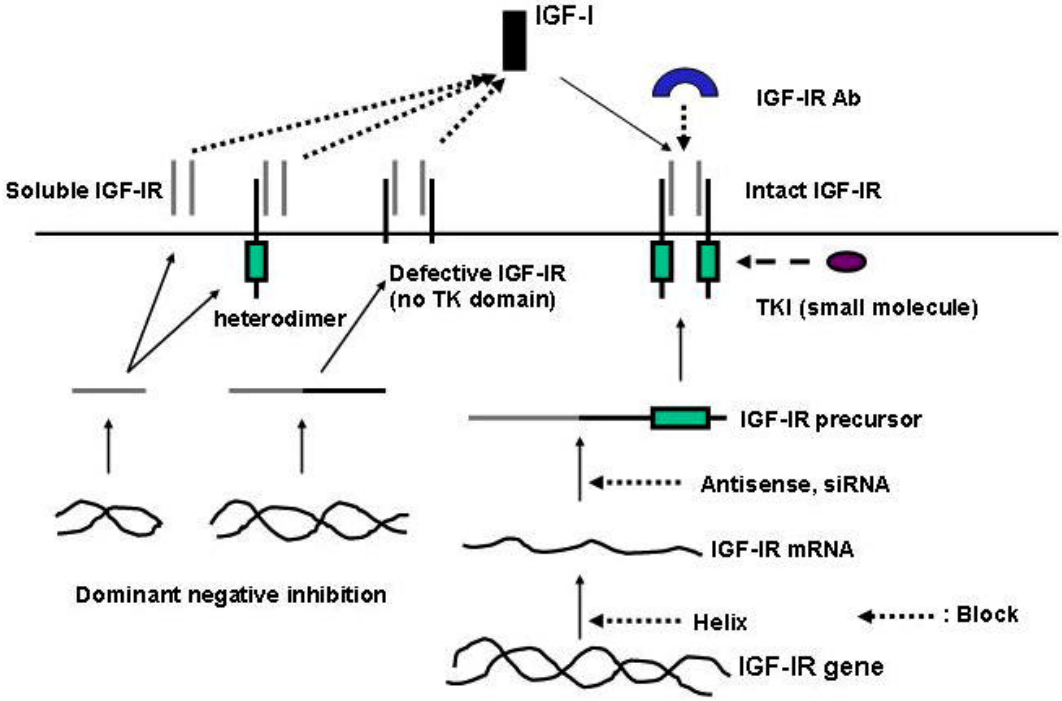

Figure 1. Summary of the IGF-IR blockade cancer treatment strategy.

antisense IGF-I and B7 into poorly immunogenic hepatoma and colon carcinoma cells induced a tumor specific antitumor immune response, which was mediated by CD3+ CD8+ T cells, and resulted in established tumor regression (Liu Y, 2000).

The role of IGF-IR blockade has been extensively studied in several cancers including breast, cervix, lung, pancreatic, brain, and colon cancers. ER negative breast cancer cells carrying antisense IGF-IR showed significant delays in cell growth, soft agar colony formation, tumor formation, and reduced metastasis in SCID mice (Chernicky et al, 2000). The same group demonstrated that a murine mammary carcinoma cell line (EMT6) carrying antisense IGF-IR showed decreased IGF-IR mRNA, and decreased tissue-type and urokinase-type plasminogen activator levels, the latter two of which have important roles in cancer invasion and metastasis (Chernicky et al, 2002). Moreover, the transfection of several cervix cancer cell lines with antisense IGF-IR reduced tumorigenesis regardless of HPV type (Nakamura et al, 2000).

Down-regulation of IGF-IR using an antisense strategy has also been shown to increase the sensitivity of cancer cells to standard anticancer treatments, for example, Ewing's sarcoma cells expressing antisense IGF-IR showed significant increases in sensitivity to doxorubicin (Scotlandi et al, 2002), IGF-IR downregulation also reduced the chemoresistance of prostate carcinoma cells, and IGF-IR antisense plasmid or oligonucleotide transfection into a chemoresistant prostate cancer cell line (DU145) reduced IGF-IR expression by 30-50% and increased sensitivity to cisplatin, mitoxantrone, or paclitaxel (Hellawell et al, 2003).

The ATM gene is mutated in AT (ataxia telangiectasia) cells and this is associated with the extreme radiosensitivity of these cells. The treatment of AT cells with ATM cDNA reversed this radiation sensitivity and increased both IGF-IR promoter activity and IGF-IR protein expression. This increase in IGF-IR levels was associated with an almost normalization of AT cell radiosensitivity. In addition, specific inhibition of IGF-IR in ATM cDNA complemented AT cells prevented this reversal of radiosensitivity. These results suggest that reduced IGF-IR function plays a direct and important role in AT radiosensitivity enhancement (Peretz et al, 2001). IGF-IR downregulation also enhanced melanoma cell sensitivity to radiation associated with a blockade of ATM activation, along with alterations in the cell cycle and the repair of radiation-induced DNA damage (Macaulay et al, 2001).

2. The use of antisense oligonucleotides (AS ODNs)

IGF-IR expression can also be effectively blocked by treating with antisense ODN to IGF-IR. In a C6 rat glioblastoma cell model, treatment with AS ODN to IGF-IR induced apoptosis, which was related to the level of IGF-IR. Furthermore, IGF-IR downregulation by ODN was also found to induce a systemic immune response and established tumor regression (Resnicoff et al, 1995). Moreover, the implantation of IGF-IR AS ODN by encapsulation in a diffusion chamber induced intracranial tumor regression in a C6 model (Resnicoff et al, 1996).

A pilot clinical study of antisense ODN against IGF-IR was conducted in malignant astrocytoma patients. Surgically obtained autologous glioma cells from sites of relapse in previously treated brain tumor patients were treated with an IGF-IR/AS ODN and encapsulated in a diffusion chamber and implanted in the rectus sheath. Of the 12 patients treated, 2 achieved CR and 6 a PR; however, response durations were short (2-27 weeks). Primary tumors at autopsy demonstrated marked

lymphocyte infiltration in four patients, which suggested that immune response had been induced by AS ODN, in addition, microvessel thrombosis in six patients contributed to the antitumor effect (Andrews et al, 2001).

The antitumor effect of AS ODNs depends on their ability to bind IGF-IR mRNA, a process that is strongly dependant on secondary structure. Recently, a scanning oligonucleotide array technique enabled the selection of an AS ODN which had a high heteroduplex yield with IGF-IR mRNA. Highly hybridizing AS ODNs were found to effectively down-regulate IGF-IR within tumor cells (Bohula et al, 2003). It is expected that this technique will increase the therapeutic potential of IGF-IR AS ODNs.

3. Viruses expressing antisense/ribozyme IGF-IR

In order to develop a clinical approach using an antisense strategy, we constructed an adenovirus expressing an antisense cDNA of IGF-IR corresponding to 321 bp of the IGF-IR open reading frame including an ATG initiation codon (Ad-IGF-IR/as). A single transduction with Ad-IGF-IR/as reduced IGF-IR number by about 50% in human lung cancer cell lines. This modest reduction in IGF-IR expression in NCI-H460 cells by Ad-IGF-IR/as markedly suppressed colony formation by nearly 10 fold in a soft agar clonogenic assay. Intraperitoneal treatment with Ad-IGF-IR/as in nude mice bearing an intraperitoneal lung cancer xenografts resulted in significantly better survival versus nude mice treated with a control virus. Thus, this study demonstrated the potential therapeutic effect of ad-IGF-IR/as on *in vitro* tumorigenicity, and on established a human lung cancer xenograft (Lee et al, 1996). Moreover, Samani et al (2001) found that a retrovirus expressing antisense (the first 309bp) of IGF-IR successfully reduced IGF-IR expression by 70% in a highly metastatic tumor cell-line (H-59, Lewis lung carcinoma sub-line). Treatment reduced its soft agar colony forming ability and reduced hepatic metastasis and increased survival. IGF-IR expression can also be reduced by ribozyme cleavage. Adeno-associated virus expressing hammerhead ribozyme targeting IGF-IR(IGF-IR Rz) successfully reduced IGF-IR expression and pathologic retinal neovascularization (Shaw et al, 2003).

B. Dominant negative strategy

1. Truncated IGF-IR expressed on cell surfaces versus soluble IGF-IR

Two different dominant negative strategies have been proposed. One strategy involves the production of a truncated IGF-IR with an intact α subunit and the transmembrane portion of the β subunit either lacking or with a mutated tyrosine kinase domain. This defective receptor was found to be presented on the cell surface and to form a dimer with wild type IGF-IR monomer or with another defective monomer. The binding IGF-I to the aberrant receptors does not result in signal transmission (Prager et al, 1994; Burgard et al, 1995; Li et al, 1996). Another strategy involves the production of a defective α subunit that can be released from the cell (soluble IGF-IR), which can then compete for IGF-I in the extracellular environment or form a defective dimer with intact IGF-IR monomer (Reiss et al, 2001). This soluble form of IGF-IR has a definite advantage due to its potential for a bystander effect as it neutralizes IGF-I by binding in the extracellular environment and thus has effects on neighboring cells not expressing the mutant receptor.

2. Plasmids expressing dominant negative IGF-IR (dn IGF-IR)

Most studies on dominant negative IGF-IR blockade have used expression vectors that produce defective IGF-IR. The stable transfection of an intact α subunit and a truncated β subunit of IGF-IR (952STOP) inhibited tumorigenicity in a rat tumor cell line (Prager et al, 1994). Moreover, IGF-IR receptors in mouse embryo fibroblasts (R-cells) transfected with IGF-IR carrying the Y950F mutation were found to have lost the ability to transmit a mitogenic signal or to transform R-cells (Miura et al, 1995). The transfection of IGF-IR truncated at codon 486 effectively blocked IGF-IR function (D'Ambrosio et al, 1996, Dunn et al, 1998). This IGF-IR 486STOP was found to be secreted in the extracellular environment and induced a strong bystander effect by neutralizing IGF-I, and thus inhibited adhesion, invasion, and metastasis in a breast cancer model. Reiss et al (1998) also showed that transfection with IGF-IR/486STOP induced massive apoptosis, and inhibited tumor growth and metastasis with a strong bystander effect. The same group suggested that this effect was via a complex mechanism involving the retention of the soluble IGF-IR fragment in the cytoplasm where it binds with endogenous wild type IGF-IR (Reiss et al, 2001). The soluble IGF-IR thus induced IGF-IR blockade by successfully competing with wild type IGF-IR for IGF-I as well as forming a defective heterodimer.

We also investigated this dominant negative IGF-IR strategy using IGF-IR/482 (stop at codon 482) in a tet(tetracycline)-repression expression vector (Adachi et al, 2002). Soft agar assays showed that the number of HT29dn colonies was suppressed by 2 to 3 orders of magnitude when IGF-IR/482st was expressed versus the same cells in which dn receptor expression was suppressed. Moreover, IGF-Ir/482st increased apoptosis 2 - 4 fold compared to controls, and chemotherapy (cisplatin and 5-FU)-induced apoptosis was significantly up-regulated in the presence of IGF-IR/482st. IGF-IR/482st blocks IGF-IR signaling mainly by modulating the PI3-K/Akt pathway. In addition, IGF-IR/482st has a pronounced bystander effect, which was confirmed using a double chamber system by Western blotting. IGF-IR/482st effectively reduced the tumorigenicity of HT29dn cells *in vivo*, and more strikingly, the induction of IGF-IR/482st on tumor cells resulted in the rapid shrinkage of SC tumors in nude mice, suggesting that IGF-IR blockade might be an effective therapeutic strategy for clinically evident tumors. In this study, IGF-IR/482st + 5-FU combination therapy maximally suppressed SC tumor growth. The number of apoptotic cells was significantly increased in IGF-IR/dn expressing HT29dn tumors. These above results suggest that IGF-IR/482st has significant

potential for both the prevention and treatment for human cancer cells.

3. Viral expression of dominant negative IGF-IR

Although the dominant negative blockade of IGF-IR achieved by plasmid transfection was found to have a strong therapeutic effect in laboratory systems, this method is encumbered by a variety of obstacles likely to prevent it from becoming a practical cancer gene therapy. To overcome these limitations, we constructed two adenoviruses expressing IGF-Ir/dns, namely, Ad-IGF-IR/482st, and Ad-IGF-IR/950st (Adachi et al 2002; Lee et al 2003; Min et al 2003). Both Ad-IGF-IR/dns can induce truncated receptors in a dose-dependent fashion, roughly in proportional to the dose of adenovirus present in colon, pancreas, and lung cancer cell lines. As expected, in these experiments defective IGF-IR from Ad-IGF-IR/950 was expressed on cell surfaces and the soluble IGF-IR was secreted after treatment with adenovirus transfected with Ad-IGF-IR/482st (**Figure 2A, B**). The blockade of IGF-I signaling by Ad-IGF-IR/dns (482st and 950st) effectively blocked IGF-I induced DNA synthesis, an index of mitogenesis. This finding suggests that truncated IGF-IRs compete with normal IGF-IR for ligands. Both Ad-IGF-IR/dns induced a marked suppression of NCI-H460 colony formation in a soft agar assay, suggesting that Ad-IGF-IR/dn transduction reduces tumorigenic potential.

Three pancreatic cancer cell lines (PANC-1, BxPC-3, and AsPC-1) infected with Ad-IGF-IR/482st showed marked reduction in viability. Moreover, Ad-IGF-IR/dns upregulated stressor (serum starvation or 5% ethanol) induced apoptosis, and enhanced chemotherapy (5-FU)- and radiation- induced apoptosis in pancreatic and colon cancer cells (BxPC-3 and HT29) (Adachi et al, 2002; Min et al, 2003).

IGF-induced phosphorylated Akt levels were reduced by both Ad-IGF-IR/dns in all colon, pancreatic, and lung cancer cells analyzed (Figure 2C). In AsPC-1 and BxPC-3 cells, Ad-IGF-IR/dn also blocked the IGF-I induced phosphorylation of p38 MAPK, but it did not influence ERK-1 or -2 phosphorylation significantly. IGF-IR/482st blocked the IGF-I-induced phosphorylation of Akt in both of these pancreatic cancer cells.

Gene therapeutic strategies based on IGF-IR/482st should show an enhanced antitumor effect due to its bystander effect. This was confirmed by conditioned media transfer. As Ad-IGF-IR/dns can markedly reduce mitogenesis and induce apoptosis *in vitro*, we investigated their *in vivo* efficacies in mouse tumor models. Intratumoral injections of Ad-IGF-IR/dn retarded and shrunk established HT29 (Adachi et al, 2002), BxPC-3(Min et al, 2003), and NCI-H460 tumors (Lee et al, 2003).

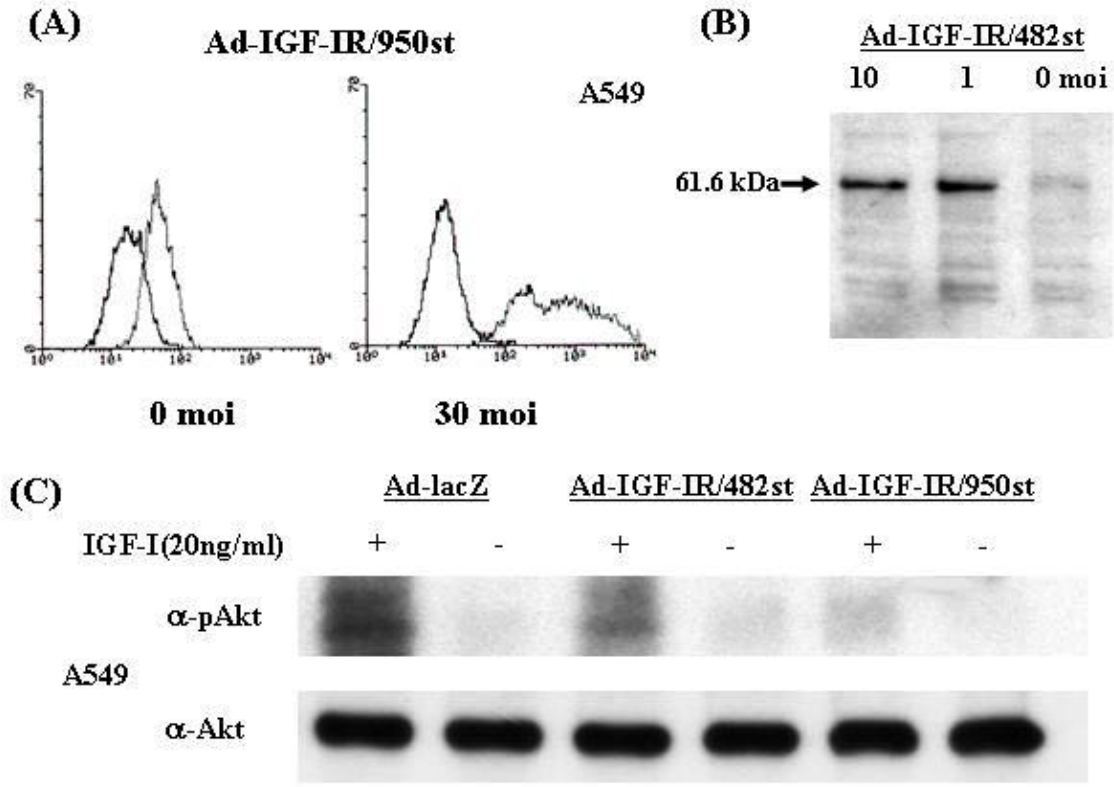

Figure 2. Dominant negative inhibition of IGF-IR by adenovirus expressing defective IGF-IR (**A**) Changes in IGF-IR expression on A549 (lung cancer cell line) cell surfaces after transduction with ad-IGF-IR/950st. (**B**) Western blot for IGR-IR conducted on concentrated ad-IGF-IR/482st transduced cell (A549) medium. The band at 61.6kDa represents soluble IGF-IR (truncated α subunit of IGF-IR). (**C**) Ad-IGF-IR/950st and ad-IGF-IR/482st effectively inhibited IGF-I-induced Akt kinase activation (Reproduced from Lee et al, 2003 with kind permission from Cancer Gene Therapy).

Moreover, the tumor suppressing effect of Ad-IGF-IR/482st was greater than that of Ad-IGF-IR/950st, which was attributed to the bystander effect of IGF-IR/482st.

Ad-IGF-IR/482st + 5-FU combination therapy for SC BxPC-3 tumors in mice was found to be more effective than either monotherapy alone. One-third of mice with these tumors were cured when treated with this regimen, whereas none were cured by treating with either agent alone. This indicates that Ad-IGF-IR/482st has potential to enhance the effectiveness of standard cancer therapies.

In addition, the antitumor effect of adenovirus-IGF-IR/dn was remarkably enhanced by cotreatment with the conditionally replicating adenovirus CRAD: Δ24RGD. Δ24RGD is a type of oncolytic adenovirus (CRAD) which produces a mutant E1 protein that lacks the ability to bind retinoblastoma protein, but which retains its viral replication competence. Moreover, theoretically this virus can only replicate in cancer cells with a defective pRb/p16 pathway. Conventional replication defective adenoviruses have a deletion in E1, which is essential for viral replication, however this E1 deleted virus can become replication competent when cotransduced with a CRAD, as CRAD supplies E1 in trans. The cotransduction of ad-IGF-IR/dn and Δ24RGD induced ad-IGF-IR/dn replication in the tumor mass and increased ad-IGF-IR/dn transduction efficiency. Thus this cotransduction remarkably increased the expression of defective IGF-IR in ad-IGF-IR/950 transduced cells or the amount of soluble IGF-IR produced by ad-IGF-IR/482 transduced cells (**Figure 3A, B**). Consequently, the intratumoral injection of Δ24RGD + ad-IGF-IR/482 induced more growth suppression of established lung cancer xenograft than injections with either agent alone (**Figure 4**) (Lee et al, 2004).

4. Myristylated COOH terminus of IGF-IR

The COOH terminus of IGR-IR generates a proapoptotic signal, and the transfection of ovarian cancer cells (CaOV-3) with a plasmid encoding last 112 amino acids of IGF-IR carrying the myristylation signal, induced growth suppression and apoptosis by counteracting the antiapoptotic signal produced by IGF-IR (Hongo et al, 1998).

5. Defective IGF-IR (soluble form) protein

Recombinant soluble IGF-IR protein was also found to effectively inhibit the IGF-I pathway. The injection of purified soluble IGF-IR protein into human ovarian cancer cells induced cancer apoptosis and retarded tumor growth. This finding suggests that peptide therapy based on soluble IGF-IR may have the advantages of repeatability and the bystander effect (Hongo et al, 2003).

Synthetic peptide from the C-terminus of IGF-IR (1282 to 1290) linked with stearic acid at its NH-terminus also inhibited DNA synthesis, cell growth *in vitro* and *in vivo*, and induced apoptosis (Reiss et al, 1999).

C. Triplex formation

The blocking of IGF-IR transcription by triple helix (triplex) formation is an alternative method for suppressing IGF-IR expression, however the effects of exogenous ODNs are very transient. Sequence specific, stable triple-helix structures can be formed by hydrogen bonding between polypurine or polypyrimidine-rich ODNs and the polypurine tracts of ds DNA. Rininsland (Rininsland et al, 1997) developed a means of producing a third strand, which can form a triple helix with the target gene and prevent the passage of RNA polymerase along the target DNA. They designed a vector to produce a triplex at the homopurine-homopyrimidine sequence 3' to the termination codon of the IGF-IR gene. The transcription vector (pTH-AG-IGFIR) containing the appropriate triple helix forming sequence with a homopurine target sequence in the 3' untranslated region of IGF-IR effectively suppressed the transcription of IGF-IR by inhibiting RNA polymerase passage. C6 rat glioblastoma cells transfected with this vector showed a dramatic reduction in tumorigenicity *in vivo*.

The therapeutic advantage of the triplex strategy is that only two targets (the two copies of the genes encoding IGF-IR) exist in diploid cells as opposed to the numerous copies of IGF-IR mRNA targeted by antisense, dominant negative, and siRNA strategies (Salisbury et al, 2003).

D. The use of siRNAs to IGF-IR

The phenomenon of sequence specific gene silencing due to RNA interference (RNAi) was first discovered in the nematode worm C. elegans as a response to dsRNA (siRNA) (Fire et al, 1998). The suppressive effect of siRNA on gene function was at least 10 times greater than that of sense or antisense RNA. Double strand siRNA, a hybrid consisting of a sense and antisense strand of endogenous mRNA, can initiate a cellular response that results in the sequence specific degradation of homologous single-strand RNA (Hannon, 2002). The term posttranscriptional indicates that RNA synthesis is not affected, but rather the RNA transcript is specifically degraded. This RNA interference apparently represents an old evolutionarily conserved defense mechanism. RNA sequence specific degradation by short sequence (19-21nt in size) siRNAs has a wide application range, as follows; First, this siRNA phenomenon can be used to selectively block a given gene to investigate its function. Second, siRNA may represent a completely new anticancer strategy. During carcinogenesis, genes (e.g., tumor suppressor gene) in cancer cells lose some aspect of their normal functionality and/or gain a function or activate of normally dormant gene (oncogene). Furthermore, to maintain its malignant phenotype, a cancer cell requires continuous stimulation by certain stimuli (e.g., growth factors). Moreover, activated oncogenes and growth factor loops also represent potential targets for RNAi strategies.

SiRNA against IGF-IR has already been described in the literature. Bohula et al (2003) described an siRNA sequence that could effectively block IGF-IR mRNA. Using a scanning oligonucleotide array technique, they selected an antisense ODN that would selectively bind IGF-IR but not insulin receptor. Furthermore, they also demonstrated that siRNAs homologous with accessible IGF-IR regional targets (determined by examining the secondary structure of IGF-IR) induce strong sequence-

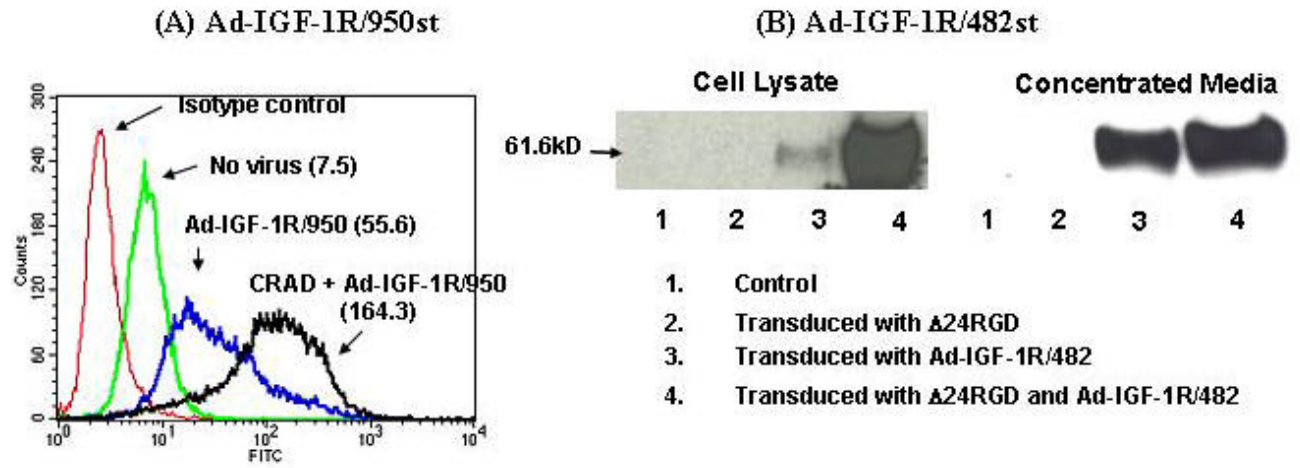

Figure 3. Combined treatment with ad-IGF-IR/dn and CRAD induced the replication ad-IGF-IR/dn in human lung cancer cells and enhanced IGF-IR/dn gene transfer rate. (**A**) Increased expression of IGF-IR on human lung cancer cell surfaces (NCI H460) after combined transduction with ad-IGF-IR/950st and Δ24RGD. (**B**) Increased production of soluble IGF-IR (truncated α chain) from Δ24RGD and ad-IGF-IR/482st cotransduced NCI H460 cells activation (Reproduced from Lee et al, 2004 with kind permission from Cancer Research).

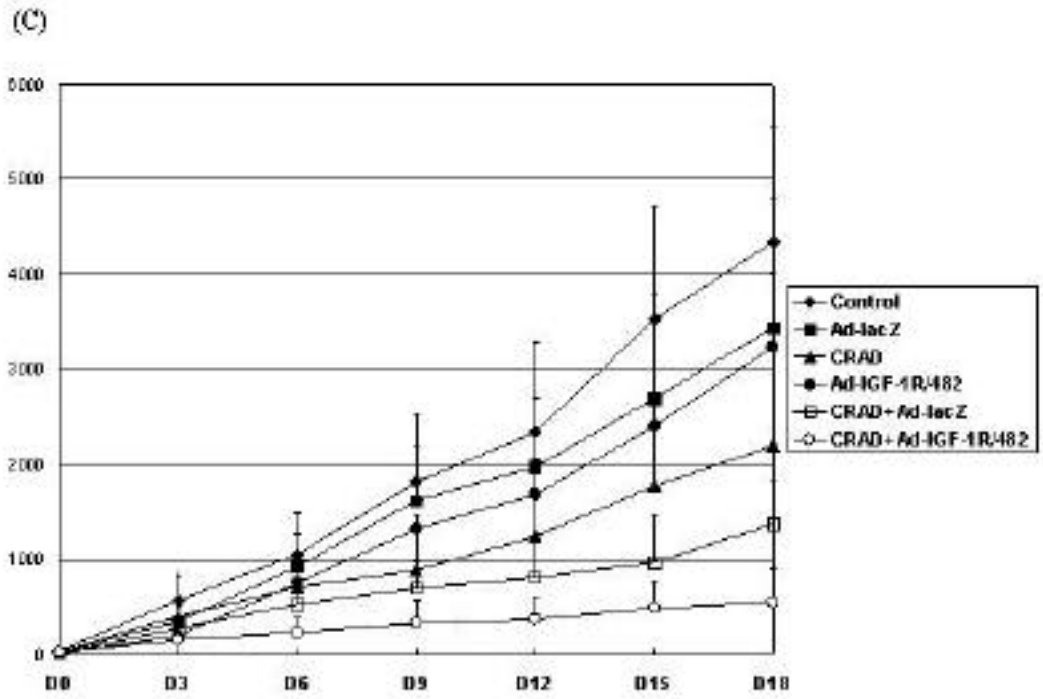

Figure 4. Treatment of lung cancer xenografts with combined ad-IGF-IR/482st and Δ24RGD enhanced *in vivo* antitumor versus treatment with these agents in isolation

specific IGF-IR gene silencing. This finding will surely facilitate the development of a siRNA strategy to block IGF-IR.

E. Antibodies to IGF-IR

IGF-IR blocking using monoclonal antibody has been extensively investigated, initially using αIR3 (IGF-IR blocking monoclonal antibody). Blocking IGF-IR with αIR3 was found to inhibit breast cancer cell-line growth in the presence but not in the absence of serum (Arteaga et al, 1989), and to suppress tumor formation in athymic mice (Arteaga et al, 1989).αIR3 inhibited Ewing's sarcoma (ES) cell growth and *in vitro* migration ability (Yee et al, 1990), and their *in vivo* tumorigenicity and metastatic ability (Scotlandi et al, 1998). IGF-IR blockade by αIR3 also increased ES sensitivity to doxorubicin and vincristine by enhancing the number of cells in the G1-phase and by increasing apoptosis (Benini S et al, 2001). 1H7 antibody, a monoclonal antibody to α subunit of IGF-IR, was found to inhibit the binding of IGF-I and IGF-II to IGF-IR and to inhibit the basal and IGF-I and IGF-II stimulated DNA synthesis in NIH3T3 cells expressing human IGF-IR (Li S et al, 1993). However, the effects of these commercially available antibodies were insufficient to allow them to be used as anticancer drugs. Thus, several

new, potent, specific IGF-IR antibodies have been recently introduced.

Recombinant single chain antibody against human IGF-IR (αIGF-IR scFv-Fc), cloned from hybridoma producing 1H7 monoclonal antibody (Li et al, 2000), has a 10-fold higher affinity for IGF-IR than αIR3, and was found to inhibit the growth of MCF-7 cells in athymic nude mice. Treatment of MCF-7 cells with this antibody downregulated IGF-IR via a lysosomal/endocytic pathway and rendered the cells refractory to IGF-I (Sachdev et al, 2003).

In addition, the antagonistic monoclonal antibody, EM164, was found to bind IGF-IR specifically without binding insulin receptor and to inhibit cell growth (Maloney et al, 2003). EM164 effectively inhibited the IGF-I induced autophosphorylation of IGF-IR and also inhibited IGF-I, IGF-II, or serum induced growth stimulation in several cancer cell lines.

Furthermore, EM164 was found to more potently suppress growth than other commercially available antibodies. It also effectively caused the regression of established human pancreatic xenografts. Moreover, combination therapy with EM164 and gemcitabine strongly enhanced the antitumor effect on established human pancreatic xenografts.

A12, another fully human antibody to IGF-IR, was introduced recently. A12 binds to IGF-IR specifically with high affinity and effectively blocks the two downstream pathways of IGF-I (MAPK and phosphatidylinositol 3'-kinase/Akt). A12 was found to reduce IGF-IR density on tumor cell surfaces by causing IGF-IR internalization and degradation. And, in several human cancer xenografts, A12 induced apoptosis and subsequent significant growth suppression (Burtrum et al, 2003).

F. IGF-IR tyrosine kinase inhibitor

Although several effective tyrosine kinase inhibitors, such as gefitinib (EGFR tyrosine kinase inhibitor) and imatinib (Bcr-Abl cytoplasmic tyrosine kinase inhibitor), are available, the structural homology between IGF-IR and the insulin receptor presents a significant obstacle for the development of an effective IGF-IR tyrosine kinase inhibitor. The β chain tyrosine kinase domain amino acids IGF-IR share 84% homology with insulin receptor (Ullrich et al, 1986). However, the elucidation of the three dimensional structure of IGF-IR may enable the development an effective and specific IGF-IR tyrosine kinase inhibitor by allowing precise 3-dimensional differences between it and insulin receptor to be identified (Garett et al, 1998; Adams et al, 2000; Favelyukis et al, 2001; Pautsch et al, 2001; De Meyts et al, 2002).

The tyrphostins are a family of synthetic protein kinase inhibitors derived from benzylidene malonitrile, and can inhibit receptor autophosphorylation. Most tyrphostins were found not to affect IGF-IR or insulin receptor binding. However, two (AG1024 and AG1034) were found to have significantly lower IC50's for IGF-IR than the insulin receptor. Moreover, AG1024 and AG1034 inhibited IGF-I-stimulated cell proliferation and blocked IGF-IR autophosphorylation and tyrosine kinase activity. However, these compounds are not suitable for human use, because they still have significant insulin receptor blocking activity (Parrizas et al, 1997).

AG538 is another potential IGF-IR tyrosine kinase inhibitor, which acts as a substrate-competitive inhibitor of IGF-IR (Blum et al, 2000). Recently this group described new more stable catechol bioisosteres of AG538, which inhibit IGF-IR kinase activity at submicromolar concentrations by substrate competitive inhibition. Moreover, these agents effectively blocked IGF-I induced IGF-IR autophosphorylation, IRS-1 phosphorylation, and PKB activation, and suppressed the soft agar clonogenicities of several tumor cell-lines (Blum et al, 2003).

Recently two pyrrolo[2,3-d]pyrimidine tyrosine kinase inhibitors, were introduced to selectively block IGF-IR tyrosine kinase activity. NVP-ADW742 is a selective IGF-IR tyrosine kinase inhibitor (over 16-fold more potent at blocking IGF-IR than the insulin receptor (IC50 for IGF-IR 0.17 μM vs 2.8 μM for IR). In addition, NVP-ADW alone showed *in vitro* and *in vivo* antitumor effects against multiple myeloma and other hematologic malignancies. NVP-ADW742 also sensitized cancer cells to other anticancer agents (i.e., doxorubicine, melphalan, dexamethasone, TRAIL, and PS-341) (Mitsiades et al, 2004).

NVP-AEW541 is another selective IGF-IR tyrosine kinase inhibitor. This small molecule also has IGF-IR selectivity versus the insulin receptor (IC50 to IGF-IR 0.086 μM vs. 2.3 μM for IR) as determined by receptor autophosphorylation. NVP-AEW541 can effectively block IGF-IR phosphorylation and the subsequent signaling pathways, and in a fibrosarcoma model, NVP-AEW541 effectively suppressed tumor growth (Garcia-Echeverria et al, 2004).

III. Discussion

IGF-IR is an attractive target for cancer treatment because it is present in many cancers, and its blockade induces tumor specific antitumor effects and systemic responses. However, the methods currently available for targeting IGF-IR, while rapidly improving, are still not ideal for clinical applications. With regard to genetic approaches, we believe that adenoviral vectors have some advantages over plasmid or AS ODN strategies though it also has significant limitations. Very significantly, there is a huge amount of evidence that combinations of current treatment modalities, such as chemotherapy, radiation therapy, and immunotherapy, with IGF-IR blockade may be particularly effective. Finally, the development of a sensitive and specific IGF-R tyrosine kinase inhibitor would be expected to have a substantial impact on cancer treatment as was demonstrated by the efficacy of this class of drugs targeting other receptors.

References

Adachi Y, Lee CT, Coffee K, Yamagata N, Ohm JE, Park KH, Dikov MM, Nadaf SR, Arteaga CL, Carbone DP (**2002)** Effects of genetic blockade of the insulin-like growth factor receptor in human colon cancer cell lines. **Gastroenterology** 123, 1191-1204.

Adams TE, Epa VC, Garrett TPJ, Ward CW (**2000)** Structure and function of the type 1 insulin-like growth factor receptor. **Cell Mol Life Sci** 57, 1050-1093.

Andrews DW, Resnicoff M, Flanders AE, Kenyon L, Curtis M, Merli G, Baserga R, Iliakis G, Aiken RD (**2001)** Results of a pilot study involving the use of an antisense oligonucleotide directed against the insulin-like growth factor I receptor in malignant astrocytomas. **J Clin Oncol** 19, 2189-2200.

Ankrapp DP, Bevan DR (**1993)** Insulin-like growth factor-I and human lung fibroblast-derived insulin-like growth factor-I stimulate the proliferation of human lung carcinoma cells *in vitro*. **Cancer Res** 53, 3399-3404.

Arteaga CL, Kitten LJ, Coronado EB, Jacobs S, Kull FCJr, Allred DC, Osborne CK (**1989)** Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic nude mice. **J Clin Invest** 84, 1418-1423.

Arteaga CL, Osborne CK (**1989)** Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. **Cancer Res** 49, 6237-6241.

Baserga R (**1994)** Oncogenes and the strategy of growth factors. **Cell** 79, 927-930.

Baserga R (**1995)** The insulin-like growth factor I receptor, a key to tumor growth? **Cancer Res** 55, 249-252.

Benini S, Manara MC, Baldini N, Cerisano V, Serra M, Mercuri M, Lollini PL, Nanni P, Picci P, Scotlandi K (**2001)** Inhibition of insulin-like growth factor I receptor increases the antitumor activity of doxorubicin and vincristine against Ewing's sarcoma cells. **Clin Cancer Res** 7, 1790-1797.

Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M (**1995)** Insulin-like growth factor I overexpression in human pancreatic cancer, evidence for autocrine and paracrine roles. **Cancer Res** 55, 2007-2011.

Blum G, Gazit A, Levitzki A (**2000)** Substrate competitive inhibitors of IGF-1 receptor kinase. **Biochemistry** 39, 15705-15712.

Blum G, Gazit A, Levitzki A (**2003)** Development of new insulin-like growth factor-1 receptor kinase inhibitors using catechol mimics. **J Biol Chem** 278, 40442-40454.

Bohula EA, Salisbury AJ, Sohail M, Playford MP, Riedemann J, Southern EM, Macaulay VM (**2003)** The efficacy of small interfering RNAs targeted to the type 1 insulin-like growth factor receptor (IGF1R) is influenced by secondary structure in the IGF1R transcript. **J Biol Chem** 287, 15991-15997.

Burgaud JL, Resnicoff M, Baserga R (**1995)** Mutant IGF-I receptors as dominant negatives for growth and transformation. **Biochem Biophys Res Commun** 214, 475-481.

Burtrum D, Zhu Z, Lu D, Anderson DM, Prewett M, Pereira DS, Bassi R, Abdullah R, Hooper AT, Koo H, Jimenez X, Johnson D, Apblett R, Kussi P, Bohlen P, Witte L, Hicklin DJ, Ludwig DL (**2003)** A fully human monoclonal antibody to the insulin-like growth factor I receptor blocks ligand-dependent signaling and inhibits human tumor growth *in vivo*. **Cancer Res** 63, 8912-8921.

Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, Hennekens CH, Pollak M (**1998)** Plasma insulin-like growth factor-I and prostate cancer risk, a prospective study. **Science** 279, 563-566.

Chernicky CL, Tan H, Yi L, De Mola JRL, Ilan J (**2002)** Treatment of murine breast cancer cells with antisense RNA to the type I insulin-like growth factor receptor decreases the level of plasminogen activator transcripts, inhibits cell growth *in vitro*, and reduces tumorgenesis *in vivo*. **J Clin Pathol, Mol Pathol** 55, 102-109.

Chernicky CL, Yi L, Tan H, Gan SU, Ilan J (**2000)** Treatment of human breast cancer cells with antisense RNA to the type I insulin-like growth factor receptor inhibits cell growth, suppresses tumorigenesis, alters the metastatic potential, and prolonged survival *in vivo*. **Cancer Gene Ther** 7, 384-395.

D'Ambrosio C, Ferber A, Resnicoff M, Baserga R (**1996)** A soluble insulin-like growth factor I receptor that induces apoptosis of tumor cells *in vivo* and inhibits tumorigenesis. **Cancer Res** 56, 4013-4020.

Datta, SR Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg ME (**1997)** Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. **Cell** 91, 231-241.

De Meyts P, Whittaker J (**2002)** Structural biology of insulin and IGF1 receptors, implications for drug design. **Nat Rev Drug Discov** 1, 769-783.

Dunn SE, Ehrlich M, Sharp NJ, Reiss K, Solomon G, Hawkins R, Baserga R, Barrett JC (**1998)** A dominant negative mutant of the insulin-like growth factor-I receptor inhibits the adhesion, invasion, and metastasis of breast cancer. **Cancer Res** 58, 3353-3361.

Favelyukis S, Till JH, Hubbard SR, Miller WT (**2001)** Structure and autoregulation of the insulin-like growth factor 1 receptor kinase. **Nat Struct Biol** 8, 1058-1063.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (**1998)** Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. **Nature** 391, 806-811.

Freier S, Weiss O, Eran M, Flyvbjerg A, Dahan R, Nephesh I, Safra T, Shiloni E, Raz I (**1999)** Expression of the insulin-like growth factors and their receptors in adenocarcinoma of the colon. **Gut** 44, 704-708.

Garcia-Echeverria C, Pearson MA, Marti A, Meyer T, Mestan J, Zimmermann J, Gao J, Brueggen J, Capraro H, Cozens R, Evans DB, Fabbro D, Furet P, Porta DG, Liebetanz J, Martiny-Baron G, Ruetz S, Hofman F (**2004)** *In vivo* antitumor effect activity of NVP-AEW541-A novel, potent, and selective inhibitor of the IGF-IR kinase. **Cancer Cell** 5, 231-239.

Garrett TPJ, McKern NM, Lou MZ, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW (**1998)** Crystal structure of the first three domains of the type-I insulin-like growth factor receptor. **Nature** 394, 395-399.

Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M (**1998)** Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. **Lancet** 351, 1393-1396.

Hannon GJ (**2002)** RNA interference. **Nature** 418,244-251.

Hellawell GO, Ferguson DJ, Brewster SF, Macaulay VM (**2003)** Chemosensitization of human prostate cancer using antisense agents targeting the type 1 insulin-like growth factor receptor. **BJU Int** 91, 271-277.

Hongo A, Kuramoto H, Nakamura Y, Hasegawa K, Nakamura K, Kodama J, Hiramatsu Y (**2003)** Antitumor effects of a soluble insulin-like growth factor I receptor in human ovarian cancer cells, Advantage of a recombinant protein administration in vivo. **Cancer Res** 63, 7834-7839.

Hongo A, Yumet G, Resnicoff M, Romano G, O'Connor R, Baserga R (**1998)** Inhibition of tumorigenesis and induction of apoptosis in human tumor cells by the stable expression of a myristylated COOH terminus of the insulin-like growth factor I receptor. **Cancer Res** 58, 2477-2484.

Kulik GA, Klippel A, Weber MJ (**1997)** Antiapoptotic signaling by the insulin-like growth factor I receptor, phosphatidylinositol 3-kinase, and Akt. **Mol Cell Biol** 17, 1595-1606.

Lahm H, Amstad P, Wyniger J, Yilmaz A, Fischer JR, Schreyer M, Givel JC (**1994)** Blockade of the insulin-like growth-factor-I receptor inhibits growth of human colorectal cancer cells, evidence of a functional IGF-II-mediated autocrine loop. **Int J Cancer** 58, 452-459.

Lee CT, Park KH, Adachi Y, Seol JY, Yoo CG, Kim YW, Han SK, Shim YS, Coffee K, Dikov MM, Carbone DP (**2003)** Recombinant adenoviruses expressing dominant negative insulin-like growth factor-I receptor demonstrate antitumor effects on lung cancer. **Cancer Gene Ther** 10, 57-63.

Lee CT, Park KH, Yanagisawa K, Adachi Y, Ohm JE, Nadaf S, Dikov MM, Curiel DT, Carbone DP (**2004)** Combination therapy with conditionally replicating adenovirus and replication defective adenovirus. **Cancer Res** 64, 6660-6665.

Lee CT, Wu S, Gabrilovich D, Chen H, Nadaf-Rahrov S, Ciernik IF, Carbone DP (**1996)** Antitumor effects of an adenovirus expressing antisense insulin-like growth factor I receptor on human lung cancer cell lines. **Cancer Res** 56, 3038-3041.

Li S, Resnicoff M, Baserga R (**1996)** Effect of mutations at serines 1280-1283 on the mitogenic and transforming activities of the insulin-like growth factor I receptor. **J Biol Chem** 271, 12254-12260.

Li SL, Kato J, Paz IB, Kasuya J, Fujita-Yamaguchi Y (**1993)** Two new monoclonal antibodies against the α subunit of the human insulin-like growth factor I receptor. **Biochem Biophys Res Commun** 196, 92-98.

Li SL, Liang SJ, Guo N, Wu AM, Fujita-Yamaguchi Y (**2000)** Single-chain antibodies against human insulin-like growth factor I receptor, expression, purification, and effect on tumor growth. **Cancer Immunol Immunother** 49, 243-252.

Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A (**1993)** Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r) **Cell** 75, 59-72.

Liu X, Turbyville T, Fritz A, Whitesell L (**1998)** Inhibition of insulin-like growth factor I receptor expression in neuroblastoma cells induces the regression of established tumors in mice. **Cancer Res** 58, 5432-5438.

Liu Y, Wang H, Zhao J, Ma J, Wei L. Wu S, Xie T, Shen F, Trojan J, Habib N, Anthony DD, Wu M, Guo Y (**2000)** Enhancement of immunogenicity of tumor cells by cotransfection with genes encoding antisense insulin-like growth factor-1 and B7.1 molecules. **Cancer Gene Ther** 7, 456-465.

Long L, Rubin R, Baserga R, Brodt P (**1995)** Loss of the metastatic phenotype in murine carcinoma cells expressing an antisense RNA to the insulin-like growth factor receptor. **Cancer Res** 55, 1006-1009.

Macaulay VM, Salisbury AJ, Bohula EA, Playford MP, Smorodinsky NI, Shiloh Y (**2001)** Downregulation of the type 1 insulin-like growth factor receptor in mouse melanoma cells associated with enhanced radiosensitivity and impaired activation of Atm kinase. **Oncogene** 20, 4029-4040.

Maloney EK, McLaughlin JL, Dagdigian NE, Garrett LM, Connors KM, Zhou XM, Blatter WA, Chittenden T, Singh R (**2003)** An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. **Cancer Res** 63, 5073-5083.

Min Y, Adachi Y, Yamamoto H, Ito H, Itoh F, Lee CT, Nadaf S, Carbone DP, Imai K (**2003)** Genetic Blockade of the Insulin-like Growth Factor-I Receptor, A Promising Strategy for Human Pancreatic Cancer. **Cancer Res** 63, 6432-6441.

Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA, Garcia-Echeverria C, Pearson MA, Hofmann F, Anderson KC, Kung AL (**2004)** Inhibition of the insulin-like growth factor-1 tyrosine kinase activity as a therapeutic strategy for multiple myeloma, other hematologic malignancies, and solid tumors. **Cancer Cell** 5, 221-230

Miura M, Li S, Baserga R (**1995)** Effect of a mutation at tyrosine 950 of the insulin-like growth factor I receptor on the growth and transformation of cells. **Cancer Res** 55, 663-667.

Nakamura K, Hongo A, Kodama J, Miyagi Y, Yoshinouchi M, Kudo T (**2000)** Down-regulation of the insulin-like growth factor I receptor by antisense RNA can reverse the transformed phenotype of human cervical cancer cell line. **Cancer Res** 60, 760-765.

Nakanishi Y, Mulshine JL, Kasprzyk PG, Natale RB, Maneckjee R, Avis I, Treston AM, Gazdar AF, Minna JD, Cuttitta F (**1988)** Insulin-like growth factor-I can mediate autocrine proliferation of human small cell lung cancer cell lines *in vitro*. **J Clin Invest** 82, 354-359.

Parrizas M, Gazit A, Levitzki A, Wertheimer E, LeRoith D (**1997)** Specific inhibition of insulin-like growth factor-1 and insulin receptor tyrosine kinase activity and biological function by tyrphostins. **Endocrinology** 138, 1427-1433.

Pautsch A, Zoephel A, Ahorn H, Spevak W, Hauptmann R, Nar H (**2001)** Crystal structure of bisphosphorylated IGF-1 receptor kinase, insight into domain movements upon kinase activation. **Structure** 9, 955-965.

Peretz S, Jensen R, Baserga R, Glazer PM (**2001)** ATM-dependent expression of the insulin-like growth factor-I receptor in a pathway regulating radiation response. **Proc Natl Acad Sci USA** 98, 1676-1681

Peruzzi F, Prisco M, Dews M, Salomoni P, Grassilli E, Romano G, Calabretta B, Baserga R (**1999)** Multiple signaling pathways of the insulin-like growth factor 1 receptor in protection from apoptosis. **Mol Cell Biol** 19, 7203-7215.

Prager D, Li HL, Asa S, Melmed S (**1994)** Dominant negative inhibition of tumorigenesis *in vivo* by human insulin- like growth factor I receptor mutant. **Proc Natl Acad Sci USA** 91, 2181-2185.

Reiss K, D'Ambrosio C, Tu X, Tu C, Baserga R (**1998)** Inhibition of tumor growth by a dominant negative mutant of the insulin-like growth factor I receptor with a bystander effect. **Clin Cancer Res** 4, 2647-2655.

Reiss K, Tu X, Romano G, Peruzzi F, Wang JY, Baserga R (**2001**) Intracellular association of a mutant insulin-like growth factor receptor with endogenous receptors. **Clin Cancer Res** 7, 2134-2144.

Reiss K, Yumet G, Shan S, Huang Z, Alnemri E, Srinivasula SM, Wang JY, Morrione A, Baserga R (**1999)** Synthetic peptide sequence from the C-terminus of the insulin-like growth factor-I receptor that induces apoptosis and inhibition of tumor growth. **J Cell Physiol** 181, 124-135.

Remacle-Bonnet M, Garrouste F, el Atiq F, Roccabianca M, Marvaldi J, Pommier G (**1992)** des-(1-3)-IGF-I, an insulin-like growth factor analog used to mimic a potential IGF-II autocrine loop, promotes the differentiation of human colon-carcinoma cells. **Int J Cancer** 52, 910-917.

Resnicoff M, Coppola D, Sell C, Rubin R, Ferrone S, Baserga R (**1994)** Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type 1 insulin-like growth factor receptor. **Cancer Res** 54, 4848-4850.

Resnicoff M, Burgaud JL, Rotman HL, Abraham D, Baserga R (**1995)** Correlation between apoptosis, tumorigenesis, and levels of insulin-like growth factor I receptors. **Cancer Res** 55, 3739-3741.

Resnicoff M, Tjuvajev J, Rotman HL, Abraham D, Curtis M, Aiken R, Baserga R (**1996)** Regression of C6 rat brain tumors by cells expressing an antisense insulin-like growth factor I receptor RNA. **J Exp Ther Oncol** 1, 385-389.

Rininsland F, Johnson TR, Chernicky CL, Schulze E, Burfeind P, Ilan J, Ilan J (**1997)** Suppression of insulin-like growth factor I receptor by a triple-helix strategy inhibits IGF-I transcription and tumorigenic potential of rate C6 glioblastoma cells. **Proc Natl Acad Sci USA** 94, 5854-5859.

Sachdev D, Li SL, Hartell JS, Fujita-Yamaguchi Y, Miller JS, Yee D (**2003)** A chimeric humanized single-chain antibody against the type I insulin-like growth factor (IGF) receptor renders breast cancer cells refractory to the mitogenic effects of IGF-I. **Cancer Res** 63, 627-635.

Salisbury AJ, Macaulay VM (**2003)** Development of molecular agents for IGF receptor targeting. **Horm Metab Res** 35, 843-849.

Samani AA, Fallavollita L, Jaalouk DE, Galipeau J, Brodt P (**2001)** Inhibition of carcinoma cell growth and metastasis by a vesicular stomatitis virus G-pseudotyped retrovector expressing type I insulin-like growth factor receptor antisense. **Human Gene Ther** 12, 1969-1977.

Sara VR, Hall K (**1990)** Insulin-like growth factors and their binding proteins. **Physiol Rev** 70, 591-614.

Scotlandi K, Maini C, Manara MC, Benini S, Serra M, Cerisano V, Strammiello R, Baldini N, Lollini PL, Nanni P, Nicoletti G, Picci P (**2002)** Effectiveness of insulin-like growth factor I receptor antisense strategy against Ewing's sarcoma cells. **Cancer Gene Ther** 9, 296-307.

Sell C, Rubini M, Rubin R, Liu JP, Efstratiadis A, Baserga R (**1993)** Simian virus 40 large tumor antigen is unable to transform mouse embryonic fibroblasts lacking type 1 insulin-like growth factor receptor. **Proc Natl Acad Sci U S A** 90, 11217-11221.

Shaw LC, Afzal A, Lewin AS, Timmers AM, Spoerri PE, Grant MB (**2003)** Decreased expression of the insulin-like growth factor 1 receptor by ribozyme cleavage. **Invest Ophthamol Vis Sci** 44, 4105-4113.

Simmons JG, Pucilowska JB, Lund PK (**1999)** Autocrine and paracrine actions of intestinal fibroblast-derived insulin-like growth factors. **Am J Physiol** 276, G817-827.

Stiles AD, D'Ercole AJ (**1990)** The insulin-like growth factors and the lung. **Am J Respir Cell Mol Biol** 3, 93-100.

Surmacz E (**2003)** Growth factor receptors as therapeutic targets, strategies to inhibit the insulin-like growth factor I receptor. **Oncogene** 22, 6589-6597.

Tai YT, Podar K, Catley L, Tseng YH, Akiyama M, Shringarpure R, Burger R, Hideshima T, Chauhan D, Mitsiades N, Richardson P, Munshi NC, Kahn CR, Mitsiades C, Anderson KC (**2003)** Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of β1-integrin and phosphatidylinositol 3'-kinase/AKT signaling. **Cancer Res** 63, 5850-5858.

Tanno S, Mitsuuchi Y, Altomare DA, Xiao GH, Testa JR (**2001)** AKT activation up-regulates insulin-like growth factor I receptor expression and promotes invasiveness of human pancreatic cancer cells. Cancer Res 61, 589-593.

Trojan J, Duc HT, Upegui-Gonzalez LC, Hor F, Guo Y, Anthony D, Ilan J (**1996)** Presence of MHC-I and B-7 molecules in rat and human glioma cells expressing antisense IGF-I mRNA. **Neurosci Lett** 212, 9-12.

Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita-Yamaguchi Y (**1986)** Insulin-like growth factor I receptor primary structure, comparison with insulin receptor suggests structural determinants that define functional specificity. **Embo J** 5, 2503-2512.

Yee D, Favoni RE, Lebovic GS, Lombana F, Powell DR, Reynolds CP, Rosen N (**1990)** Insulin-like growth factor I expression by tumors of neuroectodermal origin with the t(11;22) chromosomal translocation. A potential autocrine growth factor. **J Clin Invest** 86, 1806-1814.

Yu H, Rohan T (**2000)** Role of the insulin-like growth factor family in cancer development and progression. **J Natl Cancer Inst** 92, 1472-1489.