The Efficacy of IGF-I Receptor Monoclonal Antibody against Human Gastrointestinal Carcinomas is Independent of k-ras Mutation Status

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Abstract

Purpose: Insulin-like growth factor (IGF)-I receptor (IGF-IR) signaling is required for carcinogenicity and proliferation of gastrointestinal cancers. We have previously shown successful targeting therapy for colorectal, pancreatic, gastric, and esophageal carcinomas using recombinant adenoviruses expressing dominant negative IGF-IR. Mutation in k-ras is one of key factors in gastrointestinal cancers. In this study, we sought to evaluate the effect of a new monoclonal antibody for IGF-IR, figitumumab (CP-751,871), on the progression of human gastrointestinal carcinomas with/without k-ras mutation.

Experimental Design: We assessed the effect of figitumumab on signal transduction, proliferation, and survival in six gastrointestinal cancer cell lines with/without k-ras mutation, including colorectal and pancreatic adenocarcinoma, esophageal squamous cell carcinoma, and hepatoma. Combination effects of figitumumab and chemotherapy were also studied. Then figitumumab was evaluated in the treatment of xenografts in nude mice.

Results: Figitumumab blocked autophosphorylation of IGF-IR and its downstream signals. The antibody suppressed proliferation and tumorigenicity in all cell lines. Figitumumab inhibited survival by itself and up-regulated chemotherapy (5-FU and gemcitabine) induced apoptosis. Moreover, the combination of this agent and chemotherapy was effective against tumors in mice. The effect of figitumumab was not influenced by the mutation status of k-ras. Figitumumab reduced expression of IGF-IR but not insulin receptor in these xenografted tumors. The drug did not affect murine body weight or blood concentrations of glucose, insulin, IGF binding protein 3, and growth hormone.

Conclusions: IGF-IR might be a good molecular therapeutic target and figitumumab may thus have therapeutic value in human gastrointestinal malignancies even in the presence of k-ras mutations. Clin Cancer Res; 17(15); 5048–59. ©2011 AACR.

Introduction

Gastrointestinal (GI) cancers encompass a variety of diseases, many of whose prognoses are poor. Although only colorectal cancer is listed in the top 10 for incidence rates of cancer in the USA, 4 GI cancers, including colorectal, pancreatic, liver and the biliary tract, and esophageal, are in the top 10 for death rates from cancer (1). In Japan, there are 5 GI cancers, including colorectal, gastric, pancreatic, hepatic, and biliary tract, in the top 10 for death rates in 2007. Therefore, we must seek new therapeutic options for GI cancers.

Signals from a variety of growth factors are required for tumorigenesis and cancer development in human malignancies (2, 3). Recently, advances in molecular cancer research have brought new therapeutic forces from the bench into clinic. One group of new targets is the receptor tyrosine kinases for which specific small molecule tyrosine kinase inhibitors (TKIs) or blocking monoclonal antibodies (mAbs) exist. Type I insulin-like growth factor (IGF)-I receptor (IGF-IR) could be the next important molecular target (3, 4).

Binding of the ligands, IGF-I and IGF-II, to IGF-IR causes receptor autophosphorylation and activates multiple signaling pathways, including the mitogen-activated protein kinase (MAPK, extracellular signal-regulated kinase [ERK]) and phosphatidylinositotide 3-kinase (PI3-K)/Akt-1 pathways (5–7). These can stimulate tumor progression and cellular differentiation (8). IGF-IR axis function is also regulated by IGF binding proteins (IGFBPs) and type 2 IGF receptor (IGF-II; refs. 9–11).
IGF-IR signaling is important for tumorigenicity and progression of many cancers and k-ras mutation is one of key factors in gastrointestinal cancers. Drugs targeting for IGF-IR pathways is in trial evaluation. In this report, we revealed that the IGF-IR monoclonal antibody, figitumumab (CP-751,871), inhibited signal transduction, proliferation, and survival of 6 gastrointestinal cancer cell lines. Moreover, in addition to its monotherapy, the combination of figitumumab and chemotherapy was highly effective against these xenograft tumors on mice. The effect of figitumumab is not influenced by the mutation status of k-ras. This study suggests that figitumumab may have therapeutic value in human gastrointestinal carcinomas even in the presence of k-ras mutations.

IGFs stimulate the proliferation of GI cancer cells and blocking of IGF-IR signaling inhibits tumor growth (9, 12–20). High serum concentration of IGF-I increases the risk of developing several cancers (10). IGF-I can also antagonize the antiproliferative effects of cyclooxygenase-2 (COX-2) inhibitors (21). Soluble IGF-IRI salvages Apc (Min/+) intestinal adenoma progression induced by loss of IGF-II imprinting (11). IGF-IR is also important for tumor maintenance in addition to carcinogenicity (22, 23). Intestinal fibroblast-derived IGF-II has been shown to stimulate proliferation of intestinal epithelial cells (24). IGF-II, in conjunction with IGF-IR, IGF-I, COX-2, and matrilysin (matrix metalloproteinase-7), seems to play a key role in the early stages of colorectal carcinogenesis (25, 26). IGF-IR signaling is also critical in tumor dissemination through the control of adhesion, migration, and metastasis. Matrilysin can cleave all 6 IGFBPs and can thus cause activate IGF signals (27, 28). We have previously reported a positive feedback loop between the IGF/IGF-IR axis and matrilysin in the invasiveness and progression of GI cancers (29).

The insulin receptor (InsR) is also another key molecule of the IGF signaling pathway and leads cell proliferation as well as affecting glucose metabolism. In addition to insulin, InsR can also bind IGF-II and initiate mitogenic signals (30). IGF-IR and InsR can form hybrid receptors that bind IGFs at physiologic concentrations. InsR and these hybrid receptors may also be involved in tumor as both insulin and IGF-I contribute to the development and progression of adenomas (31).

There are several possible approaches to blocking the IGF-IR axis. Humanized or human mAbs and TKIs for IGF-IR are available and some are in phase trials (32–34). We have constructed 2 dominant negative inhibitors for IGF-IR (CP-751,871) that are active as plasmids and recombinant adenovirus vectors for several GI malignancies (15, 35–37). Figitumumab (CP-751,871) is a highly specific, fully human mAb that inhibits ligand-induced IGF-IR autophosphorylation (32). Phase 1 trials reveal that this mAb has a favorable pharmacodynamic profile and is well tolerated as a single drug without dose-limiting toxicities (38, 39). Figitumumab is also tolerable in combination with paclitaxel, carboplatin, or docetaxel (40, 41). There are only 3 reports about the efficacy of figitumumab on human GI malignancies, 1 colorectal cancer cell line, colo205, and 2 clinical studies for patients with solid tumors, including esophageal, gastric, and colon cancers and GI stromal tumor (32, 38, 42). As GI cancers are heterogeneous diseases, we decided to analyze the utility of this compound in several human GI cancer cell lines.

On the other hand, cetuximab, which is a mAb for epidermal growth factor receptor, is a useful drug for patients with colorectal cancer, however, it is not effective for tumors bearing k-ras mutations (43, 44). Mutation in k-ras is a critical genetic change, as the incidence of k-ras mutation is 40% to 45% in colon cancer and around 90% in pancreatic cancer. However, there is no report regarding the efficacy of IGF-IR targeting therapies for k-ras mutated cancers.

In this study, we assessed the impact of figitumumab on signaling blockade, growth, apoptosis-induction, and in vivo therapeutic efficacy in s.c. xenografts for GI cancers. We also analyzed the effects of this drug in cell lines with k-ras mutations. These observations strengthen the rationale for using figitumumab alone or in combination with chemotherapy in the molecular targeted therapy of GI cancers.

**Materials and Methods**

**Materials, cell lines, and mice**

Anti-Akt1(c-20), anti-ERK1(K-23), anti-phospho-ERK1 (E-4), anti-IGF-I(G-17), anti-IGF-IIR(2C8), and anti-IGF-IR(R204) were purchased from Santa Cruz Biotechnology and anti-phospho-Akt(Ser473), anti-phospho-p44/42-MAPK(Thr202/Tyr204), and PathScan Multiplex Western Cocktail-I were from Cell-Signaling Technology. 5-Fluorouracil (5-FU) was purchased from Sigma. Recombinant human IGF-I and IGF-II was purchased from R&D systems. All human GI cancer cell lines, colon adenocarcinomas, HT29 and DLD-1; pancreatic adenocarcinomas, BxPC3 and MIAPaca2; esophageal squamous cell carcinoma (ESCC), TE-1; and hepatocellular carcinoma, PLC/PRF/5 were obtained from Japanese Cancer Collection of Research Bioresources Cell Bank. Cells were passaged in RPMI1640 and DMEM with 10% FBS. Specific-pathogen-free female BALB/cAnNcrj-nu mice, 6-weeks-old, were purchased from Charles River. The care and use of mice were according to our university’s guidelines.

A new monoclonal antibody for IGF-IR, figitumumab (CP-751,871), was kindly provided by Pfizer.

**Western blotting**

To analyze the duration of efficacy of this antibody, cells were cultured with media containing 0.1% bovine serum albumin and were incubated several hours with 1 μg/mL figitumumab before ligand stimulation. To assess the concentration of figitumumab required to block
ligand-induced signal transduction, cells were cultured with media containing 0.1% bovine serum albumin and were incubated 0.5 to 3 hours with several concentrations of this mAb before stimulation.

Cells were treated with 20 ng/mL IGF-I, 40 ng/mL IGF-II, or 10 nmol/L insulin 5 minutes. Cell lysates were prepared as described previously (35). Equal aliquots of lysates (100 µg) were separated by 4% to 20% SDS-PAGE and immunoblotted onto polyvinylidene Hybond-P membrane (Amersham). Analysis was performed using indicated antibodies, and bands visualized by ECL (Amersham).

Colony-forming activity

Cells (3 × 10³/plate) were seeded onto 60 mm culture plates and incubated for 24 hours. The cells were then treated with figitumumab and were incubated for 14 days. After air drying, cells were fixed with methanol and stained with Giemsa solution. Colonies containing 50 cells or more were counted.

Assessment for apoptosis

The caspase-3 colorimetric protease assay was performed following the manufacturer’s protocol (MBL). In brief, the caspase-3 activity of lysates (100 µg) was measured by colorimetric reaction at 400 nm. Early apoptosis were quantified by staining with Anexin-V-FITC, according to the manufacturer’s protocol (BD Biosciences) and measured by flow cytometry. TUNEL assays were performed with in situ apoptosis detection kit (Takara), following the manufacturer’s protocol.

In vivo therapeutic efficacy in established tumors

A total of 1 × 10⁶ GI cancer cells were s.c. injected into nude mice. After tumors were palpable, animals were treated with intraperitoneal (i.p.) injection of 125 µg figitumumab, twice a week or control. Tumor diameters were serially measured with calipers and tumor volume was calculated using the formula: tumor volume (mm³) = (width² × length)/2.

After GI tumors were palpable, animals were treated with i.p. injection of 125 µg figitumumab, twice a week or control. Both groups were then divided into pair-matched cytotoxic drugs, 50 mg/kg 5-FU (administered i.p. once per week) or 100 mg/kg gemcitabine (i.p., twice a week), treated and control groups. Mice were euthanized when tumors reached 2 cm in size or they developed clinically evident symptoms.

Immunohistochemical analysis

Sections (5 µm) from formalin-fixed, paraffin-embedded tumor xenografts were prepared. After removed paraffin, the sections were pretreated with Dako-Cytomation Target Retrieval Solution (Dako) in a microwave (10 minutes). Then, endogenous peroxidase activity was blocked. Antibodies were applied after blocking with normal goat serum. Sections were incubated with the anti-rabbit secondary antibody (Santa Cruz Biotechnology) and a streptavidin-HRP (Dako) followed by exposure to the diaminobenzidine tetrahydrochloride substrate (Dako).

ELISA

Serum concentrations of IGF-I, GH, insulin, and IGFBP-3 were measured using the following ELISA kits, following the manufacturer’s protocols: mouse IGF-I Quantikine (R&D Systems), mouse growth hormone kit (Usn Life Science & Technology), mouse insulin kit (Shibayagi), and mouse- and rat-IGFBP-3 ELISA (Mediagnost).

Statistical analysis

The results are presented as mean ± SE for each sample. The statistical significance of differences was determined by Student’s 2-tailed t test in 2 groups and done by 1-way ANOVA in multiple groups, and 2-factor factorial ANOVA. P values of less than 0.05 were considered to indicate statistical significance.

Results

Blockade of signal transduction

To investigate the effect of figitumumab on the IGF/receptor axis, we examined 2 GI cancer cell lines, a pancreatic cancer cell line, BxPC3 and a colorectal cancer cell line, HT29, which we used to evaluate the efficacy of IGF-IR blockade in the previous studies (36, 37). First, we assessed effects of figitumumab in BxPC3 on activation of IGF-IR and its downstream signals by immunoblotting. Low concentration of 0.1 µg/mL figitumumab blocked IGF-I–induced phosphorylation of both IGF-IR and Akt-1 and reduced phosphorylation of ERKs (Fig. 1A).

Similarly, in HT29 cells, figitumumab eliminated ligand-induced IGF-IR autophosphorylation and phosphorylation of both downstream, Akt-1 and ERKs (Fig. 1B). The results indicate that this mAb might effectively inhibit the IGF axis in both BxPC3 and HT29.

Then, the effect of figitumumab on a hepaloma cell line, PLC/PRF/5, was assessed (Fig. 1C). This mAb blocked both IGF-IR downstream signals of Akt-1 and ERKs with a dose dependency. One µg/mL figitumumab blocked autophosphorylation of IGF-IR and the downstream signals from 1 to 48 hours.

The in vitro effect on cell growth and survival

Then we analyzed the effect of this drug on in vitro growth and survival. One µg/mL figitumumab dramatically reduced the in vitro tumorigenicity of 3 cell lines as assessed by the colony formation assay (Fig. 2A).

Annexin-V assays revealed that this mAb up-regulated ethanol-induced early apoptosis synergistically in HT29 (Fig. 2B). Then we evaluated the effect of this drug on chemotherapy-induced apoptosis. Caspase-3 assay revealed that figitumumab enhanced 5-FU induced apoptosis synergistically in both HT29 and BxPC3 (Fig. 2C). The antibody strengthened additively the effect of gemcitabine...
in BxPC3 and that of 5-FU in PLC/PRF/5 (Fig. 2C). These data suggest that figitumumab might have several in vitro antitumor effects on GI cancer cells.

Figitumumab suppresses xenograft tumors

To analyze the effect of this drug on in vivo GI cancer, HT29 cells were inoculated s.c. in nude mice and allowed to

Figure 1. Two gastrointestinal cancer cell lines, pancreatic adenocarcinoma, BxPC3 (A), and colorectal adenocarcinoma, HT29 (B) were cultured with different doses of figitumumab 30 minutes, then were stimulated with 20 μg/mL IGF-I 5 minutes. Western blotting shows that CP-751,871 blocks IGF-I–induced autophosphorylation of IGF-IR completely in both cells. Figitumumab terminated IGF-I–induced activation of Akt-1, but not ERKs completely. C, in hepatocellular carcinoma, PLC/PRF/S, ligand induced both phosphorylation of Akt and ERKs were blocked by 3 hours treatment with this mAb. With incubation with 1 μg/mL figitumumab from 1 to 48 hours, this agent effectively blocked IGF-I–stimulated autophosphorylation of IGF-IR and both activation of Akt and ERKs in PLC/PRF/S.

Figure 2. The effects of figitumumab on colony formation and apoptotic induction. A, colony formation assay shows that 1 μg/mL CP-751,871 reduced the number of colony of 3 GI cancer cell lines. B, Anexin-V assay revealed that CP-751,871 strengthen ethanol induced apoptosis synergistically in HT29. C, caspase-3 assay shows that CP-781871 enhanced 5-FU induced apoptosis synergistically in HT29 and BxPC3, and did additively in PLC/PRF/S. Cont, control; CP, CP-751,871 (figitumumab); Gem, gemcitabine.
form evident tumors. HT29 tumor-bearing mice were treated with i.p. injections of 125 μg figitumumab (twice a week) along with 5-FU or PBS. Although either 5-FU or figitumumab tended to suppress tumor growth, the combination therapy was much more effective than either treatment alone (Fig. 3A). Then, BxPC3 cells were inoculated in nude mice. Figitumumab monotherapy reduced the tumor growth rate of BxPC3; however, the combination with this drug and gemcitabine most effectively suppressed growth among all tested groups (Fig. 3B). However, both murine body weight and serum glucose concentration on sacrifice were not different among the 4 groups of mice.

Serum concentrations of IGF axis ligands were assessed by ELISA at the time of sacrifice. As sample number and volume were limited, we analyzed those in 2 groups, figitumumab or control (n = 8). Concentrations of serum IGF-I, growth hormone, insulin, and IGFBP3 were not significantly different between the 2 groups (Fig. 3C).

Resected tumor samples were investigated by immunohistochemical and TUNEL assays (Fig. 3D). The expression of IGF-IR was suppressed in BxPC3 by figitumumab monotherapy and in the both cell lines by the combination therapy. Although all treatments suppressed InsR in HT29, only the combination up-regulated InsR in BxPC3. Both 5-FU and figitumumab induced apoptosis in HT29.
xenografts, and the number of apoptotic cells was greatest in the combination group. In BxPC3 xenografts, figitumumab up-regulated gemcitabine-induced apoptosis, although each individual treatment did not induce apoptosis significantly.

The results indicate that figitumumab might be effective for GI cancers, HT29 and BxPC3; however, both cell lines have wild-type k-ras so we asked whether the same effects could be observed in k-ras mutant cell lines.

Blockade of signal transduction in k-ras mutated cells

It has been reported that k-ras mutation is one of key genetic events in GI malignancies, and is associated with significant resistance to molecular targeted therapies such as cetuximab (44). There is therefore a dearth of novel and effective therapies in this subset of tumors. To assess the effect of figitumumab on the IGF/IGF-IR axis in GI cancers with k-ras mutation, we examined 3 GI cancer cell lines, DLD-1, MIAPaca2, and TE-1 with this mutation. One μg/mL figitumumab blocked autophosphorylation of IGF-IR from 1 to 48 hours in all 3 cell lines (Fig. 4A). Downstream signals of Akt-1 and the ERKs were also blocked by 1 μg/mL figitumumab. Although the effect of this mAb on the ERKs showed dose dependency in all 3 cell lines, a low dose of 0.1 μg/mL figitumumab blocked Akt effectively (Fig. 4B). These data show that figitumumab can block IGF-I signals in GI cancers with k-ras mutations.

In addition to IGF-I, 1 μg/mL figitumumab blocked IGF-II induced both phosphorylation of Akt and ERKs in DLD-1 (Fig. 4C). The results indicate that this mAb can block IGF signals effectively for GI malignancies having a mutation in k-ras.

The effect of this antibody on insulin signaling was limited. High doses of 10 μg/mL figitumumab could block insulin-induced autophosphorylation of InsR and activation of Akt-1, but not that of ERKs in DLD1 (Fig. 4D). One μg/mL figitumumab did not inhibit insulin signals between 30 minutes and 48 hours exposures in DLD1.

The in vitro effect on cell growth and survival for k-ras mutated cells

Next, we assessed the effect of this drug on the in vitro growth and survival of k-ras mutated GI cancers. Colony formation assays showed that this mAb reduced the in vitro tumorigenicity of both DLD1 and MIAPaca2 in a dose-dependent fashion (Fig. 5A). One μg/mL figitumumab also effectively reduced colony formation of TE1.

Caspase-3 assays revealed that this antibody enhanced 5-FU induced apoptosis synergistically in DLD1 and additively in the other cell lines, MIAPaca2 and TE1 (Fig. 5B).
Figure 4. The effect of signal transduction on gastrointestinal cancer cells with k-ras mutation. A, with incubation with 1 μg/mL figitumumab from 1 to 48 hours, this mAb effectively blocked IGF-I–stimulated autophosphorylation of IGF-IR and both activation of Akt and ERKs in 3 cell lines; colorectal adenocarcinoma, DLD-1; pancreatic adenocarcinoma, MIAPaca2; and esophageal squamous cell carcinoma, TE1. B, ligand induced phospholyration of downstream, Akt and ERKs, was blocked by 3 hours treatment with figitumumab, the former tend to be seen in lower dose of figitumumab than the latter. C, in DLD-1, this mAb blocked IGF-II stimulated both phosphorylation of Akt and ERKs. D, to block 10 nmol/L insulin-induced signal transduction, more than 10 μg/mL figitumumab (3 hours incubation) are needed in DLD-1.
This drug strengthened the effect of gemcitabine additively in MIAPaca2. These results suggest that figitumumab has significant antitumor effects on GI cancer cells with k-ras mutations.

**Figitumumab suppressed k-ras mutated tumors in mice**

To assess the *in vivo* effect of this drug on GI cancers with k-ras mutations, DLD1 cells were inoculated s.c. in nude mice and allowed to form evident tumors. DLD1 tumor-bearing mice were treated with an i.p. injection of 125 μg figitumumab (twice a week) or control. This mAb suppressed tumor growth significantly (Fig. 6A). Then, the combination effect of this antibody and 5-FU was assessed. This combination suppressed tumor growth more effectively than monotherapy, however these additive effects were limited. None of these treatments affected murine body weight on sacrifice.

The *in vivo* effect of figitumumab on another k-ras mutated cell line, MIAPaca2, was then assessed. This drug inhibited dramatically tumor growth of MIAPaca2 xenografts (Fig. 6B). Both monotherapies of figitumumab and gemcitabine clearly reduced tumor growth (*P* = 0.0036 and 0.0012 compared to control, respectively), however the combination inhibited growth more effectively (*P* < 0.0001). Although treatment with gemcitabine was associated with reduced murine weight on sacrifice, the mAb alone did not affect animal weight. Figitumumab increased serum glucose concentrations on sacrifice more than gemcitabine monotherapy.

To assess the effect of treatments on both cell proliferation and apoptosis, resected tumor tissues were analyzed (Fig. 6C). In DLD1 tumors, only the combination therapy effectively reduced cell proliferation. In MIAPaca2 tumors, gemcitabine down-regulated proliferation, which was enhanced by figitumumab. In DLD1 tumors, 5-FU induced apoptosis and this antibody increased this effect. In MIAPaca2 tumors, gemcitabine induced apoptosis and this mAb up-regulated this effect. These results suggest that the antitumor effects of figitumumab are observed even in the presence of k-ras mutation in GI cancers.

**Discussion**

GI carcinomas are composed of a variety of histological types, and patients with these cancers show vastly different clinical courses. GI cancers are often diagnosed in advanced stages having lymph node/distant metastases and peritoneal dissemination, and in these cases there are extremely limited...
Figure 6. The effects of figitumumab on k-ras mutated GI cancer on mice. A, figitumumab suppressed tumor growth rate of DLD-1 on mice (n = 8, P = 0.0053). Tumor volume of mice treated with combination of this mAb and 5-FU showed least of all groups (P = 0.0151, the combination vs. control, each group n = 8), however there are no significant differences between each monotherapy and the combination. Body weight of mice on sacrifice was not influenced by treatments. B, figitumumab inhibited tumor growth rate of MIAPaca2 on mice (n = 8, P = 0.0288). The combination of this antibody and gemcitabine suppressed tumor volume most effectively of all (P < 0.0001, the combination vs. control, each group n = 8), however there are no significant differences between each monotherapy and the combination. Gemcitabine decreased murine weight on sacrifice and figitumumab single therapy up-regulated blood glucose on sacrifice. C, the combination treatment reduced Ki-67 label in DLD1. Gemcitabine alone reduce tumor cell growth and figitumumab enhanced this effect. In both tumors, 5-FU induced TUNEL positive area and figitumumab strengthened this effect. Post hoc t test was by Fischer’s PLSD. Cont, control; CP, CP-751,871 (figitumumab); Gem, gemcitabine.
options. GI malignancies are thus higher in overall mortality rates compared to their incidence rates (1). IGF-mediated growth-responsiveness is found in most GI cancer cells, including esophagus, intestine, pancreas, and liver (12, 15, 16, 19). High expression of both IGF-IR and the ligands in tumor tissues has indicated continuous activation of this system by paracrine and autocrine loops (19, 45). The expression of IGF-IR/IGF-II might be useful for the predictive marker of recurrence and poor prognosis in ESCC (15). The functional significance of IGF-IR has been showed as cancer growth inhibition by anti-IGF-IR antibodies (19, 32). Here, we used a new IGF-IR mAb, figitumumab, for the accurate dissection of the responsible signaling pathways. The results indicate that this strategy is promising for the treatment of GI tumors, not only as a monotherapy but especially in combination with cytotoxic drugs. We first showed that figitumumab enhanced the effects of both 5-FU and gemcitabine. Resistance to chemotherapy is a serious problem in patients with GI malignancies and this approach has the potential for overcoming this difficulty. It is important to note that our studies show that figitumumab is effective against a wide range of GI carcinomas, and these effects are not unique to 1 or 2 unusual cell lines.

Cetuximab is a powerful molecular targeted drug for patients with colorectal cancer, however, it shows limited or no efficacy for k-ras mutated cancers (43, 44). Although IGF-IR might be the next important target in human GI carcinomas, there is no information about IGF-IR-targeted therapy in k-ras mutated cancers. In this study, figitumumab showed antitumor effects for k-ras mutated cancers as well as wild-type ones both in vitro and in vivo. Our previous data in GI carcinomas indicated that IGF-IR blockade inhibited Akt signals more than ERK signaling, so the PI3-K/Akt pathway might play a more important role than the ras/ERK pathway in the downstream signals of the IGF/IGF-IR axis (15, 35–37). This could explain why figitumumab is active for k-ras mutated GI cancer cells, however further studies are needed to analyze this mechanism precisely. Although we did not assess the efficacy of this drug for patients with GI cancers with and without k-ras mutations, these data might support the use of this mAb in this clinical setting. Further studies will be needed to clarify this hypothesis.

A major obstacle to the targeting of IGF-IR is the close homology of the IGF-IR and the IR kinase domains (46). Therefore, it is important that any strategy designed to block IGF signaling has specificity for IGF-IR and without a detrimental influence on IR signals. We show here that figitumumab does not suppress insulin-induced InsR- or Akt-phosphorylation in DLD1 indicating a high degree of receptor selectivity at tumor-active doses with this molecular-targeted therapy. Plasma concentrations of glucose, IGF-I, insulin, growth hormone, IGFBP3 were not influenced by this mAb. Moreover, body weight was monitored and not significantly affected by this therapy. This lack of effect on the insulin pathway and clinical toxicity leads us to believe that these combinations will have minimal adverse effects in clinical applications.

As figitumumab is of the IgG2 subtype, which is usually a poor activator of cellular immune responses, ongoing clinical trials may clarify whether this agent has significantly different properties from the IgG1 class from which most other mAbs targeting IGF-IR are derived. According to several clinical studies, the main adverse effects of IGF-IR mAb are hyperglycemia, mild skin toxicities, and fatigue (41, 47, 48). IGF-IR mAb has been reported to cause hyperglycemia in about 20% of patients but this has generally been tolerable, mild to moderate, reversible, and manageable with usual oral hypoglycemic drugs. Patients with previous glucose intolerance or with steroids usage might be at increased risk of hyperglycemia.

It is also important to define surrogate markers of response for modern targeted agents. Although we could not assess this completely in these murine models, several possibilities have been put forward. One is that circulating tumor cells would be a marker, as treatment with figitumumab decreased both total circulating tumor cell count and IGF-IR–positive circulating tumor cell count (41). Another is that high concentration of serum-free IGF-I may be a marker of high responder of patients with non small cell lung carcinoma treated with figitumumab.

Recently, a new role of the IGF-IR has been identified that there is significant cross-talk between the IGF-IR and other tyrosine kinase receptors. For example, many patients with breast cancer who achieve an initial response to trastuzumab acquire secondary resistance. One mechanism of resistance has been showed to be overexpression of IGF-IR (49) and another is the formation of IGF-IR/Her2 heterodimers (50). These data suggest that IGF-IR blockade may be specifically effective for breast cancer patients with trastuzumab-resistant tumors.

Thus, in this study, we show that figitumumab suppresses the tumorigenicity and survival of GI cancers with or without k-ras mutations, by blocking Akt and ERK activation, both in vitro and in animal models. It also enhances chemotherapy efficacy without significantly influencing InsR signals at therapeutically effective doses. This study thus validates IGF-IR as a therapeutic target in GI carcinomas and suggest that figitumumab may be a promising antitumor therapeutic for these diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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