Cancer Therapy: Preclinical

Monoclonal Antibodies to Fibroblast Growth Factor Receptor 2 Effectively Inhibit Growth of Gastric Tumor Xenografts

Wei-meng Zhao1, Lihong Wang1, Hangil Park1, Sophea Chhim1, Melanie Tanphanich1, Masakazu Yashiro2, and K. Jin Kim1

Abstract

Purpose: Overexpression of fibroblast growth factor receptor 2 (FGFR2) may be a causative factor of a number of human tumors, especially gastric tumors of the poorly differentiated type. We investigated whether monoclonal antibodies (mAbs) directed against FGFR2 can inhibit the growth of tumors in xenograft models.

Experimental Design: We generated and characterized 3 mAbs that recognize different epitopes on FGFR2: GAL-FR21, GAL-FR22, and GAL-FR23. The ability of the mAbs to recognize the FGFR2IIlb and FGFR2IIlc isoforms of FGFR2 was determined, as was their ability to block binding of FGF ligands to FGFR2. The capability of the mAbs to inhibit FGF-induced FGFR2 phosphorylation and to downmodulate FGFR2 was also investigated. Finally, the ability of the anti-FGFR2 mAbs to inhibit tumor growth was determined by establishing xenografts of SNU-16 and OCUM-2M human gastric tumor cell lines in nude mice, treating with each mAb (0.5–5 mg/kg intraperitoneally twice weekly) and monitoring tumor size.

Results: Of the 3 mAbs, GAL-FR21 binds only the FGFR2IIlb isoform, whereas GAL-FR22 and GAL-FR23 bind to both the FGFR2IIlb and FGFR2IIlc forms, with binding regions respectively in the D3, D2-D3, and D1 domains of FGFR2. GAL-FR21 and GAL-FR22 blocked the binding of FGF2, FGF7, and FGF10 to FGFR2IIlb. GAL-FR21 inhibited FGF2 and FGF7 induced phosphorylation of FGFR2, and both mAbs downmodulated FGFR2 expression on SNU-16 cells. These mAbs effectively inhibited growth of established SNU-16 and OCUM-2M xenografts in mice.


The fibroblast growth factor (FGF) family plays important roles in embryonic development, tissue repair, angiogenesis, and the growth of certain tumors (1, 2). Although the family has 22 known members in humans, there are only 4 cellular FGF receptors, designated FGFR1–4, with the various FGFs binding the different FGFRs to varying extents (3, 4). The FGF receptors are structurally related transmembrane tyrosine kinases: each FGFR consists of an extracellular domain (ECD) comprising 3 immunoglobulin (Ig)-like domains (D1, D2, and D3), a single transmembrane helix, and an intracellular kinase domain (5). The FGFRs are characterized by multiple alternative splicing of their mRNAs, leading to a variety of isoforms (3). Notably, there are forms containing all 3 Ig domains (α-isoforms) or only the 2 Ig domains D2 and D3 without D1 (β-isoforms). Of special importance in FGFR2, all forms contain the first half of D3 denoted IIIa, whereas 2 alternative exons can be utilized for the second half of D3, leading to IIlb and IIlc forms. These are respectively denoted FGFR2IIlb and FGFR2IIlc; the corresponding β forms are denoted as FGFR2(β)IIlb and FGFR2(β)IIlc. Importantly, the FGFR2IIlb form is a high-affinity receptor for FGF1 and FGF2 as well as for FGF7 (keratinocyte growth factor; KGF) and FGF10, whereas FGFR2IIlc binds both FGF1 and FGF2 well but does not bind KGF (6). Indeed, FGFR2IIlb is the main receptor for KGF (3) and is thus also designated as KGFR.

The FGFRs and their isoforms are differentially expressed in various tissues. Generally, FGFR2IIlb (and the IIlb forms of FGFR1 and FGFR3) are expressed in epithelial tissues, whereas FGFR2IIlc is expressed in mesenchymal tissues (7), although this correlation is not strict (8). Certain FGF ligands of these receptors have an opposite pattern of expression. Thus, FGF3 and FGF7 are expressed in mesenchymal tissues, so may be paracrine effectors of epithelial cells expressing FGFR2IIlb, whereas the FGF4 subfamily members FGF4–6 bind to FGFR2IIlc and are expressed in both epithelial and mesenchymal lineages so...
may have either autocrine or paracrine functions (3). Because of the expression patterns of the isoforms of FGFR2 and their ligands, FGFR2 plays a role in epithelial–mesenchymal interactions (9), so it is not surprising that knock out of FGFR2 IIb in mice leads to embryonic defects and lethality (10).

Several FGFs are believed to play a role in cancer, both by stimulating angiogenesis and tumor cells directly (2, 11). Correspondingly, FGFR2 IIb is expressed or overexpressed in many types of tumors (12, 13). For example, FGF7 and FGFR2 IIb are overexpressed in many pancreatic cancers (14) and their coexpression correlates with poor prognosis (15). Somatic mutations of the FGFR2 gene were found in 10% to 12% of large panels of endometrial carcinomas, (15). Somatic mutations of the FGFR2 gene were found in (14) and their coexpression correlates with poor prognosis (25). The association of FGFR2 overexpression or mutation with many types of cancer suggests that FGFR2 may be an excellent therapeutic target. Several small molecule antagonists of FGFR2 have been reported (26–28), but these agents also have inhibitory activity toward other tyrosine kinase receptors, increasing their potential for toxicity if used to treat human patients. On the other hand, highly specific monoclonal antibodies (mAbs) against various growth factors or their receptors including VEGF, EGF receptor, and HER2 are now being used to treat various types of cancer with considerable success, suggesting a mAb approach to targeting FGFR2. However, only a few anti-FGFR2 mAbs have been reported (29, 30), perhaps because the very high sequence homology between mouse and human FGFR2 makes development of such mAbs difficult; moreover, the ability of the reported mAbs to inhibit tumor growth was not established. In this study, we have therefore generated and characterized several anti-FGFR2 mAbs and shown that they block the functional activity of FGFR2 in vitro and inhibit growth of FGFR2 overexpressing gastric tumor xenografts in vivo.

**Materials and Methods**

**Cell lines**

SNU-16 (ATCC CRL 5974) and KATO-III (HTB-103) were obtained from ATCC. OCUM-2M was from Osaka City University (Osaka, Japan). Upon receipt, the cell lines were expanded and frozen in vials, and a new vial was thawed for each series of experiments.

**Reagents**

The α-isoform (D1, D2, and D3 domains) and β-isoform (D2 and D3 domains) of human FGFR2 IIb and FGFR2 IIc were expressed as immunoadhesin molecules by respectively fusing the ECD residues 1–378 (α-isoform) and 152–378 (β-isoform) of FGFR2 IIb or 1–377 (α-isoform) and 152–377 (β-isoform) of FGFR2 IIc to the human Ig Fc region (residues 216 to 446) in the pDisplay vector (Invitrogen). Each FGFR2-Fc protein was expressed by transfecting human 293F cells (Invitrogen), and the FGFR2-Fc was purified from culture medium using a protein A/G column. Fc fusion proteins of the other FGFRs as well as FGFR2 IIb-Fc with the S252W mutation were similarly generated and produced. Mouse FGFR2 IIb-Fc was purchased (R&D Systems).

**Generation of mAbs to FGFR2**

Female 6-week-old Balb/c mice (Charles River Laboratories) were immunized with human FGFR2 IIb-Fc or FGFR2 IIc-Fc in monophosphoryl lipid A/trehalose dicorynomycolate (Sigma) in their rear footpads about 20 times once per week. Sera titers were determined by an FGFR ELISA described later. Three days after the final injection,
popliteal lymphoid cells were fused with P3/X63-Ag8U1 mouse myeloma cells using an electrofusion device according to the manufacturer’s instructions (Cyto Pulse Sciences). Two weeks after the fusion, hybridoma culture supernatants were screened first for FGFR2IIIb-Fc binding by the ELISA described below. Positive hybridomas were further screened for their ability to bind to FGFR2-positive tumor cell lines. Selected hybridomas were cloned twice by limiting dilution. The isotype of the mAbs produced by these selected clones was determined using isotype-specific antibodies (BioRad).

**ELISA**

Each step of each assay was performed by room temperature incubation with the appropriate reagent for 1 hour, except the initial plate-coating step was done overnight at 4°C. Between each step, plates were washed 3 times in PBS containing 0.05% Tween 20. Data points were generally in triplicate. To determine binding of the mAbs to FGFR2 isoforms, ELISA plates were coated with goat anti-human IgG-Fc (2 μg/mL; Jackson ImmunoResearch Labs) first, followed by blocking with 2% bovine serum albumin (BSA). The plates were then incubated with the α- and β-isoforms of FGFR2IIIb-Fc and FGFR2IIc-Fc (0.5 μg/mL) or control human IgG, followed by incubation with various concentrations of mAbs. The bound murine mAb was detected by the addition of horseradish peroxidase, HRP-goat anti-mouse IgG and TMB substrate (tetramethylbenzidine; Sigma-Aldrich). To determine the ability of the mAbs to block ligand binding to FGFR2, plates were coated with 50 μg/mL of heparin (Sigma), to which FGFs are known to bind (1). The plates were then incubated with 0.2 μg/mL of human FGF2, FGF7, or FGF10 (R&D Systems) and blocked with 2% BSA. The plates were next incubated with FGFR2IIb-Fc (0.5 μg/mL) in the presence of various concentrations of mAbs, and the bound FGFR2IIib-Fc was detected with HRP-goat anti-human IgG-Fc and substrate.

**Flow cytometry**

Appropriate cells (2 × 10^5) were washed twice in cell-sorting buffer (CSB; PBS/1% FBS/0.02% NaN₃), resuspended in 50 μL of CSB and incubated with 1 μg/50 μL of an anti-FGFR2 mAb for 1 hour at 4°C on a shaker. Cells were then washed twice in CSB and the bound antibodies were detected by incubation with Alexa 488-goat anti-mouse IgG (Invitrogen) for 1 hour on ice. After washing twice in CSB, cells were analyzed on a FACScan.

**FGFR2 phosphorylation assay**

SNU-16 cells were grown in RPMI medium with 10% FCS and then in serum-free RPMI/0.1% BSA for 24 hours. The cells were resuspended at 3 × 10^5 cells per well in RPMI/0.1% BSA and incubated with mAb for 30 minutes, followed by addition of FGF2 (100 ng/mL) or FGF7 (10 ng/mL) for 10 minutes. Cells were then placed on ice and washed with ice-cold PBS. Cell lysates were prepared and the level of phosphorylated FGFR2 determined using a Phospho-FGF R2α DuoSet IC kit (R&D Systems, DYC684-2) for ELISA according to the manufacturer’s instructions.

**Down modulation of FGFR2 expression**

SNU-16 cells (5 × 10^5 per well) were treated with mAbs (10 μg/mL) or control human IgG, followed by incubation with various concentrations of mAbs. The bound murine mAb was detected by the addition of horseradish peroxidase, HRP-goat anti-mouse IgG and TMB substrate (tetramethylbenzidine; Sigma-Aldrich). To determine the ability of the mAbs to block ligand binding to FGFR2, plates were coated with 50 μg/mL of heparin (Sigma), to which FGFs are known to bind (1). The plates were then incubated with 0.2 μg/mL of human FGF2, FGF7, or FGF10 (R&D Systems) and blocked with 2% BSA. The plates were next incubated with FGFR2IIb-Fc (0.5 μg/mL) in the presence of various concentrations of mAbs, and the bound FGFR2IIib-Fc was detected with HRP-goat anti-human IgG-Fc and substrate.

**Flow cytometry**

Appropriate cells (2 × 10^5) were washed twice in cell-sorting buffer (CSB; PBS/1% FBS/0.02% NaN₃), resuspended in 50 μL of CSB and incubated with 1 μg/50 μL of an anti-FGFR2 mAb for 1 hour at 4°C on a shaker. Cells were then washed twice in CSB and the bound antibodies were detected by incubation with Alexa 488-goat anti-mouse IgG (Invitrogen) for 1 hour on ice. After washing twice in CSB, cells were analyzed on a FACScan.

**Xenograft models**

Animal experiments were conducted in accordance with U.S. Public Health Service policy. SNU-16 or a subclone of OCUM-2M cells grown in complete DMEM medium were harvested in PBS. Female 5- to 6-week-old athymic nude mice were injected subcutaneously with 5 × 10^6 cells in 0.1 mL of PBS containing 50 μL of matrigel (BD Biosciences) in the dorsal area. When the tumor sizes reached ~150 mm³, mice were grouped randomly (n = 5–7 per group) and appropriate doses of mAbs (0.5–5 mg/kg) in a volume of 0.1 mL were administered intraperitoneally twice per week. Tumor volumes were determined twice weekly by measuring in 2 dimensions, length (a) and width (b), and calculating volume as V = a²b/2. Statistical analysis was performed by Student’s t test applied to the final timepoint.

**Results**

**Generation and binding properties of anti-FGFR2 mAbs**

After screening several thousand hybridomas from 5 fusions in an FGFR2IIib-Fc binding ELISA followed by flow cytometry on SNU-16 cells (31), we selected 3 mAbs (GAL-FR21, GAL-FR22 and GAL-FR23) for further analysis. GAL-FR21 is of the IgG1 isotype whereas GAL-FR22 and GAL-FR23 are of the IgG2b isotype.

GAL-FR21 bound to only FGFR2IIib-Fc whereas GAL-FR22 and GAL-FR23 bound to both FGFR2IIib-Fc and FGFR2IIlc-Fc in ELISA (Fig. 1A, B). GAL-FR21 bound to denatured FGFR2IIib-Fc in a Western blot (data not shown), suggesting that GAL-FR21 recognizes a linear epitope. Although the binding affinities of the mAbs have not been determined precisely by BioCore or similar methods, the low EC₅₀ for binding in the ELISA (approximately 20, 5 and 10 pmol/L for GAL-FR21, GAL-FR22, and GAL-FR23, respectively) suggest the affinities are very high. In the same ELISA format, none of the mAbs displayed detectable binding affinity to FGFR1, FGFR3, or FGFR4. Because the mAbs were generated against soluble forms of FGFR2, we needed to verify that they bind to FGFR2 in its native, membrane-bound form. In flow cytometry, all the mAbs
3 mAbs strongly stained SNU-16 gastric tumor cells over-expressing FGFR2IIIb (Fig. 2A) whereas only GAL-FR22 and GAL-FR23 stained 293F cells transfected with FGFR2IIIc (Fig. 2B); none of the mAbs stained the host 293F cells (not shown). The mAbs stained OCLM-2M gastric tumor cells (32) and another gastric tumor line KATO-III very similarly to SNU-16 cells (not shown). Finally, when FGFR2IIb containing the S252W mutation associated with Apert syndrome and certain tumors was expressed on 293F cells by transfection, all the mAbs bound to the mutant FGFR2IIb as well as normal FGFR2IIb (Fig. 2C).

To help interpret the results of the xenograft experiments described below, we determined the ability of GAL-FR21 and GAL-FR22 to bind to mouse FGFR2IIb. In an ELISA assay, GAL-FR21 bound to mouse FGFR2IIb within several-fold as well as human FGFR2IIb (Fig. 1C), whereas GAL-FR22 exhibited weak binding to mouse FGFR2IIb (Fig. 1D). The cross-reactivity of GAL-FR21 with mouse FGFR2IIb may seem surprising because the mAb was generated by immunization with human FGFR2IIb. However, human and mouse FGFR2 have high sequence homology, and the very low level expression of FGFR2 in tissues of the adult animal may make it relatively easy to break tolerance. In any case, the good reactivity of GAL-FR21 with FGFR2IIb from mouse and probably other species will facilitate animal studies of this mAb.

To further define the binding region of the mAbs, we compared their binding to the β-isofoms (ECD domains 2 and 3) with binding to the α-isofoms (ECD domains 1–3) of FGFR2IIb and FGFR2IIc. Results shown in Figure 3A demonstrate that GAL-FR21 bound to both α- and β-isofoms of FGFR2IIb but not FGFR2IIc. This suggests that the GAL-FR21 binding region does not involve D1 but may be in the D3 domain, where the sequence difference between FGFR2IIb and FGFR2IIc is located. GAL-FR22 bound to α and β forms of both

![Fig. 2](image-url)
FGFR2IIIb and FGFR2IIIfc, indicating that the binding region of GAL-FR22 does not involve D1 but is contained in D2-D3IIIa, where FGFR2IIb and FGFR2IIIfc share the same sequences. In contrast, GAL-FR23 bound only to the α-β-α-β II domain of both receptors, implying the binding region is located in the D1 domain. Hence, GAL-FR21, GAL-FR22, and GAL-FR23 must bind 3 different epitopes of FGFR2. This conclusion is supported by the fact that in a competitive binding ELISA, none of the mAbs competed with each other for binding to FGFR2 (Fig. 3B).

**Ligand blocking activities of the anti-FGFR2 mAbs in vitro**

X-ray crystallography shows that FGF ligands interact extensively with the D2-D3 region of the FGF receptors (5). Thus GAL-FR21 and GAL-FR22, which have binding regions in the D2 and D3 domains, have the potential to block ligand/receptor binding. Because all the 3 mAbs bind to FGFR2IIb, we investigated the blocking activities of these mAbs toward the binding of FGF2, FGF7, and FGF10 to FGFR2IIb. For this purpose, the FGF ligands bound to heparin-coated ELISA plates were incubated with soluble FGFR2IIb-Fc with or without the respective mAbs, and the bound FGFR2IIb-Fc was detected by the addition of HRP-goat anti-human IgG, taking advantage of the Fc tag in the fusion protein. As shown in Figure 4, GAL-FR21 and GAL-FR22 effectively blocked the binding of FGFR2IIb to each of FGF2, FGF7, and FGF10 with IC50s of approximately 1.5 to 3 μg/mL. In contrast, as expected from the location of its epitope in D1, GAL-FR23 did not inhibit binding of the tested FGF10 to FGFR2IIb.

**Anti-FGFR2 mAbs inhibit ligand-induced FGFR2IIb phosphorylation**

We further investigated the blocking activities of the anti-FGFR2 mAbs using a functional in vitro assay, FGF-induced FGFR2IIb phosphorylation. In this experiment, SNU-16 cells were serum starved overnight and treated with 10 μg/mL of mAb for 30 minutes, followed by the addition of FGF2 (100 ng/mL) or FGF7 (10 ng/mL) in the presence of heparin for 10 minutes. Phosphorylated FGFR2 in the cell lysates was detected using an ELISA specific for p-FGFR2. GAL-FR21, and GAL-FR22 by themselves demonstrated weak agonist activity in this assay (compare the level of p-FGFR2 in the absence of ligand when cells were treated with GAL-FR21 or GAL-FR22 vs. control mAb in Fig. 5A). Thus, we were not able to demonstrate the complete inhibition of FGF-induced FGFR2 phosphorylation by these mAbs. The level of phosphorylation was the same in the presence of GAL-FR21 whether or not FGF7 was added (Fig. 5A), indicating effective blocking of FGF7-induced phosphorylation by this mAb. In contrast, GAL-FR22 did not block FGF7-induced phosphorylation in the conditions used (Fig. 5A). It is possible that low residual binding of FGF7 to FGFR2IIb in the presence of GAL-FR22 is sufficient to induce phosphorylation of FGFR2IIb, whereas this is evidently not true for GAL-FR21. However, both GAL-FR21 and GAL-FR22 significantly inhibited FGF2-induced phosphorylation of FGFR2IIb (Fig. 5B).
with GAL-FR21 more effective than GAL-FR22 and with GAL-FR23 having no effect as expected. Despite their inhibitory effect on ligand-induced phosphorylation, none of the mAbs inhibited proliferation of SNU-16 (or OCUM-2M) cells in vitro (data not shown).

Anti-FGFR2 mAbs down-modulate FGFR2 expression

To further define the functional activity of GAL-FR21 and GAL-FR22, we investigated whether binding of the mAbs to FGFR2 reduces membrane FGFR2 expression. SNU-16 cells treated overnight with the mAbs were collected and the level of FGFR2 on the cell membrane was analyzed by flow cytometry using the GAL-FR23 mAb (Fig. 5C), which does not compete with GAL-FR21 or GAL-FR22 for binding (Fig. 3B). We also determined the level of total cellular FGFR2 by immunoblotting of cell lysates with GAL-FR21 (Fig. 5D), which binds to denatured FGFR2IIIb. Treatment with GAL-FR21 or GAL-FR22 down-modulated membrane expression of FGFR2 by approximately 50% (Fig. 5C). The total level of FGFR2 determined in the immunoblot analysis was also substantially reduced compared to the control (Fig. 5D), in the case of GAL-FR21 by 8 but not 2 hours of treatment. As a positive control, we also treated cells with FGF2, which reduced membrane and total FGFR2 even more strongly than the mAbs. Downmodulation of receptors by their ligands is of course a well-known phenomenon.

Anti-FGFR2 mAbs demonstrated potent anti-tumor activity in vivo

As noted previously, GAL-FR21 and GAL-FR22 bind very well to the gastric cancer cell lines SNU-16 and OCUM-2M, which overexpress FGFR2 (33). Thus, we investigated the anti-tumor activities of the anti-FGFR2 mAbs on xenografts of these cell lines. Antibody was administered intraperitoneally twice weekly at dose levels of 0.5 to 5 mg/kg after tumor size had reached ~150 mm^3. The GAL-FR21 and GAL-FR22 mAbs inhibited growth of the SNU-16 xenografts essentially completely at a dose level of 1 mg/kg and in some cases tumor regression was observed (Fig. 6A; P = 0.0014 for GAL-FR21 vs. mIgG and P = 0.0005 for GAL-
FR2 vs. mlG). The inhibitory effect of GAL-FR21 on the xenografts was essentially independent of dose level from 5 mg/kg down to the quite low dose level of 0.5 mg/kg (10 μg per injection; Fig. 6B). The mAbs GAL-FR21 and GAL-FR22 tested at 2.5 mg/kg also inhibited growth of OCUM-2M xenografts (Fig. 6C; P = 0.003 for GAL-FR21 or GAL-FR22 vs. mlG). Because the antitumor activities of these mAbs were so potent, we also measured total body weight at the end of the experiment as an indication of potential toxicity. After subtracting the tumor weight, there were no appreciable differences between the average body weights of the mice treated with any of the mAbs or with PBS. Nor did the mAb-treated mice exhibit any other obvious signs of toxicity, such as lethargy or failure to feed, although histopathology was not conducted. These results are especially meaningful for GAL-FR21, which binds well to mouse FGFR2.

Discussion

Gastric cancer is a fairly common type of malignancy in Western countries, with about 21,000 new cases in the United States in 2009, very comparable to the incidence of ovarian cancer or liver cancer (34). For reasons that are not well understood, gastric cancer is much more common in Asian countries, making it the second leading cause of cancer death worldwide (35). The prognosis for gastric cancer is poor unless it is detected at an early stage, so the 5-year survival rate in the U.S. is only about 25% (35). Surgery is a common treatment for gastric cancer but is rarely curative when the cancer is advanced, and chemotherapy, often platinum-based, is also not very effective. No targeted therapies have been approved for gastric cancer, although several small-molecule tyrosine kinase inhibitors, including sunitinib, are being tested for this indication, according to the clinicaltrials.gov web site. Indeed, in a recent phase III trial in gastric cancer, the anti-VEGF mAb bevacizumab (Avastin) did not prolong survival. Hence, drugs directed against other molecular targets in gastric tumors are certainly needed.

Gastric cancer is classified into 2 major types (36): well differentiated and poorly differentiated. The poorly differentiated form, which is also called diffuse or infiltrative, itself has several subtypes (36): scirrhous, signet ring cell carcinoma, and mucinous adenocarcinoma. FGFR2 overexpression is preferentially associated with the poorly differentiated type of gastric carcinoma: In a series of gastric cancer patients, 20 of 38 tumors of the poorly differentiated type overexpressed FGFR2, but none of 11 tumors of the well-differentiated type overexpressed FGFR2, but not proliferation of 3 nonscirrhous gastric cancer lines, and strongly inhibited growth of OCUM-2MD3 xenografts in mice (27). And the FGFR2 inhibitor KI23057 suppressed proliferation of the scirrhous gastric carcinoma cell lines OCUM-2MD3 and OCUM-8 overexpressing FGFR2, but not proliferation of 3 nonscirrhous gastric cancer lines, and strongly inhibited growth of OCUM-2MD3 xenografts in mice (33). The small molecule FGFR2 inhibitor PD173074 potently inhibited this phosphorylation and the growth of the SNU-16, OCUM-2M, and KATO-III cell lines in vitro and induced apoptosis of SNU-16 and OCUM-2M cells (33). Similarly, the small molecule FGFR2 inhibitor KI23057 suppressed proliferation of the scirrhous gastric carcinoma cell lines OCUM-2MD3 and OCUM-8 overexpressing FGFR2, but not proliferation of 3 nonscirrhous gastric cancer lines, and strongly inhibited growth of OCUM-2MD3 xenografts in mice (27). And the FGFR2 inhibitor AZD2171 potently inhibited FGFR2 phosphorylation, cell growth in vitro, and xenograft growth in vivo of the OCUM-2M and KATO-III cell lines (28). However, all these agents inhibit other tyrosine kinase receptors in addition to FGFR2, including FGFR1, FGFR3, VEGFR-1, VEGFR-2, VEGFR-3, and/or PDGF-βR. Besides increasing the potential for toxicity of these agents when used in human patients, this lack of specificity somewhat weakens the evidence they provide for FGFR2 as the cause of the cancerous phenotype of the tested cell lines. Partly filling this gap, FGFR2-specific siRNA also strongly inhibited growth of the OCUM-2M and KATO-III cell lines (28, 33). Importantly, the ability of the GAL-FR21 and GAL-FR22 mAbs, which are highly specific for FGFR2, to almost completely inhibit the growth of SNU-16 and OCUM-2M xenografts provides decisive additional evidence for the causative role of FGFR2.

We have not fully established the mechanism of action of the anti-FGFR2 mAbs against SNU-16 and OCUM-2M tumor xenografts. Both mAbs downmodulate FGFR2 expression on the SNU-16 cells, which may reduce cell signaling from the activated FGFR2 that is driving cell proliferation. It is also possible that cross-linking of the FGFR2 by the mAbs directly transmits a proapoptotic signal. However, the mAbs do not inhibit cell proliferation in vitro, in contrast with their potent growth inhibitory effects in vivo. This phenomenon has also been seen with mAbs to other growth factors or their receptors. For example, the L2G7 mAb to hepatocyte growth factor (37) strongly inhibits growth of xenografts from several tumor cell lines against which it has little direct effect in vitro (our unpublished data). The greater vulnerability of the cells to anti-FGFR2 mAbs when growing in vivo

than in vitro is probably a result of the increased stresses they are subjected to as tumors, for example hypoxia, but this question should be the subject of further investigation.

Several other potential modes of action probably do not make a major contribution to the anti-tumor effects of the mAbs. ADCC (antibody-dependent cell-mediated cytotoxicity) is unlikely to be an important factor because GAL-FR21, which is of the IgG1 isotype that mediates ADCC poorly (38), inhibits tumor growth as well as GAL-FR22, which is of the IgG2b isotype known to mediate ADCC well. Anti-angiogenic effects are also probably not important: whereas certain FGFs, such as FGF2, are potent angiogenic factors (2), FGF2 is only one of the receptors for these factors. Moreover, GAL-FR22 only weakly binds the mouse FGF2 expressed on endothelial cells of growing blood vessels in the xenografts, but inhibits tumor growth as well as GAL-FR21, which binds mouse FGF2 strongly. In addition, although tested in different xenograft models, the antitumor effects of GAL-FR21 and GAL-FR22 are stronger than those generally seen with the murine precursor anti-body of bevacizumab (39), an anti-angiogenic mAb that inhibits VEGF. Finally, although the mAbs effectively block binding of the FGF2, FGF7, and FGF10 ligands to FGFR2, this is also unlikely to contribute to their antitumor effect in the models used here. Indeed, FGFR2 is already maximally activated in OCM-2M cells in the absence of ligand, and although FG/7 does stimulate FGFR2 phosphorylation in SNu-16 cells, this does not further stimulate downstream signaling pathways (33). Hence, blocking of FGF7 or other FGF ligands should have little effect on proliferation of these gastric tumor cell lines. However, the ability of the mAbs to inhibit ligand binding may be more important in treatment of other types of tumors, for example pancreatic or prostate, where FGF7 and/or FGF10 are believed to play a role in tumorigenicity (40, 41).

The findings reported here are very analogous to the recent demonstration that a mAb directed to another of the FGF receptors, FGFR3, blocks ligand binding and inhibits growth of bladder tumor xenografts (42). The ability of the GAL-FR21 and GAL-FR22 mAbs to inhibit growth of xenografts from 2 gastric tumor cell lines without apparent toxicity suggests that it will be worthwhile to humanize one of these mAbs and then test the humanized mAb in clinical trials for the treatment of the poorly differentiated form of gastric carcinoma and potentially other types of cancer.

Disclosure of Potential Conflicts of Interest

K. J. Kim is the owner of 40% of Galaxy Biotech, LLC; W.-M. Zhao, L. Wang, H. Park, S. Chhim, and M. Tangphanich own between 1% and 5% of Galaxy Biotech, LLC.

Grant Support

NIH grants 5R44CA101283-03 and 1R43CA141748-01.

Received 03/01/2010; revised 06/23/2010; accepted 07/14/2010; published OnlineFirst 07/29/2010.

References


Downloaded from cincancersres.aacrjournals.org on December 31, 2017. © 2010 American Association for Cancer Research.


Clinical Cancer Research

Monoclonal Antibodies to Fibroblast Growth Factor Receptor 2 Effectively Inhibit Growth of Gastric Tumor Xenografts

Wei-meng Zhao, Lihong Wang, Hangil Park, et al.


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-10-0531</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cited articles</td>
<td>This article cites 39 articles, 14 of which you can access for free at: <a href="http://clincancerres.aacrjournals.org/content/16/23/5750.full#ref-list-1">http://clincancerres.aacrjournals.org/content/16/23/5750.full#ref-list-1</a></td>
</tr>
<tr>
<td>Citing articles</td>
<td>This article has been cited by 3 HighWire-hosted articles. Access the articles at: <a href="http://clincancerres.aacrjournals.org/content/16/23/5750.full#related-urls">http://clincancerres.aacrjournals.org/content/16/23/5750.full#related-urls</a></td>
</tr>
<tr>
<td>E-mail alerts</td>
<td>Sign up to receive free email-alerts related to this article or journal.</td>
</tr>
<tr>
<td>Reprints and Subscriptions</td>
<td>To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at <a href="mailto:pubs@aacr.org">pubs@aacr.org</a>.</td>
</tr>
<tr>
<td>Permissions</td>
<td>To request permission to re-use all or part of this article, use this link <a href="http://clincancerres.aacrjournals.org/content/16/23/5750">http://clincancerres.aacrjournals.org/content/16/23/5750</a>. Click on &quot;Request Permissions&quot; which will take you to the Copyright Clearance Center's (CCC) Rightslink site.</td>
</tr>
</tbody>
</table>