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

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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 FGFR2IIb and FGFR2IIc 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 FGFR2IIb isoform, whereas GAL-FR22 and GAL-FR23 bind to both the FGFR2IIb and FGFR2IIc 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 FGFR2IIb. 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.

Conclusions: Anti-FGFR2 mAbs GAL-FR21 and GAL-FR22 have potential for the treatment of gastric and other tumors.

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-isoisforms) or only the 2 Ig domains D2 and D3 without D1 (β-isoisoms). 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 IIIb and IIIc forms. These are respectively denoted FGFR2IIb and FGFR2IIc; the corresponding β forms are denoted as FGFR2(β)IIb and FGFR2(β)IIc. Importantly, the FGFR2IIb form is a high-affinity receptor for FGF1 and FGF2 as well as for FGF7 (keratinocyte growth factor; KGF) and FGF10, whereas FGFR2IIc binds both FGF1 and FGF2 well but does not bind KGF (6). Indeed, FGFR2IIb 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, FGFR2IIb (and the IIb forms of FGFR1 and FGFR3) are expressed in epithelial tissues, whereas FGFR2IIc 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, FG3 and FGF7 are expressed in mesenchymal tissues, so may be paracrine effectors of epithelial cells expressing FGFR2IIb, whereas the FG4 subfamily members FGF4–6 bind to FGFR2IIc and are expressed in both epithelial and mesenchymal lineages so

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found, a Ser-252 Trp substitution, is known to also be strongly associated with the congenital skeletal disorder Apert syndrome (18) and increases ligand binding and receptor activation (19). FGFR2IIb RNA was expressed in 16 of 20 epithelial ovarian cancers (EOCs) but not in normal ovarian surface epithelium (20); and the FGFR2IIb ligands FGF1, FGF7, and FGF10 induced proliferation, motility, and protection from cell death in EOC cell lines (21), suggesting that FGFR2IIb may contribute to the malignant phenotype in ovarian cancer. The FGFR2 gene is also amplified in a subset of breast cancers (22), and genome-wide association studies of breast cancer patients have demonstrated the FGFR2 locus to be strongly associated with susceptibility to breast cancer (23, 24). Of particular interest, amplification and overexpression of FGFR2 is strongly associated with the poorly differentiated, diffuse type of gastric cancer, which has an especially unfavorable 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 FGFR2IIb and FGFR2IIc were expressed as immunoadhesin molecules by respectively fusing the ECD residues 1–378 (α-isoform) and 152–378 (β-isoform) of FGFR2IIb or 1–377 (α-isoform) and 152–377 (β-isoform) of FGFR2IIc 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). Mouse FGFR2IIb-Fc was purchased from R&D Systems.

**Generation of mAbs to FGFR2**

Female 6-week-old Balb/c mice (Charles River Laboratories) were immunized with human FGFR2IIb-Fc or FGFR2IIc-Fc in monophosphoryl lipid A/trehalose dicorymycolate (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 FGFR2IIIb-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 × 10⁶) 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 × 10⁵ 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 × 10⁵ per well) were treated with mAbs (10 μg/mL) or FGF2 (100 ng/mL) in RPMI with 10% FCS for 20 hours and the level of cell surface FGFR2 determined by flow cytometry as described above, using biotinylated GAL-FR23 mAb followed by streptavidin-R-phycocerythrin for detection. In addition, total cell lysate was prepared after various periods of treatment with the mAbs, separated by SDS gel electrophoresis and processed for Western blotting. The FGFR2IIIb band was detected by incubation with 0.2 μg/mL of GAL-FR21 mAb, followed by HRP-goat anti-mouse IgG.

** Xenograft models**

Animal experiments were conducted in accordance with U.S. Public Health Service policy. SNU-16 or a subclone of OCM1-2M cells grown in complete DMEM medium were harvested in PBS. Female 5- to 6-week-old athymic nude mice were injected subcutaneously with 5 × 10⁵ 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 = ab²/2. 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 FGFR2IIb-Fc and FGFR2IIc-Fc in ELISA (Fig. 1A, B). GAL-FR21 bound to denatured FGFR2IIb-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
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 FGFR2IIib as well as normal FGFR2IIib (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 FGFR2IIib. In an ELISA assay, GAL-FR21 bound to mouse FGFR2IIib within several-fold as well as human FGFR2IIib (Fig. 1C), whereas GAL-FR22 exhibited weak binding to mouse FGFR2IIib (Fig. 1D). The cross-reactivity of GAL-FR21 with mouse FGFR2IIib may seem surprising because the mAb was generated by immunization with human FGFR2IIib. 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 FGFR2IIib 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 FGFR2IIib and FGFR2IIic. Results shown in Figure 3A demonstrate that GAL-FR21 bound to both α- and β-isofoms of FGFR2IIib but not FGFR2IIic. This suggests that the GAL-FR21 binding region does not involve D1 but may be in the D3 domain, where the sequence difference between FGFR2IIib and FGFR2IIic is located. GAL-FR22 bound to α and β forms of both...
FGFR2IIIb and FGFR2IIIc, indicating that the binding region of GAL-FR22 does not involve D1 but is contained in D2-D3IIIa, where FGFR2IIIb and FGFR2IIIc share the same sequences. In contrast, GAL-FR23 bound only to the α-b receptor 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 mAb 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 FGFR2IIib, we investigated the blocking activities of these mAbs toward the binding of FGF2, FGF7, and FGF10 to FGFR2IIib. For this purpose, the FGF ligands bound to heparin-coated ELISA plates were incubated with soluble FGFR2IIib-Fc with or without the respective mAbs, and the bound FGFR2IIib-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 FGFR2IIib 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 FGFR2IIib.

Anti-FGFR2 mAbs inhibit ligand-induced FGFR2IIib phosphorylation

We further investigated the blocking activities of the anti-FGFR2 mAbs using a functional in vitro assay. FGF-induced FGFR2IIib 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 FGFR2IIib in the presence of GAL-FR22 is sufficient to induce phosphorylation of FGFR2IIib, whereas this is evidently not true for GAL-FR21. However, both GAL-FR21 and GAL-FR22 significantly inhibited FGF2-induced phosphorylation of FGFR2IIib (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 mm³. 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.\(^1\) 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 did, suggesting that the overall incidence of FGFR2 overexpression in gastric cancer may be as high as 40% (25). Although some tumors of each of the poorly differentiated subtypes overexpressed FGFR2, not enough data were presented to determine whether the incidence of FGFR2 overexpression differs between the subtypes (25). The FGFR2-overexpressing cell lines SNU-16 and OCUM-2M used here for xenograft studies originated from poorly differentiated gastric tumors (31, 32), with OCUM-2M known to come from the scirrhous subtype. The poorly differentiated type of gastric cancer has a worse prognosis than the well-differentiated type (36), so the existence of the well-defined target FGFR2 in this type is fortunate.

Importantly, FGFR2 overexpression appears to be an oncogenic driver in the gastric tumors where it appears, rather than a mere marker, as shown by the effect of FGFR2 antagonists. The overexpression of FGFR2 in SNU-16, OCUM-2M, and KATO-III cells is associated with high basal levels of FGFR2 activation site phosphorylation (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 K23057 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-R. 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

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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), FGFR2 is only one of the receptors for these factors. Moreover, GAL-FR22 only weakly binds the mouse FGFR2 expressed on endothelial cells of growing blood vessels in the xenografts, but inhibits tumor growth as well as GAL-FR21, which binds mouse FGFR2 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 antibody 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 FG7 does stimulate FGFR2 phosphorylation in SNU-16 cells, this does not further stimulate downstream signaling pathways (33). Hence, blocking of FGFR2 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 FG7 and/or FG10 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. Tanphanich own between 1% and 5% of Galaxy Biotech, LLC.

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