Mouse-Human Chimeric Anti-Epidermal Growth Factor Receptor Antibody C225 Inhibits the Growth of Human Renal Cell Carcinoma Xenografts in Nude Mice

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ABSTRACT

The epidermal growth factor (EGF) receptor and its ligand transforming growth factor-α (TGF-α) are overexpressed in human renal cell carcinoma (RCC). The chimeric anti-EGF receptor monoclonal antibody C225 was used to determine the effects of blocking the EGF receptor on RCC growth both in vitro and in vivo. A panel of RCC cell lines all tested positive at various levels for EGF receptor cell surface expression. C225 inhibited DNA synthesis of cultured A498, Caki-1, SK-RC-4, SK-RC-29, and SW839 cells in a dose-dependent manner, ranging from 20 to 45% inhibition compared with untreated controls. C225 also inhibited exogenous ligand-stimulated tyrosine phosphorylation of EGF receptor on RCC cells. The antitumor effects of C225 on RCC tumor growth were evaluated in ascites, s.c., and orthotopic RCC xenograft models. Mice treated with C225 in a Caki-1 ascites xenograft model showed a significant increase in survival (P = 0.002). All control mice died with ascites tumors by week 9, whereas >70% of C225-treated mice survived beyond 12 weeks. C225 also inhibited the growth of s.c. SK-RC-29 tumors in a dose-dependent manner. Mice treated with C225 (1 mg/dose) displayed a significant decrease in tumor volume compared with mice treated with control antibody (P < 0.05) or vehicle alone (P < 0.01). Lastly, C225 inhibited the growth and metastasis of RCC tumors growing orthotopically in the renal subcapsule of nude mice. Histological examination of RCC tumors from mice treated with C225 showed a substantial decrease in proliferating cell nuclear antigen staining and an increase in tumor cell apoptosis. These data suggest that C225 affects growth of RCC tumors by inhibiting EGF receptor-dependent proliferation and demonstrate the potential for therapeutic application of C225 in the treatment of human renal cancer.

INTRODUCTION

RCC is the most common malignant tumor arising from the kidney with an estimated incidence of >30,000 new cases per year in the United States (1). RCC is an insidious cancer with 25% of patients at presentation with stage IV disease (2). The prognostic factors for patients with RCC carcinoma are regional node involvement and distant metastatic dissemination of the tumor. The presence or absence of metastasis at the time of diagnosis is the main factor determining survival (3). Radical nephrectomy is the main treatment for localized RCC (1). Radiotherapy and available chemotherapeutic agents are ineffective against metastatic RCC. Additional treatments for metastatic RCC include the biological agents IL-2 and INF-α. In a minority of patients, dramatic complete or partial responses have been achieved with IL-2, IFN-α, or a combination of both agents (1, 4-6). However, these therapies suffer from severe toxicity, are effective in only a small percentage of patients, and are rarely durable. Development of new therapies that would provide more effective responses for a larger number of patients is clearly needed for the treatment of human RCC.

The EGF receptor is a transmembrane protein tyrosine kinase encoded by the c-erb-B proto-oncogene and expressed on many normal and malignant cells (7, 8). The receptor is a member of a large family of tyrosine kinases that also includes Her-2/new/erb B-2, erb B-3, and erb B-4 (9-11). The EGF receptor is frequently overexpressed in many human epithelial tumors including breast carcinoma (12), head and neck carcinoma (13), bladder carcinoma (14), glioblastoma (15), and lung carcinoma (16). High levels of EGF receptor expression predict a poor clinical prognosis for many of these cancers (17-19). The EGF receptor is also overexpressed in 40-85% of primary RCCs, and its expression correlates with high tumor grade and poor clinical outcome (12, 14, 20). Furthermore, increased EGF receptor expression in RCC is often associated with increased production of TGF-α by the same tumor cells, implicating an autocrine stimulatory pathway for tumor cell growth. These studies suggest that receptor blockade may be a means of limiting tumor progression by inhibiting tumor cell growth, inducing terminal differentiation, or causing apoptosis (21-23).

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2 The abbreviations used are: RCC, renal cell carcinoma; IL, interleukin; EGF, epidermal growth factor; hu IgG1, human IgG1; TGF-α, transforming growth factor α; MAb, monoclonal antibody cMAb, chimeric monoclonal antibody; FBS, fetal bovine serum; HRP, horseradish peroxidase; PCNA, proliferating cell nuclear antigen; IC50, IgG concentration representing 50% of the maximum antibody inhibition.
The mouse anti-EGF receptor MAb C225, and its mouse-human chimeric version C225, bind the receptor with affinity comparable to the natural ligand and compete for ligand binding (24–26). Blockade of ligand binding to EGF receptor by MAb C225 inhibits activation of the receptor tyrosine kinase and retards cell cycle progression with accumulations of cells in G1 (27). Several studies have shown that C225 is capable of inhibiting growth of EGF receptor-expressing tumor cells, including renal tumor lines, in vitro (22, 28–31), and treatment with C225 results in marked inhibition of tumor growth in nude mice bearing s.c. xenografts of cancer cell lines (31–33). Moreover, treatment with C225 in combination with the chemotherapeutic drugs is effective in completely eradicating tumors that are well established in nude mice (34). The objective of this study was to determine the potential therapeutic utility of the cMAb C225 to inhibit growth of RCC cells in vitro and affect the growth of RCC xenograft tumors in vivo.

**MATERIALS AND METHODS**

**Animals.** Female nu/nu mice, 5–6 weeks of age, were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Mice were housed under pathogen-free conditions in microisolator cages with laboratory chow and water available *ad libitum*. All experiments and procedures were performed in accordance with the United States Department of Agriculture, Department of Health and Human Services, and NIH policies regarding the humane care and use of laboratory animals.

**Cell Lines.** RCC cell lines A498, Caki-1, and SW839 were obtained from the American Type Culture Collection (Rockville, MD). Cell lines SK-RC-4 and SK-RC-29 were established and characterized as described previously (35). Cell line SN12-PM6 was obtained from Dr. I. J. Fidler (M. D. Anderson Cancer Center, Houston, TX). The cell line RC29-MK1 was established by passing SK-RC-29 cells in the renal subcapsule of athymic mice. RCC cell lines were maintained in DMEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% FBS (HyClone, Lenexa, KY) and 2 mM l-glutamine (Life Technologies, Inc.). Cells were cultured at 37°C in a 5% CO2 atmosphere and routinely passaged by Tryptsin-EDTA (Life Technologies, Inc.) treatment. RCC cell lines were routinely checked for *Mycoplasma* contamination.

**Antibodies.** Clinical grade anti-EGF receptor MAb C225 was supplied by the ImClone Systems, Inc. manufacturing facility. The antibody was purified by a multistep chromatography process to a purity >99% as determined by SDS-PAGE. The negative control hu IgG1 was purified via affinity chromatography of polyclonal human IgG (Jackson ImmunoResearch, West Grove, PA) on a goat anti-human IgG1 (Sigma) coupled to Sepharose resin (Pharmacia, Piscataway, NJ).

**Flow Cytometry.** Subconfluent cultures of RCC cell lines grown in 100-mm² plates were washed in ice-cold HBSS and detached by incubating the monolayers in HBSS with EDTA. Aliquots of 10⁶ cells were incubated for 1 h on ice with primary antibody (C225 or control IgG) diluted in PBS with 1% BSA, 0.02% sodium azide (flow buffer). Cells were washed twice with flow buffer and then incubated for 30 min on ice with FITC-labeled goat anti-human IgG Fc-specific secondary antibody (Jackson Immunoresearch Laboratories) diluted in flow buffer. Cells were washed as above and analyzed for FITC emission intensity at 520 nm on an Epics Elite XL (Coulter, Hialeah, FL) flow cytometer. Dead cells and debris were eliminated from the analysis on the basis of forward and sideways light scatter.

**Phosphorylation Assay.** Phosphorylation assays were performed by seeding RCC cells at 10⁴/100-mm² tissue culture plate in DMEM containing 0.5% FBS in the presence or absence of 30 nM C225. After culturing for 24 h, cells were then stimulated with 5 ng/ml EGF or 5 ng/ml TGF-α (Sigma) for 20 min at 37°C. Monolayers were then washed with ice-cold PBS containing 1 mM sodium orthovanadate. Cells were lysed, immunoprecipitated with anti-EGF receptor Ab (Upstate Biotechnology, Inc., Lake Placid, NY) and subjected to Western blot analysis. The phosphorylation patterns were determined by probing the blots with an HRP-conjugated anti-phosphotyrosine MAb (UBI), followed by detection using the ECL method (Amersham, Arlington Heights, IL). Loading equivalence of EGF receptor in each lane was verified by stripping and re-probing blots with an anti-EGF receptor antibody (UBI).

**Cell Proliferation Assay.** Inhibition of RCC cell growth in vitro was determined by a [³H]thymidine incorporation proliferation assay. RCC cells were plated in complete media (10⁴ cells/well) until 96-well tissue culture plates with C225 or controls in DMEM containing 0.5% FBS. After 24 h, 0.5 μCi/well [³H]thymidine (New England Nuclear) was added, and cultures were incubated an additional 24 h. Cells were then harvested, and the incorporated radioactivity was determined by liquid scintillation counting (Wallach, Gaithersburg, MD). The percentage of inhibition of cell proliferation was calculated as (1 – (cpm C225/cpm hu IgG1)) × 100.

**Treatment of RCC Xenografts in Athymic Nude Mice.** Ascites tumors were established by injecting athymic nude mice i.p. with pristane 7 days prior to i.p. injection of 5 x 10⁶ Caki-1 cells. Twenty-four h after tumor cell injection, groups of 10 animals received i.p. injections of either C225 (0.5–1 mg/dose), hu IgG1 control antibody (1 mg/dose) or PBS twice weekly for 5 weeks. Animal survival was followed for a total of 12 weeks.

s.c. RCC tumors were established by injecting athymic nude mice s.c. in the right flank with 2 x 10⁶ SK-RC-29 cells mixed in Matrigel (Collaborative Research Biochemicals, Bedford, MA). Tumors were allowed to reach 200 mm³ in size, and then groups of 10 animals received i.p. injections of either C225 (0.5–1 mg/dose), hu IgG1 (1 mg/dose), or PBS every 3 days for 5 weeks of total treatment. Tumors were measured twice each week with calipers, and tumor volumes were calculated by the formula (π/6 w₃ x w₂ x w₁), where w₁ represents the smallest tumor diameter and w₂ represents the largest tumor diameter (36). Tumor volume ratios were calculated using the equation: (tumor volume at day x)/(tumor volume at start of treatment) = tumor volume ratio (37).

Orthotopic RCC tumors were established by injecting 10⁶ RC29-MK1 cells into the renal subcapsule of the left kidney of athymic nude mice. After 3 days, groups of 10 animals received i.p. injections of either C225 (0.5–1 mg/dose), hu IgG1 (1 mg/dose), or PBS every 3 days for 4 weeks of total treatment. At the completion of the treatment schedule, mice were sacrificed, and kidneys weights were measured. Organs from mice were harvested for histological examination.
Histology. RCC tumors, kidneys, and lungs were surgically removed, fixed in 10% neutral buffered formalin, embedded in paraffin, or flash-frozen and sectioned onto slides at 6-μm thickness. All tumors were stained histologically with H&E. Immunostaining was performed with anti-EGF receptor (Calbiochem, San Diego, CA) or anti-PCNA (Novocastra, Newcastle upon Tyne, United Kingdom) antibodies at concentrations of 10 and 2 μg/ml, respectively. Biotinylated secondary antibodies (Tago, Camarillo, CA) were used at 1 μg/ml. Detection was performed with HRP-Streptavidin and dianaminobenidine (Link and Label kit; BioGenex, San Ramon, CA) according to the manufacturer’s instructions. Sections were counterstained with either hematoxylin or eosin before coverslipping. Apoptotic cells were visualized using a TdT-mediated dUTP nick end labeling kit (Boehringer-Mannheim, Indianapolis, IN). Light and fluorescent images of immunostained tissue were viewed on a Zeiss Axioskop and digitized using a SONY camera and Scion CG-7 framegrabber.

Statistical Analysis. Survival of mice was assessed using a Mann-Whitney U test. Tumor volumes, as well as expression of PCNA levels, were analyzed using Student’s t test. Analyses were computed using the SigmaStat statistical package version 1.0 (Jandel Scientific, San Rafael, CA).

RESULTS

EGF Receptor Expression on Renal Carcinoma Cell Lines. Expression of EGF receptor on the cell surface of RCC cell lines was evaluated by staining with MAb C225 and flow cytometry analysis. All RCC cell lines expressed varied levels of EGF receptor (Fig. 1). SK-RC-29 cells displayed the highest level of EGF receptor on the cell surface, whereas Caki-1 cells displayed the lowest level of EGF receptor expression. No staining of RCC cell lines was observed with the hu IgG1 control.

C225 Inhibits Ligand-stimulated EGF Receptor Tyrosine Phosphorylation in Renal Carcinoma Cells in Vitro. Activation of the EGF receptor by exogenous ligand stimulation in the presence or absence of C225 was evaluated in a receptor phosphorylation assay. Under normal culture conditions, a low basal level of EGF receptor tyrosine phosphorylation was observed in Caki-1, SK-RC-4, SK-RC-29, and SN12-PM6 cells (Fig. 2). Addition of exogenous EGF ligand to cultures increased tyrosine phosphorylation of EGF receptor in all cell lines, whereas in cell line SN12-PM6, EGF receptor phosphorylation was not stimulated by exogenous TGF-α. Treatment of cells with an excess of C225 (30 nM) significantly inhibited EGF receptor tyrosine phosphorylation in those cells responsive to exogenous EGF or TGF-α stimulation. Treatment of Caki-1, SK-RC-4, SK-RC-29, and SN12-PM6 cells with C225 did not completely inhibit baseline phosphorylation of EGF receptor, suggesting a ligand binding independent level of receptor phosphorylation in these cells.

C225 Inhibits RCC Proliferation in Vitro. To determine whether C225 could inhibit the growth of RCC cell lines in vitro, cells were incubated with various concentrations of C225 and assessed for [3H]thymidine incorporation into DNA. C225 inhibited DNA synthesis of A498, Caki-1, SK-RC-4, SK-RC-29, and SW839 cells in a dose-dependent manner (Fig. 3). Inhibition of RCC cell line growth by C225 ranged from 20 to 45% compared with untreated controls with the IC50 ranging from 0.05 to 4 nM. Growth of SN12-PM6 cells was not affected by C225 treatment. Furthermore, exogenous EGF or TGF-α treatment of SN12-PM6 cells did not stimulate DNA synthesis in these cells.

Increased Survival of Mice Treated with C225 in a RCC Ascites Tumor Model. The effects of C225 on RCC tumor growth in vivo were evaluated in an ascites xenograft model. Athymic mice were injected i.p. with Caki-1 cells and then treated with either C225 (1 mg) or hu IgG1 control (1 mg) twice weekly for 5 weeks. Mice treated with C225 displayed a significant increase in survival (P < 0.05) compared with control mice (Fig. 4). All control mice died with ascites tumors by day 64, whereas >70% of C225 treated mice survived beyond 12 weeks. Postmortem evaluation showed that control mice had large production of hemorrhagic, malignant ascites fluid containing RCC cells and large tumor masses attached to the abdominal wall, bladder, spleen, and liver. Mice treated with C225 had little or no ascitic fluid accumulation and only local growth of small RCC tumors on the abdominal wall.

C225 Inhibits RCC Tumor Growth in a s.c. Xenograft Model. The effect of C225 on the growth of RCC tumors was further evaluated in a s.c. tumor model. Athymic mice were injected with SK-RC-29 cells and then treated with C225 at various doses, control hu IgG1 (1 mg/dose), or vehicle alone.

Fig. 1 Flow cytometry analysis of EGF receptor expression on human RCC cell lines. RCC cell lines were incubated for 1 h with C225 (black line) or control IgG (gray line). Cells were then washed in 1% BSA/PBS and incubated for 30 min on ice with FITC-labeled goat anti-human IgG Fc-specific secondary antibody. Cells were washed again and analyzed by flow cytometry.
C225 inhibited the growth of s.c. SK-RC-29 tumors in a dose-dependent manner (Fig. 5). Mice treated with C225 (1 mg/dose) displayed a significant decrease in tumor volume compared with mice treated with hu IgG1 or vehicle alone ($P < 0.05$ and $P < 0.01$, respectively). Tumor volumes in mice treated with 0.5 mg of C225 were not statistically different from control mice. Although C225 treatment inhibited the growth of established SK-RC-29 tumors, no complete tumor regressions were observed. Withdrawal of C225 treatment resulted in growth of RCC tumors with kinetics similar to controls after a lag period of ~3 weeks (data not shown).

Histological examination of RCC s.c. tumors demonstrated dramatic differences in tumors from C225-treated versus control animals. H&E staining of tumor sections from C225-treated animals revealed a marked decrease in cellularity compared with controls, with necrotic regions replaced by collagen and fibrous tissue (Fig. 6, A and B). Immunostaining with an anti-EGF receptor polyclonal antibody demonstrated consistent EGF receptor expression in RCC tumors from both treated and control groups (Fig. 6, C and D). A marked decrease in cell proliferation was seen in C225-treated tumors, as measured by anti-PCNA immunostaining (Fig. 6, E and F). Also, an increase in tumor cell apoptosis was observed in the tumors of these C225-treated animals (Fig. 6, G and H).

C225 Inhibits the Growth of RCC Tumors in an Orthotopic Renal Subcapsule Model. The effects of C225 on RCC tumor growth were also evaluated in a renal subcapsule orthotopic model. Orthotopic tumors were established by implanting RC29-MK1 cells in the renal subcapsule of athymic mice. Mice were treated with C225 (0.5-1 mg), hu IgG1 (1 mg), or vehicle alone every 3 days for the duration of the experiment. After 30 days, severe renal failure and mortality was observed in control mice, and kidneys were removed from all groups for examination. Large tumor masses were observed in the injected kidney of control animals, and in most cases, dissemination of the tumor was observed in the adjacent kidney (Fig. 7, A and B). Mice treated with C225 had significantly ($P < 0.01$) reduced tumor masses as determined by kidney weight (Table 1). Tumor dissemination to the adjacent kidney was observed only in 6 of 10 of the animals treated with C225. However, tumor masses in the adjacent kidney were markedly lower than in control animals. Lungs from all mice were resected and fixed in Bouin’s solution to determine the extent of RCC tumor metastasis. Lungs from control animals showed extensive dissemination of RCC metastases (Fig. 7C, arrows), whereas no evidence of RCC metastasis was found in the lungs of C225-treated mice (Fig. 7D). Numerous clusters of large, round cells were seen on H&E stains of sectioned lung tissue from control but not from C225-treated mice (Fig. 7, E and F). Immunostaining with a monoclonal antibody specific for human EGF receptor index:

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\text{Columns, } \%\text{ inhibition} = \left[1 - \left(\frac{\text{cpm C225}}{\text{cpm hu IgG1}}\right)\right] \times 100.
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Increased survival in athymic mice bearing ascites RCC xenografts. Ascites tumors were established by injecting athymic nude mice with pristane i.p. 7 days before i.p. injection of $5 \times 10^6$ Caki-1 cells. Twenty-four h after tumor cell injection, groups of 10 animals received i.p. injections of either C225 (0.5–1 mg/dose), control antibody (1 mg/dose), or PBS twice a week for 5 weeks. Animal survival was followed for a total of 12 weeks. A, survival time in days. B, representative sample of mice from hu IgG1 group (left) and from the C225 treatment group (right).

cated that these metastatic foci were human EGF receptor positive RCC tumor cells (Fig. 7E, inset).

**DISCUSSION**

Growth factors and growth factor receptors play an important role in regulating the growth of malignant cells (7, 38, 39). Increased expression of growth factors or their receptors may enhance the growth of RCC cells. Several investigators have reported the overexpression of TGF-α and the EGF receptor in human RCC and have shown an association of this overexpression with poor prognosis (8, 20, 28, 40). The present study was undertaken to assess the ability of an anti-EGF receptor MAb to inhibit the growth of RCC tumors in vivo. This hypothesis was based on previous observations that some RCC cell lines exhibit a TGF-α/EGF receptor autocrine loop mechanism of RCC cell growth that could be inhibited by EGF receptor blockade (8).

The results of the present study demonstrate that blockade

![Fig. 4](image-url)  
**Fig. 4** Increased survival in athymic mice bearing ascites RCC xenografts. Ascites tumors were established by injecting athymic nude mice with pristane i.p. 7 days before i.p. injection of $5 \times 10^6$ Caki-1 cells. Twenty-four h after tumor cell injection, groups of 10 animals received i.p. injections of either C225 (0.5–1 mg/dose), control antibody (1 mg/dose), or PBS twice a week for 5 weeks. Animal survival was followed for a total of 12 weeks. A, survival time in days. B, representative sample of mice from hu IgG1 group (left) and from the C225 treatment group (right).

![Fig. 5](image-url)  
**Fig. 5** Growth of RCC s.c. xenografts in nude mice is inhibited by C225. s.c. RCC tumors were established by injecting athymic nude mice s.c. in the right flank with $2 \times 10^6$ SK-RC-29 cells mixed in Matrigel. After tumors reached 200 mm$^3$ in size, animals were randomized into groups of 10 that received i.p. injections of either C225 (0.5–1 mg/dose), hu IgG1 (1 mg/dose), or PBS every 3 days for 5 weeks total treatment. Tumors were measured twice each week. A, mean tumor growth curves for C225 treatment and control groups; bars, SD. B, tumor volume ratios for animals in C225-treated and control groups at day 54; bars, SD; *, $P < 0.05$. C, representative mice from the hu IgG1 treatment group and the C225 treatment group.
of the EGF receptor by the cMAb C225 inhibits the growth of RCC cells in vitro and growth of RCC tumors in athymic mice. The in vitro results showed at best only a modest inhibition (20–40%) in the growth of cultured RCC cells by C225. In contrast, C225 was very effective in inhibiting the growth of RCC tumors in an ascites, s.c., and orthotopic tumor model. These results are consistent with results obtained using the anti-EGF receptor MAb 225 and cMAb C225 in other tumor models where effective antitumor responses were observed in vivo with only partial in vitro inhibition (31–34, 41). The effect of C225 in the RCC xenograft models appeared to be mainly cytostatic with respect to tumor growth. Although C225 treatment inhibited the growth of RCC tumors in the various tumor models, the treatment did not completely eliminate tumor bur-
den as evidenced by the regrowth of tumors if C225 treatment was withdrawn.

Some additional important effects were observed in the various RCC models. In the ascites model, control animals produced large tumors with accumulation of hemorrhagic ascites and dissemination of tumor cells to several organs. In addition to prolonged survival, C225-treated animals exhibited only a localized tumor mass with no evidence of ascitic fluid accumulation or dissemination of tumor cells to other organs. Histological examination of s.c. RCC tumors from C225-treated animals showed a dramatic decrease in mitotic tumor cells and an increase in tumor necrosis and fibrosis. The orthotopic RCC model more closely mimics human disease. In this model, RCC tumors grow very aggressively in the organ of origin and metastasize to the adjacent kidney and lungs. C225 treatment in the orthotopic model markedly slowed the growth of the primary tumor and completely inhibited tumor cell dissemination to the lungs. These findings suggest that, in addition to effecting the growth of localized tumor, C225 may also inhibit the dissemination and/or growth of distant metastases.

The mechanism of the inhibitory effect of C225 on tumor growth has been extensively investigated in other tumor models. Treatment of cells with anti-EGF receptor murine MAb 225 leads to inhibition of EGF receptor downstream signaling and perturbation of cell cycle progression, resulting in the accumulation of cells in G1 (42). The mechanism of G1 arrest in cells treated with anti-EGF receptor MAb 225 is due to an increase in the cell cycle inhibitor p27KIP1, which inactivates CDK2 kinase (42, 43). The extent to which anti-EGF receptor blockade inhibits cell cycle progression appears to be dependent on the particular tumor cell line used in each study. For example, treatment of colon carcinoma cells DiFi with MAb 225 dramatically inhibits the growth of these cells and induces programmed cell death (44). In other tumor cells, EGF receptor blockade leads to a steady accumulation of cells in G1 but does not induce apoptosis (27, 45). The results of the present study are compatible with these and other previous observations in human tumor models. Analysis of RCC tumors from mice treated with C225 showed a decrease in the number of mitotic tumor cells as measured by PCNA staining. However, C225-treated RCC tumors also showed evidence of apoptosis and tumor necrosis, suggesting that the effect of C225 in vivo was not entirely cytostatic.

Because the effect of C225 on cultured RCC cells is less dramatic than that seen on tumor cells in vivo, it is likely that these cells are either more susceptible to EGF receptor blockade in situ or that additional mechanisms are responsible for these effects. One possible explanation for the enhanced effects of C225 in vivo is suggested by the recent findings of Petit et al. (46). In their studies, C225 was shown to dramatically affect the angiogenic potential of A431 cells by down-regulating vascular endothelial growth factor expression and thus, decreasing the angiogenesis associated with growth of these tumors in vivo. Consistent with these data, we have found that C225 down-regulates vascular endothelial growth factor expression in RCC cell lines.3 Presently, we are analyzing the antiangiogenic effects of C225 treatment on RCC tumors growing in vivo. RCC is a highly vascularized tumor that may contribute to its growth characteristics and to the development of metastasis. Angiogenic factors and their receptors may provide additional novel targets for consideration as treatment strategies for RCC.

Mortality in RCC is associated with metastatic disease that is refractory to radiotherapy and available chemotherapeutic drugs. Presently, no antineoplastic agent has produced response rates to the extent that justify its use as a single agent in RCC. Recently, the investigation of immunomodulators such as IFN-α and IL-2 has shown promising responses in some patients (4–6). However, the response rates (10–20%) and duration of responses (~2 years, 5%) for patients treated with IFN-α and/or IL-2 indicate the need for additional treatment modalities. Clearly, a better understanding of the biology of RCC is still required to develop adequate strategies for this human cancer. Results of the present study suggest that blockade of the EGF receptor be a useful strategy for treatment of RCC. In this respect, Phase II trials are planned to evaluate C225 as a treatment for human RCC in a clinical setting.

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