In Vivo Enhancement of Tumor Radioresponse by C225 Antiepidermal Growth Factor Receptor Antibody

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ABSTRACT

Overexpression of epidermal growth factor receptor (EGFR) has been correlated with tumor resistance to cytotoxic agents, including radiation (T. Akimoto et al., Clin. Cancer Res., 5: 2884–2890, 1999), and thus is a candidate target for anticancer treatment. This study investigated whether treatment with C225 anti-EGFR antibody would improve tumor response to radiotherapy. Nude mice bearing 8-mm-diameter A431 tumor xenografts in the hind leg were treated with C225 antibody, 18 Gy of single-dose local tumor irradiation, or both. C225 was given i.p. at a dose of 1 mg/mouse 6 h before irradiation or 6 h before and 3 and 6 days after irradiation. Delay in tumor growth was the treatment end point. C225 dramatically improved the efficacy of local tumor irradiation, particularly when multiple injections of C225 were administered. Tumor radioresistance was enhanced by a factor of 1.59 by a single dose and by a factor of 3.62 by 3 doses of C225. Histological analyses of tumors revealed that C225 caused a striking increase in central tumor necrosis associated with hemorrhage and vascular thrombosis when combined with radiotherapy. In addition, C225 induced heavy tumor infiltration with granulocytes, increased tumor cell terminal differentiation, and inhibited tumor angiogenesis. We conclude that C225 anti-EGFR antibody enhances tumor radioresponse by multiple mechanisms that may involve direct and indirect actions on tumor cell survival.

INTRODUCTION

EGFR is a transmembrane protein involved in signaling pathways essential for cell survival. Overexpression of this receptor often accompanies development and growth of malignant tumors. There is increasing evidence that high expression of EGFR is correlated with aggressive tumor growth, as well as with poor clinical outcome of common cancers in humans, including breast, cervix, lung, and head and neck carcinomas (1–5).

There is also increasing evidence that links EGFR with resistance to chemotherapeutic drugs and radiation. Suggestions for a causal relationship are the observations that transfection of EGFR into human breast cancer cells increased the resistance of these cells to cytotoxic drugs (6) and that the blockade of the EGFR-mediated signaling pathway with antibodies to EGFR enhanced the sensitivity of tumor cells to a number of chemotherapeutic agents (1, 7–9). Recent studies have shown that anti-EGFR antibodies can be highly effective in the treatment of human tumor xenografts when combined with chemotherapeutic drugs (1, 7–11).

A number of studies have shown a positive relationship between EGFR expression and cell or tumor resistance to radiation (12, 13). Sheridan et al. found that cell cultures derived from head and neck carcinoma patients expressing high levels of EGFR were more radioresistant than those expressing low levels of EGFR. Our study demonstrated that the expression of EGFR varied greatly, by 21-fold, among murine carcinomas of different histology and that the magnitude of the EGFR expression positively correlated with increased tumor radioresistance (14). Addition of EGF to cell cultures protected cells against radiation (15), whereas treatment of cell cultures with antibodies to EGFR yielded the opposite effect, i.e., sensitization to radiation (16). Huang et al. reported that treatment of cultured human head and neck carcinoma cells with C225 antibody, a mouse-human chimeric anti-EGFR mAb, enhanced the radioresponse of these cells in vitro; the enhancement was attributed to increased radiation-induced apoptosis.

Recently, C225 antibody has undergone extensive exploration of its therapeutic potential (1, 11, 18–20). In vitro studies demonstrated that the antibody can be either inhibitory for cell growth or cytotoxic (1, 20, 21) and can enhance the cytotoxic effect of chemotherapeutic drugs (1, 8, 9) or ionizing radiation (17). The antibody is also highly effective against tumor xenografts, particularly when combined with chemotherapeutic drugs (8, 9, 11). Because no information is available on the in vivo efficacy of C225 when combined with radiotherapy, the present study investigated whether C225 affects the response of human tumor xenografts to local tumor irradiation.

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4 The abbreviations used are: EGFR, epidermal growth factor receptor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; mAb, monoclonal antibody.
MATERIALS AND METHODS

Mice and Tumors. Male nude (nu/nu) mice were bred and maintained in our own specific pathogen-free mouse colony and housed five in each cage. Animals used in this study were maintained in facilities approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture and Department of Health and Human Services. Solitary tumors were produced by inoculation of $10^6$ cells into the muscle of the right hind leg of 3–4-month-old mice. Tumor cell suspensions were prepared from A431 cells grown as monolayers in vitro. Experiments were initiated when the tumors had grown to 8 mm in diameter.

C225 Monoclonal Antibody. Human mouse chimeric anti-EGFR mAb C225 was derived from murine mAb 225 clone and was provided by Dr. Harlan Warksal from ImClone Systems, Inc. (New York, NY). C225 fully retains the activity of murine mAb 225 in blocking EGF or transforming growth factor α for receptor binding and produces a similar spectrum of antitumor activity on a variety of cultured and xenografted human cell lines (22, 23). The dose of C225 used in the present experiments was 1 mg/mouse and was administered i.p. in a volume of 0.45 ml.

Tumor Irradiation. Unanesthetized mice were immobilized in a jig, and tumors were centered in a 3-cm diameter circular field. A single 18-Gy dose of gamma radiation was locally delivered using a dual-source $^{137}$Cs unit at a dose rate of 6.25 Gy/min. The tumor-bearing mice were treated with either C225 or local tumor irradiation alone, or with C225 6 h before or 6 h before and 3 and 6 days after local tumor irradiation. Untreated tumor-bearing mice served as controls.

Measures of Tumor Response. The effect of radiation alone, C225 alone, and the combination of the two treatments on tumor response was assessed by tumor growth delay. Three orthogonal tumor diameters were measured using Vernier calipers at 2–3-day intervals until the tumors grew to at least 12 mm in mean diameter. Tumor growth was measured for up to 120 days. The degree of growth delay was expressed either as the absolute or normalized growth delay. Absolute growth delay was defined as the time in days for tumors in the treatment arms to grow from 8 to 12 mm in diameter minus the time in days for the tumors in the untreated control group to reach the same size. Normalized growth delay was defined as the time for tumors in groups treated with a combined regimen to grow from 8 to 12 mm minus the time to reach the same size in mice treated with C225 alone.

Analysis of Apoptosis and Necrosis. Mice were killed by CO$_2$ inhalation at different times after treatment, and the tumors were immediately excised and placed in neutral buffered formalin. The tissues were embedded in paraffin blocks, and 4-µm sections were cut and stained with H&E. The apoptotic cells were scored on coded slides at ×400 magnification. The morphological features used to identify apoptosis in murine tumors have been previously described, illustrated, and associated with positive terminal deoxynucleotidyl transferase-mediated nick end labeling staining (24, 25). Five fields of nonnecrotic areas were selected randomly across each tumor section, and in each field, apoptotic cells were expressed as a percentage based on the scoring of 1500 nuclei at each time interval after treatment. The percentage of cells that were necrotic was determined using a Chalkey point counter with 25 random points (26). At a magnification of ×200, the number of points that fell on necrosis were counted in 20 fields distributed evenly across the area of the tumor section. Thus, the percentage of necrosis was based on scoring 500 points per section as either necrotic or nonnecrotic. The light-microscopic features used to identify necrosis included increased cell size, indistinct cell border, eosinophilic cytoplasm, loss or condensation of the nucleus, and associated inflammation.

Tumor Angiogenesis. An intradermal assay (27, 28) was used to assess the effect of C225 on tumor angiogenesis. Mice were anesthetized with Nembutal (0.06 mg/g body weight), and a triangular skin flap was constructed at the right abdominal region by making a skin incision along the midline of the abdomen and extending it to the right groin. The skin flap was separated from the s.c. tissue by a gentle pull laterally and then was examined for the area with the fewest tiny blood vessels using a dissecting microscope with a magnification of ×20. After the number of blood vessels was recorded at the tumor cell injection site, $10^6$ A431 cells were injected intradermally in a volume of 0.03 ml of PBS using a 30-gauge needle. The skin flap was then brought back to the midline and closed using surgical clips. One day after the injection of tumor cells, the mice were given C225 at an i.p. dose of 1 mg/mouse. The number of blood vessels as well as tumor size was determined at 2, 4, 6, 8, and 10 days after tumor cell injection. This was performed under a dissecting microscope (×20) in anesthetized animals in which the skin flap was reopened by removing the surgical clips and pulling the flap laterally. The tumor volume was calculated using the formula for elliptical mass ($\pi/6 \pi abc$).

RESULTS

To determine the effect of C225 on tumor radioresponse, mice bearing 8-mm-diameter A431 tumor xenografts were treated with C225, 18 Gy of local tumor irradiation, or both. C225, at a dose of 1 mg/mouse, was given i.p. either as a single injection 6 h before tumor irradiation or in three injections administered 3 days apart, the first one preceding tumor irradiation by 6 h. Tumor growth delay was the treatment end point. The results presented in Table 1 and Fig. 1 show that the combined treatment with C225 and 18 Gy delayed tumor growth more than was the additive effect of individual treatments. The growth of tumors from 8 to 12 mm in diameter was delayed by 5.5 ± 2.8 days after one dose of C225 ($P = 0.17$), by 7.4 ± 3.5 days after three doses of C225 ($P = 0.07$), by 19.3 ± 3.4 days after 18 Gy ($P < 0.01$), by 36.2 ± 3.3 days after one dose of C225 plus 18 Gy ($P < 0.01$), and by 77.3 ± 10.1 days after three injections of C225 plus 18 Gy ($P < 0.01$). In this latter group, one of seven tumors was permanently eradicated and was excluded from the analysis. Thus, treatment with C225 greatly potentiated the efficacy of local tumor irradiation, with three doses of C225 being more effective than one dose. The enhancement factors, estimates of the additional effect of C225 over and above the effect of radiation alone, were 1.59 for a single dose of C225 plus 18 Gy and 3.62 for three doses of C225 plus 18 Gy.

In this study, a single dose of 18 Gy was used because it caused significant tumor growth delay in a previous pilot study.
The dose of 1 mg of C225 per mouse was selected based on our previous studies on its antitumor efficacy (8, 29). This dose caused no mouse morbidity. A 6-h interval for C225 administration before tumor irradiation was used to allow sufficient time for the antibody to redistribute within the tumor. The half-life of the antibody in mice is 72 h (29).

It was recently reported that C225 could enhance in vitro radioresponse of head and neck carcinoma cells; this effect was attributed to an increase in cell sensitivity to radiation-induced apoptosis (17). To determine whether this mechanism was involved in the observed enhancement of A431 tumor radioresponse in vivo, apoptotic indices were determined in untreated tumors and in tumors treated with a single dose of C225, 18 Gy of local tumor irradiation, or C225 plus radiation, in which the antibody was administered 6 h before irradiation of 8-mm-diameter A431 tumors. Apoptosis and necrosis were quantified at 4 h, 1 day, 3 days, and 7 days after local tumor irradiation. The results presented in Table 2 show that the mean background value of apoptosis was less than 1% and that neither C225, radiation, nor their combination increased this value significantly. The apoptotic index in the treated groups in no case exceeded 2%. These findings suggest that the induction of apoptosis may not account for the observed tumor radioenhancement of A431 tumor in vivo.

In contrast to apoptosis, an increase in the extent of necrosis was a dominant histological feature after tumor treatment with C225 plus irradiation (Table 2). In untreated tumors of 8 mm in diameter, the percentage of necrosis was 10.4 ± 2.1%. The amount of necrosis in tumors treated with C225 alone was higher within 3 days after the antibody administration, but not significantly. It ranged from 13.3 ± 4.6% (P = 0.57) at 1 day to 26.9 ± 10.0% (P = 0.16) at 3 days after the injection of C225. However, on day 7 after the treatment with C225, the percentage of necrosis was 55.5 ± 8.3% (P = 0.04). Tumors treated with radiation only showed a progressive increase in the amount of necrosis within 7 days after irradiation: 21.7 ± 5.0% at 4 h (P = 0.11), 41.1 ± 3.5% at 1 day (P < 0.01), 64.8 ± 8.3% at 3 days (P = 0.01), and 74.8 ± 5.5% at 7 days (P < 0.01). The largest increase in the amount of necrosis resulted from the combined C225 plus radiation treatment. The percentage of necrosis was 59.8 ± 6.9% at 4 h after irradiation (P = 0.03), 57.8 ± 2.0% at 1 day (P < 0.01), 80.1 ± 6.2% at 3 days (P < 0.01), and 85.9 ± 4.0% at 7 days (P < 0.01) after irradiation. The presence of massive necrosis by 4 h after the combined treatment suggests that the treatment severely disrupted tumor vascularity very quickly. Fig. 2 illustrates that at 7 days after the combined treatment, tumor tissue remained viable only at the tumor periphery.

There were other histological changes in tumors treated with C225, particularly in combination with local tumor irradiation. C225 plus radiation significantly increased the percentage of necrosis in A431 tumors. It ranged from 13.3 ± 4.6% (P = 0.57) at 1 day to 26.9 ± 10.0% (P = 0.16) at 3 days after the injection of C225. However, on day 7 after the treatment with C225, the percentage of necrosis was 55.5 ± 8.3% (P = 0.04). Tumors treated with radiation only showed a progressive increase in the amount of necrosis within 7 days after irradiation: 21.7 ± 5.0% at 4 h (P = 0.11), 41.1 ± 3.5% at 1 day (P < 0.01), 64.8 ± 8.3% at 3 days (P = 0.01), and 74.8 ± 5.5% at 7 days (P < 0.01). The largest increase in the amount of necrosis resulted from the combined C225 plus radiation treatment. The percentage of necrosis was 59.8 ± 6.9% at 4 h after irradiation (P = 0.03), 57.8 ± 2.0% at 1 day (P < 0.01), 80.1 ± 6.2% at 3 days (P < 0.01), and 85.9 ± 4.0% at 7 days (P < 0.01) after irradiation. The presence of massive necrosis by 4 h after the combined treatment suggests that the treatment severely disrupted tumor vascularity very quickly. Fig. 2 illustrates that at 7 days after the combined treatment, tumor tissue remained viable only at the tumor periphery.

There were other histological changes in tumors treated with C225, particularly in combination with local tumor irradiation.
These include tumor infiltration with granulocytes, hemorrhage, vascular thrombosis, and increased cell differentiation. The histological appearance of untreated tumors is shown in Fig. 3A. In treated tumors, the infiltration with granulocytes, mainly neutrophils, took place primarily in perivascular areas but was present throughout the tumor tissue (Fig. 3B). Some degree of granulocyte accumulation was present in untreated tumors as well but was confined to the stromal capsule surrounding these xenografts. The occlusion of blood vessels with intravascular thrombi (Fig. 3C) was frequently associated with hemorrhage into the surrounding tumor areas. Cell differentiation was more frequent and involved larger areas than in untreated tumors (Fig. 3D). These changes increased progressively during the 7-day observation period.

Because inhibition of tumor angiogenesis was recently found to increase tumor radiosensitivity (30, 31) and because C225 was suggested to possess antiangiogenic activity (32, 33), we tested whether C225 inhibits the formation of blood vessels induced by A431 tumor cells. We used an intradermal assay, developed in our laboratory (27), for studying angiogenesis in mice. The mice were injected intradermally with 10^6 A431 cells, and the number of vessels at the injection site was determined 2, 4, 6, 8, and 10 days later. C225, at a dose of 1 mg/mouse, was given i.p. 1 day after tumor cell inoculation. Fig. 4 shows that C225 significantly reduced the number of newly formed blood vessels (P < 0.02) and that this reduction was associated with significant tumor growth retardation (P < 0.03). These P values are for the day 10 point only.

### DISCUSSION

Aberrant signal transduction pathways, including the pathway in which EGFR is involved, are being increasingly explored as promising targets in cancer therapy. One of these approaches consists of blocking EGFR by such anti-EGFR antibodies as C225 (1, 8, 9, 11).

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| Table 2 | Effect of C225, radiation, or both on apoptosis and necrosis in A431 tumor xenografts^a^ |
|----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Time after irradiation^b^ | Apoptosis (% | Necrosis (%) | Apoptosis (% | Necrosis (%) | Apoptosis (% | Necrosis (%) | Apoptosis (% | Necrosis (%) | Apoptosis (% | Necrosis (%) |
| 0 h | 0.7 ± 0.4 | 10.4 ± 2.1 | 0.8 ± 0.2 | 19.9 ± 6.2 | 2.2 ± 0.6 | 21.7 ± 5.0 | 0.9 ± 0.6 | 59.8 ± 6.9 |
| 4 h | 1.2 ± 0.4 | 13.3 ± 4.6 | 0.9 ± 0.5 | 26.9 ± 10.0 | 1.5 ± 0.3 | 64.8 ± 8.3 | 1.3 ± 0.4 | 80.1 ± 6.2 |
| 1 day | 0.5 ± 0.1 | 55.5 ± 8.3 | 0.2 ± 0.1 | 74.8 ± 5.5 | 0.3 ± 0.2 | 85.9 ± 4.0 |
| 3 days | 0.7 ± 0.2 | 20.2 ± 2.6 | 1.0 ± 0.1 | 41.1 ± 3.5 | 1.0 ± 0.4 | 57.8 ± 2.0 |
| 7 days | 0.5 ± 0.1 | 55.5 ± 8.3 | 0.2 ± 0.1 | 74.8 ± 5.5 | 0.3 ± 0.2 | 85.9 ± 4.0 |

^a^ Mice bearing 8-mm tumors in the right hind leg were given i.p. 1 mg of C225 antibody, 18 Gy of local tumor irradiation or the antibody plus 18 Gy. When the two agents were combined, C225 was given 6 h before tumor irradiation. Groups consisted of 3–7 mice each.

^b^ The times indicated are in relation to the time of local tumor irradiation. In the case of C225 treatment only, 6 h needs to be added to these times. For example, 0 h analysis for apoptosis or necrosis is actually 6 h after administration of C225.

^c^ Mean ± SE.
Fig. 3  Histological features (H&E staining) of A431 xenografts treated with C225 antibody (1 mg i.p. per mouse). Untreated tumors (A) are densely cellular, and mitotic figures can be seen (long arrow), as well as rare microareas of terminal cell differentiation and necrotic disintegration (short arrow; magnification, ×250). B, tumor infiltration with granulocytes (magnification, ×250). C, intravascular thrombosis (magnification, ×400). D, terminal differentiation (magnification, ×250).
can be cytotoxic or inhibit proliferation of tumor cells (1, 8, 9, 20–22), and it can enhance the cytotoxicity of chemotherapeutic drugs (1, 8, 9, 11) and of ionizing radiation (17). In this report, we demonstrate that C225 greatly enhanced the in vivo tumor response to radiation. The effect was greater than the sum of growth delays caused by the individual treatments.

The combined antibody-radiation treatment consisted of 18 Gy of single-dose tumor irradiation and systemic application of a single dose or multiple (three) doses of C225. Whereas a single dose of C225 on its own caused some, but not significant, growth delay of a relatively large tumor mass (8 mm in diameter), it greatly enhanced tumor radioresponse. The enhancement factor was 1.59. Treatment with C225 preceded radiation delivery by 6 h. However, the augmentation of tumor radioresponse was particularly dramatic, reaching an enhancement factor of 3.62, when three doses of C225 were combined with irradiation. One dose of C225 was given 6 h before irradiation, one 3 days after irradiation, and one 6 days after irradiation.

A preliminary assessment of potential mechanisms of interaction revealed several findings. We first considered a possibility that C225 increased susceptibility of cells to radiation-induced apoptosis. Induction of apoptosis is commonly regarded as underlying the cytotoxicity of agents that inhibit EGFR tyrosine kinase (34, 35) or block EGFR (16, 36, 37), including C225 antibodies (21). Also, the ability of anti-EGFR antibodies to enhance in vitro response of tumor cells to cytotoxic drugs (1, 8, 9, 11) or ionizing radiation (16, 17) was reported to be due to augmentation of drug- or radiation-induced apoptosis. However, our present findings (Table 2) do not support induction of apoptosis as an underlying mechanism of antitumor action of either C225, irradiation, or the combination of the two in this particular tumor. The percentage of apoptosis within 7 days after all of these treatments was not significantly increased above the background level of 1%.

Second, C225 induced a significant increase in the amount of tumor necrosis (Table 2), which progressed within the observation period of 7 days. In some tumors, necrosis was very extensive, occupying the whole tumor except for a tiny peripheral rim (illustrated in Fig. 2). Cell death by necrosis in treated tumors could have resulted from direct damage to tumor cells, indirectly through the damage of tumor vascularization, or more likely both. The involvement of the vascular effect was supported by histological features that included intravascular thrombosis, tumor hemorrhage, and massive necrosis positioned more centrally within the tumor.

Similar results were observed using DiFi colon adenocarcinoma cells (21). Exposure of DiFi cells to C225 antibody resulted in typical apoptosis in tissue culture: the antibody activated the caspase cascade, induced DNA laddering, and caused cell membrane blebbing (21). Also, treatment of nude mice bearing DiFi cell xenografts resulted in complete regression of these xenografts. However, no signs of increased apoptosis were observed in the xenograft tissue specimens. Instead, the xenografts were found to be necrotic and infiltrated with inflammatory cells. It appears that there might be differences in either induction or detection of C225-induced apoptosis between in vitro and in vivo settings, and the possibility that C225 increases tumor radioresponse in vivo by enhancing induction of apoptosis followed by necrosis cannot be ruled out. It may be that massive necrosis induced by radiation masked the apoptotic response. Whether these effects are specific to the A431 tumor xenografts is being currently explored by using other tumors expressing EGFR.

A third possibility considered was that C225 exerted antitumor effects and enhanced tumor radioresponse by interfering with tumor neovascularization. EGFR ligands, EGF, and transforming growth factor α were reported to play a role in tumor angiogenesis, directly (38) or indirectly by interaction with VEGF (39). Our present study demonstrated that C225 significantly inhibited formation of new vessels at the site of A431 tumor cell inoculation (Fig. 4). This inhibition of neangiogenesis was associated with significant delay in tumor growth. This delay could have resulted either from a direct effect of C225 on tumor angiogenesis or from inhibition of tumor cell proliferation, thus indirectly inhibiting angiogenesis. The direct inhibition effect of C225 is supported by a recent study showing that the antibody inhibited mRNA and protein production of the VEGF, interleukin 8, and basic fibroblast growth factor angiogenic factors in cultured bladder cancer cells, as well as in xenografts derived from these cells (33).

Thus, the observed C225 antitumor effect is mediated, at least in part, by inhibition of tumor angiogenesis. However, it is not clear whether this inhibition underlies the C225-induced enhancement of A431 tumor radioresponse. That this mechanism was likely involved is suggested by a number of recent reports that treatment with such antiangiogenesis agents as angiostatin and TNP-470 increases tumor radioresponse in preclinical tumor models (30, 31, 40–42). The effect was attributed primarily to the damage of endothelial cells (31, 40–42). Inter-
estingly, VEGF was recently reported to be radioprotective for endothelial cells (30). This protection was abolished by an anti-VEGF antibody, and furthermore, treatment of tumor-bearing mice with this antibody enhanced tumor radioreponse (30). Because C225 inhibits VEGF production (32, 33), it is possible that it enhanced tumor radioreponse of A431 tumors by a mechanism similar to that reported by Gorski et al. (30).

Another interesting histological feature associated with C225 treatment was tumor infiltration with granulocytes. The infiltrating cells were present throughout tumor tissue but were particularly pronounced in the perivascular region. Most likely, the granulocytic response was related to clearing dead tumor cells, but a possibility exists that these infiltrating cells were involved in tumor cell kill on their own. This possibility is suggested by a recent observation of Stockmeyer et al. (43) that granulocytes primed with the granulocyte colony-stimulating factor were cytolytic to a number of breast cancer cell lines expressing HER-2/neu in the presence of anti-HER-2/neu monoclonal antibodies. It is then also possible that heavy perivascular infiltration with granulocytes could damage vascular walls and indirectly contribute to tumor cell kill and development of the severe necrosis discussed above.

Treatment with C225 was associated with increased terminal cell differentiation. Microrregions of differentiation were seen throughout the tumor and were especially pronounced when the antibody was combined with irradiation. Terminal cell differentiation was recently reported to occur in cultured tumor cells when exposed to anti-EGF antibodies (ICR63 or ICR80) or tyrosine kinase inhibitors (37). It is also possible that C225 delayed the onset of cell repopulation in irradiated tumors and thus greatly postponed the regrowth of treated tumors. This repopulation-inhibitory effect could account for the dramatic retardation in tumor growth when C225 was administered both before and after radiation treatment.

Overall, our observations showed that C225 antibody greatly increased tumor response to local tumor irradiation. Multiple administrations of C225 were more effective than a single dose of C225 given several hours before irradiation. The study also showed that a number of mechanisms were likely involved, including inhibition of tumor angiogenesis, vascular damage, and tumor infiltration with polymorphonuclear cells. These findings suggest that anti-EGFR antibodies have high potential to improve the efficacy of radiotherapy.

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