Human glioblastoma xenografts overexpressing a tumorspecific mutant epidermal growth factor receptor factor sensitized to cisplatin by the AG1478 tyrosine kinase inhibitor

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Object. Activation of signaling by the epidermal growth factor receptor (EGFR) through gene amplification or rearrangement is common in human malignancy, especially in a large fraction of de novo glioblastomas multiforme (GBMs). The most common mutant EGFR, (ΔEGFR, also known as de2-7 EGFR and EGFRvIII) lacks a portion of the extracellular domain, enhances tumorigenicity in vivo, and causes resistance to the chemotherapeutic drug cisplatin (CDDP). This resistance is due to the suppression of CDDP-induced apoptosis by the constitutively active tyrosine kinase activity of the receptor. The authors have investigated whether inhibition of ΔEGFR signaling by the tyrosine kinase inhibitor, tyrphostin AG1478, could sensitize tumor xenografts to CDDP and, thereby, enhance its therapeutic efficacy in animals.

Methods. Nude mice were inoculated either subcutaneously or intracerebrally with human GBM cells expressing ΔEGFR and were then systemically treated with CDDP and/or AG1478. Tumor volumes were monitored and tumor sections were analyzed by using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) assays or MIB-1 staining.

Expression of ΔEGFR, but not wild-type EGFR, conferred CDDP resistance to the cells in vivo. Inhibition of receptor signaling by the EGFR-specific tyrosine kinase inhibitor, AG1478, sensitized the xenografts to the cytotoxic effects of CDDP. This combined CDDP/AG1478 treatment significantly suppressed growth of subcutaneous xenografts in nude mice in a synergistic manner (p < 0.01 compared with vehicle control) without causing generalized toxicity, whereas treatments with CDDP or AG1478 alone were ineffective. The synergistic growth suppression by the CDDP/AG1478 combination was not observed in xenografts overexpressing wild-type EGFR or kinase-deficient ΔEGFR. The combined CDDP/AG1478 treatment induced tumor growth suppression, which correlated with increased apoptosis and reduced proliferation. This treatment also extended the life span of mice bearing intracerebral xenografts (p < 0.01 compared with controls).

Conclusions. The results of this study may provide the basis for the development of a novel and safe therapeutic strategy for the very aggressive ΔEGFR-expressing GBM.

Key Words • glioblastoma multiforme • epidermal growth factor receptor • mutant receptor • drug resistance • tyrosine kinase inhibitor • combined drug therapy

Malignant gliomas, the most frequently occurring form of primary brain tumors, remain intractable and the prognosis for patients with the most malignant glioma, GBM, is similar to that of patients with metastatic brain tumors. Malignant gliomas often fail to respond to adjuvant radio- and chemotherapy, and complete surgical resection is difficult, illustrating the need for improved treatment. Initiation and malignant progression of gliomas involves the accumulation of genetic alterations that cause activation of oncogenes, including EGFR, CDK4, CDK6, and MDM2 genes, or inactivation of tumor suppressor genes such as TP53, CDKN2A, RB, and PTEN. Alterations of such genes, which appear to represent components of signal transduction, cell cycle regulatory or apoptotic pathways, bestow a growth advantage on tumor cells, and chemotherapeutic drug resistance may arise as a consequence of such genetic variations.

Amplification and rearrangement of the EGFR gene is common in GBMs and often results in a truncation of the extracellular domain of the receptor due to a genomic deletion of exons 2 through 7, which generates a mutant receptor called ΔEGFR (also known as de2-7 EGFR, or EGFRvIII). Several clinical and histopathological studies have shown that the presence of EGFR amplifi-
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cation correlates with a shorter interval to disease relapse and lower rates of survival in patients receiving adjuvant therapies. This specific rearrangement has also been described in lung, breast, and prostate cancers. Overexpression of ΔEGFR in GBM cells has been shown to enhance tumorigenicity and increase cell proliferation, and reduce cell death. This receptor also confers resistance to CDDP and other cytotoxic drugs through modulation of Bcl-X<sub>L</sub> expression and the consequent inhibition of apoptosis induction. These effects were attributed to the ligand-independent, constitutive activation of ΔEGFR tyrosine kinase because overexpression of a kinase-deficient ΔEGFR or of the wtEGFR had no such effects. These observations suggest that, although expression of and constitutive signaling from ΔEGFR grant a growth advantage to aggressive GBMs, it may also provide a potential target for their treatment.

The tyrosine kinase inhibitor, tyrphostin AG1478, competes with adenosine triphosphate and has been reported to be selectively inhibitory toward EGFR compared with PDGF, P210<sub>Bcr-Abl</sub>, or HER2-Neu. It has also been shown to effect a greater inhibition of ΔEGFR than wtEGFR. It effectively decreases ΔEGFR autophosphorylation, thereby causing a downregulation of Bcl-X<sub>L</sub> expression and, thus, synergistically enhancing the cytotoxic effect of CDDP in vitro. This suggested an approach to reverse the ΔEGFR-mediated drug-resistant phenotype by pharmacological interference with receptor signaling. In the present report we demonstrate that the expression of ΔEGFR did confer drug resistance in vivo to human tumor xenografts. Combined treatment with CDDP and AG1478 significantly suppressed the growth in a synergistic manner and without generalized toxicity of human GBM xenografts expressing ΔEGFR in mice. These results suggest a potential therapeutic strategy for the most aggressive type of GBM expressing ΔEGFR that combines conventional chemotherapy with a directed tyrosine kinase inhibitor.

**Materials and Methods**

**Cell Lines**

The human GBM cell line U87MG, which expresses low levels of wtEGFR, its sublines U87MG-ΔEGFR, U87MG-DK, and U87MG-wtEGFR, which overexpress ΔEGFR, a kinase-deficient mutant of ΔEGFR, and exogenous wtEGFR, respectively, were described previously. All cells were cultured in a manner previously described.

**In Vivo Treatments**

Human GBM cells were suspended in 0.1 ml PBS and injected subcutaneously into the right flank of 4- to 5-week-old BALB/c female nude mice. For treatment of established xenografts, the tumors were permitted to establish and grow until tumor volumes reached 50 to 80 mm<sup>3</sup>. For intracranial stereotactic inoculations, 5 × 10<sup>5</sup> cells in 5 μl of PBS were implanted in the right corpus striatum of the mouse brain in a manner previously described. For combined drug treatment experiments, either CDDP (3 mg/kg; that is, 100 μl of 600 μg/ml CDDP for a 20-g mouse) or sterile normal saline (100 μl) was administered intraperitoneally every other day during treatment. Mice were also treated with either AG1478<sup>29</sup> (400 μg) in 20 μl DMSO or with 20 μl DMSO daily beginning 2 days before initiation of the CDDP treatment. The growth of tumors was measured as described in a previous publication. Systemic toxicity due to drug treatments was assessed by changes in body weight and by organ inspection at autopsy. All treatment protocols were approved by the animal care and use committee at the University of California, San Diego.

**Apoptosis Assay**

In vivo TUNEL assays for detection of apoptotic cells in xenografts were performed according to a protocol previously described. The apoptotic index was calculated as the ratio of apoptotic cell number to total tumor cell number by examination of 10 hpf. More than 1000 tumor cells were examined and scored in areas of the tumor judged to be most representative.

**Proliferation Assay**

Subcutaneous tumors in nude mice were resected, fixed in 10% formalin, and embedded in paraffin. Thin sections were deparaffinized, rehydrated, and baked in a microwave oven in citrate buffer (0.01 M sodium citrate, pH 6) for antigen retrieval. Sections were then blocked by soaking them in a blocking buffer (2% bovine serum albumin, and 0.1% saponin in PBS) and were incubated with anti–human MIB-1 antibody (1:100 dilution in the blocking buffer) at room temperature overnight. After the sections were rinsed in PBS, goat anti–mouse immunoglobulin G labeled with Alexa fluor 488 probe (1:2000 dilution) was applied for 1 hour at room temperature. The tissue sections were rinsed in PBS, counterstained with Hoechst 33258, and mounted for analysis by fluorescence microscopy. The percentage of tumor nuclei reactive to the MIB-1 antibody was estimated following examination of 10 hpf. An average 1000 cells were examined and scored in areas of the tumor deemed the most representative.

**Western Blotting Analysis**

Whole-tumor lysates were prepared in radioimmunoprecipitation buffer containing 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and inhibitors of proteases and phosphatases as previously described. Equal amounts of protein (20 μg) samples were separated on sodium dodecyl sulfate–polyacrylamide gels, and transferred to nitrocellulose membranes. Proteins on the membranes were probed with antibodies to EGFR (C13), or phosphotyrosine (4G10), and detected by chemiluminescence.

**Statistical Analysis**

The data were analyzed for significance by using the Student t-test, except for in vivo survival assays, for which we used Cox–Mantel analysis.

**Sources of Supplies and Equipment**

The BALB/c nude mice were acquired from Simonsen Laboratories, Inc. (Gilroy, CA). The CDDP was purchased from Sigma Chemical Co. (St. Louis, MO) and the AG1478 from Calbiochem (La Jolla, CA). For the proliferation assay, we obtained anti–human MIB-1 from PharMingen (San Diego, CA) and Alexa fluor 488 from Molecular Probes (Eugene, OR). For the Western blot analysis, we purchased an antibody to phosphotyrosine, 4G10, from Upstate Biotechnology (Lake Placid, NY) and nitrocellulose membranes from BioRad (Hercules, CA). Chemiluminescence was performed using supplies provided by Amersham (Piscataway, NJ).

**Results**

In Nude Mice ΔEGFR Confers Resistance to CDDP in Human GBM Xenografts

Because ΔEGFR expression causes chemoresistance in U87MG cells in vitro as well as in another human glioma, U178MG (unpublished data), we determined whether the same phenotype was present in human xenografts in vivo. Mice bearing established subcutaneous tumor x-
nografts derived from either U87MG, U87MG.DEGFR, U87MG.DK, or U87MG.wtEGFR cells were treated every other day with CDDP, which was administered intraperitoneally, until control tumors in the mice treated with normal saline reached maximum size (approximately 2500 mm$^3$) as described in Materials and Methods. Low-dose CDDP treatment (0.3 mg/kg) was ineffective in the inhibition of growth of U87MG-derived xenografts (data not shown). Treatment with CDDP at a dose of 3 mg/kg significantly suppressed the growth of tumors derived from U87MG (p < 0.001, Student t-test), U87MG.DK (p < 0.05), and U87MG.wtEGFR (p < 0.05) cells without causing generalized toxicity, whereas it had no effect on the growth of U87MG.DEGFR–derived tumors (Fig. 1). Higher doses (5 mg/kg) of CDDP suppressed the growth of U87MG.DEGFR xenografts as well as other tumors, but also resulted in a significant loss of body weight (approximately 20% of the original body weight) in tumor-bearing mice. Although there were no deaths during the course of treatment, the dramatic drop in body weight indicated that this dose was beyond the maximum tolerated dose, and we adopted a CDDP dose of 3 mg/kg for further experiments. It was apparent that DEGFR signaling caused CDDP resistance in vivo.

**Inhibition of DEGFR Signaling by AG1478 Causes in Vivo Sensitization to CDDP**

We next determined whether the tyrosine kinase inhibitor AG1478, which has some selectivity for DEGFR in vitro, could enhance the antitumor effects of CDDP treatment on xenografts derived from U87MG.DEGFR cells in nude mice. The AG1478 treatment of U87MG. DEGFR cells in vitro had already been shown to inhibit constitutive tyrosine autophosphorylation of DEGFR in a reversible manner (unpublished data). Mice bearing established subcutaneous U87MG.DEGFR xenografts were treated systemically with either CDDP alone (3 mg/kg; every other day, for a total of five times), AG1478 alone (400 μg daily), or both as described in Materials and Methods. At a higher dose (800 μg/body) AG1478 treatment caused loss of body weight, especially when combined with CDDP, whereas at a low dose (100 μg/body) AG1478 had little effect on tumor growth (data not
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shown). In animals treated with the mock vehicle control or with either CDDP or AG1478 alone, tumor growth rates were similar to untreated controls. In contrast, combined CDDP/AG1478 treatment significantly suppressed in a synergistic manner the growth of U87MG.\(\Delta EGFR\) xenografts (animals given the combined drug treatment compared with controls: 76\% reduction in tumor volume, \(p < 0.01\); compared with CDDP alone or AG1478 alone: \(p < 0.05\) (Fig. 2A and B). Similar but slightly weaker tumor suppression was effected by using the CDDP analog carboplatin (20 mg/kg) (data not shown). The suppression of tumor growth was dose dependent for both AG1478 and CDDP in the combined drug treatment; treatment at lower doses (AG1478 100 \(\mu\)g/body or CDDP 1 mg/kg) had less tumor-suppressive effects, whereas treatment at higher doses (AG1478 800 \(\mu\)g/body or CDDP 5 mg/kg) caused systemic toxicity (data not shown). There was no significant loss in body weight or any obvious neurological sequelae in the tumor-bearing mice or in control mice without tumors, in response to any of the treatments at the doses used (Fig. 2C). Histologically, the livers, kidneys, and brains of control mice without tumors that underwent treatment displayed no obvious pathological changes, regardless of the treatment used (data not shown). Histopathological examination of treated tumors demonstrated focal vacuole formation and degenerative changes in those treated with the CDDP/AG1478 combination, but not in other treated groups (Fig. 2D). Western blot analysis revealed that activation of \(\Delta EGFR\) by tyrosine phosphorylation was detectably reduced in some tumors harvested from animals after treatment with AG1478 and CDDP, whereas expression of \(\Delta EGFR\) protein was unaltered (Fig. 2E). In contrast with U87MG.\(\Delta EGFR\) tumors, the addition of AG1478 did not enhance CDDP-induced growth suppression of xenografts derived from U87MG.wtEGFR or U87MG.DK cells, even though treatment with AG1478 alone resulted in marginal effects consistent with the original derivation of the compound as a \(\Delta EGFR\) inhibitor. This suggests that the synergistic growth inhibition induced by the AG1478/CDDP combination was selective for the mutant receptor (Fig. 3).

We also determined the effect of the combined drug treatment on tumor formation in nude mice. The mice were inoculated subcutaneously with U87MG.\(\Delta EGFR\) cells and treated concurrently with CDDP (3 mg/kg, every other day for a total of nine times) and AG1478 (400 \(\mu\)g/day, daily). Although small nodules developed 4 days after tumor cell inoculation in all treatment groups, the tumors in mice treated with the CDDP/AG1478 combination remained small (< 400 mm\(^3\)) until Day 14; tumors in the other treatment groups grew at a faster rate (Fig. 4). Tumor formation and growth were significantly suppressed only in the combined drug treatment group (34.9\% of tumor volume in vehicle control group, \(p < 0.001\)). These results suggest that the combination of tyrophostin and CDDP suppresses the initial and final phases of growth of U87MG.\(\Delta EGFR\) tumors in vivo.

**Increased Apoptosis and Reduced Proliferation of Tumors in Response to Combined Drug Treatment**

To determine the underlying mechanisms of tumor-growth suppression by the combined CDDP/AG1478 treatment, we next examined the rates of apoptosis and proliferation in treated tumors. The combined drug treatment increased apoptosis in established xenografts, as demonstrated by TUNEL assays (threefold more than the vehicle control; \(p < 0.001\)) (Fig. 5A). The combined drug treatment also resulted in a significant reduction in the proliferation rate, as determined by MIB-1 staining (\(p < 0.05\)) (Fig. 5B).

**Survival of Mice Bearing Intracranial Xenografts is Extended by Combined Drug Treatment**

We examined the potency of the combined CDDP/AG1478 treatment for intracerebral xenografts. Mice were inoculated using stereotactic guidance in the brain with U87MG.\(\Delta EGFR\) cells and were then treated systemically with five injections of CDDP and daily AG1478 doses beginning from Day 2 after inoculation. The combined CDDP/AG1478 treatment significantly extended the survival of mice bearing intracerebral xenografts, when compared with either no treatment, treatment with vehicle, or treatment with either CDDP or AG1478 alone (\(p < 0.01\), Cox–Mantel analysis) (Table 1).

**Discussion**

Evidence is accumulating that \(\Delta EGFR\) may account for several of the poor characteristics of GBMs. It enhances the tumorigenicity of glioma cells in vitro and in vivo.\(^{3,12,20,27}\) It also causes drug resistance and, thus, could be an obstacle to adjuvant chemotherapy.\(^{23}\) Correspondingly, expression of \(\Delta EGFR\) in GBMs correlates with a poor prognosis in patients.\(^{8}\) In the present studies, we reasoned that inhibition of this tumor-specific mutant receptor could provide a novel therapeutic approach. We found that a \(\Delta EGFR\) inhibitor combined with a conventional chemotherapeutic agent significantly suppressed growth rates of subcutaneous human GBM xenografts expressing \(\Delta EGFR\) and extended the survival of animals bearing such intracranial tumors without causing generalized toxicity.

The constitutively active tyrosine kinase activity of \(\Delta EGFR\) is essential for the augmentation of mutant receptor-mediated tumor growth,\(^{13}\) which is effected through enhancing proliferation and reducing apoptosis.\(^{22}\) Previously we showed that AG1478 selectively inhibits tyrosine autophosphorylation of \(\Delta EGFR\) and results in reduced DNA synthesis and proliferation rates in GBM cells in vitro.\(^{11}\) Indeed, in some tumors harvested from animals after treatment with AG1478 and CDDP, reduced \(\Delta EGFR\) phosphorylation was observed in vivo as well, strongly suggesting that it is this inhibition that is the mechanism of tumor suppression by the combined treatment. Correspondingly, AG1478 exerted only minimal antitumor effects when combined with CDDP in tumors derived from U87MG cells overexpressing wtEGFR or kinase-deficient \(\Delta EGFR\), suggesting that the AG1478 effect has greater efficacy against the constitutively active kinase of \(\Delta EGFR\) in vivo as well.

Tumor growth and angiogenesis are regulated to a large extent by growth factors, receptor tyrosine kinases, and their signaling pathways. Small molecule inhibitors, which target such receptors or components of the signal-
Fig. 2. Synergistic antitumor effect of combined CDDP/AG1478 treatment on U87MG.ΔEGFR xenografts in vivo. Nude mice (four per group) were injected subcutaneously with \(5 \times 10^6\) U87MG.ΔEGFR cells and were then given intraperitoneal injections of either CDDP (3 mg/kg) or normal saline every other day, for a total of five times; (arrow in B) from Day 4 (tumor volume approximately 50 mm\(^3\)) in combination with daily administration of either AG1478 (400 μg/day) or DMSO. The experiments were repeated independently two times with similar results. A: Photographs obtained on postimplantation Day 17 showing mice bearing U87MG.ΔEGFR xenografts after treatment. B: Graph depicting growth curves of xenografts separated by treatment group. Grids designate the untreated control group; squares, the vehicle-treated (normal saline and DMSO) control group; diamonds, the CDDP (3 mg/kg)-treated group; circles, the AG1478 (400 μg/day)-treated group; and triangles, the CDDP/AG1478–treated group. **p < 0.01, combined treatment group compared with no treatment (control group); p < 0.05, combined treatment group compared with CDDP- or AG1478–treatment group. Data are shown as means ± SEMs. C: Bar graph demonstrating changes in body weight in nude mice after the course of treatments compared with body weight before treatments. Data are shown as means ± SEMs. D: Photomicrographs showing histological changes in U87MG.ΔEGFR xenografts after treatments. (1) No treatment control, (2) vehicle control, (3) CDDP alone, (4) AG1478 alone, or (5) combination of CDDP and AG1478. H & E, original magnification \(\times 100\). E: Expression and tyrosine phosphorylation of ΔEGFR in U87MG.ΔEGFR xenografts after treatments. Tumors were harvested after the treatment course and total cellular lysates were subjected to Western blot analysis followed by exposure to antiphosphotyrosine and anti-EGFR antibodies. Representative results are shown.
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**Fig. 3.** Graphs showing that combined CDDP/AG1478 treatment shows no synergism for xenografts derived from U87MG overexpressing wtEGFR or kinase-deficient EGFR. Nude mice (six per group for U87MG.wtEGFR and five per group for U87MG.DK) were injected subcutaneously with 1.5 × 10^6 U87MG.wtEGFR (A) or U87MG.DK (B) cells and treated by intraperitoneal administration of CDDP (3 mg/kg every other day, for a total of five times; arrows) and AG1478 (400 µg/day, daily; open rectangle). Data are shown as means ± SEMs. See Fig. 2 for definitions of symbols.

...ing pathways, are potential therapeutic agents for malignancies and other diseases. For example, the synthetic protein tyrosine kinase inhibitors, AG490, AG1295, and SU5416 potently and selectively inhibit Jak-2, platelet-derived growth factor receptor tyrosine kinase and the Flk-1 vascular EGFR, respectively. Some of these compounds are currently being investigated in clinical trials. The tyrohisin AG1478 has some specificity for inhibiting the tyrosine kinase activity of ΔEGFR but could not induce apoptosis in vitro or inhibit growth of tumors expressing ΔEGFR in vivo alone (Fig. 2). Previously, we showed that exposure to AG1478 in combination with the chemotherapeutic agent CDDP produced synergistic apoptotic effects in vitro. Here, we have demonstrated that this combined drug treatment effectively suppresses the growth of ΔEGFR-expressing tumors and prolongs the survival of tumor-bearing animals in vivo.

Although the combined drug treatment was effective, providing a preclinical proof of principle for the approach, it should be noted that the tumors eventually grew and durable remissions were not achieved. This may have resulted from a rapid degradation or inactivation of AG1478 in the animals, because its half-life in the mouse bloodstream is less than an hour (unpublished data). The blood–brain barrier, which may reduce CDDP and AG1478 penetration, could also account for incomplete growth inhibition of the intracranial tumors. Studies in which radioisotope labeling is used are now underway to evaluate the pharmacokinetics of AG1478 in vivo and to detect the level of its penetration through the blood–brain barrier (E Nice, et al., personal communication, 2000). Efforts at continuous administration of AG1478 by using osmotic pumps or biodegradable wafers may further refine drug delivery. Finally, other molecules with greater potency or specificity for kinase inhibition can also be developed to enhance therapeutic efficacy.

Our results indicate that ΔEGFR may represent a useful tumor-specific target for treatment. Because ΔEGFR is a mutant transmembrane receptor molecule, it could also serve as a target for immunotherapeutic approaches. A receptor tyrosine kinase that is frequently overexpressed on the cell surface of tumors from breast and lung, HER-2 has been a target for the human anti–HER-2 antibody, Herceptin, which has been demonstrated to enhance the antitumor activity of Taxol and doxorubicin in breast cancer xenografts and in clinical trials. Several monoclonal antibodies raised against the ΔEGFR are currently being evaluated for their efficacy in anti–ΔEGFR tumor treatment. One such antibody, L8A4, has been shown to bind specifically to ΔEGFR on the cell surface and, subsequently, to be effectively internalized, thereby having potential use for specific immunolabeling or delivery of

**Fig. 4.** Synergistic suppression of tumor formation by combined CDDP/AG1478 treatment in vivo. Nude mice (five per treatment group except the CDDP/AG1478-treated group in which there were six animals) were injected subcutaneously with 1.5 × 10^6 U87MG.ΔEGFR cells and treated with CDDP (3 mg/kg, every other day; arrows) and AG1478 (400 µg/day, daily; open rectangle) beginning on the same day. Data are shown as means ± SEMs. ***p < 0.001 (combined CDDP/AG1478-treated group compared with control group). See Fig. 2 for definitions of symbols.
FIG. 5. Bar graphs demonstrating that combined CDDP/AG1478 treatment results in an increased apoptosis rate and a reduction of proliferation rate in tumor cells of the U87MG.ΔEGFR xenografts. Nude mice with subcutaneous U87MG xenografts were treated with vehicle, CDDP alone, AG1478 alone, or a combination of CDDP and AG1478, as described in Fig. 2. A: Paraffin-embedded tumor sections were subjected to TUNEL assay as described in Materials and Methods. Both TUNEL-positive cells and Hoechst 33258–stained nuclei were counted in 10 hpf (magnification ×800) per tumor. Data represent a ratio of TUNEL-positivity in treated tumors to that in vehicle-treated control tumors, in which the average TUNEL positivity in control tumors is assigned a score of 100% (mean ± SD). ***p < 0.001. B: Paraffin-embedded tumor sections were stained with MIB-1 antibody together with Hoechst 33258 as described in Materials and Methods. The MIB-1–positive cells and the Hoechst 33258–stained nuclei were counted in 10 hpf (magnification ×800) per tumor. The data represent a ratio of MIB-1 positivity in treated tumors to that in vehicle-treated control tumors, in which the average MIB-1 positivity in control tumors is assigned a score of 100% (mean ± SD). *p < 0.05. The experiments were repeated independently two times with similar results.

conjugated toxins. Indeed, the ΔEGFR-specific antibody Y10 was shown to inhibit ΔERFR-expressing tumors effectively in animals and to prolong their survival. A peptide encompassing the fusion junction of the mutant receptor has also been used as an antitumor vaccine in animals. Thus, there are now several approaches to exploit this receptor for therapy.

Conclusions

The results of the present study demonstrate that a small molecule that inhibits the tyrosine kinase activity of ΔEGFR also inhibits the growth of human GBM cells expressing ΔEGFR in vivo when combined with apoptosis-inducing chemotherapeutic agents. These results provide a basis for the development of combined treatments for intractable malignant gliomas, especially those of the de novo type, as well as subsets of lung, breast, and prostate cancers that have been reported to express ΔEGFR.

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