The Protein Tyrosine Phosphatase TCPTP Suppresses the Tumorigenicity of Glioblastoma Cells Expressing a Mutant Epidermal Growth Factor Receptor*

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Glioblastoma multiforme (GBM) is the most aggressive type of glioma and GBMs frequently contain amplifications or mutations of the EGFR gene. The most common mutation results in a truncated receptor tyrosine kinase known as ΔEGFR that signals constitutively and promotes GBM growth. Here, we report that the 45-kDa variant of the protein tyrosine phosphatase TCPTP (TC45) can recognize ΔEGFR as a cellular substrate. TC45 dephosphorylated ΔEGFR in U87MG glioblastoma cells and inhibited mitogen-activated protein kinase (MAPK) ERK2 and phosphatidylinositol 3-kinase (PI3K) signaling. In contrast, the substrate-trapping TC45-D182A mutant, which is capable of forming stable complexes with TC45 substrates, suppressed the activation of ERK2 but not phosphatidylinositol 3-kinase. TC45 inhibited the proliferation and anchorage-independent growth of ΔEGFR cells but TC45-D182A only inhibited cellular proliferation. Notably, neither TC45 nor TC45-D182A inhibited the proliferation of U87MG cells that did not express ΔEGFR. ΔEGFR activity was necessary for the activation of ERK2, and pharmacological inhibition of ERK2 inhibited the proliferation of ΔEGFR-expressing U87MG cells. Expression of either TC45 or TC45-D182A also suppressed the growth of ΔEGFR-expressing U87MG cells in vivo and prolonged the survival of mice implanted intracerebrally with these tumor cells. These results indicate that TC45 can inhibit the ΔEGFR-mediated activation of ERK2 and suppress the tumorigenicity of ΔEGFR-expressing glioblastoma cells in vivo.
cancer. We have generated stable TC45-producing cell lines in ΔEGFR-expressing U87MG glioblastoma cells to examine whether TC45 can suppress ΔEGFR-mediated growth and tumorigenicity. TC45 dephosphorylated ΔEGFR to inhibit the ΔEGFR-mediated proliferation of glioblastoma cells and the growth of tumor xenografts in nude mice. Although TC45 inhibited the activation of both PI3K/Akt and ERK2, our studies indicate that the suppression of ERK2 may be sufficient to inhibit the proliferation of ΔEGFR-expressing U87MG cells in vitro and in vivo. Our studies highlight the importance of the MAPK ERK2 in ΔEGFR-mediated proliferation and shed light on the ΔEGFR-mediated signaling cascades that promote GBM growth.

EXPERIMENTAL PROCEDURES

Materials—P.D.98059 and monoclonal anti-EGFR Ab-1 were purchased from Calbiochem Oncogene Research Products (Cambridge, MA), AG1478 from Sigma, polyclonal anti-EGFR used for immunoblotting from Santa Cruz Biotechnology (Santa Cruz, CA), monoclonal PI3K pS5 antibody from BD Transduction Laboratories (San Diego, CA), and monoclonal phospho-ERK2, polyclonal phospho-Akt (Ser-473), and Akt antibodies from New England BioLabs (Beverly, MA). ERK2 1B3B9 antibody was provided by M. Weber (University of Virginia) and the monoclonal phospho-EGFR G104 and TCPTP CF4 antibodies by N. K. Tonks (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Plasmid Constructs—The TC45 and TC45-D182A cDNAs were excised from the expression vector pCG. To generate the TC45 and TC45-D182A pWZL-Hygro constructs, the TC45 and TC45-D182A cDNAs were cloned into the HindIII site immediately 3′ to the termination codons. The 5′ oligonucleotides incorporated a SpeI site immediately 5′ to the initiating codons and a BamHI site immediately 3′ to the termination codons. The 5′ oligonucleotide used was 5′-GGTCCCCAATAGTATTGCCACCACTTGGAG-GACCCCATGGCCTCGAATGCCCACCACCATCGAG-3′. The 3′ oligonucleotide was 5′-CCATCGTCATTA-CTATGCTTCGCTACTTTGCGCTT-3′. SpeIBamHI-digested PCR products were cloned into the XbaIBamHI site of the mammalian expression vector pC7. To generate the TC45 and TC45-D182A pWZL-Hygro constructs, the TC45 and TC45-D182A cDNAs were excised with SalI and EcoRI from the respective pBluescript constructs and cloned into the same sites of the retroviral expression vector pWZL-Hygro. To create the ΔEGFR-pCDNA3.1 construct, the ΔEGFR cDNA was excised by SalI and EcoRI digestion from the ΔEGFR-pCDNA3.1 construct. The ΔEGFR cDNA was blunt-ended, digested with XhoI and cloned into the EcoRV and XhoI sites of pCDNA3.1 (Invitrogen, San Diego, CA). The structures of the recombinant plasmids generated were confirmed by restriction endonuclease analysis and the fidelity of the cloned cDNAs confirmed by sequencing.

Cell Culture, Electroporations, and Flow Cytometry—293 cells (ATCC) and human U87MG glioblastoma cells (ATCC) were cultured at 37 °C and 5% CO2 in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. ΔEGFR-expressing U87MG glioblastoma cells (ΔEGFR-U87MG) have been described previously (9–11) and were cultured in the same medium plus 200 μg/ml G418 (Life Technologies, Inc.). Where indicated, cells were serum-starved for 24 h in Dulbecco’s modified Eagle’s medium containing 0.1% fetal bovine serum. 293 cells were electroporated as described previously (14) with 1 μg of ΔEGFR-pCDNA3.1 and 20 μg of either pCG, TC45-pCG, or TC45-D182A-pCG and processed at 24 h post-transfection. Fluorescence-activated cell sorting analysis was performed using propidium iodide (Sigma)-stained cells.

Generation of Cell Lines—pWZL(Hygro) vector control, TC45, and TC45-D182A encoding retroviruses were generated as described previously (19). Briefly, the BING amphotropic virus-packaging cell line was electroporated with pWZL(Hygro) vector control, TC45-pWZL(Hygro), or TC45-D182A-pWZL(Hygro) DNA. Virus-containing supernatants were harvested and added to cultures of U87MG or ΔEGFR-U87MG cells. Drug selection was in medium containing 100 μg/ml hygromycin B (Life Technologies, Inc.) for U87MG cells and in the same medium plus 200 μg/ml G418 (Life Technologies, Inc.) for the ΔEGFR-U87MG cells. Drug-resistant colonies were selected 10–16 days after infection.

Cell Proliferation, Soft Agar, Tumorigenicity, and Survival Studies—Cellular proliferation was measured using the MTT assay as described by the manufacturer (Roche Diagnostics, Mannheim, Germany), and soft agar assays were performed as described previously (20). After 3–4 weeks, colonies in soft agar were stained with crystal violet and photographed. For tumorigenicity and survival studies, TC45 (clones 8 and 16 pooled) or TC45-D182A (clones 11 and 14 pooled) ΔEGFR-U87MG cells (5×103) were inoculated into the brains of 4–5-week-old female BALB/c nude mice as described previously (10). For tumorigenicity studies the brains were resected at 14 days post-implantation, and frozen sections were stained with hematoxylin.

Immunoprecipitations—Cells were collected in lysis buffer (50 mM Tris (pH 7.5), 1% (w/v) Triton X-100, 200 mM NaCl, 50 mM NaF, leupeptin (5 μg/ml), pepstatin (1 μg/ml), 1 mM benzamidine, 2 mM phenylmethanesulfonyl fluoride) and processed as described previously (15). TCPTP was immunoprecipitated with CF4 antibody in the presence of 5 mM iodoacetic acid, whereas ΔEGFR was immunoprecipitated from serum-starved cells with anti-EGFR Ab-1 in the presence of 2 mM sodium orthovanadate.

RESULTS

ΔEGFR Is a Cellular Substrate of TC45—All protein tyrosine phosphatases contain an Asp residue that is essential for catalysis and mutation of this residue (Asp-182 in TC45) to alanine generates mutants that can form stable complexes with tyrosine phosphorylated substrates (15, 21). To determine whether TC45 can also recognize ΔEGFR as a cellular substrate, we transiently co-expressed ΔEGFR with either wild type TC45 or the D182A mutant in 293 cells. We assessed the ability of TC45 to dephosphorylate ΔEGFR and whether TC45-D182A was capable of forming a stable complex with the tyrosine-phosphorylated ΔEGFR. As seen in lysates of these co-transfected cells, TC45 dephosphorylated ΔEGFR, whereas TC45-D182A protected ΔEGFR from dephosphorylation by endogenous phosphatases (Fig. 1). Moreover, the tyrosine phosphorylated ΔEGFR could be co-immunoprecipitated with the TC45-D182A mutant but not with wild type TC45 (Fig. 1). These results indicate that the TC45-D182A-trapping mutant and the tyrosine-phosphorylated ΔEGFR receptor is a substrate for TC45 (15). To determine whether TC45 can also recognize ΔEGFR as a cellular substrate, we transiently co-expressed ΔEGFR with either wild type TC45 or the D182A mutant in 293 cells. We assessed the ability of TC45 to dephosphorylate ΔEGFR and whether TC45-D182A was capable of forming a stable complex with the tyrosine-phosphorylated ΔEGFR. As seen in lysates of these co-transfected cells, TC45 dephosphorylated ΔEGFR, whereas TC45-D182A protected ΔEGFR from dephosphorylation by endogenous phosphatases (Fig. 1). Moreover, the tyrosine phosphorylated ΔEGFR could be co-immunoprecipitated with the TC45-D182A mutant but not with wild type TC45 (Fig. 1). These results indicate that the TC45-D182A-trapping mutant and the tyrosine-phosphorylated ΔEGFR interact through the active site of the phosphatase. Similar results were also obtained when ΔEGFR and TC45 were co-expressed in U251MG glioblastoma cells (data not shown). These results are consistent with ΔEGFR being a direct substrate of TC45, as we have demonstrated previously for the wild type EGFR receptor (14, 15).

Effect of TC45 on ΔEGFR-mediated Proliferation—To assess the ability of TC45 to regulate ΔEGFR signaling and function, we stably expressed TC45 or the TC45-D182A mutant in either U87MG human glioblastoma cells or in U87MG cells that had been generated previously to stably express ΔEGFR (ΔEGFR-U87MG) to levels similar to those of GBMs (10, 11). To increase the gene delivery efficiency and to attain stable gene integration, vector control amphotropic retroviruses or those expressing TC45 or TC45-D182A were used to infect U87MG or...
∆EGFR-U87MG cells. After approximately 2 weeks of selection in hygromycin B, very few colonies were observed in the TC45 or TC45-D182A retrovirus-infected ∆EGFR-U87MG cells relative to vector control (Fig. 2A). In contrast, no significant difference was observed for the vector control, TC45, or TC45-D182A infected U87MG cells (Fig. 2A). The infection of the U87MG and ∆EGFR-U87MG cells was undertaken at the same time, with the same retroviral preparations, and similar levels of TC45 and TC45-D182A were expressed in the pooled colonies (data not shown). These results indicate that TC45 and TC45-D182A may inhibit the proliferation of ∆EGFR-U87MG cells.

To further analyze the effects of TC45 and TC45-D182A on cellular proliferation, at least three hygromycin B-resistant clones from vector control, TC45, or TC45-D182A U87MG and ∆EGFR-U87MG populations were isolated and characterized. U87MG and ∆EGFR-U87MG cells express relatively high amounts of the endogenous 48-kDa TCPTP variant but low levels of endogenous TC45 (Fig. 2B). The U87MG and ∆EGFR-U87MG stable clones overexpressed similar amounts (∼5-fold) of either TC45 or TC45-D182A (Fig. 2B). Indeed, the level of TC45 overexpression was similar to the amount of endogenous 48-kDa TCPTP in these cells (Fig. 2B) and was similar to the endogenous levels of TC45 in other cell types such as HepG2 hepatoma cells (data not shown). The results presented hereon are from one clone for each of the vector control, TC45, or TC45-D182A U87MG, or ∆EGFR-U87MG cell lines. Similar results were obtained for at least two other clones for each of the cell lines generated. We found that expression of either TC45 or the TC45-D182A substrate-trapping mutant significantly inhibited the proliferation of ∆EGFR-U87MG cells as compared with parental or vector control cells (Fig. 2C). In contrast, neither TC45 nor TC45-D182A had any significant effect on the proliferation of U87MG cells not expressing ∆EGFR (Fig. 2C). These results are consistent with the observations made after retroviral infection (Fig. 2A). Moreover, consistent with an effect on cellular proliferation we found that the expression of either TC45 or TC45-D182A altered markedly the cell cycle progression of the ∆EGFR-U87MG cells (Fig. 3). Expression of either TC45 or TC45-D182A in ∆EGFR-U87MG cells led to a reduction of S and G2/M cell populations and a concomitant increase in G1/G0 populations as compared with parental or vector control cells (Fig. 3). The results indicate that the expression of either TC45 or TC45-D182A leads to a delay in G1/S cell cycle progression. Previous studies have shown that the pharmacological inhibition of ∆EGFR can suppress the proliferation of ∆EGFR-U87MG cells (22). In this study we find that the treatment of ∆EGFR-U87MG cells with AG1478, a pharmacological inhibitor of ∆EGFR, also results in decreased S and G2/M and increased G1/G0 cell populations (Fig. 3). As such, TC45 and TC45-D182A may exert their selective effects on the proliferation of ∆EGFR-U87MG cells by acting specifically on ∆EGFR or ∆EGFR-induced signaling cascades to cause a delay in G1/S progression.

Effect of TC45 on ∆EGFR-mediated Signaling—We examined the tyrosine phosphorylation status of ∆EGFR in the lysates of TC45 and TC45-D182A ∆EGFR-U87MG stable cell lines. Consistent with our co-transfection experiments in 293 cells (Fig. 1), we found that TC45 almost completely dephosphorylated ∆EGFR in the ∆EGFR-U87MG cells (Fig. 4A). Similar results were obtained when cells were lysed directly in Laemmli sample buffer (to prevent any post-lysis dephosphorylation) (data not shown) indicating that the dephosphorylation was occurring in a cellular context. In contrast, the TC45-D182A substrate-trapping mutant formed a complex with the tyrosine-phosphorylated ∆EGFR and protected it from dephosphorylation (Fig. 4A), as we found in the 293 cells (Fig. 1) and as we have reported previously for the wild type EGF receptor (14, 15). These results indicate that ∆EGFR is a substrate for TC45 and that the wild type and mutant phosphatases have the potential to regulate cellular proliferation via the modulation of ∆EGFR signaling.
Previously we have shown that transiently expressed TC45 and TC45-D182A act on endogenous wild type EGFR receptor in COS1 cells to inhibit the recruitment of the PI3K p85 regulatory subunit and consequent activation of PI3K and downstream Akt (14). The wild type TC45 dephosphorylates the EGFR receptor to inhibit recruitment, whereas TC45-D182A forms a complex with the tyrosine-phosphorylated EGFR receptor and inhibits recruitment in a competitive manner (14). We assessed the status of PI3K recruitment and downstream activation of Akt in the TC45 and TC45-D182A ΔEGFR-U87MG stable cell lines. Effects on PI3K recruitment were assessed by monitoring for the presence of p85 in ΔEGFR immunoprecipitates. We found that TC45 inhibited almost completely the association of p85 with ΔEGFR, whereas the TC45-D182A mutant had no significant effect on p85 recruitment (Fig. 4B). The activation of protein kinase Akt was then monitored in cell lysates using antibodies specific for Akt phosphorylated on Ser-473 (P-Akt). P-Akt immunoblots were stripped and reprobed with antibodies specific for Akt.

Nevertheless, the ability of TC45 to inhibit the ΔEGFR-mediated activation of PI3K/Akt is not likely to account for its effects on the proliferation of ΔEGFR-U87MG cells because both TC45 and TC45-D182A inhibited proliferation (Fig. 2), but only TC45 inhibited PI3K/Akt (Fig. 4). The MAPK pathway is central to the proliferation of cells downstream of growth factor receptors, cytokine receptors, and integrins (23, 24), and in many tumor cells, the activation of the MAPK ERK2 is essential for cellular proliferation (25). We examined whether ΔEGFR-mediated ERK2 activation was necessary for the proliferation of ΔEGFR-U87MG cells (Fig. 5). First, we found that pharmacological inhibition of ΔEGFR with AG1478 ablated ΔEGFR PTK activity and consequent autophosphorylation and diminished the activation of ERK2 (Fig. 5A). These results indicate that the activation of ERK2 in ΔEGFR-U87MG cells is dependent on ΔEGFR activity. Second, we found that pharmacological inhibition of ERK2 with the MEK inhibitor PD98059 inhibited significantly cellular proliferation (Fig. 5B). These results demonstrate that the ΔEGFR-mediated activation of ERK2 is necessary for the proliferation of ΔEGFR-U87MG cells (Fig. 5C). We determined whether the wild type and mutant phosphatases exerted their effects on ΔEGFR-U87MG proliferation by inhibiting the ΔEGFR-mediated activation of ERK2. We assessed the activation status of ERK2 in lysates of TC45- and TC45-D182A-expressing ΔEGFR-U87MG cells using both ERK2 phosphorylation specific antibodies and by monitoring the shift in ERK2 electrophoretic mobility that occurs upon phosphorylation and activation. We found that both TC45 and TC45-D182A inhibited significantly the activation of ERK2 (Fig. 4C). These results indicate that TC45 and TC45-D182A may act on ΔEGFR to inhibit the downstream activation of ERK2 and the consequent proliferation of ΔEGFR-U87MG cells.

Effect of TC45 on Anchorage-independent Growth—We next determined the effects of TC45 and TC45-D182A on the anchorage-independent growth of ΔEGFR-U87MG cells by ascertaining the ability of our stable cell lines to form colonies in soft agar (Fig. 6). Although no significant difference was observed between ΔEGFR-U87MG parental and pWZL vector control cells (data not shown), the expression of TC45 inhibited completely the formation of colonies in soft agar (Fig. 6). However, TC45 had no effect on the formation of colonies by U87MG parental cells (data not shown). These results indicate that TC45 and TC45-D182A may act on ΔEGFR to inhibit the downstream activation of ERK2 and the consequent proliferation of ΔEGFR-U87MG cells.
inhibits the anchorage independence of ΔEGFR-U87MG cells and correlates with the differential effects of TC45 and TC45-D182A on the ΔEGFR-mediated activation of PI3K. As such, these results suggest that the ΔEGFR-mediated activation of PI3K may be necessary for the anchorage-independent growth of ΔEGFR-U87MG cells.

Effect of TC45 on Tumorigenicity and the Survival of Mice—ΔEGFR enhances the tumorigenicity of U87MG glioblastoma cells in vivo and ΔEGFR PTK activity, autophosphorylation, and signaling are required for this growth advantage (10, 11). Our studies indicate that the MAPK ERK2 and PI3K may be required differentially for the proliferation and anchorage independence of ΔEGFR-expressing U87MG glioblastoma cells in vitro. To examine the relative importance of these pathways in vivo, we assessed the tumorigenicity of our TC45- and TC45-D182A-expressing ΔEGFR-U87MG cell lines. The glioblastoma cells were injected stereotactically into the brains of nude mice. We found that the expression of either TC45 or TC45-D182A in the ΔEGFR-U87MG cells prolonged similarly the survival of mice implanted with these tumor cells as compared with parental or vector control ΔEGFR cells. Consistent with these results we found that TC45- and TC45-D182A-expressing tumors were smaller than those generated by either ΔEGFR-U87MG parental or vector control cells (Fig. 7). These results indicate that TC45 has the potential to inhibit the growth of ΔEGFR-expressing glioblastomas in vivo.

DISCUSSION

Despite the fact that the ΔEGFR oncogene is expressed in roughly half of all de novo GBMs, little is known about the signal transduction processes that ΔEGFR utilizes to increase cellular proliferation and enhance tumor growth (1). Constitutive ΔEGFR PTK activity and tyrosine phosphorylation-dependent signaling are necessary for the ΔEGFR-mediated growth of glioblastoma cells in vivo (10, 11). As such, the dephosphorylation of ΔEGFR by tyrosine-specific phosphatases would negate the contribution of ΔEGFR to gliomagenesis. In this study we have demonstrated that TC45 dephosphorylates and suppresses the tumorigenic potential of ΔEGFR in glioblastoma cells.

ΔEGFR autophosphorylates on at least five tyrosines in the intracellular C-terminal portion of the receptor (10, 11). As for many other receptor PTKs, including the wild type EGF receptor, these phosphotyrosines act as docking sites for the recruitment of Src homology 2 and phosphotyrosine binding domain-containing proteins for the activation of downstream signaling cascades (26). Whereas the wild type TC45 dephosphorylated ΔEGFR in human glioblastoma cells, the TC45-D182A mutant formed a complex with the tyrosine-phosphorylated receptor. We found that both TC45 and TC45-D182A inhibited the activation of ERK2 in ΔEGFR-expressing U87MG cells, but neither had any effect on the activation of ERK2 in U87MG cells not expressing ΔEGFR. ΔEGFR activity was necessary for the activation of ERK2, and this was essential for the proliferation of ΔEGFR-U87MG cells. These results indicate that TC45 and the D182A mutant may exert selective effects on ERK2 because of their specific recognition of ΔEGFR as a cellular substrate. Previous studies have shown that in COS1 cells, TC45 does not inhibit the EGF-induced activation of ERK2 (14). This apparent discrepancy may occur because different cell types were used for the respective studies. Alternatively, these differences may result from the wild type EGF receptor and ΔEGFR utilizing different mechanisms for the activation of ERK2. The manner by which ERK2 is activated may depend on the extent of EGF receptor activation (27), and although constitutive, the level of ΔEGFR autophosphorylation is significantly lower than that observed for EGF-stimulated wild type receptor (9, 28). Nevertheless, consistent with their effects on ERK2, we found that TC45 and TC45-D182A suppressed significantly the proliferation of ΔEGFR-expressing U87MG glioblastoma cells and caused a delay in G1/S cell cycle progression. Previous studies have demonstrated that ΔEGFR activity is necessary for the proliferation of ΔEGFR-U87MG cells in vitro (22). We report that the pharmacological inhibition of ΔEGFR causes a delay in G1/S cell cycle progression similar to the effects of TC45 and TC45-D182A expression. Previous studies have also shown that the expression of ΔEGFR in U87MG cells enhances Ras activation, which is upstream of the MAPK ERK2, and G1/S progression as measured by BrdUrd incorporation (11). As such ΔEGFR would appear to mediate the progression of cells through G1/S via the activation of ERK2. Thus, we propose that TC45 may inhibit selectively the G1/S progression and proliferation of ΔEGFR-expressing U87MG cells by dephosphorylating and maintaining the ΔEGFR in an inactive state and suppressing the downstream activation of ERK2. However, we cannot exclude the possibility that at least part of the suppression of the ΔEGFR-mediated activation of ERK2 may occur downstream of ΔEGFR. We have shown previously that TC45 can recognize the adaptor protein p50Shc as a cellular substrate (15). In ΔEGFR-U87MG cells the ΔEGFR protein associates constitutively with the adaptor proteins Shc and Grb2, which can allow for the recruitment of Ras and the

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**Fig. 6.** TC45 suppresses the anchorage-independent growth of ΔEGFR-U87MG cells pWZL vector control, TC45, and TC45-D182A (TC45D). ΔEGFR-U87MG cells were plated in triplicate in soft agar and incubated for up to 4 weeks. The colonies were stained with crystal violet, counted, and photographed. Shown is a representative experiment of three independent experiments.

**Fig. 7.** TC45 suppresses the tumorigenicity of ΔEGFR-U87MG cells. ΔEGFR-U87MG cells and pWZL control, TC45, and TC45-D182A (TC45D) ΔEGFR-U87MG cells were inoculated stereotactically into the brains of nude mice. Mouse survival was assessed, or brains were resected at 14 days post-implantation and analyzed by hematoxylin staining.
activation of MAPK signaling cascades (11). We have yet to determine whether p52

Sacred activation of ERK2 or whether p52

Sacred may serve as a substrate for TC45 in the ΔEGFR-U87MG cells.

The PI3K/Akt signal transduction pathway has been implicated in many receptor PTK-mediated processes including the regulation of cellular proliferation, migration and survival (29, 30). We found that in addition to inhibiting ERK2 activation, TC45 also inhibited the recruitment of the p85 regulatory subunit of PI3K and the downstream activation of the protein kinase Akt in ΔEGFR-U87MG cells. As in the case of wild type EGFR receptor signaling (14–16), we propose that TC45 acts upstream of PI3K on ΔEGFR to inhibit PI3K recruitment and the concomitant activation of PI3K and Akt. In contrast, the TC45-D182A substrate-trapping mutant had no effect on the recruitment of PI3K or the activation of Akt. That the D182A mutant was unable to inhibit PI3K recruitment can most likely be attributed to substrate trapping by TC45-D182A being less efficient than dephosphorylation. Since TC45 and TC45-D182A suppressed equally the proliferation of ΔEGFR-U87MG cells, these results suggest that the proliferation of ΔEGFR-U87MG cells may be independent of ΔEGFR-mediated PI3K/Akt signaling.

Although ΔEGFR-mediated PI3K/Akt signaling may not be essential for proliferation, our studies indicate that it may be required for anchorage-independent growth in soft agar. We found that expression of TC45 inhibited completely the formation of ΔEGFR-U87MG colonies in soft agar. In contrast to TC45, we found that the D182A mutant had no effect on the number of colonies formed by the ΔEGFR-expressing cells. Given that TC45-D182A had no effect on PI3K/Akt activation, these results suggest that the inhibition of PI3K/Akt may be responsible for the dramatic effect of TC45 on anchorage-independent growth. Consistent with this conclusion, others have shown that PI3K signaling is necessary for the anchorage-independent growth of glioblastoma cells (20) and that ΔEGFR-mediated PI3K signaling in NIH3T3 cells is necessary for anchorage independence (7). However, it is important to note that the colonies formed by the TC45-D182A-expressing cells were smaller than those of vector control ΔEGFR-U87MG cells, indicating that ERK2 signaling and cellular proliferation may also contribute to the growth of cells in soft agar.

Our studies indicate that TC45 and TC45-D182A differentially regulate ΔEGFR-mediated signaling processes in glioblastoma cells. Whereas TC45 inhibited both ERK2 activation and PI3K/Akt signaling, the TC45-D182A mutant suppressed only ERK2. Ultimately, the real contribution of a signaling pathway to the tumorigenic potential of ΔEGFR can only be assessed in vivo. We found that in intracerebral xenografts of our stable ΔEGFR-U87MG cell lines, TC45 and TC45-D182A suppressed similarly the growth of tumors and prolonged similarly the survival of mice. Taken together with our in vitro studies, these results indicate that the ΔEGFR-mediated activation of ERK2 and the consequent cellular proliferation contribute to the growth of glioblastomas in vivo.

Holland et al. (31) have shown recently that constitutively active Ras, and hence the activation of MAPK signaling cases, together with constitutively active Akt can induce, in mice, high grade gliomas with features of GBMs. Although our studies do not preclude a role for the PI3K/Akt pathway in tumorigenesis, we certainly indicate that the inhibition of ΔEGFR-mediated PI3K, in addition to ERK2, does not add considerably to the suppression of tumorigenesis. As such, our findings suggest that the inhibition of ERK2 may be sufficient to impede the growth of ΔEGFR-expressing GBMs, and rational GBM therapies may include inhibitors of ERK2. Moreover, agents that stimulate the expression, activation, or constitutive cytoplasmic localization of TC45 may also provide an alternative strategy for the treatment of ΔEGFR-expressing GBMs.

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REFERENCES

1. Holland, E. C. (2001) Nat. Rev. Genet. 2, 120–129

2. Maher, E. A., Furnari, F. B., Bachoo, R. M., Rowitch, D. H., Louis, D. N., Cavenee, W. K., and DePinho, R. A. (2001) Genes Dev. 15, 1311–1333

3. Prenzel, N., Fischer, O. M., Streit, S., Hart, S., and Ullrich, A. (2001) Endocr. Relat. Cancer 8, 11–31

4. Wikström, C. J., McLendon, R. E., Friedman, A. H., and Bigner, D. D. (1997) Cancer Res. 57, 4330–4411

5. Wikström, C. J., Hale, I. P., Batra, S. K., Hill, M. L., Humphrey, P. A., Kurpad, S. N., McLendon, R. E., Moschetta, D., Pegram, C. N., Reist, C. J., Travek, S. T., Wong, A. J., Zalatsky, M. R., and Bigner, D. D. (1999) Cancer Res. 59, 3140–3148

6. Olapade-Olola, E. O., Moschetta, D. K., MacKay, E. H., Horsburgh, T., Sandkuh, D. P., Terry, T. B., Wong, A. J., and Habib, F. K. (2000) Br. J. Cancer 82, 186–194

7. Moscatello, D. K., Holgado-Madruga, M., Emlet, D. R., Montgomery, R. B., and Wong, A. J. (1998) J. Biol. Chem. 273, 200–206

8. Lee, S. H., Kim, M. S., Kwon, H. C., Park, I. C., Park, M. J., Lee, C. T., Kim, Y. W., Kim, C. M., and Hong, S. I. (2000) Int. J. Mol. Med. 6, 559–563

9. Nishikawa, R., Xi, D. X., Harmon, R. C., Lazar, C. S., Gill, G. N., Cavenee, W. K., and Huang, H. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 91, 7727–7731

10. Huang, H. S., Nagane, M., Klingbeil, C. K., Lin, H., Nishikawa, R., Xi, D. X., Huang, C. M., Gill, G. N., Wiley, H. S., and Cavenee, W. K. (1997) J. Biol. Chem. 272, 2927–2935

11. Prigent, S. A., Nagane, M., Lin, H., Huvark, I., Boss, G. R., Feramisco, J. R., Cavenee, W. K., and Huang, H. S. (1996) J. Biol. Chem. 271, 25639–25645

12. Nagane, M., Coufal, F., Lin, H., Bogler, O., Cavenee, W. K., and Huang, H. J. (1996) Cancer Res. 56, 5079–5086

13. Ibarra-Sanchez, M., Simoncic, P. D., Nestel, F. R., Duplay, P., Lapp, W. S., and James, C. D. (1994) Genes Dev. 8, 1311

14. Tiganis, T., Kemp, B. E., and Tonks, N. K. (1999) J. Biol. Chem. 274, 27768–27775

15. Tiganis, T., Nep, B. E., and Tonks, N. K. (1999) J. Biol. Chem. 274, 21548–21557

16. Garton, A. J., and Tonks, N. K. (1999) J. Biol. Chem. 274, 3859–3865

17. Tiganis, T., Flint, A. J., Adam, S. A., and Tonks, N. K. (1997) J. Biol. Chem. 272, 21548–21557

18. Tanaka, M., and Herr, W. (1990) Cell 60, 375–386

19. Garton, A. J., and Tonks, N. K. (1999) J. Biol. Chem. 274, 3861–3868

20. Li, D. M., and Sun, H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 15406–15411

21. Flint, A. J., Tiganis, T., Barford, D., and Tonks, N. K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1680–1685

22. Han, Y., Caday, C. G., Nanda, A., Cavenee, W. K., and Huang, H. J. (1996) Cancer Res. 56, 5079–5086

23. Widmann, C., Gibson, S., Jarpe, M. B., and Johnson, G. L. (1999) Physiol. Rev. 79, 143–189

24. Robinson, M. J., and Cobb, M. H. (1997) Curr. Opin. Cell Biol. 9, 180–186

25. Hosotani, T., Tamimura, S., Watanabe, K., Katoaka, T., and Kohno, M. (2001) J. Biol. Chem. 276, 2686–2692

26. Schlessinger, J. (2000) Cell 103, 211–225

27. Wennaström, S., and Downward, J. (1999) Mol. Cell. Biol. 19, 4279–4288

28. Ekstrand, A. J., Longo, M., Hamid, M. L., Olson, J. J., Liu, L., Collins, V. P., and James, C. D. (1994) Oncogene 9, 2313–2320

29. Blume-Jensen, P., and Hunter, T. (2001) Nature 411, 355–365

30. Rameh, L. E., and Cantley, L. C. (1999) J. Biol. Chem. 274, 8347–8350

31. Holland, E. C., Celestino, J., Dai, C., Schaefer, L., Sawaya, R. E., and Fuller, G. N. (2000) Nat. Genet. 25, 55–57
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