Insulin-like Growth Factor Receptor I Mediates Resistance to Anti-Epidermal Growth Factor Receptor Therapy in Primary Human Glioblastoma Cells through Continued Activation of Phosphoinositide 3-Kinase Signaling

Arnab Chakravarti, Jay S. Loeffler, and Nicholas J. Dyson

ABSTRACT

Overexpression of the epidermal growth factor receptor (EGFR) has been shown previously to correlate with enhanced malignant potential of many human tumor types, including glioblastoma multiforme (GBM). Anti-EGFR targeting has been demonstrated to enhance apoptosis and reduce both cellular invasion and angiogenic potential. It remains unclear whether absolute EGFR expression levels are sufficient to predict which tumors will respond best to anti-EGFR therapy. We have identified two primary GBM cell lines with equivalent EGFR expression levels with very different sensitivities to the EGFR receptor tyrosine kinase inhibitor, AG1478. This was apparent despite similar reductions in EGFR signaling in both cell lines, as measured by phospho-EGFR levels. AG1478 enhanced both spontaneous and radiation-induced apoptosis and reduced invasive potential in the GBM\textsubscript{C}, but not in the GBM\textsubscript{R}, cell line. The resistant GBM\textsubscript{R} cell line demonstrated an up-regulation of insulin-like growth factor receptor 1 (IGF-I) levels on AG1478 administration. This resulted in sustained signaling through the phosphoinositide 3-kinase pathway, resulting in potent antiapoptotic and proinvasive effects. Cotargeting IGF-I with EGFR greatly enhanced both spontaneous and radiation-induced apoptosis of the GBM\textsubscript{R} cells and reduced their invasive potential. Akt1 and p70\textsuperscript{S6} appeared to be important downstream targets of IGF-I-mediated resistance to anti-EGFR targeting. These findings suggest that IGF-I signaling through phosphoinositide 3-kinase may represent a novel and potentially important mechanism of resistance to anti-EGFR therapy.

INTRODUCTION

The EGFR\textsuperscript{1} is a type I RTK commonly overexpressed in a wide variety of human cancers. The EGFR family members consist of four known family members (ErB1, ErB2, ErB3, and ErB4), which regulate a number of important downstream signaling pathways (1–4). The receptor consists of three major domains: an extracellular ligand-binding domain, a transmembrane lipophilic segment, and a cytoplasmic protein tyrosine kinase domain (1–5). On binding of its known family members (ErB1, ErB2, ErB3, and ErB4), which regulate a number of important downstream signaling pathways (1–4). The receptor consists of three major domains: an extracellular ligand-binding domain, a transmembrane lipophilic segment, and a cytoplasmic protein tyrosine kinase domain (1–5). On binding of its known family members, the receptor undergoes dimerization, which activates the intrinsic protein tyrosine kinase via dimerization-induced apoptosis of the GBM\textsubscript{C}, but not in the GBM\textsubscript{R}, cell line. The resistant GBM\textsubscript{R} cell line demonstrated an up-regulation of insulin-like growth factor receptor 1 (IGF-I) levels on AG1478 administration. This resulted in sustained signaling through the phosphoinositide 3-kinase pathway, resulting in potent antiapoptotic and proinvasive effects. Cotargeting IGF-I with EGFR greatly enhanced both spontaneous and radiation-induced apoptosis of the GBM\textsubscript{R} cells and reduced their invasive potential. Akt1 and p70\textsuperscript{S6} appeared to be important downstream targets of IGF-I-mediated resistance to anti-EGFR targeting. These findings suggest that IGF-I signaling through phosphoinositide 3-kinase may represent a novel and potentially important mechanism of resistance to anti-EGFR therapy.

Received 8/27/01; accepted 11/1/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by Grant K08CA21613, the Massachusetts General Hospital Brian Silber Memorial Fund (both to A. C.), and Grant RO1CA64402 (to N. J. D.), all from the NIH/National Cancer Institute.

2 To whom requests for reprints should be addressed, at Massachusetts General Hospital, Laboratory of Molecular Oncology, 13th Street, Building 149, Room 7330, Charlestown, MA 02129. Fax: (617) 726-5637; E-mail: achakravarti@partners.org.

3 The abbreviations used are: EGFR, epidermal growth factor receptor; RTK, receptor tyrosine kinase; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; PI3-K, phosphoinositide-3 kinase; IGF-I, insulin-like growth factor receptor I; GBM, glioblastoma multiforme; IGF-I, insulin-like growth factor I; MEK, mitogen-activated protein/extracellular signal-regulated kinase pathway; TBST, Tris buffer solution with 0.2% Triton; mTOR, mammalian target of rapamycin.

MATERIALS AND METHODS

Cell Culture. The two cell lines used in this study were established in culture from glioblastoma specimens using techniques described in detail elsewhere (37). Briefly, the GBM\textsubscript{C} and GBM\textsubscript{R} were obtained during open resection, mechanically dissociated, and the dispersed cells and fragments were cultured and passaged as described (37). The selective EGFR tyrosine kinase inhibitor, AG1478, and the IGF-I tyrosine kinase inhibitor, AG1024, were purchased from Calbiochem. AG1478 is reported to have an IC\textsubscript{50} > 100 \textmu M versus her-2 and platelet-derived growth factor receptor kinase (Calbiochem product data sheet). Likewise, AG1024 demonstrates lower IC\textsubscript{50} values for IGF-I than insulin RTK activity. AG1478 and AG1024 doses of 20 \textmu M were both found to diminish EGFR and IGF-I tyrosine kinase activities by >95%, respec-
IGFR-I Inhibitors, PD98059, were purchased from Cell Signaling Technology. Doses of 50 μM PD98059 and LY294002 were the lowest doses that resulted in >95% reduction of phospho-MAPK and phospho-Akt levels under normal culture conditions and, therefore, were used. Higher doses did not appear to have additional effect in this regard. Antisense Akt (sequence = AGC1TCTTTC-CGAGTGC) was provided as a generous gift of Dr. Brett Monia, Sigma Pharmaceuticals (ISIS 28935), as was the scrambled sequence (ISIS 29848).

Western Blot Analysis. Lysates were generated by placing these cells in RIPA lysis buffer. Bradford assays were performed to determine total protein concentrations, which were normalized to 1 μg/ml for all samples. Samples were then incubated in sample buffer and heated to 95°C for 5 min. The samples were run on 12% polyacrylamide gels for total MAPK (p44/p42) and phospho-EGFR (p44/p42) and on 10% gels for total Akt, phospho-Akt, p70s6k, and phospho p70s6k. Immunoprecipitation/Western analysis for phospho-IGFR-I was performed as described previously (38). Protein lysates (15 μl) in sample buffer from each tissue were loaded within each well. Gels were run at constant current (40 mA) for 3–4 h for maximum separation. Western transfer was performed for 4 h at constant voltage (40 V) using polyvinylidene difluoride membrane presoaked in methanol. The membrane was then blocked for 1 h in 5% milk in 0.2% TBST. The membranes were then washed in 0.2% TBST x 3 for 15 min each. The membranes were then incubated overnight with primary antibodies directed against either phospho-EGFR, total MAPK (p44/p42), phospho-MAPK (p44/p42); total Akt; phospho-Akt, Akt 1, 2, or 3 isoforms; p70s6k, or phospho-p70s6k. Subsequently, the membranes were washed in 0.2% TBST x 3 for 15 min each. The membrane was then incubated with secondary antibody (antirabbit) for 45 min. Chemiluminescent (Bio-Rad) detection was then used to detect phospho-MAPK (p44/p42) and phospho-Akt expression. The expression levels of both were quantitated using densitometry. The primary antibodies directed against phospho-EGFR, total MAPK (p44/p42), phospho-MAPK (p44/p42), total Akt, phospho-Akt, p70s6k, and phospho p70s6k were purchased from Cell Signaling Technology. Primary antibodies directed against Akt 1, 2, and 3 isoforms were purchased from Santa Cruz Biotechnology.

Apoptosis Assay. The cells were examined for their ability to undergo both spontaneous apoptosis and apoptosis in response to radiation +/- selective pharmacological inhibitors, including: AG1478 (selective EGFR tyrosine kinase inhibitor), AG1024 (selective IGFR-I inhibitor), PD98059 (selective MEK1 inhibitor), LY294002 (selective PI3-K inhibitor), rapamycin (p70s6k inhibitor), and antisense Akt1(ISIS®). For the radiation response assay, the cells were irradiated to a single dose of 8 Gy +/- above irradiators, then apoptosis was assessed at various time points from 2 to 24 h after treatment to assess peak apoptotic index. Controls in each case corresponded to untreated cells. Apoptosis was assayed by detection of membrane externalization of phosphatidylserine with Annexin V-FITC conjugate (Caltag). To assess peak apoptotic index, cells were harvested at various intervals after treatment and resuspended in PBS solution. Both adherent and floating cells were harvested for the apoptosis assay. The cells were then suspended in 1–2 ml of FITC-Annexin V solution. Propidium iodide was added to a final concentration of 1 μg/ml. This was analyzed by flow cytometry using blue light excitation, and green fluorescence of FITC was measured at 530 +/- 20, and red fluorescence was measured at >600.

Invasion Chamber Assay. Invasion assays were performed using the Matrigel® (Becton Dickinson) basement membrane chamber assay. Before use, the inserts were rehydrated for 1.5–2 h with 0.5 ml of warm DMEM containing 0.1% BSA. Rehydrated chambers (containing Matrigel® Matrix) were placed into the wells of a 24-well companion plate. Each well contained 0.5 ml of media with 5 x 10⁴ cells plated. The chambers were incubated for 24 h at 37°C in a 5% CO₂ atmosphere. After 48 h, the chambers were scrubbed with a cotton swab to remove the matrix. Cells on the undersurface of the membrane were fixed and stained in solution containing methylene blue dissolved in 70% ethanol and counted on examination by light microscopy. Control chambers included untreated cells. The remaining chambers contained cells treated by specific pharmacological inhibitors. The percentage of invasive cells was calculated by comparing the number of invasive cells after pharmacological inhibition divided by the number of invasive cells in the control chambers (untreated cells).

RESULTS

Primary GBM Cell Lines Can Have Different Responses to EGFR Blockade, Despite Equivalent EGFR Expression Levels. In a survey of primary GBM cell lines, we observed that two lines (referred to as GBM_S and GBM_R in this study) demonstrated equivalent expression levels of total EGFR and phospho-EGFR, as well as similar deficiencies in the p53/pRB pathway (phospho-EGFR levels shown in Fig. 1A). However, on administration of the potent EGFR tyrosine kinase inhibitor, AG1478, these two cell lines demonstrated significant differences in both spontaneous and radiation-induced apoptosis, as well as a change in invasive potential (Fig. 2, A–E). In the GBM_S cell line, there was significant enhancement of both spontaneous and treatment-induced apoptosis after AG1478 administration. In contrast, in the GBM_R cell line, there was no apparent enhancement of either spontaneous or radiation-induced apoptosis after AG1478 administration. The percentage of invasive cells likewise decreased more significantly in the GBM_R versus the GBM_S cell line after AG1478. To verify activity of AG1478 against the EGFR tyrosine kinase, levels of phospho-EGFR were measured after AG1478 treatment. It was evident that in both cell lines, the decrease in phospho-EGFR levels after AG1478 was equivalent (Fig. 1A). Therefore, the differences in antitumor effect on EGFR blockade in these two GBM cell lines did not appear to stem from lack of activity of AG1478 on the EGFR tyrosine kinase.

Differences in Downstream Signaling between GBM_S and GBM_R Cell Lines on Inhibition of EGFR. The downstream effects of EGFR signaling are known to be mediated, in a large measure, by the MAPK (p44/p42) and the PI3-K pathways. Hence, we investigated whether EGFR blockade failed to reduce activity along one or both of these pathways in the GBM_R cell line to account for their resistance...
to EGFR inhibition. For both the GBM<sub>S</sub> and GBM<sub>R</sub> cell lines, comparable reduction in phospho-MAPK levels was evident after AG1478 (Fig. 1B). This appears to suggest that resistance to the effects of EGFR blockade was not mediated through MAPK (p44/p42) signaling for the GBM<sub>R</sub> cells. Next, we examined activity along the PI3-K pathway (via phospho-Akt levels) in the two cell lines after AG1478 administration. Interestingly, in the GBM<sub>S</sub> cell line, there was a substantial decrease in phospho-Akt levels on AG1478 administration, whereas in the GBM<sub>R</sub> cell line, no such decrease was evident (Fig. 1C).

**IGFR-I Can Compensate for Loss of EGFR Signaling in GBM Cell Lines.** We explored the underlying reasons for continued PI3-K signaling in the GBM<sub>R</sub> cell line after AG1478. PTEN, a suspected down-regulator of PI3-K activation of Akt, demonstrated normal expression levels in the GBM<sub>R</sub> cell line, making its absence an unlikely cause. Next, we investigated the expression patterns of a panel of known RTKs expressed in GBM tumors that signal through PI3-K: IGFR-I, PDGFR, TrkA, and other Erb-family members. In the GBM<sub>S</sub> cell line, only expression of Erb-1 (EGFR) was found among these. However, in the GBM<sub>R</sub> cell line, coexpression of both EGFR and IGFR-I was found (Figs. 1A and 3A). Treatment of this cell line with AG1478 resulted in increased levels of both IGFR-I and phospho-IGFR-I (Fig. 3, A and B), suggesting a possible compensatory response for loss of EGFR function. To test this hypothesis, we used dual targeting of EGFR and IGFR-I to determine whether we could reduce the malignant phenotype of the GBM<sub>R</sub> cell lines. Indeed, dual targeting increased both spontaneous and radiation-induced apoptosis (Fig. 4, A and B). Furthermore, the invasive potential of these cells decreased substantially. In fact, when IGFR-I was inhibited by AG1024 without coinhibition of EGFR, there was still a significant enhancement of apoptosis and reduction of invasive potential evident, unlike that with EGFR inhibition alone (Fig. 4, C and D). This suggests that when coexpressed with EGFR, the IGFR-I pathway may provide dominant prosurvival and proinvasive signaling in GBM<sub>R</sub>. As would be expected, AG1024 failed to demonstrate any effect on the GBM<sub>S</sub> cell line, which does not express IGFR-I (Fig. 4, E–G).

We tested this hypothesis in yet another manner. The GBM<sub>R</sub> cell lines were cultured in serum-free media with increasing concentrations of exogenous EGF and IGF-I added. It was evident that IGF-I was a more potent inducer of phospo-Akt levels than EGF, whereas EGF appeared to be a stronger inducer of phospho-MAPK (p44/p42) levels (Fig. 3, C and D). Apoptosis and invasion assays were then performed. It was evident that exogenous IGF-I was a more potent suppressor of spontaneous, as well as radiation-induced, apoptosis than exogenous EGF in the GBM<sub>R</sub> cell line (Fig. 5, A and B). Furthermore, exogenous IGF-I stimulation enhanced the invasive potential of GBM<sub>R</sub> cells to a greater extent than did EGF (Fig. 5C).
IGFR-I-mediated Resistance to EGFR Blockade Is Dependent on Akt1 and p70S6K-mediated PI3-K Signaling. We next investigated whether enhanced signaling through PI3-K was directly responsible for IGFR-I-mediated resistance to EGFR blockade in the GBM R cell line. When the selective PI3-K inhibitor, LY294002, was administered to the GBM R cell line. When the selective PI3-K inhibitor, LY294002, was administered to the GBM R cell line with AG1024 and EGFR inhibition with AG1478 significantly enhances levels of both spontaneous and radiation-induced apoptosis, respectively, in the GBM R cell line ($P < 0.0001$).

In C and D, IGFR-I blockade alone with AG1024 results in enhanced spontaneous and radiation-induced apoptosis, respectively, compared with EGFR blockade alone with AG1478 ($P = 0.0002$ and $< 0.0001$, respectively). In E-G, AG1024 has no effect on either enhancing spontaneous apoptosis, radiation-induced apoptosis, or in reducing invasive potential, respectively, in GBM S cells ($P = 0.0002$ and $< 0.0001$, respectively). In B, AG1024 has no effect on either enhancing spontaneous apoptosis, radiation-induced apoptosis, or in reducing invasive potential, respectively, in GBM S cells ($P = 0.0002$ and $< 0.0001$, respectively). In E-G, AG1024 has no effect on either enhancing spontaneous apoptosis, radiation-induced apoptosis, or in reducing invasive potential, respectively, in GBM S cells ($P = 0.0002$ and $< 0.0001$, respectively). In E-G, AG1024 has no effect on either enhancing spontaneous apoptosis, radiation-induced apoptosis, or in reducing invasive potential, respectively, in GBM S cells ($P = 0.0002$ and $< 0.0001$, respectively).
**IGFR-I MEDIATES RESISTANCE TO ANTI-EGFR THERAPY**

![Image](image_url)

**Fig. 5.** Exogenous IGF-I generates both a stronger antiapoptotic and proinvasion effect than exogenous EGF in the GBM R cell line. In A, exogenous IGF-I has a significantly stronger antiapoptotic effect than exogenous EGF for the GBM R cell line ($P < 0.0001$). In B, exogenous IGF-I is a stronger suppressor of radiation-induced apoptosis than exogenous EGF for the GBM R cell line ($P < 0.0001$). Fig. 1C suggests that exogenous IGF-I produces stronger proinvasive signaling than EGF for the GBM R cell line ($P = 0.05$). Bars, 95% confidence interval.

respectively). Additionally, combined AG1024 and AG1478 reduced phospho-Akt levels in the GBM R cell line greater than that seen with AG1478 alone (data not shown) for the GBM R cell line. In contrast, the selective MEK1 inhibitor, PD98059, +/− AG1478 failed to enhance apoptosis as significantly. Reduction of invasive potential was also more pronounced with LY294002 than with PD98059 administration. This suggests, at least causally, that in the GBM R cell line, IGFR-I-mediated compensation for loss of EGFR function is mediated in a large measure through the PI3-K pathway. To more directly evaluate causality, the GBM R cell line was cultured in serum-free media with exogenous IGF-I +/− LY294002/PD98059. LY294002, unlike PD98059, was clearly able to abrogate the antiapoptotic and proinvasive effects of exogenous IGF-I (Fig. 6, A–C). This confirms in a more direct manner that IGFR-I-mediated compensation of EGFR blockade (in this case, absence of ligands that bind EGFR) is dependent more on PI3-K than MAPK signaling.

We next investigated whether Akt or p70S6K, two of the prominent known targets of PI3-K, was involved in compensatory IGFR-I-mediated prosurvival, proinvasion signaling in response to EGFR blockade. It was found that that the GBM R cell line expressed Akt1 but not Akt 2 or 3. These cells, in the presence of exogenous IGF-I, were then treated by either antisense Akt1 or rapamycin, which is a known antagonist of p70S6K, to determine which of these downstream effectors of PI3-K is most essential in mediating antiapoptotic/proinvasion response in response to IGF-I stimulation. Fig. 6D demonstrates reduced Akt1 levels in GBM R cells on administration of antisense Akt1 but not with the scrambled sequence. Both antisense Akt1 and rapamycin resulted in a reduction of the antiapoptotic effects of exogenous IGF-I to a similar degree. Both antisense Akt-1 and rapamycin suppressed IGF-I-mediated cellular invasion. However, in each case, inhibition of PI3-K by LY294002 resulted in greater enhancement of apoptosis, especially radiation-induced apoptosis, and reduction of invasive potential than combined inhibition of Akt1 and p70S6K, suggesting that IGFR-I-mediated activation of PI3-K has other contributing downstream mediators.

Akt1 and p70S6K also appear to be critical for prosurvival and proinvasive signaling in GBM R cell line, which expresses EGFR but lacks IGFR-I expression. Increasing concentrations of exogenous EGF reduced both spontaneous and treatment-induced apoptosis (Fig. 7, A and B). However, in the presence of exogenous EGF, antisense Akt1 and rapamycin both were able to: (a) partially counter these effects and release GBM R cells from EGF-mediated inhibition of both spontaneous and radiation-induced apoptosis; and (b) reduce invasive capability of these cells (Fig. 7C). However, the additive proapoptotic effects, especially after radiation, were significantly less with combined Akt1 and p70S6K antagonism than with PI3-K inhibition. This suggests that EGF-, like IGF-I-, mediated activation of PI3-K may generate additional antiapoptotic signals that are not exclusively through Akt1 and p70S6K.

**DISCUSSION**

Antagonism of the EGFR pathway holds much promise in the management of many types of human cancers, including gliomas. Most human gliomas have measurable expression of EGFR, and a subset of these have overexpression, which in most cases, is secondary to gene amplification (chromosome 7; Refs. 33 and 34). Furthermore, ~25% of all GBMs express a mutant form of EGFR, termed the vIII mutant, which lacks the extracellular binding domain and is constitutively active (34). Preliminary data suggest that established glioma cell lines (e.g., U87) are sensitive to the effects of EGFR blockade and are more prone to undergo apoptosis in response to radiation (36). The issue of whether all EGFR-expressing gliomas would be equally sensitive to the effects of EGFR antagonism regardless of expression levels has been difficult to address with the relatively limited number of available EGFR-expressing established glioma cell lines and, therefore, has been poorly understood.

Clinical data on squamous cell carcinomas of the head and neck suggest that there may, indeed, be heterogeneity in response to EGFR antagonism among EGFR-expressing tumors (19). We find that, despite having equivalent EGFR expression levels, primary glioma cell lines can have very different sensitivities to EGFR antagonism. The results described here suggest that signaling through the IGFR-I represents at least one mechanism by which tumor cells can become resistant to anti-EGFR therapy.

It is known that on binding to its substrate, IGF-I, IGFR-I activates the intrinsic tyrosine kinase, resulting in receptor autophosphorylation and the presentation of suitable binding sites for substrates containing either Src homology 2 or phosphotyrosine binding domains (39). Activation of the SHC/Grb2/SOS/Ras/raf/MEK/MAPK and IRS/PI3-
K/Akt/p70s6k pathways is a known consequence of IGFR-I-mediated signal transduction, leading to a host of effects, including: proliferation, tumorigenesis, and inhibition of apoptosis. It appears that in the GBMR cell line, IGFR-I-mediated PI3-K activation results in potent antiapoptotic and proinvasive effects.

Several studies have shown previously that the PI3-K pathway is critical in mediating neuronal cell survival, as well as the survival of other specific tumor subtypes (40–43). The downstream mediators of PI3-K function are not entirely clear but are becoming better understood. It is thought that the protein-serine/threonine kinases, 3'-phosphoinositide-dependent kinase-1 and Akt/protein kinase B, are two important mediators of PI3-K signaling (44–49).

Akt promotes cell survival in a number of different ways. It phosphorylates the proapoptotic protein BAD so that it is unable to bind and inactivate the antiapoptotic protein, Bcl-xl. Akt also serves to inhibit apoptosis by inhibiting caspase 9 and FKHLR1 (forkhead transcription factor) and by activating IkB kinase (45, 49–51). In addition, Akt has been shown to be the key mediator by which growth factor-growth factor receptor interactions affect the phosphorylation state of mTOR. It has been shown that mTOR has at least two important targets, including 4E-BP1/PHAS-1, which inhibits initiation of translation through its association with eIF-4F (26). The second target of mTOR is the kinase p70s6k (45). In a recent study, it was demonstrated that p70s6k may, in fact, be a more potent kinase for BAD than Akt in response to IGF-I stimulation and, hence, may play an important antiapoptotic role (50).

Given that p70s6k can be phosphorylated by Akt-dependent and independent mechanisms (through phosphoinositide-dependent kinase-1), we proceeded to examine the relative importance of Akt-dependent p70s6k signaling. We found that inhibition of the Akt-dependent pathway by rapamycin antagonizes the antiapoptotic, proinvasive effect of exogenous IGF-I. Although antisense Akt1 also appears to increase apoptosis in both normal astrocytes and tumor cells, antagonism of p70s6k gives this effect almost exclusively in tumor cells (data not shown), suggesting that targeting downstream effectors of Akt, such as p70s6k, may provide a greater therapeutic gain in cases where continued signaling through PI3-K generates resistance to anti-EGFR therapy.

IGFR-I-mediated activation of Akt and p70s6k also appears to play an important role in resistance to radiation-induced apoptosis in primary glioma cell lines. Additionally intriguing is the observation that direct inhibition of PI3-K signaling mediated by IGF-I and EGF (by LY294002) appears to generate a significantly stronger proapoptotic effect after radiation than combined antagonism of Akt1 and p70s6k, which suggests that PI3-K may induce other, heretofore unidentified, downstream prosurvival pathways that are Akt independent.

Our data suggest that simple measurement of absolute EGFR expression levels may be inadequate by itself in predicting which tumors...
ACKNOWLEDGMENTS

We thank Dr. Peter McL. Black, Department of Neurosurgery, Brigham and Women’s Hospital/Harvard Medical School, for kindly providing the primary glioblastoma cell lines used in this study.

REFERENCES


Insulin-like Growth Factor Receptor I Mediates Resistance to Anti-Epidermal Growth Factor Receptor Therapy in Primary Human Glioblastoma Cells through Continued Activation of Phosphoinositide 3-Kinase Signaling

Arnab Chakravarti, Jay S. Loeffler and Nicholas J. Dyson


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/1/200

Cited articles
This article cites 49 articles, 25 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/1/200.full#ref-list-1

Citing articles
This article has been cited by 85 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/1/200.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.