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EGFR-Targeted Therapy in Malignant Glioma: Novel Aspects and Mechanisms of Drug Resistance

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Abstract

Glioblastoma, GBM, is the most frequent brain malignancy in adults. Patients with these tumors survive only, approximately, one year after diagnosis and rarely survive beyond two years. This poor prognosis is, in part, due to our insufficient understanding of the complex aggressive nature of these tumors and the lack of effective therapy. In GBM, over-expression of EGFR and/or its constitutively activated variant EGFRvIII is a major characteristic and is associated with tumorigenesis and more aggressive phenotypes, such as, invasiveness and therapeutic resistance. Consequently, both have been major targets for GBM therapy, however, clinical trials of EGFR- and EGFRvIII-targeted therapies have yielded unsatisfactory results and the molecular basis for the poor results is still unclear. Thus, in this review, we will summarize results of recent clinical trials and recent advances made in the understanding of the EGFR/EGFRvIII pathways with a key focus on those associated with intrinsic resistance of GBM to EGFR-targeted therapy. For example, emerging evidence indicates an important role that PTEN plays in predicting GBM response to EGFR-targeted therapy. Aberrant Akt/mTOR pathway has been shown to contribute to the resistant phenotype. Also, several studies have reported that EGFR/EGFRvIII's cross-talk with the oncogenic transcription factorSTAT3 and receptor tyrosine kinases, (c-Met and PDGFR) potentially lead to GBM resistance to anti-EGFR therapy. Other emerging mechanisms, including one involving HMG-CoA reductase, will also be discussed in this mini-review. These recent findings have provided new insight into the highly complex and interactive nature of the EGFR pathway and generated rationales for novel combinational targeted therapies for these tumors.

Keywords

EGFR/EGFRvIII; glioblastoma; malignant glioma; drug resistance; PTEN; PI3-K/Akt/mTOR; c-Met; STAT3

INTRODUCTION

Gliomas account for approximately 80% of primary brain cancers in adults. Glioblastoma multiforme (GBM) is the most frequent type of gliomas and the most malignant form that is associated with dismal prognosis. Patients with these tumors survive only, approximately, one year after diagnosis and rarely survive beyond two years [1]. This poor prognosis is, in part, due to our insufficient understanding of the complex aggressive nature of these tumors and the lack of effective therapy. As such, vigorous efforts are ongoing to either improve

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current therapy or to identify and strike new molecular targets for GBM therapy [2]. A number of genes and pathways have emerged as attractive therapeutic targets for GBM, such as, PI3-K/mTOR, PDGFR, VEGF/angiogenesis [3-5], Hedgehog-GLI1 [6] and EGFR/EGFRvIII [5, 7-10].

Given that the genes encoding EGFR and its constitutively activated variant EGFRvIII are frequently amplified and/or over-expressed in GBMs, mono and combinational EGFR-targeted therapies have attracted much attention and are being extensively evaluated pre-clinically and clinically in GBM. Although the pre-clinical studies have shown encouraging results, clinical trials have consistently yielded limited survival benefits [10]. This poor outcome has prompted extensive investigations that aim to shed light on the complex and interactive nature of the EGFR/EGFRvIII signaling pathways and to elucidate molecular mechanisms underlying GBM resistance to EGFR-targeted therapy. This review will thus summarize recent advances made in the fundamental understanding the EGFR- and EGFRvIII-mediated signaling, outcome of clinical trials with EGFR-targeted agents in malignant gliomas, and the potential mechanisms that underlie the resistance of GBM to these therapies.

THE EGFR PATHWAY

Overexpression of EGFR and/or its constitutively activated variant EGFRvIII is frequently found in many human cancers, including GBMs, and is a hallmark for more aggressive tumors that are highly invasive and more resistant to therapy [11, 12]. EGFRvIII is a product of rearrangement with an in-frame deletion of 801 bp of the coding sequence of the extracellular domain, resulting in a deletion of residues 6 through 273 and a glycine insertion as residue 6 [13-16]. EGFR gene amplification is the most frequent genetic alteration in primary GBMs and approximately, half of these tumors carry the rearranged EGFRvIII gene [13-17]. Although EGFRvIII overexpression is mostly concurrent with EGFR gene amplification, it has been reported in a small proportion of primary GBMs without EGFR gene amplification [18]. GBM cells expressing EGFRvIII are more tumorigenic in nude mice compared to those with the wild-type EGFR [19]. Importantly, both EGFR- and EGFRvIII-mediated pathways are of a high degree of biological complexity and consisted of two major signaling modes, namely, the cell-surface and nuclear modes [11, 20, 21].

In the cell-surface signaling mode (Fig. **1a**), both EGFR and EGFRvIII function as receptor tyrosine kinases (RTKs) that activate a number of signaling modules, such as, those mediated by PLC-γ, Ras, PI3-K and Janus-activated kinase 2 (JAK2), leading to tumorigenesis and more aggressive tumor behaviors [11, 12]. With regard to signal transducer and activator of transcription 3 (STAT3), cell-surface EGFR and EGFRvIII physically associate with and phosphorylate STAT3 at Y705 and in turn, phosphorylated STAT3 dimerizes and translocates into the cell nucleus to regulate gene expression. The tumor suppressor gene, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), antagonizes the PI3-K pathway and subsequently inhibits the downstream effectors of PI3-K, Akt and mTOR. Interestingly, EGFR also exerts kinase-independent function [22]. In this context, EGFR interacts with and stabilizes sodium/glucose cotransporter 1, thereby maintaining intracellular glucose levels and preventing autophagic cell death [22]. Together, EGFR and EGFRvIII mediate a web of complex signaling networks and impact many important cellular processed.

Evidence to date indicates that cell-surface EGFR and EGFRvIII differ in their ability to activate their downstream pathways. However, the results are somewhat inconsistent and controversial. For example, Huang *et al.* [23] conducted a large-scale analysis of

phosphotyrosine-mediated signaling pathways using U87MG GBM cells stably expressing EGFRvIII and subsequently found that EGFRvIII preferentially activates PI3-K/Akt over the Ras/MAPK and STAT3 pathways. This observation corroborate the finding reported by Mellinghoff *et al.* [5] that GBMs with concurrent expression of EGFRvIII and PTEN had a better response to the EGFR kinase inhibitor erlotinib. However, Progent *et al.* [24] reported that the increased tumorigenic potential of EGFRvIII-expressing GBM, relative to those with EGFR, was associated with Ras/MAPK hyperactivation. Currently, this issue has not been resolved and is likely dependent on cellular context.

In the nuclear signaling mode (Fig. **1b**), EGFR has three key functions: (i) gene transactivation [25-28], (ii) tyrosine phosphorylation [29], and (iii) protein-protein interactions [30, 31]. EGFR ligands, oxidative stress and radiation-induced DNA damage stimulate EGFR nuclear transport [11]. Nuclear EGFR is localized on the inner nuclear membrane [32, 33] and in the nucleoplasm [27, 28, 34, 35]. The effect of cetuximab on EGFR nuclear translocalization has been investigated. Liao and Carpenter [36] showed that cetuximab activates EGFR nuclear transport. In contrast, Dittmann *et al.* [31] reported that cetuximab inhibits radiation-induced EGFR nuclear translocalization. *Via* its gene transactivation domain, nuclear EGFR activates gene expression [27]. Because of its lack of a DNA-binding domain, nuclear EGFR interacts with DNA-binding transcription factors, STAT3, E2F1 and STAT5, to induce expression of iNOS, B-Myb and aurora A genes, respectively, in breast cancer [25, 26, 28]. Nuclear EGFR retains its tyrosine kinase activity and phosphorylates proliferating cell nuclear antigen (PCNA) to promote cell proliferation [29]. Moreover, nuclear EGFR undergoes protein-protein interactions with DNA-PK to facilitate repair of radiation-induced DNA double-strand breaks in bronchial carcinoma [30, 31].

In GBMs, the nuclear EGFR and nuclear EGFRvIII pathways have been recently investigated. The report by de la Iglesia *et al.* [37] showed that EGFRvIII is detected in the nucleus of normal astrocytes and primary GBMs. While the consequence of nuclear EGFRvIII was not elucidated, nuclear EGFRvIII appears to interact with STAT3 in normal astrocytes, leading to their malignant transformation [37]. Most recently, our laboratory showed conclusive evidences for the existence of nuclear EGFR and EGFRvIII in GBM cells and its functional interaction with nuclear STAT3 to activate COX-2 gene expression, thus linking EGFR/EGFRvIII to the inflammatory pathway [38]. Nuclear translocalization of both receptors depends on nuclear localization signals located within the juxtamembrane region and when deleted, both receptors fail to enter the cell nucleus. Evidence also suggest a role that nuclear EGFR may play in gliomagenesis [38]. Collectively, the EGFR- and EGFRvIII-mediated pathways are critical for cancer biology and potentially associated with increased proliferation, invasion/metastasis, radio-resistance, and shortened patient survival. These pathways are also highly complex with a profound potential to interact with other important pathways in cancers.

PROGNOSTIC VALUE OF EGFR AND EGFRVIII IN MALIGNANT GLIOMAS

It remains inconclusive regarding the prognostic value of EGFR and EGFRvIII in malignant gliomas. Shinojima *et al.* [18] evaluated 87 newly diagnosed GBM patients and found EGFR amplification to be an independent, unfavorable predictor for overall survival. In this cohort, EGFRvIII overexpression in the presence of EGFR amplification is the strongest indicator of a poor survival prognosis. In contrast, a number of other studies [39-41] did not observe an association of EGFR amplification with survival in GBM patients. Similarly, Heimberger *et al.* [42] concluded that overexpression of EGFR and EGFRvIII are not independent predictors of overall survival in a cohort of 54 GBM patients who did not have extensive tumor resection. Analysis of 44 GBM patients by Aldape *et al.* [43] indicated that EGFRvIII

was not predictive of patient survival. It is still unknown whether nuclear EGFRvIII is a prognostic factor for GBM. In other cancer types, high levels of nuclear EGFR predict poor overall survival of patients with breast carcinomas [35], oropharyngeal squamous cell carcinomas [35, 44] and ovarian cancer [45]. Taken together, future investigations are needed to clarify the role of EGFR and EGFRvIII in prognostic prediction of patient survival in malignant gliomas. The predictive value of EGFR and EGFRvIII in EGFR-targeted therapy is discussed in the next section.

EGFR-TARGETED THERAPY IN MALIGNANT GLIOMAS

Five anti-EGFR agents have been approved by the FDA for treating cancer patients, including, three small molecule inhibitors and two antibodies. Chemical structures of the three small molecular weight EGFR inhibitors are listed in Fig. (**2**). (1) Gefitinib (ZD1839; Iressa) is a small molecular weight EGFR kinase inhibitor that has been approved for locally advanced and metastatic non-small cell lung cancer, NSCLC. (2) Erlotinib (OSI-774; Tarceva), a small molecule EGFR kinase inhibitor, was approved to treat metastatic NSCLC. It has been also approved to be used in combination with gemcitabine for pancreatic cancer that cannot be removed by surgery or has metastasized. (3) Lapatinib (GW572016; Tykerb/Tyverb) is an EGFR/Her-2-dual targeting small molecule inhibitor approved to be combined with other drugs to treat advanced or metastatic breast cancer [46]. It is used in patients whose cancer is Her-2 positive and has failed to respond to other drugs. (4) Cetuximab (C225; Erbitux) is a humanized monoclonal antibody that recognizes the extracellular domain of both EGFR [46] and EGFRvIII [47]. It has been approved for squamous cell carcinoma of the head and neck that has metastasized or recurred after other chemotherapy. It is also used with radiation therapy, as a first-time treatment for advanced squamous cell carcinoma of the head and neck. Cetuximab is also approved for treating metastatic colorectal cancer that has metastasized, after other chemotherapy has failed and for combined used with irinotecan for metastatic colorectal cancer patients who have not responded to irinotecan alone. (5) Panitumumab (ABX-EGF; Vectibix) is a human monoclonal antibody raised against the extracellular domain of EGFR [48]. It has been approved to treat colorectal cancer that has failed other therapies and has metastasized. All these agents, except panitumumab, have been evaluated in phases I & II clinical trials in patients with malignant gliomas. The clinical experiences with these EGFR-targeted therapies are summarized below and in Table **1**.

Gefitinib (ZD1839; Iressa)

A phase II clinical trial led by Rich *et al.* [49] examined the effects of gefitinib as single agent in 57 patients with recurrent GBM and found 6-month EFS to be 13%. The median EFS (event-free survival) time was 8.1 week and the median OS (overall survival) was 39.4 weeks. In this unselected cohort, EGFR expression did not correlate with EFS or OS. Another single agent phase II trial conducted by Franceschi *et al.* [50] reported that 6-month PFS (progression-free survival) was 14.3%, similar to what was reported by Rich *et al.* [49] and that there was no correlation between EGFR/p-Akt expression and PFS/OS. Gefitinib-based combination therapy has also been tested clinically in patients with malignant gliomas. Reardon *et al.* [51] conducted a phase I trial consisted of 34 patients with recurrent GBMs and AAs to examine the efficacy of combined uses of gefitinib and sirolimus (mTOR inhibitor). This study reported encouraging results with two patients (6%) achieving a partial radiographic response and 13 patients (38%) having stable disease. Gefitinib has also been combined with another mTOR inhibitor, everolimus, in a recent phase I/II trial led by Kriesl *et al.* [52]. This trial enrolled 22 GBM patients and reported disappointing results with only one patient with PSF beyond 6 months. Corroborating results from previous trials, this study

observed no correlation between EGFR status and patient response. The study did not find a predictive value for PTEN status in the patient cohort.

A phase I study by Prados *et al.* [53] compared the effects of Iressa alone and Iressa plus TMZ on 26 GBM patients and found the combinations to be generally safe and recommended phase-2 dose of gefitinib when used in combination with TMZ. Furthermore, gefitinib was evaluated in combination with fractionated stereotactic radiosurgery in a phase I clinical trial of 15 patients with recurrent GBMs and AAs [54]. Schwer *et al.* reported promising results in which 6-month PFS and 1-year OS were 63% and 40%, respectively. The median OS for the 11 GBM patients was 21 months (range, 9-33 months).

Erlotinib (OSI-774; Tarceva)

A 2004 phase II trial led by Raizer *et al.*[1] (meeting abstract) have examined the effects of erlotinib as single agent in 67 patients with recurrent GBMs. This study found erlotinib to have limited activity as a single agent. Another erlotinib single agent phase II trial[2] (meeting abstract) enrolled 58 patients with recurrent GBM and reported 6-month PSF of 17%. No correlation between EGFR status and response rates was found in this cohort. Gefitinib has also been used in combination with sirolimus in a recent Phase II trial led by Reardon *et al.* [55]. This study included 32 patients with recurrent GBM in which 47% of the patients had stable disease. The 6-month PFS was only 3.1% for all patients, but was better for patients not on EIAEDs. The observation with the negative impact of EIAEDs on gefitinib efficacy is consistent with the notions that erlotinib is a substrate of CYP3A [56] and EIAEDs induce CYP3A. These investigators also found no correlation between EGFR/PTEN and patient response, except for p-Akt that was of borderline significance.

Two clinical trials have examined the effects of combination of erlotinib with TMZ in GBM patients. A phase I trial by Prados *et al.* [57] included 83 GBM patients and reported 6-month PFS to be 10.5%. Co-administration of EIAEDs was found to have reduced exposure to erlotinib, compared to erlotinib alone (33%-71% reduction). Furthermore, Brown *et al.* [58] conducted a phase I/II trials with 97 newly diagnosed GBM patients and found the cohort to be sensitive to erlotinib, in contrast to the disappointing outcome from patients with recurrent GBMs. Furthermore, a phase II trial by van den Bent *et al.* [59] was recently completed in 110 patients with recurrent GBMs. This study has compared the effects of erlotinib alone and TMZ (or BCNU). Unfortunately, erlotinib appeared to have insufficient single-agent activity in unselected GBM as indicated by the 6-month PFS of 11.4% in the erlotinib arm, compared to 24% in the other arm with TMZ or BCNU. No clear biomarker was identified to associate with improved outcome to erlotinib. Furthermore, erlotinib was also used in combination with carboplatin to treat 43 recurrent GBM patients in a phase II study led by de Groot *et al.* [60]. Although this combination was well tolerated but only yielded modest activity in unselected patients with 6-month PFS of 14%. Similar to other reports, no correlation was observed between EGFR/Akt/PTEN expression and PFS/OS.

Krishnan *et al.* [61] examined the effects of combined use of radiotherapy and erlotinib in 19 GBM patients in a phase I trial. Median survival was 55 weeks. Most recently, Sathornsumetee *et al.*[3] (meeting abstract) completed a single-arm phase II trial to evaluate the efficacy of erlotinib plus bevacizumab (Avastin, anti-VEGF antibody) in 56 patients with recurrent GBMs and AAs. They have reported encouraging outcomes with 6-month PFS of 25% for GBMs and 50% for AAs. It is worth noting that in May 2009, bevacizumab was approved by FDA to treat GBM that have progressed. This approval was based on the promising results of two clinical trials, NCT00345163 conducted by Friedman *et al.* with 167 patients [62] and a NCI study 06-C-0064E (56 patients). Overall, responses were observed in 20-26% of patients and the median duration of response was approximately 4 months.

Lapatinib (GW572016; Tykerb/Tyverb)

Lapatinib targets both EGFR and Her-2 [46]. The phase I/II trial by Thiessen *et al.* [63] enrolled a total of 24 patients with recurrent GBM. Accrual was ceased because of the lack of phase II efficacy. Overall, lapatinib did not show significant activity in unselected GBM patients. Lapatinib plasma clearance was increased by approximately ten-fold when given with EIAEDs. Similar to other reports, EGFRvIII and PTEN co-expression did not predict a favorable response. Another phase II trial (NCT00103129) using lapatinib was recently completed in recurrent GBMs and gliosarcomas, and the results are being prepared.

Cetuximab (C225; Erbitux)

Cetuximab is a humanized monoclonal antibody that recognizes the extracellular domain of both EGFR [46] and EGFRvIII [47]. A single-arm phase I/II trial by Combs *et al.*[4] (meeting abstract) evaluated the efficacy of cetuximab+radiation therapy+TMZ combination in 17 GBM patients. The results are encouraging with 6-month PFS of 81%, 12-month PFS of 37% and 12-month OS of 87%. A stratified phase II trial based on EGFR copy number was recently completed by Neyns *et al.* [64]. In this study with cetuximab as single agent, a total of 55 GBM patients were evaluated in which 28 and 27 patients were with and without an increased EGFR copy number, respectively. However, no significant correlation was found between response, survival and EGFR copy number. Another recently completed phase II trial evaluated the efficacy of cetuximab+bevacizumab+irinotecan combination in 32 patients with recurrent GBMs[5] (meeting abstract). In this study, Lassen *et al.* found the combination to be well tolerated but have similar benefits compared to bevacizumab +irinotecan combination. Further evaluation of this regimen is not planned by the investigators.

THE EFFICIENCY OF EGFR-TARGETED AGENTS IN PENETRATING BLOOD-BRAIN BARRIER (BBB)

Brain tumor chemotherapy often encounters a major hurdle, BBB, which serves as a physical barrier against systemically administered anti-cancer agents. The information with regard to the efficiency of EGFR-targeted agents in penetrating BBB and concentrating in the GBM tumors are limited and somewhat, controversial. This can be due to the following reasons. Although pre-clinical pharmacokinetic studies provided some information using non-tumor bearing animal models, these results do not always corroborate the clinical data. In addition, only a small proportion of the clinical trials in malignant gliomas concurrently conducted pharmacokinetic studies and most of these studies collected blood samples to determine plasma clearance of the agents rather than analyzing drug concentrations in the intracerebral fluids and/or glioma tissues. Therefore, the degrees of anti-EGFR agents in penetrating BBB and accumulating in GBM tumors are often estimated from indirect evidence, such as, the extent of inhibition of EGFR phosphorylation in the tumors, disease progression and patient survival.

Gefitinib

It is a widely accepted concept that compounds with certain biochemical properties, including higher lipophilicity, are expected to penetrate BBB [65]. Since gefitinib is highly water-soluble, it was initially predicted to have a low capacity to penetrate BBB. Consistent with this notion, AstraZeneca initially reported that only very limited amount of radiolabeled [^{14}C]-gefitinib was detected in the CNS in non-tumor-bearing rats. In contrast to these predictions and results, a 2002 study by Heimberger *et al.* [66] showed that intracranial EGFR-expressing GBM xenografts responded to orally administrated gefitinib. In 2003, another study led by Cappuzzo *et al.* [67] showed in a total of four NSCLC patients that

their brain metastases completely or partially responded to gefitinib treatments. The observed penetration of gefitinib through BBB may likely be the consequences of the altered BBB integrity as a result of intracranial tumors and/or prior exposures to chemotherapy. This speculation is supported by a study conducted by Hofer *et al.* [68] showing a 10-13-fold higher gefitinib concentrations in the GBM tissues than in plasma from two patients receiving gefitinib treatments and prior first-line chemotherapy. The reasonable extent of gefitinib accumulation in GBM tissues may also be due to the fact that CYP3A, the key cytochrome P450 enzyme that metabolizes gefitinib [56], is expressed at a low level in GBM tissues [68]. Conversely, enzyme-inducing antiepileptic drugs (EIAEDs) induce CYP3A and Reardon *et al.* [51] reported that GBM exposure to gefitinib was significantly lowered by the concurrent use of EIAEDs. Therefore, EIAEDs-induced inactivation of gefitinib may contribute to the observed lack of clinical efficacy of gefitinib in inhibiting EGFR phosphorylation in GBM tumors [69].

In addition to CYP3A, gefitinib appears to be a substrate of the drug efflux protein p-glycoprotein that is highly expression in BBB[6] (meeting report) [70]. Interestingly, it has been reported that gefitinib inhibits the activity of another drug efflux protein, breast cancer resistance protein (BCRP, ABCG2), and reverses the resistance of non-glioma cancer cells to a series of anti-cancer agents, 7-ethyl-10-hydroxycamptothecin (SN-38), topotecan, and mitoxantrone [71]. Together, these findings indicate that gefitinib can penetrate BBB of GBM-carrying animals and GBM patients; however, its overall efficiency in BBB penetration and accumulation in GBM may be compromised by the concurrent use of EIAEDs that induce CYP3A and by drug efflux protein p-glycoprotein that is expressed in BBB.

Erlotinib

There is limited information with regard to the efficiency of erlotinib in crossing BBB. Sakaria *et al.* [72] reported that erlotinib sensitized intracranial GBM tumors to radiation therapy in nude mice. In the clinical setting, however, Lassman *et al.* [69] reported that erlotinib showed a very low efficiency in penetrating BBB and in inhibiting tumoric EGFR phosphorylation. This is consistent with the facts that BBB expresses high levels of drug efflux proteins, such as, p-glycoprotein, BCRP and multidrug resistance protein 2 (MRP2; ABCC2) [70, 73][6] (meeting report) and that erlotinib is a substrate of these proteins [70]. Furthermore, erlotinib is also a substrate of CYP3A [56] and this can potentially render erlotinib sensitive to EIAEDs-mediated inactivation. Corroborating this observation, Prado *et al.* [57] showed that co-administration of EIAEDs reduced the exposure of GBM to erlotinib. Another clinical trial led by van den Bent *et al.* [59] also demonstrated that the use of EIAEDs significantly increased erlotinib clearance. A recent phase I trial with erlotinib and sirolimus by Reardon *et al.* [55] reported that PFS was better for GBM patients not on EIAEDs; however, median OS did not differ significantly by EIAED status. Another GBM trial with bevacizumab plus erlotinib reported no survival difference between EIAED and non-EIAED groups[3] (meeting abstract).

Lapatinib, Cetuximab, and Panitumumab

Very little is known about the extent to which these three agents penetrate BBB. The phase I/II trial by Thiessen *et al.* [63] concluded that lapatinib did not show significant activity in GBM patients and that lapatinib clearance was significantly increased by the concurrent use of EIAEDs. Corroborating this observation, lapatinib has been shown to be a substrate of drug efflux proteins within BBB, including, p-glycoprotein and BCRP [74, 75]. For cetuximab, Eller *et al.* [76] showed that intracranially grown GBM xenografts responded to systemically administered cetuximab treatments. In this animal study, radiation therapy was observed to augment the efficacy of cetuximab. Thus far, no information is available on the

ability of panitumumab to cross BBB, despite a study [77] reported that panitumumab, in combination with AMG 102 (a HGF neutralizing antibody), was effective in targeting subcutaneous GBM xenografts.

MOLECULAR MECHANISMS UNDERLYING GLIOMA RESISTANCE TO EGFR-TARGETED THERAPY

Although the gain-of-function mutations within the EGFR kinase domain is commonly found in lung cancer [78, 79] and lead to their hyper-sensitivity to EGFR inhibitors, these somatic mutations are not present in gliomas [80]. Thus, extensive investigations are being directed at identifying other mechanisms that may account for GBM resistance to EGFR-targeted therapy. One of the key directions has been focused on the PTEN-Akt-mTOR signaling axis. The tumor suppressor gene PTEN is frequently mutated and/or deleted in GBMs and consequently, renders the PI3-K downstream effectors (Akt and mTOR) hyperactive in these tumors. Accumulating evidence suggests EGFRvIII and PTEN co-expression to be a predictor for the responsiveness of GBM to EGFR inhibitory agents [5]. However, there are also reports showing a lack of correlation between EGFRvIII-PTEN status and GBM response to the therapy, suggesting other resistance factors may also be involved. For example, several studies have provided evidence supporting the notion that inhibition of a dominant oncogene, such as EGFR/EGFRvIII, by targeted therapy can alter the hierarchy of RTKs and non-receptor TKs resulting in the activation of other TKs, such as, c-mesenchymal-epithelial transition factor (c-Met), platelet-derived growth factor receptor (PDGFR) and JAK2, in order to facilitate tumor survival [77, 81, 82]. Another potential mechanism of resistance can be derived from HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway that produces metabolites to activate EGFR signaling [83, 84]. Our laboratory has recently shown that the JAK2-STAT3 pathway is constitutively activated in the majority of GBMs and that STAT3 undergoes multi-level interactions with EGFR, leading to the resistance of GBM cells to Iressa [81]. Detailed discussions for each of above mentioned mechanisms are provided below.

PTEN-PI3-K-Akt-mTOR Signaling Axis

GBMs commonly contain mutations and deletion of the PTEN tumor suppressor gene [85]. The PTEN gene encodes a lipid phosphatase that metabolizes phosphatidylinositol 3,4,5-trisphosphate, the product of PI3-K, and thereby antagonizes PI3K-mediated signaling pathway. Consistent with this notion, loss of PTEN correlates with increased activity of PI3-K's downstream effector Akt and the substrate of Akt, mTOR (mammalian target of rapamycin) [86].

Emerging evidence indicates that PTEN expression is a molecular determinant of the response of EGFRvIII-expressing GBMs to EGFR kinase inhibitors. In the study led by Mellinghoff *et al.* [5] which contained a total of 82 patients from two institutes, recurrent malignant gliomas co-expressing EGFRvIII and PTEN demonstrated significantly better clinical response to erlotinib. This clinical association was confirmed using *in vitro* experiments with cultured GBM cells [5]. In agreement with this finding, a phase I trial led by Haas-Kogan *et al.* [87] reported that GBMs with high levels of EGFR and low levels of phosphorylated Akt had a better response to erlotinib. Furthermore, Sarkaria *et al.* [88] showed in serially passaged GBM xenografts that erlotinib-sensitive tumors commonly express PTEN and amplified EGFR. In contrast to these observations, a phase II trial with GBM patients reported that the expression of PTEN was not associated with patient response to erlotinib while low p-Akt expression was of borderline significance to an improved outcome [59]. Results of another phase I/II trial showed that EGFRvIII and PTEN

co-expression did not predict better responsiveness of GBM patients to lapatinib and that lapatinib did not yield significant activity in GBM patients [63].

Wang *et al.* [89] reported that rapamycin enhanced the sensitivity of PTEN-deficient GBM cells to erlotinib treatment, providing a rationale for the combination of mTOR and EGFR kinase inhibitors in treating GBM with PTEN deficiency. In light of this interesting *in vitro* observation, the efficacy of this combination has been evaluated clinically in GBM patients. Results of a recent phase II clinical trial of erlotinib plus sirolimus showed that the combination was well tolerated in adult patients with heavily pre-treated, recurrent GBM, but unfortunately, yielded negligible activity among these unselected GBM patients [55]. This study led by Reardon *et al.* also reported that tumor markers, including, EGFR, EGFRvIII, and PTEN failed to show an association with PFS, except for increased p-Akt expression which only achieved borderline significance. Taken together, these observations clearly indicate that the role of PTEN expression as a determinant of GBM responsiveness to EGFR-targeted therapy is still unclear and that the combination of mTOR- and EGFR-targeted therapies is in need of improvement.

c-Met and PDGFR

The c-Met RTK has been shown to be co-activated in GBM cells with increased levels of EGFR/EGFRvIII [23, 77, 82]. This co-expression/co-activation has been shown to be a result of transcription-independent and -dependent mechanisms. In a transcription-independent fashion, activated EGFR associates with c-Met and the association facilitates c-Met phosphorylation in the absence of its only known ligand, HGF [90]. In support of this finding, c-Met is constitutively phosphorylated in the absence of HGF in human cancer cells. On the other hand, HGF transcriptionally activates the expression of EGFR ligands, TGF-α and heparin binding-EGF, leading to EGFR activation [91].

Combination of the c-Met inhibitor (SU11272) and erlotinib has been shown to yield significantly higher anti-proliferative effects than single agents on GBM cells with c-Met/EGFR co-activation [23, 82]. Using xenograft models, Lal *et al.* [92] showed that a neutralizing anti-HGF monoclonal antibody (L2G7) synergizes with erlotinib to inhibit the growth of the PTEN-null/HGF(+)/c-Met(+)/EGFRvIII(+) U87MG GBM tumors. Similar positive results were reported by Pillary *et al.* [77] using the humanized HGF-specific antibody AMG 102 (Amgen) in combination with panitumumab. The AMG 102/479-panitumumab combination will be evaluated in a phase I trial in colon cancer, colorectal cancer, gastrointestinal cancer, metastatic colorectal cancer and rectal cancer with wild-type KRAS (clinical trial # NCT00788957; http://ClinicalTrials.gov).

It is worth noting that there are several other anti-HGF and anti-c-Met agents that are under various phases of clinical trials in different cancer types. For example, the c-Met kinase inhibitor foretinib (GSK1363089; XL880; GSK) is being evaluated in papillary renal cell carcinoma, metastatic gastric cancer and hepatocellular carcinoma (NCT00920192). Another c-Met kinase inhibitor, PF-02341066 by Pfizer, will be tested in adults with lymphoma and in young patients with CNS cancers and large cell lymphoma (clinical trial # NCT00939770). AMG 208 (Amgen), a small molecule inhibitor of c-Met, will be evaluated in a phase I trial in solid tumors (clinical trial # NCT00813384). Interestingly, concurrent activation of c-Met and PDGFR appears to be a frequent event in GBM and this has been suspected to be a mechanism for GBM resistance to EGFR kinase inhibitors [23, 82]. Stommel *et al.* [82] showed that the combination of erlotinib, SU11274 (c-Met inhibitor) and imatinib (Gleevec; abl-PDGFR inhibitor) significantly inhibits the *in vitro* growth of GBM cells, compared to single agents and dual-drug combinations.

HMG-CoA Reductase

HMG-CoA (3-hydroxy-3-mehylglutaryl CoA) reductase is the rate-limiting step of the mevalonate pathway that catalyzes the conversion HMG-CoA to mevalonate [93]. The mevalonate pathway produces end products, such as, dolichol, cholesterol, geranylgeranyl pyrophosphate and farnesyl pyrophosphate that can directly affect EGFR activity, as well as, indirectly modulate the activity of EGFR-mediated downstream molecules [84]. Dolichol is involved in N-linked glycosylation of several RTKs [94] and the ligand-binding domain of EGFR is glycosylated to allow for ligand-binding, cell-surface localization and proper conformation [95]. Cholesterol modulates EGFR kinase activity [83]. Finally, the EGFR downstream signaling molecule, Ras, is post-translationally modified by geranylgeranyl transferase and farnesyl transferase that use geranylgeranyl pyrophosphate and farnesyl pyrophosphate, respectively. Although the levels of HMG-CoA reductase in tumors have not been shown to be elevated compared to normal tissues, the growth of some cancer cells appears to be more dependent on the metabolites of the mevalonate pathway. This is likely due to the fact that cancer cells, but not normal tissues, frequently express high levels of EGFR and Ras, whose activity is enhanced by these metabolites.

Inhibitors that target HMG-CoA reductase, also known as statins used to reduce cholesterol levels, have been shown to demonstrate anti-cancer activity [96]. For example, a Japanese-conducted randomized control trial of 81 patients with hepatocellular carcinoma showed that the HMG-CoA reductase inhibitor, pravastatin, prolonged the survival of 5-FU-treated patients from 9 months to 18 months [94]. However, no significant benefit was reported in a phase I/II trial led by Larner *et al.* [97] using lovastatin in patients with malignant gliomas. While the mechanism underlying statins-mediated anti-cancer effects remain unclear, statins are known to inhibit EGFR autophosphorylation and thereby, target EGFR-expressing cancer cells. For example, several studies reported that combined targeting of HMG-CoA reductase and EGFR yielded synergistic killing effects in several cancer types, including GBM [98]. Combination of lovastatin and EGFR kinase inhibitory agents, AG1478 and Iressa, led to growth-inhibitory effects on breast cancer, colon carcinoma and NSCLC [99, 100]. The combination of cetuximab and the HMG-CoA reductase inhibitor (fluvastatin) suppressed the growth of hepatocellular cancer [101]. Lovastatin significantly enhanced the sensitivity of GBM cells to gefitinib [98]. The synergistic effects of Iressa with lovastatin were observed in GBM cells with EGFR or EGFRvIII expression independent of PTEN status, thereby providing a rationale for combining HMG-CoA reductase- and EGFR-targeted therapies as a novel therapy for GBM [98].

STAT3 IN GBM RESISTANCE TO EGFR-TARGETED THERAPY

STAT3 and Oncogenesis

STAT3 is a transcription factor that has been shown to induce oncogenesis of normal fibroblasts [102] and cancers of the prostate [103] and skin [104, 105]. STAT3 also transforms mouse bone marrow cells into highly aggressive T cell leukemia in mice [106]. In contrast, activated STAT3 has been shown to suppress c-myc-, but not RasV12-mediated malignant transformation of mouse embryonic fibroblasts [106]. In normal brain, the role of STAT3 as an oncogene appears to depend on genetic status of PTEN and EGFR [37]. In PTEN-proficient mouse astrocytes, STAT3 behaves as a tumor suppressor and conversely, dual-suppression of PTEN and STAT3 leads to their malignant transformation. In contrast to the tumor suppressive role in PTEN-positive astrocytes, STAT3 promotes EGFRvIII-induced glial transformation by forming a complex with EGFRvIII in the nucleus [37]. The oncogenic role of STAT3 in gliomas is further supported by the notion that STAT3 activation is rarely detected in normal brain tissues [81, 107].

STAT3 in Human Cancers

In cancerous cells, STAT3 activation has been consistently shown to associate with more malignant cancer biology and poor prognosis [28, 81, 107-110]. STAT3 is highly activated in malignant gliomas [81, 107] and the extent of STAT3 activation correlates with glioma grade [81]. STAT3 can be activated *via* phosphorylation at Y705 and/or S727. As described in Fig. (**3a**), STAT3 Y705 is directly phosphorylated by RTKs (EGFR and EGFRvIII) and non-receptor TK (JAK2). Inactive JAK2 is constitutively bound to G-protein coupled receptors (IL-R, LIF-R, gp130) and autophosphorylates upon receptor activation. In addition, JAK2 can be phosphorylated at Y1007/1008 directly by RTKs (EGFR/EGFRvIII and PDGFR) and non-receptor TK, Src [111, 112]. As described in Fig. (**3b**), in addition to Y705 phosphorylation, STAT3 can be activated *via* S727 phosphorylation through EGF- and IL-6-dependent mechanisms [103, 113-116]. Despite both Y705 and S727 phosphorylation can activate the transcriptional activity of STAT3, little information is available with regard to the relationship between p-STAT3 (Y705) and p-STAT3 (S727). Interestingly, a recent study by Qin *et al.* [103] showed that activation of STAT3 through a phosphomimetic S727 promotes prostate tumorigenesis independent of Y705 phosphorylation.

As summarized in Fig. (**4**), activated STAT3 dimerizes and translocates into the nucleus to activate expression of genes that are important for G1 cell cycle progression (cyclin D1), oncogenesis (c-Myc, c-fos and iNOS), anti-apoptosis (Bcl-XL, Mcl-1, pim-1), EMT (TWIST), metastasis (MMP-1/2), angiogenesis (VEGF and iNOS) [28, 117-123] and immuno-suppression (IL-23) [124]. Together, these findings clearly indicate that STAT3 is highly activated in many human cancers, including, malignant gliomas and that it is an important molecule that converges signals of several pathways and mediates many important cellular processes.

STAT3/JAK2 Inhibitors

Given the pivotal and central role that STAT3 plays in many human cancers, STAT3 has emerged as a major molecular target for cancer therapy [125]. Several anti-STAT3 agents are under pre-clinical and clinical evaluation, and can be classified into two major categories: (1) direct STAT3 inhibitors and (2) indirect inhibitors that target STAT3's upstream activating kinase, JAK2. For example, STA-21 small molecule compound directly inhibits STAT3 and targets breast cancer cells *in vitro* [126]. Platinum compounds, IS3 295 and CPA-7, directly inhibit STAT3's DNA-binding ability, in which CPA-7 demonstrates anti-metastasis activity toward colon tumors [127]. Another direct STAT3 inhibitor, STAT3 decoy, is consisted of double-stranded decoy oligodeoxynucleotides which closely correspond to the STAT3 binding site within the c-fos promoter. STAT3 decoy shows promising anti-tumor effects in head and neck squamous cell carcinomas [128] and GBM cells [129]. Indirect STAT3 inhibitors JSI-124, AG490 and WP1066 target JAK2 and suppress the growth of GBM cells and/or xenografts [81, 130, 131]. LLL3, a structural analogue of STA-21, showed anti-GBM activity [132]. INCB018424 (Incyte), an orally active small molecular weight JAK2 inhibitor, has been examined in metastatic prostate cancer and the results are forthcoming (clinical trial # NCT00638378). INCB018424 is being evaluated in clinical trials for multiple myeloma (clinical trial # NCT00639002). A phase 0 clinical trial is recruiting head and neck cancer patients to determine the effects of STAT3 decoy (clinical trial # NCT00696176). Three phase I trials are enrolling patients with relapsed/refractory non-Hodgkin's lymphoma or multiple myeloma (NCT00511082) and with advanced solid tumors (NCT00955812) to evaluate the efficacy of a STAT3 small molecule inhibitor OPB-31121.

EGFR-STAT3 Interactions in GBM Resistance to EGFR-targeted Therapy

EGFR physically interacts and functionally cooperates with STAT3, at both cytoplasmic and nuclear levels. At the cytoplasmic level, *via* the two docking autophosphorylated tyrosines (Y1068 and Y1086), cell-surface EGFR interacts with STAT3 SH2 domain [133]. This interaction leads to phosphorylation of STAT3 at Y705 and its activation. Cell-surface EGFRvIII also interacts with and phosphorylates STAT3. Importantly, we and others showed in cancers of breast, colon and skin that cell-surface EGFR cooperates with STAT3 to induce expression of TWIST (to facilitate EMT), VEGF (to promote angiogenesis) and Eme1 endonuclease (to reduce drug-induced DNA damage) [121, 123, 134]. In primary breast carcinomas, co-expression of EGFR and activated STAT3 (Y705) is frequent, 39% [123]. In primary gliomas, the extent of concurrent EGFR/EGFRvIII expression and STAT3 activation was also a frequent event and positively correlates with glioma grade [81]. The few reports with respect to the ability of EGFR versus EGFRvIII to activate STAT3 in GBM have shown rather inconsistent results. For example, a study reported that the PI3K pathway is dominant over the MAPK and STAT3 pathways in GBM with a high level of EGFRvIII expression [23]. However, another study reports that STAT3 is more activated in EGFRvIII-carrying GBM than those with EGFR and mixed expression [135].

At the nuclear level, EGFR interacts with STAT3 to activate expression of iNOS gene in carcinomas of breast and epidermoid [28]. Nuclear EGFRvIII interacts with STAT3 in normal astrocytes and such interaction contributes to their malignant transformation into glioma [37]. It is speculated that nuclear EGFRvIII-STAT3 interaction involves the tyrosine kinase function of EGFRvIII, albeit the exact effect of nuclear EGFRvIII on STAT3 remains unknown. Most recently, we found nuclear EGFR-EGFRvIII and nuclear STAT3 cooperate to activate expression of pro-inflammatory gene, COX-2, in malignant gliomas [38]. Together, these findings indicate that EGFR/EGFRvIII and STAT3 pathways significantly interact at multiple levels, leading to gene activation and more aggressive tumor behaviors.

The multi-level interactions between EGFR and STAT3 emerge as a potential mechanism underlying the resistance of GBM to EGFR-targeted therapy. In primary specimens and cancer cell lines, STAT3 activation is paradoxically sustained when EGFR is inhibited [28, 81, 136]. The STAT3-activating kinase, JAK2, is activated in GBM cell lines and combined inhibition of JAK2 and EGFR/EGFRvIII abolishes STAT3 activation and synergistically suppresses the growth of EGFR- and EGFRvIII-expressing cell lines of breast cancer [28] and epidermoid carcinoma [28, 137], and GBM [81]. These encouraging *in vitro* observations provide a rationale to evaluate the efficacy of combination of EGFR and STAT3/JAK2 inhibitors in targeting GBM *in vivo*.

CONCLUDING REMARKS

GBM is the most common brain cancer in adults and unfortunately, is also the most aggressive type and the least responsive to various therapies. Overexpression of EGFR and/or EGFRvIII is frequently found in GBM and is generally associated with more malignant phenotype and poor clinical outcome. Consequently, EGFR-targeted therapy emerges as a promising anti-GBM therapy. However, the clinical efficacy of EGFR-targeted therapy has been only modest in GBM patients. Although intrinsic drug resistance is known to be a major obstacle for EGFR-targeted therapy, the underlying mechanisms are still poorly understood, despite extensive investigations are being conducted to shed light on these mechanisms. Experiences drawn from clinical trials indicate that mono and combination EGFR-targeted therapies encountered many challenges, including, insufficient penetration through BBB, drug inactivated induced by concurrent uses of EIAEDs, drug efflux at BBB, inability to inhibit tumoric EGFR kinase activity, and the lack of a consistent association between biomarkers and patient response. Therefore, it remains an important task to better

our understanding of the complex and interactive nature of the EGFR- and EGFRvIII-mediated signaling networks, to identify the alternative signaling pathways that GBMs activate while the EGFR activity is inhibited by EGFR-targeted agents, and to identify other underlying mechanisms in order to improve the observed modest efficacy of EGFR-targeted therapy in GBM patients.

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ABBREVIATIONS

AA	anaplastic astrocytoma
AO	anaplastic oligodendroglioma
BBB	blood-brain barrier
BCRP	breast cancer resistance protein
BCNU	bis-chloronitrosourea
B-Myb	B-myeloblastosis viral oncogene homolog
CNS	central nervous system
c-Met	c-mesenchymal-epithelial transition factor
COX-2	cyclooxygenase-2
DNA-PK	DNA-dependent protein kinase
EIAED	enzyme-inducing antiepileptic drug
EFS	event-free survival
EGFR	epidermal growth factor receptor
EGFRvIII	epidermal growth factor receptor variant III
EMT	epithelial-mesenchymal transition
E2F1	electro-acoustic 2 factor 1
GLI1	glioma-associated oncogene homologue 1
GBM	glioblastoma multiforme
HGF	hepatocyte growth factor
HMG-CoA	3-hydroxy-3-mehylglutaryl CoA
IL-6	interleukin-6
iNOS	inducible nitric oxide synthase
JAK2	Janus-activated kinase 2
Mcl-1	myeloid cell leukemia sequence 1
MMP	matrix metalloproteinase
MRP	multidrug resistance protein
mTOR	mammalian target of rapamycin
NSCLC	non-small cell lung cancer

OS	overall survival
PCNA	proliferating cell nuclear antigen
PDGFR	platelet-derived growth factor receptor
PFS	progression-free survival
PR	partial response
PTEN	phosphatase and tensin homolog deleted on chromosome 10
RTK	receptor tyrosine kinase
SD	stable disease
STAT3	signal transducer and activator of transcription 3
STAT35	signal transducer and activator of transcription 5
TGF-α	tumor transforming factor-α
TMZ	temozolomide
VEGF	vascular endothelial growth factor

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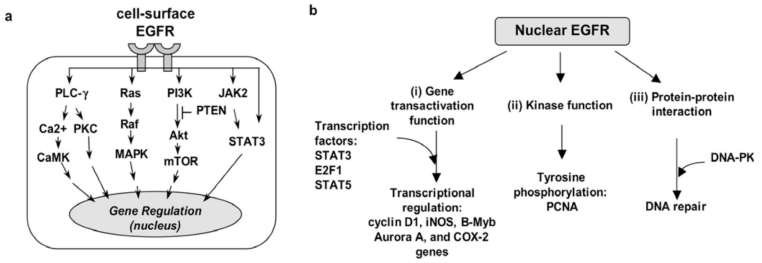

Fig. 1. The EGFR signaling pathway is of critical importance to human cancers and of a high complexity

The EGFR signaling pathway exerts its biological effects *via* two major modes of actions, namely, the cytoplasmic/ traditional (**a**) and the nuclear (**b**) signaling modes.

a. The cytoplasmic/traditional EGFR pathway is consisted of five major modules: PLC-γ-CaMK/PKC, Ras-Raf-MAPK, PI-3K-Akt-mTOR, JAK2/STAT3 and STAT3. Activation of these signaling modules often leads to tumorigenesis, tumor proliferation, metastasis, chemoresistance, and radio-resistance.

b. Nuclear EGFR has three key functions: (i) gene transactivation, (ii) tyrosine kinase, and (iii) protein-protein interaction.

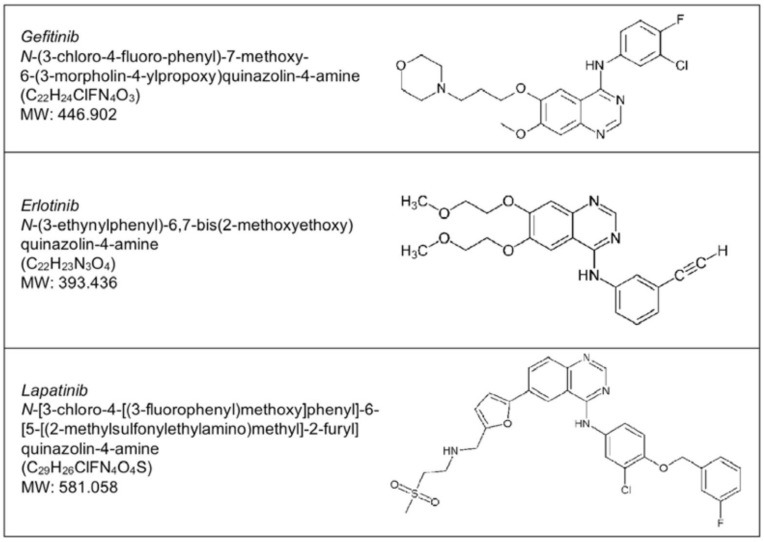

Fig. 2.
Chemical structures of three small molecule EGFR inhibitors.

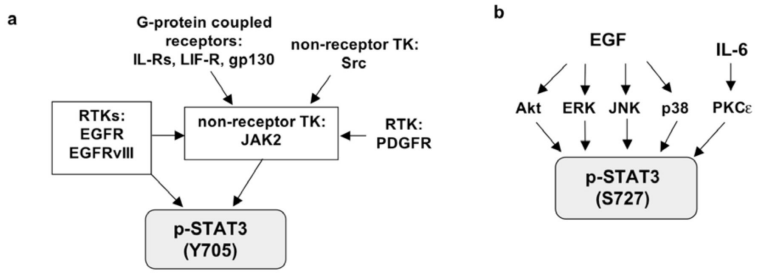

Fig. 3. Signaling pathways that lead to STAT3 activation

a. STAT3 can be phosphorylated at Y705 directly by RTKs (EGFR and EGFRvIII) and non-receptor TK (JAK2) and becomes activated. Inactive JAK2 is constitutively bound to the G-protein coupled receptors (IL-R, LIF-R, gp130) and becomes auto-phosphorylated at Y1007/1008 upon receptor activation. In addition, JAK2 can be phosphorylated at Y1007/1008 directly by RTKs (EGFR/EGFRvIII and PDGFR) and non-receptor TK, Src.
b. STAT3 activation *via* S727 phosphorylation can be initiated by stimulation of EGF (*via* Akt, ERK, JNK and p38) and IL-6 (*via* PKCε).

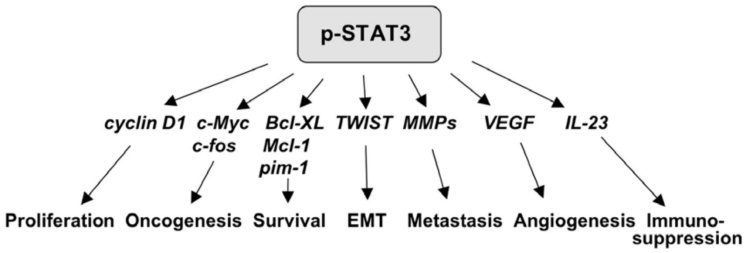

Fig. 4.

Activated STAT3 modulates expression of many important genes involved in oncogenesis, as well as, various important cellular processes in human cancers.

Table 1

Overview of Outcome of Clinical Trials Using EGFR-Targeted Therapy in Malignant Gliomas

Agent	Targets	Phase	Study Design	Outcomes	References
Small Molecules Gefitinib (Iressa)	EGFR	II	single agent 53 recurrent GBM	6-month EFS: 13% median OS: 39.4 wks median EFS: 8.1 wks No correlation between EGFR and OS or EFS	Rich 2004 [49]
		II	single agent 28 GBM, AO & AA	6-month PFS: 14.3% median OS: 24.6 wks No correlation between EGFR/p-Akt and response	Franceschi 2007 [50]
		I	gefitinib+sirolimus 34 recurrent GBM & AA	6-month PFS: 23.5% median PFS: 27.4 wks PR: 14%, SD: 38%	Reardon 2006 [51]
		I/II	gefitinib+everolimus 22 GBM	6-month PFS, 4.5% median PFS: 2.6 months median OS: 5.8 months PR: 14%, SD: 38% No correlation between EGFR/PTEN and response	Kriesl 2009 [52]
		I	gefitinib+TMZ 26 GBM	Recommendations for phase-2 doses	Prados 2008 [53]
		I	radiosurgery 15 recurrent GBM & AA	6-month PFS: 63% median PFS: 7 months median OS: 29 months (all pt's) median OS: 21 months (GBM)	Schwer 2008 [54]
Erlotinib (Tarceva)	EGFR	II	single agent 67 recurrent GBM & AA	median PFS: 12 wks (GBM) median PFS: 8.6 wks (AA) limited activity as single agent	Raizer 2004[1]
		II	single agent 58 recurrent GBM	6-month PFS: 17% median OS: 10 months No correlation between EGFR and response	Cloughesy 2005[2]
		II	erlotinib+sirolimus 32 recurrent GBM	6-month PFS: 3.1% negligible activity p-AKT, but not EGFR/EGFRvIII/ PTEN correlates with response.	Reardon 2009 [55]
		I	Arm 1: erlotinib alone Arm 2: erlotinib+TMZ 83 GBM	Recommendations for phase-2 doses	Prados 2006 [57]
		I/II	erlotinib+TMZ+RT 97 newly diagnosed GBM	median OS: 15.3 months biomarkers: pt's not sensitive to er lotinib No correlation between EGFR/EGFRvIII/ PTEN and response	Brown 2008 [58]
		II	Arm 1: erlotinib alone Arm 2: TMZ or BCNU 110 recurrent GBM	6-month PFS: 11.4% (Arm 1) 6-month PFS: 24% (Arm 2) Limited activity of erlotinib No correlation between EGFR/EGFRvIII PTEN/p-Akt and response to erlotinib	Van den Bent 2009 [59]
		II	erlotinib+carboplatin 43 recurrent GBM	6-month PSF: 14% median PSF: 9 wks median PS: 30 wks No correlation between EGFR/PTEN/ Akt and PFS or OS	de Groot 2008 [60]
		I	erlotiniib+RT 19 GBM	median OS: 55 wks	Krishnan 2006 [61]

Agent	Targets	Phase	Study Design	Outcomes	References
		II	erlotinib+ bevacizumab 56 recurrent GBM & AA	6-month PFS: 25% (GBM) 6-month PFS: 50% (AA) Full results to be reported	Sathornsumetee 2009[3]
Lapatinib (Tykerb/Tyverb)	EGFR/HER2	I/II	single agent 7 recurrent GBM (I) 17 recurrent GBM (II)	No significant lapatinib activity No correlation between EG FRvIII/PTEN and response	Thiessen 2009 [63]
Antibodies Cetuximab (Erbitux)	EGFR	I/II	cetuximab+RT+TMZ 17 GBM	6-month PFS: 81% 12-month PFS: 37% 12-month OS: 87%	Combs 2009[4]
		II	single agent Arm 1: 28 GBM with EGFR amplification Arm 2: 27 GBM with no EGFR amplification	No significant cetuximab activity No correlation between EGFR and response	Neyns 2009 [64]
		II	cetuximab+ bevacizumab+ irinotecan 32 recurrent GBM	Response rates similar to bevacizu mab+irinotecan	Lassen 2008[5]

EFS: event-free survival, OS: overall survival, PFS: progression-free survival, PR: partial response, SD: stable disease.

AA: anaplastic astrocytoma, AO: anaplastic oligodendrogliomas, RT: radiation therapy.