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Advances in the Development of EGFR Targeted Therapies for the Treatment of Glioblastoma

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1. Introduction

The epidermal growth factor receptor (EGFR) is receptor tyrosine kinase (RTK) dysregulated in glioblastoma (GBM) through overexpression, mutation or inappropriate expression of ligand. Activation of the EGFR by these mechanisms contributes to the development and progression of GBM by engaging downstream targets, such as the PI3K pathway. The de2-7 EGFR (or EGFRvIII), a naturally occurring mutation of the EGFR frequently expressed in GBM, preferentially activates this pathway. Clinical trials with EGFR-specific tyrosine kinase inhibitors (TKIs) have been disappointing with very little antitumor activity observed. The outcome of controlled clinical trials with EGFR-specific antibodies is yet to be reported. Encouraging preclinical and preliminary clinical data suggests that the combination of EGFR therapeutics and compounds that target molecules downstream of EGFR might have increased efficacy. Finally, the identification of biomarkers that predict those patients most likely to respond to EGFR inhibition is desperately needed.

2. Expression of EGFR and its ligands in GBM

The EGFR is frequently expressed in GBM (Jungbluth et al., 2003), the most common and deadly form of malignant brain cancer (DeAngelis, 2001). Extensive co-expression of EGFR ligands such as EGF and TGF-α has also been reported (Ekstrand et al., 1991), suggesting the existence of a robust autocrine loop in many cases of GBM. Furthermore, overexpression of the EGFR has been reported in up to 60% of GBM cases depending on the technique used (Libermann et al., 1985; Schlegel et al., 1994; Jungbluth et al., 2003), with overexpression leading to ligand-independent activation of the receptor (Thomas et al., 2003). The activation and subsequent phosphorylation of EGFR stimulates several downstream pathways including Ras/MAPK, PI3K/Akt, PLC-gamma and STAT3 (Halatsch et al., 2006; Nakamura, 2007). All four pathways contribute to the tumorigenicity of GBM, but the PI3K/Akt pathway appears to have a central role in the development and maintenance of this cancer (Chakravarti et al., 2004). Indeed, inactivation/deletion/mutation of PTEN, an endogenous inhibitor of the PI3K pathway, is also a common event in GBM (Rasheed et al., 1997). Of note, there is an emerging role for EGFR-mediated activation of STAT3 in the development of GBM (Weissenberger et al., 2004; Mizoguchi et al., 2006; Sherry et al., 2009).

3. Amplification of the EGFR gene in GBM

Amplification of the *EGFR* gene was the first reported genetic alteration in GBM (Libermann et al., 1985). Subsequent studies have confirmed that approximately 40% of GBMs display amplification of the *EGFR* gene (Wong et al., 1987). Gene amplification invariably leads to overexpression of the EGFR at the cell surface (Wong et al., 1987), although given that overexpression of the receptor occurs in 60% of GBMs, gene amplification is not the only route to increased expression. The majority of GBMs develop rapidly, without evidence of pre-existing malignant lesion, and are known as primary (or *de novo*) GBMs, while secondary GBMs arise from low-grade diffuse astrocytomas or anaplastic astrocytomas (Furnari et al., 2007; Ohgaki & Kleihues, 2007). *EGFR* gene amplification is more commonly associated with primary GBMs than secondary GBMs, where it occurs at a frequency of less than 10% (Watanabe et al., 1996; Ohgaki & Kleihues, 2007). Overexpressed EGFR not only activates in a ligand-independent manner, but shows enhanced signaling through the STATs, including STAT3 (Thomas et al., 2003; Pedersen et al., 2005), which in turn can induce expression of IL-6. Since IL-6 autocrine loops and amplification of the *IL6* gene have been reported at high frequency in GBM (Weissenberger et al., 2004; Tchirkov et al., 2007), this could be an important, but largely overlooked, consequence of EGFR overexpression.

Recent studies have shown that GBM can be classified into at least 4 distinct molecular sub-types; classical, pro-neural, neural and mesenchymal (Brennan et al., 2009; Verhaak et al., 2010). Pro-neural GBMs largely constitutes the secondary GBMs and therefore does not display EGFR amplification and/or overexpression. In contrast nearly all GBM in the classical sub-type overexpress EGFR (Verhaak et al., 2010). Furthermore, the GBM specific mutation, de2-7 EGFR, is found almost exclusively in the classical sub-type. Neural and mesenchymal GBMs have variable levels of EGFR with some showing increased expression and others decreased expression.

4. Mutations of EGFR described in GBM

Amplification of the *EGFR* gene in GBM is associated with gene rearrangements. The first rearrangement to be described in detail was an extracellular domain (ECD) deletion producing a mutant known as the de2-7 EGFR (or EGFRvIII) (Sugawa et al., 1990; Yamazaki et al., 1990; Ekstrand et al., 1992; Wong et al., 1992). Several other deletion mutants have since been described and categorized (Table 1) (Ekstrand et al., 1992; Frederick et al., 2000). Numerous subsequent studies have shown that the most common mutation is the de2-7 EGFR, occurring in about 50% of cases where the *EGFR* gene is amplified (Wikstrand et al., 1998; Frederick et al., 2000; Pedersen et al., 2001). This cancer-specific EGFR mutant has a specific deletion between exons 2 and 7 of *EGFR* (Sugawa et al., 1990). The truncation of exons 2–7 leads to the elimination of 267 amino acids from the ECD and the insertion of a novel glycine at the fusion junction. This renders the mutant EGFR unable to bind any known ligand (Sugawa et al., 1990; Wikstrand et al., 1998; Pedersen et al., 2001). Despite this, the de2-7 EGFR is capable of low-level constitutive signaling, which is augmented by the mutant receptor's impaired internalization and downregulation (Nishikawa et al., 1994; Schmidt et al., 2003).

GBM cell lines transfected with the de2-7 EGFR display enhanced tumorigenicity when grown as xenografts in nude mice, but only a marginal effect on growth is observed *in vitro*

(Nishikawa et al., 1994). Furthermore, expression of the de2-7 EGFR is consistently lost when GBM cell lines are established *in vitro* using serum, yet is retained if GBM samples are implanted and subsequently passaged directly in nude mice (Sarkaria et al., 2007). Taken together, this indicates that the de2-7 EGFR contributes primarily to aspects of *in vivo* growth. While the increased tumorigenicity mediated by the de2-7 EGFR is primarily due to the receptor's constitutive tyrosine kinase activity (Huang et al., 1997), attempts to identify intracellular molecules and signaling pathways associated with its growth advantage remain ongoing. Transfection of the de2-7 EGFR into U87MG human GBM cells results in an increase in PI3K activity that is important to the growth advantage mediated by the mutant receptor, an observation confirmed by several papers (Moscatello et al., 1998; Li et al., 2004; Luwor et al., 2004). U87MG cells transfected with the de2-7 EGFR also co-express wild-type (wt) EGFR, a scenario that probably mimics the situation in GBM patients. The significance of a possible interaction between the de2-7 EGFR and wtEGFR is not fully known, but it has been shown that the de2-7 EGFR can directly activate PI3K in the absence of the wtEGFR in non-GBM cell lines (Moscatello et al., 1998). We reported that the de2-7 EGFR and the wt EGFR can heterodimerize leading to increased PI3K signaling (Luwor et al., 2004), suggesting that an interaction between the mutant and wt receptor could enhance de2-7 EGFR signaling. One clear consequence of PI3K activation by de2-7 EGFR is the increased production of VEGF, both under normoxic and hypoxic conditions (Feldkamp et al., 1999), ascribing a pro-angiogenic function to this receptor.

Mutation	Frequency (%)	Biological Effect
Δ6–273	30	Ligand-independent, failure to down-regulate
Δ521–603	<10	Unknown
Δ959–1186	<10	Increased ligand-dependant kinase activity
Δ959–1030 and Δ959-1043	<10	Increased basal activity especially Δ959–1030 but responds to ligand
R84K	<5	Increased basal activity but responds to ligand
T239P	<5	Increased basal activity but responds to ligand
A265V/D/T	<5	Increased basal activity but responds to ligand
G574V	<1	Increased basal activity but responds to ligand
Kinase mutation (L861Q)	<1	Increased basal activity, failure to downregulate

Table 1. Selected mutations of the EGFR expressed in GBM

Very recently de2-7 EGFR has been shown to stimulate the production of cytokines, including IL-6 and LIF, which signal through the gp130 complex (Inda et al., 2010). Importantly these cytokines were shown to activate the wtEGFR when it is overexpressed in neighboring GBM cells, through a mechanism involving cross-talk between gp130 and

EGFR. Activation of the wtEGFR in this manner leads to enhanced proliferation. Thus, de2-7 EGFR contributes to the growth of surrounding GBM cells through this field effect. This work also shows the functional link between EGFR and IL-6. More generally this indicates that the de2-7 EGFR actively contributes to the heterogeneity of GBM by acting indirectly with neighboring de2-7 EGFR negative cells (Inda et al., 2010). This hypothesis is entirely consistent with the observation that wtEGFR amplification and de2-7 EGFR expression are usually seen together. It may also explain why the pronounced growth advantage mediated by the de2-7 EGFR does not lead in patients to a homogenous population of cells in patients all expressing the receptor.

Lee *et al* sequenced the entire *EGFR* gene in a panel of eight GBM cell lines and 132 GBM samples (Table 1) (Lee et al., 2006). Interestingly, they identified a series of missense mutations in the ECD of the EGFR expressed in 14% of the GBM samples and 12% of the cell lines (Lee et al., 2006). In general, the missense mutations were found to be independent of the de2-7 EGFR but were associated with *EGFR* gene amplification; approximately 60% of samples with missense mutations also had *EGFR* gene amplification. Subsequent studies showed that these single amino acid mutations led to ligand-independent activation of the EGFR, and unlike the wtEGFR, were transforming in NR6 cells; a variant of mouse 3T3 cells lacking the EGFR (Lee et al., 2006). However, unlike the de2-7 EGFR, these mutants could also respond to ligand stimulation. Recently, we extended these studies and showed that some of these mutations also provide a significant advantage to *in vivo* growth (Ymer et al., 2011).

The presence of activating kinase mutations, such as those commonly found in lung cancer (Sharma et al., 2007), is extremely rare in GBM, with only one sample displaying this type of mutation (Lee et al., 2006). Interestingly, a subsequent analysis of 119 lung cancer samples failed to find a single missense mutation in the ECD, although 13% of the samples contained kinase domain mutations, as expected (Lee et al., 2006). Thus, mutations of the EGFR in GBM appear to cluster in the ECD and lead to ligand independence. Therefore, the lessons learned with respect to EGFR therapeutics for the treatment of lung cancer are probably of minimal value in the context of GBM. Finally, these mutations further emphasize just how frequently the EGFR is perturbed in GBM; in fact, taking into account EGFR autocrine loops, activation of the EGFR probably occurs in over 70% of GBMs.

5. EGFR as a therapeutic target in GBM

Given that the EGFR is activated or dysregulated in a large percentage of GBM cases it is a rational target for therapeutic intervention in this disease. There are two major classes of EGFR inhibitors either currently approved or being evaluated for the treatment of various cancers; antibodies that target the ECD and small molecule TKIs that target the intracellular kinase domain (Marshall, 2006). No specific agent from either class has been approved for the treatment of GBM, but as described below, a number of clinical trials have been reported or are ongoing.

5.1 Antibodies directed to the EGFR

Overexpression of the de2-7 EGFR on the cell surface, the unique junctional peptide created by the deletion and the aggressive phenotype associated with this receptor, suggests that targeting the de2-7 EGFR with antibodies that are cancer-specific is an attractive therapeutic

strategy. In fact, antibodies specific for the de2-7 EGFR have been generated; the monoclonal antibodies (mAbs) DH8.3 (Hills et al., 1995), L8A4 and Y10 (Wikstrand et al., 1995) all preferentially recognize the unique junctional peptide. DH8.3 and Y10 have been shown to specifically target cells expressing the de2-7 EGFR (i.e. they do not bind the wtEGFR) (Hills et al., 1995; Wikstrand et al., 1995). Although DH8.3 has been shown to target de2-7 EGFR-expressing xenografts *in vivo* (Johns et al., 2002), its efficacy has not been reported. The Y10 antibody has been shown to have *in vivo* antitumor activity against murine B16 melanoma cells transfected with a murine homolog of the human de2-7 EGFR (Sampson et al., 2000), an unusual system for its evaluation since expression of the de2-7 EGFR has not been reported in melanoma. Furthermore, the antitumor activity seen was completely dependent on the Fc function of the Y10 antibody and not a direct inhibitory effect on de2-7 EGFR signaling (Sampson et al., 2000). There have been no reports to date of clinical trials using de2-7 EGFR-specific antibodies. These antibodies are internalized and could be used for delivery of radiotherapy or cytotoxics given their specificity (Foulon et al., 2000), but this approach has not been examined clinically.

Numerous therapeutic antibodies to the wtEGFR have been described and several have been used in the context of GBM (Quang & Brady, 2004; Belda-Iniesta et al., 2006). These antibodies all function in a similar manner by interacting with the L2 domain of the EGFR and inhibiting the binding of ligand. Structural studies with Cetuximab suggest that these antibodies also prevent EGFR dimerization, a crucial step in its activation (Li et al., 2005). Antibodies directed to the wtEGFR do show antitumor activity in GBM xenograft models even when the tumors are grown intracranially (Eller et al., 2002; Perera et al., 2005). Furthermore, these antibodies can bind the de2-7 EGFR and can inhibit GBM cells co-expressing the de2-7 and wtEGFR (Perera et al., 2005). However, the large intratumoral pressure found in GBM, the 'remnants' of a blood brain barrier (BBB) and the inefficient nature of GBM vascularization have all raised concerns about the effective targeting of antibodies to GBM following systemic administration. Despite these concerns, three antibodies targeting wtEGFR are have been tested in GBM using systemic administration, including mAb 425. ^{131}I-mAb 425 has been used in several clinical trials and clearly demonstrates targeting to GBM following systemic administration (Quang & Brady, 2004). Unfortunately, this antibody is of murine origin and can only be administered on a few occasions before immune responses render it ineffective.

Cetuximab is a chimeric antibody directed to the EGFR that has been approved in several human cancers including that of the colon (Moosmann & Heinemann, 2007). There are anecdotal reports of this antibody being used for the treatment of GBM, but no rigorously controlled studies have been implemented to date (Belda-Iniesta et al., 2006). Preclinical *in vitro* and *in vivo* studies suggest that cetuximab and related antibodies have significant antitumor activity in GBM, encouraging the establishment of a Phase I/II clinical trial (Combs et al., 2006). In this trial, cetuximab will be co-administered with a combination of temozolomide and radiotherapy, which is the standard of care following initial resection of a GBM (Trial Number: NCT00311857). Outcomes from this trial should be reported shortly. Recently cetuximab was trialed with a combination with bevacizumab (i.e. avastin) and irinotecan but did not increase the efficacy of this combination in GBM patients (Hasselbalch et al., 2010). There are several other EGFR-directed antibodies either approved or in development including Panitumumab (Rivera et al., 2008); however, there have been no reports of their systematic evaluation in GBM.

A novel EGFR antibody (mAb 806) was generated against cells expressing the de2-7 EGFR but, unexpectedly, was also found to bind to a small proportion of the wtEGFR in GBM samples that overexpress the receptor (Jungbluth et al., 2003). mAb 806 does not bind the unique junctional peptide found in the de2-7 EGFR; rather, it binds to a short cysteine loop on the ECD that is only transiently exposed as the wtEGFR moves from its inactive to active conformation (Johns et al., 2004). The loop is constitutively exposed in the de2-7 EGFR, consistent with our original desire to generate a de2-7 EGFR-specific antibody. Thus, mAb 806 reactivity is found only in cells with favorable conditions for receptor activation, such as the presence of mutations (e.g. de2-7 EGFR), overexpression of the wt receptor or increased presence of EGFR ligands. In the case of EGFR overexpression, there is increased activation as a result of ligand-independent EGFR activation and changes in glycosylation (Johns et al., 2005). These conditions are common in malignant cells but are rare in normal tissues, thereby allowing mAb 806 to preferentially target malignancy such as GBM but not normal organs such as the liver. Our recent Phase I clinical trial confirmed that a chimeric version of mAb 806 did not bind normal tissue and could target GBM following systemic administration (Scott et al., 2007). Since mAb 806, unlike Cetuximab, does not target organs such as the liver it is easier to deliver a therapeutically relevant dose to the site of the GBM. Furthermore, the lack of normal tissue uptake will allow the labeling of mAb 806 with cytotoxic compounds or radioisotopes to enhance its already substantial antitumor efficacy (Johns et al., 2007). This antibody has been licensed and has re-entered clinical trial.

5.2 Tyrosine kinase inhibitors that target the EGFR

TKIs are small molecules that specifically target the kinase domain of tyrosine kinases, preventing binding of ATP and subsequent activation (Marshall, 2006). Two EGFR-specific TKIs have been approved for the treatment of certain cancers (Mendelsohn & Baselga, 2006), while several others are in clinical trials. Similar to EGFR-specific antibodies, these compounds have shown antitumor activity in *in vitro* and *in vivo* preclinical models (Mendelsohn & Baselga, 2006). They are active against both the wtEGFR and the de2-7 EGFR (Stea et al., 2003; Halatsch et al., 2006; Sarkaria et al., 2007), although they might be less effective against the latter, especially if the de2-7 EGFR is expressed in the absence of the wtEGFR (Learn et al., 2004). Given that there are fewer concerns with regard to delivery of TKIs to the site of GBM when compared with antibodies, the development of these reagents for the treatment of GBM is more advanced. In fact, excellent targeting of these agents to the site of GBM has been well demonstrated (Hofer et al., 2006; Hegi et al., 2011).

The two clinically approved EGFR TKIs, erlotinib and gefitinib, have been used as monotherapy or in combination with temozolomide and/or radiotherapy in clinical trials of GBM patients (Table 2 lists selected Phase II trials). No significant clinical activity has been observed for either erlotinib or gefitinib in either primary or recurrent GBM when used as monotherapies (Table 2). Surgery, followed by temozolomide/radiotherapy is standard of care in primary GBM. The addition or erlotinib to this standard of care has been assessed in 3 different Phase II trials (Table 2). Two of these trials failed to show any benefit when erlotinib was added to standard of care and was associated with significant toxicity (Brown et al., 2008; Peereboom et al., 2010). In a third trial however, the authors reported encouraging PFS and median survival when compared to their previous studies using other agents (Prados et al., 2009). All three studies were conducted in newly diagnosed patients.

Why these trials have produced conflicting outcomes is not clear, but a comprehensive Phase III study is probably required to finally determine if erlotinib improves standard therapy. Overall though the data suggests that erlotinib's benefits are relatively small at best.

Trial	Patients (*n*)	Objective Response[#]	PFS	Median OS (months)
Gefitinib (single agent, recurrent)				
Rich *et al* (Rich et al., 2004)	**53**	**0**	**13% (6 months)**	**10**
Franceschi *et al* (Franceschi et al., 2007) *Gefitinib (single agent, primary)*	**16**	**0**	**12% (6 months)**	**6**
Uhm *et al* (Uhm et al., 2011)	**98**	**N/A**	**17% (12 months)**	**12**
Erlotinib (single agent, recurrent)				
Raizer *et al* (*Raizer et al., 2010*)	**38**	**0**	**3% (6 months)**	**6**
Erlotinib (plus standard care, primary)				
Pereeboom *et al* (Pereeboom et al., 2010)	**27**	**5 patients remain on study**	**3 months (median)**	**9[†]**
Prados *et al* (Prados et al., 2009)	**65**	**N/A**	**8 months (median)**	**19**
Brown *et al* (Brown et al., 2008)	**97**	**N/A**	**7 months (median)**	**15[†]**

Abbreviations: CR, complete response; PR, partial response; N/A not available; PFS, progression-free survival; OS, overall survival. *Abstract form only. #Excludes stable disease. [†]Significant toxicity

Table 2. Selected Phase II clinical trials of EGFR TKI's in GBM

A group of GBM patients treated with erlotinib or gefitinib who had responded or failed to respond to therapy was analyzed retrospectively to determine factors that might explain the different responses (Mellinghoff et al., 2005). The majority of responders expressed the de2-7 EGFR. Furthermore, loss of PTEN, an endogenous inhibitor of PI3K that leads to reduced phosphorylated AKT (Akt), was highly predictive of treatment failure. Not surprisingly, the co-expression of de2-7 EGFR and PTEN was predictive of response to EGFR-specific TKI therapy. A panel of human GBMs directly established as xenografts in nude mice retains expression of de2-7 EGFR in some cases (Sarkaria et al., 2006). This model system also confirmed that the presence of de2-7 EGFR and PTEN in a xenograft was predictive of response (Sarkaria et al., 2007). The mechanistic reasons for this observation remain unknown, but it provides a potential method for screening for patients likely to respond to TKI therapy. Finally, none of the current trials have shown a correlation between response to EGFR TKIs and *EGFR* gene amplification (Brown et al., 2008), although one immunohistochemical study reported a correlation between response to erlotinib and high

expression of EGFR in combination with low levels of pAkt (Haas-Kogan et al., 2005). Once again this supports the notion that activated EGFR in the presence of low pAkt may be indicative of increased levels of response to EGFR inhibition.

Recently bevacizumab (i.e. avastin) was approved for the treatment of recurrent GBM. A Phase II trial of erlotinib and bevacizumab showed that this combination was adequately tolerated, but provided no additional benefit to that seen in other bevacizumab containing regimens (Sathornsumetee et al., 2010). mTOR is downstream of the PI3K/Akt pathway and therefore represents a therapeutic target in GBM. The combination of sirolimus (a mTor inhibitor), and erlotinib has been reported in recurrent GBM patients (Reardon et al., 2010). While the combination was well tolerated, it displayed negligible anti-tumor activity.

6. Future developments

Both Cetuximab and Panitumumab have been approved for the treatment of colon cancer and erlotinib and gefitinib are approved for non-small cell lung cancer (NSCLC), validating the EGFR as a genuine therapeutic target (Van den Eynde et al., 2011). Given the dysregulation of the EGFR in GBM through the range of mechanisms described above, the failure of these agents to show significant anti-tumor activity in this cancer is somewhat surprising. The possibility that the BBB is responsible for this lack of activity has been conclusively discounted for EGFR TKI's and appears not to be a factor for anti-EGFR antibodies, although formal demonstration of this is still required for antibodies. Future research should focus the identification of patients more likely to respond to EGFR inhibition and identifying other targeted therapies that might work synergistically with EGFR inhibitors. Further classification of GBM into unique molecular sub-types beyond the four already described (Verhaak et al., 2010) will hopefully help identify EGFR levels of tumor subtypes more likely to respond to EGFR inhibition.

6.1 Lessons from other cancers

In NSCLC the sub-set of patients most likely to respond to EGFR TKI's are those containing activating mutations in the kinase domain of *EGFR (da Cunha Santos et al., 2011)*. Significant clinical and laboratory evidence suggests that these mutations lead to "oncogenic addiction"; the phenomenon whereby a tumor becomes largely dependent on a single activated kinase. Interestingly, *EGFR* amplification is not strongly associated with response to TKI's in NSCLC (De Luca & Normanno, 2010). In contrast, EGFR activation in GBM is most often driven by gene amplification and/or mutations found in the ECD domain of the receptor (Lee et al., 2006), although a recent report described several c-terminal deleted EGFR molecules that are constitutively active (Pines et al., 2010). The clinical data in GBM implies that mutations of this nature do not lead to EGFR addiction. The reason for this remains speculative but suggests that the primary role of EGFR in GBM may not be tumor proliferation or survival but some other important biological function that supports, but is not essential to, GBM growth. A recent paper showing that inactive EGFR has a critical role in upregulating glucose transport highlights other unanticipated roles for this receptor (Weihua et al., 2008). Importantly inhibition of EGFR does not block this activity, rather EGFR needed to be removed from the cell surface. This result suggests at least one mechanism by which the presence of inactive EGFR may contribute to GBM development in

a non-critical manner. Indeed, we have very recently shown that de2-7 EGFR has a role in regulating GBM response to low glucose (Cvrljevic et al, *in press*).

EGFR antibodies do not have any efficacy in colon cancer in patients containing mutations in Ras or Raf (Laurent-Puig et al., 2009). While mutations in these molecules are comparatively rare in GBM (McLendon et al., 2008), and thus cannot be used to stratify patients, the pathways associated with Ras/Raf can be activated by other RTKs on the cell surface. The activation of Ras/Raf by these RTKs might cause *de novo* resistance to EGFR therapeutics. Raf can be targeted directly by TKIs already in the clinic, however Ras cannot be inhibited by this approach. However, the signalling of both kinases can be targeted by blocking downstream targets such as ERK and MEK (Pratilas & Solit, 2010). If the toxicity issues can be managed, targeting these kinases in combination with EGFR inhibitors is a logical next step in GBM. This is not a problem in NSCLC as EGFR and ras mutations are mutually exclusive in this cancer type.

Recent data suggests that mutations in PI3K may also cause resistance to EGFR antibodies in colon cancer (Weickhardt et al., 2010). This is highly relevant to GBM as the PI3K pathway appears to be dysregulated in most GBMs through direct mutation of PI3K, the deletion or mutation of the negative PI3K regulator PTEN or activation through other RTKs (McLendon et al., 2008). Indeed, as discussed above, the absence of PTEN was associated with clinical resistance to EGFR TKIs in GBM patients expressing the de2-7 EGFR while responsiveness was associated with co-expression of PTEN and de2-7 EGFR. The recent translation of PI3K inhibitors into the clinic provides a strategy for overcoming PI3K-mediated resistance to EGFR inhibitors. All the evidence suggests that this combinational approach might finally unleash the potential of EGFR inhibitors for the treatment of GBM (Fan & Weiss, 2010); once again management of toxicity remains as a concern.

6.2 Combination of EGFR inhibitors with other targeted therapies

Since most RTKs can activate Ras/Raf and/or PI3K, the dual inhibition of EGFR and additional activated RTKs is an obvious therapeutic approach. Stommel *et al* clearly showed that multiple RTKs can be activated in GBM and that these RTKs activate overlapping downstream pathways causing resistance to TKIs that only targeting a single RTK (Stommel et al., 2007). The RTK c-Met is also commonly activated in GBM through a number of mechanisms and also has an important role in angiogenesis (Abounader & Laterra, 2005). We recently showed that co-expression of de2-7 EGFR and c-Met in GBM xenografts causes therapeutic resistant to single agents directed to either of these RTK. However, the combination of EGFR and c-Met inhibitors produced synergistic anti-tumor activity (Pillay et al., 2009), confirming that dual inhibition of RTKs is a valid approach in GBM. A number of other RTKs have been shown to be activated in GBM including the FGFR family, Axl, ErbB2/3/4, EphA2/7, VEGFR2 and PDGFRα/β (Ren et al., 2007; Pillay et al., 2009). Given the range of potential RTKs activated in a given patient, the most effective therapeutic strategy may have to be determined by screening patient tissues for RTK mutation and/or activation (i.e. phosphorylation) before commencing treatment.

We recently showed that EGFR and Src-family kinases (SFK) are frequently coactivated in GBM (Lu et al., 2009). Furthermore, the de2-7 EGFR physically associated with SFKs and

this interaction increased tumor growth and invasion. This association was also confirmed in clinical samples. Treatment of GBM xenografts with the EGFR antibody mAb 806 and dasatinib, a SFK inhibitor, resulted in synergistic anti-tumor activity compared to either agent alone (Lu et al., 2009). These results suggest that the combination of EGFR inhibition and SFK blockade may be efficacious in GBM. As such, a trial of evaluating the combination of erlotinib and dasatinib has commenced in recurrent GBM (Trial No. NCT00609999).

7. Conclusion

The seemingly central role of EGFR in GBM biology would suggest that it should be an excellent therapeutic target in GBM; clinical trials clearly show that this is not the case when EGFR is targeted alone or in combination with standard GBM therapy. The further development of EGFR inhibitors in GBM must be underpinned by additional studies into the biology of EGFR in this cancer and the identification of signalling events associated with resistance to EGFR therapeutics. On-going rational trials using EGFR inhibitors in combination with other TKIs are justified, possibly underpinned by some basic stratification of patients based on mutations present in tumors. Finally, a prospective study formally proving that therapeutic antibodies do actually enter the GBMs and bind target cells would be informative. In conclusion, inhibition of the EGFR pathway in GBM is ineffective as a therapeutic strategy even when used in combination with current therapies. However, their effectiveness in combination with other targeted therapeutics should form the next stage of their development as a therapeutic target.

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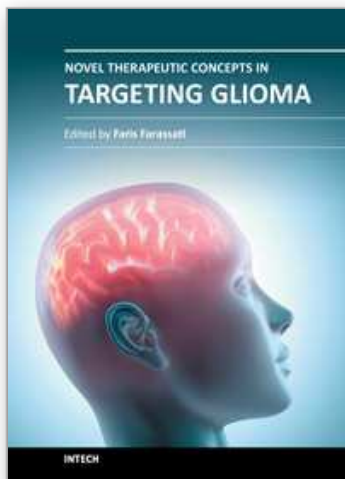
Novel Therapeutic Concepts in Targeting Glioma

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