The Molecular Basis of Resistance to the Antiproliferative Effect of EGFR Inhibition in Human Glioblastoma Multiforme Cell Lines

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1. Introduction

Dysregulated epidermal growth factor receptor (HER1/EGFR) is found in about 50% of glioblastoma, the most common primary brain tumor (Karpel-Massler et al., 2009). Despite recent improvements of the standard of care which currently comprises gross total tumor resection, irradiation and concomitant and adjuvant chemotherapy with temozolomide, the prognosis of patients with this disease remains dismal. A variety of therapeutic strategies was developed in order to improve the clinical outcome of patients with glioblastoma. One such approach involves HER1/EGFR-targeted agents among which small-molecule tyrosine kinase (TK) inhibitors (e.g., erlotinib) represent the clinically most advanced HER1/EGFR-targeted compounds (Halatsch et al., 2006). While experimental studies with erlotinib in the setting of glioblastoma showed promising results (Lal et al., 2002; Halatsch et al., 2004), clinical translation failed to prove a significant benefit (van den Bent et al., 2009). This finding might be partly explained by the fact that erlotinib was shown to exert largely variable antiproliferative effects on different human glioblastoma cell lines in vitro and in vivo (Halatsch et al., 2004). Based on the observation that there is no established correlation between HER1/EGFR baseline expression and erlotinib-induced antiproliferative effects, it seems likely that more complex genetic constellations form the molecular basis of the erlotinib-sensitive and erlotinib-resistant glioblastoma phenotypes. Identification of the molecular pattern determining the erlotinib-resistant phenotype may allow the development of a therapeutic concept to overcome resistance towards erlotinib using multi-targeting that includes those genes that confer resistance. By analyzing a set of erlotinib-sensitive, intermediately responsive and erlotinib-resistant glioblastoma cell lines in an expression analysis of 244 prospectively selected genes whose products, among others, constitute the HER1/EGFR signaling pathway, expression of two genes, FK506-binding protein 14 (FKBP14) and Ras-related C3 botulinum toxin substrate 1 (RAC1) were identified to significantly correlate with the erlotinib-resistant glioblastoma phenotype (Halatsch et al., 2008). Thus, interference with these genes may enhance the antiproliferative efficacy of erlotinib against glioblastoma.
2. HER1/EGFR-targeted therapy in glioblastoma

2.1 HER1/EGFR – a promising target
HER1/EGFR plays an important role in the regulation of diverse cellular functions such as proliferation or differentiation (Wells, 1999). It belongs to the HER family of receptors and contains an extracellular ligand-binding site, a transmembrane portion, and an intracellular tyrosine kinase (TK) domain. Activation of HER1/EGFR is triggered by binding of e.g. epidermal growth factor (EGF) or transforming growth factor-α (TGF-α) to the ligand-binding site. As a consequence, autophosphorylation of specific tyrosine residues within the cytoplasmic catalytic kinase domain of the receptor takes place, initiating further downward signaling via the ras-raf-mitogen-activated protein kinase (MAPK) or the phosphatidylinositol 3-kinase (PI3-K)/Akt pathways (Arteaga, 2003; Scagliotti et al., 2004). Dysregulation of HER1/EGFR was shown to be associated with a variety of neoplastic disorders including glioblastoma (Earp et al., 2003). This finding can be explained by the fact that aberrant alteration of downstream signal transduction results in a shift of the cellular homeostasis towards increased proliferation, tumorigenesis, angiogenesis or invasion and/or inhibition of apoptosis, thus towards a neoplastic cellular phenotype (Halatsch et al., 2006). Gene amplification represents one of the mechanisms that lead to dysregulation of HER1/EGFR-mediated signaling and HER1/EGFR overexpression as shown for 40-50% of glioblastoma (Salomon et al., 1995). Mutational changes of the intrinsic receptor structure constitute another mechanism that may result in uncontrolled HER1/EGFR signaling. The most frequent mutant form of HER1/EGFR, termed EGFR variant III (EGFRvIII), results from an in-frame deletion of 801 base pairs in the DNA sequence encoding the extracellular ligand-binding domain of HER1/EGFR. This truncated receptor variant constitutes approximately 60% of all mutants and is characterized by continuous activation independent of ligand-binding (Frederick et al., 2000; Karpel-Massler et al., 2010).

2.2 HER1/EGFR-targeted small molecule TK inhibitors – preclinical studies
The accumulation of evidence for HER1/EGFR to play a pro-oncogenic role in glioblastoma led to the development of a multitude of HER1/EGFR-targeted therapeutic strategies such as vaccination therapy, HER1/EGFR-targeted antibodies or small-molecule TK inhibitors (Karpel-Massler et al., 2009). In glioblastoma, most clinical experience exists with small-molecule TK inhibitors such as erlotinib. The mode of action of erlotinib is based on binding to the intracellular catalytic TK domain of HER1/EGFR in competition with adenosine triphosphate, thereby inhibiting autophosphorylation of the receptor and further downstream signaling (Halatsch et al., 2006). In preclinical studies, erlotinib was shown to exert a variety of interesting antineoplastic effects in the setting of glioblastoma. Griffiero et al. showed that treatment with erlotinib at a concentration of 5 µM significantly reduced cellular viability of six human glioblastoma-derived tumor-initiating cell lines (Griffiero et al., 2003). This effect was shown to correlate with decreased EGF-induced phosphorylation of HER1/EGFR and subsequent inhibition of the MAPK signaling pathway. In a different study, Lal et al. showed that exposure of transformed D54-MG glioblastoma cells (D54-EGFRvIII) to 20 µM of erlotinib resulted in a significant downregulation of certain genes encoding pro-invasive proteins and to significantly inhibit the invasiveness of D54-EGFRvIII cells (Lal et al., 2002). In addition, Halatsch et al. showed that the extent of erlotinib-mediated inhibition of anchorage-independent growth of glioblastoma-derived cell lines...
correlates inversely with the cellular capability to induce HER1/EGFR mRNA, emphasizing the important role of HER1/EGFR in the pathogenesis of glioblastoma (Halatsch et al., 2004).

2.3 HER1/EGFR-targeted small molecule TK inhibitors – clinical situation

The promising findings shown by experimental studies led to the conductance of several clinical trials examining the effects of erlotinib in the setting of recurrent or newly diagnosed glioblastoma. Erlotinib was shown to fit a reasonable safety profile and was generally well tolerated as shown by phase I clinical trials (Krishnan et al., 2006; Prados et al., 2006). With regard to its clinical efficacy, varying results were derived from early phase clinical trials. In a phase II trial, the effects of erlotinib applied at a dose of 150 mg/d on 42 patients with recurrent glioblastoma and 43 patients with non-progressive glioblastoma following radiotherapy were examined (Raizer et al., 2010). Median overall survival and progression-free survival for the patients with recurrent glioblastoma were reported as 6 months and 2 months, respectively. The patients with non-progressive glioblastoma post radiotherapy reached a median overall survival of 14 months. Thus, erlotinib had minimal efficacy in patients with recurrent glioblastoma and at best slight efficacy in patients with non-progressive glioblastoma post radiotherapy compared to historical controls. Other investigators showed for 48 patients with recurrent glioblastoma who were treated with erlotinib a median overall survival and 6-month progression-free survival that exceeded historical data of patients receiving standard chemotherapy (Yung et al., 2010). However, this study had to be discontinued due to an insufficient number of responses after a planned interim analysis. In a randomized controlled phase II clinical trial, it was finally shown for patients with recurrent glioblastoma that erlotinib monotherapy was inferior to the treatment with temozolomide or BCNU in terms of clinical efficacy (van den Bent et al., 2009). Only 11.4% of the patients treated with erlotinib were free of progression after 6 months compared to 24.1% of the patients in the control group. In addition, no significant differences in median overall survival were found among the different treatment groups.

2.4 Erlotinib and standard radio-/chemotherapy

Several studies were conducted to evaluate whether treatment with erlotinib might provide a clinical benefit when combined with conventional radiochemotherapy. In a phase I/II trial, 89 patients with newly diagnosed glioblastoma were treated with erlotinib at a dose of 150 mg/d starting 1 week prior to fractionated radiotherapy (60 Gy total dose) and temozolomide at a dose of 75 mg/m²/d (Brown et al., 2008). The treatment with erlotinib was continued during radiotherapy and accompanied by up to 6 cycles of temozolomide at a dose of 200 mg/m²/d for 5 days every 4 weeks subsequent to the completion of radiotherapy. Median overall survival was 15.7 months which, however, was not significantly different from that of the radiotherapy plus temozolomide arm from the EORTC 26981/22981-NCIC trial (Mirimanoff et al., 2006).

In a different phase II clinical trial, 65 patients with newly diagnosed glioblastoma or gliosarcoma were treated with erlotinib and fractionated radiotherapy with concomitant and adjuvant temozolomide (Prados et al., 2009). Median progression-free survival and median overall survival were reported as 8.2 months and 19.3 months, respectively, and were thus markedly prolonged when compared to a combined historical control. In contrast,
rather negative results were reported in 27 patients with newly diagnosed glioblastoma that were treated with a maximum dose of 150 mg/d erlotinib and radiotherapy (60 Gy in 30 fractions) with concurrent (75 mg/m²/d for 42 days) and adjuvant (12 four-week cycles comprising each 5 days of 150-200 mg/m²/d) temozolomide (Peereboom et al., 2010). Due to lack of efficacy and unacceptable toxicity, this trial had to be closed preterm. Median overall survival and median progression-free survival were reported as 8.6 months and 2.8 months, respectively. Progressive disease was found in 22 patients (67%). In addition, 4 patients (15%) had an adverse event. Notably, three treatment-related deaths occurred. Based on these results, a combined treatment with erlotinib, temozolomide and radiotherapy appears antagonistic and dangerous.

### 2.5 Erlotinib and other agents

The fact that treatment with erlotinib alone or together with conventional adjuvant therapies does not seem to provide a substantial benefit in this disease emphasizes the necessity for a change of strategy. Combining HER1/EGFR TK inhibitors with other targeted agents represents one such new promising approach.

Inhibition of downstream key regulators such as mammalian target of rapamycin (mTOR) and PI3-K in addition to the treatment with HER1/EGFR TK inhibitors was shown to be favorable in preclinical studies. Combined treatment of phosphatase and tensin homolog deleted on chromosome 10 (PTEN)-deficient U87 and SF295 glioblastoma cells with erlotinib and rapamycin, an mTOR inhibitor, resulted in a significantly increased anti-proliferative effect when compared to cells receiving either agent alone (Wang et al., 2006). Similar results were reported by a different group (Fan et al., 2007). Moreover, in this study, an even more pronounced antiproliferative efficacy was observed when PI3-K was also inhibited by using a dual mTOR/PI3-K inhibitor (PI-103) compared to the treatment with erlotinib and either additional inhibition of mTOR or PI3-K. In the clinical setting, 22 patients with recurrent glioblastoma treated with erlotinib or gefitinib in combination with sirolimus were shown to have a 6-month progression-free survival of 25% (Doherty et al., 2006). However, a phase II clinical trial evaluating the therapeutic efficacy of a treatment with 150 mg/d (450 mg/d in patients on enzyme-inducing anti-epileptic drugs [EIAEDs]) erlotinib and 5 mg/d (10 mg/d in patients on EIAEDs) sirolimus in 32 patients with recurrent glioblastoma reported negligible anti-tumor activity (Reardon et al., 2010). Complete or partial responses were not observed, and median overall survival and median progression-free survival were 33.8 weeks and 6.9 weeks, respectively.

Recently, the clinical efficacy of a combination therapy with erlotinib and bevacizumab was examined in the setting of recurrent high-grade glioma (Sathornsumetee et al., 2010). Twenty-five patients were treated with bevacizumab at a dose of 10 mg/kg i.v. biweekly and erlotinib at a dose of 200 mg/d (500 mg/d in patients on EIAEDs). Overall survival and median progression-free survival were favorable (42 weeks and 18 weeks, respectively). In addition, in nearly half of the patients a radiographic response was observed. However, a similar progression-free survival and radiographic response were derived from historical data of patients receiving single-agent therapy with bevacizumab.

Overall, despite promising results reported by some early phase clinical trials, combining HER1/EGFR TK inhibitors with other agents has so far been not overly successful in the treatment of patients with glioblastoma. Novel targets need to be identified to overcome...
resistance towards HER1/EGFR TK inhibitors and to substantially enhance the latter’s antineoplastic effects by compounds without overlapping toxicity profile.

3. **FKBP14 and RAC1 – two candidate genes for conferring resistance towards erlotinib**

The fact that alterations of HER1/EGFR and its pathway are so frequently encountered in glioblastoma while erlotinib failed in the clinical setting reflects the complex architecture of tumor-driving signaling pathway networks. This phenomenon is highlighted by findings derived from an experimental study showing that erlotinib exerts highly variable antiproliferative effects on different human glioblastoma cell lines independent of baseline HER1/EGFR expression levels (Halatsch et al., 2004). By analyzing HER1/EGFR pathway gene expression profiles of glioblastoma cell lines displaying an erlotinib-responsive, somewhat responsive and erlotinib-resistant phenotype, FKBP14 and RAC1 were identified as candidate genes conferring resistance to erlotinib (Halatsch et al., 2008). This fact may render FKBP14 and RAC1 potential therapeutic targets in the vicinity of the EGFR signaling pathway, and interference with FKBP14 and/or RAC1 may allow enhancing the efficacy of erlotinib against glioblastoma. RAC1 is an important contributor to cell survival in glioma and plays an established role in tumorigenesis as well as in tumor maintenance in this setting (Senger et al., 2002). With respect to FKBP14, less is known about its function. However, data available so far do indicate a tumor-promoting role for its gene product. FKBP1s are encoded by a multigene family and exert anti-apoptotic effects. In addition, FKBP12, a member of this family was reported to be overexpressed in childhood astrocytomas (Khatua et al., 2003). Further studies are warranted to evaluate the potential benefit of a combinatorial therapeutic regime including erlotinib and an inhibitor of RAC1 and/or FKBP14.

4. **Conclusion**

With respect to the treatment of glioblastoma, erlotinib has so far failed to shape clinical practice. A consistent therapeutic benefit could be noted neither when erlotinib was applied alone nor when it was administered in combination with standard radio-/chemotherapy. Recently, the suspicion was raised that erlotinib might even compromise patients’ safety when added to temozolomide and radiotherapy (Peereboom et al., 2010). The discrepancy between the promising results erlotinib achieved in experimental studies and its clinical failure might be partly explained by insufficient penetration of the blood brain barrier and the heterogeneity of tumor cells. In addition, another important factor underlying glioblastoma resistance towards erlotinib could be simultaneous activation of multiple receptor tyrosine kinases (RTKs) with complex converging interactions or other key factors mediating pro-neoplastic signaling. Therefore, interception of HER1/EGFR-mediated signaling by erlotinib might be compensated, leading to maintenance of the cancerous cellular phenotype. Multiple PI3-K-activating RTKs were shown to be simultaneously activated in a variety of different glioma cell lines (Stommel et al., 2007). In this study, shut-down of either the hepatocyte growth factor receptor or HER1/EGFR alone did not significantly inhibit downstream signaling, while the inhibition of both markedly decreased PI3-K activation and cell survival.
In concordance with this notion, two molecular determinants, RAC1 and FKBP14, are overexpressed in glioblastoma cell lines with erlotinib-resistant phenotype, thus representing candidate genes for conferring resistance towards erlotinib. Further studies are under way examining the potential benefit of a multitargeted therapeutic strategy including erlotinib as well as RAC1 and/or FKBP14 inhibitors.

The current clinical situation of patients with glioblastoma does not allow us to take a rest. A continuous search for novel targets and more efficient combination therapies is of ultimate urge and requires all our efforts.

5. References


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Brain Tumors: Current and Emerging Therapeutic Strategies focuses on tumor models, the molecular mechanisms involved in the pathogenesis of this disease, and on the new diagnostic and treatment strategies utilized to stage and treat this malignancy. A special section on immunotherapy and gene therapy provides the most up-to-date information on the pre-clinical and clinical advances of this therapeutic venue. Each chapter in Brain Tumors: Current and Emerging Therapeutic Strategies is authored by international experts with extensive experience in the areas covered.

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