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The TICking clock of EGFR therapy resistance in glioblastoma: Target Independence or target Compensation

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ABSTRACT

Targeted therapy against driver mutations responsible for cancer progression has been shown to be effective in many tumor types. For glioblastoma (GBM), the epidermal growth factor receptor (EGFR) gene is the most frequently mutated oncogenic driver and has therefore been considered an attractive target for therapy. However, so far responses to EGFR-pathway inhibitors have been disappointing. We performed an exhaustive analysis of the mechanisms that might account for therapy resistance against EGFR inhibition. We define two major mechanisms of resistance and propose modalities to overcome them. The first resistance mechanism concerns target independence. In this case, cells have lost expression of the EGFR protein and experience no negative impact of EGFR targeting. Loss of extrachromosomally encoded EGFR as present in double minute DNA is a frequent mechanism for this type of drug resistance. The second mechanism concerns target compensation. In this case, cells will counteract EGFR inhibition by activation of compensatory pathways that render them independent of EGFR signaling. Compensatory pathway candidates are platelet-derived growth factor β (PDGFβ), Insulin-like growth factor 1 (IGFR1) and cMET and their downstream targets, all not commonly mutated at the time of diagnosis alongside EGFR mutation. Given that both mechanisms make cells independent of EGFR expression, other means have to be found to eradicate drug resistant cells. To this end we suggest

Abbreviations: AKT, (PKB) Protein kinase B; ATP, Adenosine triphosphate; Axl, Tyrosine-protein kinase receptor UFO; Bcl-2, B-cell lymphoma 2; BH3, Bcl-2 homology domain 3; BIM, Bcl-2-like protein 11; BRD4, Bromodomain-containing protein 4; CDKN2A, Cyclin-dependent kinase inhibitor 2A; c-Myc, Avian myelocytomatosis virus oncogene cellular homolog; CSC, Cancer stem cell; DRD2, Dopamine receptor D2; ECM, Extracellular matrix; EGFR, Epidermal growth factor receptor; EGFRvIII, Epidermal growth factor receptor variant III; ERBB2, (HER2) Erythroblastic oncogene B 2; ERBB3, (HER3) Erythroblastic oncogene B 3; ERK, (MAPK) Extracellular signal-regulated kinases; FAK, (PTK2) Focal adhesion kinase; FDA, Food and Drug Administration; FGFR, Fibroblast growth factor receptor; GAS6, Growth arrest-specific 6; GBM, Glioblastoma; GNAI2, Guanine nucleotide-binding protein G(i), alpha-2 subunit; HDAC, Histone deacetylase; IGFR-1, Insulin-like growth factor 1; IL-6, Interleukin-6; JNK, c-Jun N-terminal kinase; MEK, (MAPKK) Mitogen-activated protein kinase; MET, (HGFR) Hepatocyte growth factor receptor; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 1; NF1, Neurofibromin 1; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; PDGFR, Platelet-derived growth factor receptor; PDGFRβ, Beta-type platelet-derived growth factor receptor; PI3K, Phosphatidylinositol 3-kinase; PIK3CA, Phosphoinositide-3-kinase, catalytic, alpha polypeptide; PIK3R1, Phosphoinositide-3-kinase regulatory subunit 1; PIP2, Phosphatidylinositol (4,5)-bisphosphate; PIP3, Phosphatidylinositol (3,4,5)-trisphosphate; PKCα, Protein kinase C alpha; PML, Promyelocytic leukemia protein; PTEN, Phosphatase and tensin homolog; Rap1, Ras-proximate-1; RAS, Rat sarcoma viral oncogene homologs; Rb, Retinoblastoma; RET, (Rearranged During Transfection) receptor tyrosine kinase; Rictor, Rapamycin-insensitive companion of mTOR; ROS1, Proto-oncogene tyrosine-protein kinase ROS; RTK, Receptor tyrosine kinase; SFK, Src family kinase; SHH, Sonic hedgehog; shRNA, Short hairpin RNA; SRC, Proto-oncogene tyrosine-protein kinase Src; TCGA, The cancer genome atlas; TERT, Telomerase reverse transcriptase; TK, (RTK) Tyrosine kinase; TNFα, Tumor necrosis factor alpha; TP53, Tumor protein p53; uPA, Urokinase-type plasminogen activator; uPAR, Urokinase-type plasminogen activator receptor; VEGFR, Vascular endothelial growth factor receptor

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rational strategies which include the use of multi-target therapies that hit truncation mutations (mechanism 1) or multi-target therapies to co-inhibit compensatory proteins (mechanism 2).

1. Introduction

GBM is an aggressive and highly diffuse glioma amongst brain tumors. The current standard therapy for GBM patients consists of a maximal safe surgical resection followed by concurrent radiotherapy and temozolomide followed by adjuvant temozolomide (Stupp et al., 2009). GBM remains an incurable disease with a median overall survival of 12–15 months after standard therapy and recurrence is inevitable (Thakkar et al., 2014; Wirsching et al., 2016). The reason for this, as with many cancers, is resistance to therapy. Over the years advances have been made in the identification of molecular targets and the development of targeted drugs such as small-molecule- and antibody inhibitors in many tumor types (Padma, 2015). However, early clinical data of these agents in GBM have been largely disappointing, as only a small percentage of the patients show clinical benefit from these targeted therapies (Prados et al., 2009; Reardon et al., 2010; Sathornsumetee et al., 2010). The reason for this is that patients who initially respond well to targeted therapy eventually acquire drug resistance over time (Masui et al., 2013; Sierra et al., 2010). Also, a subset of GBM patients does not respond to targeted therapy at all, demonstrating intrinsic resistance to therapy (Ramirez et al., 2013). Here we provide a comprehensive list of cellular responses that contribute to resistance to EGFR-targeted therapy in GBM. We describe the various underlying mechanisms that have been shown to confer resistance to EGFR-targeted therapy in GBM and propose modalities to overcome them in the near future.

2. Targeting the EGF receptor in GBM

Recent advances in our understanding of the molecular pathology of gliomas have identified various common genetic alterations. This resulted in the disruption of key signaling pathways that drive gliomagenesis and disease progression. The Cancer Genome Atlas (TCGA) contains a series of genomic, epigenomic, transcriptomic and proteomic analyses of more than 500 GBM tumors, thereby providing a comprehensive molecular characterization of GBM. Targeted therapy has become the desired approach against specific driver mutations in many tumor types (Burger et al., 2011; T.C.G.A Network et al., 2008).

The epidermal growth factor receptor (EGFR) is most often mutated in GBM (26%) and is thereby considered an important target. The deletion of exons 2–7 of the EGFR gene is another common genetic aberration in GBM. This deletion results in a constitutively active, ligand-independent mutant known as EGFRvIII. EGFR mutations in GBM such as the vIII, R108 K, A289 V, G598 V are extracellular domain mutations that have been shown to be poorly inhibited by EGFR inhibitors that target the active kinase conformation (e.g. erlotinib) (Aljohani et al., 2015; Chang et al., 2015; Lee et al., 2006; Vivanco et al., 2012; Wee and Wang, 2017). This is in contrast to lung cancers that similar to GBM, frequently show kinase domain mutations such as in EGFR and are sensitive to several of these small molecule inhibitors (Bonavida and Tivnan, 2016; Huang and Fu, 2015). For example, the T790 M mutation which enhanced the affinity of the adenosine triphosphate (ATP) binding pocket for ATP, thereby preventing its binding to the inhibitor (Pao et al., 2005; Wu et al., 2016), is seen in lung cancers but not in gliomas. However, other secondary mutations, such as D761Y and T854 A, have also been associated with acquired resistance to EGFR inhibition in lung cancer.

3. Definition of two types of therapy resistance mechanisms

Due to the EGFR mutations that occur GBM as mentioned earlier, many therapies against this target were developed. These therapies included small-molecule tyrosine kinase inhibitors (TKIs), antibody-drug conjugates (ADCs), vaccination, and Chimeric antigen receptor (CAR) T-Cell approaches. These approaches were mainly developed to target EGFRvIII, the most common mutation of EGFR in GBM. Unfortunately, most strategies used so far have shown disappointing clinical results (Eskilsson et al., 2017; Gao et al., 2018; Padfield et al., 2015). In recent years, a large number of studies have been performed to decipher and elucidate the various molecular mechanisms underlying resistance to EGFR inhibition (Table 1). The two main mechanisms of resistance to EGFR inhibitors are (a) target independence through alterations in structure or expression of the drug target, and (b) target compensation where alternative signaling pathways are activated. These mechanisms are shown in Fig. 1 and explained in detail below.

3.1. Mechanism 1: target independence

Target independence after small molecule therapy. In 2014, Nathanson et al., discovered that the dynamic regulation of EGFRvIII expression by small circular extra-chromosomal DNA fragments can mediate resistance to EGFR inhibition in GBM. The authors demonstrated that erlotinib-treated GBM cells reversibly suppress mutant EGFR from extrachromosomal DNA, rendering GBM cells resistant to EGFR inhibition. Upon drug withdrawal, EGFR mutations re-emerge on extrachromosomal DNA, resulting in the upregulation of EGFRvIII and the re-sensitization of GBM cells to erlotinib-induced cell death. Furthermore, treatment with lapatinib, another EGFR inhibitor, showed a clear reduction in the EGFRvIII copy number in 6 patients (Nathanson et al., 2014).

Target independence after CAR T therapy. In a recent study, O'Rourke et al., studied CAR T cells directed against the EGFRvIII which were administered to patients with EGFRvIII expressing recurrent GBMs. The authors observed loss or decreased expression of EGFRvIII in tumors resected after CAR T infusion. Apart from the loss of antigen containing cells, the infusion of CAR T-EGFRvIII cells also resulted in a more immunosuppressive tumor microenvironment. The authors suggested that targeting other antigens, in addition to EGFRvIII, using CAR T cells in combination with drugs that target immunosuppressive molecules, might improve the clinical efficacy of this therapy. Analysis of recurrent GBM has shown loss of this mutation in 50% of these tumors prior to EGFR targeted therapy. This might explain the failure of the CAR T vaccine (van den Bent et al., 2015; Wang et al., 2016; Weller et al., 2017).

3.2. Mechanism 2: target compensation

Target compensation through downstream signaling via bypass pathways. In a phase II trial, the activation of the EGFR/PI3K pathway in recurrent GBM under treatment with the EGFR inhibitor gefitinib was evaluated. While EGFR was efficiently dephosphorylated in gefitinib-treated patients, no significant effect was detectable on downstream pathway components (Hegi et al., 2011). Concurrent activation of multiple RTK families is common in GBM in response to EGFR inhibition including erythroblastic leukemia viral oncogene homolog (ERBB)2, ERBB3, hepatocyte growth factor receptor (HGFR/MET), platelet-derived growth factor receptor (PDGFR), insulin-like growth

Table 1
Molecular mechanisms underlying resistance to EGFR-inhibition in GBM and lung cancer.

Target	Drug(s)	Cancer type	Resistance mechanism	Reference	Overcoming resistance mechanism	Resistance gene	Comutation* with target #	Comutation with target %	Acquired/ intrinsic**
Mechanism: Target independence									
EGFR	Erlotinib	GBM	Loss of EGFRvIII extrachomosomal DNA	Nathanson et al., 2014	Pulsatile intermittent EGFR inhibition	N/A	N/A	N/A	N/A
EGFR	CART-EGFRvIII cells	GBM	Loss of EGFRvIII, Immunosuppression	O'Rourke et al., 2017	Not tested	N/A	N/A	N/A	N/A
Mechanism: Target compensation									
EGFR	Erlotinib, Gefitinib, Afatinib	NSCLC	T790 M EGFR mutation	Pao et al., 2005; Wu et al., 2016	Third generation EGFR TKIs (mutation-specific)	N/A	N/A	N/A	N/A
EGFR	Erlotinib	GBM	EGFRvIII upregulation	Schulte et al., 2013	EGFR + PI3K/p110δ inhibition	PTEN	26	4.3	Partly intrinsic
EGFR	Cetuximab	GBM (CSCs)	ERBB2/ERBB3 signaling	Clark et al., 2012	EGFR + ERBB2 inhibition	ERBB2	0	0.0	Aquired
EGFR	Panitumumab	GBM	ERBB4 activation	Donoghue et al., 2018	Pan-ERBB inhibition	ERBB3	6	1.0	Partly intrinsic
						ERBB4	0	0.0	Aquired
EGFR	Gefitinib	GBM	MET activation	Jun et al., 2012	EGFR + MET inhibition	MET	1	0.2	Aquired
EGFR	Erlotinib, Lapatinib	GBM	PDGFRβ upregulation	Akhavan et al., 2013	EGFR + PDGFRβ inhibition	PDGFR beta	0	0.0	Aquired
EGFR	AG1478	GBM	IGFR-1 upregulation	Chakravarti et al., 2002	EGFR + IGFR-1 inhibition	IGFR-1	1	0.2	Aquired
EGFR	Gefitinib	GBM	ROS1 upregulation	Aljohani et al., 2015	EGFR + ROS1 inhibition	N/A	N/A	N/A	N/A
EGFR	Erlotinib	GBM	TNF upregulation	Guo et al., 2017	EGFR + TNF/JNK/Axl/MAPK inhibition	TNF(RSF1B)	0	0.0	Aquired
						TNF(SF9)	2	0.3	Aquired
EGFR	Iressa	GBM	Notch signaling	Staberg et al., 2016	EGFR + Notch inhibition	NOTCH2-4	3	0.5	Aquired
EGFR	AG1478	GBM	DRD2 signaling	Li et al., 2014	EGFR + DRD2 inhibition	DDR2	1	0.2	Aquired
EGFR	Erlotinib, Gefitinib	GBM	Loss/mutation of PTEN	Mellinghoff et al., 2005; Wang et al., 2006	EGFR + mTOR inhibition	PTEN	26	4.3	Partly intrinsic
EGFR	Erlotinib, Dasatinib	GBM	PTEN Y240 phosphorylation	Fenton et al., 2012	EGFR + FGFR or SFK inhibition	FGFR1-4/PRAG1	13/2	2.2/0.3	Partly intrinsic
EGFR	Erlotinib	Lung Cancer	Loss of NF1	de Bruin et al., 2014	EGFR + MEK inhibition	NF1	4	0.7	Aquired
EGFR	Erlotinib	GBM	Rictor acetylation	Masui et al., 2015	EGFR + mTORC2 or MyC inhibition	PTEN	26	4.3	Partly intrinsic
EGFR	Gefitinib	GBM	uPA upregulation	Wykosky et al., 2015	EGFR + MEK inhibition or BH3 mimetics	MAP2K2 (MEK2)	4	0.7	Aquired
EGFR	Gefitinib, Erlotinib, Lapatinib	GBM	IL-6 upregulation	Zanca et al., 2017	EGFR + NF-κB/BRD4/survivin inhibition	IL6R/NFKB1,2;REL,A,B/ BRD4/BIRC5 (survivin)	2/3/2/0	0.3/0.5/0.3/0.0	Partly intrinsic
EGFR	PD153035	GBM (CSCs)	β1-integrin signaling	Srikanth et al., 2013	EGFR + FAK inhibition	B1-integrin (ITGB1 + 3)	1	0.2	Aquired
EGFR	Erlotinib	GBM	PML upregulation	Iwanami et al., 2013	EGFR + PML inhibition	PML	0	0.0	Aquired
EGFR/RTKs	ZD6474	GBM	Induction of autophagy	Shen et al., 2013	EGFR + autophagy inhibition	N/A	N/A	N/A	N/A
EGFR	Erlotinib	GBM (CSCs)	Hedgehog signaling	Eimer et al., 2012	EGFR + SHH inhibition	SMO/Ptch1-2	0/2	0/0.4	Aquired
EGFR	BMS-599626, Gefitinib, others	GBM	NRG1 upregulation	Lee et al, 2018	NRG1 inhibition	NRG1	0	0.0	Aquired

GSCs is defined here as GBM cells with stem-like properties.
* in a TCGA cohord of 604 GBM or 566 Lung cancer patients.
** partially intrinsic is defined as comutated with EGFR in at least 1% of all patients.
NA, not applicable.

factor 1 (IGFR-1) and proto-oncogene tyrosine kinase (ROS1); these contribute as compensatory downstream signaling pathways (Padfield et al., 2015; Stommel et al., 2007). In vitro experiments in GBM cells expressing EGFRvIII were intrinsically more resistant to the EGFR inhibitor gefitinib than GBM cells expressing wild-type EGFR. Although increasing the dose and exposure to gefitinib showed inhibition of EGFRvIII phosphorylation, downstream signaling by Akt and DNA synthesis remained unaffected. The difference between wild type EGFR sensitivity and EGFRvIII resistance to gefitinib might be a result of differential regulation of Akt and other signaling pathways (Learn et al., 2004). Schulte et al. (2013) demonstrated that resistance to erlotinib in GBM cells was associated with a strong upregulation of EGFRvIII and knockdown of EGFRvIII re-sensitized drug-resistant cells to erlotinib. Upregulation of EGFRvIII induced the expression of the regulatory subunit of PI3K (PI3Kp110δ) and subsequently activated the PI3K pathway, thereby mediating resistance to EGFR inhibition (Schulte et al., 2013). Specific inhibition of p110δ through CAL-101 or possibly other mediators of PI3K pathway, in combination with EGFR inhibition, may overcome resistance to EGFR inhibition in GBM with the EGFRvIII mutation.

In 2012, Clark et al., reported that GBM cells with stem-cell like properties (GBM CSCs) exhibited intrinsic resistance to EGFR inhibition. This was mediated by the compensatory activation of other members of ERBB receptor family, namely ERBB2 and ERBB3. In this study, multiple GBM CSC lines were continuously proliferating and maintained stem-cell like properties despite EGFR signal inhibition. Clark et al., went on to demonstrate that downstream activation of the PI3K and RAS pathways remained active in GBM CSCs through the activation of ERBB2 and ERBB3. The authors also observed that in contrast to the EGFR inhibitor cetuximab, the dual inhibitor lapatinib targeting EGFR and ERBB3 was significantly more effective at decreasing downstream signaling and preventing proliferation of GBM CSCs (Clark et al., 2012). Similarly, Donoghue et al., reported that compensatory activation of ERBB4 by the ligand heregulin-1β also promotes resistance to EGFR-targeted therapy. The authors also demonstrated that compared to EGFR inhibitors, pan-ERBB inhibitors (e.g. dacomitinib) were more efficient and prevented recurrence in GBM xenograft models (Donoghue et al., 2018).

In response to EGFR inhibition, activation of the MET receptor tyrosine kinase has been shown to maintain downstream signaling in GBM. Jun et al. (2012) reported that in vivo experiments, transcriptional activation of MET was associated with resistance to EGFR inhibition in GBM (Jun et al., 2012). This finding is supported by previous studies that showed the inhibition of MET by SU11274 in combination with EGFR inhibition, that enhanced cytotoxicity and was necessary to disrupt downstream PI3K signaling in GBM cells (Huang et al., 2007; Stommel et al., 2007). EGFRvIII is known to suppress PDGFRβ expression via mTORC1 and extracellular signal-regulated kinases (ERK)-dependent mechanisms. However, EGFR inhibition caused de-repression of PDGFRβ, rendering GBM more dependent on PDGFRβ signaling for growth and survival. Consequently, combined inhibition of EGFR witherlotinib, and PDGFRβ by AG1295, resulted in a more potent anti-proliferative activity in GBM cells than either inhibitor alone (Akhavan et al., 2013).

IGFR-1 signaling is another compensatory pathway through which GBM cells can become resistant to EGFR-targeted therapy. Chakravarti et al., reported that treatment with the EGFR inhibitor AG1478 in GBM cell lines resulted in increased expression of IGFR-1. Dual-targeting of IGFR-1 and EGFR with the inhibitor AG1024 not only enhanced cytotoxicity but also reduced the invasive potential of GBM cells (Chakravarti et al., 2002). ROS1 is another compensatory pathway that has also been implicated in mediating drug resistance to EGFR inhibition in GBM. Aljohani et al., demonstrated that in gefitinib-resistant clones of U87 GBM cells, ROS1 was highly expressed at both the mRNA and protein levels. Treatment with a ROS1 inhibitor re-sensitized these cells to gefitinib, confirming that the acquired resistance was mediated by ROS1. Dual-targeting of EGFR and ROS1 resulted in an efficient inhibition of downstream signaling as well as a significant increase in cell death via apoptosis (Aljohani et al., 2015).

Target compensation through alternative pathways. In a recent study, Guo et al., revealed the role of the tumor necrosis factor alpha (TNFα) - c-Jun N-terminal kinase (JNK) - tyrosine-protein kinase receptor UFO (Axl) - MAPK signaling pathway in mediating intrinsic resistance to EGFR inhibition in GBM cells. They demonstrated that inhibition of EGFR, both wild-type and mutant, triggered a rapid adaptive response driven by increased production and secretion of TNFα. Increased levels of TNFα leads to the activation of JNK, which in turn increases the expression of the ligand growth arrest-specific 6 (GAS6) for the Axl receptor. Axl signaling is known to activate MAPK, which is a key downstream effector of the RAS pathway. Interruption of this adaptive pathway by the inhibition of either TNFα, JNK, Axl or MAPK in combination with an EGFR inhibitor showed that resistant GBM cells became sensitive to EGFR inhibition (Guo et al., 2017).

Cross-talk between EGFR and the Notch signaling pathway has also been observed in GBM. For example, EGFR gene amplification in GBM is correlated with an overexpression of notch-regulated genes and conversely the activation of Notch signaling has showed an upregulation of EGFR through a p53-depentent mechanism. Similar to EGFR, Notch signaling has been linked to both the PI3K and RAS signaling pathways. Staberg et al., reported that dual-targeting of EGFR and the Notch pathway increased the sensitivity of GBM cells compared to monotherapy. Hence, the authors demonstrated that the enhanced anti-proliferative effects of dual-therapy were a result of more effective inhibition of the PI3K and RAS pathways. This suggests that activation of the Notch pathway can confer resistance to EGFR inhibition by maintaining compensatory downstream signaling (Staberg et al., 2016). Genome-wide small hairpin RNA (shRNA) screens are used to identify critical effectors and pathways mediating resistance in cancer. These screens were also applied to study GBM resistance to EGFR inhibition. It was discovered that dopamine receptor D2 (DRD2) signaling also contributed to the proliferation of GBM cells. Compared to non-neoplastic cerebrum, GBM was shown to have an increased expression of DRD2, both at the mRNA and protein levels suggesting the presence of mitogenic signaling between EGFR and DRD2 (Li et al., 2014). In vitro as well as in vivo experiments with a dual-targeted therapy of DRD2 and EGFR inhibitors (haloperidol and AG1478, respectively) resulted in synergistic growth inhibition and MAPK suppression. These findings

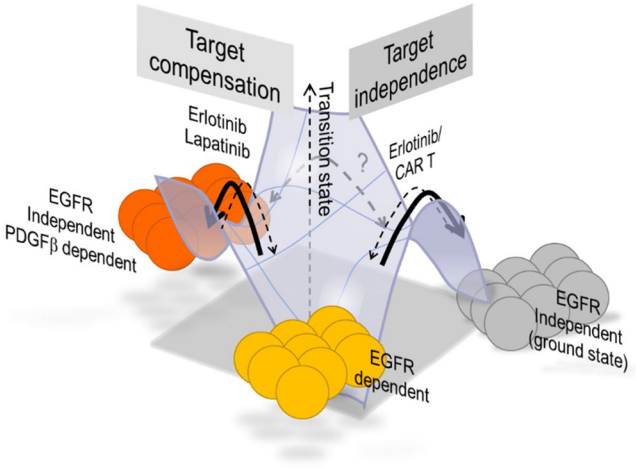

Fig. 1. The two main types of resistance mechanisms to EGFR inhibition. Populations of cells within a tumor could either be EGFR sensitive (yellow), or EGFR insensitive through EGFR independence (grey) or through upregulation of a redundant pathway such as the PDGFβ pathway (orange) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

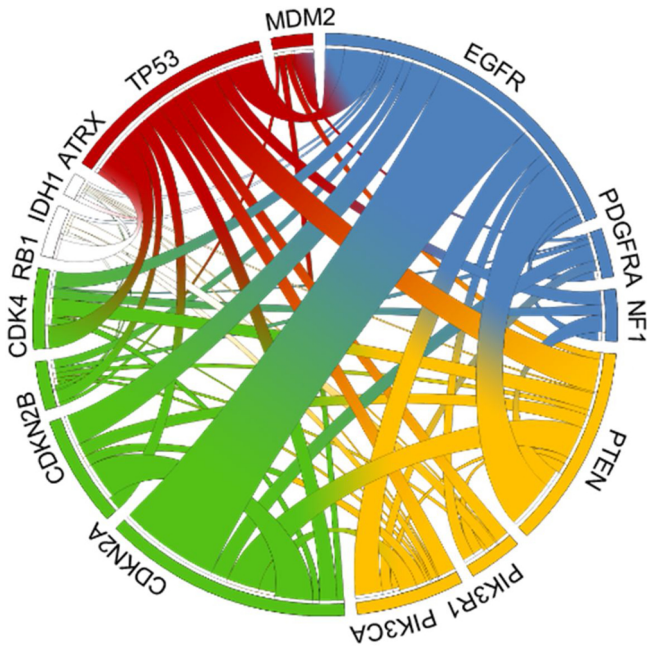

Fig. 2. Circos plot showing the relative frequencies of co-mutations in GBM. Note that many GBM tumors are EGFR mutated/amplified in combination with CDKN2 A and PTEN mutations (Source: The Cancer Genome Atlas, TCGA, Brennan et al., 2013).

suggest that DRD2 activity can maintain the downstream signalling pathway of RAS through guanine nucleotide-binding protein G(i), α-2 subunit (GNAI2) - ras-proximate-1 (Rap1) and MAPK regulation. (Li et al., 2014).

Target compensation through alterations of downstream effectors/repressors. The tumor suppressor PTEN negatively regulates the PI3K pathway by the dephosphorylation of PIP3 to phosphatidylinositol (4,5)-biphosphate (PIP2). In 40–50% of GBM cases there is a loss of PTEN that limits effective treatment against GBM via EGFR inhibition. In the analysis of recurrent malignant gliomas treated with EGFR inhibitors, loss of PTEN significantly correlated with poor response to EGFR inhibition. Drug resistance was mediated by the uncoupling of EGFR inhibition from downstream inhibition of the PI3K pathway via Akt dependent and independent mechanisms. (Mellinghoff et al., 2005). In a subsequent study, Wang and colleagues demonstrated that inhibition of mTOR restores sensitivity of PTEN-deficient gliomas to erlotinib. In addition a dual-target inhibitor of EGFR and mTOR, rapamycin, effectively inhibited the downstream PI3K signaling pathways as well as tumor growth (Wang et al., 2006). Furthermore, Fenton et al., showed that in GBM expressing wild-type PTEN, fibroblast growth factor receptor (FGFR)/Src-family kinase (SFK)-mediated inactivation of PTEN can occur by preventing the phosphorylation of the tyrosine residue Y240 in this protein. These findings suggest the PI3K signaling pathway has a critical role in resistance to EGFR inhibitors in GBM (Fenton et al., 2012).

Loss of function of the NF1 gene can also induce intrinsic resistance to EGFR inhibitors via the downstream RAS signaling pathway. This has previously been shown in lung cancer where reduced expression of NF1 caused resistance to the EGFR inhibitor, erlotinib. Future studies in GBM could evaluate the relationship between NF1 mutation and resistance to EGFR inhibitors (de Bruin et al., 2014).

Next to playing a pivotal role in tumorigenesis and cancer progression, reprogramming of cellular metabolism has also been shown to promote resistance to therapy. It is known that glucose or acetate can promote EGFRvIII signaling through mTORC2 in GBM. This is caused by the acetylation of the core subunit of mTORC2, Rictor. Once Rictor is activated by the upstream signaling pathway RTK, mTORC2 forms an auto-activation loop. The auto-activation loop can be formed by promoting glucose uptake and acetyl-coA production through its downstream effector c-Myc. Next to this it can also be formed by an inactivating class IIa histone deacetylase (HDAC), which regulates Rictor hence suppressing mTORC2. Inactivation of HDAC occurs via protein kinase C alpha (PKCα)-mediated phosphorylation and mTORC2 is allowed to promote intrinsic resistance in GBM cells. Deacetylation of Rictor by the introduction of a class IIa HDAC construct to GBM cells sensitized the cells to EGFR-target therapy (Masui et al., 2015).

Target compensation through survival pathways. The suppression of B-cell lymphoma 2 (Bcl-2)-like protein 11 (BIM) via urokinase-type plasminogen activator (uPA) and urokinase-type plasminogen activator receptor (uPAR) signaling was identified as a mechanism of acquired resistance to EGFR inhibition. Furthermore, increased signaling through uPAR activates the MEK/MAPK pathway. This plays an important role in mediating uPAR-dependent anti-apoptotic signaling by suppressing BIM via phosphorylation and subsequent degradation. Inhibiting MEK or treatment with a Bcl-2 homology domain 3 (BH3)-mimetic drug will counteract the activity of anti-apoptotic Bcl-2 family members. Treating EGFR inhibition-resistant GBM cells with a MEK inhibitor or BH3-mimetic drug showed restored sensitivity (Wykosky et al., 2015).

Activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling via Interleukin-6 (IL-6) has also been shown to confer resistance to EGFR inhibition. IL-6 secretion by EGFRvIII-positive GBM cells activates NF-κB signaling, resulting in a Bromodomain-containing protein 4 (BRD4)-dependent expression of the pro-survival protein survivin (BIRC5). Additionally, the inhibition of NF-κB, BRD4 or BIRC5 restored sensitivity to EGFR inhibitors in vivo GBM models (Zanca et al., 2017).

Srikanth et al., identified another resistance mechanism to EGFR inhibitors in GBM CSCs. This was mediated via focal adhesion kinase (FAK) activation by β1-integrin. β1-integrin is a transmembrane protein that facilitates extracellular matrix (ECM) adhesion. Apart from sharing common receptor downstream signaling elements with EGFR, such as PI3K and RAS, β1-integrin signaling can also directly activate EGFR in a ligand-independent manner. In this study, the authors showed that β1-integrin is highly expressed in GBM CSCs and that its expression correlates with poor survival. More importantly, the authors also demonstrated that overexpression of β1-integrin renders GBM CSCs resistant to EGFR inhibition. Moreover, combined inhibition of EGFR and FAK overcomes this resistance mechanism through an enhanced induction of apoptosis (Huang and Fu, 2015; Srikanth et al., 2013).

EGFR inhibitors, which block downstream mTOR signaling, are known to activate the expression of promyelocytic leukemia protein (PML) in GBM. In turn, PML contributes to resistance to EGFR inhibitors by preventing cell death. Next to negatively regulating downstream effectors of the PI3K pathway, PML has also been shown to repress the transcriptional activity of the EGFR promoter. Furthermore, abrogation of PML expression by gene silencing sensitized GBM cells to erlotinib-induced cell death, through inhibition of Akt/mTOR signaling (Iwanami et al., 2013).

Shen et al., demonstrated that inhibition of EGFR induced autophagy through PI3K/Akt/mTOR pathway as an adaptive response, along with vascular endothelial growth factor receptor (VEGFR) and RET TKs. This contributed to resistance to EGFR inhibition in GBM. Autophagy is a lysosomal degradation pathway that allows the recycling of cellular components to sustain cellular metabolism under deprivation conditions. Therefore, combined therapy by inhibiting EGFR, VEGFR and RET TKs together with an inhibitor against autophagy caused significant tumor growth regression in GBM xenograft models, compared to monotherapy (Shen et al., 2013).

The sonic hedgehog pathway (SHH signaling), which plays a crucial role during embryonic development, is activated in GBM CSCs and has been shown to increase their viability. Targeting EGFR alone did not have an effect on the self-renewal capacity of sphere-generating GBM

CSCs. Combined inhibition of EGFR with erlotinib, and the SHH signaling with cyclopamine, abrogated their sphere-initiating ability and enhanced the anti-proliferative effects of erlotinib on GBM CSC proliferation (Eimer et al., 2012).The existence of multiple mechanisms underlying resistance to EGFR inhibition has been described and the current challenge is to implement this knowledge to overcome resistance to EGFR-inhibitors and improve the clinical outcome for GBM.

Target compensation through co-mutations. Resistance to EGFR inhibition can either be activated at the time of the administration of the therapy or induced by the therapy itself (Asić, 2016). The genes or pathways that are causally involved in compensatory resistance might already have been mutated in primary tumors at the time of diagnosis. To analyze this, the genetic information present in the TCGA database (Ceccarelli et al., 2016) is useful and showed that only in a fraction of cases such mutations were present (Table 1). This indicates that target-compensation through mutations is quite uncommon at the time of diagnosis, however, subpopulations of cells that contain mutations leading to resistance could be preferentially expanded through cellular/clonal selection in due course, giving rise to selection of therapy resistant tumor clones.

3.3. FUTURE PERSPECTIVES: Overcoming Resistance of EGFR therapy

Avoiding target independence through dosing/epigenetic therapy. Tumor heterogeneity could be the molecular mechanism underlying target independence as different levels of EGFRvIII occur frequently within the tumor. EGFRvIII-mediated signaling could be required for tuning the tumor to the microenvironment and nutrient supply (Nathanson et al., 2014). Spatiotemporal epigenetic repression and extrachromosomal stochastic distribution could fine tune these requirements on a local level. This provides a source of heterogeneity throughout the tumor. This would argue that in the absence of therapy pressure, an equilibrium could be established leading to sub-fractions of cells that express and are dependent on EGFRvIII. In order to overcome this phenomenon, re-sensitization of tumors using drug scheduling could be a plausible approach (Nathanson et al., 2014). Pulsatile intermittent treatment with higher doses of an EGFR-inhibitor or a “drug holiday” can result in a more efficient inhibition of the target, delay of therapy resistance and reduction in toxicity compared to continuous dosing. In addition, it might be possible to target the mechanisms that drive target compensation through the use of drugs that affect epigenetic adaptation (Liau et al., 2017).

Avoiding target independence through targeting truncation mutations. In addition to this strategy to counter target independence, common driver mutations that frequently co-occur with EGFR mutation/gene amplification might provide good targets for combination therapy (Fig. 2). Important candidates for co-targeting are cyclin-dependent kinase inhibitor 2 A (CDKN2 A) (molecular target: cyclin-dependent kinase (CDK) 4/6) and PTEN (molecular target: PI3K). For targeting CDK4/6 the FDA-approved oral drugs palbociclib, abemaciclib, and ribocyclib (Asghar et al., 2015; Fry et al., 2004; Toogood et al., 2005) may be considered. Of these drugs, abemaciclib has been shown to have relatively improved blood brain barrier crossing capabilities (Heffron, 2016). In combination with PI3K inhibition, inhibitors undergoing advanced clinical trials include GDC-0084, buparlisib, PX-866, pilaralisib and XL765 (Burger et al., 2011; Heffron et al., 2016; Ihle et al., 2004; Yu et al., 2014); of these drugs, GDC-0084 may be preferred due to its’ relative good blood brain barrier crossing capabilities (Heffron et al., 2016).

Avoiding target compensation through multitarget therapy. As can be observed from the summary of resistance through signaling compensation, combining EGFR inhibitors with inhibitors that target resistance-causing or other downstream molecules can lead to more efficient therapies as compared to EGFR-targeted monotherapy (Table 1). It is known that kinase inhibitors have the ability to bind to many protein kinases (Anastassiadis et al., 2011; Davis et al., 2011; Klaeger et al., 2017). The consequence of this promiscuous behavior has only been exploited retrospectively (i.e. evidence based) but not prospectively (though combination of available bio-activity data). Simultaneous targeting of multiple mutated kinases by a single drug can lead to more effective therapies. However, this polypharmacology approach has not been explored.

In addition to polypharmacological small molecule therapy, polytarget therapies such as antibody drug conjugates (ADCs) and bispecific CAR-T cells might provide excellent strategies to move forward. Several GBM specific epitopes have already been identified for this purpose (such as EphA2 and IL13RA2, Yi et al., 2018; Bielamowicz et al., 2018; Chow et al., 2013; Hatano et al., 2005; Krenciute et al., 2016). In all these cases of multi-target therapies, the most optimal balance between efficacy and toxicity should be determined.

4. Final remarks

Given the multiplicity of distinct drug resistance mechanisms and their possible combinations, it will remain a challenge for the near future to tune inhibition of multiple targets that are necessary for effective EGFR therapies. The use of multi-targeted therapies might enable such a strategy by selecting the desired combinations of targeted therapeutics in individual tumors. These efforts require an integrative approach to define common vulnerabilities that can be matched with the most optimal blood brain barrier-crossing therapies and biomarkers that can predict therapy outcome. These efforts should lead to novel, rational and more effective and personalized therapeutic strategies for the treatment of GBM.

Conflict of interest disclosure

The authors made no disclosures.

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