

Epidermal growth factor receptor in glioblastoma (Review)

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Abstract. Mutations in the epidermal growth factor receptor (EGFR) are commonly occurring in glioblastoma. Enhanced activation of EGFR can occur through a variety of different mechanisms, both ligand-dependent and ligand-independent. Numerous evidence has suggested that EGFR is overexpressed in most of primary glioblastomas and some of the secondary glioblastomas and is characteristic of more aggressive glioblastoma phenotypes. Additionally, recent studies have revealed that wild-type EGFR, and to a greater extent hyper-activating EGFR mutants induced a substantial upregulation of Fyn expression. Furthermore, it was determined that Fyn expression is upregulated across a panel of patient-derived glioblastoma stem cells (GSCs) relative to normal progenitor controls. Moreover, researchers are continuously involved in elucidation of novel mechanism linking EGFR EGFR-expressing glioblastoma. The present review highlights current aspects of EGFR receptor in glioblastoma and concludes that the concept of EGFR signaling and related receptors and associated factors is evolving, however, it needs detailed evaluation for future clinical applications in cancer patients.

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1. Introduction

Epidermal growth factor receptor (EGFR), also referred to as HER1/ErbB1, belongs to a larger family of ErbB receptors with tyrosine kinase activity (1,2). Other members of the HER family include ErbB2/HER2, ErbB3/HER3 and ErbB4/HER4. EGFR is frequently overexpressed and/or hyper-activated in human malignancies, including glioblastoma, and therefore EGFR-directed therapeutic strategies are often utilized. Increased activation of EGFR can occur through a variety of different mechanisms, both ligand-dependent and ligand-independent (3-5). Among these mechanisms include: Aberrant enhancement of ligand production; constitutive receptor activation by multiple exon deletion or missense mutations (6); crosstalk with other receptors; increased receptor protein level via gene amplification; and malfunction in receptor degradation. EGFR overexpression and activation are known to significantly impact cancer cell hallmark traits, such as increased cell survival, proliferation and invasion (7) (Fig. 1).

2. EGFR alterations in glioblastoma

EGFR is overexpressed in ~60% of primary glioblastomas versus only 10% of secondary glioblastomas and is characteristic of more aggressive glioblastoma phenotypes. In addition to overexpression, several alternative mechanisms account for aberrant induction of EGFR activation in glioblastoma, including enhanced autocrine expression of cognate ligands (8). Gene amplification and mutation of EGFR also enhance EGFR activation and occur in upwards of 57% of glioblastomas as determined by the TCGA dataset (9). From a subtype perspective, classical glioblastoma are synonymous with focal amplification of EGFR (~95%), whereas mesenchymal, neural and proneural glioblastomas are associated with reduced rates of EGFR amplification at 29, 67 and 17%, respectively. Mutations of EGFR occur in roughly one-third of all classical tumors and often in mesenchymal, proneural and neural glioblastomas as well (10). Of these mutations, extracellular domain EGFR mutations are most commonly observed in glioblastoma (11).

The most frequently occurring EGFR mutation in glioblastoma, EGFR Δ III, arises from an in-frame deletion of 801 bp in the DNA sequence encoding the extracellular domain, rendering a truncated yet constitutively active form of the receptor (12) EGFR Δ III is a cancer specific mutation, as it

not detected in normal tissues, making it an attractive target for therapeutic intervention. Several different studies (12-14) have indicated that EGFR Δ III is expressed in ~50% of glioblastomas that amplify wild-type EGFR (13). Additionally, data mined from the TCGA indicates that EGFR Δ III is most commonly present in the classical tumors (23%), where EGFR amplification is most prevalent.

Despite being constitutively active, EGFR Δ III sustains a low-level signal capable of evading internalization and downregulation, which primarily result from inefficient dimerization (14). In contrast, wild-type EGFR is rapidly degraded following acute stimulation with ligand (15). Though low-level in nature, constitutive signaling downstream of EGFR Δ III leads to increased glioblastoma cell survival *in vivo* through selective augmentation of various mitogenic factors, namely Akt and repression of apoptosis via enhanced Bcl2 family member expression (16).

EGFR Δ III has also been associated with transformative properties, as INK4A/Arf depleted astrocytes and neural stem cells form high grade tumors *in vivo* when expressing EGFR Δ III (17). Given this, EGFR Δ III may act as a critical initiating event in tumor development. Not only is EGFR Δ III likely an important factor in gliomagenesis, but the tumorigenic potential of glioma cells *in vivo* are significantly increased by EGFR Δ III expression when compared to xenografts expressing the wild-type EGFR (18,19). Studies have also shown that EGFR Δ III-expressing glioblastoma cells are approvingly resilient towards both chemotherapy as well as radiation (19-21). Interestingly, recent reports indicate that co-expression of EGFR Δ III and the GSC marker CD133⁺ defines a population of GSCs harboring the greatest tumor-initiating ability, thus further defining its importance in glioblastoma. Taken together, it is not surprising that EGFR Δ III expression has been strongly associated with a poor survival prognosis for patients whose tumors amplify EGFR (21). In addition to EGFR Δ III, sequence analysis of the EGFR coding region in a cohort of 151 glioblastoma tumors and cell lines identified a number of novel ectodomain missense mutations (22). Approximately 14% of glioblastoma patient samples and 13% of glioblastoma cell lines displayed this form of mutation. Using missense mutants encoding R108K, T263P, A289V, G598V, and L861Q it was determined that these mutations were: i) hyper-phosphorylated receptor in the absence of ligand; ii) accompanied by an increased EGFR gene dosage; and iii) exhibited a stronger transforming phenotype relative to wild-type EGFR as determined by anchorage-independent growth in NIH-3T3 cells. Importantly, of the missense mutations evaluated, EGFR-R108K shares the greatest degree of signaling and behavioral homology to EGFR Δ III, particularly as it relates to therapeutic resistance (23).

3. EGFR therapies in glioblastoma

Overexpression of EGFR has been noted in multiple epithelial tumors, supporting the notion that deregulated EGFR expression and signaling are pivotal events in the origin of human cancers (24). This led to the development of multiple inhibitors of EGFR, including EGFR-targeted monoclonal antibodies (mAb) such as mAb C225 69 and mAb 528 (25). Mechanistically, EGFR-directed mAbs compete with

cognate ligands for binding, effectively down-regulating receptor expression and leading to inhibition of cell growth by induction of cell cycle arrest (26). Initially, mAb C225, dubbed cetuximab, demonstrated promising antitumor effects in cell cultures and xenograft models, leading to its implementation as a therapeutic agent (27). Since, cetuximab has been approved for use in metastatic colorectal cancer (CRC) as well as squamous cell carcinoma of the head and neck (HNSCC) (28,29). Cetuximab has additionally been under evaluation in progressive non-small cell lung cancer (NSCLC), where activating mutations of EGFR commonly occur (30). Notably, preclinical studies in glioblastoma cell cultures and mouse models have demonstrated the antitumor and radio-sensitizing effects of cetuximab in this setting (31). Preclinical data also suggest that cetuximab is active against EGFR Δ III, where it binds to and engenders receptor internalization, rendering a reduction in kinase activation (32). Though cetuximab has displayed promising effects in clinical trials involving CRC, HNSCC and NSCLC, phase I/II trials in patients with recurrent glioblastoma have failed to confer any efficacious advantages over standard of care regimens (33). Insufficient intratumoral accumulation of cetuximab was cited in the failed inhibition of EGFR autophosphorylation and degradation in these studies.

Small molecule tyrosine kinase inhibitors (TKIs) that competitively target receptor catalytic activity via the EGFR kinase domain adenosine triphosphate (ATP)-binding pocket, present another approach to targeting EGFR (34). Despite being low in molecular weight and more likely to penetrate the BBB, the specificity of these inhibitors is diminished by the fact that the EGFR ATP-binding pocket shares homology with that of other RTKs, resulting in off-target effects (35). Three TKIs of EGFR (gefitinib, erlotinib and lapatinib) have previously received regulatory approval for use in NSCLC and breast cancer (36). In contrast, several phase II clinical trials evaluating gefitinib, erlotinib or lapatinib in newly diagnosed or recurrent glioblastoma have yielded minimal clinical activity as either a monotherapy or in combination regimens (37-39). The lack of clinical effects were attributed to insufficient inhibition of Akt activation, which correlated most strongly with EGFR Δ III expression and loss of PTEN. Collectively, these findings highlight the need for novel therapeutic targets capable of improving clinical responses in this deadly disease.

4. Dysregulated EGFR signaling networks in glioblastoma

The EGFR family is a complex system involved in growth factor cellular signaling. Phosphorylation of EGFR at the plasma membrane leads to the recruitment of multiple effector proteins via recognition and binding of Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains to phosphotyrosine motifs on the receptor. Formation of the EGFR signaling complex, in turn, triggers a variety signaling cascades involved in tumor cell proliferation, angiogenesis, motility, differentiation, and survival (Fig. 1) (40). Interestingly, similar substrates are activated downstream of EGFR and EGFR Δ III, but with differing levels of intensity. These pathways include the phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription 3 (STAT3) pathways and Src family kinases (41).

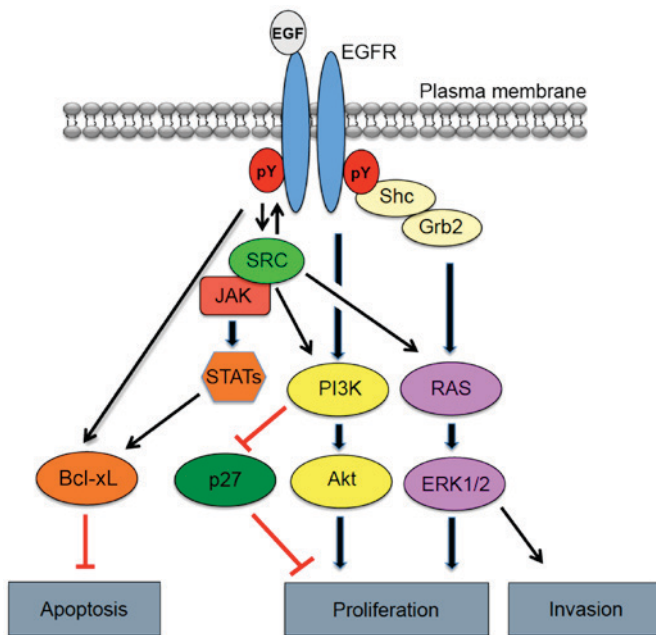


Figure 1. Epidermal growth factor receptor (EGFR) signaling and related pathways in cancer. Illustration of the gene products involved in transferring signals from the outside of the cell to the nucleus mediated by EGFR, notably through SRC. Grb2, growth factor receptor-bound protein 2; STAT, signal transducer and activator of transcription; PI3K, phosphoinositide 3-kinase.

5. PI3K

The class IA PI3Ks form heterodimers that are recruited to trigger RTKs and adaptor proteins through regulatory subunits, including p85a, p55a and p50a, or PIK3R1; p85b or PIKR2; and p55y or PIKR3. p85a associates with EGFR either through ErbB3 heterodimerization or through phosphorylation of EGFR by the SFK c-Src (42). p85a association with EGFR results in a conformational change in p85a, releasing the inhibition of the catalytic subunit p110 of PI3K. PI3K then localizes to the plasma membrane, where it functions to catalyze the formation of phosphatidylinositol 3,4,5-trisphosphate (PIP3) via the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2). The resulting PIP3 is a critical activator of Akt, which consequently phosphorylates, or inhibits, numerous target proteins involved in regulating cellular metabolism, motility and protein synthesis (43). Akt activation additionally results in phosphorylation of Bad, a Bcl family member, which when phosphorylated fails to inhibit the survival protein Bcl-xL, thus precluding apoptotic induction (44). Activation of PI3K can also arise from point mutations, of which ~15% have been catalogued in glioblastoma tumors. These mutations occur most commonly in the adaptor-binding domain (ABD) and less frequently in the C2 helical and kinase domains of the catalytic subunit (PIK3CA) (45). Though mutations in the regulatory subunit (PI3KR1) are uncommon, prior sequencing analysis from the TCGA indicated the presence of 9 such mutations occurring among a cohort of 91 glioblastoma samples. As a result, aberrant PI3K activation and subsequent activation of Akt is observed in upwards of 85% of glioblastoma samples (46). PI3K signaling is negatively regulated by various proteins, most notably PTEN; PTEN, however, is commonly inactivated

(~50%) in glioblastoma by either epigenetic silencing or deletion mutation (37). Loss of PTEN, therefore, disrupts the PI3K:PTEN balance resulting in increased Akt activation and uncontrolled cell growth. Given the frequency of PI3K pathway aberrations occurring in glioblastoma, inhibition of its signaling components is an important contribution for a therapeutic avenue. Based on this, the rapamycin analogs, everolimus (Afinitor) and temsirolimus (Torisel), both of which inhibit mammalian target of rapamycin complex 1 (mTORC1) are regulatory-approved for treatment of advanced renal cell carcinoma and have been evaluated in glioblastoma patients. Unfortunately, the clinical application of rapamycin analogs has yielded infrequent and short-lived responses in glioblastoma. Additionally, the PKC/PI3K/AKT inhibitor, enzastaurin, was the first targeted therapy for glioblastoma evaluated in a phase III clinical trial (47).

6. MAPK

Following EGFR activation, the MAPK signaling pathway is triggered by the growth factor receptor-bound protein 2 (Grb2) binding directly to EGFR via Y1068 and Y1086 or indirectly by SHC binding Y1173 and Y1143 (48). Grb2 also houses 2 SH3 domains, allowing for interactions with proline-rich sequences, namely those of son of sevenless (SOS) (49). The Grb2/Shc/EGFR interaction precedes recruitment of SOS to the plasma membrane. SOS is a guanine nucleotide exchange factor, which functions to promote the conversion of Ras-GDP to the active Ras-GTP. Subsequently, Ras activates Raf, a serine-threonine protein kinase, which then phosphorylates and activates MEK1/2, resulting in activation of ERK1/2 (MAPK) (50).

7. Conclusions

The present study concludes that the concept of EGFR signaling and related receptors and associated factors is evolving, however, it needs detailed evaluation for future clinical applications in cancer patients.

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