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REVIEW ARTICLE

Epidermal growth factor receptor: a key manipulator in molecular pathways of malignant glioma

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Abstract

The epidermal growth factor receptor (EGFR) is a member of the ErbB/EGFR family, including EGFR/Her1, ErbB2/Her2, ErbB-3/Her3, and ErbB-4/Her4. EGFR exerts its effects through the receptor tyrosine kinase phosphorylation and activation of important downstream signaling pathways in normal and neoplastic cells, mainly the Ras GTPase/MAP kinase (MAPK), STAT3, and phosphatidylinositide 3 kinase-AKT pathways. EGFR deregulation is common in malignant glioma, especially primary glioblastoma, and exists in three forms: gene overexpression (amplification), autocrine effects of EGFR activation, and activating receptor mutation (EGFRvIII). However, some EGFR abnormalities have also been found in low-grade gliomas, including the nuclear localization of EGFR, expression in the microfoci of anaplastic transformation, and association with neovascularization in the mesenchyma of the glioma, which suggests that some unknown EGFR-related mechanisms are possibly responsible for its central role in the initiation and progression of malignant glioma. Uncovering these mechanisms will have potential value in the development of radiotherapy, chemotherapy, and EGFR-targeted therapy for glioma.

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Gliomas represent the largest group (accounting for about 40%) of primary central nervous system (CNS) tumors, representing approximately 2% of all systemic tumors in adults. According to the World Health Organization (WHO) CNS tumor classification (2007), gliomas can currently be classified into distinct subtypes, mainly: pilocytic astrocytoma, diffuse glioma (astrocytoma, oligodendroglioma, ependymoma), anaplastic gliomas, and glioblastoma (GBM), based on their clinical, histopathological, and molecular characteristics. The grading system of CNS tumors is also stratified according to their biological behavior, from Grade I to IV. For example, Grade I refers to benign biological behavior, and Grades II, III, and IV denote behavior with malignant potential, malignancy, and high-grade malignancy, respectively. The gliomas (Grades II–IV) all show malignant biological behavior, and are therefore commonly known as malignant gliomas [1–3].

Although consistent efforts are focused on understanding the pathogenesis, progression, and therapeutics of malignant gliomas, these factors pose significant challenges to clinicians. However, recent understanding of CNS tumors has been revolutionized by a series of genomic studies [4–5]. For example, mutations in the isocitrate dehydrogenase (IDH1/2) gene are thought to occur early in gliomagenesis and are associated with tumor progression; although these mutations are found in 70–80% of Grade II and III gliomas and secondary GBMs, they rarely occur in primary GBM [6–7]. Loss-of-expression mutations of the alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene has a similar distribution pattern in gliomas as the IDH mutations, which possibly lead to a better prognosis [8]. Malignant gliomas bearing the 1p and 19q co-deletion are associated with longer survival, however, a glioma of the same grade but without such genetic alteration could not be differentiated histopathologically [9]. The expression of FOXG1 is notably higher in glioma tissues than in the control brain tissues, and is positively correlated with histological malignancy [10]. In addition, the O^6-methylguanine-DNA methyltransferase methylation, v-RAF murine sarcoma viral oncogene homolog B1 (BRAF) alterations, and TP53 mutation were commonly found in low-grade astrocytomas. Mutations of telomerase reverse transcriptase (TERT) have been found in ma-

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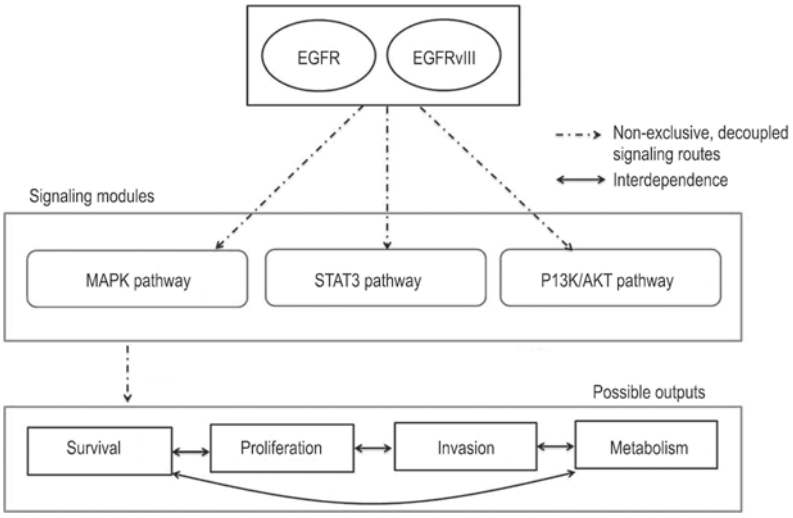

Fig. 1 Examples of modularity in the EGFR-driven signaling system

jority of the primary GBMs, but are less common in lower-grade gliomas and secondary GBMs [11–12]. Epidermal growth factor receptor (EGFR) variant III (EGFRvIII) was found in 20–30% of the GBMs [13]. As described above, identification of a series of driver mutations in gliomas has broadened our understanding of the field, thus, helping to better assess the prognosis of patients, revealing new insights into gliomagenesis, and establishing possible and specific strategies for molecular-targeted treatment in future.

EGFR (ErbB-1; HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor (EGF) family. The EGFR gene is located at chromosome 7p11.2, encoding 1186 amino acid residues with a molecular weight of 170 KDa. EGFR belongs to the ErbB family of receptors, along with three related receptor tyrosine kinases: HER2/c-neu (ErbB-2), Her3 (ErbB-3), and Her4 (ErbB-4) [14–15]. EGFR is activated by binding of its main ligands, EGF and transforming growth factor-α (TGFα), as well as with some other ligands, including amphiregulin, epigen, heparin-binding EGF-like growth factor, epiregulin, and betacellulin. The ligand binding results in an active dimeric conformation of EGFR (homodimerization/heterodimerization). Subsequently, the catalytic intracellular domain is activated by phosphorylation of tyrosine residues, and proteins containing an Src homology domain 2 (SH2) region recognize the tyrosine phosphate residues and bind directly to the activated receptor. Such proteins then become activated and transfer the signal to downstream effectors [16] through the Ras GTPase/MAP kinase (MAPK), STAT3, and phosphatidylinositide 3 kinase (PI3K)-AKT pathways (Fig. 1) [17].

Along with the activation of these molecular pathways, DNA synthesis and cell proliferation are initiated, and receptor tyrosine phosphorylation also simultaneously initiates the recruitment of ubiquitin ligases as a negative regulatory mechanism [18]. Since the essential role of EGFR in epithelial development was discovered, mutations leading to EGFR over-expression have been associated with a number of neoplasms, including lung cancer, anal cancer, breast cancer, and GBM. Somatic mutations of EGFR usually result in constant activation, leading to uncontrolled cell division. Mutations, amplifications, or misregulations of EGFR or family members are detected in about 30% of all human epithelial cancers.

There are three main mechanisms of the deregulation of EGFR. The first mechanism is EGFR overexpression, and increased EGFR abundance is found in primary GBM through gene amplification and/or increased translation of this gene. Amplification of the EGFR gene has been reported in 40–70% of primary GBMs [19–20], implicating its possible role in driving gliomagenesis in primary GBMs, although some cases of GBM with EGFR overexpression without amplification have been reported [21]. The second mechanism of EGFR deregulation is an autocrine mechanism. EGFR overexpression is sometimes accompanied with an increased abundance of its cognate ligands such as EGF and TGF-α, forming uncontrolled autocrine cycles, resulting in long-lasting EGFR signal activation [22]. The third mechanism is through activating receptor mutations. Among these, 20–30% of primary GBMs express a variant mutation form of EGFR, EGFRvIII, due to the deletion of exons 2–7, which results in a constitutively active receptor that is unable to bind to a ligand and leads to continuous activation of cell growth and anti-apoptotic pathways [13]. Activation of EGFR in gliomas also occurs through gain-of-function mutations and via double minute chromosomes [23]. One study also demonstrated that a cell line stably expressing EGFRvIII and EGFL858R displayed decreased growth and migration ability compared with wild type EGFR-expressing cells, suggesting that there are distinct functional differences between differ-

ent EGFR mutation forms. The functional differences between different mutations highlight the necessity for the development of mutation-specific targeted therapies [24]. The Cancer Genome Atlas consortium identified EGFR as the fourth most highly mutated gene based on the results from a cohort of 91 GBM cases [25]. The mutation forms of EGFR may occur at the extracellular domains (EGFRvI and EGFRvII) and/or at the intra-cytoplasmic tail of the receptor (EGFRvIV, EGFRvV) [26–30]. The most frequent EGFRvIII mutation form is mainly observed in GBMs, with a much lower incidence in non-small cell carcinoma and other human cancers [30–32]. The oncogenic mechanism of EGFR mutant forms involves a series of signaling networks. For example, defects in receptor internalization result in its constitutive localization to the plasma membrane and sustained unattenuated signals [33]; phosphorylation of EGFRvIII induces a stable increase in the phosphorylation level that is distinct from that in the wild type EGFR [34]; activation of the PI3K pathway, which is negatively regulated by phosphoinositide phosphatases, including Src homology 2 domain-containing inositol phosphatases (SHIP-1 and -2); through the Erk1/Erk2 MAPK pathway by receptor dimerization, EGFR transphosphorylation, and activation, which are triggered by Grb2 binding directly to the receptor at residue Y1068 and indirectly through Src homology domain-containing adaptor protein C binding at residues Y1173 and Y1148 of the EGFRvIII mutant [35]; or through the signal transducer and activator of transcription (STAT) pathway. STAT proteins are a family of latent transcription factors that are recruited to ligand-bound EGFR dimers in combination with SH2 domains. The kinase domain of EGFR may phosphorylate the STATs, inducing their homo- or heterodimers, via SH2-phosphotyrosine interactions [36–37] and exert biological effects by colocalization of EGFRvIII and STAT3 in the nucleus of glioma cells [38]. Considering the important role of EGFR and related molecular pathways, the possibility of a new molecular classification of gliomas based on EGFR and platelet-derived growth factor receptor A (PDGFRA) expression has been recently explored [39]. EGFR-related molecular pathways are considered to play a key role in the poor prognosis of gliomas. Gene coexpression modules around EGFR (EM, 29 genes) or PDGFRA (PM, 40 genes) in 1369 adult diffuse gliomas (WHO Grades II–IV) were examined. Based on the EM and PM expression signatures, three subtypes were categorized: EM, PM, and EM (low) PM (low) gliomas, in a morphology-independent manner. Besides their distinct patterns of genomic alterations, EM gliomas were found to be associated with old age, poor prognosis, and strong expression of neural stem cell and astrogenesis genes. The EM/PM-based molecular classification scheme is applicable to adult low-grade and high-grade diffuse gliomas, and outperforms existing classification schemes in assigning diffuse gliomas to subtypes with distinct transcriptomic and genomic profiles. This EM/PM-based molecular classification provides a new molecular diagnostic framework to improve our current knowledge on the biology of malignant glioma.

It is generally recognized that EGFR abnormality is seldom found in low-grade gliomas; however, these have nevertheless been reported in several studies. In a group of 145 glioma cases, including pilocytic astrocytoma, astrocyma, anaplastic astrocytoma, and GBM cases, Carvalho *et al* [40] demonstrated EGFR overexpression and EGFR amplification in 50% and 20% of astrocytomas, respectively, whereas the EGFRvIII mutation was only found in GBMs (34.5%, $P = 0.005$). Among EGFR-amplified GBM cases, 59% also showed EGFRvIII expression ($P < 0.001$). Furthermore, cytoplasmic accumulation of EGFR protein was also found in 75% of astrocytomas detected by immunohistochemistry, and 21% of the astrocytomas showed nuclear localization of EGFR. The detection of EGFR alterations in all grades of astrocytoma implicates its key role in the progression of gliomas. In addition, Pedeutour-Braccini *et al* [41] evaluated a group of Grade II gliomas to search for high-grade glioma components within the Grade II tumor tissue; microfoci with high cellular density, high vascular density, or minimal endothelial proliferation were determined, which were referred to as the GII+ phenotype. Furthermore, cell proliferation, hypoxia, vascularization, and alterations of tumorigenic pathways were examined in the hypercellular foci of 16 GII+ cases by immunohistochemistry of Ki-67, CD31, HIF-1-alpha, EGFR, P-AKT, P53, and MDM2, and with fluorescence *in situ* hybridization of EGFR, MDM2, and PDGFRA. Ki-67 and CD31 expression was higher in the foci than in the tumor background in all cases. Aberrant expression of protein markers and genomic aberrations were also observed in some foci, and EGFR overexpression was detected in 7/16 cases, which was distinct from the tumor background. Survival of patients was shorter among GII+ cases than for all GII cases. These foci were thought to be an early histological hallmark of anaplastic transformation, which is supported by molecular aberrations. Further molecular analysis is needed to elucidate the pathogenesis of low-grade glioma progressing to high-grade glioma.

Besides the genetic abnormality of EGFR in the glioma parenchyma, the neovascularization of the glioma interstitia is also related to EGFR mutation. A recent study [42] using the LN229 GBM cell line transfected with EGFR wild type and EGFRvIII mRNA showed upregulation of the mRNA and protein expression levels of angiopoietin-like 4 factor by EGFRvIII overexpression. However, knockdown of this factor using shRNA significantly decreased the microvascular density in the transplanted tumor and inhibited its growth both *in vitro* and *in vivo*.

Further work demonstrated that the ERK pathway and its downstream regulated c-Myc pathway may be responsible for these effects.

In the last decade of molecular biological research, there have been many crucial findings relevant to gliomas, including the discovery that many of the genes involved in the initiation and progression of malignant gliomas are involved in EGFR signaling networks, which in turn govern the rampant tumor proliferation, invasive growth, and microvascular hyperplasia. Along with clinical applications of next-generation sequencing [43], the picture of the molecular pathogenesis and progression of malignant gliomas will undoubtedly become clear, with the hope of generating new pathways for drug or biomarker development to treat malignant gliomas in the future.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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