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EGFR Expression and Mechanism of Action in Glioblastoma

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ABSTRACT
Epidermal growth factor receptor (EGFR) is frequently abnormal in about 60% of glioblastoma (GBM) patients, mainly manifested by gene amplification and EGFRvIII mutation, resulting in continuous activation of downstream signaling pathways , driving tumor cell proliferation, invasion, migration and stem cell characteristics maintenance. Multiple factors influence these pathways by regulating EGFR activity and stability through different mechanisms. In tumor microenvironment, secreted protein POSTN inhibits EGFR degradation, inflammatory factor PTX3 activates PI3K pathway, and JAK2/STAT3/STAT6 pathway induces M2-type macrophage polarization, which jointly promote GBM progression. In addition, EGFR signaling promotes tumor stem cells to differentiate into endothelial-like cells through RTK/PI3K/AKT pathway, forming vascular mimicry (VM) and accelerating tumor invasion and metastasis. Therapeutic strategies targeting EGFR include monoclonal antibodies and small molecule tyrosine kinase inhibitors, but have limited efficacy and resistance; CAR-T cell therapy targeting EGFR faces challenges such as tumor microenvironment inhibition. So further understanding of EGFR regulatory networks and drug resistance mechanisms is essential to optimize GBM therapy.

KEYWORDS
EGFR; Glioblastoma; Signal pathway; Therapy

1 Introduction

Glioblastoma (GBM) is the most common primary solid brain tumor in adults . The median survival time was only 14.6~16.8 months, and the overall survival rate at 24 months was 27%~30% [1]. EGFR is a glycoprotein encoded by proto-oncogene, which is activated after binding with ligand and amplified through cascade effect signal pathway, thus producing a series of regulation on cell proliferation, differentiation, apoptosis and other life processes [2]. Against the backdrop of high mortality rates associated with traditional treatments, molecular biology approaches are of particular value. This article will describe the mechanism of EGFR in GBM and its application in therapy.

2 EGFR

EGFR is widely distributed on glial cells, fibroblasts, mammalian epithelial cells and other cell surfaces. It is encoded by protooncogene c-erbB-1. The gene product is a polypeptide composed of 1210 amino acid residues. After cleavage processing, the N terminal sequence is cut off to become a glycoprotein containing 1186 amino acid residues. Its molecular weight is 180kDa. EGFR encodes proteins with tyrosine kinase activity that are activated by binding to ligands. In approximately 60% of patients with GBM, there is enhanced signaling mediated by EGFR , which recruits and regulates multiple downstream pathways such as RAS/ MAPK, PI3K/ AKT, JAK2/ STAT and PKC pathways [3-5]. EGFR gene abnormal expression patterns in different tumors, this difference suggests that EGFR can be used as a potential target of GBM for tumor therapy and drug development. EGFR has been confirmed to be involved in the development of GBM, providing new ideas and broad prospects for the treatment of GBM.

3 Mechanism of EGFR in GBM

Gliomas can be divided into IDH wild-type and IDH mutant according to the mutation of isocitrate dehydrogenase (IDH). Glioblastoma is mostly IDH wild-type. In IDH wild-type glioblastoma, IDH gene does not mutate, but EGFR gene abnormality occurs. EGFR gene abnormalities are often manifested as gene amplification and gene mutation in IDH wild-type glioblastoma, among which gene amplification is more common, and EGFRvIII is the main type of gene mutation. Although gene amplification and EGFRvIII mutation have been identified as the main causes of GBM, their regulatory mechanisms are still unclear. Therefore, this article will focus on its regulatory mechanism.

3.1 Gene amplification and mutation

EGFR gene amplification is often accompanied by overexpression. Recent studies have found that EGFR amplification can be found in about 40% of primary GBM, while EGFR overexpression is found in> 60% of GBM [6]. However, multiple

studies have shown that both EGFR overexpression and EGFR amplification occur mostly in primary GBM [7]. Ren et al. believed that EGFR may form autocrine or paracrine loop by binding with its ligand epidermal growth factor or transforming growth factor [8], leading to malignant uncontrolled proliferation [9]. EGFR vIII contains no ligand binding domain and remains activated in the absence of upstream EGF stimulation. EGFR pathway activation induced by EGF stimulation and EGFR vIII can downregulate H2AZK4/7AC [10], H2AZ is the most conserved variant of H2A and is associated with chromatin integrity and transcriptional regulation [11]. However, whether H2AZK4/7AC is related to chromatin integrity and transcriptional regulation needs further study.

3.2 EGFR-related signaling pathways

3.2.1 EGFR downstream signaling pathways and molecular markers

PI3K/ AKT pathway is the most classical pathway in EGFR downstream pathway. Many molecules regulate the development of GBM through PI3K/AKT pathway. PI3K/AKT/mTOR pathway in GBM overactivates EGFR to activate PI3K, PI3K is involved in AKT phosphorylation, mTORC2 also adds phosphate groups to AKT, the latter two together make AKT fully activated [12], and can continue to activate downstream pathways. The PI3K/AKT/mTOR pathway regulates cell quiescence, proliferation, cancer, and longevity. In addition, studies have found that EGF binds to EGFR, activates EGFR-ERK pathway, maintains PD-L1 stability by up-regulating CSN6, and then up-regulates PD-L1 expression at the post-transcriptional level [13]. CSN6 and PD-L1 are expected to be biomarkers for the diagnosis of GBM.

The JAK/STAT pathway is also one of the downstream pathways of EGFR. This signaling pathway consists of tyrosine kinase-related receptors that receive signals, Janus kinases that transmit signals, tyrosine kinases JAK, and transcription factors that produce effects [14-15].

RAS-RAF-MAPK pathway is one of the downstream signaling pathways after EGFR activation, which plays an important role in tumor cell growth by regulating cell cycle progression and proliferation. Studies have shown that EGFR promotes cell proliferation and survival by activating RAS and RAF, which in turn activates downstream MAPK signaling pathways [16-17].

3.2.2 Factors affecting EGFR

After EphB3 expression was down-regulated, EGFR expression was up-regulated, i.e. EphB3 was negatively correlated with EGFR expression level. EphB3 regulates PI3K/AKT pathway by regulating EGFR, affecting invasion, migration and proliferation of GBM [18]. However, the mechanism by which EphB3 down-regulates EGFR overexpression remains unclear.

DMTN can promote PI3K-AKT and MAPK pathway activation by regulating EGFR [19]. It has been reported that EGFR internalizes into early endosomes when stimulated by EGF, and then recirculates to cell membranes or is degraded by lysosomes [20, 21]. DMTN was found to interact with EGFR, and DMTN may interact with EGFR through region 226-336, thus regulating downstream pathways [17].

Zinc finger BED domain protein 1 (ZBED1) is a member of the ZBED family. Numerous pieces of evidence indicate that the ZBED family plays a role in tumors through its transcriptional regulatory properties. Compared with the adjacent tissues of Glioma, ZBED1 is generally highly expressed in glioblastoma, especially in Glioma Stem Cells (GSCs), and the expression of ZBED1 is positively correlated with SOX2, a dry marker of glioblastoma . Since GSCs play a key role in the tumorigenesis process of GBM, ZBED1 may affect the occurrence and development of GBM by influencing GSCs. Whether ZBED1 affects the transcriptional process of GSCs or GBM remains to be further studied.

HECTD3 activates the EGFR pathway through polyubiquitination modification of PARP1. E3 ubiquitin ligase HECTD3 is a member of the HECTD ubiquitin ligase family, homologous to the carboxy terminus of E6-related proteins, and contains DOC and HECT domains [22-23]. Studies have shown that HECTD3 can bind to PARP1 and mediate K63-linked polyubiquitination of PARP1. After ubiquitination, ubiquitin molecules are recruited to lysine at positions 209 and 221 (ubiquitin binding sites) of PARP1 to stabilize PARP1 expression and activate EGFR downstream signaling pathways, thus promoting GBM occurrence [24].

4 EGFR regulatory mechanism in GBM

EGFR can regulate the progression of GBM by affecting EGFR downstream signaling pathways, and can also promote the progression of GBM by forming vascular mimicry.

Glioblastoma stem cells can produce a secreted protein (POSTN), which is an important component of tumor microenvironment. POSTN activates EGFR downstream signaling pathway PI3K-Akt pathway, inhibits EGFR degradation, and promotes GBMs cell proliferation, invasion, migration, and dryness maintenance [25].

The human STAT family currently consists of seven members (STAT1, STAT2, STAT3, STAT4, STAT5B, STAT6). The JAK

family consists of four members (JAK1, JAK2, JAK3, TYK2). The JAK2/STAT3/STAT6 pathway promotes macrophage polarization toward M2, thereby facilitating glioblastoma progression [26].

Pentraxin 3 (PTX3) is a soluble inflammatory factor in tumor microenvironment. PTX3 inhibition activates the PI3K/Akt/mTOR pathway by negative feedback. PTX3 also activates PI3K/Akt/mTOR pathway in M2 macrophages, thus regulating the development of GBM.

VM is defined by a fluid-conducting, matrix-embedded meshwork that is independent of endothelial cells (ECs), but instead is formed by certain types of tumor cells through their acquirement of plasticity to mimic endothelial function.The more VM channels there are, the more they promote the invasion and metastasis of GBM.

RTK/PI3K/Akt/mTOR pathway can influence VM formation and development through multiple pathways. Glioblastoma stem cells have strong plasticity and can differentiate into endothelial-like cells. MMPs-dependent matrix degradation and extracellular matrix recombination reshape the tumor microenvironment. At the same time, cell migration and expression of adhesion molecules can promote the assembly of vascular structures. RTK/PI3K/Akt/mTOR pathway can positively regulate the differentiation of stem cells into endothelial-like cells, matrix degradation and extracellular matrix reconstruction, and assembly of vascular-like structures [27, 28].

5 EGFR use in drug therapy

5.1 Anti-EGFR molecular targeted drugs

5.1.1 Monoclonal antibodies

Monoclonal antibodies include panitumumab, nimotuzumab, cetuximab, mAb806, etc. It acts on transmembrane receptors on the surface of GBM cells, or acts on GBM extracellular growth factors, blocking their ligand activities, inducing apoptosis and anti-angiogenesis, thus inhibiting GBM cells [29]. Nimotuzumab and panitumumab are humanized EGFR monoclonal antibodies that competitively bind to EGFR extracellular ligand binding domains after administration, blocking downstream signaling pathways, thereby inhibiting GBM proliferation [30-32]. Cetuximab exerts its antitumor effect by binding to EGFR and blocking tyrosine site phosphorylation caused by ligand in patients, thereby inhibiting downstream pathway activation [33]. mAb806 binds specifically to EGFR gene mutant proteins, inhibiting downstream signaling pathways and thus exerting antitumor effects [34].

5.1.2 Small molecule tyrosine kinase inhibitors (TKIs)

TKIs theoretically act on EGFR signaling, but have a significant effect only on NSCLC and little effect on GBM. Existing studies have limited explanations for its resistance mechanism, but there are still some studies that may be potential therapeutic targets for GBM. TKIs include gefitinib, erlotinib, oxitinib, etc. The LRIG gene family was discovered during the study of EGFR negative feedback mechanism, which includes three members of LRIG1, 2 and 3 [35]. Gefitinib is an EGFR inhibitor. It has been found that overexpression of LRIG2 and extracellular segment of LRIG2 enhances U87 and U251 to resist the therapeutic effect of gefitinib. Overexpression of LRIG2 and extracellular segment can significantly enhance the survival rate of glioblastoma cells, increase cell mitochondrial membrane potential and reduce apoptosis, thus resisting the therapeutic effect of gefitinib [36]. It has been found that osimertinib has certain inhibitory effect on EGFR A289V mutant U87 and U251 cells in vitro. Erlotinib slightly inhibited EGFR A289V mutant U87 cells to some extent, while U251 cells were relatively insensitive to erlotinib [37]. In addition, experiments have shown that CSN6 promotes GBM cell proliferation and migration by regulating EGFR-mediated signaling pathways. Erlotinib antagonizes CSN6's effect on cell proliferation, thereby altering the expression levels of EGFR and downstream target proteins AKT and ERK. The cells used in this experiment were CSN6 overexpressing cell lines (U-87MG-CSN 6 cells and LN-229-CSN6 cells) and their control cells [38]. Erlotinib may affect EGFR signaling pathway via CSN6 in U87 cells, but not U251 cells.

5.2 EGFR in CAR-T therapy

Chimeric antigen receptor (CAR)-T cell therapy is an adoptive cell transfer therapy (ACT), that is, by inducing, activating and expanding lymphocytes or their precursor cells with specific immunity in vitro, and then transferring them to those with low cellular immunity, the body can restore normal anti-tumor immune response, thus controlling and eliminating tumors. CAR-T therapy uses genetic engineering technology to modify CAR genes, T cells fused with CAR containing specific antigen recognition domains and T cell activation signals, which can exert targeted killing effect after activation [39]. CAR-T therapy remains a major challenge due to Tumor microenvironment (TME) inhibition of T cell immune killing function [40]. Compared with normal macrophages, tumor-associated macrophages (TAMs) are stimulated and regulated by many factors in tumor microenvironment, resulting in their phenotype biased towards M2 type. This makes TAMs have

the characteristics of promoting immune microenvironment suppression [41]. Because TAMs play an important role in tumor genesis and development, tumor treatment strategies targeting TAMs have attracted much attention.

6 EGFR application prospects in GBM

EGFR mutations not only have an important impact in GBM, but also play an important role in non-small cell lung cancer (NSCLC). The target response rate of NSCLC is very high, but the target response rate of GBM is extremely low. It is speculated that there may be the following reasons. First, in NSCLC, targeted drugs can easily reach the primary tumor site and act on the corresponding target, while GBM is difficult to reach the primary lesion in the brain due to the barrier of blood-brain barrier. Second, GBM has stronger drug resistance than NSCLC. Even if NSCLC forms brain metastases, it can be targeted by ositinib, which has the ability to penetrate the blood-brain barrier, while the therapeutic effect of ositinib on GBM is very limited. Therefore, if GBM drugs can overcome drug resistance and blood-brain barrier barriers, it will provide a new direction for the treatment of GBM patients.

6.1 Application of PROTAC degradation agent

PROTAC is an artificial molecule that specifically binds to a target protein at one end and is linked to E3 ubiquitin ligase at the other end. It binds to a "death tag"_multiple ubiquitin molecules to complete polyubiquitination, thereby degrading the target protein. PROTAC does not require specific and continuously activated binding sites to bind to target proteins, which greatly increases the number of proteins it can bind to. GBM targeted drugs currently used in clinical practice have poor therapeutic effect due to drug resistance, so combining PROTAC with EGFR protein and degrading GBM cells through polyubiquitination may achieve the effect of treating GBM. PROTAC degraders have been shown to be useful in the treatment of NSCLC, and PROTAC induced EGFR protein degradation results in cell cycle arrest at G1 , as well as degradation of EGFR via ubiquitin/proteasome pathway and autophagy/lysosomal pathway [42]. In breast cancer, PROTAC can affect signaling pathways by degrading estrogen receptors, thereby achieving breast cancer treatment [43]. For the application of PROTAC, one of the major difficulties in GBM compared with lung cancer and breast cancer may be whether PROTAC can break through the blood-brain barrier.

6.2 Nanomaterials could be a new way to cross the blood-brain barrier

Current nanocarriers include metal nanoparticles, liposomes, dendrimers, etc., where metal nanoparticles can be coupled to drugs for delivery [44]. If PROTAC can be coupled to metal nanoparticles, it can solve the problems of GBM resistance and blood-brain barrier barrier proposed above. Whether this method is feasible or not needs further clinical verification.

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