Undergraduate Research Journal

Issue 16, Volume 2 April 2024

EGFR Mutations and Signaling Pathways in Glioblastoma: Implications for Pathogenesis and Therapeutic Targeting

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ACKNOWLEDGEMENTS

This paper is dedicated to my beloved aunt, Mrs. Suvarchala Pulivarthi, whose courageous battle with glioblastoma serves as a poignant reminder of the urgent need for continued research and advancements in the field. This paper was written under the guidance of Michael Thompson.

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06 March 2024

Abstract

A cancerous tumor in the brain known as glioblastoma multiforme (GBM) originates from astrocytes of the central nervous system. Consequently, GBM poses significant challenges to the oncology community because of its aggressive characteristics and poor prognosis. GBM hallmarks include fast growth, invasiveness, and high rates of recurrence. This tumor is highly heterogeneous with different genetic and molecular features found within the tumor cells. There is an ongoing obstacle to conceptualizing effective management for this grappling disease. This is largely due to the tumor displaying intra-heterogeneity, in addition to a plethora of differences in the tumor's microenvironment. The heterogeneity exhibited by this tumor not only makes it more resistant to treatment but also influences its ability to evolve. The Epidermal Growth Factor Receptor (EGFR) which falls under the Human Epidermal Growth Factor (ErbB) family is a transmembrane receptor that assists in understanding complex molecular pathways involved in GBM formation. EGFR mutations have been shown to affect signaling cascades including Ras/Raf/MEK/ERK, PI3K/Akt/mTOR, JAK/STAT, PLC/PKC among others transforming cellular machinery involved in cell survival, proliferation and invasion. Knowledge about EGFR's aberrant mutations can be useful for developing novel therapeutic strategies aimed at EGFR inhibition in GBM therapy. This gives hope for patients with this challenging disease to have better outcomes.

Keywords: Glioblastoma multiforme (GBM), Astrocytes, Central Nervous System, Heterogeneity, Epidermal Growth Factor Receptor (EGFR), Tumor Progression, Signaling Cascades, Pathogenesis

EGFR Mutations and Signaling Pathways in Glioblastoma: Implications for Pathogenesis and Therapeutic Targeting

Glioblastoma multiforme (GBM) is a highly aggressive and malignant brain tumor that arises from glial cells, specifically astrocytes, within the central nervous system (Wirsching et al., 2016). GBM is one of the most challenging and lethal forms of cancer encountered in clinical Oncology. GBM is characterized by its rapid progression, invasive behavior, and resistance to conventional treatment, making it notoriously challenging for patients and physicians alike to manage, leading to poor patient outcomes. The term “multiforme” refers to the heterogeneous nature of GBM, as the tumor cells exhibit a large array of genetic and molecular characteristics (Batash et al., 2017). The aggressive nature of GBM is underscored by its ability to infiltrate surrounding brain tissue, demonstrating that surgical resection can be challenging and increase the rate of recurrence (Pan & Magge, 2020). Despite advancements in treatment modalities, including surgery, radiation therapy, and chemotherapy, the median survival rate for GBM remains dishearteningly low.

Epidemiological research demonstrates that GBM accounts for a significant proportion, approximately 15%, of primary brain tumors, with an incidence rate that increases with age. The average age adjusted incidence rate of GBM between 2012 and 2016 was 3.22 per 100,000 individuals in the United States (Khabibov et al., 2022). While GBM can occur across all age groups, it is predominantly diagnosed in older individuals, ranging between ages 45-70 (Davis, 2016). Prognosis of GBM is bleak, with a median survival of approximately 12 to 15 months following diagnosis, even with aggressive treatment approaches. Factors of prognosis can include a patient's age, performance status, extent of surgical resection, molecular characteristics of tumor, and response to therapy.

Despite extensive research efforts, the etiology of GBM remains elusive, with ionizing radiation exposure being the only confirmed risk factor (Khabibov et al., 2022). The heterogeneous nature of GBM presents a formidable challenge in its management and treatment. Understanding this heterogeneity is paramount to deciphering the underlying mechanisms driving tumor aggressiveness, treatment resistance, and recurrence. The dire need for personalized medicine approaches tailored to the unique molecular profiles of individual tumors is significant, and unraveling GBM's heterogeneous nature holds promise for the development of more effective therapeutic strategies and improved outcomes for patients battling this devastating disease.

Molecular Pathogenesis of Glioblastoma

Astrocytes, a type of glial cell, are prevalent within the central nervous system (CNS) and serve as the source of GBM (W. Wu et al., 2021). Astrocytes, a crucial subtype of glial cells, play various essential roles in CNS function including homeostasis, supporting neurons structurally, regulating neurotransmitter levels, safeguarding blood-brain barrier, and repairing injury to the brain through physical trauma, stroke, or neurodegenerative diseases. However, in the context of GBM, astrocytes transform into malignant cells known as astrocytomas. This transformation, referred to as gliomagenesis, is marked by uncontrolled proliferation, resistance to apoptosis, and the acquisition of invasive properties (Hanif et al., 2017). Multiple factors, including genetic mutations, epigenetic alterations, and microenvironmental cues, can trigger this oncogenic change in astrocytes.

While traditionally viewed as supportive cells, astrocytes have garnered attention from researchers due to mounting evidence suggesting their involvement in promoting a microenvironment that is stable for driving the spread of cancer to the brain. One mechanism

implicated in this process involves the release of fatty acids by astrocytes, which activates the PPAR-gamma signaling pathway in cancer cells. This activation creates an optimal environment for cancer cell survival and replication, potentially facilitating their migration and colonization within the brain. Thus, the transition of astrocytes from supportive to potentially tumor-promoting entities underscores the complexity of their role in brain health and disease.

Key genetic mutations that are implicated in GBM include alterations in genes such as EGFR, PTEN, TP53, and IDH1/2 (Liu et al., 2016). These mutations are known to disrupt vital signaling pathways in the cell, such as the PI3K/AKT/mTOR and MAPK/ERK pathways, which lead to uncontrolled cell proliferation, enhanced survival, and increased invasion capacity to infiltrate surrounding brain tissue (Behrooz et al., 2022). Moreover, epigenetic modifications such as DNA methylation, histone modifications, and microRNA dysregulation are the main mechanisms of gene expression regulation in GBM. DNA methylation, which is the addition of a methyl group to cysteine residues in DNA, can lead to gene silencing by inhibiting transcription factor binding or recruiting repressive chromatin remodeling complexes, such as histone deacetylases and methyl-binding proteins (A. Liu et al., 2016). Histone modifications, such as acetylation, methylation, phosphorylation, and ubiquitination, alter chromatin structure and accessibility, thereby regulating gene expression (McCornack et al., 2023). MicroRNAs, small noncoding RNAs, can post-transcriptionally regulate gene expression by binding to target messenger RNAs (mRNAs), leading to mRNA degradation or translational repression (Y. Liu et al., 2021). These modifications result in the silencing of tumor suppressor genes or other genes involved in the regulation in the cell cycle progression, apoptosis, and DNA repair. Dysregulation of epigenetic regulators can facilitate transcription of genes that promote tumorigenesis (Wu et al., 2021).

The aggressive behavior of GBM can be pointed predominantly to the intrinsic intra-tumor heterogeneity, or the differences in individual tumors. On the other hand, the inter-tumor heterogeneity, or population level differences, of GBM is relatively homogenous (Aldape et al., 2015). GBM presents 2.2 somatic mutations per megabase 74 kB, compared to other cancers such as lung cancer with more than 8 somatic mutations/Mb in 75, and melanoma with above 12 somatic mutations/Mb in 75 for melanoma (Vivanco et al., 2012). Instead, GBM presents diverse genetic subclones of the tumor representing inter-heterogeneity. GBM displays mosaic amplifications of excessive expression and activation through mutations that affect different RTKs, receptor tyrosine kinases, a hallmark of 50% of GBM prognosis in individuals (Becker et al., 2021). Overall, understanding this complex heterogeneity is crucial for developing effective treatment strategies tailored to individual patients and improving outcomes for those affected with this challenging disease.

Epidermal Growth Factor Receptor (EGFR) in Cancer

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane receptor protein that belongs to the Human Epidermal Growth Factor (ErbB) family of receptor tyrosine kinases (Rodriguez et al., 2023). The activation of EGFR occurs when specific ligands, such as epidermal growth factor, EGF, bind to the extracellular domain of EGFR. This binding leads to a cascade of intracellular signaling events. Ligand binding induces a conformational change in the receptor, leading to the formation of receptor dimers, forming either homodimers with other EGFR molecules or heterodimers with other members of the ErbB receptor family. EGFR's intracellular domain has tyrosine kinase activity. Upon dimerization, the tyrosine kinase domains cross-phosphorylate each other on specific tyrosine residues. These auto-phosphorylated tyrosine residues on EGFR serve to be docking sites for various signaling proteins and become activated

through phosphorylation. EGFR activation can lead to the activation of pathways such as the Ras/Raf/MEK/ERK pathway, PI3K/AKT pathway, and the JAK/STAT pathway (Gan et al., 2013). This can impact several downstream signaling pathways that converge on the cell's nucleus to influence the activation or repression of specific genes that are involved in cell growth, proliferation, survival, etc. This protein emerged to be frequently mutated in the realm of a plethora of human cancers, thus assuming a pivotal role as a prime therapeutic target (Rodriguez et al., 2023).

Unlike other cancers that were studied in relation to EGFR, which demonstrated mutations occurring in the intracellular tyrosine kinase domain, Glioblastoma multiforme (GBM), a highly aggressive and malignant type of brain tumor arising from the glial cells of the central nervous system, portrays mutations that are present exclusively in the extracellular domain of EGFR. These mutations impede EGFR's ability to differentiate between two crucial ligands, Epiregulin (EREG), a low affinity ligand, and EGF, a high affinity ligand, in cellular assays; therefore, affecting its predestined response in a normal cellular environment. This altercation leads to aberrant signaling cascades, contributing to dysregulated growth and increased proliferation of glioblastoma cells.

Prevalence of EGFR mutations in GBM

Increased activation of EGFR can occur through a variety of different mechanisms, both ligand-dependent and ligand-independent. In addition to EGFR's inability to differentiate a high affinity ligand like EGF and a low affinity ligand like EGFR, GBM presents EGFR mutations that can express a plethora of genetic alterations affecting the receptor's function (Hu et al., 2022). Current research on mutations of GBM are related to deletion and point mutations. EGFR deletions in GBM include *EGFRvI* (N-terminal deletion), *vII* (deletion of exons 14–15), *vIII*

(deletion of exons 2–7), *vIV* (deletion of exons 25–27), *vV* (deletion of exons 25–28), among which *vII* and *vIII* are oncogenic (An et al., 2018). EGFRvII and EGFRvIII are specifically highlighted as oncogenic, meaning they have the potential to drive tumorigenesis or contribute to the development and progression of cancer. The repercussions of deleting specific exons is crucial to the structural integrity of EGFR. For instance, the deletion of exons 2-7 in vII, disrupt crucial domains in ligand binding, leading to constitutive activation of the receptor. Moreover, point mutations in the extracellular region of EGFR such as R108K, A289V/D/T, G598D and other extracellular domain mutations are identified in 24% GBM samples (An et al., 2018). These point mutations are known to keep EGFR in an active conformation.

The most frequently occurring EGFR mutation in glioblastoma, EGFRΔIII, typically in large measure occurring after amplification of the wild type EGFR, 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 (Liu et al., 2016). Increased receptor levels via gene amplification in cancer cells can allow for more binding sites to be present for ligands of EGFR, thereby enhancing receptor activation. Several different studies have indicated that EGFRΔIII is expressed in approximately 50% of glioblastomas that amplify wild-type EGFR. This signifies a strong synergistic relationship between the gene amplification of EGFR and the variant EGFRΔIII (Q. Wu et al., 2021).

Compared to EGFRWT (EGFR wild type), EGFRvIII lacks amino acids 6–273, and deletion of those 268 amino acids creates a junction site with a new glycine residue between amino acids 5 and 274 (An et al., 2018). The constitutive activity of EGFRvIII, despite exhibiting a weaker intrinsic kinase activity compared to EGFR wild type, is due to the structural and functional alterations resulting in the deletion mutation. One such alteration can be pointed

to EGFRvIII lacking a ligand binding domain, thus disrupting the typical regulatory mechanisms of EGFR. Autophosphorylation occurs normally in activated EGFR receptors upon the binding of a ligand, contributing to the formation of dimers. In the context of EGFRvIII, this process continues to occur, as the mutant EGFR variants are phosphorylated by the wild type EGFR molecules (Hanif et al., 2017). This implies that the sustained growth advantage of EGFRvIII-transduced cells results from the coordination with wildtype EGFR.

Tumorigenesis and Angiogenesis: Synergistic Effects on GBM's Heterogeneity

The clonal heterogeneity of EGFRs in GBM highlights not only genetic diversity but also functional differences between amplified wild-type EGFR (wtEGFR) and mutated EGFR variants. This variability fits the pathophysiology of GBM, especially when considering the growth and evolution of the tumor. Because the normal brain has a large amount of vasculature, tumors may not require angiogenesis in their early stages. Angiogenesis is a process by which pre-existing blood vessels give rise to new ones (A. Liu et al., 2016). Signaling chemicals released by tumor cells cause surrounding blood vessels to proliferate and expand in the direction of the tumor, creating new blood vessels. Tumor growth, invasion, and metastasis are encouraged by this mechanism, which eases the transport of nutrients and oxygen from the newly formed blood vessels to reach the tumor and permit the evacuation of waste materials (Pan & Magge, 2020). EGFR mutation and amplification can initiate signaling pathways that enhance invasion and promote angiogenesis when tumors grow in GBM prognosis, enabling tumors to proliferate and endure hypoxic environments. As tumors develop in GBM prognosis, EGFR mutation and amplification can trigger signaling pathways that improve invasion and encourage angiogenesis, allowing tumors to grow and survive in hypoxic conditions. Thus, local environmental factors may have an impact on the appearance of EGFR mutations, particularly EGFRvIII, which may

then lead to the focused development of angiogenesis and tumor progression (Eskilsson et al., 2018). The complicated relationship between the dynamics of the tumor microenvironment and EGFR mutations highlights the intricacy of GBM pathogenesis and development.

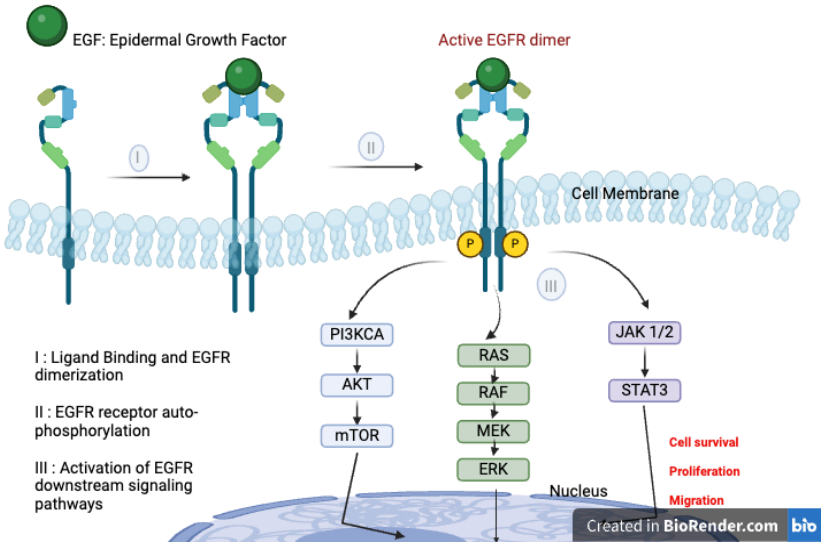
EGFR Signaling Pathways in Glioblastoma

Activation of EGFR in the plasma membrane triggers several downstream signaling pathways including the RAS/mitogen-activated protein kinase(MAPK)/extracellular signal regulated kinase (ERK) pathway, phosphoinositide-3-kinase(PI3K)/protein kinase B (AKT) pathway, the Janus Kinase (JAK)/ signal transducer and activator of transcription (STAT) pathway, and protein kinase C (PKC) pathway. EGFR can also localize in non-plasma membrane components, including in the nucleus and the mitochondria. Nuclear localized EGFR interacts with DNA repair proteins, transcription factors, and chromatin factors, thus influencing gene expression and DNA repair mechanisms. Mitochondrial-localized EGFR interacts with mitochondrial function and metabolism, contributing to tumor growth survival and adaptation in hypoxic conditions (An et al., 2018). The EGFR variant most predominant in GBM, EGFRvIII, exhibits aberrant signaling properties in comparison to the wild type EGFR. Although sharing similar pathways like the ones listed above, this variant poses various different functional properties that set it apart from the wild type, including enhanced activation of certain downstream effectors and increased oncogenic potential.

The RAS/MAPK/ERK pathway, depicted by Figure 1, is essential to cell functions of growth and survival. RAS proteins can be analogous to switches in the cell. These switches are regulated by two proteins; SOS is the protein that turns them “on,” and NF1 is a protein that turns them “off”. In a healthy cell, when EGFR is activated, it recruits a protein called GRB2, which then activates RAS. The activation of RAS exacerbates a downstream signaling cascade,

as it allows for the activation of other proteins such as ERK1/2(Q. Wu et al., 2021). ERK1/2 are then able to move into the nucleus of the cell and control the activity of genes that are involved in cell growth and proliferation, among other processes. In GBM, the pathway is overactive. Mutation of RAS is rare in GBM (only two percent), high RAS activity in the tumor is frequently observed (An et al., 2018). Instead, researchers postulate that this could be due to mutations present in NF1, a protein known to regulate the activity of RAS proteins. The RAS-GAP NF1 is mutated or deleted in 18% of GBM patients. Tumors with NF1 mutation/deletion show activation of RAS, measured by p-ERK and p-MEK (An et al., 2018). These results indicate that the EGFR/RAS/MEK/ERK pathway plays an important role in pathogenesis.

Figure 1



Note: This figure depicts EGFR activation and its pathway in a healthy (non-cancerous) cell. Created with BioRender.com

Similarly, the PI3K/AKT pathway, demonstrated by Figure 1, is a cellular control center that regulates cell growth, survival, and metabolism. AKT substrates, proteins critical in regulating cell proliferation and survival, include tuberous sclerosis complex (TSC), BCL2

associated death protein (BAD), Beclin 1, Caspase-9, inhibitor of nuclear factor kappa-B (NFκB) kinase subunit alpha (IKKα), transcription factors cAMP response element-binding protein (CREB) and forkhead homeobox type O (FOXO) (Khabibov et al., 2022). AKT also promotes metabolism by facilitating membrane localization and expression of glucose transporters, by phosphorylating critical enzymes in metabolism such as fructose-2,6 bisphosphatase, and ATP-citrate lyase (ACLY), which enables production of acetyl Co-A production (An et al., 2018). This pathway has a control mechanism, referred to as the protein, PTEN, that can switch off this pathway by turning an intermediate activated molecule, PPI3, in the downregulated stream of signaling cascades to a molecule that was activated in the beginning, PPI2 of the pathway's genesis (Ding et al., 2022). In GBM, this pathway is also seen to be overactive, due to the mutations present among PTEN.

The JAK/STAT pathway, shown through Figure 1, is a vital communication course that aids cells to communicate with cytokines—proteins that are involved in immune responses. JAK proteins get activated through the interaction of cytokines, which are released through EGFR activation. These cytokines bind to their respective receptors, which in turn activate JAKs, initiating the downstream signaling cascade of events in this pathway. The activation of JAKs phosphorylate other proteins, such as STATs, that readily form pairs and move to the nucleus of the cell. The paired STATs can then regulate the transcription of specific genes that regulate cellular processes such as cell growth, inflammation, stem cell characteristics, and cell movement. The crossplay with other proteins such as AKT also affects the activity of STAT proteins. AKT can activate another protein, EZH2, which further activates STAT3, demonstrating that EGFR activation can indirectly affect STAT (H. Liu et al., 2020). EGFR can also directly activate STAT3 through phosphorylation at a specific amino acid residue on its polypeptide

chain, Y705 (Kenchappa et al., 2022). STAT3 is vital to understanding the tumorigenesis of GBM. In a healthy cell, STAT3 is known to suppress the transformation of normal cells into cancerous ones, when the PTEN gene (as part of the PI3K/AKT pathway) is intact (Becker et al., 2021). However, in most cases of GBM, PTEN and EGFR are often mutated, resulting in STAT3 being constitutively activated through phosphorylation of EGFR, driving cells to become cancerous.

The PLC/PKC pathway is also integral to the continuation of cellular processes like cell proliferation, survival, and motility. Activation of EGFR can recruit and activate an enzyme called phospholipase C (PLC)(Becker et al., 2021). PLC is able to break down a molecule called phosphatidylinositol 4,5-bisphosphate (PIP2) into two smaller molecules: inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). DAG is able to activate another enzyme called protein kinase C, PKC. In GBM, PKC enzymes such as PKCα, PKCη, and PKCδ are particularly notable, contributing to tumorigenesis (An et al., 2018). The activation of PKC enzymes influence the activity of various proteins in the vell, such as those involved in cell regulation (e.g. p53 and p21), cell growth and proliferation (e.g. RAS-RAF1 and GSK3), cell motility (e.g. integrins), cell survival (e.g. BCL2 and BAD), and inflammation (e.g.NFκB) (An et al., 2018).

Current treatment approaches for GBM and their limitations

The implementation of cancer vaccine therapy demonstrated significant potential in preventative and therapeutic realms. More specifically in GBM, cancer vaccines are specifically engineered to selectively target tumor-specific antigens to stimulate an immune response against malignant tumors (Angom et al., 2023). The process of leukapheresis collects a patient's own T cells, and gene editing mechanisms, such as CRISPR/Cas9 or viral vectors, are utilized to genetically modify them. Expanded in vitro, the modified T cells are able to generate a large

proportion of CAR T cells (chimeric antigen receptor T cells), which are infused back into the patient where they are trafficked to the tumor antigen site. The immune response is generated as these cells become active and release cytotoxic molecules, such as perforin and granzymes and recruitment of other immune cells. EGFRvIII, a mutant variant of EGFR that remains active and consistently found in 20-30% of GBM cases, is a tumor specific antigen that is currently researched in a great deal (Eskilsson et al., 2018). This allows this variant to be recognizable by the body's immune system. Immunotherapeutic approaches such as chimeric antigen receptor (CARs) T cell therapy genetically engineer T cells and enable them to express CARs, synthetic receptors designed to recognize specific antigens on cancer cells, to target the tumor-specific antigen, EGFRvIII (Angom et al., 2023).

The use of small molecule tyrosine kinase inhibitors (TKIs) as a therapeutic approach in targeting EGFR of GBM is also apparent. Small TKIs such as erlotinib, gefitinib, among others, competitively bind to the tyrosine kinase domain of EGFR, thus preventing the receptor from autophosphorylating and activating downstream signaling pathways (Binder et al., 2018). TKIs are orally administered in the form of tablets or capsules, and patients take them usually once daily. In preclinical and clinical studies, TKIs like erlotinib and gefitinib have shown effectiveness in inhibiting the growth of various cancer cell types by inducing cell arrest and apoptosis. However, their efficacy in treating GBM has been limited, with response rates ranging up to to only 25% in some cases (Angom et al., 2023). Erlotinib and gefitinib are known to affect EGFR activity in patients with lung cancer, whose activating mutations typically lie in exons 19 and 21 of the tyrosine kinase domain (Verreault et al., 2022). These mutations do not exist in GBM, potentially contributing to the lack of survival benefit in patients treated with erlotinib or gefitinib (Angom et al., 2023).

FDA-approved anti EGFR antibodies, cetuximab and panitumumab, bind to the L2 domain of EGFR, preventing the binding of ligands and activation of downstream signaling pathways (C. Wu et al., 2022). However, these antibodies were not able to effectively target the mutant version of EGFR, EGFRvIII. Soon after, an unconventional antibody was introduced in preclinical studies as mAb806, which was specifically designed to target overly expressed EGFR and co-expression of EGFR and EGFRvIII. This antibody has shown promising results in GBM patients where it potently kills EGFRvIII expressing tumors in preclinical studies (Becker et al., 2021). Antibodies can also be conjugated with toxins or radioactive isotopes which enhance the ability to enhance eradication of tumors (Wieduwilt & Moasser, 2008). Bispecific antibodies (bisAbs) contain two different binding specificities fused into one molecule. They can be engineered to bispecific Tcell engagers (BiTEs), which bind to the CD3 T cell coreceptor to recruit cytotoxic T cells. A BiTE named bscEGFRvIIIxCD3, designed to redirect Tcells to tumors expressing EGFRvIII, showed potent killing of EGFRvIII-expressing GBM in vitro and in mice. Injection of bscEGFRvIIIxCD3 intravenously achieved complete cure in up to 75% in NSG mice with U87.EGFRvIII intracranial xenografts. Whether this BiTE can be used in patients awaits further study (An et al., 2018).

Vaccines are known to stimulate the immune system, allowing for recognition and target to attack the tumor cells expressing specific antigens. A vaccine known as Rindopepimut (CDX110) targets the mutated version of EGFR, EGFRvIII. CDX-110 consists of a 14-mer peptide that spans the mutation site of EGFRvIII which is attached to immune adjuvant keyhole limpet hemocyanin (KLH). The vaccine was found to be safe, immunogenic, and tumor-specific in phase I of the clinical trial and in accordance with administered TMZ, prolonged survival in GBM patients in phase II (Verreault et al., 2022). However, as part of phase III, the vaccine

failed the double-blind randomization trial. Besides vaccines, RNA-based therapies targeting EGFR/EGFRvIII include antisense oligonucleotides, RNA interference (RNAi), ribozymes and adjuvant microRNA (miRNA) based therapies (An et al., 2018). The common goal is to reduce the mRNA levels of EGFR/EGFRvIII to inhibit tumorigenesis. Although in vitro studies have shown decreases in mRNA levels of EGFR/EGFRvIII, thus limiting expression and tumor proliferation, in vivo studies have yet to match their efficacy.

Factors that contribute to treatment resistance of EGFR/EGFRvIII in GBM can be attributed to a plethora of reasons. The most significantly studied phenomenon is the blood-brain barrier (BBB), a specialized interface separating the bloodstream from the brain tissue, which regulates the passage of substances into and out of the brain (Haar et al., 2012). Many chemicals and antibodies targeting EGFR/EGFRvIII are not efficient enough at crossing the BBB, thus hindering their efficacy. Moreover, as EGFR/EGFRvIII are located upstream of downstream signaling cascades, mutations that are apparent in downstream molecules and upregulation of other tyrosine kinases like the IGF-1 receptor (IGF-1R), MET, and PDGFRβ can bypass the inhibition to EGFR/EGFRvIII, thus driving metastasis of the tumor (Barzegar Behrooz et al., 2022). PTEN is a downstream protein that negatively regulates the PI3K signaling pathway. It has been observed that patients with amplified EGFR and intact PTEN have shown a decrease in tumor progression. However, patients who have been identified to have a loss of PTEN functionality, lead to resistance to EGFR inhibitors (Yalamarty et al., 2023). Moreover, phosphorylation of PTEN at sites such as Y240 by Src Family Kinases (SFK) and Fibroblast Growth Factor Receptor (FGFR) contribute to the resistance against EGFR inhibitors.

The high level of heterogeneity of GBM is displayed by numerous cells expressing an amplification of various RTKs, like EGFR, MET, or PDGA(An et al., 2018). This signifies that

targeting one RTK may not be sufficient enough to treat GBM. Moreover, as RTK amplifications are commonly found among extrachromosomal DNA double minute structures, tyrosine kinase inhibitors, TKIs, may initially demonstrate the elimination of these segments containing EGFRvIII, resulting in a tumor shrinkage. However, once the treatment is halted, a re-emergence of EGRvIII containing extrachromosomal DNA is often observed. The resurgence of tumorigenesis ensues, and drug resistance is molded. The tumor's microenvironment, particularly immune and stromal cells, such as microglia and or macrophages, play a vital role in the metastasis of GBM. The crosstalk between immune cells, stromal cells, and the tumor to regulate immune cell infiltration remains a question yet to be answered. Exploring these limitations will lead to more effective therapeutic strategies for GBM.

Conclusion

This paper offers a comprehensive review of the role of EGFR (Epidermal Growth Factor Receptor) in GBM. Moreover, EGFR is most often co-expressed and amplified with the mutated variant, EGFRvIII, which contributes to tumor metastasis by dysregulating downstream signaling pathways, such as Ras/Raf/MEK/ERK, JAK/STAT, PI3K/Akt/mTOR, and PLC/PKC. The vast and high levels of tumor intra-tumor heterogeneity contribute to diverse genetic alterations and extrachromosomal DNA containing various RTK amplifications of EGFR, PDGA, and MET which pose treatment ineffectiveness (Angom et al., 2023). Additionally, immunotherapy targeting such as chimeric antigen receptor (CARs) T-cell therapy and antibodies targeting EGFRvIII have been shown to effectively kill EGFRvIII positive tumor cells. However, the remainder of oversimplified and mutated EGFR tumors remain and contribute to tumorigenesis. In conclusion, inefficient blood brain penetration, intratumor heterogeneity, compensatory signaling pathways, and secondary mutations contribute to the resistance of therapy. A

combination of therapies are necessary to develop a better outcome beneficial to patients with GBM.

This has significant implications for future research in the therapeutic development to treat GBM. This paper stands to emphasize the dire need of further exploration of drug resistance mechanisms, such as the dynamics of extrachromosomal DNA and RTK amplifications as a limitation of TKIs, tyrosine kinase inhibitors, and the crosstalk in the tumor's microenvironment of neighboring stromal and immune cells. The exemplary heterogeneous nature of GBM underscores the importance of personalized medicine approaches. Molecular profiling and targeted therapies tailored to individual patients on their specific molecular profiles allows for increased survival rates and a potential cure. In conclusion, this paper establishes the groundwork for developing precision medicine strategies and enhancing outcomes for patients battling Glioblastoma Multiforme (GBM) by elucidating the complex interactions between EGFR mutations and signaling pathways in GBM and suggesting potential future directions for therapeutic targeting.

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