# Gene Fusion in Malignant Glioma: An Emerging Target for Next-Generation Personalized Treatment ⦿ CrossMark

Tao Xu[*,1], Hongxiang Wang[*,1], Xiaoquan Huang[†], Weiqing Li[‡,2], Qilin Huang[*], Yong Yan[*] and Juxiang Chen[*]

[*]Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China; [†]Center of Evidence-based Medicine, Zhongshan Hospital, Fudan University, Shanghai 200032, China; [‡]Department of Pathology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

## Abstract

Malignant gliomas are heterogeneous diseases in genetic basis. The development of sequencing techniques has identified many gene rearrangements encoding novel oncogenic fusions in malignant glioma to date. Understanding the gene fusions and how they regulate cellular processes in different subtypes of glioma will shed light on genomic diagnostic approaches for personalized treatment. By now, studies of gene fusions in glioma remain limited, and no medication has been approved for treating the malignancy harboring gene fusions. This review will discuss the current characterization of gene fusions occurring in both adult and pediatric malignant gliomas, their roles in oncogenesis, and the potential clinical implication as therapeutic targets.

## Introduction

Gene fusions are hybrid of two coding or regulatory DNA sequences between genes. They are pathognomonic mutations resulting from genomic rearrangements such as translocations, deletions, duplications, or inversions. This phenomenon was first described in chronic myeloid leukemia (CML) when researchers used quinacrine fluorescence and Giemsa staining, finding a cellular oncogene ABL1 on chromosome 9 fused to BCR on chromosome 22, forming BCR-ABL1 fusion gene on the newly formed Philadelphia chromosome. Later studies found that this gene fusion had changed this previously deadly type of cancer into the nearly cured disease using tyrosine kinase inhibitors [1–5]. Since then, with the development of biomedical technologies, more and more gene fusions were detected with further understanding of their mechanisms and biological functions. At present, more than 10,000 fusion genes in cancer have been found and indexed in the Mitelman Database of Chromosome Aberrations and Gene Fusions (2017) (http://cgap.nci.nih.gov/Chromosomes/Mitelman).

Despite most of fusions appearing to be passenger mutations without oncogenic functions, some of the fusions do constitute strong driver alterations in the initial steps of tumor development, while other fusions may play important roles in tumor progression [6–8]. For example, *EML4-ALK* fusion causes transformation of non–small-cell lung cancer, and *COL1A1-PDGFRB* fusion contributed directly to growth and progression of dermatofibrosarcoma protuberans [9–12].

The deep insight into oncogenic gene fusions will not only help extend our understanding of tumor biology but also provide robust therapeutic targets for selected tumor types [13]. The tyrosine kinase inhibitor (TKI) imatinib, designed for treating CML with *BCR-ABL1* fusion, was approved by US Food and Drug Administration (FDA) in 2001 and achieved excellent efficacy with improved lifespan and quality of life of patients [14]. The successful clinical practice has spurred interest in developing drugs targeting the products of gene fusions in multiple tumors. Subsequently, several new kinase inhibitors were approved by the FDA, such as crizotinib and ceritinib for the treatment of non–small cell lung cancer with ALK fusions, and

Address all correspondence to: Juxiang Chen, Department of Neurosurgery, Changzheng Hospital, Second Military Medical University, 415 Fengyang Road, Shanghai 200003, China.
E-mail: juxiangchen@smmu.edu.cn
[1] Authors contributed equally to this review.
[2] Current Address: Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78,216, USA.

ponatinib used to treat both CML and acute lymphoblastic leukemia patients with *BCR-ABL1* fusion [7].

Gliomas are the most common primary brain tumors with high recurrence and mortality rates [15]. Over the past decade, molecular targeted therapy has underscored the promise of managing the malignant gliomas. One of the challenges in understanding and treating gliomas has been the clinical and molecular heterogeneity associated with this disease [16]. Currently, genomic and transcriptomic analyses are defining the molecular architecture of gliomas and have uncovered a subset of patients carrying gene fusions [8]. Despite relatively low-frequency rate in gliomas, gene fusion has a disproportionate importance in investigating the mechanism of tumorigenesis and provides potential targeted therapies for selected patients with specific fusions [8]. In this review, we summarize the current characterization of gene fusions occurring in both adult and pediatric malignant gliomas and discuss the potential clinical implication of oncogenic fusions as therapeutic targets.

## Types of Gene Fusions
Gene fusions are two or more genes that form chimeric genes involving parts of each gene. The formation of fusion genes in different cells can occur through different mechanisms [17]. Previous studies have demonstrated that fusion transcripts can be not only produced by chromosomal rearrangement at the DNA level but also formed through trans-splicing and cis-splicing between neighboring genes.

There are four basic types of chromosomal rearrangements that could lead to gene fusions (Figure 1): (1) a genomic region between two genes located on the same strand is deleted, and a fusion gene arises through deletion; (2) a genomic region is duplicated one or more times on chromosomal is the tandem duplication; (3) a chromosomal translocation involves the translocation of genomic regions on different chromosomes; and (4) two genes located on opposite strands of a chromosome form an infusion gene, and inversion event is suspected to happen [17].

## Detection of Gene Fusions
Advances in sequencing technologies have refined the "driver" mutation in tumors and facilitated a move toward "precision medicine." These technologies were discussed below with their own advantages and disadvantages summarized in Table 1.

### *Immunohistochemistry (IHC)*
IHC is the most commonly used method in most pathology laboratories [18]. It is inexpensive, is easy to perform and requires only a small biopsy sample, and that tissue morphology is retained during analysis. However, it cannot detect unknown fusions, while the technique is time consuming with relatively low efficacy [19]. Also, not all fusion genes have primary antibodies with high affinity and specificity. Therefore, IHC is not applicable to genes without well-performing antibodies.
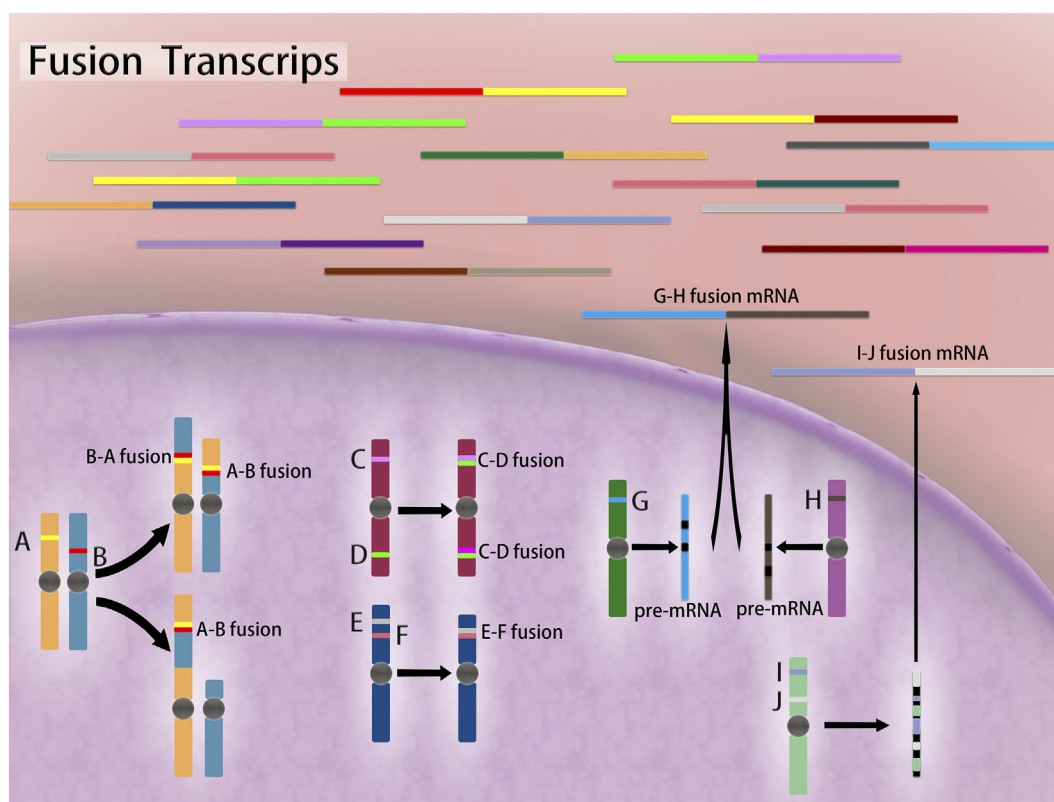


**Figure 1.** Gene fusions leading to chimeric transcripts through a variety of mechanisms. Fusion genes can form at chromosomal level, where translocation, insertion, inversion, and deletion occur and produce B-A and A-B fusion mRNAs, A-B fusion mRNA, C-D fusion mRNA, and E-F fusion mRNA, respectively. Fusion transcripts can also be caused by trans-splicing (G-H fusion mRNA) or cis-splicing (I-J fusion mRNA) events at posttranscriptional level. Black blocks represent introns. Cyan block in the pre-mRNA represents intergenic region.

**Table 1.** Different Detection Methods of Gene Fusions

| Detection Methods | Advantages | Disadvantages |
| --- | --- | --- |
| FISH | Gold standard; rapid; capable of detecting rearrangements without knowing fusion partners | Expensive; not uniformly available in all laboratories; low multiplexing ability |
| IHC | Low cost; easy to perform; small sample requirement, retained tissue morphology; widespread availability | Time consuming; possible false-negative findings because of low sensitivity; low multiplexing ability |
| RT-PCR | Rapid; sensitive; low detection limit; identification of specific fusion partners | Unable to detect unknown fusion partners; require specialized equipment and lab settings; rely on the quality of RNA; low multiplexing ability |
| Gene array | Genome-wide profile and high-resolution analysis without prior cell culturing | Need confirmation using RT-PCR or FISH to avoid false-positive findings |
| WGS | Comprehensive and unbiased characterization of genomic alterations, capable of detecting novel fusions. | Short read length; technical artifacts; limited coverage; high false-positive signal; expensive |
| RNA-Seq | Low cost; quick turnaround time; detect multiple alternative splice variants caused by a fusion event | Unable to monitor nontranscribed regions; complicated data analysis |

### Reverse Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR is a fast and sensitive method that is routinely used in detecting low levels of gene fusions. The rapid turnaround time, low amount of tissue requirement, and identification of specific fusion partners are advantages of RT-PCR. However, the requirement of fusion-specific primers results in inability to detect rearrangements involving unknown fusion partners [20]. Another limitation of RT-PCR is that when applied in formalin-fixed, paraffin-embedded tissues, false-negative results might be due to highly degraded RNA available [18].

### Fluorescence In Situ Hybridization (FISH)

FISH is a rapid, expensive, gold standard method that can detect gene fusions on both DNA and RNA levels [21]. The advantage of FISH is that it can be performed on formalin-fixed, paraffin-embedded tissues and nondividing cells (interphase nuclei), and it is also capable of detecting rearrangements without prior knowledge of 5′ fusion partners compared with RT-PCR [7,18,22]. However, this technique is not uniformly available in all laboratories [18]; thus, it is used as a confirmatory tool rather than a screening tool [23–25]. A lot of gene fusions were identified by using FISH probes, e.g., *ETV6/AML1* fusion in acute lymphoblastic leukemia [26], *JAZF1-JJAZ1* fusion in endometrial stromal tumors [27], and *EML4-ALK* in lung cancer [28].

Currently, as researches in oncogenic fusion continue, the need to detect multiple targets at the same time is becoming more and more urgent, calling for high-throughput methods such as gene array and next-generation sequencing (NGS). The low multiplexing ability makes IHC, PT-PCR, and FISH only as orthogonal confirmatory methods for detection of gene fusions.

### Gene Array

The usage of gene array-based technologies provided new options for detection of gene fusions from 1990s [29]. This technique could enable a genome-wide profile and offer high-resolution analysis on the expression level of DNA or exons without the requirement of prior cell culturing [30]. Numerous novel gene fusions were detected in different cancer types using high-throughput array-based technologies, like *PAX3-NCOA1* in alveolar rhabdomyosarcoma tumors [31] and *HEY1-NCOA2* in mesenchymal chondrosarcoma [32]. However, this method cannot be used alone because of the sheer number of breakpoints found typically in malignancies and by extensive constitutional copy number variation; thus, it always needs confirmation by FISH or RT-PCR to avoid false-positive findings [33]. Also, not all gene fusions will lead to abnormalities in expression of involved parts of genes; these fusions are hard to be detected by gene array. With the development of deep sequencing, gene array-based technology is not used as widely as before.

### Next-Generation Sequencing

NGS has provided a radically new means to identify novel or known fusions either in genomic DNA or transcriptome sequencing datasets. This technique contains several subtypes involving whole genome sequencing (WGS), whole exome sequencing, and whole transcriptome sequencing (RNA-Seq). WGS and RNA-Seq are the most commonly used NGS in this field and have achieved important discoveries [34–38].

WGS offers the large comprehensive and unbiased characterization of genomic alterations in genomes, especially cancer genomes [39]. Despite being the most powerful sequencing technology, WGS falls short because of to the short read length, technical artifacts, limited coverage, and high false-positive signal resulting from sequencing errors [40–42]. Besides, despite significantly decreased cost of NGS in the last few years, the cost of WGS is still more expensive than RNA-Seq [43].

RNA-Seq is the most commonly selected technique for transcript detection of fusion gene owing to its low cost and quick turnaround time. More importantly, it can detect multiple alternative splice variants caused by a fusion event [39] and focuses on the expressed regions of the genome, which makes the detected fusions more biologically relevant in general. However, RNA-Seq technology only sequences about 2% of the entire genome compared with WGS [44], and it cannot monitor fusion events involving nontranscribed regions [45], which is a double-edged sword that detecting fusion events do not occur at the DNA level [41].

In general, it is reasonable to believe that development and progression of fusion gene detection technologies will contribute to the future studies on diagnosis and personalized treatment of human cancers.

## The Landscape of Gene Fusions in Subtypes of Malignant Gliomas

### Glioblastoma (GBM)

GBM accounts for 82% of malignant gliomas [46]. Patients with GBMs have a uniformly poor prognosis, with a median survival of approximately 14 to 16 months after multimodal treatments [47]. Considering the poor prognosis and therapeutic effect, it is of significance to develop novel and effective treatment strategies. Gene fusions are frequent genomic abnormalities in GBM; previous studies have shown that two major specific genomic hotspots where fusion frequently occurs are on chromosomes 7p and 12q, while other regions with a higher frequency of fusions are on chromosomes 1, 4,

**Table 2.** Recurrent gene fusions with verified biological functions in malignant gliomas

| Tumor Subtype | Fusion gene symbol | Incidence Rate | Activated Signaling Pathway | Prognostic or Diagnostic role | Target Medicine |
|---|---|---|---|---|---|
| Glioblastoma | *FGFR1-TACC1* | 1.1% | | | Ponatinib BGJ398 |
| | *FGFR3-TACC3* | 1.2%-8.3% | MAPK, ERK, PI3K, and STAT3 | | Erdafitinib AZD4547 |
| | *EGFR-SEPT14* | 4% | STAT3 | | Lapatinib |
| | *EGFR-PSPH* | 2.2% | | | Erlotinib |
| | *NTRK* fusions | 1.2-1.7 % | NGF/TrkA- | | Entrectinib Larotrectinib |
| | *PTPRZ1-MET* | 3% | | Unfavorable prognosis | |
| pHGG | *NTRK* fusions | 4% in DIPGs, 10% in NBS-pHGGs 40% in NBS-pHGGs within 3 years old | PI3K, MAPK | | Entrectinib |
| | *MET* fusions | 3-7% in pGBM, 10% in pHGG | MAPK | | Foretinib Crizotinib |
| Ependymoma | *RELA fusion* | 70% in pediatric supra-tentorial ependymoma patients | NF-κB | Unfavorable prognosis | |

6, and 19 [48]. Although fusions occur in approximately 30% to 50% of GBM patient samples, only a few of them are found in more than one patient with oncogenic biological function (Table 2). Potentially druggable gene fusions in all GBMs are discussed below, some of which have shown good preliminary antitumor effects in preclinical and clinical trials.

*Fibroblast Growth Factor Receptor (FGFR) Fusions.* The tyrosine kinase coding domains of *FGFR* genes (*FGFR1* to *FGFR3*) were found to be fused to the transforming acidic coiled-coil (*TACC*) coding domains of *TACC1* or *TACC3*, respectively, forming the *FGFR-TACC* fusions [49]. TACC proteins are characterized by a coiled-coil domain at the C-terminus, known as the TACC domain, which regulates localization to the centrosome and mitotic spindle [50,51]. It is hypothesized that TACC proteins are oncogenic in several human cancers including GBM [52,53]. The FGFR family plays an important role in cell growth, differentiation, and angiogenesis, and its activation usually contributes to carcinogenic events [54]. The oncogenic activities of the FGFR-TACC fusion protein are greater than the sum of its parts when introduced into astrocytes or stereotactically transduced in the mouse brain [49].

*FGFR-TACC1* fusion was found in 1 out of 88 primary GBMs [49]. The incidence of *FGFR3-TACC3* is considerably higher than the frequency of *FGFR1-TACC1*. The *FGFR3-TACC3* fusion gene is a consequence of a 70-kb tandem duplication on chromosome 4q16.3 [49] and 1.2% to 8.3% of GBM patients harboring *FGFR3-TACC* [54–57]. FGFR3-TACC3 fusion protein displays oncogenic activity that promotes tumorigenesis and enhances tumor progression [49,56]. The abnormal expression of FGFR3-TACC3 in certain cell type was reported to activate mitogen-activated protein kinase (MAPK), extracellular signal–regulated kinase (ERK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), and signal transducer and activator of transcription 3 (STAT3) pathways (Figure 2) [56,58,59]. *FGFR-TACC* fusions may identify a subset of GBM patients who would benefit from targeted FGFR kinase inhibition.

Drugs targeting *FGFR-TACC* fusion genes have shown promising therapeutic potential in several types of cancer models, while clinical trials are still ongoing. To date, preclinical studies have shown a reduction of tumor growth and an increase of apoptosis upon treatment with the pan-FGFR inhibitor ponatinib in the U87MG cell line and the mouse xenograft model, suggesting a potential application of ponatinib as a chemotherapeutic option against GBM cells [60]. A phase II clinical trial studying ponatinib on recurrent GBM is now ongoing (NCT02478164); however, it is not specifically designed for FGFR fusion–positive patients.

Erdafitinib (JNJ-42756493), an oral ATP-competitive pan-FGFR selective inhibitor, inhibits tyrosine phosphorylation of activated FGFR at nanomolar concentrations [61]. It was applied to treat two GBM patients with *FGFR3-TACC3* in a phase I trial and showed a tolerable toxicity and clear antitumor activity [61]. BGJ398, an FGFR1-3 kinase inhibitor, is evaluated in a phase II study in recurrent GBM with *FGFR-TACC* fusions and/or *FGFR* mutation (NCT01975701). Another clinical trial involving AZD4547 (a selective inhibitor of *FGFR* tyrosine kinase family) in recurrent glioma patients with *FGFR* fusion is under recruiting (NCT02824133). Given the encouraging outcome of *FGFR* inhibition in preclinical studies and earlier trials, further studies are warranted to identify the most effective *FGFR* inhibitors targeting *FGFR-TACC* fusion so as to translate them into clinical trials.

*Epidermal Growth Factor Receptor (EGFR) Fusions.* EGFR is a transmembrane protein which serves as a receptor for the epidermal growth factor family [62]. Overexpression of EGFR is frequently found in GBM, and EGFR-targeted therapy represents a promising anti-GBM therapy [63]. Besides the *FGFR3-TACC3* fusions, *EGFR* emerged as the most frequent recurrent in-frame fusion in GBM [64]. *EGFR* is commonly fused to intron 9 of *SEPT14* or to the gene *PSPH*, and most of them presented a carboxyl-terminal truncation [65].

*EGFR-SEPT14* fusion gene is an in-frame fusion with C-terminal deletion of *EGFR* [48] and was found in about 4% of GBMs [64]. This fusion gene has been shown to activate STAT3 signaling, thus increasing tumor proliferation, migration, and stemness [64]. *EGFR-PSPH* fusion was also found in GBM, and the frequency of which was 2.2% (3/135), whereas signaling of *EGFR-PSPH* fusions remains unclear [64]. Interestingly, most of the *EGFR-SEPT14* or -*PSPH* fusions lack *EGFRvIII* (exon 2-7 deletion) expression, which may be the reason that the C-terminal deletion of *EGFR* via gene fusion could serve as an alternative mechanism of *EGFR* activation [65].

Several modes of targeted therapies have been used to target *EGFR* including TKIs, antibody-based therapy, immunotherapy, and preclinical trials of RNA therapies. TKIs are small-molecule inhibitors, which bind to the ligand-binding site on the extracellular domain, and are the most clinically advanced EGFR-targeting therapy to date [66]. Preclinical studies suggest a beneficial role when *EGFR-SEPT14* was targeted by TKIs like lapatinib and erlotinib; tumors with *EGFR* genomic alterations show delay on growth. However, clinical trials of EGFR inhibitors failed to show a definitive survival superiority in GBM, probably because of nonspecific patient selection; only a small portion of study populations were harboring
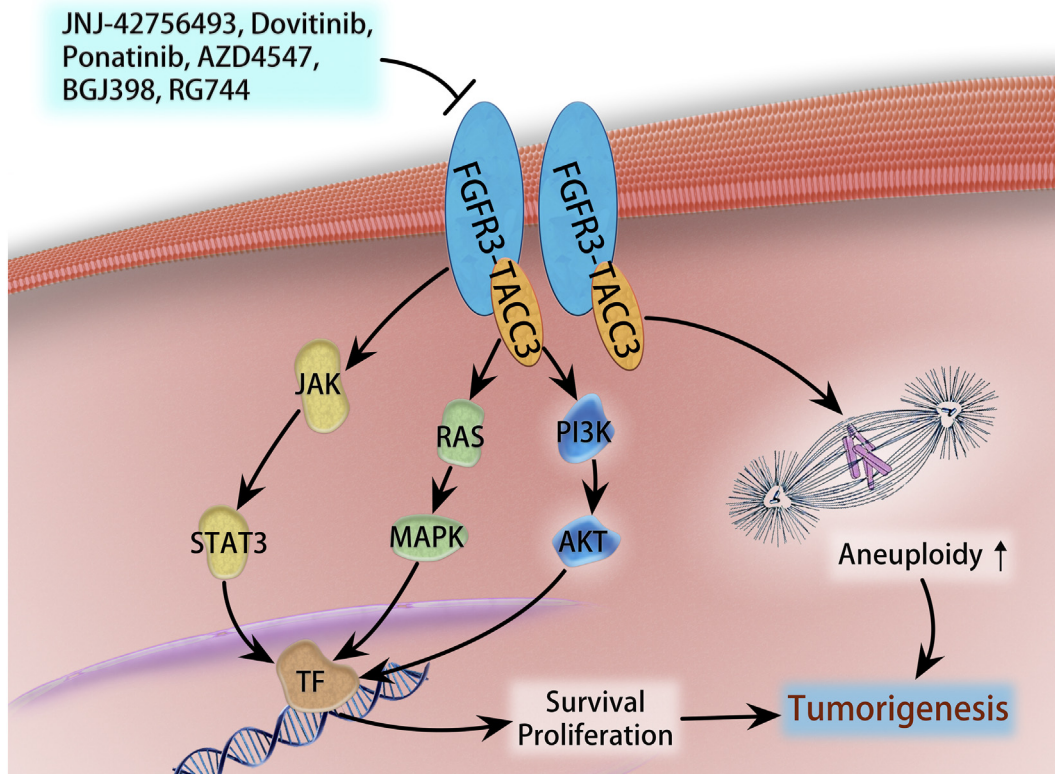
**Figure 2.** FGFR3-TACC3 fusion-mediated oncogenic pathways in GBM. FGFR3-TACC3 protein can constitutively and automatically activate tyrosine kinase domain, which exerts oncogenic function weakly through PI3K/ATK, RAS/MAPK, and STAT3 signaling and may localize to mitotic spindle poles to trigger aneuploidy.

*EGFR* fusions [67–70]. Future studies may focus on targeting patients purely with *EGFR*-fusions based on individualized genomic examination.

*Neurotrophic Tyrosine Kinase Receptor Type (NTRK) Fusions.* *NTRK* encodes the tropomyosin receptor kinase (Trk) receptor family. TrkA, B, and C receptors are encoded by *NTRK1, NTRK2,* and *NTRK3* genes, respectively. The rearrangements of the Trk receptor family play important roles in the oncogenesis in many types of tumor including glioma as well as NSCLC, colon cancer, and papillary thyroid cancer. Several drugs targeting cancers harboring these gene rearrangements are within clinical trials, yet none has been approved by the FDA so far [71].

*NTRK1* is a well-known oncogene that is found commonly in many human cancers but largely lacked in GBMs. In the RNA-Seq data of The Cancer Genome Atlas (TCGA), 2 out of 162 GBM (1.2%) patients were found to have *NTRK1* fused with two genes, neurofascin (*NFASC*) and brevican (*BCAN*), both of which are expressed in neuronal tissues. The fusion gene showed elevated expression of *NTRK1*, as well as neuro growth factor (NGF)–triggered activation of the NGF/TrkA- downstream pathway. Transduction of the *NFASC-NTRK1* fusion gene in cell model increased proliferation both *in vitro* and *in vivo* [72]. What's more, both patients with *NTRK* fusion showed *EGFR* amplification (less than two-folds), indicating some potential linkage between these two genes. In another study involving 115 GBMs, Zongli et al. found two novel *NTRK1* fusions in two separate GBM patients, *CHTOP-NTRK1* and *ARHGEF2-NTRK1*, both of which are in-frame fusions. Interestingly, the *CHTOP-NTRK1* case harbored IDH1 R132

mutation, while the *ARHGEF2-NTRK1* showed EGFR amplification [73]; the biological functions of these two novel fusions are not clear yet.

Although more data need to be accumulated to support the efficiency of Trk inhibitors in clinical trials, it is very promising that targeting the Trk family of receptor tyrosine kinases will help glioma patients with *NTRK* fusions. Entrectinib (RXDX-101) is a pan-Trk inhibitor with good blood-brain barrier penetration. In an open-label, multicenter, global, Phase II basket study (STARTRK-2) examining the use of entrectinib in patients having different types of tumors with the Trk gene rearrangements, the only pontine astrocytoma patient harboring *BCAN-NTRK1* fusion showed 45% tumor volume reduction after dosing entrectinib [74]. Larotrectinib (LOXO-101) is a selective pan-TRK inhibitor that has no significant activity outside of the Trk family. Preliminary results from NAVIGATE Phase 2 larotrectinib trial showed its potential role in treating *NTRK* fusion-positive recurrent GBM. A 35-year-old woman with recurrent multifocal GBM harboring in-frame *EML4-NTRK3* fusion was treated with larotrectinib [75]. The periventricular tumor shrank significantly (from 67 × 52 mm to 8 × 4 mm), while tumor at frontal and occipital lobes had no shrinkage. The trial is continuing to further understand this biological effect.

*Other Fusions.* There are several other gene fusions detected; however, the functional outcomes of these fusions are yet to be studied. The *FIG-ROS1* fusion was the first identified fusion gene in GBM cell lines; an intrachromosomal homozygous deletion on 6q21 caused the *FIG-ROS* locus, this fusion encodes an in-frame fusion protein that has an active kinase activity, suggesting an oncogenic role

[76]. Nameeta et al. explored genomic data of 185 GBM patient samples from both TCGA and Ivy center and found in total 27 genes that were fusion partners in more than one patient sample, including some novel finding of fusion involving noncoding genes. For example, noncoding RNA *RP11-745C15.2* was found fusing with *LANCL2* gene in two patients, one in TCGA database and the other in Ivy database. The same noncoding RNA was fused with *EGFR* in two other TCGA patients. Both fusions lead to C-terminal truncation of the fused gene [48]. In a study carried out based on Chinese Glioma Genome Atlas database, a novel *PTPRZ1-MET* fusion was found in 3% (3/99) of GBM patients. This fusion can induce elevation expression and phosphorylation of MET oncoprotein, and was associated with poor prognosis [77]. Currently, there have been no studies featuring targeting these gene fusions in adult GBM patients.

### Pediatric High-Grade Glioma (pHGG)

pHGGs account for approximately 8% to 12% of all childhood central nervous system tumors, with a distinct biological entity compared with adult HGGs [78]. Most of the pHGGs are histologically anaplastic astrocytoma or GBM; the general prognosis is poor, especially for tumors that are not amenable to resection like diffuse intrinsic pontine glioma (DIPG) [79]. Gene fusions were commonly seen in pHGG including DIPG, although most of them were not recurrent, with uncertain influence on tumor initiating and progressing (Table 2).

*NTRK Fusions.* In a study done by Wu et al. analyzing transcriptome and WGS data from 127 pHGG patients, the *NTRK* fusions, which were detected also in adult GBM with a low frequency as described above, were found in 4% of DIPGs, 10% of non–brain stem pHGGs, and, most notably, 40% of non–brain stem pHGGs in infants within 3 years old [80].

A total of five *NTRK* fusion genes were found: *TPM3-NTRK1*, *BTBD1-NTRK3*, *ETV6-NTRK3*, *VCL-NTRK2*, and *AGBL4-NTRK2*. The first two fusion proteins even showed the ability to induce high-grade astrocytoma in *Tp53*-null mice models with PI3K and MAPK pathway activation. Taken together, these results indicated that *NTRK* fusion genes may act as important tumorigenic drivers in infant pHGGs. Based on these findings, a phase I/Ib study is conducted in pediatric population to evaluate entrectinib in primary central nervous system tumors (NCT02650401).

*MET Fusions.* MET gene encodes the protein c-Met, which possesses tyrosine kinase activity and is involved in different types of cancers. *MET* amplification has been described in about 3% to 7% of pediatric GBMs [81]. A recently published study from the International Cancer Genome Consortium PedBrain Tumor Project found an up to 10% chance of *MET* fusions with activated MAPK signaling.

The whole-genome sequencing of tumor and blood DNA was done in 53 pediatric GBM samples, as well as 5 pediatric GBM cell lines; researchers found 5 partners that fused with MET. They were *TFG-MET*, *CLIP2-MET*, *PTPRZ1(exon1)-MET*, *PTPRZ1(exon1-2)-MET*, and *PTPRZ1(exon1-8)-MET*. What's more, all these samples with *MET* fusion also harbor impaired cell cycle regulation resulting from *Tp53* mutation or *CKDN2A* and *CDKN2B* deletion.

The *TGF-MET* fusion showed strong oncogenicity when induced to neonatal mice with impaired cell cycle (Ckdn2a-dificient or p53-null) in very short periods; histopathology confirmed that the tumor was high-grade glioma expressing fusion protein as well as phosphorylated MET and ERK. Notably, foretinib, an experimental inhibitor targeting MET and vascular endothelial growth factor receptor 2, decelerated MET fusion–driven tumor growth *in vivo*. The therapeutic effect of foretinib was further confirmed in another *CLIP2-MET* fusion SCID mice model.

Based on these findings, crizotinib, an FDA-approved inhibitor targeting ALK as well as MET and ROS1, was used in an 8-year-old GBM patient with *PTPRZ1-MET* fusion; this therapy led to substantial tumor shrinkage at 2 months, but treatment-resistant lesions were also detected with rapid progression that finally caused death of this patient, indicating that pediatric GBM remains a heterogenetic lesion that needs combined therapies on multitargets to help maintain a long-term response [82].

*DHX57-TMEM178-MAP4K3 Fusion.* Carvalho et al. found and validated a novel fusion complex including three genes in a DNA copy number profiling study of 100 pHGG cases. The fusion gene *DHX57-TMEM178-MAP4K3* was found on chromosome 2p22.1 of an anaplastic astrocytoma case; it comprised exons 1-12 of *DHX57*, exons 2-4 of *TMEM178*, and exons 13-34 of *MAP4K3*. The resulting fusion gene contains key regulatory domains from all three proteins. Although the biological function is yet to be studied, the oncogenic capacity of all three components draws the hypothesis that this *DHX57-TMEM178-MAP4K3* gene may be associated with tumor cell growth and proliferation [83].

### Ependymomas

Ependymomas are glial tumors that arise from the cells lining the ventricles and central canal within the spinal cord. They can arise on various age groups and make up 3.6% of malignant primary central nervous system tumors [46]. Ependymomas have a variable prognosis, probably because of their different genomic landscape including transcriptional profiles, DNA copy number alterations, et al. The molecular classification of ependymoma involving nine subgroups helps make therapeutic strategies and prognosis prediction than classical histopathology [84]. Gene fusions were used to characterize two specific subgroups which were discussed below.

*V-rel Avian Reticuloendotheliosis Viral Oncogene Homolog A (RELA) Fusion.* By whole-genome sequencing in 41 tumors with matched normal blood and RNA sequencing in 77 tumors, Parker et al. found that a novel fusion of two genes in chromosome 11q13.1, *RELA* and a poorly characterized gene, *C11orf95*. This fusion resulted from chromothripsis, leading to constitutive aberrant activation of the nuclear factor-kB (NF-κB) signaling pathway (Table 2). After inducing the fusion gene to the neural stem cell, it rapidly generated tumor in xenograft models [85].

Another study from an independent cohort including 500 ependymoma data confirmed that *C11orf95-RELA* fusion is mostly seen in pediatric patients with a median age of 8 years old, but only in supratentorial ependymomas and not in posterior fossa or spinal ependymomas [84]. The incidence is more than 70% [84–86], and most tumors harboring fusion are World Health Organization (WHO) grade II to III tumors. About 15% patients with *C11orf95-RELA* fusion also have homozygous deletions of CDKN2A, which indicates poor prognosis for ependymomas [84,87]. In the newly released 2016 WHO Classification of Tumors of Central Nervous System, ependymomas with *C11orf95-RELA* fusion were put into a specific diagnostic entity [88], indicating high-risk biological behavior, with a 10-year overall survival of around 50% and a 10-year progression-free survival of around 20% [84].

*YAP1 Fusions.* *YAP1* fusions were found in the same studies with *RELA* fusion; it is regarded as the character of another subgroup of supratentorial ependymoma, which is distinct from *RELA* fusion–positive ones [84]. There are two partners found to be fused with *YAP1* gene in chromosome 11. The most common fusion partner is *MAMLD1*, identified in 86% (6/7), followed by *FAM118B*, identified in 14% (1/7) of the cases. The exact function of these fusions is still not clear and remains to be investigated. Unlike *RELA* fusion, tumors harboring *YAP1* fusion showed no evidence of chromothripsis and had relatively stable genomes. *YAP1* fusion was found mostly in ependymoma patients with a median age at diagnosis of 1.4 years, with a relatively favorable prognosis and with a 5-year progression-free survival of 66% and overall survival of 100% [84]. The detailed mechanism why the fusion is related with good prognosis is not clear yet.

## Conclusion and Perspectives

Malignant gliomas are characterized by extensive genomic instability [89], while most of them are associated with a corresponding increase in fusion transcript frequency [8,90]. The advent of high-throughput technologies, especially NGS, has revolutionized the detection of gene fusions, with an overwhelming majority identified in the past 5 years [7]. Discovery of gene fusions in gliomas benefits from the new wave of technology, as fusions are identified in approximately 30% to 50% of GBMs and 47% of DIPGs and non–brain stem HGGs [48,80]. To capture exactly what proportion oncogenic drivers account for among all gene fusions, the significant organization is urgently required with the rapid increase of gene fusion data. Excitedly, concerted efforts have been made to develop computational algorithms and tools involving Oncofuse and Pegasus for separating driver fusions from passenger mutations with sequencing data [91,92]. Furthermore, bioinformatics approaches also have elucidated many aspects of oncogenic gene fusions, from the genetic changes and a causative importance of fusion events to the structural properties and regulatory functions of fusion proteins [90]. Thus, the computational study will catalyze advancements in oncogenic gene fusion identification and guide experimental validation studies in glioma.

Fusions with well-characterized kinases make up a significant part of oncogenic drivers, which is attributed to their susceptibility to kinase inhibitors [57,93]. Recently, a deep-sequencing data study reported 4.4% of GBMs and 1.5% low-grade gliomas harboring potentially druggable kinase fusions [8]. Consistently, a more recent analysis of RNA-seq confirmed a similar prevalence (9.7%) of potentially targetable fusions in gliomas, of which 11% (24/226) of GBMs, 12% (5/42) of anaplastic astrocytomas, and 8% (2/25) of grade II astrocytomas carried targetable fusions [94]. As demonstrated, the portion of kinase gene involved in the fusion encoded the intact domain which is conserved across all fusions and essential for activity [95]. It is reasonable that drugs targeting primary oncoproteins have great promise to be applied directly to clinical samples which are addicted to the oncogenic fusion kinases in malignant gliomas (Figure 3). Several drugs have been granted approval by FDA as standard therapy for tumor
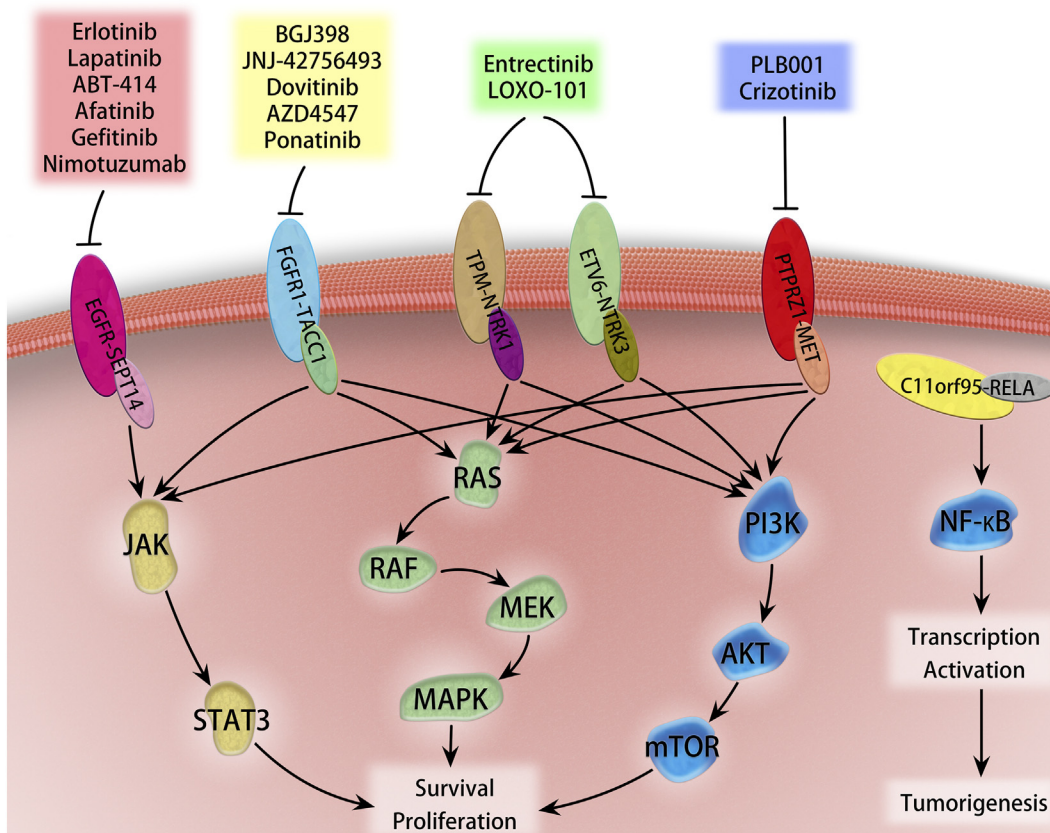


**Figure 3.** Overview of downstream signaling linked to selected driver fusions and potential intervention of specific targeting inhibitors. Glioma-associated oncogenic fusion proteins commonly activate canonical pathways, such as JAK/STAT3, ERK/MAKP, PI3K/ATK, and NF-κB, involved in tumor survival, proliferation, and tumorigenesis. Some of the proteins have been explored as therapeutic targets to inhibit glioma.

**Table 3.** Gene Fusions as Therapeutic Targets in Malignant Gliomas

| Fusion Gene | Medicine | Clinical Trial Status |
|---|---|---|
| *FGFR* fusion | BGJ398 | Phase II study in recurrent GBM patients with *FGFR-TACC* fusions and/or FGFR mutation. (ongoing, NCT01975701) |
| | Erdafitinib (JNJ-42756493) | Phase I study in adult participants with advanced or refractory solid tumors or lymphoma. (ongoing, NCT01703481) |
| | | - Two GBM patients with *FGFR3-TACC3* showed clear anti-tumor activity. |
| | AZD4547 | Phase I/II study in relapsed/refractory glioma patients positive for an *FGFR* fusion (recruiting, NCT02824133) |
| *NTRK* fusion | Entrectinib (RXDX-101) | Phase II basket study in patients having different types of tumors with the TRK gene rearrangements (recruiting, NCT02568267) |
| | | - The only pontine astrocytoma patient harboring *BCAN-NTRK1* fusion showed 45% tumor volume reduction after dosing entrectinib; |
| | | Phase I/Ib study in children and adolescents with recurrent or refractory solid tumors and primary central nervous system tumors, with or without *TRK, ROS1,* or *ALK* fusions (recruiting, NCT02650401). |
| | Larotrectinib (LOXO-101) | Phase II study in patients with advanced solid tumors harboring NTRK fusions (recruiting, NCT02576431) |
| | | - A 35-year-old woman with recurrent multifocal GBM harboring in-frame *EML4-NTRK3* fusion was treated with larotrectinib. The periventricular tumor shrank significantly (67 × 52 mm to 8 × 4 mm), while tumor at frontal and occipital lobes had no shrinkage. |
| *MET* fusion | PLB1001 | Phase I study in recurrent high-grade glioma patients with *PTPRZ1-MET* fusion gene (recruiting, NCT02978261) |
| | Crizotinib | Single case experience. |
| | | - Used in an 8-year-old GBM patient with *PTPRZ1-MET* fusion, led to substantial tumor shrinkage at 2 months, but treatment-resistant lesions were also detected with rapid progression that finally caused death of this patient. |

patients harboring specific gene fusions [96], and in malignant glioma patients, clinical trials are still ongoing, with some exciting preliminary findings (Table 3). The successful targeting of oncogenic fusion kinases is now driving a major paradigm shift in oncology, whereby somatic genetic alterations rather than the histological subtype can provide the guidance for selecting therapeutic strategies. Although the frequency of recurrent fusion transcripts is generally substantially low [7], the subset of different types of glioma stratified by the druggable oncogenic fusions will benefit from the precise treatment approaches.

The revised 2016 WHO Classification of Tumors of the Central Nervous System combines biology-driven molecular marker diagnostics in addition to classical histology [88], formulating a concept for how glioma diagnoses should be structured in the molecular era and facilitating management of outcome prediction and treatment decisions. Different from many cancer-associated mutations with a variable and heterogeneous nature occurring in various morphologically and clinically distinct tumors, gene fusions, for the most part, are typically disease specific, which implicates its promising application in cancer diagnosis [42]. Recurrent gene fusions, such as *ERG, ETV1, TFE3, NUT, POU5F1, NFIB, PLAG1, PAX8,* and *RELA*, have been emerging as required diagnostic biomarkers in different type of cancers including ependymoma [13]. It is the inexorable trend to establish more gene fusions as clinical biomarkers across different subtypes of gliomas to identify patients with specific promising therapeutic targets.

Taken together, data-intensive studies are playing the key role in detecting gene fusions, while further functional characterization of these fusions should be a high priority. A more detailed understanding of its diverse regulatory pathway and involvement in cellular processes will provide valuable insight into malignant glioma biology. The integration of diagnosis, stratification, and targeted treatment based on gene fusion will direct the future avenues of next-generation personalized treatment for glioma patients.

## Conflicts of Interests
The authors declare no conflict of interest.

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