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Identification of the clinical and genetic characteristics of gliomas with gene fusions by integrated genomic and transcriptomic analysis

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Abstract

The identification of oncogenic gene fusions in diffuse gliomas may serve as potential therapeutic targets and prognostic indicators, representing a novel strategy for treating gliomas consistent with the principles of personalized medicine. This study identified detectable oncogene fusions in glioma patients through an integrated analysis of genomic and transcriptomic data, which encompassed whole exon sequencing and next-generation RNA sequencing. In addition, this study also conducted a comparison of the genetic characteristics, tumor microenvironment, mutation burden and survival between glioma patients with or without gene fusions. A total of 68 glioma patients were enrolled in this study, including glioblastoma (GBM), low grade glioma (LGG) and diffuse midline glioma (DMG). 14 cases of GBM patients (51.9%, 14/27) were found to harbor the following 70 oncogenic gene fusions: ROS1 ($n=8$), NTRK ($n=5$), KIF5 ($n=5$), RET ($n=3$) and other infrequent gene fusions ($n=49$). A total of 11 gene fusions were identified in 8 LGG patients (32.0%, 8/25) and seven gene fusions were identified in one DMG patient (16.7%, 1/6). In GBM patient group, five genes including HOXA3, ACTB, CDK5, GNA12 and CARD11 exhibited a statistically significant higher copy number amplification frequency in the GBM group without gene fusions compared to that in the GBM group with gene fusions. In LGG patient group, CDK5 gene was also found to exhibit a statistically significant higher amplification frequency in the LGG group without gene fusions. In addition, KMT2D exhibited a statistically significant higher mutation frequency in the LGG group with gene fusions compared to that in the LGG group without gene fusions. Comparison of the other genetic characteristics including immune cell infiltration score, tumor mutation burden (TMB), and microsatellite instability (MSI). The results showed no statistically significant differences were observed between fusion and non-fusion group of GBM and LGG. The survival analysis revealed that GBM patients without gene fusions exhibited a longer median survival (737 days) compared to GBM patients with gene fusions (642 days), but without a statistical significancy. Our study has identified a set of gene fusions present in gliomas, including a number of novel gene fusions that have not been previously reported. We have also elucidated

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the underlying genetic characteristics of glioma with gene fusions. Collectively, our findings have the potential to inform future clinical treatment strategies for patients with glioma.

Keywords Oncogenic gene fusions, Gliomas, Whole exon sequencing, Next-generation RNA sequencing, Survival

Background

Malignant gliomas, particularly glioblastomas (GBM), are highly aggressive primary brain tumors in central nervous system [1, 2]. GBM represents one of the most clinically and genetically heterogeneous groups of neoplasms, which present significant challenges in terms of treatment. The potential therapeutic options for glioma include surgical resection, radiotherapy, alkylating chemotherapy, bevacizumab and tumor treating fields [3, 4]. However, despite receiving standard therapy for GBM, the 5-year overall survival rate remains dismal at a mere 6.8% [4, 5]. The lack of effective treatments has prompted the field to explore innovative therapeutics, such as targeted therapy aimed at gene fusions.

Gene fusions resulting from chromosomal rearrangements have the potential to generate chimeric proteins with modified functions, which may contribute to cancer development [6, 7]. Although the majority of fusion events are considered to be passenger mutations, a subset is expected to play pivotal roles in tumorigenesis and progression [8]. For example, a subset of patients with non-small cell lung cancer has been found to harbor ALK fusions, and the use of ALK inhibitors has shown improved outcomes in those with EML4–ALK-positive tumors [9, 10]. The transcription of a constitutively active tyrosine receptor kinase (TRK) protein, BCR–ABL, was identified as an oncogenic driver for chronic myelogenous leukemia. Inhibition of this aberrant kinase with imatinib provided an effective therapy against a previously fatal disease [11].

Gene fusions have also long been recognized as a common occurrence in gliomas. The first reported gene fusion discovered in glioma was FIG–ROS, identified in vitro glioma cells [12]. Previous studies have also documented several specific gene fusions, including FGFR3–TACC3 and PTPRZ1–MET [13, 14]. Despite the relatively low frequency of gene fusion in gliomas, they still present potential therapeutic targets for selected patients with specific fusions. Recently, the development of next-generation sequencing (NGS) allowed identification of many gene rearrangements encoding novel oncogenic fusions in gliomas. Transcriptome sequencing has already been able to identify gene fusions in approximately 30–50% of gliomas [15].

The objective of this study was to identify detectable oncogene fusions in glioma patients through an integrated analysis of genomic and transcriptomic data, which included whole exon sequencing and next-generation RNA sequencing. In addition, this article examined the general clinicopathological, genetic characteristics and overall survival of gliomas that harbor gene fusions.

Materials and methods

Patient recruiting

A total of 68 gliomas were surgically removed and analyzed by integrated genomic and transcriptomic analysis (whole exon sequencing and next generation RNA sequencing) at Nanfang hospital (Guangzhou, China) from 2019 to 2021. Tumors were evaluated histopathologically and categorized according to the WHO classification system (2016). This study was approved by the institutional review board of the Nanfang hospital. All patients signed informed consent forms for the use of their tumor tissue samples in clinical research.

Sample extraction and sequencing

Whole exome sequencing (WES) and analysis were performed at the Genomics Laboratory of GenomicCare Biotechnology (Shanghai, China). For frozen tissue or blood, DNA was extracted from thawed materials using the Maxwell RSC Blood DNA Kit (cat# AS1400, Promega, Madison, WI, USA) on a Maxwell RSC system (cat# AS4500, Promega). For formalin-fixed, paraffin-embedded (FFPE) tissue, DNA was extracted using the MagMAX FFPE DNA/RNA Ultra Kit (cat# A31881, ThermoFisher, Waltham, MA, USA) on a KingFisher Flex system (ThermoFisher). The extracted DNA was sheared using a Covaris L220 sonicator, then the exome DNA was captured using the SureSelect Human All Exon V7 kit (cat# 5991-9039EN, Agilent), prepared to library using the SureSelectXT Low Input Target Enrichment and Library Preparation system (cat# G9703-90000, Agilent, Santa Clara, CA USA), and sequenced on an Illumina NovaSeq-6000 sequencer (Illumina, San Diego, CA, USA) to generate 2×150 bp paired end reads. Image analysis and base calling was done using onboard RTA3 software (Illumina).

RNA from FFPE sample was purified using the MagMAX FFPE DNA/RNA Ultra Kit (cat# A31881, ThermoFisher) on a KingFisher Flex system (ThermoFisher), and used as the template to synthesize cDNA using NEBNext RNA First Strand Synthesis Module (cat# E7525S, NEB, Waltham, MA, USA) and NEBNext mRNA Second Strand Synthesis Module (cat# E6111S, NEB)

sequentially. The library preparation, sequencing, and base calling were done similarly as above in the WES section.

RNA fusion analysis

Transcripts were assigned using StringTie2(version 1.3.5) and fusion genes were identified using STAR–FUSION (version 1.8.0) with default parameters set by the softwares, except requiring at least 3 supporting reads during the fusion gene calling to increase the confidence. In addition, genes annotated as 'probably false positive' by FusionHub (https://fusionhub.persistent.co.in/) were also excluded. To be reported, gene fusions were required to (1) be within ± 15 bp of the exon boundaries of two genes, with at least one of the partners being in our list of reportable genes; (2) have five or more supporting reads; (3) have breakpoints farther than 100 kb apart, with the exception of FGFR3–TACC3, which is a known clinically significant gene fusion between genes that are closer than 100 kb; and (4) be in-frame.

Somatic variant identification

After removing adapters and low-quality reads, the commercial Sentieon (version 201911) running environment with default parameters was implemented to process the following steps sequentially: reads alignment to NCBI human genome reference assembly hg19 using the Burrows-Wheeler Aligner (BWA) algorithm, duplication sorting, realignment and recalibration, and somatic mutation calling including single nucleotide variations (SNVs) and short insertion/deletions (INDELs). During the mutation calling stage, the reads from the tumor sample were compared with the paired blood from the same patient to generate the somatic mutation list. The called somatic mutations were then filtered, meaning to retain only the mutations with the variant allele frequency (VAF) > = 0.05 and supported by at least three reads, and annotated using the Variant Effect Predictor (VEP) package.

Germline variant identification

Germline variants were defined as rare variants in peripheral whole blood detected by WES and GATK HaplotypeCaller using the below filters: (1) average sequencing coverage > 10x; (2) frequency < 2% in all populations in Exome Aggregation Consortium (ExAC) which includes exomes from 60706 humans; and (3) frequency < 2% in East Asian population in ExAC which includes exomes from 4327 East Asians.

Tumor mutation burden (TMB)

TMB was defined as the total number of somatic nonsynonymous mutations (SNVs or indels) in the tumor exome for each patient. The number was divided by the total size of the targeted regions to give the TMB score in counts/Mb. In this study, the Agilent SureSelect Human All Exon V7 kit was used and its estimated total targeting size (exome) is 35 Mb.

Copy number variation (CNV)

By following the ExomeCNV package, a normalized depth-of-coverage ratio approach was used to identify CNV from the WES result of paired samples. Standard normal distribution was used to account for five sources of bias that would affect raw read counts, which include the size of exonic regions, batch effect, the quantity and quality of the sequencing data, local GC content, and genomic mappability. Genes with haploid copy number > = 3 or < = 1.2 were defined as amplified or deleted, respectively, and a minimum tumor content (purity) of 20% is required.

Microsatellite instability (MSI)

All autosomal microsatellite tracts containing five or more repeating subunits 1-5 bp in length in GRCh37/hg19 were identified using MISA (http://pgrc.ipk-gatersleben.de/misa/misa.html). A MSI analysis tool MSIsensor was used for MSI calling. It computes length distributions of microsatellites per site in the paired tumor and normal samples, then uses this information to statistically compare the observed distributions in the two samples. Patients with > = 3.5% unstable microsatellite sites were defined as MSI-high according to the MSIsensor validation data.

Immune cell infiltration score

RNA expression count data was used to calculate normalized expression data by DESeq2. The normalized expression data is used to generate the immune infiltration score using the CIBERSORT. Then the resulting immune cell infiltration score is visualized by R package ggplot2.

Statistical analysis and survival analysis

Unless specified otherwise, Pearson Chi-square test or Fisher's exact test were used for *P* value calculations for categorical variables, while two-sided Mann–Whitney *U* test was used between two continuous variables for all figures. Kaplan–Meier curves were used and the significance was estimated with the log-rank test. Overall survival was defined as the interval from the date of initial surgical resection to the date of death. The mean follow-up duration of the patients was 34.2 months. All statistical analyses were performed using the SPSS software (version 21.0; IBM Statistics, Armonk, NY). All statistical

tests were two-tailed and $p < 0.05$ was considered statistically significant.

Results

Overall characteristics of all glioma patients including in the study

In this study, a total of 68 glioma patients were enrolled after obtaining informed consent and approval from the medical ethical committee of Nanfang Hospital (NFEC-2021-364). The baseline characteristics of all patients are presented in Table 1. Among the patients enrolled in this study, the majority ($n=65$, 95.6%) were primary cases. The two most prevalent histopathological types observed were glioblastoma ($n=27$, 39.7%) and diffuse astrocytoma ($n=20$, 29.4%). In addition, other types of gliomas including diffuse midline glioma ($n=6$, 8.8%), anaplastic astrocytoma ($n=6$, 8.8%), oligodendroglioma ($n=4$, 5.9%), anaplastic oligodendroglioma ($n=4$, 5.9%), and pilocytic astrocytoma ($n=1$, 1.5%) were included to facilitate detection of gene mutation characteristics and screening for gene fusions. Both whole exon sequencing and transcriptomic sequencing were performed in all 68 cases of gliomas.

Table 1 Baseline characteristics of glioma patients ($n=68$)

Variables	*n* (%)
Age	
>60 years	15 (22.1%)
<60 years	53 (77.9%)
Gender	
Male	42 (61.8%)
Female	26 (38.2%)
IDH1/2 status	
Mutant	27 (39.7%)
Wild type	41 (60.3%)
Primary or Recurrent	
Primary	65 (95.6%)
Recurrent	3 (4.4%)
MGMT promoter Methylation	
Methylated	32 (47.1%)
unMethylated	36 (52.9%)
Tumor Histopathological Type	
Glioblastoma (GBM)	27 (39.7%)
Diffuse Astrocytoma	20 (29.4%)
Diffuse Midline Glioma (DMG)	6 (8.8%)
Anaplastic astrocytoma	6 (8.8%)
Oligodendroglioma	4 (5.9%)
Anaplastic oligodendroglioma	4 (5.9%)
Pilocytic astrocytoma	1 (1.5%)
Total	68 (100.0%)

Gene fusions identified in GBM patients

A total of 27 patients diagnosed with glioblastoma (including 24 cases of conventional glioblastoma, two cases of epithelioid glioblastoma and one case of gliosarcoma) were enrolled in this study. Among these patients, 14 cases (51.9%) were found to harbor gene fusions, while no gene fusions were detected in the remaining 13 cases, and only three cases (11.1%) exhibited gene fusions exceeding 10, while all other cases demonstrated gene fusions below three. In total, 68 gene fusions were identified in 24 cases of conventional GBM patients, while 2 gene fusions were detected in one case of gliosarcoma patient and none were found in the epithelioid glioblastoma patient group. All of these gene fusions identified in GBM are depicted in Fig. 1. Among the subgroup of conventional GBM patients ($n=24$), ROS1 gene fusion ($n=8$), NTRK gene fusions ($n=5$) and KIF5 gene fusions ($n=5$) were identified as the top three, while other gene fusions such as FGFR and RET were also identified, but with low frequency. In the gliosarcoma patient subgroup, two novel gene fusions (ROS1–SLC34A2 and ALK–EML4) were identified, which have not been previously reported in studies on GBM.

Genetic characteristics of GBM patients with or without gene fusions

Furthermore, we conducted a comparison of the genetic characteristics, including somatic and germline mutations as well as copy number alterations, between GBM patients with or without gene fusions. We identified the top 20 different mutated genes between two groups and conducted statistics analysis. The top 20 different mutated somatic genes between the two groups are listed in Fig. 2A. Among them, PTEN and NOTCH1 exhibited a higher mutation frequency in the GBM group without gene fusions compared to that in the GBM group with gene fusions; however, this difference was not statistically significant. The top 20 genes with different copy number alterations between the two groups are listed in Fig. 2B. There were five genes including HOXA3, ACTB, CDK5, GNA12 and CARD11 (marked in red box) exhibited a statistically significant higher copy number amplification frequency in the GBM group without gene fusions compared to that in the GBM group with gene fusions. We also examined the top 20 germline genes with different mutations frequency between the two groups, but there was no statistically significant difference in gene mutation frequency between them (Fig. 2C). Finally, we also analyzed the immune cell infiltration score, tumor mutation burden (TMB), and microsatellite instability (MSI) between the two groups; however, no statistically significant differences were observed (Fig. 2D, E).

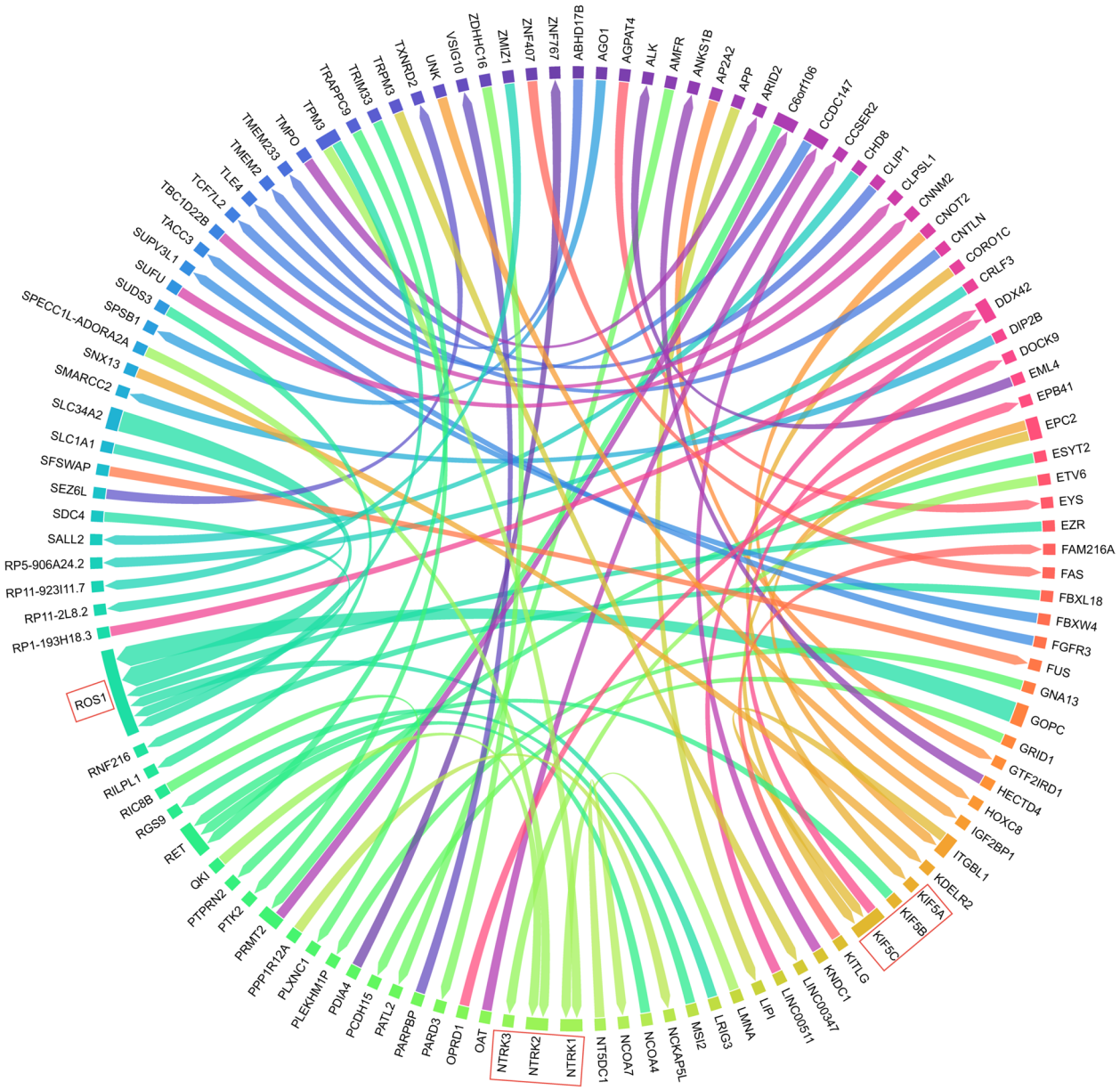

Fig. 1 circle plot depicting gene fusions detected in patients with glioblastoma multiforme (GBM). A total of 70 gene fusions were identified in all GBM patients, with ROS1, NTRK, and KIF5 being the top three gene fusions ($n=5$ each). Other gene fusions such as FGFR and RET were also detected but at a lower frequency

Gene fusions identified in LGG and DMG patients

A total of 25 patients diagnosed with low grade glioma (LGG, including WHO grade 1 and grade 2) and 6 patients diagnosed with diffuse midline glioma (DMG) were enrolled in this study. There were 11 gene fusions identified in LGG group and seven gene fusions identified in DMG group, which are shown in Fig. 3A, B. In LGG subgroup, 8 patients (32.0%) were found to harbor gene fusions. Interestingly, we identified three rare gene fusions (USP2–YAP1, YAP1–FAM118B and KIRREL3–SIK3) in a case of pilocytic astrocytoma that have not been previously reported. In DMG subgroup, only 1 patient (16.7%) were found to harbor gene fusions and all the 7 gene fusions were not reported in previous study.

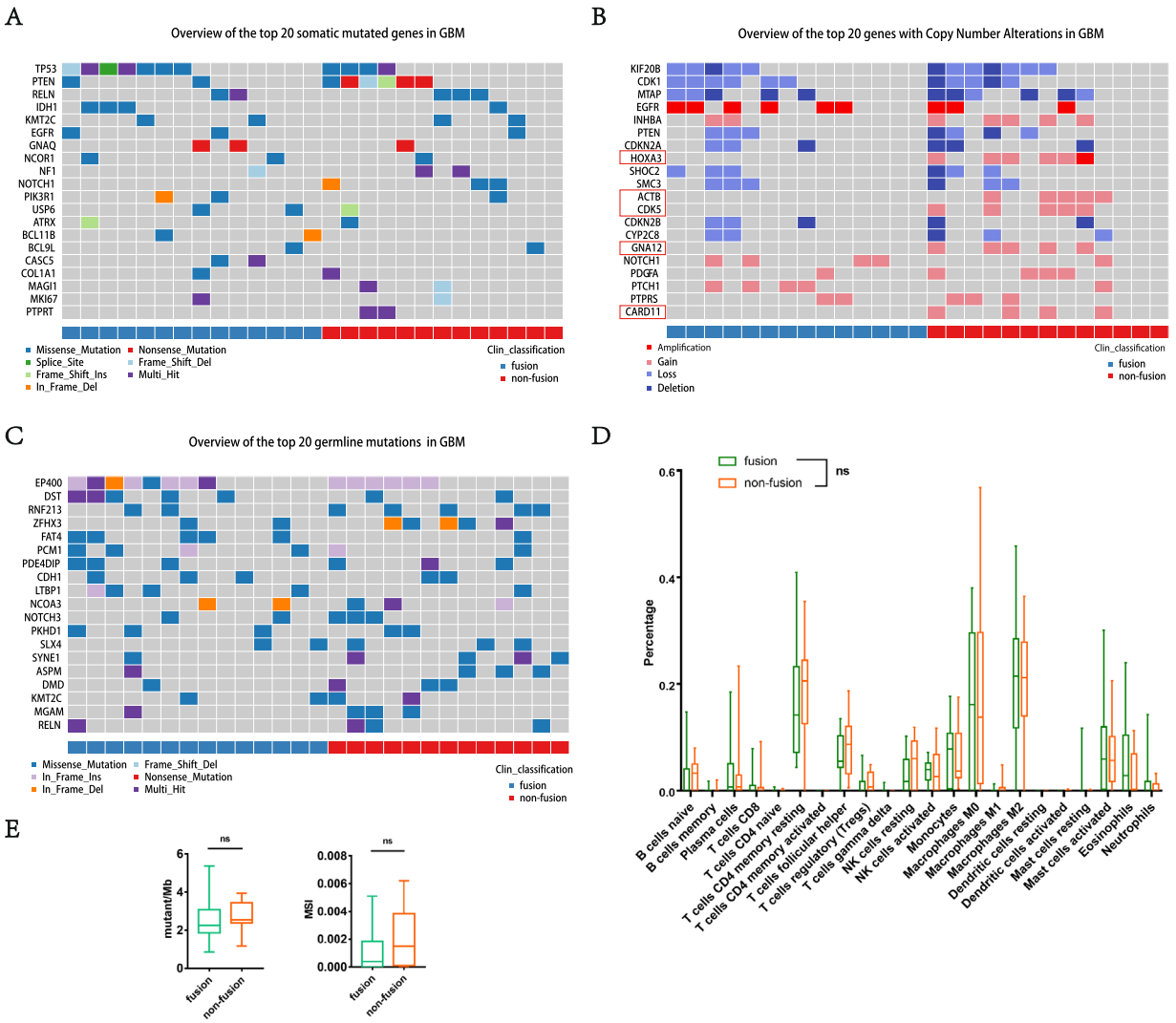

Fig. 2 Comparison of genetic background and tumor microenvironment between GBM patients with or without gene fusions. **A** The top 20 different mutated somatic genes between GBM patients with or without gene fusions. Among them, PTEN and NOTCH1 exhibited a higher mutation frequency in the GBM group without gene fusions compared to that in the GBM group with gene fusions; however, this difference was not statistically significant. **B** Top 20 genes with different copy number alterations between the two group. There were five genes including HOXA3, ACTB, CDK5, GNA12 and CARD11 (marked in red box) exhibited a statistically significant higher mutation frequency in the GBM group without gene fusions compared to that in the GBM group with gene fusions. **C** Top 20 germline genes with different mutations frequency between the two groups, but there was no statistically significant difference in gene mutation frequency between them. The immune cell infiltration score (**D**), tumor mutation burden (TMB) and microsatellite instability (MSI) (**E**) between the two groups were also analyzed, no statistically significant differences were observed

Genetic characteristics of LGG and DMG patients with or without gene fusions

We conducted a comparison of the genetic characteristics, including somatic and germline mutations as well as copy number alterations, between LGG patients with or without gene fusions. The top 20 different mutated somatic genes between the two groups are listed in Fig. 4A. Among them, KMT2D (marked in red box) exhibited a statistically significant higher mutation frequency in the LGG group with gene fusions compared to that in the LGG group without gene fusions. The top 20 genes exhibiting differential copy number alterations and the top 20 germline genes with varying mutation frequencies between the two groups are, respectively, presented in Fig. 4B, C. CDK5 (marked in red box) gene was found to exhibit a statistically significant higher amplification frequency in the LGG group without gene fusions compared to that in the LGG group with gene fusions.

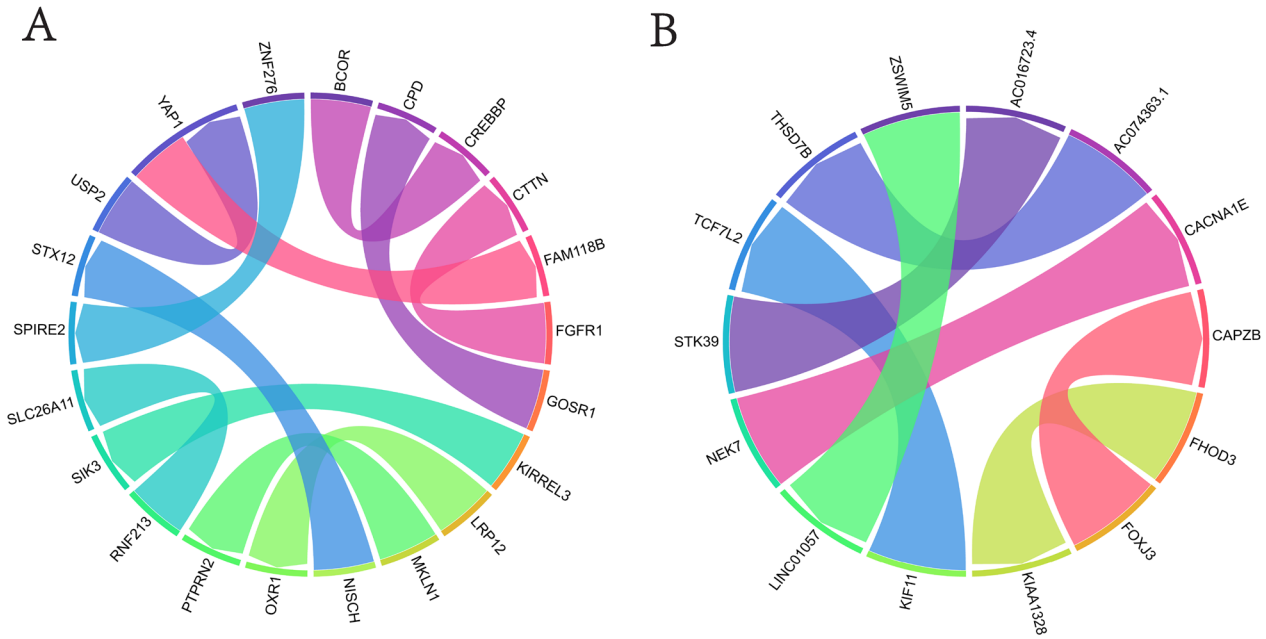

Fig. 3 The circle plot depicting gene fusions detected in patients with low grade glioma(LGG)and diffuse midline glioma (DMG). **A** In LGG subgroup, 8 patients (28.6%) were found to harbor gene fusionsand atotal of 11gene fusions were identified in all LGGpatients. **B**In DMG subgroup, only 1 patient (16.7%) were found to harbor gene fusions and all the 7 gene fusionsidentified in the researchwere not reported in previous studies

According to statistical analysis, the frequency of germline gene mutations did not differ significantly between the two groups. The immune cell infiltration score, tumor mutation burden (TMB), and microsatellite instability (MSI) between the two groups were also analyzed. However, no statistically significant differences were observed (Fig. 4D, E).

In the DMG subgroup, Fig. 5A presents the top 20 somatic genes with different mutations, Fig. 5B shows the top 20 genes exhibiting differential copy number alterations, and Fig. 5C displays the top 20 germline genes with varying mutation frequencies between DMG patients with or without gene fusions. No statistically significant differences were observed between the two groups due to the limited number of patients.

(6) Survival analysis of gliomas patients with or without gene fusions

We also conducted survival analysis in GBM patient subgroup and LGG patient subgroup. We initially conducted a survival analysis of GBM patients stratified by IDH1/2 mutation status. The findings indicated that GBM patients with IDH1/2 mutation had a longer median survival compared to those with IDH1/2 wild type, although the difference was not statistically significant (Fig. 6A). We further investigated the impact of gene fusions on the survival of GBM patients. Our findings revealed that those without gene fusions exhibited a longer median survival (737 days) compared to their counterparts with gene fusions (642 days), but without a statistical significancy (Fig. 6B). Finally, we also investigated the impact of gene fusions on the survival of LGG patients. The findings indicated that there was no statistically significant difference observed between the two groups (Fig. 6C).

Discussion

Gliomas represent the most prevalent primary malignant tumors in the central nervous system, characterized by a rather dismal prognosis, particularly for GBM. Despite the implementation of intensive multimodal therapies, the survival rate for patients with glioblastoma remains at 12.1–14.6 months, with only a small percentage (3–5%) achieving long-term survival. Gene fusions have long been recognized as a common occurrence in gliomas. Targeted therapy aimed at gene fusions in gliomas is a promising strategy for treating this devastating disease. In this research, we conducted an integrated analysis of whole exon sequencing and next-generation RNA sequencing to identify detectable oncogene fusions in glioma patients and compare the genetic characteristics difference between gliomas with and without gene fusions. In addition to some widely reported oncogenes, we have also identified several novel fusion genes and their partner genes in this research. Potentially targetable gene fusions were detected in 51.9% (14/27) of GBM, 28.6% (8/25) of LGG and 16.7% (1/6) of DMG. The

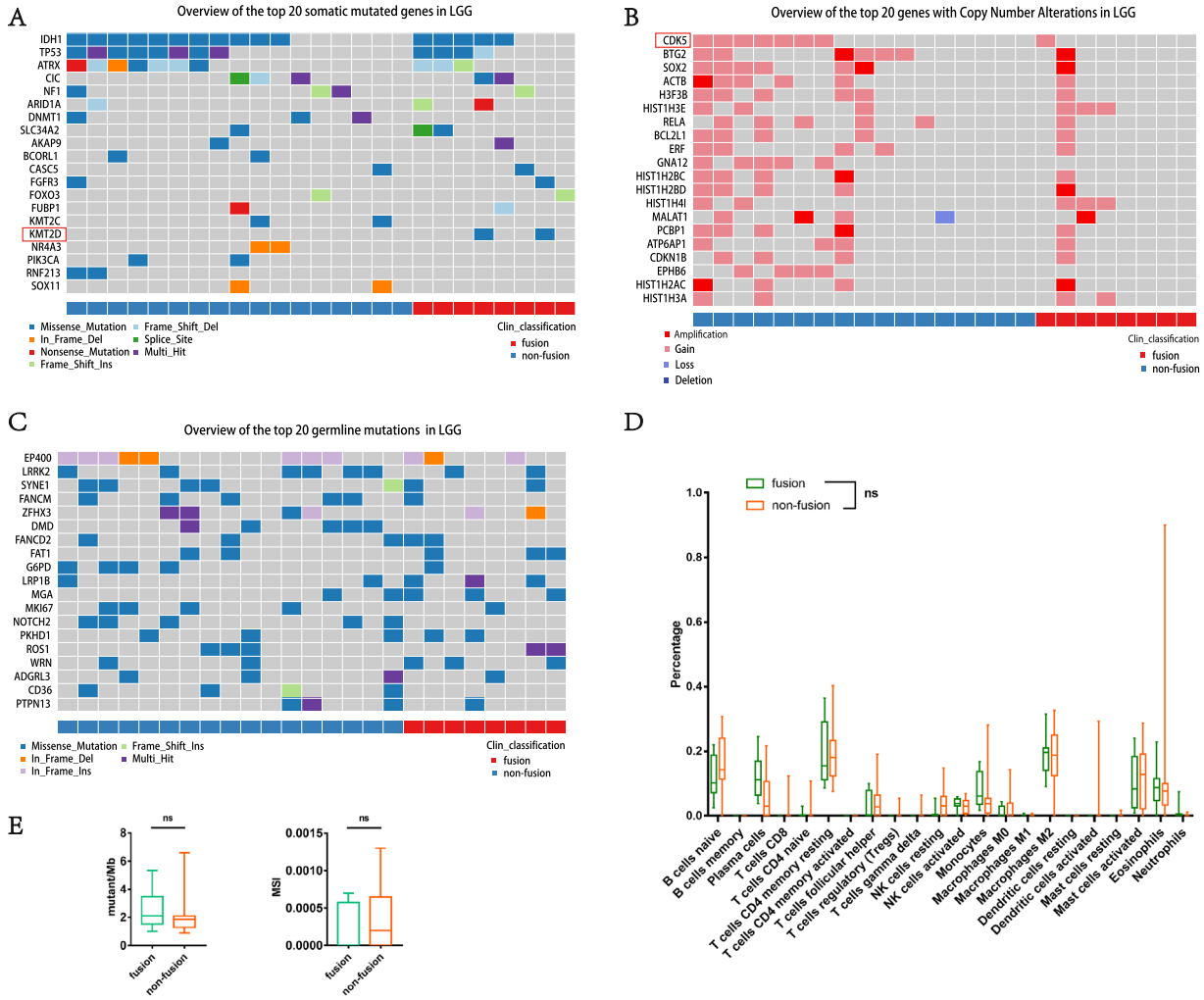

Fig. 4 The comparison of genetic background and tumor microenvironment between LGG patients with or without gene fusions. **A** The top 20 different mutated somatic genes between LGG patients with or without gene fusions. KMT2D (marked in red box) exhibited a statistically significant higher mutation frequency in the LGG group with gene fusions compared to that in the LGG group without gene fusions. **B** Top 20 genes exhibiting differential copy number alterations between the two groups. CDK5 (marked in red box) gene was found to exhibit a statistically significant higher amplification frequency in the LGG group without gene fusions compared to that in the LGG group with gene fusions. **C** Top 20 germline genes with varying mutation frequencies between the two groups. According to statistical analysis, the frequency of germline gene mutations did not differ significantly between the two groups. The immune cell infiltration score (**D**), tumor mutation burden (TMB) and microsatellite instability (MSI) (**E**) between the two groups were also analyzed. However, no statistically significant differences were observed

detection rate of gene fusions in our study exceeded that reported in previous researches, which may be attributed to the utilization of next-generation RNA sequencing for identifying detectable oncogene fusions. Another possible explanation is that the incidence of gene fusions in glioma varies depending on the specific gene fusion type and glioma subtype, and may not be as rare as previously believed.

In this study, a total of 70 gene fusions were identified in GBM patient subgroup. The most frequently detected fusion (11.4%, 8/70) was ROS1 gene fusions, which were found in 3 GBM patients. The partners fusion genes of ROS1 including SLC34A2, GOPC, SDC4, LRIG3, TPM3 and EZR. Rearrangement involving ROS1, an orphan receptor tyrosine kinase gene, was first described in a GBM cell line (U118MG) in 1987 [16]. Recently, various ROS1-fusions were identified in subsets of diverse pediatric and adult malignancies, including melanoma, cholangiocarcinoma and non–small cell lung cancer (NSCLC) [17]. Among all the ROS1 gene fusions identified in this study, GOPC–ROS1 was found to be an oncogenic fusion in a congenital glioblastoma case [18], while

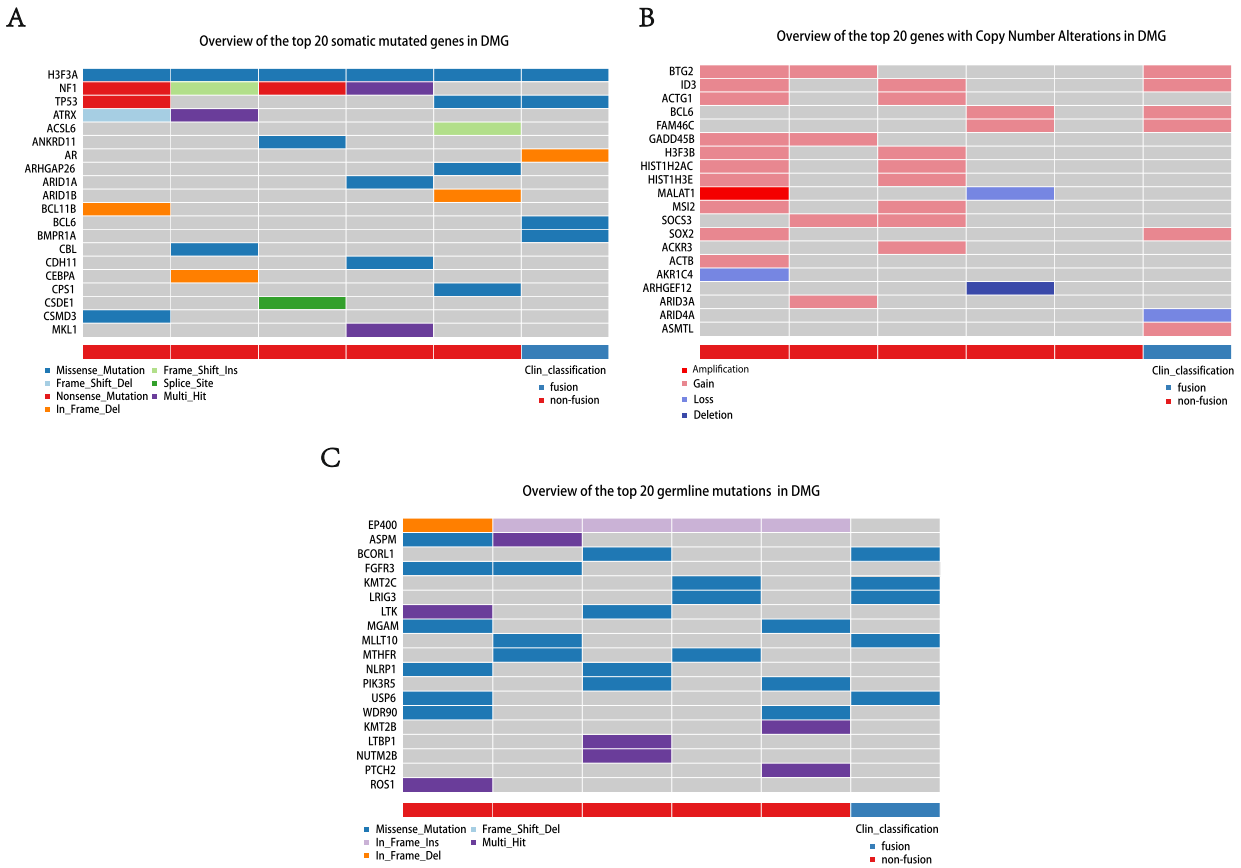

Fig. 5 Comparison of genetic background between DMG patients with or without gene fusions. **A** Top 20 somatic genes with different mutations, **B** top 20 genes exhibiting differential copy number alterations, and (**C**) top 20 germline genes with varying mutation frequencies between DMG patients with or without gene fusions. However, no statistically significant differences were observed between the two groups due to the limited number of patients

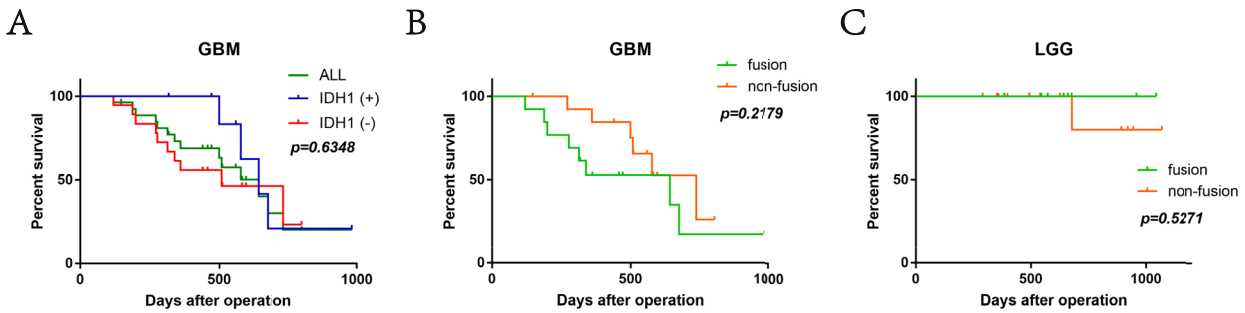

Fig. 6 Survival analysis in GBM patient subgroup and LGG patient subgroup. **A** Survival analysis of GBM patients stratified by IDH1/2 mutation status. The findings indicated that GBM patients with IDH1/2 mutation had a longer median survival compared to those with IDH1/2 wild type, although the difference was not statistically significant. **B** Survival analysis of GBM patients stratified by gene fusions status. Our findings revealed that those without gene fusions exhibited a longer median survival (737 days) compared to their counterparts with gene fusions (642 days), but without a statistical significancy. **C** Survival analysis of LGG patients stratified by gene fusions status. The findings indicated that there was no statistically significant difference observed between the two groups

other gene fusions were infrequently reported. Philipp Sievers and colleagues have also reported that GOPC–ROS1 fusion genes, along with other ROS1 fusions, represent a rare yet recurrent therapeutic target in various types of gliomas [19].

The second most common gene fusions (7.1%, 5/70) identified in GBM patient subgroup involved the Neuro Trophin Receptor Kinase genes NTRK1, NTRK2 and NTRK3. NTRK gene fusions were detected in 2 GBM patients. The NTRK gene fusions identified in this study were as follows: LMNA–NTRK1, TPM3–NTRK1, ADORA2A–NTRK2, QKI–NTRK2 and ETV6–NTRK3. The occurrence of NTRK gene fusion has been reported in 4% (out of a sample size of 57) of pediatric high-grade gliomas and ranges from 1 to 4% in adult gliomas [20]. It is noteworthy that a study revealed 40% (out of 10) of patients under the age of 3 years with non-brainstem high-grade glioma were found to harbor an NTRK fusion [21]. Larotrectinib is a pan NTRK inhibitor that exhibits CNS activity with minimal dose-limiting toxicities. A case report by Ziegler et al. demonstrated near-complete resolution of an ETV6–NTRK3 fusion-positive high-grade glioma in a 3-year-old girl treated with Larotrectinib [22]. Several clinical trials are currently underway, such as NCT02650401, NCT04655404 and NCT02637687, focusing on the treatment of NTRK fusion gliomas. Targeted therapies utilizing NTRK inhibitors have shown promising results in patients with this condition [23].

The third most common gene fusions (7.1%, 5/70) were involved Kinesin Family Member 5 genes including KIF5A, KIF5B and KIF5C, which were detected in 3 GBM patients. The gene fusions identified in this study include CORO1C–KIF5A, KIF5B–RET, KIF5C–DOCK9, EPC2–KIF5C and ITGBL1–KIF5C. All of these fusions were initially discovered and reported in patients with glioblastoma multiforme (GBM). The KIF5B–RET gene rearrangement is observed in approximately 1% of lung adenocarcinomas, whereas no studies have reported this gene fusion in gliomas [24]. A total of three novel RET gene fusions were identified, with TRIM33, NCOA4 and KIF5B as partner genes. RET rearrangement was originally identified in other types of neoplasms, such as papillary thyroid cancer and non-small cell lung cancer. Various available FDA-approved multi-kinase inhibitors such as vandetanib, cabozantinib and sorafenib could possess anti-RET activity [25]. Several drugs targeting RET fusion-positive malignancies are also undergoing clinical trials. Other GBM specific oncogene fusions including FGFR3–TACC3 [13], which were reported in previous studies, were also identified in this study. In this study, a patient was diagnosed with gliosarcoma that exhibited EML4–ALK fusion positivity, representing a rare case not previously documented. Targeted ALK inhibitors, such as ceritinib and crizotinib, hypothetically benefit glioma patients. These therapeutics have demonstrated efficacy in treating non-small cell lung cancer patients with ALK fusions [26]. However, the clinical benefit of ALK inhibitors for gliomas remains to be established through rigorous trials.

We also conducted a comparison of the genetic characteristics, tumor microenvironment, mutation burden and survival between glioma patients with or without gene fusions. No statistically significant differences were observed in the immune cell infiltration score, tumor mutation burden (TMB), and microsatellite instability (MSI) between the two groups. Furthermore, we found that CDK5 gene demonstrated with copy number amplification in non-fusion subgroup of GBM and LGG. The survival analysis revealed that the GBM patient group without gene fusion had a longer median survival time, although this difference was not statistically significant. Ha Young Woo and colleagues discovered that the PTPRZ1–MET fusion is linked to unfavorable progression-free survival in IDH-wildtype glioblastoma patients [27]. However, another valuable study has documented that the overall survival (OS) of 80 patients with FGFR3–TACC3 fusion was 31.1 months, compared to only 19.9 months in those without this fusion. Moreover, the presence of CDK4 and MDM2 amplifications in these patients was associated with longer survival times of 57.5 vs. 25.1 months and 47 vs. 28.6 months, respectively [28]. The precise significance and relevance of specific gene fusions, CDK gene amplifications, and their impact on survival necessitate further clarification through larger-scale clinical trials and validation in future basic research.

This study has certain limitations; the precise biological functions of gene fusions were not experimentally validated in vitro, and the potential technical errors associated with NGS analysis cannot be entirely excluded. In addition, the sample size of glioma patients included in this research was limited. Conducting studies on larger cohorts in the future would provide us with more robust statistical conclusions.

In summary, our study has identified a set of gene fusions present in gliomas, including a number of novel gene fusions that have not been previously reported. We have also elucidated the underlying genetic characteristics of glioma with gene fusions. Collectively, our findings have the potential to inform future clinical treatment strategies for patients with glioma.

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Author contributions
GZ. Y., WY. F. and GL.H. conceived and designed the experiments; GZ.Y., HY.Z. TS. Q., SQ. Q, and ZY. L. performed the experiments; GZ.Y. WY.F. and ST. Q. organized and analyzed the data; GZ. Y. and HY. Z. wrote the manuscript; GZ.Y. and GL.H. revised and submitted the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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