High frequency of PDGFRA and MUC family gene mutations in diffuse hemispheric glioma, H3 G34-mutant: a glimmer of hope?

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Abstract

Background: Diffuse hemispheric glioma H3 G34-mutant (G34-DHG) is a new type of pediatric-type diffuse high-grade glioma in the fifth edition of the WHO Classification of Tumors of the Central Nervous System. The current treatment for G34-DHG involves a combination of surgery and conventional radiotherapy or chemotherapy; however, the therapeutic efficacy of this approach is not satisfactory. In recent years, molecular targeted therapy and immunotherapy have achieved significant benefits in a variety of tumors. In-depth understanding of molecular changes and immune infiltration in G34-DHGs will help to establish personalized tumor treatment strategies. Here, we report the clinicopathological, molecular and immune infiltration characteristics of G34-DHG cases from our center along with cases from the HERBY Trial and the Chinese Glioma Genome Atlas database (CGGA).

Methods: Hematoxylin–eosin (HE) and immunohistochemistry (IHC) staining were used to present the clinicopathological characteristics of 10 Chinese G34-DHG patients treated at our institution. To address the molecular characteristics of G34-DHG, we performed whole-exome sequencing (WES) and RNA sequencing (RNA-seq) analyses of 5 patients from our center and 3 Chinese patients from the Chinese Glioma Genome Atlas (CGGA) database. Additionally, 7 European G34-DHG patients from the HERBY Trial were also subjected to analyses, with 7 cases of WES data and 2 cases of RNA-seq data. Six G34-DHG patients from another organization were used as external validation.

Results: WES showed a high frequency of PDGFRA mutation in G34-DHGs (12/15). We further identified frequent mutations in MUC family genes in G34-DHGs, including MUC16 (8/15) and MUC17 (8/15). Although no statistical difference was found, PDGFRA mutation tended to be an indicator for worse prognosis whereas MUC16/MUC17 mutation indicated a favorable prognosis in G34-DHGs. RNA sequencing results revealed that most G34-DHG are considered to be immune cold tumors. However, one patient in our cohort with MUC16 mutation showed significant immune infiltration, and the total overall survival of this patient reached 75 months.

Conclusions: Our results demonstrate that G34-DHG is a new high-grade glioma with high frequency of PDGFRA and MUC gene family mutations. PDGFRA may serve as an indicator of poor prognosis and an effective therapeutic target. Moreover, MUC16 tends to be a favorable prognostic factor and indicates high immune infiltration in certain
Background
Pediatric-type diffuse high-grade glioma (pHGG) is a highly aggressive brain tumor with a poor prognosis that accounts for approximately 8–15% of all central nervous system tumors in children and adolescents [1]. The widespread use of high-throughput sequencing, genetic profiling, and epigenetic analysis has greatly increased our understanding of the cell origin, pathogenesis, and biological characteristics of pHGG. Two types of heterozygous somatic mutations in \( H3F3A \), which encodes histone H3, were firstly identified in pHGG in 2012 [2]. These mutations lead to an amino acid substitution at key residues (K27M or G34R/G34V). Subsequent studies found that K27M mutation presented in both \( H3F3A \) and \( HIST1H3B/C \) genes, but G34R/V-mutant gliomas were characterized by recurrent glycine-to-arginine/valine alterations at codon 34 (G34R/V) only in the \( H3F3A \) gene. The fifth edition of the WHO Classification of Tumors of Central Nervous System in 2021 defined the standard name of G34R/V-mutant gliomas as diffuse hemispheric glioma H3 G34-mutant, classified as WHO grade 4.

Most diffuse hemispheric glioma H3 G34-mutant tumors (G34-DHGs) carry \( TP53 \) mutations and \( ATRX \) deletions [2], with hypermethylation at the \( OLIG2 \) locus, leading to the absence of \( OLIG2 \) expression [3]. The current treatment for these tumors is surgery, radiotherapy, and chemotherapy. However, the efficacy of this therapeutic strategy is not satisfactory. In-depth understanding of the molecular changes and immune infiltration in G34-DHGs will help to establish personalized tumor treatment strategies.

In this study, we conducted a comprehensive analysis of G34-DHGs at the clinicopathological and genetic level. Our research uncovered new molecular characteristics of this unique tumor, which provide potential opportunities to develop individualized therapy.

Methods
Patient cohort
The institutional ethical committee of Sun Yat-sen University Cancer Center (SYSUCC) approved this study, and written informed consent was obtained from all patients. A flow diagram of patient selection is shown in Additional file 1: Figure S1.

Ten \( H3F3A \) G34-mutated patients were selected from the clinicopathology database on the basis of \( H3F3A \) Sanger sequencing results, including nine patients with G34R mutation and one patient with G34V mutation. Pathological specimens of all patients were independently reviewed by two senior neuropathologists according to the criteria of the 2021 WHO Classification of Tumors of Central Nervous System [4]. All patients met the criteria for diffuse hemispheric glioma, H3 G34-mutant. Clinicopathological characteristics of patients including age, sex, chief complaint, site of lesion, treatment, IDH/TERT/MGMT promoter methylation status, and survival status were retrospectively reviewed.

Five cases had sufficient fresh tumor samples for whole-exome sequencing (WES) and RNA sequencing (RNA-seq); freshly frozen tumor tissues were subjected to WES and RNA-seq analyses, and corresponding blood samples were subjected to WES analyses. We also obtained WES and RNA-seq data of three Chinese patients from the Chinese Glioma Genome Atlas (CGGA) database and seven European patients from the HERBY Trial (study BO25041; clinicaltrials.gov NCT01390948).

For survival analysis, we examined overall survival (OS) of the total 20 patients with G34-DHG, along with 21 cases with \( IDH \)-mutant high-grade glioma and 34 cases with H3K27M-mutant diffuse midline glioma at our institution.

Immunohistochemistry
Immunohistochemical staining was performed using an immunostaining system (BenchMark ULTRA system, Ventana-Roche, Switzerland) with primary antibodies against GFAP (1:200, ZSGB-BIO, Beijing, China), \( OLIG2 \) (1:100, ZSGB-BIO), \( ATRX \) (1:100, ZSGB-BIO), \( IDH1 \) R132H (1:100, MX031, Fuzhou, China), \( H3K27M \) (1:1000, Millipore, Temecula, CA, USA), p53 (1:1000, Dako, Glostrup, Denmark) and Ki67 (1:200; Dako). Appropriate positive controls were included.

Methylation-specific PCR analysis
Methylation-specific PCR was conducted using an EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) to determine the methylation status of the MGMT promoter, as described previously [5].

WES
DNA was extracted from fresh tissues, paraffin-embedded tissue and peripheral blood. WES was performed using a targeted capture approach with the Agilent
SureSelect Human All Exon Kit (Santa Clara, CA, USA) followed by massively parallel sequencing of enriched fragments on the Illumina HiSeq2500 by Gene Denovo Biotechnology Co. Tumor and corresponding blood leukocyte DNA samples had an average sequencing depth of the target exonic region of $>200 \times$. For a big panel targeting 539 and a small panel targeting 135 tumor genes, the sequencing depth was 2000×.

Somatic variant identification
All the sequence reads were mapped according to the human reference genome GRCh37 using BWA-MEM with default parameters. Following the GATK standard protocol, realignment and recalibration were performed for BAM files after removing PCR duplicates. Single-nucleotide variations (SNVs) were identified with MuTect in the GATK suite. Using white blood cell data as controls, tumor sample data were then input into GATK to call somatic mutations (SNV). Finally, maftools was employed to visualize the mutational landscape of the results.

Immune infiltration score calculation
To de-convolute immune components of tumor samples, the TIMER online tool (http://cistrome.org/TIMER/) was employed to evaluate the immune cell infiltration score using default settings. The immune infiltration score was later visualized and plotted by the R studio function pheatmap.

Statistical analysis
Statistical analysis was performed using IBM-SPSS Statistics version 18.0 (IBM, NY, USA). Kaplan–Meier survival curves were generated to estimate OS. Survival differences were analyzed by the log-rank test.

Results
Clinical characteristics of G34-DHG
The clinical characteristics of the 10 patients with G34-DHG from SYSUCC are summarized in Table 1. The patient group included four male patients and six female patients. The patient age at initial diagnosis ranged from 13 to 25 years, and the median patient age was 20.5 years. The symptoms were the classic clinical features of brain tumors, including dizziness, headache, nausea, vomiting, weakness of right limbs and generalized tonic–clonic seizures dependent on the site of the lesion. All lesions were primarily located in cerebral hemispheres; the lesions were in the right hemisphere in five patients, the left hemisphere in four patients and bilateral hemisphere in one patient. Tumor invasion in the frontal lobe was detected in eight patients, and invasion in the parietal lobe was detected in three patients; temporal lobe involvement was observed in two patients and insular lobe involvement was observed in two patients. In addition, corona radiata, basal ganglia or corpus callosum were infiltrated by tumors in four patients.

For initial treatment, gross total resection (GTR) was performed in five patients, whereas four patients received subtotal resection and one patient only received biopsy. For postoperative adjuvant treatment, nine patients received adjuvant concurrent chemotherapy and radiation therapy (CCRT), and one patient who was included in a clinical trial received 12 courses of dianhydrodulcitol. Among the nine patients receiving CCRT, five patients further received maintenance temozolomide (TMZ) treatment and one patient received four courses of TMZ plus cisplatin followed by maintenance TMZ treatment.

Seven patients showed recurrent disease after the initial treatment, and four patients died. Four patients who initially received subtotal resection chose conservative treatment after recurrence, and two died at 6 and 16 months after the initial operation. The remaining three patients with recurrent disease received a second operation, and two of them further received adjuvant therapy after the second operation. Patient 9 received TG02, a novel pyrimidine-based multi-kinase inhibitor of CDKs together with JAK2 and FLT3, after STR for recurrent disease. This patient died at 1 year after recurrence with an OS of 17 months. Patient 1, who was diagnosed with methylated MGMT promoter after initially receiving GTR followed by CCRT and 12 courses of maintenance TMZ treatment, then again underwent GTR followed by radiotherapy and 12 courses of maintenance TMZ treatment for her recurrent disease; this patient achieved the longest OS of 75 months. Moreover, three patients who were also diagnosed with methylated MGMT promoter after initially receiving GTR followed by CCRT and maintenance TMZ treatment were free of recurrence after at least 18 months since the initial operation. Intriguingly, two of the three patients without recurrent disease continuously received maintenance TMZ treatment (ongoing), including patient 4, who initially received only biopsy. These results indicated that only GTR and long-term maintenance TMZ treatment might benefit patients with G34-DHG in conventional treatment.

Pathological characteristics of G34-DHG
Histological features including necrosis, nuclear atypia, mitotic activity and vascular characteristics were assessed. Histopathologic examination of the surgical specimens from our cohort showed high-grade glioma morphology (Fig. 1). All cases showed high cell density, featuring nuclear atypia, mitotic activity and cellular pleomorphism with focal gemistocytic cells and multinuclear giant cells. Most cases were glioblastoma
| Patient | Sex/age (years) | Chief complaint | Site of lesion | Initial OP and adjuvant Tx | 2nd OP and adjuvant Tx | H3F3A | MGMT promoter | IDH | TERT promoter | Morphology | Current status | OS (months) |
|---------|----------------|----------------|---------------|---------------------------|-----------------------|------|---------------|-----|---------------|-------------|--------------|-------------|
| 1       | F/14           | Headache, nausea and vomiting | Left temporal lobe | GTR + CCRT + TMZ#12 | NA | G34R | Methylated | Wildtype | Wildtype | GBM with primitive neuronal component | Death | 75 |
| 2       | F/18           | Headache, nausea and vomiting | Right frontal lobe | GTR + CCRT + TMZ#2 | NA | G34R | Methylated | Wildtype | Wildtype | GBM | Alive | 24 |
| 3       | M/16           | Headache, nausea and vomiting | Left parietal lobe and corpus callosum | STR + CCRT | Conservative | G34V | Un-methylated | Wildtype | Wildtype | GBM | Alive | 16 |
| 4       | F/20           | Weakness of right limbs | Bilateral frontal lobe and corpus callosum | Biopsy + CCRT + TMZ#24 (continuously) | NA | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 21 |
| 5       | M/25           | Generalized tonic clonic seizures | Right frontal, temporal, insular lobe and basal ganglia | GTR + CCRT + TMZ#22 (continuously) | Conservative | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 18 |
| 6       | F/23           | Headache, nausea and vomiting | Right fronto lobe, corona radiata, basal ganglia | STR + CCRT | Conservative | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 6 |
| 7       | F/23           | Headache and weakness of left limbs | Right frontal and insular lobe | STR + CCRT | Conservative | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 16 |
| 8       | M/13           | Headache and weakness of left limb | Left frontal and parietal lobe | STR + CCRT + TMZ/ DDP#4 + TMZ#6 | Conservative | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 13 |
| 9       | F/21           | Dizziness and weakness of right foot | Left frontal lobe | GTR + CCRT + TMZ#3 | Conservative | G34R | Methylated | Wildtype | Wildtype | G8M | Alive | 17 |
| 10      | M/21           | Generalized tonic clonic seizures | Left frontal and parietal lobe | GTR + Dianhydridulcitol#12 | GTR | G34R | Methylated | Wildtype | Wildtype | G8M with primitive neuronal component | Alive | 20 |

CCRT concurrent chemotherapy and radiation therapy, DDP cisplatin, GBM glioblastoma, GTR gross total resection, MGMT O6-methylguanine-DNA methyltransferase, NA not applicable, RT radiation therapy, STR subtotal resection, TMZ temozolomide
(GBM)-like with microvascular proliferating and/or palisade necrosis and two cases showed focal embryonal appearance. However, calcification, perivascular growth pattern and perineuronal satellitosis, which rarely appears in GBM, were also observed (Fig. 1E, F). All cases showed GFAP expression and negative expression of OLIG2; most cases were negative for ATRX (9/10) and most showed diffuse strong p53 positivity (8/10). The Ki67 labeling index was high, ranging from 20 to 60%. Sanger sequencing revealed that all cases were IDH wild-type and TERT promoter wild-type (Table 1). Methylation PCR revealed that most cases showed MGMT promoter methylation (8/10) (Table 1).

Mutational landscape of G34-DHGs
To investigate the mutational landscape of G34-DHGs, we performed WES on five fresh samples and matched peripheral blood leukocytes from our cohort. The average depths of targeted exome regions in tumors and matched blood samples were 400× and 100×, respectively. More than 98.69% of the targeted regions were covered sufficiently for confident variant calling (≥10× depth). We included three G34-DHG cases from the CGGA and seven G34-DHG cases from the HERBY trial (study BO25041; clinicaltrials.gov NCT01390948). The total somatic mutations of the 15 G34-DHG samples are listed in Fig. 2.

The mutational frequencies, mutation types and clinical features of the 15 G34-DHG cases are displayed in Fig. 2A. TP53 (13/15 87.0%), PDGFRα (12/15, 80.0%) and ATRX (10/15, 67.0%) were the most three frequently mutated genes. Other frequently mutated genes including MUC17 (8/15, 53.0%), MUC16 (8/15, 53.0%), MUC3B (7/15, 47.0%), MUC3A (7/15, 47.0%), OBSCN (6/15, 40.0%), SSPO (6/15, 40.0%) and DOCK3 (6/15, 40.0%) were identified by WES.

Notably, MUC family gene mutations have not been reported in G34-DHGs. The human MUC16 gene is located on chromosome 19p13.2 and the MUC17 gene is located on chromosome 7q22.1. Out of the eight cases with MUC16 mutations, 87.5% (7/8) of the cases had missense mutations, and the remaining case had a frame_shift_ins mutation. Moreover, all eight cases with MUC17 mutations had missense mutations.

Somatic SNVs and indels
A total of 8285 exonic mutations were identified in the 15 G34-DHG patients. Of these mutations, 7187 were missense mutations, 448 were nonsense mutations, 153 were frameshift deletions, 313 were frameshift insertions, 10 were in_frame_del mutations, 1 was an in_frame_ins mutation and 173 were splice site mutations. We removed 1561 silent variants with unknown function. The predominant types of nucleotide substitutions in SNVs in G34-DHGs were C > T/G > A transitions and C > A/G > T transversions.

Oncogenic pathways analysis
We identified multiple pathways of somatic mutated genes in G34-DHGs using the oncogenic pathways module of the R package maftools. Crucial signal transduction pathways included the RTK-RAS, NOTCH, WNT, Hippo, PI3K, TP53, MYC and Cell_Cycle pathways (Fig. 2B). Exome sequencing revealed that 14 of 15 cases had changes in the receptor tyrosine kinase RTK-RAS pathway, involving 42/84 pathway genes (including PDGFRα, MET, BRAF, ERF, FGFR1 and NFI). KEGG pathway analysis revealed that many of the mutated genes were involved in cancer signal transduction. Mutant
genes may promote tumor cell proliferation and escape apoptosis through cascade reaction.

Copy number alterations (CNAs)
We conducted somatic CNA analyses in the five SYSUCC cases and seven HEBRY cases. The results identified recurrent gains in chromosomes 3p26.33 (11/12), 4p12 (11/12), 9q21.13 (7/12) and 9q34.11 (4/12). Recurrent losses were identified in chromosomal regions 4p35.1 (10/12), 10q25.1 (10/12), 19p13.43 (9/12), 18q23 (8/12) and 9p21.3 (7/12). Loss of somatic CNAs on chromosome 10q25 affects the largest number of genes (1330 genes), including the MGMT locus. Gains of somatic CNAs on chromosome 3p26 affects 396 genes, including ABCC5. Studies have shown that ABCB5 is associated with chemoresistance of astrocytic tumors [6].

PDGFRA mutation and G34-DHGs
PDGFRA is an important receptor tyrosine kinase in glial development and a recurrent driver in high-grade gliomas [7–9]. PDGFRA mutation and the PDGFRA signaling pathway were reported to play potent oncogenic roles.
in G34-DHGs [10]. Our sequencing results also showed that most G34-DHGs had PDGFRA mutation (12/15).

To explore the potential pathways and genes related to PDGFRA mutation in G34-DHGs, we analyzed differentially expressed genes (DEGs) using RNA-Seq data from two G34-DHG patients with wild-type PDGFRA and eight G34-DHG patients with mutated PDGFRA. The results identified 150 DEGs (|FC| ≥ 2.0 and P < 0.05), including 95 down-regulated genes and 55 up-regulated genes. The DEGs between the two groups are shown in a heatmap in Fig. 3A. We identified the top 10 hub genes ranked by degree, including FOS, CXCL8, CXCR1, IL1B, COL1A1, MMP9, FGFR3, TNF, CCL4 and CCL3. We used MCODE in Cytoscape to identify gene modules in the PPI network and mapped the interaction network of 52 core genes in module 1 (Fig. 3B). The results indicated that the genes were mainly involved with blood microparticles, the phospholipase C-activating G protein-coupled receptor signaling pathway, substrate-specific channel activity, complement and coagulation cascades, the neuroactive ligand-receptor interaction and aldosterone-regulated sodium reabsorption.

We used the DAVID database to analyze the GO and KEGG pathways of the DEGs. KEGG pathway analysis revealed that DEGs were significantly enriched in the extracellular matrix–receptor interaction, cytokine–cytokine receptor interaction, PIK3-AKT signaling pathway and chemokine signaling pathway (Fig. 3C). The enriched GO-Biological Process terms included immune and inflammatory response (Fig. 3D).

**MUC16 mutation and immune infiltration characteristics of G34-DHGs**

Previous studies indicated that pHGGs with a high mutation load have an elevated neoantigen load and immune response [11, 12]. However, tumors with
histone H3 gene mutations are considered as immune cold tumors, which are defined as a lack of CD8 immunoreactivity and lack of tumor-infiltrating lymphocytes [13]. Using the RNA sequencing results, we next analyzed the expressions of immune-related genes in 10 patients with G34-DHG, including 5 from SYSUCC, 3 from CGGA and 2 of the HERBY cases. The 67 differentially expressed immune-related genes were classified according to CD8+ T cell, T cell (general), B cells, monocyte, tumor-associated macrophage, M1 macrophage, M2 macrophage, neutrophil, natural killer cell, dendritic cell, Th1, Th2, Th17, Treg and T cell exhaustion markers (Fig. 4). In general, consistent with HERBY Phase II Randomized Trial, we also found G34-DHG were immune cold tumors, with a few exceptions. For instance, the tumor specimen of patient 1 in

Fig. 4 Immune-related genes expression profiles for immune cells plotted as a heatmap from 10 cases with RNA-seq data
our cohort showed significant immune infiltration with substantial amounts of CD4 and CD8 T cells (Additional file 2: Figure S2). The OS of this patient reached 75 months, which was markedly longer than that of the nine patients with low immune infiltration (mean survival time: 12 months).

A previously published pan-cancer analysis of 30 solid tumor types showed that patients with MUC16 mutations showed higher tumor mutation burden and neoantigen burden, indicating increased tumor immunogenicity, which can predict immune checkpoint inhibitor treatment response. The study included 397 cases of glioblastoma and 61 of these cases (15.37%) showed MUC16 mutations [14]. Therefore, we wondered whether the MUC16 mutation in G34-DHGs might also be associated with immune infiltration.

We used TIMER 2.0 to analyze the immune cell infiltration of G34-DHGs with MUC16 mutation and G34-DHGs with wild-type MUC16. However, no connection between MUC16 mutation and immune cell infiltration was found; this may be because of the small number of cases and relatively low immune infiltration of G34 glioma. We further analyzed the DEGs between G34-DHGs with MUC16 mutation and G34-DHGs with wild-type MUC16. G Protein-Coupled Receptors (GPCR) signaling relevant proteins MTNR1B, OXTR and PDYN were all low expressed in G34-DHGs with MUC16 mutation (Fig. 5).

**Survival analysis of patients with G34-DHGs**

We performed survival analyses of the patients from our center (SYSUCC) (Fig. 6). We found that the OS of patients with H3G34-mutant DHGs was worse than that of patients with IDH-mutant high-grade gliomas, but better than that of patients with H3K27M-mutant DMGs. The mean survival times of patients with IDH-mutant high-grade gliomas, G34-DHGs and H3K27M DMGs were 58.4, 53.8 and 18.4 months, respectively (P < 0.001).

We further performed analysis in the overall patient group (patients with G34-DHGs from SYSUCC, CGGA and HERBY Trial). Age (≥ 18 years old vs. < 18 years old) and sex (male vs. female) did not influence patient prognosis. Although no statistical difference was achieved, Kaplan–Meier curve analyses showed some trends according to race, PDGFRA mutation, MUC16 mutation and MUC17 mutation. The median OS for Chinese patients was 18 months compared with 12 months for Caucasian patients (P=0.105). Patients with PDGFRA mutation tended to show a shorter OS (median OS: 11.5 months vs. 16 months), and MUC16 and MUC17 mutations both seemed favorable for prognosis (median OS: 15 months vs. 12 months and 16 months vs. 11.5 months, respectively). Importantly, although immune infiltration varied in G34 glioma patients, patient 1 in our cohort who harbored MUC16 mutation...
had obviously high immune infiltration and achieved the longest OS of 75 months.

**External validation**

To validate that PDGFRA and MUC family gene mutations status in G34-DHGs, we reviewed the reports of tumor-sequencing test from 6 external patients, among which WES, a big panel targeting 539 or a small panel targeting 135 tumor genes were respectively performed in every 2 patients (Table 2). Somatic alterations in PDGFRA were detected in all 6 external patients, including 2 patients having both missense mutations and gene amplification, 1 patient having a missense mutation only, 1 patient having both a missense mutation and a deletion-insertion variant, and 2 patients having in-frame insertions or deletions. For the 2 patients who received WES, missense mutations in MUC4 and MUC12 were detected individually. Unfortunately, the targeted sequencing panels did not include MUC family genes in the panel.

**Discussion**

Diffuse hemispheric glioma H3 G34-mutant is a newly recognized tumor type in the 2021 WHO Classification of Tumors of the Central Nervous System. This tumor is a malignant infiltrative glioma that typically occurs in the cerebral hemispheres and harbors a missense mutation in

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**Table 2** Somatic alterations in PDGFRA and MUC family in G34-DHGs of 6 external patients

| Patients | Sex/age (years) | Sequencing test | Somatic alterations in PDGFRA | Somatic alterations in MUC family | Other important molecular changes |
|----------|----------------|----------------|-----------------------------|----------------------------------|----------------------------------|
| ExP1     | M/22           | WES            | p.E387del                   | MUC4 p.V1961L                    | ATRX p.K1584Ifs, EGFR amplification |
| ExP2     | F/18           | WES            | p.L275S, Amplification      | MUC12 p.T1733A                   | ATRX p.Q2168Rfs, TP53 p.R3068p.R342 |
| ExP3     | F/37           | 539 genes panel | p.E279_A280delinsT, p.Y288C | N/A                              | TP53 p.W538p.R342, CDKN2A deletion |
| ExP4     | M/10           | 539 genes panel | p.C290G, Amplification      | N/A                              | ATRX c.S955_S956+2del, TP53 p.E171del |
| ExP5     | M/21           | 135 genes panel | p.Q246_Y249delinsH          | N/A                              | ATRX p.R781, TP53 p.V1471fs |
| ExP6     | M/32           | 135 genes panel | p.G286E                     | N/A                              | ATRX c.S567, TP53 c.S524 |
the H3F3A gene that results in a G34R/V substitution in histone H3. The histological heterogeneity of G34-DHGs has been previously reported [15]. In general, most cases show high-grade anaplastic features, with microscopic features of anaplastic astrocytoma, GBM or embryonic tumors (primitive neuroectodermal tumor-like features) or even anaplastic pleomorphic xanthoastrocytoma [16]. Therefore, misdiagnosis of cases is likely if clinicians and pathologists rely solely on histological diagnosis. Consistent with these findings, most cases in our center also presented as GBM-like and with/without a focal embryonal appearance. However, calcification, perivascular growth pattern and perineuronal satellitosis, which rarely appear in GBM or called ‘secondary structures’, were observed [3, 17]. We further analyzed the immunohistochemical and characteristics of H3G34-mutant diffuse gliomas. Lack of OLIG2 expression is one of the characteristics of this tumor and was previously reported in 100% of G34 DHGs [18]. We also observed loss of OLIG2 expression in all of our cases. In addition, loss of ATRX expression and p53 overexpression are also frequently observed in this tumor type. The H3F3A G34R/V mutation is associated with a high frequency of MGMT methylation, but this is mutually exclusive with IDH or TERT promoter mutation.

Significantly, we found frequent PDGFRA mutation (12/15) in the G34-DHGs in our study. PDGFRA is a classical receptor tyrosine kinase with five extracellular immunoglobulin-like structures and responsive to platelet-derived growth factor (PDGF) [19], and this protein plays an important role in cell determination, cell proliferation and migration during neural development and adult neurogenesis [19, 20]. In glioma, somatic alterations of PDGFRA include missense mutations, in-frame insertions or deletions, gene amplification and fusion. A high proportion of gliomas, especially TERTp wild-type GBM, are associated with somatic alterations in PDGFRA. Recurrent PDGFRA mutations have also been found in low-grade neurotumors associated with refractory seizures, called septal dysplasia neuroepithelial tumors or mucinous glial neuronal tumors [21]. PDGFRA gene amplification is present in approximately one-third of HGGs, and most tumors with PDGFRA gene amplification have genomic deletions of exons 8 and 9[22]. Ozawa et al. reported an adult case with a gene fusion between PDGFRA and VEGFR2 [23]. Point mutations are located in the extracellular domain, transmembrane domain and kinase domain of the PDGFRA receptor. These oncogenic mutations lead to constitutively activated PDGFRA, thereby activating downstream signal transduction [24]. aberrant PDGFRA signaling in gliomas leads to activation of the Ras-Raf-MEK-ERK pathway [25]. Gain-of-function PDGFRA variants trigger multiple tumor-promoting signaling cascades, including phospholipase Cγ (PLCγ), phosphatidylinositol-3-kinase (PI3K), mitogen-activated protein (MAP) kinase, and signal transduction and Activators of Transcription (STATs) [26, 27]. Targeting PDGFRA by tyrosine kinase inhibitors (TKIs) or antibodies showed promising anti-tumor effects in patients with various PDGFR-driven extracranial tumors and pre-clinical models of gliomas [28–31]. In a glioma subset with histone H3 mutation (H3K27M DMGs), PDGFRA amplification and mutation were reported together with histone H3.3 mutation. Li et al. found that 18 cases of 112 midline glioma patients had PDGFRA mutations [32]. Both our study and Chen's study found high frequencies of PDGFRA mutations in G34-DHGs [10]. PDGFRA mutation was present in 12 out of 15 cases (80%) in our study group, and Chen et al. extensively analyzed 95 cases of HGGs with H3.3G34R/V mutation and found that PDGFRA mutations presented in 44% of all tumors and 81% of recurrent tumors, they speculated that PDGFRA mutant G34 tumors have expanded astrocytic compartments that are conducive to maintaining the active chromatin conformation state of the GSX2 enhancer to maintain high PDGFRA expression and continue to promote the carcinogenic state [10]. In GBM and in IDH mutant lower-grade (WHO Grades II/III) glioma, PDGFRA amplification is associated with shorter progression-free survival and OS and it is an independent prognostic factor [33]. PDGFRA amplification was also an indicator of poor prognosis in H3K27M DMGs [34, 35]. However, the prognostic implication of PDGFRA mutation has not been extensively studied. Our data suggested that PDGFRA mutation may be an indicator for poor prognosis in G34-DHGs, but the results were not statistically significant; this may because of the relatively small number of G34 cases in our cohort.

Another important finding included the MUC family genes were also frequently mutated in G34-DHGs. The MUC gene family encodes mucins, a type of high molecular weight glycoprotein [36]. There are 21 MUC genes in the human genome that encode secreted and membrane-type mucins [37]. Increasing evidence has shown that MUC proteins play an important role in regulating tumor cell proliferation, growth, apoptosis and chemical tolerance [38, 39]. MUC gene mutation analysis showed that MUC16 (OMIM 606154) has the highest mutation frequency in G34-DHGs, followed by MUC17. A pan-cancer analysis involving somatic mutations of 10,195 samples and mRNA expression profiles of 9850 samples for 30 solid tumors found a greater abundance of immune cells in the microenvironment of MUC16-mutated tumors, and MUC16 mutation was associated with factors associated with response to immune checkpoint inhibitor therapy [14]. Tumors with
**Conclusions**

G34-DHG is a new high-grade glioma with high frequency of PDGFRA and MUC gene family mutations. PDGFRA may serve as a poor prognostic indicator and an effective therapeutic target for G34-DHGs. Moreover, MUC16 mutation tends to be a favorable prognostic factor and indicates high immune infiltration in certain patients, which may provide a new direction for immunotherapy of G34-DHGs.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12967-022-03258-1.

**Abbreviations**

G34-DHG: Diffuse hemispheric glioma H3 G34-mutant; CGGA: Chinese Glioma Genome Atlas database; HE: Hematoxylin–eosin; IHC: Immunohistochemistry; WES: Whole-exome sequencing; pHGG: Pediatric-type diffuse high-grade glioma; SYSUCC: Sun Yat-sen University Cancer Center; OS: Overall survival; GTR: Gross total resection; CCRT: Concurrent chemotherapy and radiation therapy; TMZ: Temozolomide; GBM: Glioblastoma.

**Additional file 1: Figure S1.** Diagram of the study flow. We initially examined 230 glioma cases (0–35 years old) from SYSUCC. 30 cases were excluded due to no enough tissue. IDH1/2 Sanger sequencing was performed in 200 cases, revealing 21 IDH mutated and 179 IDH wild-type cases. H3F3A Sanger sequencing revealed 34 cases with H3 K27M mutation, 9 cases with H3 G34R mutation and 1 case with H3 G34V mutation. The 10 G34R/V cases were reviewed by hematoxylin and eosin and immunohistochemical staining. Only five cases had sufficient fresh tumor samples for whole-exome and RNA sequencing. This study also included...
three cases from CGGA database and seven cases from the HERBY Trial analyzed and selected as shown.

Additional file 2: Figure S2. Substantial T cell infiltration in patient 1. (A) Hematoxylin and eosin staining and immunohistochemistry for (B) CD4 and (C) CD8

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Authors' contributions
WMH, JZ and YGM conceived and designed the experiments; HD and SA analyzed the data; WMH, HD, SZ, JZ and YGM contributed to the writing and revise of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data in the study will be deposited in the Research Data Deposit (RDD, http://www.researchdata.org.cn/) of Sun Yat-sen University Cancer Center (SYSUCC), which will be shared by request from any qualified investigator upon approval by the SYSUCC data request committee. All data generated or analysed during this study are included in this published article. And the data is available from the corresponding author on reasonable request.

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Declarations

Ethics approval and consent to participate
The present study was approved by the Ethical Committee of the Sun Yat-sen University Cancer Center (SYSUCC) and was carried out in accordance with the Declaration of Helsinki.

Consent for publication
Written informed consent was provided by all the included subjects.

Competing interests
The authors declare that they have no competing interests.

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