News Release

Title

Identification of characteristic chromosome structural abnormality in diffuse midline glioma, H3 K27M-mutant with whole-genome sequencing.

~ *H3F3A* mutant allele specific imbalance in an aggressive subtype of diffuse midline glioma, H3 K27M-mutant~

Key Points

- Diffuse midline glioma is an infiltrative glial neoplasm located in the thalamus, brain stem, or spinal cord. Most of these tumors develop in children, adolescents, and young adults with a lethal clinical course. Molecular alterations such as *H3F3A K27M* mutation frequently found in diffuse midline glioma have been well-studied; however, the prognostic markers of this type of glioma have not been identified to date.
- In our study, whole-genome sequencing for several diffuse midline glioma cases revealed that characteristic chromosome structural abnormality of chromosome 1 on which *H3F3A* gene located, along with *H3F3A K27M* mutation, was associated with poorer prognosis.
- Our study is the first study to identify the characteristic chromosomal structural abnormality which was associated with an aggressive phenotype in these types of glioma by whole-genome sequencing analyses. Further investigation of the molecular mechanisms by which this abnormality is associated with the aggressive phenotype of these cases might contribute to elucidating the tumor formation mechanism of an aggressive subset of diffuse midline glioma, H3 K27M-mutant.

Summary

Diffuse midline glioma, H3 K27M-mutant is a lethal brain tumor located in the thalamus, brain stem, or spinal cord. H3 K27M encoded by the mutation of a histone H3 gene such as *H3F3A* plays a pivotal role in the tumorigenesis of this type of glioma. Although several studies have revealed comprehensive genetic and epigenetic profiling, the prognostic factors of these tumors have not been identified to date. In various cancers, oncogenic driver genes have been found to exhibit characteristic copy number alterations termed mutant allele specific imbalance (MASI). Here, we showed that several diffuse midline gliomas, H3 K27M-mutant exhibited high variant allele frequency (VAF) of the mutated *H3F3A* gene using droplet digital polymerase chain reaction (ddPCR) assays. Whole-genome sequencing (WGS) revealed that these cases had various copy number alterations that affected the mutant and/or wild-type alleles of the *H3F3A* gene. We also found that these MASI cases showed a significantly higher Ki-67 index and poorer survival compared with those in the lower VAF cases ($P < 0.05$). Our results indicated that the MASI of the *H3F3A K27M* mutation was associated with the aggressive phenotype of the diffuse midline glioma, H3 K27M-mutant via upregulation of the H3 K27M mutant protein, resulting in downregulation of

H3K27me3 modification.

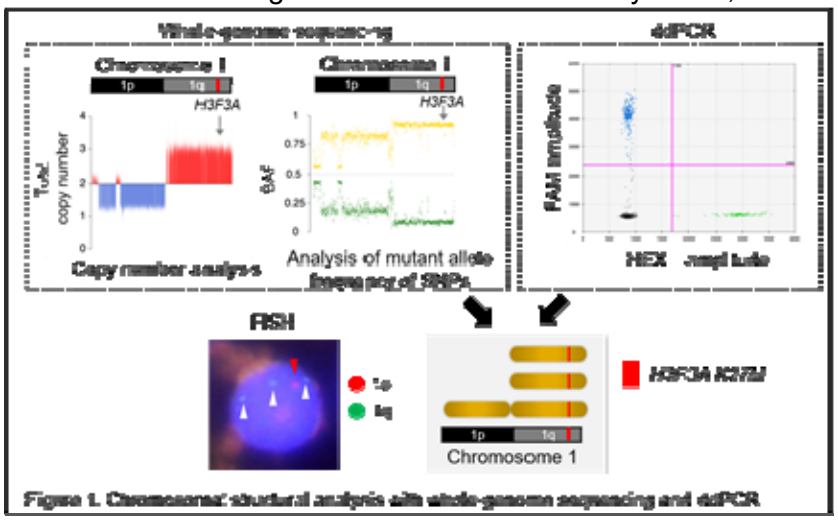
Research Background

Diffuse midline glioma is an infiltrative glial neoplasm located in the thalamus, brain stem, or spinal cord. Most of these tumors develop in children, adolescents, and young adults with a lethal clinical course. Most cases cannot achieve maximum tumor removal owing to tumor location and effective adjuvant therapies other than radiation therapy have not been identified to date. Recent comprehensive molecular analyses revealed that the Lys 27-to-methionine (K27M) mutations at one allele of the histone H3 gene such as the *H3F3A* gene were found in most of this type of gliomas. Diffuse midline glioma exhibiting heterozygous H3 K27M mutation is defined as diffuse midline glioma, H3 K27M-mutant by the 2016 World Health Organization Classification of Tumors of the Central Nervous System. Molecular mechanisms during diffuse midline glioma, H3 K27M-mutant formation have been well-studied; however, the prognostic markers of this type of glioma have not been identified to date.

Research Results

Using droplet digital PCR (ddPCR) assay, we analyzed 15 cases of diffuse midline gliomas, H3 K27M-mutant. Four out of 15 diffuse midline glioma, H3 K27M-mutant cases exhibited more than 50% variant allele frequency (VAF). These data indicated that the four cases might exhibit chromosome structural abnormality of chromosome 1 where *H3F3A* gene is located. Therefore, we performed whole-genome sequencing (WGS) using DNA derived from the four cases that exhibited more than 50% VAF. For chromosome 1, we also analyzed the B allele frequency (BAF) of the common single nucleotide polymorphisms (SNPs). We evaluated the tumor content of the tumor specimen as the frequency of H3 K27M positive cells among DAPI positive cells. By combining these data with the VAF of the *H3F3A K27M* obtained by ddPCR, we determined the most appropriate chromosomal structure model for each case.

For two cases (5 and 15), WGS revealed candidate models that exhibited an one copy loss of 1p and one or two copy gains of 1q in tumor cells. Using BAF of SNPs obtained by WGS, VAF of *H3F3A K27M* obtained by ddPCR and calculated tumor content, we found that the chromosomal structure model that exhibited three *H3F3A K27M* mutant alleles without the wild-type allele was the most appropriate for case 5 (Figure 1), while for case 15, two *H3F3A K27M* mutant alleles with one wild-type allele (a total of three



copies) was the most appropriate. For a case (case 10), we found that the chromosomal structure model of two mutant alleles without a wild-type allele was the most appropriate. For the other case (case 12), we found that the most appropriate chromosomal structure model was the partial loss of 1q containing the wild-type *H3F3A* allele. In all these cases, we confirmed that the calculated tumor contents based on the most appropriate chromosomal structure model were consistent with those of the tumor specimen. These data revealed that all four cases exhibited mutant allele specific imbalance (MASI) of *H3F3A K27M* mutant with (cases 5, 10, and 12) or without (case 15) loss of the wild-type allele. We also found consistent copy number variations on chromosome 1p and 1q in all MASI cases using the FISH assay, although FISH assay also revealed slight heterogeneous copy number variations on chromosome 1p and 1q in these cases.

Immunohistochemistry (IHC) revealed that the MASI cases (n = 4) exhibited a significantly higher expression level of H3 K27M and lower level of H3K27me3 than those in cases with lower VAF (n = 6) ($P < 0.05$ and $P < 0.01$, respectively). Additionally, the four MASI cases showed a significantly higher Ki-67 index than that in the lower VAF cases ($P < 0.05$). Next, we investigated the characteristic clinical features of the MASI cases. Compared with the lower VAF cases (n = 11), MASI cases (n = 4) exhibited a significantly poorer PFS ($P = 0.03$) and OS ($P = 0.01$) compared with the lower VAF cases (Figure 2). These data revealed that the MASI of *H3F3A K27M* was associated with the aggressive phenotype of diffuse midline glioma, H3 K27M-mutant.

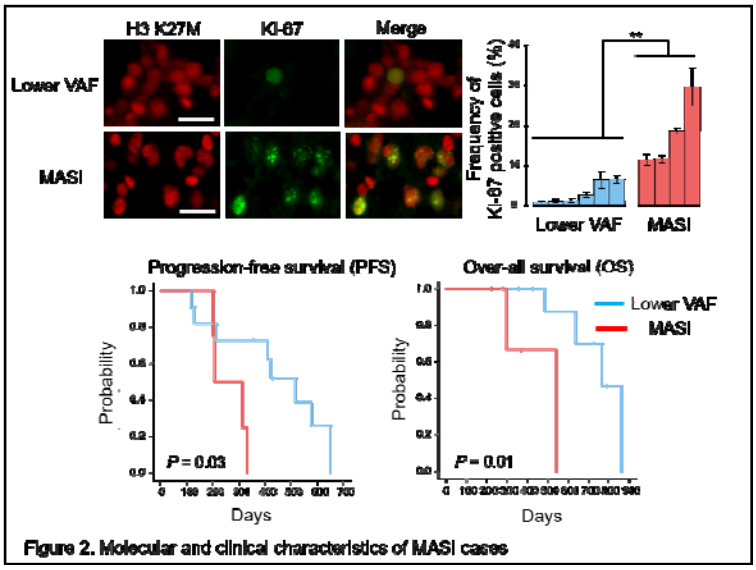


Figure 2. Molecular and clinical characteristics of MASI cases

Research Summary and Future Perspective

Several studies have reported that various cancers exhibit mutant allele specific imbalance (MASI) of driver oncogenes. The copy number gain of the mutant allele and/or loss of the wild-type allele of these genes constitutes MASI. In *KRAS*-mutant pancreatic adenocarcinomas and colorectal cancers, MASI of the *KRAS* gene has been associated with a poorer prognosis compared with tumors with heterozygous *KRAS* mutations. The MASI of the driver oncogene might be a new class of potential biomarkers for malignancy, even though the MASI of the *H3F3A* gene in the diffuse midline glioma, H3 K27M-mutant has not been identified yet. To the best of our knowledge, our study is the first study to identify the MASI of *H3F3A K27M* cases in these types of glioma by WGS analyses. Further investigation of the molecular mechanisms by which MASI of *H3F3A K27M* is associated with the aggressive phenotype of these cases might contribute to elucidating the tumor formation mechanism of an aggressive subset of diffuse midline glioma, H3 K27M-mutant.

Publication

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