Determination of frequency and clinicopathological criteria of H3 K27M mutated tumors in adult patients with midline gliomas

Background and Rationale:

Diffuse midline gliomas are aggressive tumors which are recently found to harbor a K27M mutation in H3F3A and HIST1H3B/C genes which code for mutant histone protein H3.1 and H3.3 respectively (1). This mutation is a substitution of lysine for methionine on histone H3. Diffuse midline gliomas with a H3 K27M mutation, due to their unique molecular signature and clinical features, are now recognized as a separate entity in the 2016 World Health Organization Classification of tumors of the central nervous system (2). These are found in midline locations such as brainstem, thalamus, cerebellum and spinal cord. Diffuse midline gliomas, with H3 K27M mutation, include the historical subgroup of diffuse intrinsic pontine gliomas (DIPGs). In pediatric cases of DIPGs, the presence of H3 K27M mutation confers a worse prognosis as compared to H3 wild type cases (3). More than 90% of children with DIPG die within 2 years from diagnosis (4), irrespective of the WHO grading of the tumor on pathology. Extrapolating from the clinico-pathologic features of DIPGs, all cases of diffuse midline gliomas, with H3 K27M mutations are conferred the WHO grade IV.

Historically, diffuse midline gliomas are predominantly recognized as pediatric brain tumors. With increased testing for the H3 K27M mutation in context of wider sequencing efforts and integration of tissue staining for H3 K27M, these tumors are now diagnosed across all age groups. There is still little information about the incidence or behavior of these tumors in adults, as this entity has not yet been defined yet in brain tumor registries. It is still unknown what percentage of adult gliomas in midline location harbor the H3 K27M mutation. In addition, there is a lack of clarity regarding the clinical behavior of these tumors in adults and how their behavior differs compared to non-mutant gliomas in that location. Currently, it is speculated that adult cases of diffuse midline gliomas behave as aggressively as their pediatric counterparts and merit a WHO grading of IV, irrespective of the actual grade found in microscopy. We aim to establish the frequency of H3 K27M mutations in adult midline gliomas, characterize their clinical features and outcomes and correlate their prognosis with the observed WHO histopathological grading.

Given the novelty and rarity of the H3 K27M mutation in adult brain tumors, the incidence and clinical behavior of these tumors in adults is still relatively unknown. There have been several small series of H3 K27M mutated tumors in adults, which have suggested this mutation may be more prevalent in tumors of the spinal cord or thalamus in adults as compared to pediatric patients (5-7). Two series of pathologically-midline cases showed an incidence of 50-60% H3 K27M mutations in midline gliomas in adults located in the brainstem or thalamus (7, 8).

It is still unclear what proportion of adult gliomas in other midline locations harbors the H3 K27M mutation. In addition, there is a lack of clarity regarding the clinical behavior of these tumors in adults and how their behavior differs compared to non-mutant gliomas in that location. Currently, adult cases of diffuse midline gliomas are treated as if they behave as aggressively as their pediatric counterparts and merit a WHO grading of IV, irrespective of the actual grade found in microscopy (6, 8), though not all series support this (5).

Clinical Significance:

A better understanding of the frequency, biology and clinical outcome of the recently defined pathological entity of diffuse midline gliomas (H3 K27M) is of great significance both for standard of care clinical practice as well as for clinical research. At present, patients with the diagnosis of a diffuse midline glioma are frequently excluded from clinical trials that are designed for patients with glioblastomas or anaplastic astrocytomas. This is in part due to the fact that it is unclear how survival and response to treatment compare to non-H3 K27M mutant tumors. In children with DIPG, the addition of chemotherapy to radiation has not provided significant clinical benefit; in adults it is still unclear whether the addition of chemotherapy may provide benefit for tumors in this location. Our study aims to provide a better understanding of the frequency of H3 K27M mutant gliomas and how these may compare to non-mutant tumors. These data will contribute to the growing cumulative knowledge of this new diagnostic group of gliomas for which new and better treatment options are urgently needed.

Specific Aims:

Specific Aim1. To determine the frequency of the H3 K27M mutation in all adult cases of diffuse midline gliomas at Johns Hopkins Hospital over the last 15 years. To elucidate the clinical and biological variables which determine the prognosis of adult diffuse midline gliomas.

Specific Aim 2. To compare the imaging, clinical features and outcomes of adult cases of diffuse midline gliomas with H3 K27M mutation against adult diffuse midline gliomas without H3 K27M mutations.

Specific Aim 3. To establish if there is an association with WHO grading and prognosis in adult cases of diffuse midline gliomas with H3 K27M mutation.

Inclusion Criteria

- Patients ≥18 years of age
- Patients who have their tumor tissue in the Hopkins pathology repository and have clinical data at Hopkins between 1/1/2002 and 5/31/2016 are included in this study.
- Patients must have a diagnosis of astrocytic or oligodendroglial tumor AND have a WHO grade II, III or IV AND have the tumor in brainstem, thalamus, cerebellum or spinal cord.
- Patients with established diagnosis of diffuse midline glioma, H3 K27M mutant, will also be included in this study. For these patients, we will not retest their tumor tissue.

Exclusion Criteria

- Patients <18 years of age
- Patients who do not have their tumor tissue in the Johns Hopkins pathology repository or have insufficient clinical data for analysis.

Research Plan:

850 consecutive adults with histologically-confirmed gliomas have been identified using the Johns Hopkins Hospital Cancer Registry. Brain MRI images obtained at the time of initial diagnosis or at time of first surgery were reviewed by one of two neuro-oncologists to determine the location of the gliomas. Of these 850 cases, 163 cases with a primarily midline location have been identified and categorized by location: brainstem, thalamus, basal ganglia, corpus callosum, spinal cord, or cerebellum. Clinical data are being collected on patient demographics, tumor histopathology, extent of surgical resection, chemotherapy and radiation treatment, time to progression, and date of death.

Additional formalin-fixed paraffin-embedded (FFPE) tissue is being obtained 163 cases with a radiographic midline location. Slides will be cut into 10 micron unstained slides and analyzed by the collaborating neuropathologist. These slides will stained with antibodies against H3 K27M in cases where that information is not available. In addition, IDH1 and ATRX staining of the tissue will be performed for all 120 cases. Clinical and staining data is collected and summarized in a password protected and de-identified data base.

Statistical analysis of the data will be performed in Stata/IC 15.1. Data will be summarized for all patients with midline tumors, those with H3 K27M mutations, and those without. Demographic, treatment, and survival data will be compared between H3 K27M mutated and non-mutant tumors using Pearson's chi squared test or linear regression as appropriate. Survival analysis will be performed and graphs created using GraphPad Prism version 7.03 (2017. La Jolla, CA: Graph Pad Software, Inc).

Results will be prepared into a manuscript and submitted for publication. They will also be formatted into an abstract and submitted for presentation at the Society for Neuro-Oncology Annual Meeting and the American Association of Neurology Annual Meeting.

This project has been approved by the Johns Hopkins University Institutional Review Board (IRB).

Funding Information and Budget:

This study has not yet been funded by any other funding entity.

For completion of this research project, we estimate the following budget:

Pathology services (tissue acquisition and cutting of samples)	$2,000
Additional tissue staining for research	$2,000
Publication costs (journal fees, poster printing)	$1,000
Travel for presentation of data at a scientific meeting	$1,000
Total costs	**$6,000**

References:

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