Rare Cases of Medulloblastoma with Hypermutation

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Running Title: Implications of tumor mutational burden in medulloblastoma

ABSTRACT

Medulloblastoma (MB) is the most common malignant brain tumor of childhood and is reported to have a low mutational burden. However, in this study, we identified nine MBs with high mutational burden by next generation sequencing. Of them, two had canonical mutations in the *POLE* proof-reading domain, where a large proportion of mutations in these tumor genomes contributed to signature 10. We report very rare incidences of hypermutation in MB and mechanisms driving mutagenesis. Strikingly, of the four known molecular subgroups in MB—SHH, WNT, Group 3, and Group 4—both the *POLE*-mutated MBs belonged to the SHH subgroup.

Key Words: exome, genome, hypermutation, immune checkpoint therapy, medulloblastoma, total mutational burden, whole genome sequencing

Abbreviations Key

GATK	Genome Analysis Tool Kit
EGA	European Genome Archive
HR	Homologous recombination
HRD	Homologous recombination defect
Mut/Mb	Mutations per megabase
MB	Medulloblastoma
SMI	Small molecule inhibitor
TMB	Tumor mutational burden
WGS	Whole genome sequencing

INTRODUCTION

Tumor mutational burden (TMB), defined by the number of nonsynonymous DNA mutations per megabase (Mut/Mb) of the genome's coding region, is a potential biomarker of tumor response to immune checkpoint inhibition[1,2]. High TMB tumors generate neoantigens triggering an antitumor cytotoxic T-cell response attenuated by immune checkpoints, which have been studied in various tumors including brain tumors (e.g., high-grade gliomas[3,4]). Medulloblastoma (MB) has been extensively analyzed in genomic, transcriptomic, and methylation studies classifying MB into molecular subgroups—Wingless (WNT), sonic hedgehog (SHH), Group 3, and Group 4—by clinically relevant and unique transcriptional, genomic, and epigenetic features[5–6]. As the genomic studies show that childhood MB has low TMB[7,10], patients with MB are not considered as good candidates for immune checkpoint inhibition therapy.

METHODS

Use of participants' tissues in genetic studies was approved by Institutional Review Boards of Van Andel Research Institute and Spectrum Health Helen DeVos Children's Hospital. Permission to download whole genome sequencing (WGS) data of 53 primary and recurrent MBs was obtained from the European Genome Archive (EGA). Thirty-three cases were obtained from formalin-fixed paraffin-embedded tissues from pathology diagnostic archives of Spectrum Health and Cooperative Human Tissue Network. Somatic mutations were called using Mutect2, following best practices guidelines for Genome Analysis Toolkit V4 (GATK)[8]. See details of methods in results and supplementary sections.

RESULTS

TMB range in 86 primary and recurrent MB genomes and exomes was 0.2–39.5 Mut/Mb (mean 3.1 Mut/Mb; median 1.2 Mut/Mb; Figure 1A). Whereas most MB genomes (89.5%) had a low TMB, we identified 9 (10.5%) cases as outliers, of which 5 (5.8%) had TMB> 10 Mut/Mb, meeting criteria of hypermutated tumors[9] (Figure S1A).

We evaluated mutational signatures to establish differences among low and high TMB cases[10,11]. Mutagenesis leaves marks on DNA (e.g., nucleotide substitutions), creating unique signatures. The initial definition of such mutagenic signatures reveals 21 signatures in human cancers[12]. We cataloged 486,078 exonic and intronic mutations by nucleotide context (bases immediately preceding and following it, forming a trinucleotide). Using these cataloged trinucleotides (96 subtypes), we performed linear regression analysis using deconstructSigs[13] to identify fractions of mutations contributing to previously established mutational signatures[12]. Due to few mutations, exomes were not analyzed for mutational signatures.

We found signatures 1, 10, 14, 15 and 21 in hypermutated MBs. However, no nonhypermutated MB had mutations contributing to signatures 10, 14, 15, and 21. Mutations in nonhypermutated MBs contributed to signatures 1, 3–6, 8, 9, 11, 12, 16, and 18–20, with prevalence of signatures 3 and 8 (Figure S1D), which are markers of homologous recombination (HR) defects (HRD)[14]. This prevalence of HRD signatures in MB needs further testing in large prospective cohort studies.

In T-10, with TMB of 37.5 Mut/Mb, 68% of mutations contributed to signature 10. This signature, characterized by C>A substitution in TpCpT and C>T substitutions in the TpCpG

context, is specifically associated with loss-of-function mutations in the exonuclease or proofreading domain of *POLE*[10]. T-1, with a TMB of 39.5 Mut/Mb, had 9% mutations contributing to signature 10 (Figures 1B-1C). We identified missense mutations p.R821C, p.D391E, and p.V411L in the *POLE* coding region in T-1 and T-10 (Table 1, Figure 1D, and Figure S1B).

Presence of *POLE* mutations and signature 10 in a hypermutated tumor suggests that *POLE* mutations are pathogenic. Furthermore, position V411 when mutated to leucin[9] is pathogenic. Therefore, we inferred that T-10 with V411L mutation in the "proof reading" domain of POLE was hypermutated secondary to this mutation. Indirect evidence of pathogenicity of the POLE mutation was determined by calculating its mutation allele frequency (MAF). MAF was measured as total number of sequences reads observed matching a specific DNA variant divided by overall coverage at a given genomic locus and is a surrogate measure of the proportion of DNA in the tumor carrying the variant. The MAF of the *POLE* mutation was ~49% (Figure 1E), indicating that the mutation is a somatic heterozygous variant present in almost all tumor cells.

T-1 had two different POLE mutations *D319E* and *R821C* (Figure S1B), both documented in the Catalogue of Somatic Mutations in Cancer database. However, their pathogenicity and association with hypermutation are not well documented. Only 9% of mutations contributed to signature 10 in T-1, and 44%, 14%, and 7.5% of mutations in T-1 contributed to signatures 14, 15, and 21, respectively (Figures 1B-1C). The underlying etiology of these signatures remains unknown, but they are reported in[12] hypermutated solid tumors,

indicating that although *POLE* mutations have a role in the hypermutated phenotype for T-1, multiple underlying mutagenic mechanisms may drive the hypermutation. In the remaining hypermutated tumors, mutations in T-13 and T-13-R contributed to signatures 1, 5, 12, and 16. Underlying mutagenesis driving signatures 12 and 16 remains unknown.

DISCUSSION

Hypermutation is a rare finding in MB. To our knowledge, only two other isolated cases are reported: an adult patient with SHH-MB with POLE mutation (V411L [same mutation detected in our reported case]) and a 5-year-old child with non-WNT, non-SHH MB, with a germline POLE mutation[15,16]. A recent genomic analysis of 134 pediatric MBs for TMB found that most tumors had low mutational burden with 8/134 MBs (6%) displaying a mutational burden of 6–20 Mut/Mb[17]. Our study, similarly, reports 9/86 MBs (10%) with a mutational burden of >6 Mut/Mb suggesting that although rare these cases do exist. Informatively, five of the nine had a TMB>10 Mut/Mb and two of these had somatic POLE mutations. Both of these with a very high TMB belonged to SHH-MB group suggesting a possible connection between hypermutator phenotype and SHH-MB.[18].

Although underlying causes of remaining hypermutated tumors were unclear, high TMB has clinical and biological significance[19]. A higher mutation rate in the coding region of a tumor genome is associated with generation of structurally and functionally altered epitopes or possible neoantigens[19]. Neoantigens can trigger a rapid immunologic cytotoxic CD8+ T-cell response often accompanied by several immune checkpoints to attenuate this effect[20]. Therefore,

hypermutation in tumors may indicate a sustained clinical response to immune checkpoint inhibition.

Furthermore, high mutation rates in tumors can lead to rapid generation of resistant clones when such tumors are treated with small molecule inhibitors (SMIs). This is very relevant to cases reported, as at least two of them belonged to SHH-MB, the only subgroup for which there is a known SMI[21]. More importantly, both POLE mutated tumors reported here were recurrent tumors. As there is no known therapy for patients with recurrent MBs, they are often considered for SMI therapy. Given these data, tumors with high TMB may respond better to immune checkpoint inhibitor therapy than SMIs.

We also highlight the importance of underlying mutagenesis in MB. Mutational signature analysis revealed high prevalence of signatures 3 and 8 in MB. The initial study defining mutational signatures did not report the prevalence of HRD signatures in MB[12], however, subsequently two large genomic studies reported high prevalence of signature 3 in MB[7,22]. This study further adds to the evidence and prevalence of HR defects in MB and needs further testing in prospective studies. This could be important in designing future trials, since cancers with HR defects and tumors with HRD often have good clinical response to platinum therapy and demonstrate synthetic lethality with Poly (ADP-ribose) polymerase inhibitor[23].

We conclude that hypermutations, though rare, are identified in MB and that mutational signature analysis may provide some useful insights into this disease. These observations are

important and warrant further investigation since both could have therapeutic and prognostic implications in MB treatment.

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Conflict of Interests

The authors declare no conflicts of interests.

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Figure Legends

FIGURE 1. (A) Tumor mutational burden (TMB) of medulloblastoma (MB). The figure depicts TMB in the coding region. The Y-axis depicts the total number of mutations per Mb, and the X-axis depicts each individual tumor. Panels labeled "Hypermutated Medulloblastoma T-1" and "Typical Medulloblastoma T-20" are scatter plots of somatic mutations showing their locations on the X-axis versus distance to other events on the Y-axis. (B) Fractions of mutations contributing to different signatures in T-1 and T-10, including signature 10, which occurs in both tumors. (C) Fractions of 96 substitution types contributing to the signature profile of each tumor. The X-axis depicts the 96 substitutions, and the Y-axis shows the fraction of contribution. (D) Schematic plot demonstrating functional derivatives of the POLE protein and location of mutations identified in T-10. (E) Distribution curve of mutation allele frequency (MAF), the dotted line depicts the MAF of the *POLE* mutation in the tumor.

SUPPLEMENTARY FIGURE 1. (A) Non-parametric distribution and inter-tumor variability of tumor mutational burden (TMB) among tumors. The Y-axis depicts the mutations per Mb; the small black circles depict outlier cases with higher TMB. Outliers are calculated as any data

points that lie beyond the point that is 1.5 times the interquartile range above the third quartile of distribution. Ends of the box represent the upper and lower quartiles; therefore, the box spans the interquartile range, and the median is marked by the dark horizontal line inside the box. Whiskers are the two lines outside the box that extend to the highest and the lowest observations. (B) Schematic plot demonstrating the functional derivatives of the POLE protein and location of mutations identified in T-1. (C) Mutational signatures in non–*POLE* mutated tumors with higher mutational burden. Each individual bar represents each tumor. (D) Landscape of mutational signatures in non-hypermutated medulloblastomas (MBs). The figure highlights the prevalence of signatures 3 and 8 in MBs, that is associated with homologous recombination defects in other solid tumors.