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TERT promoter mutation and aberrant hypermethylation are associated with elevated expression in medulloblastoma and characterise the majority of non-infant SHH subgroup tumours

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Running title: *TERT* alterations in medulloblastoma

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To the editor:

The childhood brain tumour medulloblastoma comprises four molecular disease subgroups (MB_{WNT}, MB_{SHH}, MB_{Group3} and MB_{Group4}). However, large-scale whole-exome sequencing investigations have not identified defining genetic lesions for the non-MB_{WNT} subgroups [8,11]. Recent studies reported in this journal and others [1,3,6,7,9] have identified frequent *TERT* promoter mutations and aberrant DNA methylation in CNS malignancies, suggesting an important mechanism in tumour development (Figure 1a). In medulloblastoma, Castelo-Branco *et al.*[3] reported a high frequency of *TERT* promoter methylation, while Killela *et al.*[6] described *TERT* promoter mutations which Koelsche *et al.*[7] and Remke *et al*.[9] subsequently reported were most frequent in adult MB_{SHH}, but rarer in childhood tumours. However, while *TERT* mutations have been associated with elevated expression in other cancers [1, 5], and account for a proportion of MB_{SHH}, the relative contribution of *TERT* methylation alterations has not yet been investigated alongside mutational analysis. Moreover, relationships between *TERT* promoter methylation and gene expression are unclear; the positive association reported across multiple malignancies by Castelo-Branco *et al.*[3] is contradicted by the inverse association described by Arita *et al.*[1] in *TERT* wild-type adult gliomas.

We therefore sought to clarify the role of *TERT* alterations in medulloblastoma, by assessing the frequency of *TERT* promoter hot-spot mutations [1,6,7,9], aberrant methylation of the critical cg11625005 *TERT* promoter CpG residue [3], and *TERT* expression, in our tumour series. We show a common, subgroup-specific, involvement of *TERT* mutations in MB_{SHH} alongside a wider involvement of *TERT* methylation across the MB subgroups, both associated with elevated *TERT* expression. Notably, in the non-infant MB_{SHH} patient group aged 4 and over at diagnosis within our cohort, we show these genetic and epigenetic aberrations occur in both childhood and adult tumours, and in a mutually exclusive fashion, representing a defining molecular alteration in >75% of this patient group.

TERT promoter mutations occurred at high frequency in both childhood (14/41 (34%)) and adult (8/11 (73%)) MB_{SHH} (Figure 1b) in our cohort, more common than any coding mutation reported in these groups to date (*TP53*, 30%; *PTCH1*, 27%; *DDX3X*, 18%; all other genes, <6% (*n=33,* data from cancer.sanger.ac.uk). The age distributions of mutated (4.7-15.5 years)

and non-mutated (5.2-15.4 years) childhood patients did not differ significantly (*p=0.27*; Mann-Whitney U test). *TERT* mutations were tumour-specific where germline DNA was available for comparison (*n=4*) and exclusive to non-infant MB_{SHH} in our investigations. Mutations were not found in MB_{WNT} (*n=16*; age range, 4.7-16.8 years), MB_{Group3} (*n=16*; 1.5-16.1 years) or MB_{Group4} (*n=20*; 2.4-15.8 years) from infants and children, or in tumours from infant MB_{SHH} (<4.0 at diagnosis; *n=17*; 0.2-3.5 years), consistent with the rarity of mutations in these subgroups reported by Remke *et al*. [9]

Aberrant *TERT* promoter methylation at cg11625005 was a feature of all medulloblastoma molecular subgroups, but varied significantly in level and incidence between tumour groups ($p=9x10^{-6}$, ANOVA); aberrant hypermethylation (with respect to normal cerebellar levels; n=17, foetal to 67 years) was observed in 63% (10/16) $MB_{WNT,}$ 69% (11/16) MB_{Group3} and in 10% (2/20) MB_{Group4}, while MB_{SHH} tumours (36%; 16/44 hypermethylated) showed greatest variation (Figure 1c). Notably, *TERT* hypermethylation showed significant age-dependent associations within the MB_{SHH} group (0% (0/6) >16 years; 52% (11/21) 4-16 years; 29% (5/17) <4 years; p=0.05, χ^2 test). Moreover, *TERT* promoter mutation and aberrant methylation at cg11625005 were mutually exclusive in non-infant MB_{SHH} within our cohort (Figure 1d) suggesting methylation alterations contribute significantly to *TERT* alteration in this group, and possible common mechanistic effects. Aberrant hypermethylation was detected in 11/17 non-mutated vs. 0/10 mutated non-infant MB_{SHH} tumours assessed (*p*=0.001; Fisher's exact test (Figure 1d)).

To assess the potential mechanistic contributions of promoter mutation and methylation to *TERT* gene expression, we next assessed their association within our cohort using expression data generated by RNA-seq. *TERT* methylation and expression showed a significant positive and linear relationship (p=0.001; Pearson's correlation test) in *TERT* wild-type tumours across all medulloblastoma subgroups, while all *TERT* mutant tumours (all MB_{SHH} > 4 years (Figure 1b)) displayed high *TERT* expression in the absence of hypermethylation (Figure 1e).

Non-coding *TERT* promoter alterations, encompassing mutually-exclusive mutation and aberrant hypermethylation, both associated with elevated *TERT* expression, are therefore a defining feature for the majority (>75%) of non-infant MB_{SHH} in our cohort, indicating a key

mechanism in their molecular pathogenesis. Moreover *TERT* hypermethylation and deregulation, in the absence of mutation, suggests a wider involvement across the other medulloblastoma molecular subtypes, notably frequent in MB_{WNT} and MB_{Group3}, but less so in MB_{Group4}, which now mandates further investigation.

Finally, our findings raise the potential importance of additional non-coding and/or epigenetic regulatory alterations in medulloblastoma, which have hitherto been overlooked by exome sequencing studies [8]. Despite current nomenclature, MB_{SHH} is not solely defined by SHH pathway activation and SHH is likely to contribute alongside other frequently disrupted pathways, with *TERT* alterations representing the most common identified to date. We believe these findings have important implications for future diagnosis, research and targeted therapy of a significant proportion of medulloblastoma patients.

Figure legend

Figure 1. *TERT* non-coding mutations, aberrant DNA hypermethylation, and expression in medulloblastoma. a. The *TERT* promoter region, showing positions of mutational hotspots and methylated regulatory CpG site, relative to the translational start site. **b.** Numbers and frequencies (%) of *TERT* mutations in medulloblastoma subgroups (subgroups determined as previously described [4,10]). Mutations were frequently and exclusively detected in the non-infant MB_{SHH} subgroups (X^2 test between MB_{SHH} age groups shown). The three different mutations are colour coded (pink, purple and gold). **c.** *TERT* promoter CpG site cg11625005 DNA methylation levels (β-value, assessed by Illumina Human Methylation 450K array [2]), are shown for normal cerebella (grey), MB_{SHH} (by age; red), MB_{WNT} (blue), MB_{Group3} (yellow) and MB_{Group4} (green). Black horizontal line, upper 99% confidence interval of mean cerebellar methylation levels, above which tumours were classed as aberrantly hyper-methylated. 'p' value, one-way analysis of variance between tumour groups shown. **d.** *TERT* promoter methylation in MB_{SHH} tumours from patients ≥4 years old at diagnosis. CpG site cg11625005 DNA methylation levels (β-value) are shown for *TERT* mutated (*n=10*) and wild-type non-infant MB_{SHH} (*n=17*) and normal cerebella (*n=17,* grey). Specific mutations are colour coded as above (b). The mean methylation level was significantly higher in wild-type tumours compared to either mutated tumours (*p=0.0006*) or the normal cerebellum (*p=0.00001*) (Student's t-test). Black horizontal line, see above (c). **e.** *TERT* expression (by RNA-seq; further details given in Supplementary Table 1) versus *TERT* promoter CpG site cg11625005 DNA methylation levels and mutation status in 51 medulloblastomas. Subgroup assignment is coloured as above (c). Mutated tumours, bold outlined boxes; wild-type tumours, circles. Dashed line, linear regression of methylation vs. expression in *TERT* wild-type tumours with associated 'p' values (Pearson's correlation). VSD, variance-stabilised transform of normalised read counts aligned to ENSG00000164362. All tumour-specific data is summarised in Supplementary Table 1.

References

1 Arita H, Narita Y, Takami H et al. (2013) TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. Acta neuropathologica 126: 939-941 Doi 10.1007/s00401-013-1203-9

2 Bibikova M, Barnes B, Tsan C et al. (2011) High density DNA methylation array with single CpG site resolution. Genomics 98: 288-295 Doi 10.1016/j.ygeno.2011.07.007

3 Castelo-Branco P, Choufani S, Mack S et al. (2013) Methylation of the TERT promoter and risk stratification of childhood brain tumours: an integrative genomic and molecular study. The lancet oncology 14: 534-542 Doi 10.1016/S1470-2045(13)70110-4

4 Hovestadt V, Remke M, Kool M et al. (2013) Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays. Acta neuropathologica 125: 913-916 Doi 10.1007/s00401-013-1126-5

5 Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA (2013) Highly recurrent TERT promoter mutations in human melanoma. Science (New York, NY) 339: 957-959 Doi 10.1126/science.1229259

6 Killela PJ, Reitman ZJ, Jiao Y et al. (2013) TERT promoter mutations occur frequently in gliomas and a subset of tumours derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A 110: 6021-6026 Doi 10.1073/pnas.1303607110

7 Koelsche C, Sahm F, Capper D et al. (2013) Distribution of TERT promoter mutations in pediatric and adult tumours of the nervous system. Acta neuropathologica 126: 907-915 Doi 10.1007/s00401-013-1195-5

8 Northcott PA, Jones DT, Kool M et al. (2012) Medulloblastomics: the end of the beginning. Nature reviews 12: 818-834 Doi 10.1038/nrc3410

9 Remke M, Ramaswamy V, Peacock J et al. (2013) TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma. Acta neuropathologica 126: 917-929 Doi 10.1007/s00401-013-1198-2

10 Schwalbe EC, Williamson D, Lindsey JC et al. (2013) DNA methylation profiling of medulloblastoma allows robust subclassification and improved outcome prediction using formalin-fixed biopsies. Acta neuropathologica 125: 359-371 Doi 10.1007/s00401-012-1077-2

11 Taylor MD, Northcott PA, Korshunov A et al. (2012) Molecular subgroups of medulloblastoma: the current consensus. Acta neuropathologica 123: 465-472 Doi 10.1007/s00401-011-0922-z

Figure 1
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