CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Northumbria Research Link

Northumbria Research Link

Citation: Lindsey, Janet, Schwalbe, Ed, Potluri, Sandeep, Bailey, Simon, Williamson, Daniel and Clifford, Steven (2014) TERT promoter mutation and aberrant hypermethylation are associated with elevated expression in medulloblastoma and characterise the majority of non-infant SHH subgroup tumours. Acta neuropathologica, 127 (2). pp. 307-9. ISSN 0001-6322

Published by: Springer

URL: http://dx.doi.org/10.1007/s00401-013-1225-3 <http://dx.doi.org/10.1007/s00401-013-1225-3>

This version was downloaded from Northumbria Research Link: http://nrl.northumbria.ac.uk/15322/

Northumbria University has developed Northumbria Research Link (NRL) to enable users to access the University's research output. Copyright © and moral rights for items on NRL are retained by the individual author(s) and/or other copyright owners. Single copies of full items can be reproduced, displayed or performed, and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided the authors, title and full bibliographic details are given, as well as a hyperlink and/or URL to the original metadata page. The content must not be changed in any way. Full items must not be sold commercially in any format or medium without formal permission of the copyright holder. The full policy is available online: http://nrl.northumbria.ac.uk/policies.html

This document may differ from the final, published version of the research and has been made available online in accordance with publisher policies. To read and/or cite from the published version of the research, please visit the publisher's website (a subscription may be required.)

www.northumbria.ac.uk/nrl

*manuscript
Click here to download manuscript: Lindsey et al manuscript v271113.docx
Click here to view linked References

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

Janet C. Lindsey[1], Ed. C. Schwalbe[1], Sandeep Potluri[1], Simon Bailey[1], Daniel Williamson[1] and Steven C. Clifford[1]*

[1]Northern Institute for Cancer Research, Newcastle University, Royal Victoria Infirmary, Newcastle upon Tyne, U.K.

Running title: *TERT* alterations in medulloblastoma

***Correspondence to:** Steven C. Clifford, Northern Institute for Cancer Research, Newcastle University, Sir James Spence Institute Level 5, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, U.K. Tel: +44 (191) 2821319, Fax: +44 (191) 2821326, e-mail: steve.clifford@ncl.ac.uk

Acknowledgements:

Funded by grants from The Brain Tumour Charity and Cancer Research UK. Tumours investigated include samples provided by the UK Children's Cancer and Leukaemia Group (CCLG) as part of CCLG-approved biological study BS-2007-04. Conducted with ethics committee approval from Newcastle / North Tyneside REC (study reference 07/Q0905/71).

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

Click here to download figure: Lindsey et al Figure 1 v271113.eps