Expression of *GNAS*, *TP53*, and *PTEN* Improves the Patient Prognostication in SHH Medulloblastoma

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Introduction

Medulloblastoma is the most frequent malignant brain tumor in children. Currently, four distinct medulloblastoma molecular subgroups have been identified: MB_{SHH}, MB_{WNT}, MB_{GRP3} and MB_{GRP4}. For medulloblastoma molecular classification, the NanoString is a high-throughput platform, highly sensitive, robust and useful for analysis of FFPE tissues. Although a 22-gene panel employing the NanoString technology was previously successfully developed for medulloblastoma molecular subgrouping, MB_{SHH} may be sectioned into distinctive subgroups according clinical and molecular characteristics. **Aim**: To apply the 22-gene panel for medulloblastoma molecular subgrouping with further key cancer-related genes in order to improve classification and subclassification of Brazilian MB_{SHH} using NanoString.

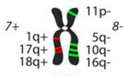
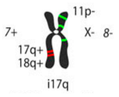
Demographics	WNT	SHH	Group 3	Group 4
Age group	3-16 yrs	<3 yrs >16 yrs	3-16 yrs	3-16 yrs
Gender: M/F	1:1	1:1	2:1	2:1
Clinical features				
Histology	Classic, Rarely LCA	Classic, LCA desmoplastic/nodular	Classic, LCA	Classic, LCA
Metastasis	Rarely M+	Uncommonly M+	Frequently M+	Frequently M+
Prognosis	Very good	Good (<3y) and intermediate	Poor	Intermediate
Genetics				
Chromosomal				
Mutations Amp/del	*CTNNB1* Mutation	*PTCH1, SMO, SUFU* Mutations *MYCN/GLI2* Amplification	*MYC* Amplification	*MYCN/CDK6* Amplification
Gene expression	WNT pathway *MYC +*	SHH pathway *MYCN +*	Phootoreceptor/ GABAergic *MYC +++*	Neuronal/ Glutametergic ↓ *MYC/MYCN*

Figure 1: Molecular subgroups of medulloblastoma. Adapted from Taylor et al., 2012 [1].

Methods

FFPE samples from 149 medulloblastoma cases from four reference centers in Brazil were enrolled. Gene expression was assessed using the 22-gene panel previously developed by Dr. Taylor's group for medulloblastoma molecular sub-grouping [2] plus 11 additional genes. Raw data was normalized by housekeeping genes, followed by class prediction with Prediction Analysis of Microarrays (PAM) in R statistical environment. MB_{SHH} sub-classification was performed by new genes low and high expression using median value of normalized expression. The molecular profile was associated with patients' clinical outcome with Kaplan-Meier and Log-Rank statistical test. R scripts were wrapped with Planemo for a local Galaxy instance in order to build a diagnostic tool of easy access for clinicians and biologists.

Results

The medulloblastoma patients were distributed into MB_{SHH} (47.7%), MB_{WNT} (16.1%), MB_{GRP3} (15.4%), and MB_{GRP4} (20.8%). The molecular distribution may be visualized on a t-SNE representation considering the 22-gene panel expression in Figure 2 A.

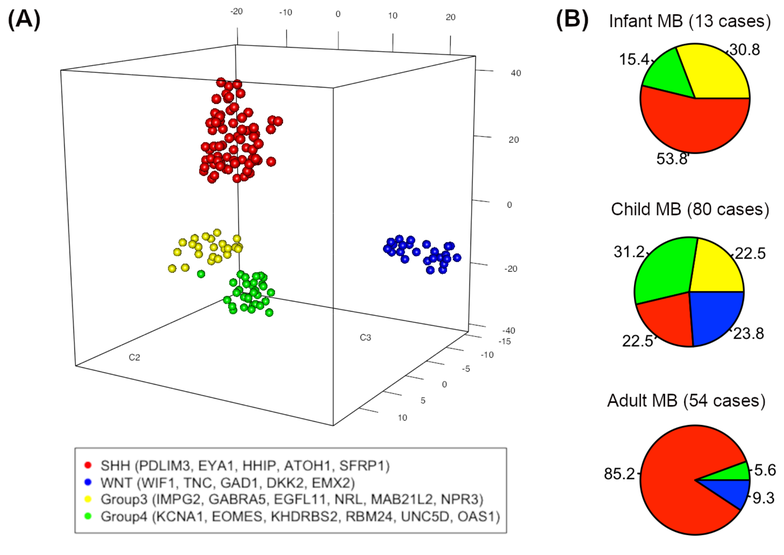


Figure 2: Cohort characterization. (A) Three components t-SNE representation of 149 Brazilian cohorts using the 22-gene panel for medulloblastoma classification. Patients are represented by spheres, colored by medulloblastoma subgroup (MB_{SHH} in red, MB_{WNT} in green, MB_{GRP3} in blue, and MB_{GRP4} in cyan). (B) Pie charts presenting the incidence of subgroups in adults and children.

All cases have been classified into the respective molecular subgroup with scores higher than 75% by PAM algorithm. *GNAS* presented the highest expression levels through all subgroups, with significantly higher expression in the MB_{SHH}. *TP53*, *MYCN*, *SOX2*, and *MET* were also upregulated in the MB_{SHH} subgroup, whereas *PTEN* was upregulated in the MB_{GRP4} group as shown in Figure 3. *GNAS*, *TP53,* and *PTEN* low expression were associated with the unfavorable patient outcome only for the MB_{SHH} subgroup (p = 0.04, 0.01 and 0.02, respectively) as shown in Figure 4.

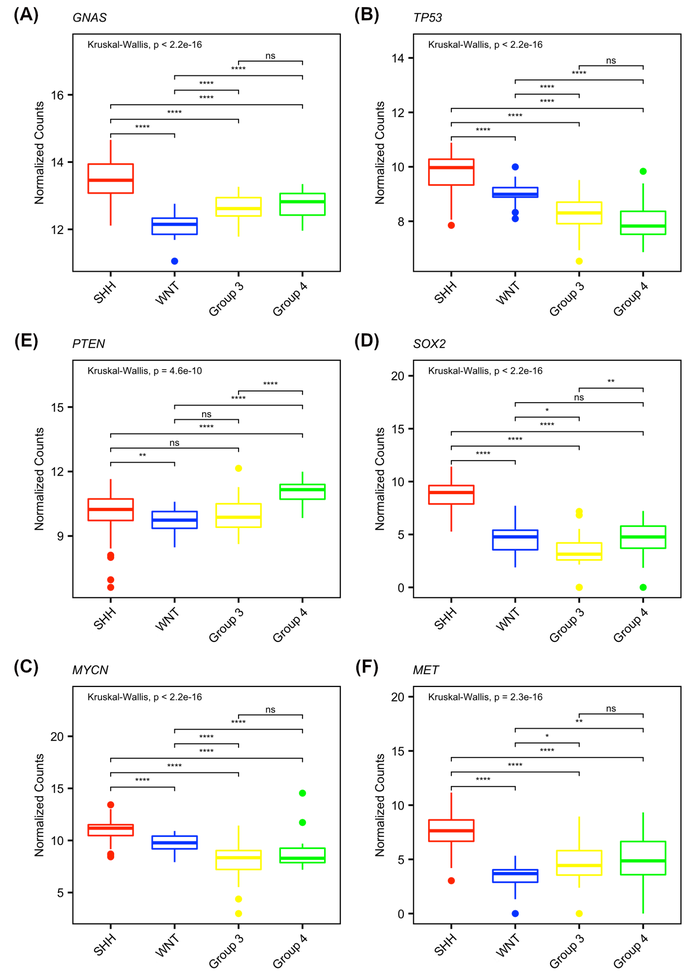


*Figure 3. Boxplot of log2 gene expression levels of the nine additional genes in the four medulloblastoma subgroups. Kruskal-Wallis and unpaired two-samples Wilcoxon tests were applied with significance threshold of p < 0.05 (ns, non significative; *p < 0.05; **p < 0.01; ***p < 0.001, ****p < 0.0001). MB_{SHH} in red, MB_{WNT} in blue, MB_{GRP3} in yellow, and MB_{GRP4} in green.*

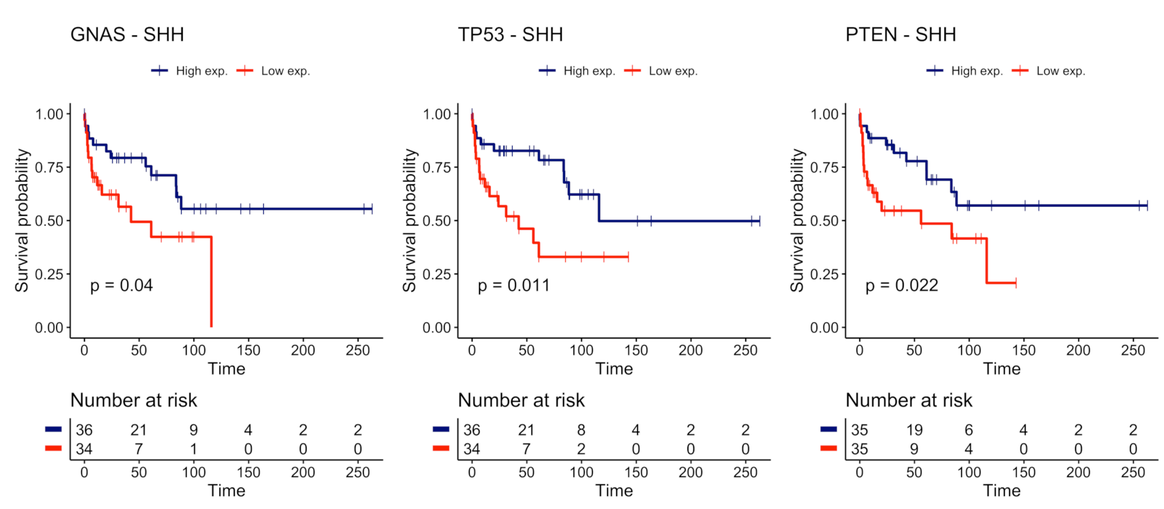


Figure 4. Kaplan-Meier plots for categories of high and low expression in MB_{SHH} patients. Median values of gene expression were applied for the classification of high and low expression levels of (A) GNAS, (B) TP53, and (C) PTEN. The significance threshold was attributed to p < 0.05 in Log Rank statistical test.

Conclusions

We have implemented the NanoString platform for molecular classification as an effective diagnostic tool for personalized medicine [3] using Galaxy. The 22-gene panel for molecular classification of medulloblastoma associated with the expression of *GNAS*, *TP53*, and *PTEN* improve the patient prognostication in MB_{SHH} subgroup and can be easily incorporated in the 22-gene panel without any additional costs.

[1] Taylor MD, et al. Acta Neuropathol. 123:465–472, 2012
[2] Northcott PA, et al. Acta Neuropathol. 123:615-26, 2012
[3] Leal LF, et al. Neuropathology. 38(5):475-483, 2018

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Acknowledgments: