Biomolecular characterization of metastatic medulloblastoma and study of telomere lengthening control

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Medulloblastoma (MDB) is a malignant embryonic brain tumor and occurs typically in pediatric patients. Medulloblastoma cells can disseminate through the cerebrospinal fluid in the leptomeningeal space; approximately 30% of children present metastasis at the onset and no gold standard treatment has been defined for these patients. Genetic, epigenetic and molecular analyses identified four molecular subgroups (WNT, SHH, group 3 and group 4), associated with prognostic stratification of patients; however, previous works in literature included only small amount of metastatic cases, not analyzed independently from the non-metastatic counterpart. Furthermore, recent studies evidenced that mechanisms of telomeres elongation can be activated in pediatric brain tumours and telomeres maintenance was enriched in SHH and group 3 non-metastatic MDB; however, elongation of telomeres was not previously investigated in metastatic medulloblastomas.

Therefore, our aim is to characterize a series of 39 pediatric MDB, selected from a cohort of 60, with leptomeningeal dissemination at the onset, studying molecular features involved in malignancy, metastasis, telomeres elongation and senescence escape. We analyzed several biomarkers and we correlated results to outcome of patients, evaluating the prognostic relevance of molecular biomarkers and subgroups. Furthermore, we analyzed the activation of mechanisms involved in control of telomeres lengthening, in order to figure out if telomeres elongation could have a role in metastatic medulloblastomas.

We show that distribution of metastatic MDB into four molecular subgroups is highly similar to the distribution of non-metastatic cases, reflecting a high molecular heterogeneity; interestingly, our molecular subgrouping system defines high-risk (group SHH, 3 and 4) and standard-risk (group WNT and Not Classifiable) patients. Furthermore, we evidence that FSTL5 over-expression is associated exclusively with groups 3/4 and with poor outcome of patients, highlighting that FSTL5 can be used to better define molecular subgroups, prognosis and risk stratification of metastatic medulloblastomas.

In addition, we analyzed H3.3 and ATRX mutations, involved in activation of the Alternative Lengthening of Telomeres (ALT) pathway, and the mutation and methylation status of TERT promoter, involved in telomerase reactivation. We evidence that metastatic MDB activate elongation of telomeres both via telomerase (14%) and via ALT mechanism (27%), triggered by ATRX mutations; interestingly, ALT pathway is highly activated in our cohort compared to MDB previously analysed in literature (<5%), highlighting the differences between metastatic and non-metastatic tumors in control of telomeres elongation and senescence escape. Furthermore, metastatic MDB show a higher reactivation of telomerase compared to pHGG (0%), triggered by TERT promoter mutations in combination with hyper-methylation. Our findings suggest that immortalization of tumor cells in metastatic MDB is a common process to escape from senescence and characterizes all molecular subgroups.

In conclusion, our results contribute to improve the current characterization of pediatric patients with metastatic medulloblastoma; however, further studies will be necessary to increase the number of cases and to analyze, with statistical significance, the molecular subgroups, FSTL5 expression, and telomeres elongation, which could be used to "personalize" treatments or develop targeted therapies, reducing the side effects of the current therapeutic protocols.

Keywords: Metastatic medulloblastoma. Molecular subgroups. WNT. SHH. Group 3. Group 4. FSTL5. Risk stratification. Telomere. Alternative lengthening of telomeres. ALT. Telomerase. TERT. ATRX. H3.3.