# Tropomodulin 1 and 2 are new favorable biomarkers in high risk neuroblastoma

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Neuroblastoma is an embryonal tumor of the peripheral sympathetic nervous system, arising during fetal or early postnatal life from cells derived from the neural crest. It is the most common solid extra cranial malignancy of childhood and it is responsible for 15% of all pediatric cancer deaths (Ishola et al., 2007). Its clinical, biological and histological heterogeneity makes it unsuitable to the classical therapy, not able to ensure an improvement in survival in the range of patients at "high risk", which remains below 40% (Garaventa et al., 2008). By a bioinformatics analysis of a large collection of microarray data, including 88 neuroblastomas, the tropomodulins family members' tropomodulin 1 and 2 were identified as favorable biomarkers. Tropomodulins (Tmods) are a conserved family of four proteins (Tmods 1-4) that cap actin filament pointed ends, stabilizing filaments and inhibiting their disassembly and turnover. Four Tmod isoforms are expressed in different tissues in vertebrates where they regulate the mechanical properties of the actin cytoscheleton, its stability, lengths and architecture, and the physiological properties of cells (Yamashiro et al., 2012). Tmod1 and Tmod2 are the only Tmods expressed in neurons and seem to negatively regulate neurite formation through the action on F-actin dynamics (Fath et al., 2011). Tmod1 and Tmod2 mRNA expression was found very high in neuroblastoma relative to the other tumors analyzed, suggesting the high specificity of these markers, and elevated expression of Tmods was associated with an higher overall survival probability. Correlation of Tmod1 and Tmod2 with age group, INSS stage and MYCN status was also assessed and revealed an inverse correlation between all these parameters and the Tmods expression. Tropomodulins expression was studied in vitro in SH-SY5Y and SK-N-SH neuroblastoma cells; in both cell lines there is a significant increase of Tmod1 and Tmod2 expression after retinoic acid differentiation, in terms of messanger RNA and protein, compared to controls. To better understand the functional role of these proteins in neuroblastoma, Tmods were down regulated with Rna interference technique, evaluating the effects on different parameters such as cell proliferation, differentiation and cell cycle. Knockdown for Tropomodulin 1 and Tropomodulin 2 induced cell proliferation arrest, as evaluated with Ki-67 cell proliferation marker. Cell cycle analysis further showed a significant arrest in $G_0/G_1$ phase. Down regulation of these actin cytoskeleton regulators also induced an initial cell differentiation, characterized by neurites elongation, without sprouting and varicosities.
Tropomodulins knockdown induces an aberrant differentiation that probably contributes to the onset and progression of tumor. In conclusion the present data indicated Tmod1 and Tmod2 as favorable biomarkers in neuroblastoma and low expression of these proteins correlates with worse disease characteristics such as MYCN amplification, age groups >18 months and INSS stage 4 in neuroblastoma patients. In our knowledge these data show for the first time a correlation between tropomodulins expression and neuroblastoma progression.

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