# EXPRESSION OF *MDM2* GENE IN NEUROBLASTOMA PRIMARY TUMORS

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Neuroblastoma (NB) is an aggressive pediatric malignancy that is mostly common diagnosed during the first year of life. NB arises as ganglioneuroma or ganglioneuroblastoma from ganglion cells of the nerve crest [1]. Despite the fact that mutations in TP53 gene arise very rarely in NB, deregulation of p53-pathway often occurs and MDM2 protein involved in this process. MDM2 protein helps to target p53 for degradation through its ubiquitin activity decreasing its tumor suppressing activity [2]. In fact, MYCN is known to be one of the *MDM2* gene expression regulators. On the other hand, hyperexpression of the MDM2 protein in the cytoplasm amplifies the stabilization and translation of the *MYCN* mRNA [3]. The aim of our study is to investigate changes in *MDM2* gene expression in NB and the association with disease outcome.

We have investigated tissue samples of 80 patients with verified diagnosis of NB. The age of patients verified from 1 month to 17 years with mean age 39.45 ± 4.81 month. For total RNA isolation was used «NucleoSpin miRNA» (Macherey-Nagel, Germany) kit. To detect the level of expression of *MDM2* gene, specific TaqMan primers and probes were used; amplification was performed on 7500 Real-Time PCR System (Applied BioSystems, USA). The real-time recording of the results was carried out in accordance with the manufacturer's recommendations. Calculations were performed using the ΔΔCt relative quantification method. To detect the *MYCN* amplification FISH analysis was performed using dual-color Vysis LSI® N-MYC Spectrum Green/CEP 2 Spectrum Orange™ (Abbott, USA). Prognostic significance of markers was verified with the ROC-curve (Receiver Operating Characteristic curve). Event free survival was evaluated by Kaplan-Meier estimator, statistical significance of parameters differences was determined using F-Cox criterion.

*MDM2* expression was higher in patients with recurrent and metastatic tumors compared to primary NB. In primary tumors, the *MDM2* mRNA expression was statistically lower in 32 and 2.6 times in comparison with recurrent and metastatic tumors respectively, $p < 0.001$. Moreover, the expression level of the mRNA *MDM2* gene varied in a quite wide range and in particular depending on the stage of the disease in the primary NB tumors. The level of *MDM2* gene expression was in 28 times higher in tumor samples obtained from patients with stage IV compared to early stages, $p < 0.001$. In additional, *MDM2* expression level was significantly lower in primary tumors without *MYCN* amplification. The *MDM2* mRNA expression was in 2.9 times higher in patient group with *MYCN* amplification in comparison with *MYCN*-negative group, $p < 0.03$. The obtained data may indicate that inactivation of p53 / MDM2 pathway in NB occurs most often during treatment and is involved in the progression of the disease and the relapse occurrence.

For the distribution of patients in two groups with high or low risk according to the level of expression of the mRNA of the *MDM2* gene, the progression free survival rate as a criterion for clinical efficacy was used. For determining of the groups to high and low *MDM2* expression levels, an optimal criterion 0.0822 a.u. was obtained. According to the ROC analysis, high expression of *MDM2* mRNA was determined as a marker for a poor outcome in NB and a risk of disease recurrence. This marker is of sufficient sensitivity (> 70%) and specificity (> 65%) and can serve as an independent marker for predicting the NB outcome and stratification of patients by risk groups.

It has been established that high expression of *MDM2* gene is associated with a decrease in the rates of progression free survival in patients with NB, regardless of the *MYCN* gene status and disease stage ($p < 0.001$). Accordingly, the 3-year-old progression free survival rate in patients with high *MDM2* mRNA expression was only 12%, whereas in patients with low expression levels reached 66%.

Such a significant difference indicates that this marker plays an essential role in the pathogenesis and progression of the NB and the disruption of MDM2 / p53 pathway leads to a poor outcome in NB patients.

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