Ministero dell'Istruzione, dell'Università e della Ricerca

Università degli Studi di Palermo

Dottorato di Ricerca in
Genomica e Proteomica nella ricerca Oncologica ed Endocrino-Metabolica

Ciclo XXIV: 2011-2013
Settore Scientifico Disciplinare: BIO/18

ONCOSUPPRESSOR ACTIVITY OF TRANSCRIPTIONAL REPRESSOR MBP-1 IN NEUROBLASTOMA LAN-5 CELLS

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***Graduation year* 2013**

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Abstract

Background: An alternative translated product of the ENO1 gene, known as MBP-1 (c-myc promoter binding protein-1), acts as a negative regulator of the c-MYC oncogene, ERBB2 and COX-2 genes (1-3). The ENO1 gene is located in chromosomal region 1p36.2 (4), within the common region of deletion detected in Neuroblastoma (NB) often associated with amplification of MYCN gene (5). Previous studies have shown that the level of MYCN expression is correlated with the growth (6-8) and invasiveness (9) of Neuroblastoma cells and its downregulation could inhibit cell proliferation and induce differentiation and apoptosis. Also it has been reported that ENO1/MBP-1 overexpression in Neuroblastoma cells significantly reduces cell growth and induces apoptosis (4).
Even though there are structural and functional similarities between c-MYC and MYCN oncogenes, there are no evidences that MBP-1 is able to interact with MYCN promoter to regulate negatively its expression and to act as an oncosuppressor protein in Neuroblastoma cells.

Methodology and Findings: We induced MBP-1 overexpression in LAN-5 Neuroblastoma cell line, characterized by MYCN amplification, and we performed luciferase reporter assays on MYCN promoter in order to verify the direct effect of MBP-1 on that promoter.
These studies have identified a promoter region involved in the negative regulation of MYCN by MBP-1, and its binding activity, within the MYCN promoter, was confirmed both *in vitro* and *in vivo,* by EMSA and chromatin-immunoprecipitation (ChIP) assays.
qRT-PCR and Western blot analysis have shown that MBP-1 overexpression in LAN-5 cells determines a decrease of MYCN expression and a significant increase of p21 and BAX levels, involved in senescence and apoptosis respectively, and γ-enolase expression, a marker of neuronal differentiation.
Scratch wound healing and proliferation assays in LAN-5 cell line have indicated that MBP-1 expression also leads to a significant reduction of cell migration and proliferation. Furthermore, by cytotoxicity assay has been shown that MBP-1 expression induces a cytotoxic and pro-apoptotic effect in MYCN-amplified cells.
The analysis of expression profiles of mRNAs and microRNAs, using microarray technology, has confirmed the data obtained from the studies conducted in LAN-5 cells expressing MBP-1 and identified pathways involved in the regulation of apoptosis, in cell growth and in cell migration.

Conclusions: The results obtained indicate that MBP-1 acts as a transcriptional repressor of MYCN gene and its expression induces senescence, apoptosis and differentiation in LAN-5 Neuroblastoma cells.

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