Gimenez M, J Cancer Sci Ther, 3:7
doi: http://dx.doi.org/10.4172/1948-5956.S1.18

OMICS Group Conferences
Accelerating Scientific Discovery

International Conference & Exhibition on
Cancer Science & Therapy
15-17 August 2011 Las Vegas, USA

Identification of altered proteins by siRNA-mediated knockdown of nucleophosmin in glioblastoma cell line

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In previous study, we reported that the protein nucleophosmin (NPM) was increased in glioblastoma multiforme (GBM) by 2D-electrophoresis analysis of tumor patient samples, when compared with normal brain tissue. NPM is a nucleolar phosphoprotein related to apoptosis, ribosome biogenesis, mitosis and DNA repair, but details about its function remain unclear. We have been investigating possible targets that can be altered when NPM gene is silenced by siRNA. The NPM knockdown was performed transfecting cells derived from GBM (U87MG) with siRNA (2, 4 and 7 days) followed by confirmation of silencing with real-time PCR and western blot. Non-transfected cells and cells transfected with siRNA scramble were used as control. We obtained a reduction of 80% in the NPM expression by siRNA after the fourth day of transfection, which was maintained until the seventh day in U87MG cell line. Peptides tagged with iTRAQ were separated by 2D-HPLC and identified by ESI-Q-TOF-MS. CID-MS/MS spectra were processed by MassLynx 4.0 and submitted to MASCOT (score >35 and $p<0.05$). We were able to identify 74 proteins. Four proteins were increased in cells transfected with siRNA-NPM and 14 presented reduction of expression (2-fold alteration in siRNA-NPM in comparison with controls). Among them, GRP78 presented a reduction of expression in siRNA-NPM1 cells. GRP78 is highly expressed in GBM patient samples and its expression level was confirmed by western blot. GRP78 is involved in ER stress response, anti-apoptotic process and chemo-resistance in cancer. The results reported here indicate that NPM may be a candidate for therapeutic target.

Biography

Marcela Gimenez is a Ph.D student from Brazil. She completed her Master Degree in Molecular and Cellular Biology at University of Sao Paulo, where develops her doctoral studies that will be concluded next year. She has experience with Biochemistry and Molecular Biology especially in proteomics and gliomas.