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Identification of Trk-specific signaling events in neuroblastoma using stable isotope labeling and phosphoproteomics

Samuel L Volchenboum
University of Chicago, USA

Neuroblastoma is the most common solid tumor in children and accounts for 15% of pediatric deaths due to cancer. Despite aggressive multi-modal treatment, more than half of children with neuroblastoma will ultimately die from their disease. Clinical markers such as age, clinical stage, and amplification of MYCN oncogene are often associated with disease aggressiveness, however, the biological pathways responsible for disease heterogeneity are yet to be established. Possible clues may lie in understanding the behavior of two very similar Trk proteins, TrkA and TrkB. While high expression of TrkA is associated with favorable outcome, high expression of TrkB is conversely associated with unfavorable and high-risk NBL. Despite divergent clinical behaviors, gene expression analysis of the model SY5Y neuroblastoma cell line stably transfected with TrkA and TrkB revealed surprisingly similar global gene expression behavior. Additionally, on a proteome level, a study using 2D gel electrophoresis with MALDI MS (Sitek et al. 2005) noted only a few differential proteins (22 and 9) between activated TrkA and TrkB in SY5Y cell lines. We hypothesize that phosphoproteomic analysis will provide insight into signaling pathway variations that could explain the dynamic differences in clinical behavior between expression of Trk A and Trk B. Here, using TrkA/TrkB transfected cell lines activated through treatment with NGF/BDNF, respectively, and inhibited by lestaurtinib, we identify phosphorylated targets of TrkA and TrkB signaling. Untreated SY5Y-TrkB samples (i.e., SY5Y stably transfected with TrkB) were compared to BDNF-, NGF- and BDNF+Inh-treated sample using SILAC labeling followed by phosphopeptide enrichment and LC-MS/MS analysis. After protein identification through MaxQuant and statistical analysis, 122 phosphoproteins were found to be over-expressed in SY5Y-TrkB BDNF samples, a significantly higher number than previous studies. A similar analysis of SY5Y-TrkA samples (none-, BDNF-, NGF- and NGF+Inhibitor-treated) revealed 143 proteins to be over-expressed in SY5Y-TrkA NGF as compared to other samples. While the overlap of proteins was few (24), comparison of these significant proteins at pathway level revealed enrichment of AKT and cdc-42 signaling pathways in SY5Y-TrkA NGF. Further, comparison of biological networks built using Ingenuity Pathway Analysis detected the presence of RNA polymerase II in SY5Y-TrkB BDNF, thereby leading credence to the model first proposed in Kramps et al. 2004 that acetylation of histone H3 and H4 along with recruitment of Pol-II could be associated with MYCN amplification. These ongoing studies are providing insight into the biological pathways modulated as a result of Trk A and Trk B receptor expression and signaling in neuroblastoma and may lead to better diagnostic and targeted therapeutic modalities.

svolchen@peds.bsd.uchicago.edu