

Neural Stem Cells, TLX, and Neuroblastoma

Akademisk Avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska Akademin vid Göteborgs Universitet kommer att offentligen försvaras i hörsal Avrid Carlsson, Academicum, Medicinaregatan 3, Gothenburg.

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Avhandlingen baseras på följande arbeten:

- I. Chavali PL, **Saini RK**, Matsumoto Y, Ågren H, and Funa K (2011). Nuclear Orphan Receptor TLX Induces Oct-3/4 for the Survival and Maintenance of Adult Hippocampal Progenitors upon Hypoxia. *J Biol Chem*, 286: 9393–9404.
- II. Chavali PL, **Saini RK**, Zhai Q, Vizlin-Hodzic D, Venkatabalasubramanian S, Hayashi A, Johansson E, Zeng Z-J, Mohlin S, Pählman S, Hansford L, Kaplan D, and Funa K. TLX activates MMP-2, promotes self-renewal of tumor spheres in neuroblastoma, and correlates with poor patient survival. *Cell Death & Dis*, *In press*.
- III. **Saini RK**, Attarha S, da Silva Santos C, Kolakowska J, Funa K, and Souchelnytskyi S. Proteomics of dedifferentiation of SK-N-BE2 neuroblastoma cells. *Cancer Science*, *Under revision*.



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Abstract

Neuroblastoma (NB) is one of the most commonly diagnosed extracranial tumors in children below five years of age. Moreover, NB accounts for almost 12-15% of all childhood cancer related fatalities. To date, the origin of NB has been linked to the neural crest derived sympathoadrenal progenitor cells. Progression and pathogenicity of the disease has been largely correlated with the ages of children and presence of undifferentiated neuroblasts and amplification of the *MYCN* oncogene. However from the clinical evidence gathered so far, *MYCN* amplification is related to poor prognosis but it occurs only in 20% of NB cases. Henceforth, there is a need for better diagnostic and predictive markers to stratify NB patients.

NB cells express a number of neural stem cell and progenitor markers such as Oct3/4, Sox2, CD133, ABCG2, and Nestin. Since self-renewal and differentiation of neural stem cells are predominantly regulated by a number of stem cell fate determinants such as Notch, Wnt, Hedgehog, PTEN, and TLX, it is speculated that deregulation of these genes may be responsible for the pathogenicity of the disease. TLX is an orphan nuclear receptor, which is predominantly expressed in the embryonic and adult forebrain, and is considered to be a crucial regulator of neurogenesis, because of its roles in neural stem cell self-renewal and maintenance. We have identified that upon hypoxia, TLX stimulates neural stem cell renewal by promoting Oct3/4 transcription in adult hippocampal progenitors. TLX is expressed at high levels in NB cell lines. In sphere forming cells generated from these cell lines, TLX was enriched and co-expressed along with the neural progenitor markers Nestin, Oct3/4, CD133, and HIF-2 α . TLX was also co-expressed with the neural progenitor markers CD15 and MMP-2 in xenografts of primary NB-tumor initiating cells (TIC) derived from patients. Thus, TLX may be involved in the tumorigenesis of NB by promoting dedifferentiation of tumor cells.

NB develops through processes which may be defined as cellular “dedifferentiation”. The ability of tumors to form spheroids is one of the manifestations of dedifferentiation and transformation. To study the mechanisms of dedifferentiation, neuroblastoma cell lines will help us to identify new diagnostic markers. We generated spheroids of the NB cell line SK-N-BE2 and performed proteomics analysis to evaluate the differential expression pattern as compared with the wild-type cells. We identified 239 proteins which were affected by the dedifferentiation process. These proteins represented several regulatory processes, such as transcription, cell cycle regulation, apoptosis, cell adhesion, metabolism, intracellular transport, stress response, and angiogenesis. An extensive analysis using Cytoscape identified “DISC-1” and “DNA-PKcs”, both of which have been previously linked to dedifferentiation and cancer. The results contribute to better understanding of mechanisms involved in Neuroblastoma pathogenesis, along with identifying possible biomarkers for the disease that may be translated to the clinic.

Keywords: Neuroblastoma, TLX, neural stem cells, dedifferentiation, DISC-1, DNA-PKcs.

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