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High-throughput mRNA sequencing in Neural cerebellar Stem Cells

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Scope of the study, results and discussion

Brief abstract

Neural cerebellar Stem Cell (NcSC) maintenance is of great interest since NcSCs can be used to treat impaired cells and tissues or improve regenerative power of degenerating cells in neurodegenerative diseases or spinal cord injuries. Under maintenance conditions, NcSCs express a number of stemness genes (e.g. Nanog, Oct4, Sox2) whose mechanisms of regulation have been investigated. However the interplay between other transcription factors and NcSC maintenance is still being charted.

Next Generation RNA sequencing (RNA-seq) is a powerful method for quantifying steady-state mRNA expression levels and detecting alternative splicing events in transcriptomes. After a typical RNA-seq experiment bioinformatics analysis is required that consists of Mapping, Assembly, Quantification of known and novel isoforms and Differential Expression analysis, in case of comparison between two groups of samples.

In this current study we investigated the role of transcription factors in NcSC isolated from murine WT postnatal cerebellum (P4), a model established in our laboratory, in comparison to their differentiated counterparts by using RNA-seq.

Since different bioinformatics tools exist we investigated several pipeline combinations by using two different mapping tools, Genomatix Mining Station and TopHat/Cufflinks, which allowed us to identify known and novel isoforms. Moreover, we used four different methods of differential expression analysis, DESeq, edgeR, Cuffdiff and Cuffdiff with trimmed reads, which allowed us to identify transcripts characterizing NcSC.

Functional analysis of differentially expressed transcripts using the DAVID database, highlighted genes involved in a number of pathways such as focal adhesion, extracellular matrix (ECM)-receptor interaction, cell cycle, DNA replication, p53, as well as cell stress response. Taking into account that the Hedgehog pathway characterizes our NcSC model we investigated transcripts implicated in the pathway. The highest expressed transcript was Forkhead Box m1 (Foxm1), part of the FOX superfamily of transcriptional regulators that play a pivotal role in cell cycle progression.

Preliminary validation experiments in our lab confirmed the high expression of Foxm1 in mRNA and protein level in NcSC. Additionally, silencing of Foxm1 in NcSC impaired the ability of NcSCs to form clones.

In this study we have provided a high-resolution analysis of NcSC transcriptome and identified a Hedgehog-related transcription factor implicated in NcSC maintenance.