M10. ISOFORM DEPENDENT FUNCTIONS OF AUTS2 DURING NEURONAL DIFFERENTIATION

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Background Disruptive mutations in the autism susceptibility candidate 2 (AUTS2) gene have been linked to a number of neurodevelopmental disorders. The individuals carrying AUTS2 mutations display similar phenotypes including intellectual disability, developmental delay and a number of dysmorphic features, including microcephaly. AUTS2 has two known isoforms, one with 19 exons and a shorter isoform that include only the last 11 exons. The long isoform was shown to be in a complex with polycomb proteins (PCGF3 and PCGF5) that activate transcription. AUTS2 is mainly expressed in the brain during development at a number of regions, including the developing cortex and cerebellum.

Methods We used two mouse cellular models to study Auts2 during neuronal differentiation: Neuro2a (N2A) and mouse embryonic stem cells (mESC). To model the human AUTS2 mutations, we used mESC line heterozygous for a gene-trap that disrupts the two isoforms of the Auts2 gene (Auts2+/-) and compared to the parental line (wild type, WT). The N2A cells were differentiated to neurons with retinoic acid. The mESC were differentiated to cortical progenitors and neurons. Auts2 expression was measured by real-time PCR, Western blots and immunofluorescence staining. Expression arrays were used to profile the global changes in gene expression in the Auts2+/- and WT cell lines in three stages of differentiation. The influence of the two AUTS2 isoforms on transcription was tested in a luciferase reporter assay with HEK293 and N2A cells. In order to identify proteins that physically interact with AUTS2, we conducted a yeast two-hybrid (YTH) screen with the two AUTS2 isoforms, validated using the Duolink in situ proximity ligation assay (PLA) in N2A cells.

Results Here, we report that the short isoform is transiently expressed during neuronal differentiation. When neuronal differentiation is initiated, there is a shift in expression from the long isoform to mutual expression of the long and short Auts2 isoform. The long isoform becomes again the dominant isoform in adult human and mouse brain. During in vitro corticogenesis, Auts2+/- cells had premature neuronal differentiation leading to increased cell death accompanied by upregulation of mesodermal genes. Reporter assays showed that AUTS2 serves as both an activator and a repressor of transcription, depending on cell type. In HEK293 cells, increase in luciferase activity was observed for both AUTS2 isoform, although the short isoform gave rise to a stronger activity. In contrast, in N2A cells the luciferase activity was decreased significantly. The polycomb group proteins, PCGF3 and PCGF5, were found to interact exclusively with the long AUTS2 isoform, whereas a splicing factor, SF3B1, with both isoforms.

Discussion Taken together, our data suggest that an isoform switch of AUTS2 mediates the regulation of genes involved in brain development and differentiation timing through selective interactions with PCGF3 and SF3B1. Thus, the disruption of AUTS2 may affect different processes including the regulation of cell-type specification as well as the balance between differentiation and proliferation.

Disclosure: Nothing to Disclose.