doi 10.18699/bgrs2024-9.1-19

Knockout or deletion of the *UBE2A* gene leads to disruption of the Rho/ROCK signaling pathway and impaired migration of neural cells differentiated from iPSCs

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Key words: UBE2A gene; X-linked Intellectual Disability type Nascimento; Rho/ROCK signalling pathway; Transcriptomics

Motivation and Aim: The X-linked intellectual disability Nascimento syndrome was first described by Rafaella Nascimento in 2006 [1]. It is characterized by delayed intellectual and cognitive development, speech impairment, and morphological abnormalities. The syndrome is associated with loss-of-function mutations in the *UBE2A* gene encoding ubiquitin-conjugating enzyme E2A, which participates in post-replicative DNA repair, regulation of gene transcription, and mitophagy. Additionally, duplication of the *UBE2A* gene has detrimental effects on mental development [2]. However, the precise role of the *UBE2A* gene and its dosage in neurogenesis remains to be elucidated.

Methods: To identify the cellular processes affected by the *UBE2A* gene dosage abnormalities in neural cells, we provided the transcriptomic analyses of neuronal precursor cells (NPCs) derived from human induced pluripotent stem cells (iPSCs). Our cell lines included iPSCs with knockout and overexpression of the *UBE2A* gene, and iPSCs of a patient with Nascimento syndrome caused by the deletion of the *UBE2A* gene [3]. We performed differential expression analysis and enrichment pathways analysis in the statistical environment R using the DESeq2 and clusterProfiler packages, respectively. The clusters of differentially expressed genes (DEGs) with shared expression profiles were identified using the 'DEGreport' package. We validated our transcriptomic results through qRT-PCR and proteomic analysis.

Results: We revealed more than 1500 up-regulated and 2000 down-regulated DEGs across the samples. These DEGs were clustered into 8 groups. The functional analysis of DEGs in NPCs derived from the patient's iPSCs revealed the abnormal expression of genes associated with cytoskeleton regulation, axon and dendrite development, and synapse regulation. Similar gene expression changes were observed in NPCs with the *UBE2A* knockout and, surprisingly, with the *UBE2A* gene overexpression. Among the common down-regulated DEGs, we identified 29 genes coding for plexins, ephrins, semaphorins, p21-activated kinases, and proteins of the LIM family. These genes are known as up-stream activators or down-stream effectors of the Rho/ROCK signaling pathway, which plays a critical role in neurodevelopment, including cell migration. To

analyze the NPC migration, we assessed the area of NPC migration from neurospheres using time-lapse microscopy. NPCs with the *UBE2A* knockout and overexpression exhibited a decreased migration area compared to normal NPCs. We hypothesize that impaired neuronal migration may contribute to the pathogenesis of Nascimento syndrome.

Conclusion: The Rho/ROCK signaling pathway demonstrated sensitivity to the *UBE2A* gene dosage abnormalities in NPCs differentiated from iPSCs. Specifically, knockout, deletion, or overexpression of the *UBE2A* gene led to a similar decrease in expression of Rho/ROCK signaling pathway genes in NPCs, resulting in impaired NPC migration.

Funding: The study is supported by RSF grant # 21-65-00017.

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