DOI 10.18699/SBB-2022-245

# Investigation of the mechanisms of neurodevelopmental disorders caused by mutations in the CNTN6 gene on the cerebral organoids model

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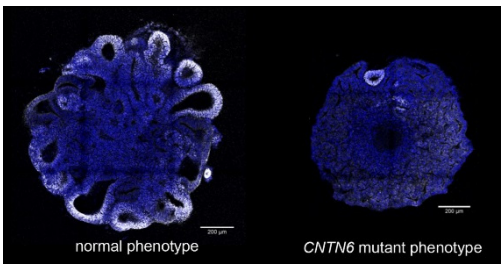
**Key words:** cerebral organoids, CNTN6, iPSC, neural differentiation, neurogenesis, mental retardation, neurodevelopmental disorders, NOTCH pathway

Hereditary intellectual disability occurs in 2-3 % of newborns. Up to 25 % of these neurodevelopmental disorders are associated with gene copy number variation. Several dozen patients have been described with CNVs that affect the *CNTN6* gene [4, 5]. This gene encodes the protein Contactin6, which is involved in the control of dendritogenesis and the synaptogenesis during the development of the mice nervous system [9]. Our attention in the last years is attracted by the patients – heterozygous carriers of the *CNTN6* gene deletion, who suffered from the severe neuropsychiatric symptoms and craniofacial dysmorphism. To study the effects of the *CNTN6* mutation, we obtained induced pluripotent stem cells (iPSCs) from skin fibroblasts of healthy donors and patients with *CNTN6* deletion. Additionally we obtained knockout lines based on iPSCs by applying the CRISPR/Cas9 system.

To simulate the early events of human neurogenesis, such as development of neuroepithelial structures and later ones (migration of neurons and the cortical plate formation), we used a unique 3D cellular model – human cerebral organoids (CO) [7]. This model system is currently used in the study of a wide range of diseases associated with brain development [3, 8].

For the first time, we have described a disturbance of neurogenesis in the *CNTN6* deletion patiences that leads to malformations during cortical development. The main features of the *CNTN6* mutant phenotype are the disturbance of the neuroepithelial structures formation and alteration of the radial glia proliferation (Fig.). In particular, knockout of the *CNTN6* gene leads to a disruption in the radial glial cells division and their transition from symmetrical proliferative to asymmetric neurogenic. The overexpression of *CNTN6* leads to the restoration of the mutant phenotype. In particular, we have shown for the first time that *CNTN6* is expressed not only in the postmitotic deep layer neurons, but also in the radial glial cells. It turned out that the level of *CNTN6* expression in neural stem cells (NSC) is higher than in terminally



differentiated neurons, which may indicate an important role of the *CNTN6* gene in the maintenance of NSC, in particular, in the regulation of their proliferation.

Based on the transcriptome analysis of COs obtained from normal and mutant iPSCs, we identified four signaling pathways that may involve the Contactin 6 protein: NOTCH, WNT, TGF-β, and BMP. In *CNTN6* knockout COs, the WNT, TGF-β, and BMP signaling pathways were activated, while the NOTCH signaling pathway was downregulated.

In addition, we discovered, for the first time, that the process of PAX6 cytoplasm-to-nucleus translocation is disrupted during iPSC to NSC differentiation of the cells with *CNTN6* homozygous mutation. This violation may be the key molecular event leading to the mutant phenotype appearance. According to the literature data, it is known that the TGF-β signaling pathway is involved in the process of the PAX6 translocation from the cytoplasm to the nucleus, in particular, through the direct binding of the SMAD3 protein to PAX6 [10]. On the other hand, the example of the TGF-β and NOTCH signaling pathways cross-regulation through the interaction of the SMAD3 protein and the intracellular domain of the NOTCH1 receptor was described in the literature [1]. Recent publications point to the extremely important role of the NOTCH signaling pathway in the evolution of the human brain. In particular, new non-canonical ligands have been described that additionally activate this signaling pathway, thus affecting the human radial glial cells proliferation [3, 6]. Interestingly, the Contactin 6 is also a non-canonical ligand of the NOTCH signaling pathway, but this interaction has so far been described only for mouse oligodendrocytes [2].

Thus, our results in the context of the literature data mentioned above indicate a completely new previously undescribed function of the *CNTN6* in early human neurogenesis.

*Acknowledgements*: This study was supported by the Russian Foundation for Basic Research (grant No. 19-29-04067). We also thank the Multiple-access Center for Microscopy of Biological Subjects (Institute of Cytology and Genetics, Novosibirsk, Russia), state project No. FWNR-2022-0019 for granting access to microscopic equipment.

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