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Functional characteristics of previously unstudied genes associated with congenital malformations of the nervous system

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Motivation and Aim: Hereditary diseases, including those associated with neuro-developmental disorders, are one of the key problems for the health care system.
For a large number of patients with undifferentiated forms of mental retardation, intellectual development delay, and autism, the molecular mechanisms underlying neurodevelopmental disorders are still unknown. Associated pathologies include developmental delay, spasticity, microcephaly, and primary epilepsy. Understanding the function of previously unstudied genes is essential for diagnosis, prognosis of the disease, and the quality of medical genetic counseling. In this project, we use modern technologies to create in vivo models and to obtain new data on molecular mechanisms leading to disorders of nervous system development.
Methods and Algorithms: To visualize the expression pattern at embryonic (E14.5, E16.5, E18.5) stages of development, the molecular biological method of RNA in situ hybridization (ISH) was used. RNA probes are pre-synthesized with cDNA of the target gene using gene-specific primers. Subsequently, the target mRNA of the gene is hybridized with RNA probes labeled with DIG-dUTP (digoxigenin). If the target mRNA is present in the sample, then the complementary nucleotide sequences of the sample and the mRNA form stable double-stranded bonds, which subsequently makes it possible to identify the location of tissue areas where the target gene is expressed.
To down-regulate the expression of studied genes specifically in cortical progenitors in vivo, we performe injections of plasmid constructs expressing shRNA targeting gene mRNA into the lateral ventricles of E13.5 mouse embryos followed by in utero electroporation for transfection. Concurrently, we co-electroporate these cells with a plasmid containing green fluorescent protein (GFP) cDNA to facilitate cell tracking. We then prepare histological slides of the electroporated cortex by isolating and fixing embryonic brain samples at days 15.5 and 17.5 of gestation and perform immunohistochemical analysis of control and KO tissues.
To analyze mitosis exit, we inject BrdU, a thymidine nucleoside analog, during IUE. BrdU is incorporated into the DNA of dividing cells at S phase of the mitotic cycle. Twenty-four hours post-electroporation, pregnant mice undergoes injection with a single dose of BrdU. Cortices are collected 2 days after IUE, fixed, and immunolabeled against EGFP, ki67, and BrdU. Quantification of both EGFP and BrdU-positive neurons relative to the total number of EGFP+ cells reveals how many neurons were born from electroporated APs and indicates whether the target gene regulates AP exit from the cell cycle. Quantification of the fraction of ki67-positive cells (a marker of undergoing

mitosis) should reveal any differences in the ability of progenitors to divide between control and KO tissues.
To study the effect of KO on cell death, we assess the expression of cleaved caspase-3: quantitative analysis of EGFP+ and caspase-3+ cells in the EGFP+ cell population reveals whether loss of a gene causes neuronal death during neurogenesis.
To study the effect of KO on migration, the distribution of migrating neurons in the layers of the developing cortex was assessed. For this purpose, sections of electroporated cortex collected on days 15.5 and 17.5 of gestation were used, as described above. Differences in the distribution pattern of GFP+ cells indicate whether KO affects the speed and direction of neuronal migration. Studying the morphology of GFP+ cells and assessing the ratio of polarized and multipolar cells show whether KO affects polarization processes in developing neurons
Results: We examined the expression pattern of two candidate genes in mouse brain sections using in situ hybridization (ISH). For one of them, ubiquitous expression was found at all developmental stages studied. For the second gene expression was found in the ventricular and subventricular zones, and weaker expression was detected in the cortical plate and in the intermedial zone.
In control electroporation experiments, approximately 20 % of all GFP-positive cells were found to be double-positive for ki67, indicating that a subset of neocortical cells incorporating the targeting constructs remained actively cycling 48 hours post-electroporation [1]. However, with shRNA electroporation, the proportion of cells persisting in the mitotic cycle doubled compared to control conditions. These observations suggest that studied gene plays a crucial role in regulating the cell cycle progression of forebrain neuronal stem cells. Downregulation of its expression results in prolonged cell cycle duration and reduced initiation of neuronal differentiation, potentially leading to a decreased number of neurons generated in the neocortex.
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References

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