МЕДИЦИНСКИ УНИВЕРСИТЕТ - ВАРНА
„Проф. д-р Параскев Стоянов“
Ул.“Марин Дринов“ 55, Варна 9002, България
Тел. : 052/ 65 00 57, Факс: 052/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

MEDICAL UNIVERSITY - VARNA
“Prof. Dr. Paraskev Stoyanov”
55, Marin Drinov Str., 9002 Varna, Bulgaria
Tel.: +359 52/ 65 00 57, Fax: + 359 52/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

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“Role of transcription factor Pax6 in the development of mouse cerebellar cortex”

Project leader: Prof. Anton Bojidarov Tonchev, MD, PhD, DSc

In the cerebellum, there are two main populations of neurons: Gamma-aminobutyric acid-ergic (GABA-ergic) – inhibitory and glutamatergic – excitatory cells. The first group of neurons arises from the embryonic ventricular zone (VZ) of the fourth ventricle and expresses the transcription factor Ptf1a. The other type of cells generates in the embryonic zone “rhombic lip” (RL) and expresses the transcription factor Pax6.

The aim of the present study is to investigate the function of Pax6 during the development of the cerebellar cortex, in particular the glutamatergic neurons in the cortex. For this purpose, cerebellar tissues of a genetically modified (transgenic) mouse are used. The results published so far are on mice with general (global) inactivation (knock-out, KO) of the Pax6 gene. These mice die at birth, which hinders the study of neurogenesis in the cerebellar cortex. In mice, neurogenesis occurs also after birth and ends by postnatal day 21 (P21). To address this perinatal mortality, the project team uses an innovative approach that includes transgenic animals with selective inactivation of Pax6 in the cerebellar cortex (Pax6 conditioned KO, Pax6cKO). These animals survive to adulthood, and at a selected time, they show a reduction in Pax6 levels in the cerebellar cortex.

Comparing mutants with healthy (control) animals of different stages will allow to be analyzed: 1) defects in the morphogenesis (foliation) of the cerebral cortex; 2) the effects of Pax6cKO on the amount of glutamatergic neurons in the cortex. These results will show for the first time the function of Pax6 for the development of the cerebellum in mammals, postnatally in vivo.

This project will show for the first time in the world the effect of loss of function of the Pax6 gene on different populations of cells in the developing postnatal cerebellum.

More specifically, a decreased expression of markers that mark glutamatergic neurons in cerebellar nuclei is expected, unipolar brush cells, and granular cells in mutants with Pax6 inactivation.

In addition, the project team’s hypothesis is that mutants with Pax6cKO will have a reduced surface area and altered shape of the cerebellar cortex foils. An altered expression of markers that mark GABAergic neurons in the cerebellum is also expected.

For the first time, we demonstrate a mouse model ($Pax6^{flox/flox}$) with selectively reduced expression of the transcription factor Pax6, in which mice can be studied postnatally (in the case of global knockout of the gene, mice die at birth). Our results show abnormal foliation of the cerebellar cortex and the presence of ectopic foci of a heterogenic population

of GABA- and glutamate-ergic neurons in the mutants, and demonstrate the essential role of Pax6 for the correct foliation of the cerebellar cortex.

In the current project we also provided evidence that heterogeneity also exists in basic histomorphological parameters of the medial (vermis) and lateral (cerebellar hemispheres) domains of the murine cerebellar cortex. We found that the cerebellar cortex of normal adult mice has on average a 42% lower surface area in the hemispheres as compared to the vermis. That surface area is unevenly distributed across the folia. In the vermis, 4 out of 10 folia compose about 55% of the total cortical surface, while in the hemispheres 3 out of 6 folia account to about 62% of the total surface. Unexpectedly, we found that the granule and molecular layers are with a lower surface area, but with a greater thickness in the hemispheres as compared to the vermis. The Purkinje cell layer had a strikingly lower (46% on average) length in the hemispheres as well compared to the length in the vermis. These results emphasize the caution needed when interpreting histomorphological changes reported in different disease models based on the mouse cerebellum.