

REVIEW

by Prof. Stefan Todorov Sivkov, MD, PhD

Department of Anatomy, Histology, Cytology and Biology

Medical University-Pleven

Subject: Dissertation for awarding PhD degree in the field of higher education 7. Health and Sports, professional field 7.1. Medicine, scientific specialty: Anatomy, Histology, and Cytology

Author: Dr. Iskren Boyanov Velikov

Topic: "ROLE OF TRANSCRIPTIONAL FACTOR ZBTB20 IN THE DEVELOPMENT OF INTERNEURONS IN THE MOUSE CEREBRAL CORTEX"

Scientific supervisor: Assoc. Prof. Irina Stoyanova-Van Der Laan, MD, PhD (Department of Anatomy and Cell Biology, Medical University-Varna)

1. **General presentation** of the procedure and the doctoral student. By order No. P-109-222/28.04.2025 of the Rector of the Medical University - Varna, I am appointed as a member of the Scientific Jury in connection with the procedure for the defense of the dissertation on the topic "Role of the transcription factor Zbtb20 in the development of the cerebellum" for the acquisition of the ONS "Doctor" in the field of higher education 7. Health and Sports, professional field 7.1. Medicine, scientific specialty Anatomy, Histology and Cytology. The author of the dissertation is Dr. Iskren Boyanov Velikov - a doctoral student in an independent form of study at the Department of "Anatomy and Medical Biology" of the Medical University - Varna with scientific supervisor Assoc. Prof. Dr. Irina Stoyanova-Van Der Laan, MD (Department of Anatomy and Cell Biology, Medical University - Varna). To prepare the review, I received the materials required by the Regulations on the conditions and procedure for acquiring scientific degrees and holding academic positions at the Medical University-Varna.

The set presented by the author Iskren Boyanov Velikov includes the following documents:

1. Application form to the rector for opening a procedure;
2. Curriculum vitae in European format;
3. Protocol of the preliminary discussion in the department;
4. Abstract;
5. Declaration of originality and authenticity of the attached documents;
6. Certificate of compliance with the minimum national requirements;
7. List of publications;
8. Dissertation;
9. Copies of publications on the topic of the dissertation;

The submitted documents meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its implementation and the Regulations of MU-Varna for admission to the defense of the ONS "Doctor".

Doctoral student Iskren Velikov was born in the town of Razgrad, where he completed his secondary education. In 2007, he graduated from the Medical University of Plovdiv, Faculty of Dental Medicine with the acquired Master's degree and professional qualification "Doctor of Dental Medicine".

He worked as a dentist in the Rural Health Department in Razgrad Region (2007-2008). From 2008 to 2009, he worked as an assistant professor at the Department of Anatomy, Histology and Embryology of the Medical University of Plovdiv. From 2009 to the present, he has been a full-time assistant professor at the Department of Anatomy and Cell Biology, Medical University of Varna. In 2013, he acquired a specialty in Anatomy, Histology and Cytology.

By order No. R-109-23 of 01.02.2019 was enrolled as a doctoral student at the Department of Anatomy and Cell Biology, Medical University-Varna. He was dismissed from doctoral studies with the right to defend by order P-109-44/14.02.2024 after successfully passing the internal defense.

2. Relevance of the topic. Neurogenesis is of interest for study in connection with the morphological changes that occur as a result of active modification of its composition. Modern molecular, genetic, physiological and anatomical methods are constantly developing. However, many processes related to neurogenesis, neuronal migration and the formation of synapses in the developing brain remain unclear. The dissertation is dedicated to studying the role of transcription factors and in particular Zbtb20 in the development of the cerebellum.

3. Knowledge of the problem. The introduction, as well as the creative use of the cited literature material, demonstrate knowledge of the state of the problem treated in the dissertation by the doctoral student. The doctoral student presents the processes of prenatal development of the cerebellum with sufficient eruditeness, with the role and fate of neuronal stem cells in neurogenesis. Spatial and temporal differences in the formation of cortical neurons are taken into account. The molecular control of the formation of the cerebellar cortex is discussed. The role of transcription factors in the development of the cerebellum is presented.

4. Characteristics and evaluation of the dissertation work and contributions. The dissertation work is structured according to generally accepted requirements - introduction, literature review, aim and objectives, material and methods, results, discussion, conclusions, literature sources. It is presented on 146 standard pages and is illustrated with 97 figures. The literature used includes 264 sources in Latin.

The Literature Review examines the features and notes the stages in embryonic development of the cerebellum in mice. Attention is paid to the morphology of neuronal and glial cells, noting the location, morphology and relationship between the different progenitors. The origin of cortical interneurons in the mature cerebellar cortex is also presented, and the spatial and temporal differences in the generation of cortical interneurons are indicated in parallel.

The migration of neurons and the formation of neuronal circuits during embryonic development of the CNS are reviewed. Newly generated neurons migrate from their germinal zone to the cerebellar cortex under the influence of controlled processes. Special attention is paid to the peculiarities of the migration of cortical interneurons. The role of the transcription factor Zbtb in a number of processes, including neurogenesis, glucose metabolism and postnatal growth, is

examined in detail. The relationship between expression and the observed phenotype in defects in Zbtb20 and certain human diseases is discussed. The data discussed in the literature review allow the doctoral student to deduce the goal of the scientific research and outline the tasks for its implementation.

Aim. The present study aims to establish the role of Zbtb20 in the development of neuronal and glial (oligodendrocyte) cell populations, as well as in the process of cerebellar foliation.

The **study material** is sufficient in volume, properly selected and structured, well documented and precisely registered, which guarantees the reliability of the results. The influence of Zbtb20 on the normal development of cortical interneurons was studied using an experimental model of a “knock-out” mouse, which lacks the functionally important BTB/POZ domain of the protein, as well as the first of the five zinc rings, replaced by a lacZ-neomycin cassette. Wild-type mice on the fourth, eighth and twelfth postnatal day were used as controls. Wild-type mice on the sixteenth prenatal and thirtieth postnatal day were also used. All experimental animals were treated according to the “German Animal Protection Act”, after receiving approval from the ethics committee.

Research methodology. The methodology is distinguished by a detailed and competent presentation of the research process. The doctoral student has used reliable and sufficient research methods to achieve the set goal, with which he receives an adequate answer to the tasks solved in the dissertation work. The obtained and prepared preparations were examined using immunofluorescence methods. Micrographs were performed with a Zeiss fluorescence microscope. Image J (Fiji) software product was used to measure areas and count cells.

The **statistical methods** were appropriately selected with the informativeness necessary for the study. Statistical processing of the data was performed with Microsoft Excel, using descriptive statistics and parametric (Student's t-test, $p < 0.05$) analysis.

Results. The obtained data are demonstrated and analyzed in the presented documentation (figures, graphs and tables). The results are presented very informatively, allowing for the presentation of the role, significance and interrelationships between the studied indicators. The photographic material is presented in a modern design and sufficiently illustrative, clearly visualizing the theses of the dissertation work.

At postnatal days 4, 8, 12 and 30, Zbtb20 is expressed by neuroglial cells, while neurons do not express Zbtb20. The absence of Zbtb20 selectively affects the proliferation of Purkinje cells in both time and space. Initially, an increased total number of cells was observed, particularly in certain folia (3, 8, 9, and 10). Towards the end of the study period (day 12) this effect subsided and in certain folias (10) the number of Purkinje cells was significantly lower than in controls. The absence of Zbtb20 did not change the total number of granule cells (Pax6+), but led to their significant reduction in folias 6 and 10 at P12. Zbtb20 probably suppresses the development of the external granular layer selectively in certain areas of the cortex

Zbtb20 does not affect the proliferation of astrocytes, but selectively suppresses the proliferation of oligodendrocytes in the postnatal period of cerebellar development

The transcription factor Zbtb20 affects the development of the cerebellar foliations. In the absence of Zbtb20, additional folds are observed in some of the cerebellar foliations at the age of 4, 8 and 12 days.

The **discussion** is characterized by completeness and thoroughness in the reasoning on the results of the study in the context of the purpose of the study and the literature data. The conclusions drawn are logical and correspond to the tasks set and the studies conducted.

Contributions. The author contributed to the creation of an original experimental setup and methodology on the cerebellum of Zbtb20 ^{-/-} mice on the fourth, eighth and twelfth postnatal day. Through it, the doctoral student for the first time performed a quantitative analysis of neuronal and glial cells.

For the first time, an analysis was made of the distribution of granular neurons in the cerebellum of Zbtb20 ^{-/-} mice.

For the first time, an analysis of glial cells (astrocytes and oligodendrocytes) was made on the cerebellum of Zbtb20 ^{-/-} mice.

For the first time, an analysis of proliferating cells was made on the cerebellum of Zbtb20 ^{-/-} mice.

For the first time, the area of the cerebellum, as well as the individual cortical layers, was measured. The measurements were made on histological preparations of Zbtb20^{-/-} mice.

Evaluation of the publications and personal contribution of the doctoral student. The main results of the dissertation were published in Trakia Journal of Sciences, 2023, in which the doctoral student is the sole author. The results of the research were also presented in six communications at scientific forums in the country. They are co-authored, and in all of them the doctoral student is the lead author, which gives me reason to believe that he has a major contribution to the results.

The **abstract** is structured correctly, reflecting the content, main results and contributions of the dissertation work.

My **recommendations** for future use of the dissertation contributions and results are for their application in the further study and expansion of this issue, in which the obtained results should also be published in independent scientific articles.

Conclusion. The dissertation work of Dr. Iskren Velikov examines a problem that is of scientific and practical interest in the field of neurogenesis. An original experimental setup and methodology were applied to establish the role of Zbtb20 through an experimental model of a "knock-out" mouse. Scientific results were obtained that enrich the knowledge on the development of cerebellar cell populations, as well as in the process of cerebellar foliation. This is a real scientific contribution. The presentation and layout of the dissertation work and the abstract is very good. A large-scale and difficult-to-perform work has been carried out. This shows that the doctoral student possesses theoretical knowledge and professional skills in the scientific specialty, as well as skills for independent scientific research. The materials and documents submitted under the procedure fully comply with the requirements of the Act on the Development of Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of this Act, the Regulations for the Acquisition of the ONS "Doctor" at the Medical University of Varna.

Based on the above, I present a positive assessment of the conducted scientific research in the dissertation work, and I propose to the esteemed scientific jury to award Dr. Iskren Velikov the educational and scientific degree "Doctor" in the field of higher education 7. Health and Sports, professional field 7.1 Medicine, scientific specialty Anatomy, Histology and Cytology.

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