Ústav
experimentální
medicíny AV ČR, v.v.i.

Review of the thesis "***Neural signalling pathways regulation at protein level***"
authored by ***Mgr. Jakub Červenka***

I thoroughly enjoyed reading the PhD thesis of Mgr. Jakub Červenka Despite the complexity of the topic, the author successfully captured my interest, compelling me to read to the very end to follow the intricate details of neural stem cell differentiation. The thesis strikes a commendable balance of information necessary for understanding the research, and I rarely found myself needing to consult additional sources. I particularly appreciated the clarity of the explanatory figures and did not encounter any significant formal errors in the thesis.

A substantial portion of the thesis is dedicated to the development of a unique modification of proteomic methods, which represents a valuable integration of whole-protein analysis with high coverage and targeted analysis of preselected proteins. This focus is understandable, as this methodological innovation appears to have been a major breakthrough within the group, likely requiring extensive optimization over several months. From a technical standpoint, I highly appreciate this elegant method, which allows for deep and informative monitoring of the differentiation process.

The subsequent section of the thesis is devoted to a detailed characterization of the neural stem cell differentiation process. Here, the author employs a broad spectrum of complementary methods to examine changes occurring not only at the protein level but also at the gene level. The discussion in this part of the work reflects the author's deep understanding, not only of the methodological aspects of the experiments but also of the molecular mechanisms underlying the differentiation process. A significant portion of the thesis also addresses the role of various forms of the VEGF molecule in neural stem cell differentiation, as well as the cells' secretome and key signaling pathways involved in this process. Collectively, this research provides a comprehensive view of the differentiation process.

The final section of the work delves into the modern understanding of Huntington's disease (HD), covering its molecular mechanisms, epidemiology, and diagnostics. In this part, the author has conducted extensive literature reviews and analysis of clinical trial data. Although no experimental work was presented in this section, including such research could have further enriched the thesis.

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The methodological advancements and experimental results from this work have been validated in two research articles and two book chapters, where the author shares detailed descriptions of the newly developed proteomic methods with the scientific community.

While reading the thesis, a few questions arose:

1. In the differentiation experiment, the author describes an increased level of cleaved CASP3 expression in the early phase of differentiation. How might you explain this alteration? What mechanisms in neural tissue development could this be associated with?

2. Are you aware of studies comparing the differentiation of normal NSCs with those modified by the HD gene? What are the key differences between them?

3. The author mentions in the discussion the lack of clinically validated biomarkers for presymptomatic HD diagnostics. The thesis also references the availability of a pig model for HD at the institution where the research was conducted. In this context, I would like to ask if you plan to, or have already begun, studies aimed at detecting such biomarkers in peripheral blood or cerebrospinal fluid of model animals.

In conclusion, I can affirm that the presented dissertation meets all the requirements set forth by the Department Board of Development and Cell Biology. Therefore, I fully recommend it for defense.

In Prague, 29.08.2024

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