## REVIEW REPORT FOR THE DISSERTATION OF PAVEL HORÁK

**REVIEWER: KATEŘINA ROHLENOVÁ**

The candidate, Mgr. Pavel Horák, submitted his thesis entitled "Regulation of expression of cancer stem cells (CSC) by epigenetic mechanisms and expression/activity of transcription factors GLI, and targeted experimental intervention against CSC tumor subpopulation as an effective anticancer therapy in selected tumor types". The thesis features (i) an introduction and literature review of the problematics of cancer stem cells, GLI transcription factors and therapeutic approaches targeting cancer stem cells; (ii) aims of the thesis; (iii) methods; (iv) a set of published results addressing the function of GLI transcription factors in cancer in the form of individual papers included in the thesis, with an extensive discussion, and (iv) conclusions.

The thesis is mostly clearly written, and formally well structured. The introduction and literature review are based on extensive review of published literature, although a part of the cited studies is not recent. This might be related to the specifics of the problematics the thesis is focused on. Besides the published papers, one scheme is included in the thesis. The aims are clearly stated. The experimental part addresses the research areas that are listed in the Aims, with one of the most important advancements being the identification of transcription factor Slug as a direct target of Hedgehog signaling pathway. An extensive discussion of the obtained results is included.

**EVALUATION:** Based on my reading of the dissertation and considering its quality as well as quality of the experimental work, I recommend the dissertation of Pavel Horák for the defense and for the award of the Ph.D. title.

Below you can find a list of my comments and questions related to the candidate's work.

1. The author lists the Aims of his research, but there is no Hypothesis directly specified in the thesis manuscript. Can you please formulate it for the public defense?
2. Considerable part of the thesis manuscript is dedicated to the discussion of the cancer stem cell problematics, yet the experimental part focuses mostly on (melanoma) cancer cells, without specifically touching cancer stem cells experimentally. Could the applicant summarize what are the most important results he obtained in the area of cancer stem cells? How exactly the other results relate to cancer stem cells? Could you suggest further, more direct experiments to study the role of Hedgehog signaling in cancer stem cells?

3. Cancer stem cells are expected to be a rare population, which makes it difficult to detect and study them. However, with the availability of single cell resolved technologies, such as single cell transcriptomics, it is feasible to detect even a very rare cell population. Can the candidate point to publications that documented the existence of cancer stem cells, either by single cell RNA sequencing, or e.g. using lineage tracing studies? Why have these methods not yet provided a convincing end to the ongoing debate about the validity/existence of cancer stem cell population?
4. The section "B" of the thesis manuscript presents Hedgehog signaling pathway but does not include any figure/scheme. To better link it to the cancer stem cell problematics, could the author prepare such a scheme for the defense and use it to briefly discuss the most important links between the Hedgehog pathway and cancer stem cells, summarizing how the pathway can be employed to target the cancer stem cell population?
5. Can you explain the criteria, based on which were the 56 cell lines used in the publication no.2 selected? Have you considered mining data available from some of the publicly available databases to support your experimental data? If not, which databases would you suggest selecting? Which new insights could such an analysis provide?
6. In the discussion, the candidate makes a parallel between cancer stem cells and iPSCs, claiming on page 156 that "iPSCs…, are notoriously difficult to detect and isolate". Could you please clarify what was meant by this? Are you suggesting that iPSCs are found naturally, i.e., without the need to be induced in vitro (in which case they should not be difficult to detect and isolate)? A brief explanation of what the iPSCs are should be presented during the defense.
7. Based on your insight in the topic, what are your experimental plans/suggestions to further explore the therapeutic potential of Hedgehog pathway in cancer stem cell targeting?

**CONFLICT OF INTEREST STATEMENT:** I have no bias or conflict of interest in relation to the candidate or his work.

In Prague, 15.6.2024.

Kateřina Rohlenová, PhD