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

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The doctorate thesis of Mgr. Pavel Horák focuses on the important aspect of tumorigenesis characterized by adopting metastatic migratory phenotype. In melanoma, processes such as invasion, epithelial-to-mesenchymal transition (EMT), metastasis, and cancer stem cell (CSC) maintenance are often regulated by transcription factors, including those in the Snail family. Slug (Snail2) protein, which typically supports migration and apoptosis resistance, plays a significant but not entirely understood role in melanoma. This study examines the transcriptional regulation of the SLUG gene, revealing that it is controlled by the Hedgehog/GLI signaling pathway, particularly by the transcription factor GLI2. The SLUG promoter contains numerous GLI-binding sites, and Slug expression is activated by GLI factors in reporter assays and inhibited by GLI and SMO inhibitors. GANT61, a GLI inhibitor, significantly lowers SLUG mRNA levels, as shown by RT-qPCR. Chromatin immunoprecipitation confirmed the binding of GLI1-3 to the SLUG promoter. Immunohistochemical analysis indicated that in metastatic melanoma, GLI2 and Slug are present in MITF-negative regions, suggesting a predominant role of GLI2 in regulating SLUG expression in melanoma. These findings elucidate a novel transcriptional activation mechanism of the SLUG gene, highlighting its primary regulation by the Hedgehog/GLI pathway in melanoma cells.

Besides mentioned work, several other papers where candidate collaborated are part of thesis:
1/ Article with Katerina Vlckova as the first author and Pavel Horák contributed with real time PCR and invasive assay;
2/ Jiri Reda article where contribution of Pavel Horák is not clear.
3/ Nadia Habel article where Pavel Horák contributed with ubiquitin related experiment.
Last article which is written in Czech language represents local scientific communication and although I acknowledge its importance I will not include it in my opponenture.

Formally, all the results are appropriately displayed, discussed, and concluded in the relevant chapters. The introduction is well-written (language wise) and provides a snapshot of the complex world of melanoma biology. However, I must stress that the structure and content of this chapter are weak at best. It contains some formal and scientific mistakes and does not provide a sufficient introduction to the major subjects of the articles presented.

For example, the sentence: "Epigenetics refers to heritable changes in gene expression that do not involve alterations to the underlying DNA sequence" does not align with the current understanding of this field. Many epigenetic changes impact DNA replication rather than gene expression. Even Wikipedia offers a more current definition: In 2008, a consensus definition of an epigenetic trait was established as a "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence." We now also know that many epigenetic changes are not heritable, among other nuances. Statements that simplify epigenetics to gene expression indicate a lack of independent thinking and scientific approach by the author.

Furthermore, the aims and articles focus on migration, invasiveness, EMT, ubiquitination, etc., yet the author does not attempt to introduce these subjects. I do not want to be overly critical, but in a thesis where the articles are simply compiled, the introduction should serve as a unifying messenger, introducing the reader to the complex environment of the author's articles. This was not achieved in this thesis. The absence of an introduction to the ubiquitin-related field is particularly disappointing,

especially since I was consulted for my expertise in this area. Lastly, complete lack of any easy to understand schemes or pictures which would introduce reader into this field is not helpful.

The author's articles are sufficient to prove that Pavel Horak has grown scientifically and learned how to present scientific work. However, I find it odd to publish the same figures in two separate articles, even though they are correctly cited. This could almost be seen as an editorial oversight in the peer review process. In my view, the same results should not be published without new analysis, as sometimes happens with publicly available "big" data.

I would like to ask the candidate a few questions regarding his articles. If these are sufficiently answered, I will not oppose the candidate's defense. These questions can be answered via email, as I cannot be present at the defense personally.

I must mention that an eight-year-long PhD is not a favorable reflection on the candidate's supervisor or the PhD programs in the Czech Republic in general. We have a responsibility to support students in this endeavor without risking their burnout and the loss of their scientific passion. That said, I do not have any information on why Mgr. Horak's PhD took so long, so I am not criticizing either side. I simply hope that, based on my own experience, these academic journeys can be made shorter.

Questions:

1/ Why did you choose to study Hedgehog pathway in melanoma. Are they any alterations which lead to significant dysregulation of this pathway in this pathology?

2/ FBXO32 has some well-established substrates like c-Myc. Did you attempt to study protein levels of these factors in your experimental model? If not what you would expect and why?

3/ *Slug* promoter is controlled via many other pathways. How do you think this pathways cooperate?

4/ If overexpressed MITF activates *Slug* promoter. Does this also impact cell migration?

5/ What are your future scientific endeavors? Are you looking for a postdoc position outside of the Czech Republic?