

PhD Thesis Review

Title : The Role of the Tumour Microenvironment in Melanoma Cell invasiveness

Author: Njainday Pulo Jobe, MSc

Opponent: Vladimir Korinek

The Thesis of Njainday Pulo Jobe is focused on several topics related to the molecular mechanisms of the interaction between the tumor cell and its environment. The first part of the Thesis characterizes the role of NG2 glycoprotein in cancer cell migration. The second part is dedicated to the analysis of the interplay between cancer-associated fibroblasts (CAFs) and melanoma cells and its influence on the invasive properties of cancer cells. Methodologically, the majority of the results were gained using cell and molecular biology, and biochemical approaches utilizing cells in culture. Moreover, in collaboration with Prof. Smetana laboratory, immunohistological staining of tumor specimens was performed. The presented work also includes unpublished data describing effects of a natural compound, curcumin, on the melanoma cell invasiveness and proliferation. The results were published in two original articles - in one of them, Njainday Pulo Jobe is the first author - and one review (the first authorship). The Thesis, written in very good English, is presented in "standard" (long) form including all (requisite) chapters, i.e. Abstract, Introduction, Aims and Objectives, Results, Discussion, Conclusions, Abbreviations and Bibliography. The published articles are included as a Supplement. The structure and content of the Thesis conforms to the rules and requests for the PhD Thesis. These mainly include the choice of the research topic and formulation of the aims and objectives, and the adequacy of the student's own research contribution (described in Results). The length of the Thesis is appropriate with respect to the objectives. It is evident that the author studied the appropriate number of bibliography sources that were properly cited. Although I have some (minor) comments to the formal quality of the Thesis, in summary, **I recommend the dissertation thesis for defense, and after successful defense to confer the title "doctor" (PhD) on the candidate Njainday Pulo Jobe.**

Questions

- (1) Question related to Figures 8a and 13a. How have been the differences between IL-8 production in 2D vs. 3D co-culture experiments generated?
 - (2) Question related to the IL-6 effects/function. On page 28/29 the author describes that IL-6 signals via the JAK/STAT pathway. Is the main "mode of action" of this interleukin considered the topic of the dissertation?
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Minor points and formal errors

At this point I remember discussions that we had with the other members of the Committee for the PhD program in Developmental and Cell Biology. I suggested (and many members agreed) that the PhD Thesis Review should - to some extent - “replicate” the review of a scientific article. Consequently, the author should correct the content of the Thesis according the reviewer’s comments and suggestions and finalize (and print) the Thesis just prior to the defense. I append some of the errors related to the content of the Thesis. The correction of these errors would definitely improve the quality of the presented work. However, I do not expect (require) the author to try to answer or explain these comments.

- (1) Introduction should include more detailed information about melanoma, tumor stroma, 3D culture systems, and curcumin. In relation to the two latter topics, parts 5.2.1 and 5.2.6 should be moved from Discussion to Introduction. Part 5.2.6 does not contain any discussion of the presented results, this part should be extended. Chapter 1.3.2.2. is too short and should also be extended.
- (2) Figures in Results are difficult to read. The figure resolution should be higher.
- (3) Inconsistent (= chaotic) usage of abbreviations - just too lengthy to explain, all “thinkable” errors.
- (4) Is it true that extracellular matrix consists of “other cells and a complex network of macromolecules” (page 21)?
- (5) Frequent usage of the term “popular”, e.g. “... the most popular cell lines...” (page 23). A more appropriate term(s) should be used (the most commonly used?).
- (6) The first sentence in Material and Methods reads: “All chemicals are from Sigma, unless otherwise stated”. However, in several paragraphs of the section Sigma is indicated as the source of various chemicals. The section does not contain description of DNA constructs, siRNA and cell transfection experiments. The source of the ROCK inhibitor (Y27632) is not indicated. The source (donator) of cell lines should be indicated in Material and Methods and not in Discussion.
- (7) Description of Figures 9, 10, 11, and 13 is not complete. What is the difference between “Ctrl neg” and “Ctrl”. Why in Figure 10d two different bars are indicated as “Ctrl”? Titles in Figures 11 and 12 are identical. Figure 14 does not contain any title; how was the IL-6/8 positivity scored in human tumor samples?

Prague, September 13, 2016

Vladimir Korinek, PhD

A handwritten signature in blue ink, likely belonging to Vladimir Korinek.