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Opponent review of Master thesis of Bc. Věra Slaninová – Regulation of cellular metabolism by the Notch receptor signaling pathway

In her Master thesis, Věra Slaninová tested a potential regulation of selected metabolic genes by the Notch signaling pathway. The thesis is written in English on 67 pages, with 83 references and 8 pages of supplementary material; it complies with all formal requirements. It is clearly formatted, well written with very good English. The introduction on 15 pages is sufficiently deep and clearly introduces the reader into the problems of metabolism related to cancer and to the Notch signaling. The text has relatively few factual and grammar mistakes (for example on page 4 – "*In the 20th decade of the 20th century...*"). The methodology is described into all necessary details; only the section "Function of genes studied and their connection to diseases" does not fit well into the methods. All results are presented in a quite clear way. Conclusions and discussion are of the appropriate length and depth demonstrating a good understanding of the author of her work, although these are the sections which I have certain concerns about as mentioned below in notes and questions.
In summary, I am very satisfied with the content and the organization of the thesis.

Notes and questions:

1. Page 1 – "*However, as proposed by Otto Warburg nearly 80 years ago, metabolic changes might be the primary cause of cancerous growth ...*" Is this proposition supported by any experimental evidence from the following 80 years? No other citation is added in further introduction. What is the opinion of the author of this thesis about this proposition? I mean the primary cause vs. a necessary adaptation to cancer growth; are the metabolic changes sufficient to trigger uncontrolled proliferation?

2. Page 14 – "*It is a known cause of carcinomas of T-cells, breast tissue, colon,...*" Can a "carcinoma" arise from T-cells?

3. While I am impressed by the amount of work and convinced about the quality of the results I have a trouble with the way of their interpretation. From the thesis alone I have a feeling that the author is strongly convinced (see last paragraph on page 15) about the hypothesis of Notch signaling being a cause of Warburg effect through a direct regulation of certain metabolic genes. On page 2, the author writes that "*By describing the regulation of new Notch target genes involved in metabolism we may also provide an explanation how the Notch pathway triggers the Warburg effect in cancer cells*". This sentence suggests that it has been already shown that Notch triggers the Warburg effect but no citation or evidence follows. Can the author clarify this?

4. The main goal of this work is then to prove this hypothesis – on page 16, the author states in Aims: "*In this thesis I am trying to prove the connection ...*" From the general point of view, this is quite dangerous for a scientist and we all (including myself) incline to do this, to prove an attractive hypothesis instead of testing if our hypothesis is true or false. Some of the interpretations and conclusions in the thesis have these tendencies. For example on page 63 where the author discusses a transcriptional response in vitro: "*What is surprising is the fact that some genes were downregulated following Notch activation (hairy and Hex-A) despite the fact that in luciferase assay and in in situ hybridizations in wing discs higher expression of these genes was observed.*" Do the luciferase assays and in situ hybridization fit better the hypothesis? In the luciferase assay the short piece of regulatory sequence lacks the complexity and crosstalks with the other regulators.

5. In vitro assays use an EDTA treatment to stimulate the Notch signaling which might have a rather broader effect on cells, at least I cannot avoid such feeling (reasoning for such treatment is not described in details in the thesis). On page 46, the author writes that "*we observed changes in expression of all genes ...*". Since the changes were rather mild and varied, did the author tried to test the expression of some Notch non-responsive genes to see that the observed changes are not results of the EDTA treatment itself? In addition, most of the data in vitro are not so convincing. The transcriptional changes are usually quite mild (often less than two fold) with big errors bars and usually achieved only in one out of three cell lines.

6. In vitro and in vivo experiments suggest a locus with CG13334 to be the most responsive. Interestingly, the neighboring gene CG42808 seems to be regulated in similar way. What is known about this gene?

7. The verification in vivo uses wing imaginal discs. I think that there are quite well defined borders of Notch activation and suppressions in the developing wing discs. Are these areas somehow reflected in the in situ hybridization signals of the selected metabolic genes which are supposed to be regulated by the Notch signaling? What could be the role of such metabolic regulation in certain areas of the developing discs?

8. For further work, I would recommend not to choose only the genes which have the most straightforward connection to Warburg effect and instead of trying to prove the connection about Notch activation and Warburg effect, I would rather unbiasedly characterize the connection between Notch signaling and metabolism which may be much more complex and colorful. It seems that in the last section of results with the first measurements of metabolic changes upon Notch activation (although again by the EDTA treatment), the author is really trying hard to prove the Warburg effect. Those downregulations and negative results from Warburg-effect point of view might have a meaning.

I am not usually so meticulous in my reviews but please take it as a sign of high quality thesis written by a young lady with big scientific potentials – I would not read her work four times, some part even more times if it would not be worthy to prepare such review – I hope that it will contribute to Věra's scientific growth. I certainly recommend her work for a successful defense to obtain a Master degree with grade from rather higher levels.

In České Budějovice, May 22, 2012

Mgr. Tomáš Doležal, Ph.D.

Evaluation of the Master thesis by Bc. Věra Slaninová entitled "Regulation of cellular metabolim by the Notch receptor signalling pathway"

The master thesis by Věra Slaninová is written in English on 80 printed pages including the list of references. Formally, the work follows the common structure required by the Faculty of Science.

By the first reading, I was quite impressed by the way the thesis is written and level of English which is much above the average of the Czech undergraduate students, and in fact well above the language skills of this reviewer. Not only the grammar is very good, but the text quality is excellent in general – the logic flow, paragraphing, etc. (only a few mistyping and errors).

The thesis starts with detail introduction – well understandable even for people working in different fields, followed by aims, material & method description, results and discussion. All these chapters are provided with sufficient amount of figures and tables. I only had a problem with fig. 21, where the description under chart was not easy to understand.

This work is also very good from experimental point of view. It is based on staggering amount of data and apparently hard systematic work of the author. In fact it contains 45 figures. In this thesis Věra combinded several difficult techniques – chip-chip, qPCR, *in situ* hybridization, luciferase reporters in 3 different cell lines and metabolism measurements. This work really exceeds the regular thesis.

Minor errors, and questions:

- Ptc-gal4 --- I did not find any description of this driver (or I missed that, then sorry).
- fig. 21, where the description under chart was not easy to understand.

A few typos, e.g.:

- Last reference (83) – the first author should be cited in the text, not the PI. (or if the PI is mentioned, then it should be something like: the group of... found (83))

My questions are mostly naive ones:

1. Induction with PBS and EDTA „...as a compromise between good induction and possible starving effect..." you used 15 min, instead of 30 min.
    a. Can you, please, comment more on actual decision? Why not 10 min – apparently you have to decide for one protocol and stick to that, but more detail explanation how this was chosen would be interesting for me.
    b. Did you have any controls for starving? Such as starving effect only? And is it doable to design such controls?
2. Promoter sequences.
    a. The Su(H) enhancer sites you were mutating are not exactly identical. Can you say, that some of sequences are better while others are less efficient?
    b. You have mutated Su(H) enhancers. Is it possible to use opposite strategy – take those enhancers that worked well and use them in luciferase reporters to determine „surrounding" necessary for up-regulation? Or is the Su(H) enhancer site sufficient?

In summary, I consider this master thesis to be excellent. . I estimate, taht this work exceeds the regular thesis twice, in terms of the amount of work, data and also writing qualities. I congratulate Ms. Slaninova on a job well done and I recommend it for defense.

In České Budějovice, 25.5.2012

DAVID DOLEŽEL