MASARYKOVA UNIVERZITA

Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

Habilitation Thesis Reviewer's Report

Masaryk University Faculty	Přírodovědecká fakulta
Procedure field	Animal Physiology
Applicant	RNDr. Alena Hyršlová Vaculová, Ph.D.
Applicant's home unit, institution	Fyziologie živočichů
Habilitation thesis	Novel molecular mechanisms of cancer cell death regulation
Reviewer	**Prof Ing Jiri Neuzil, PhD**
Reviewer's home unit, institution	**Institute of Biotechnology, Czech Academy of Sciences, Prumyslova 595, 252 50 Prague-West, Czech Republic**

In this habilitation thesis, Dr. Alena Hyrslova-Vaculova has focused her research on novel aspects of programmed cell death, in particular on apoptosis induced by external stimuli, presented primarily by the death ligand TRAIL, and its synergic or additive effects when combined with other inducers of apoptosis, such as 5-fluorouracil, the cisplatin analogue LA-12 and several other agents. In general, this is a very important aspect of cell biology overlapping with oncology, with potential translational aspects, since cancer is a pathology that still has not been harnessed. As such, any new discoveries, particularly those that can be translated towards the clinic, are beneficial.

The Thesis is composed of several major parts. It starts with the Abstract, in which the candidate summarizes the contents of the Thesis, which is consistent with the contents of the papers published in this area of research by the author (see below). In my opinion, while the Abstract

does correspond to the major focus of the research work of the candidate, it should be somewhat more detailed so that one could already have a better idea of the work, even without having to go through the individual papers. However, this is only a minor critical point.

In the Introduction part, the candidate presents an overview of apoptosis, the major form of programmed cell death. The first subchapter, called 'Apoptosis and cancer' is far too brief, Given the fact that the candidate puts an impact on the importance of apoptosis in cancer suppression (which is the general direction of her research area), I would expect more detailed insight into this topic and its better description, rather than a very brief overview on just a few pages. Needless to say, there is a very extensive literature on apoptosis and its role in cancer (suppression). Further in this chapter, the following sentence is included: "Evasion of apoptosis has been proposed as an important mechanism implicated in malignant transformation, and one of the hallmarks of cancer (Fig. 1) (Hanahan & Weinberg, 2000; Hanahan & Weinberg, 2011)." In particular due to apoptosis being a hallmark of cancer, the author ought to give more attention to this aspect. Again, this is not a major critical point, rather a suggestion how to make the thesis more balanced.

A lot of attention is given to the characteristics of the death ligand TRAIL, which is one of the triggers of external apoptotic signalling, and which is a major focus of the candidate's research. In fact, vast majority of the thesis is devoted to TRAIL. While chapter 1 (apoptosis in general terms) is on three pages, the part occupied by TRAIL and its role in apoptosis spreads over 22 pages. Again, while it makes sense to cover TRAIL in such a detail, some balance in the depths of Chapter 1 and Chapter 2 would be good.

I must say that the thesis is generally very well written and, despite the minor criticism above (and, for that matter, also below), it is well undertaken and the author shows great expertise and knowledge in the topic at hand. There are a few parts where I would welcome a bit better way of covering the topic, such that reading these parts would be easier. For example, in Chapter "2.2.2. Sensitization to TRAIL-induced apoptosis – combined therapy" on page 11, the candidate gives an account of agents that TRAIL has been shown to cooperate with in order to achieve a better therapeutic outcome. Both paragraphs of the Chapter give a number of compounds followed in each case by a reference. This is way too difficult to read and I feel that the text presents a bit of a riddle here. In fact, it hurts the eyes to try to decipher the contents these two paragraphs. While it is important to document how TRAIL may or does overcome

resistance of cancer to established therapies and/or to show that TRAIL has a synergistic effect with other therapeutics, all this should be better documented in the form of a table, which would be much easier to read. If in a tabular form, I would be very interested to find out which compounds TRAIL synergizes with – however, as it is written, I am not attracted to read the text, I am afraid to have to say.

In Figure 5 on page 12, the candidate presents an overview of extrinsic and intrinsic pathways involved in apoptosis and the points where intervention is possible (or where intervention has been documented). I understand that this Scheme was taken from a paper by Fox & MacFarlane (2016). To me, also given the fact that this is a habilitation thesis, the scheme is too simplistic and it is far from being exhaustive given the interrogation points where therapeutic effects have been shown. Certainly, there are many more points of intervention and many more compounds that promote apoptosis leading to tumour suppression. For example, the number of compounds mentioned in Chapter 2.2.2.1 that synergize with TRAIL (or overcome resistance to chemotherapy) is rather high. I think that a Scheme such as in Figure 5, could be re-drawn by the author and, also, should be more comprehensive.

Habilitation is a process that is based on the track record and the quality of research over a considerable (or whole) professional career of the applicant. Here, I am very happy to highlight the research of the candidate, with 30 papers to date, with the first one published in 2002. What is also highly commendable is the consistence of the research direction of the candidate. Majority of her papers are within the area of apoptosis and the role of death ligands in the process. One of the real highlights is also her performance during her post-doctoral stay in the Karolinska Institutet, one of the top research institutions in the area of biomedicine. Here she co-authored a paper in *Nat Cell Biol*, which is an exceptionally great achievement. Overall, the papers of the candidate are in pretty good journals that correspond to the quality of the research, which is high. Once again, it is the consistency of the focus of the candidate that makes me feel very good about her research presented in the habilitation thesis. I am convinced that the well deserved award of the title of Associate Professor will motivate Dr. Alena Vaculova-Hyrslova to go another major step in her research career and become a world-acclaimed scientist in an area of research that is very important for the mankind.

Reviewer's questions for the habilitation thesis defence

1. One of the major goals of researchers trying to understand the molecular mechanism of apoptosis is the eventual translation of their research to give benefit to patients. Can the candidate give more insight into the idea that TRAIL may be utilised as an 'off-the-shelf' anti-cancer therapeutics and what are the hurdles to achieve this?
2. It is my understanding that it has been shown in the literature that while TRAIL may be selectively apoptogenic towards cancer cells *in vivo*, this is not the case for FasL, which upon injection into mice kills them within a few hours due to massive hepatotoxicity. Why is TRAIL, unlike FasL, selective for cancer cells when the induction of apoptosis by the two death ligands is similar and the signalling cascade also involves formation of DISC and activation of caspase-8?
3. Can the candidate discuss with some more depth the role of decoy receptors for TRAIL in cancer and how do these receptors make cells resistant to TRAIL expressed in cancer? Is expression of decoy receptors cancer type-specific? Does expression of decoy receptors in non-malignant cells make them resistant against TRAIL-induced apoptosis?
4. The habilitation thesis comprises 19 publications. Having gone through all of them, I noted that they are based on experiments with cultured cells. I appreciate the depth of these papers with which the various caveats of TRAIL-induced apoptosis are studied. There is one point that I really want to make, which is the lack of *in vivo* experiments. So, the question is: why has the applicant never attempted to verify the signalling pathways she was researching on an *in vitro* level also using mouse models of cancer? The institution where the candidate has carried out her research can certainly accommodate mouse models of cancer. If not, then there are collaborators the candidate has been working with that can help with *in vivo* experiments.
5. Can the candidate elaborate on the future application of the combined effect of LA-12 and TRAIL? It would have been good to document the relevant, very nice *in vitro* results also in the case of a mouse model of cancer, for example using the p53-wild type and $p53^{-/-}$ colorectal carcinoma cells. This would give the thesis itself a higher translational value; notwithstanding this, it seems as a good future focus of the candidate to continue in this direction.

6. One point that I am also interested in is linked to the future plans of the applicant. So, can the candidate give an account of her plans for the next few years in terms of research as well as training of PhD students?

Conclusion

The habilitation thesis entitled "**Novel molecular mechanisms of cancer cell death regulation**" by Alena Hyrslova-Vaculova ***fulfils*** requirements expected of a habilitation thesis in the field of **Animal Physiology**.

In Southport, Qld, Australia on August 31, 2019