Evaluation of the Master thesis

Author: Radka Hobizalová, BSc.

Title: Functional analysis of Salp25D, a homologue of peroxiredoxin, from castor bean tick *Ixodes ricinus*

Supervisor: Nataliia Rudenko, PhD.

Co-supervisor: Maryna Golovchenko, MSc.

Faculty guarantee: Prof. RNDr. Libor Grubhoffer, CSc.

Reviewed by: Radek Šíma, PhD., Institute of Parasitology, BC ASCR

Master thesis of Radka Hobizalová is aimed at functional analysis of Salp25D from *Ixodes ricinus*, an antioxidant protein originally described in salivary glands of closely related tick *Ixodes scapularis*.

The master thesis is written in English and extents 64 printed pages including the list of references and appendix. Formally, the work follows the common structure required by the Faculty of Science.

The **Introduction** is very well written and introduces reader into the topic. The author describes in details various sources of reactive oxygen species and most important molecules used by organisms to fight the oxidative stress. In the following chapter, author summarizes recent knowledge about oxidative stress and antioxidant defense in ticks. The last section is dedicated to anti-tick vaccines. I appreciate the author's ability to present these complex topics in a clear and readable form.

Goals of the work are clear and well defined.

Materials and Methods are clearly structured and individual methods are explained sufficiently.

Results are summarized on 11 pages, including 2 tables, 15 figures and 4 graphs. Author successfully cloned and expressed recombinant Salp25D, proved its ability to protect DNA from hydroxyl radical mediated damage and showed positive effect of Salp25D on viability of borrelia spirochetes in the presence of oxidative stress. Author didn't prove the activity of recombinant Salp25D in the reaction with reduced glutathione. Author also prepared dsRNA for Salp25D gene silencing. Its knock-down effect in adult ticks was proved by real-time PCR.

Discussion is on high professional level, author interprets her results with published data. I haven't major comments to this chapter.

<u>Questions and critical comments</u>:

1) p.14: Author suggests that heme in ticks is degraded by the action of heme oxygenase. To my (poor) knowledge, nobody has proved heme oxygenase activity in ticks. Moreover, gene for heme

oxygenase hasn't been annotated in the genome of *I. scapularis* so far. Please could you comment on this?

2) p.36: Amount of total RNA used in RT-PCR reaction isn't mentioned.

3) DNA sequencing methodology is missing in the Material a Methods section.

4) Author classifies Salp25D as a homologue of peroxiredoxin. But in the original paper, this protein is described as a glutathione peroxidase homologue. What is the true classification of this protein?

5) p.43: Author used reduced glutathione as a donor of electrons in the assay testing rSalp25D activity. According to author, Salp25D belongs to peroxiredoxin class. It's known that most of the peroxiredoxins utilize thioredoxin as a donor of electrons. Why did you use glutathione instead of thioredoxin in this reaction? Is it a possible reason why this assay failed?

6) Figures 19, 23 and 24 have poor quality.

7) Did you observe any effect on tick fitness after Salp25D gene knock-down in comparison with GFP control? Do you plan any further experiments in this direction?

(please comment highlighted questions/notes)

Final evaluation: Radka Hobizalová prepared excellent master thesis. In contains minimum of mistakes and the level of her English is much above the average of the Czech undergraduate students. Radka proved her talent for scientific work and I am sure that she will employ gained experience in her future research career. I'm happy to **recommend** her master thesis for defense and suggest the grade **1**.

České Budějovice

January 17th, 2013

Radek Šíma

UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE

Faculty of Science

STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Bc. Radka Hobizalová, BSc.

Thesis title: Functional analysis of Salp25D, a homologue of peroxiredoxin, from castor bean tick Ixodes ricinus

Supervisor: Nataliia Rudenko, PhD.

Reviewer: Jan Lopatar, PhD.
Reviewer` affiliation: Dept of Fundamental Neurosciences, University of Lausanne, Switzerland

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced extents of the thesis divisions** (recommended extent of the theoretical part is max. 1/3 of the total extent), **logical structure of the thesis**	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Adequacy and clarity of the results and conclusions	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	3
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	3
Formal requirements – points in total		24
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the filed and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		27
POINTS IN TOTAL (MAX/AWARDED)	51	51

Suggestions and questions, to which the student has to answer during the defense:

Comment: In the first sentence on page 4 you chose the word "detoxification" when you talk about the breakdown of H_2O_2 by the enzyme catalase. While it is true that H_2O_2 has predominantly harmful and detrimental effects on cellular structures, there are situations when H_2O_2 (and other ROS for that matter) is actually a physiologically relevant agent (via activation or inactivation of several transcription factors, kinases/phosphatases or ion channels) which can regulate cell proliferation, immune response, and is also an important neuromodulator.

Question: There are several H_2O_2-metabolising enzymes, in particular PRx, GPx or CAT. How would you explain this functional overlap?

Question: With respect to the degradation of heme, can you comment on the products of the degradation of heme? Are they harmless or harmful in terms of their redox abilities?

Question: On page 49 when you talk about the distribution of Salp25D in tick's organ systems you mention the gut, the ovaries and the salivary glands as organs that come into contact with host blood antigens. Can you elaborate as to how the ovaries might come into contact with host blood antigens?

Comment and question: You assume that Salp25D is released into the saliva. Based on literature search (ref 108) you propose other, non-standard means of secretion of Salp25D into the saliva, since no N-secretory peptide was identified in Salp25D. However, in your thesis you only isolated the salivary glands, not pure saliva. How can you be certain that Salp25D is indeed released in any way from the salivary glands into the saliva? Is it possible to isolate pure saliva from the tick to test your hypothesis?

Question: Considering that the expression pattern of Salp25D is highest in the gut, why does its name suggest a predominantly salivary protein?

Question: Even though the pattern in Borrelia viability assays that you used was very similar, you obtained different results in control groups (22.3 % dead borrelia in Live/Dead assay *vs* 9.2 % when FACS was used). How do you explain this discrepancy?

Eventual mistakes, which have to be corrected:

None

Eventual additional comments of the supervisor on the student and the thesis:

Conclusion:

The author of this thesis used a variety of biochemical, molecular biological and fluorescent approaches to study the role of Salp25D. The author successfully expressed a recombinant version of Salp25D. That it was a functional peroxiredoxin was confirmed when (i) the addition of Salp25D inhibited metal-catalysed oxidation and DNA nicking and when (ii) Salp25D rescued a high percentage of borrelia exposed to oxidative stress. The author further nicely excluded the possibility of Salp25D possessing GPx activity. At the end, the author set out to explore the phenotype of Salp25D gene silencing, and successfully started to achieve this goal by optimizing the procedure. I can see that the author of this thesis has acquired both writing skills as well as has mastered many important techniques which I believe will help her in her future steps as a scientist.

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1 .[2]

In **Lausanne, Switzerland** date **18.1.2013**

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signature

[2] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.