Přírodovědecká fakulta
Faculty of Science
Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR THESIS

Name of the student: Armig Kabrelian

Thesis title: Antiviral activity of selected IFN-β-stimulated genes
Supervisor: Prof. RNDr. Libor Grubhoffer, CSc

Referee: RNDr. Jindřich Chmelař, PhD.
Referee`s affiliation: Dept. of Medical Biology, Faculty of Science, University of South Bohemia

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	2.5
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	1
Formal requirements – points in total		14.5

(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	1.5
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	0.5
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	2.5
Experimental difficulty of the thesis, independence in experimental work	0-3	2

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

Quality of experimental data presentation	0-3	1.5
The use of up-to-date techniques	0-3	2.5
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	1
Practical requirements – points in total		15.5

POINTS IN TOTAL (MAX/AWARDED)	48	30

Comments of the reviewer on the student and the thesis:

Questions that should be answered are in italics.

Abstract:

Abstract displays signs of discussion and description of methods, which should not be. Abstract should introduce the project, content of the work and obtained results.

Formal requirements: The work has 56 pages, which is very good extent. Individual sections vary significantly with longest section Methods (17pg) to very short Discussion (3pg). However, the formal side is ok.

Introduction:

Introduction is rather brief, which in some parts is contra-productive as the statements are too vague, raising many questions. The titles of chapters are insufficient, for example the title Vectors – one could expect large chapter about all disease vectors, but it is about TBEV vectors and this should be in the title, i.e. "Vectors of TBEV". The immunology chapters are very unsatisfactory as it is only very, very brief introduction to the fact that vertebrates have some immune system with cells and antibodies. It should rather describe antiviral immunity in more details. Directly after general introduction of immune system, there is a chapter about interferons. This chapter is not precise and sometimes you contradict yourself (see questions). At the end of the introduction, you correctly introduce your proteins of interest and their role in antiviral defense. This chapter is ok, however, very few references are used, and often you take information only from one source, which is not good.

Comments and questions to individual chapters:

Chapter "Vectors" is quite rudimentary with only two references. The chapter should be more clear about what ticks transmit what in which parts of the world. *Which ticks in Figure 2 serve as vectors of TBEV?*

Description of immune system too vague, imprecise and chaotic. Information is given very selectively without explaining, why specifically certain information is relevant to the study. The description of immune system should be more relevant to the aim of the work.

You mention natural killer cells as phagocytes. Is it true? Which immune cells are phagocytes? Can adaptive immune response be quicker than innate?

You write that type I interferons regulate immune system and that type II (IFN-g) has some antiviral and antitumor effects. One sentence later you write that only type I were shown to restrict viral infection. *Which statement is correct?*

What do you mean by intracellular signaling with interferons (page 5)? Do peptidic

hormones or cytokines have intracellular receptors?

You write: "...leading to dramatic changes...that involve the activation of specific signaling receptors and the transcription of several antiviral genes..." *Which receptors and which genes?* When you write sentences like this, you need to be more specific and use some references. Otherwise, such vague statements just fill the space without any useful information.

Aims:

Two aims are mentioned – one is very specific, to clone the genes via Gateway cloning and the second is more vague, to test antiviral effect in DAOY cells. It would be good to mention, how you wanted to test.

Materials and methods:

Methods are the best section of the thesis. This section has detailed descriptions, sometimes not necessary, because it only repeats the manufacturer's manuals (RNA isolation, plasmid isolation etc.), but for bachelor student, it is good to write down whole protocol personally and get familiar with the techniques. There are few mistakes, but nothing serious. It is obvious that you performed many experiments and gained some experience, especially in PCR and molecular cloning.

Comments and questions:

- *Cell concentration formula by Bürker chamber does not seem correct. What is the correct formula?*
- 3.6. You write that "the isolated DNA band was loaded on a 1,2% agarose gel". *What does it mean?*
- There is a mistake in table 6 annealing temperature.
- "This method was achieved..." better "was done or performed". *Please explain in detail Gateway cloning. I don't understand, why you transfer between different vectors and how adaptor fragments attL, R, P, and B are related.*
- *Table 10 – what was the template for PCR?*
- *You were using High GC Enhancer in reactions. What is GC content of your desired genes?*

3.9. You describe cell concentration for 24 well plates, but you describe also that 96 well plates were used. For them, however, no information about cell concentration is given.

- 3.12 – You mention virus media. *Is it medium with or without virus?*

Results:

Most of the results are unfortunately negative, but this is not an issue in bachelor thesis. However, there is a lot of other issues, mainly errors in the description that need to be addressed.

Comments and questions:

- Figure 7. You should show only zoomed, relevant part of the gel. The ladder size should be described directly in the figure, not at the side with completely different size. This holds true for all gel figures.
- Figure 8. You mention the same size of PCR products for Figure 7 and 8. *Is it correct or the amplicon should be bigger? What should be its actual size?*
- Figure 7-9: Insufficiently separated gels, if you want to distinguish the size of the two different amplicons.
- Alignments in figures 10 and 11 are not sufficiently described. You should explain in

the legend or in the text, why you can claim that there is a match between the sequences. Reader does not have to be familiar with Geneious software.

- Figure 12: Nicely separated gel, however the two different products seem to be of much more similar size than at Figure 7. *How is it possible?*
- Page 32: You write "...the practical obtained IFI6/27 genes after being cloned into donor vector, match the literature database". *What literature database do you mean?*
- Table 21 . When you want to show the concentrations and purity of the plasmids, you should mention the name of the construct, i.e. vector+cloned gene of interest.
- You mention the purity. *What the two ratios in table 21 mean*?
- 4.2.1. This chapter looks more like methods description. P-value is not usually mentioned, it is sufficient to state only whether p is < or> than 0,05, 0,01 etc. This is usually expressed by asterisks above respective bars in graphs. Moreover, you do not mention, between which groups there was statistic significance, you mention only p-value without any other information.
- Figure 15 - there should be indication of statistically significant results. Used statistical test (Student's t-test) is not correctly used. *What test should have been used here?*
- I would rather use infected/uninfected cells than TBEV/mock or mock cells. Mock is usually used, when you use something that should imitate experimental treatment, for example in RNAi, you use scrambled RNA as mock.
- The English is sometimes grammatically and syntactically incorrect. For example the sentence: "Therefore, indicating the possible antiviral effect of IFI6." lacks the verb. This is general comment to the English language whole thesis, but it is not something serious.
- Figure 20: Double delta Ct method description or the relevant reference are missing. What were the values normalized to, i.e. fold change comparing to what?
- *According to Figure 18, are the qPCR data for IFI6 reliable or usable at all?*
- Protein detection by antibodies was rather unsuccessful, which would not be the problem. What is much worse is that in all SDS-PAGE figures you state that you used DNA marker. Actually, you just copied the sentence from figure 7 and pasted to all figures with gels, no matter whether it was protein or DNA. This is very bad approach and such things actually tell a lot about your overall reliability, so don't do that anymore. Never!
- I think that the use of 10-245 kDa marker is not ideal in combination with such thick gel. It works, but the description of the ladder (B part of figure 21, 22 and 26-29) does not correspond with the actual membrane and it is difficult to understand, where the specific molecular weights are. *BTW, why we do not see 3 highest bands of the ladder? Where did they disappear?*
- You write at figure 23 that there is low signal of IFI6. According to the three pictures, I would say the opposite. The signal (red channel) looks quite strong and present among all the cells. However, I would expect that you show the difference between treated cells with overexpressed genes and controls. *What information should we get from figure 23?*
- Figure 24.- T7-IFI6-poly(T) is said to be 900bp long. *Please explain, why is it that long, while the gene itself has only about 400bp.*
- *Why cell viability assay was not performed with mRNA transfected cells? Why there is no positive control in figure 25 (similarly to figure 16)?*
- Western blot figures are a bit confusing to me. *What should we actually see, if the*

experiments were successful? Please draw an ideal western blot with ideal bands in every lane and show it in your presentation.

Discussion:

The discussion is poor and short, with only 4 references. First paragraph summarizes, why this topic was chosen, second paragraph states that you successfully cloned the genes. Then there is description of negative transfection results and an unintelligible passage about qPCR and Western blots (page 43, line 10-14), followed by a tautology (page 43 the end of second paragraph) – the...method showed a weak signal...indicating that the signal was too low... The only discussion is that the negative results could be due to unsuccessful transfections or bad antibodies. When you cite Gjermandsen 2000, it almost seems that you will discuss the influence of different cell lines, but you turn the sentence again to the statement that overexpression did not work and in qPCR, there was a pipetting error. This information comes unexpectedly, undermining all qPCR results. Last sentence of the discussion tells us that the optimization of the transfection needs to be further optimized. Such nonsenses should definitely be avoided.

References:

The citations should be either in round or in square brackets, not both. Total of 20 references, including one textbook is very low even for a bachelor thesis. I believe there is much richer choice of relevant scientific literature that could significantly contribute to the quality and informative value of the thesis.

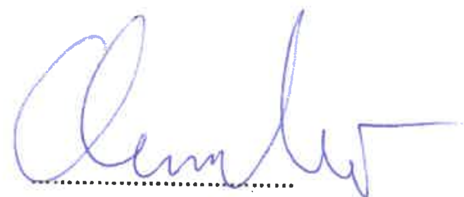
Overall, the impression from the work is that it was written in haste. The work with literature is poor and it is visible in both introduction and discussion, which are sloppy, incorrect and imprecise. Vague statements are often covering the lack of necessary specific knowledge. This is not acceptable in scientific writing, which is actually based on specific and detailed knowledge of the topic, on the ability to compare with other works. Bachelor thesis is usually the first encounter of a student with scientific writing, so the attempt doesn't have to be perfect, however, the effort must be noticeable.

I appreciate the methods description, which includes some minor mistakes, but overall it is very good.

Conclusion:

In conclusion, even despite many comments I r e c o m m e n d the thesis for the defence and I suggest the grade 2-3, depending on the presentation.[2]

In **České Budějovice** date **12.9.2018**



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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. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).