RNDr. Zdeněk Franta, Ph.D.
Faculty of Science.
University of South Bohemia
České Budějovice

Bachelor thesis evaluation: Anda Radoš
Theme: OASL protein isoforms in human neural cell lines infected by tick-borne encephalitis

The bachelor thesis of Anda Radoš monitors the time-dependent expression profile of three OASL isoforms (a, b, d) in three different human brain cell lines (9U373, DAOY, SK-N-SH) upon infection with TBEV virus. The thesis is written on 47 pages and consists of 7 chapters (introduction, goals, materials and methods, results, discussion, conclusion and references). Although the thesis deals with a quite interesting topic, my first impression after reading was that the thesis was written in a very short time. It contains several typos (qRT vs qPCR, mL vs ml), auto correction errors? (eg prodigy vs progeny) and differences in the cited literature (missing authors on page 44 or web source). Also the overall quality of presented pictures is a bit weak. Most of the pictures (eg. 1, 2, 4, 6, 9, 11, 12) are blurry due to magnification, legends to the pictures (3, 6) are not on the same page. The author also included list of abbreviations (page 11), which is a great idea especially in the topic of immunology but unfortunately the list is a bit chaotic. The individual abbreviations are not listed in the alphabetical order and many of them are missing (eg DAYO, FBS, DMEM, IMDM etc).

The first chapter, named Introduction is the biggest part of the thesis (18 pages). It starts with the short overview of TBEV, describes virus cycle, its neuropathology, and replication. Follows a brief introduction to innate as well as acquired immunity and Toll like receptors with major focus on OAS-Like proteins and their antiviral role.
I have just two comments here:
1 – Instead of a plain text dividing TBEV into different groups, subgroups etc., I would rather suggest to include a table. Such a table will, in my opinion, be easier for the reader to follow the TBEV classification.
2 – On page 2 author says, that the TBEV transmission can occur also on the immune host. I was wondering, where the virus locates and how such a transmission occurs.

Chapter two: Materials and methods chapter covers some cell culture skills as well as total RNA isolation, cDNA synthesis and PCR experiments. The methods included in this chapter belong among basic molecular approaches and their understanding and maintaining will help the candidate in her future scientific career. In general, I would like to say that every M&M part shall be written in the way that any researcher can repeat the experiment. Here you use many un precise/unclear formulations, which make it hard to repeat the experiments. For example: You should state how many days and at what time points you sub-cultured cells instead of "for several days", what was the real concentration of trypsin (in mg or units) instead of "0,25% trypsin", or for how long were cells incubated instead of "several minutes"
My questions/suggestion to the candidate:
1 – On the page 20 you says that "a small amount of medium was added to inactivate trypsin" could you explain how this works?
2 – If you have problems with the insufficient amount of total RNA (table 3 and 6). Why did you make 2 different setups for RT-PCR and qRT-PCR respectively? You could easily use the same samples (RNA) for both experiments. Why did you use bigger wells (more cells) for more sensitive method (qRT-PCR), where you theoretically need less material?

3 – I did not really understand your calculations in table 1 and 2. Why the cell concentrations were so different? (1 120 000 vs 160 000) it cannot be just due to the 2ml volume difference.
4 – Why did you freeze samples from 24-well plate prior RNA isolation?
5 – Why did you do an extra DNAse step (after the isolation), if you already performed one during the RNA isolation?
6 – Could you comment on very low A260/A230 ratio? Did you do any ethanol precipitation to clear your RNA samples?
7 – What happened to your SK-N-SH cell at 24 and 72 hpi or what was the isolated RNA?
8 – Could you please comment on the calculations in table 6? Seems to me that the concentrations are wrong
9 – On the page 27 you say that 2µl of RNA were used for negative control, but from table 7 it is obvious that you use 4µl of RNA in test sample. You should use the same amount of RNA in both cases
10 – I am missing more data for the qPCR analysis. If you are using SYBR, you should also do the melting curve analysis, to be sure that detected signal is really specific and not due to any primer dimers. I am also missing more data in the table 9. Especially in the case of qRT PCR primers you should state the quantitative coefficient as well as coef. of determination, cause both parameters are very important for relative quantification.
11 – Why did you decide for one step RT/qRT PCR? I think it would be more convenient to do the two step approach especially in case you need to repeat any experiment.

Results chapter has 8 pages and shows the major findings for both RT PCR as well as qRT PCR approaches. I have following questions to the candidate.
1 – I am missing any RNA gels in this chapter. To see the RNA integrity is in my opinion a crucial step in any consequent experiment and should be present in this chapter.
2 – What reference gene did you use for the RT PCR experiment? It is in my opinion very hard to comment any data, if you do not see a reference gene control. You simply cannot be sure, if the absence of band is due to no expression or bad quality of RNA/cDNA sample.
3 – You say that OASLd is present only at 24hpi in U373 cells, but similar size samples are also present in NC (24 hpi) and at 48hpi. Did you tried to verify these bands?
4 – Why you did not include the 72hpi interval in you qRT-PCR analysis?
5 – Could you comment on the error bars, for OASLa in figure 15 and 17? Both does not correspond to the text on page 33.

In the next chapter (discussion) author compares her results to the literature. I would be a bit careful about same data interpretation (eg there is no expression of any OASL isoform in SK-N-SH cell, page 38), because of the missing controls.

Despite of all my critical comments and questions I think that the Bachelor thesis of Anda Radoš meets all requirements of Faculty of Science at University of South Bohemia and therefore I recommend it for the defense. The final mark will depend on the performance of candidate during the defense and her answers to my questions.

In České Budějovice 13.9.2017

Zdeněk Franta