

Opponent's report on the PhD thesis

Name of opponent: Prof. RNDr. Daniel Rösel, PhD.

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Thesis title: **Translational control of ERK and AMPK signaling activity in melanoma**

In the thesis, the author investigates how eIF4F contributes to the regulation of the oncogenic ERK and AMPK signaling pathways in the context of a cellular model of melanoma with activating BRAFV600E or NRAS mutations. To achieve this goal, analyses of the activity and expression of signaling pathway proteins were performed after affecting cells with specific inhibitors. Within the part of the dissertation dealing with AMPK signaling, inhibitor-based analyses are further supported by knockdown experiments. The author shows that eIF4F restricts both pathways through translational control, specifically increasing ERK activity through downregulation of short-lived DUSP6 and activating AMPK through downregulation of PP2A and its adaptor UHRF1. These findings suggest that translational control could be pharmacologically exploited to treat melanoma.

Quality assessment of the thesis

Quality of the dissertation

I enjoyed reading the introduction, the result section sometimes include sentences which are hard to read but overall, I consider the thesis as well-written. The quality of the presented results exceeds the requirements for awarding PhD.

The student contribution

I understand that all of the work described in the results section of the thesis was done by the student. This amount of work is certainly more than sufficient and scientifically sound.

The student is also a co-author of 3 scientific research publications. On two of which she is the first author or has shared first authorship. Her contribution to all three publications is clearly described in the thesis. I would emphasize that she participated experimentally in all of them.

Student capacity for independent research

Based on the text of the written thesis, especially the introduction, results, and discussion parts, I consider the student to be fully capable of independent creative research.

Specific comments and questions

Comments:

Blots typically lack quantification analyses. Although conclusions are usually convincingly supported by blot images, densitometric analysis presented e.g. as profile plots would support the presented conclusions. The blot legends also lack information on how many times the experiment was performed.

Effect of Rocaglamide A on c-Fos expression is analyzed only on the protein level, though the results are interpreted as transcriptional regulation. Reporter system and/or qPCR should be used to confirm this.



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It is almost impossible on my screen to see any positive signal in pictures documenting PP2A-AMPK interaction using proximity ligation assay (Figure 30A). Higher contrast should have been used in the green channel.

I disagree with the conclusion regarding the effect of Rocaglamide A on cell viability based on the data in Figure 36B, which is interpreted as cell growth arrest. The high decrease in crystal violet staining in treated cells after 24/48 hours is, in my opinion, more indicative of cell death. Although I am not an expert in the field, I would similarly interpret the data from Annexin staining as a cytotoxic and not just antiproliferative effect of Rocaglamide A.

Questions:

In the introduction, the term of MAPK fitness zone is used in connection to therapy resistance. In this context, what molecular mechanisms are responsible for cell death when the MAPK signaling pathway is activated above its optimal range?

What is the difference in regulation of c-Fos and c-Jun by ERK signaling? What are the consequences of these differences for AP1 driven transcription?

Unlike other tested DUSPs, DUSP5 shows increased expression upon Rocaglamide A treatment. How do you explain this?

Conclusions

In my opinion, the quality of the written thesis and publications, co-authored by the student, clearly demonstrate her ability to work independently on a scientific project and to lead such a project. I therefore recommend that the thesis be accepted as the basis for awarding the PhD. degree.

In Prague 28.8.2026

Prof. RNDr. Daniel Rösel, PhD.