REVIEW OF PhD THESIS

Author: **BORIS RJABČENKO**

Title: **SENSING OF MPyV INFECTION BY INNATE IMMUNITY SENSORS**

The doctoral thesis submitted by Boris Rjabčenko presents his results obtained during the PhD studies under supervision of Doc. RNDr. Jitka Forstová, CSc.

The thesis is conceived as a short commentary to four papers published in peer-review journals with a high impact factor that were authored (1) or co-authored (3) by Boris Rjabčenko. Additionally, the candidate has contributed to 5 more publications that were not included in the thesis.

The major focus of the thesis consists in a detailed analysis of the mechanisms of delayed recognition of MPyV by cellular cytoplasmic DNA sensor resulting in the induction of IFNβ. Further attention is paid to the contribution of MPyV-induced TLR4-mediated changes of cytokine/chemokine production to the phenotype of cancer-associated fibroblasts (CAFs) and their invasiveness. The candidate has significantly contributed to all parts of the studies – conception, design and performance of experiments, data analysis and manuscript preparation.

In general, the thesis is written in an interesting and comprehensible way (despite the complicated topic) and there are not too many typographical errors. It includes an interesting, even funny introduction, a review of relevant processes implicated in innate immune responses, aims and results of the studies included in the thesis, and a general discussion with conclusions.

I have the following questions and comments:

1. You were not able to detect measurable 2´3'-cGAMP levels in the nuclei of infected cells. Is it possible that the compound would diffuse out from nuclei during the isolation procedure? Alternatively, did you consider a possible role of other cGAMP isomers? Would they be detected by your approaches?

2. In your work, you have nicely identified p204, murine orthologue of human IFI16, and cGAS as receptors for detection of MPyV DNA at late times of replication cycle, resulting in activation of STING, IRF-3 phosphorylation and expression of IFNβ in mouse 3T6 fibroblasts. Yet, in the paper you consider levels of induction of interferon mRNA as relatively limited. Do you suspect an existence of additional mechanisms how MPyV could interfere with induction of IFNβ? Could you speculate about possibility of other mechanisms of viral interference induced by polyomaviruses?

3. You have not found any effects of TLR4-induced IL-6 production on infectivity of MPyV in MEFs. Did you analyze the whole replication cycle and the role of different m.o.i.? Do you plan to analyze these effects in other cell types? Would you expect a different outcome?

4. Polyomaviruses use different types of gangliosides as receptors for the entry into the cells. Could you comment on the specific factors determining tissue tropism and the pathogenic mechanisms of individual polyoma viruses and their significance for human pathology?

Minor comment:

On p. 14, CD4 T-cells and CTLs should not be missing among the important cellular sources of IFNγ.

In summary, the thesis of Boris Rjabčenko includes original data published in respected peer-reviewed journals and fulfills requirements for a doctoral thesis. I fully recommend it to be accepted for defense and, based on the outcome of the thesis defense, for awarding of the PhD degree.

MUDr. Zora Mělková, PhD

Prague, September 7, 2021