Reviewer's evaluation of the PhD Thesis submitted by Boris Ryabchenko

The PhD Thesis entitled "Sensing of MPyV infection by innate immunity sensors" encompasses just 47 pages, however its integral part is a supplemental file containing within its 71 pages three publications with Boris Ryabchenko as a co-author and one this year published paper in The FEBS Journal with his first authorship with total IF over 21). The Thesis is focused on polyomaviruses or more precisely on mouse polyomavirus MPyV, respectively on MPyV-triggered signaling in in infected cells (mouse fibroblasts). The Introduction covers within its 18 pages basal information on polyomaviruses family, their structural aspects, life cycle and most notably interaction with cellular viral infection counter-acting signaling pathways (type I interferons and IL-6). The introduction is rather informative and is supported by nine relevant, well-fitting figures.

Following sections in this a bit unusual Thesis setup cover in five pages Thesis aims and overview of main result from the supplementary file (four publications) including two illustrative figures. The last major section contains in nine pages discussion of published as well as unpublished data and final remarks. Discussion relevantly touches and if possible links major outcomes from four attached publications – naked viral DNA activated type I interferon response, relevance of hydrophobic domains in accessory structural viral proteins VP2 for MPyV multiplication, activation of IFNβ production by sensing viral DNA mainly in cytoplasm as well as virion-triggered TLR4 signaling resulting in IL6 production by infected cells.

The first out of four attached papers published in DNA Cell Biology in 2013 deals with naked plasmid DNA -triggered type I interferon response apparently in TLR9-independent (and likely dependent on DAI-induced signaling - https://doi.org/10.1093/nar/gkz768) manner triggered just by electroporation/nucleofection and not by Turbofect-mediated transfection. The second communication published in The FEBS Journal deals with the essential predominantly ER membrane-attaching/perforating role hydrophobic domains HD1 and mainly HD2 in VP2 protein as hydrophilic substitutions in HD2 abolish its intracellular trafficking and thus replication/multiplication as well. The third Boris Ryabchenko's first author publication again in The FEBS Journal nicely dissects mechanisms of activation of type I interferons expression and signaling both in the nucleus and in cytoplasm. In the nucleus IFI16 homolog protein p204 recognizes likely replicating viral DNA and in the cytoplasm DNA sensing cyclic GMP-AMP synthase (cGAS) activate via STING-IRF3 axis expression of IFNβ. Interestingly, as the data obtained using cGAS knockout mouse fibroblasts documented, sensing from nucleus extruded viral DNA as well as along with replication stress released micronuclei by cGAS is essential for triggering interferon response in infected cells. The fourth recent publication in the Cancers journal deals with another aspect of MPyV-infected mouse fibroblasts – triggering IL-6 production in TLR4-dependent manner, which apparently transforms surrounding cells in cancer-associated fibroblasts (CAFs)-like cells with enhanced migratory phenotype.

<u>Questions & comments:</u>

1. In DNA Cell Biology publication is shown that even transient transfection of VP2 or VP3 capsid proteins triggers mouse fibroblast demise within 24 hrs and for this cell death inducing activity seems to be essential functional HD2 domain (The FEBS Journal paper). Is it known which cellular membrane is targeted by VP2/3 and which mode of regulated cell death (RCD) is triggered? Is RCD also triggered during viral infection of fibroblasts and does MPyV genome contains a RCD-modulating gene?
2. The 2021 FEBS Journal paper exploiting knockout cells nicely documents essential role of cGAS-triggered signaling in the activation of IFNβ expression in infected fibroblasts. cGAS is apparently in addition to cytoplasm also located in the nucleus, however in nucleus is likely

not active as nuclear levels of its product second messenger cGAMP are quite low. As cGAS knockout fibroblasts are defective in triggering IFNβ production it might be informative to re-express regular or nucleus-targeting (i.e. with added strong NLS) cGAS in these cells and analyze IFNβ production upon MPyV infection of these cells.

3. Does this cytoplasmic viral DNA/micronuclei trigger also other responses as AIM2-dependent IL-1b processing/pyroptosis or DAI-dependent RIP3 kinase activation and necroptotic signaling?
4. TLR4-induced IL-6 expression might in infected fibroblasts via Stat3 activation attenuate type I interferons-induced signaling (see https://doi.org/10.3389/fimmu.2019.01448). Did you or would you look at levels of e.g. IFNb and MX1 expression in MPyV – infected cells treated with TLR4 agonist or inhibitor?

In this formally unusual two-volume PhD Thesis Boris Ryabchenko presented interesting collection of data from MPyV life cycle and its interaction with cellular systems resulting if four publications in high quality journals. The published data, their relevant and well-presented discussion undoubtedly represent more than solid research achievement and strong foundation for flawless defense of this PhD Thesis and I believe well-deserved PhD title.

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In Vestec, 31.8.2021 RNDr. Ladislav Andera, CSc.