REVIEW OF PhD THESIS

Author: **HELENA FARKAŠOVÁ**
Title: **HOST-VIRUS INTERACTIONS OF MAMMALIAN ENDOGENOUS RETROVIRUSES**

The doctoral thesis submitted by Helena Farkašová presents her results obtained during the PhD studies in the Laboratory of Viral and Cellular Genetics under supervision of Daniel Elleder, MD, PhD. at the Institute of Molecular Genetics of the Academy of Science of the Czech Republic.
In the thesis, Helena Farkašová describes experiments by which she significantly contributed to the discovery and further studies of four endogenous retroviruses: identification and phylogenetic analysis of two new retroviruses (lentivirus ELVgv and deltaretrovirus MINERVa), analysis of virus-host interactions of the previously described but not fully characterized gammaretrovirus CrERV, and isolation of peptides interfering with virus entry in CHOK1 cells, originating from a putative new gammaretrovirus. The experiments resulted in four papers in peer-review journals with a high impact factor, with Helena Farkašová being the first author in two cases and a second author in the other two.

The first part of the thesis consists in a computational screen and identification of the two new endogenous retroviruses: an endogenous Lentivirus in Galeopterus variegatus – ELVgv, and a deltaretrovirus in the Miniopteridae bats – MINERVa (Miniopterus endogenous retrovirus). The presence of the viruses was confirmed by sequencing of selected parts of the genome of several individuals and related species. Using bioinformatics, phylogenetic trees were constructed and time of the insertion was estimated.

Major part of the thesis involves isolation of a molecular clone and study of virus-host interactions of the Cervid endogenous retrovirus – CrERV. First, the virus production from mule deer cells OHK by a co-culture experiment was repeated in a way similar to previously described Deer kindney virus – DKV, probably the identical retrovirus. The virus production was characterized by RT activity in the culture supenatant and then viral particles were isolated by a gradient centrifugation. A complete sequence was obtained by sequencing of the provirus from the infected human cells. Using bioinformatic tools, a similarity of this isolate to the other known copies of CrERV and its position in gammaretrovirus phylogeny were assessed. Further, integration site polymorphism was characterized in different animals and various Cervidae species.

Additional experiments were performed using an elegant approach consisting in the construction of a mutant virus, CrERV-mut, containing silent mutations to allow distinction of the incoming virus from the endogenous copies of CrERV in OHK cells. This mutant was able to infect and produce reverse transcripts in human HEK 293T cells, but not in OHK cells, the primary deer cells. From these experiments, it was concluded that the infection was blocked in the early stages of the virus replication cycle, at the level of receptor binding or entry into the cell, or at a later step before reverse transcription. However, nucleofection of the molecular clone nor infection with a VSV-G pseudotyped CrERV did not lead to virion release in OHK cells, suggesting also a possibility of a block in later stages of the virus replication cycle.

The last part of the thesis is dedicated to the analysis of the mechanism of Chinese Hamster ovary cells (CHOK1) resistance to virus infection. Virus-conditioned medium was fractionated and individual fractions tested for their inhibitory effects on amphotropic MLV replication. Fractions with a highest inhibitory activity were analyzed by mass spectrometry, leading to identification of peptides similar but not identical to envelope proteins of the FeLV. This result suggests a presence a new endogenous gammaretrovirus producing receptor-blocking peptides in CHOK1 cells.

The thesis is divided in classical sections with results and discussion being combined. The individual sections are subdivided in chapters according to the viruses. In general, the thesis is well written and there are not too many typographical errors. However, the content section does not include all the subchapters of the thesis. Additionally, I find the format of part 4.2.2.1 describing individual experiments with different viral pseudotypes as relatively odd. Finally, I would appreciate a short, specific introduction to individual experiments and a more elaborated discussion of individual topics.

I have several questions and comments:

CrERV was obtained by a co-culture with human cells without any specific induction or stimulation. Would it be possible to increase virus production by some kind of stimulation? Is it known what affects the extent of virus release? Could the low level of virus production suggest existence of a block at the later step, after the provirus formation, or it would rather reflect a low number of proviral sequences capable of expression?

The time-course of infection with CrERV and PERV was quite comparable, but the level of infection was much lower in CrERV (m.o.i. suggested to be less than 0.001). Then, a 30-day infection resulted in about 1 copy of env/cell, as determined in the whole cell lysates (Fig. 26). Since the primers used were specific for env, as described in the thesis, it seems that the number corresponds to the total number of DNA copies. Therefore, the number of infected cells with the integrated provirus was probably much lower. Would you please comment? Also in respect to the receptor interference suspected to take place in experiments in chronically infected cells?

It was mentioned in the text that the titer of CrERV-ind was low. Was it also the case for CrERV-mut? How were the virus stocks grown, isolated and titrated? What was their final titer and fitness? Could you please comment on the differences and the importance for the experimental outcome among determination of RT activity, viral antigen concentration and virus titer?

What was the viability, growth rate and metabolic state of OHK cells before the infection in the individual experiments? Were the cells split or treated with a fresh serum and medium in a constant time before the infection to assure their comparable state?

CrERV-mut was able to infect and produce reverse transcripts of the pol gene in human cells HEK 293T, but not in the mule deer cells OHK cells nor in chronically infected human cells. I would like to know if you have characterized a longer time course in human cells after the acute infection and what was the percentage of

infected cells with an integrated provirus. Was there any clonal selection? On the other hand, do you know if chronically infected human cells might produce interferons or virus-induced factors other than those hypothesized to cause receptor interference?

Despite the fact that the virus obtained by co-culture was able to replicate in human cells, is it possible that it would still contain a mutation that is not important in human cells but would be critical in mule deer cells OHK? E.g. some endogenous factor might compensate for the mutation in human cells but not in mule deer cells.

Did you try to determine virus adsorption on the cells?

In the introduction, you mention that the major disadvantage for the host cell is when the provirus integrates into the exon or intron in the plus orientation and therefore it disrupts expression of the endogenous gene or causes an aberrant splicing. However, this type of insertion should not be advantageous for the virus either. Still, HIV-1 is known to preferentially integrate in the actively transcribed genes, often resulting in transcriptional interference and silencing of the virus. Could you please compare characteristics of the integration sites for an endogenous and an exogenous virus?

In summary, the thesis of Helena Farkašová includes original data published in good 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.

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