Abstract

The course of polyomavirus infection can be regulated in the nucleus by a number of cellular proteins. Replication of mouse polyomavirus (MPyV) in the cell nucleus occurs in close proximity to PML nuclear bodies, which also contain the DAXX protein. DAXX is a restriction factor for many viruses, but its positive effect on the replication of some herpesviruses and papillomaviruses has also been described. The first part of this work focused on the influence of DAXX protein on MPyV replication cycle. A decrease in viral DNA replication and lower levels of both early and late viral transcripts were observed in cells with suppressed DAXX expression. The results indicate a positive effect of DAXX protein on MPyV replication. Another goal was to prepare a *DAXX* KO cell line to study the effect of DAXX on BK polyomavirus infection. The modification was carried out using the CRISPR/Cas9 system, and potential *DAXX* KO cell clones were isolated, in which the deletion of the *DAXX* gene will be further verified.

The second part of this thesis was focused on the interactions of MPyV large T antigen (LT) with cellular proteins. Based on the data obtained from the study of MPyV LT antigen interactome (experiments performed by Mgr. Karolína Štaflová, Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic, group of Ing. Iva Pichová, CSc.) the mutual localization of LT and selected cellular proteins was examined. Colocalization of LT with BAF57, BAG2, PRC1, WDR48 and MKK3 proteins was observed. In the case of NONO and SMC4 proteins, colocalization did not occur. From the listed interaction partners of LT, we further focused on the MKK3 kinase, which is part of the p38 MAP kinase pathway. MKK3 colocalized with LT in both infected and uninfected cells expressing LT. A reduced amount of MKK3 protein and, conversely, an increased activation of p38 kinase were detected in infected cells. Knockdown of MKK3 expression by siRNA did not affect the number of cells infected with MPyV or the amount of infectious virions produced. The results suggest that MKK3 kinase does not have a significant effect on MPyV infection.

Keywords: mouse polyomavirus, DAXX, large T antigen, MKK3, MAP kinase, p38