

# REGULATION OF BK VIRUS DNA REPLICATION BY TRANSCRIPTION FACTORS AND NONCODING RNAs

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## ABSTRACT

Human polyomavirus BK (BKV) asymptotically infects 80~90% of people during early childhood, and establishes a life-long persistent infection without causing overt clinical symptoms. High level BKV replication occurs predominantly in urogenital tracts of immune-suppressed patients following renal transplantation and bone marrow transplantation, which cause polyomavirus associated nephropathy (PVAN) and hemorrhagic cystitis, respectively. PVAN has become a leading cause for renal transplantation failures since tacrolimus and mycophenolate mofetil began to be widely used in transplantation patients in 1995. Epidemiological studies indicate that around 30% of kidney transplantation patients are at risk of developing PVAN and 50% of PVAN patients are at risk of transplantation failure.

This dissertation explores the mechanisms by which the genomic noncoding control region (NCCR) regulates BKV DNA replication in cell culture and might be related to the establishment of BKV persistent infection and pathogenesis of PVAN.

These studies indicate that cellular transcription factor NFI interacts with the BKV NCCR and stimulates BKV DNA replication *in vivo* and *in vitro*. Also, the data reveal that isotypes NFIA and NFIB strongly interact with BKV large T antigen (Tag) and NFIC interacts with DNA polymerase- $\alpha$  primase (pol- $\alpha$  primase), suggesting NFI-family transcription factors may stimulate BKV DNA replication through recruitment of Tag and pol- $\alpha$  primase. In contrast, ectopic expression of PCAF/GCN5 histone acetyltransferases inhibit BKV DNA replication. Tag has a site

for acetylation by PCAF/GCN5, but inhibition of BKV DNA replication by PCAF/GCN5 is not due to acetylation of Tag, suggesting PCAF/GCN5 target other component(s) of DNA replication machinery. Possible targets include nucleosomes associated with the NCCR and other components of the replication machinery. A search for these targets is proposed, and possible functions of acetylation on BKV Tag are discussed.

BKV DNA does not replicate in murine cells. We and our collaborators have found that this host-restriction of BKV DNA replication involves not only incompatibility of BKV Tag with mouse pol- $\alpha$  primase, but also inhibitory small noncoding RNAs in murine cells, termed srRNAs, that act through BKV NCCR. Specific srRNAs were sequenced and cloned. *In vitro* transcribed srRNAs inhibit BKV replication *in vitro*; and ectopic expression of a specific srRNA strongly inhibits BKV DNA replication *in vivo* in human cells. Surprisingly, srRNAs from human cancer cells stimulate BKV DNA replication *in vitro*, suggesting cell type specific expression of srRNAs has distinct role in regulation of BKV DNA replication. We propose that differential expression of srRNAs may have implication in the viral tropism, establishment of persistent infection and reactivation.