BOVINE LEUKEMIA VIRUS ANTISENSE TRANSCRIPTION REGULATES VIRAL REPLICATION, AFFECTS GENE EXPRESSION AND DIRECTS HOST CELL FATE

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Background:
The BLV model contributes to a better understanding of the mechanisms of HTLV-1 induced diseases. While only non-human primates indeed recapitulate most aspects of viral infection, replication and pathogenesis, ethical restrictions and biological constraints are limitations of these models. In this context, BLV infection of sheep may be helpful as all animals develop leukemia within a mean latency period of 2 years.

The objective of this study is to address the following question: "Is antisense transcription required for viral gene expression, infection, replication and pathogenesis?"

Methods:
A reverse genetics approach generated BLV mutants with impaired antisense transcription. The mutations exploit a mechanistic difference in transcription initiation between the 5' and 3' promoters. A series of mutants were constructed, and bi-directional promoter activities were assessed by dual luciferase-based assays and RT-qPCR. Cell fusion infectivity was evaluated through syncytia formation with CC81 cells and with OVK cells transduced with a LTR-luciferase reporter. After inoculation into sheep, proviral replication was characterized by qPCR, RT-qPCR, confocal microscopy, flow cytometry. The transcriptome of peripheral blood B cells was determined by RNA sequencing. Immunochemistry was performed with lymphoid organs.

Results:
A single, two-nucleotide mutation (AS1) efficiently inhibited 3'LTR promoter activity and antisense transcription. This mutation did significantly affect neither sense expression nor cell-fusion ability. The AS1 mutant was infectious upon inoculation of sheep. However, viral replication in peripheral blood and lymphoid organs was significantly reduced compared to wild-type (WT) levels. RNA sequencing and bioinformatics characterized the molecular pathways associated with impaired antisense transcription. Among the differentially expressed genes, the hemicentin gene transcription was mostly affected. Immunohistochemistry analyzes revealed that the infection by the AS1 mutant is associated with increased proliferation and reduced migration to lymphoid organs.

Conclusion:
We have demonstrated the key role of antisense transcription in viral replication, cell proliferation and homing.

Disclosure of Interest Statement:
Nothing to disclose.