DISCOVERY AND FUNCTION OF microRNAs ENCODED BY HUMAN POLYOMAVIRUSES

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INTRODUCTION. We have previously shown that the primate polyomavirus, SV40, encodes a pre-microRNA (pre-miRNA) late during infection that is processed into multiple miRNAs (1, 2). The SV40-encoded miRNAs function to downregulate early viral gene expression by directing their RISC-mediated cleavage. JCV and BKV are human homologs of SV40 that cause disease in immunocompromised individuals; BKV induces nephritis in some kidney transplant recipients and JCV causes progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease found in patients suffering with late stages of AIDS (3). We demonstrate that JCV and BKV encode miRNAs functionally conserved with the SV40 miRNAs.

METHODS. Using vMir2.1 (1), a pre-miRNA prediction algorithm, we obtained candidate pre-miRNAs from the genomes of BKV and JCV using a vMir cutoff score of 175. We harvested total RNA from BKV and JCV infected cells as well as PML specimens obtained posthumously. Samples were run on 15% TBE-Urea polyacrylamide gels, transferred, and hybridized with radio-labeled probes against the predicted miRNAs. miRNA mediated cleavage fragments from JCV and BKV were identified by modified RLM-5’-RACE protocol. Briefly, a 5’RACE adaptor was ligated to total RNA from infected cells. Reverse transcription followed by nested PCR was performed using 5’ RACE adaptor primers and target JCV and BKV primers. PCR products were cloned using TOPO Cloning Kit (Invitrogen). Twelve clones each from JCV and BKV were sequenced.

RESULTS. Of the four predicted BKV and two JCV pre-miRNAs, one pre-miRNA from each virus was confirmed by northern blot to generate miRNAs. Both the 5’ and 3’ arms of the pre-miRNA give rise to mature miRNAs demonstrating striking similarities to the SV40 miRNAs. Control probes against the terminal loops and flanking regions confirm that the observed bands are not
non-specific degradation products but in fact are bona fide miRNAs. Both pre-
mirnas are encoded on the late strand of their respective genomes and are
perfectly complimentary to early transcripts. To test whether both arms are
functionally active at cleaving early transcripts, 5’RACE analysis was performed
to map the 5’ ends of the predicted cleavage fragments. For both viruses, 11/12
clones mapped the 5’ends to the middle of the miRNA homology regions, with
the more abundant 5P miRNA exhibiting the most activity. Finally, JCV
miRNAs were detected in three out of four brain samples of JCV+ patients
diagnosed with PML. These data suggest a likely a role for the JCV miRNAs in
PML progression.

**DISCUSSION.** Using a combined computational/Northern blot approach, we
now show that the human polyomaviruses BKV and JCV also encode miRNAs.
These miRNAs share several atypical properties in common with their homologs
encoded by SV40, including robust expression levels and a high ratio of pre-
mirna:miRNA. We show that the miRNAs generated from both arms of the pre-
mirna hairpin are active at directing the cleavage of the early mRNAs. These
results suggest, that despite being only ~60% identical to the SV40 miRNAs, the
primary target (the early mRNAs) and function (downregulation of early gene
expression) are evolutionarily conserved amongst the primate polyomaviruses.
Lastly, we show that PML lesions from human brain tissue express robust
amounts of the JCV mirna, comparable to the levels detected in cultured cells
infected *in vitro*. This result validates the conclusions drawn from our in vitro
studies and suggests that the JCV miRNA is a candidate target for future
therapeutic intervention.

**REFERENCES**
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