Viral Predictors of BK Polyomavirus Associated Hemorrhagic Cystitis

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Introduction: BK Polyomavirus (BKPyV) associated hemorrhagic cystitis (HC) is a common complication in individuals who have received a hematopoietic stem cell transplant. Interestingly, though viruria occurs in 80% of these individuals, only 25% will go on to develop hemorrhagic cystitis. The mechanisms underlying these variable clinical outcomes remain poorly understood. Multiple studies of other viruses have demonstrated that genetic variation within a viral genome can result in considerable variation in pathogenic potential, host cell tropism, and replication capacity. Further investigation is needed to uncover how variation within the BKPyV genome impacts its propensity to cause HC. To this end, a recent study by our group utilized a novel method to determine circulating BKPyV genotype prevalence in a cohort of children who received an allogeneic hematopoietic stem cell transplant. The current study utilized the same method to determine BKPyV genotypes in a separate validation cohort.

Methods: Viral DNA was extracted from urine samples obtained from a cohort of adolescents who received an allogeneic hematopoietic stem cell transplant at the Cincinnati Children's Hospital and the Children's Hospital of Philadelphia. Any circular DNA present in the sample was amplified by rolling circle amplification and then linearized in preparation for polymerase chain reaction (PCR) amplification. PCR amplification of the full-length BKPyV genome was attempted first. Then, a nested PCR amplification of the portion of the BKPyV genome encoding viral protein 1 (VP1) was performed if the full-length PCR was negative. PCR products were separated by size using gel electrophoresis. DNA bands corresponding to the full-length BKPyV genome or the VP1 portion of the genome were purified from the agarose gel and sent for next-generation sequencing (NGS). Phylogenetic analysis of the viral sequences was performed using Unipro UGENE, Clustal X, and NJPlot to determine the viral genotype.

Results: 62 urine samples from the current cohort were fully processed. Of these, 24 (39%) were positive for the full-length BKPyV genome. One additional sample was positive for only the VP1 portion of the genome, yielding a total positive rate of 25/62 (40%). All 25 samples are currently being processed for next-generation sequencing. Sequence data will be analyzed to determine genotypes once NGS data are available.

Conclusion: BKPyV DNA was able to be amplified from 40% of the 62 urine samples processed. Phylogenetic analysis will reveal if the circulating genotypes in this current cohort validate the findings of the aforementioned previous study by our group.

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