BK virus genotypes associated with hemorrhagic cystitis in post-HSCT pediatric patients

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Introduction: BK polyomavirus (BKPyV) is a widely seroprevalent virus that remains asymptomatic in healthy individuals but causes symptomatic disease under immunosuppression and is associated with the development of hemorrhagic cystitis (HC) after hematopoietic stem cell transplant (HSCT). Previous studies suggest differences in subtype specific genotypes of BKPyV that precede HC.

Methods: To investigate potential viral genetic factors linked to HC, urine samples were collected 30 days post-allogeneic HSCT from pediatric patients at CCHMC/CHOP with and without HC. From each sample, viral load was obtained. DNA was extracted, amplified using an in-house double-amplification technique including rolling circle amplification and full-length (FL) PCR, linearized, and run through gel electrophoresis to visualize a 5kb band. Bands were excised and extracted from gel. Nested Viral Protein 1 (VP1) PCR was performed on samples without visible 5kb bands. 1.5kb bands were excised and extracted from gel. Gel extractions were sequenced using next-generation sequencing (NGS). Raw reads were aligned in UGENE to generate consensus sequences and viral subtype was determined using phylogenetic tree analysis in ClustalX. Statistical analysis was performed to determine subtype differences in patients with and without HC. APOBEC3-induced hypermutations and signature patterns were detected using Hypermut and VESPA, respectively.

Results: 40 urine samples were collected. 31 samples (77.5%) were amplified (28 FL and 4 VP1 region genomes). Phylogenetic tree analysis showed that 24 (77.4%) genomes were subtype Ia and 7 (22.6%) genomes were subtype Ib1. 9 samples (1 subtype Ib1, 8 subtype Ia) developed HC. Odds ratio with relative risk was performed for subtype diversity in HC onset. Subtype Ia exhibited higher incidence of HC compared to subtype Ib1 (RR = 2.33, OR = 3.0, Fisher's exact p = 0.639). Logistic regression revealed that subtype Ib1 yields 67% lower odds of developing HC than subtype Ia (OR = 0.33, 95% CI: 0.03–3.26, p = 0.345). These results necessitate further validation with larger sample sizes, as p-values do not support statistical significance. Two-tailed T-test analysis showed that urine viral load did not differ between subtypes 1a and 1b1 (M_{1a}: 1.58×10^8, M_{1b1}: 2.01×10^8; t = -0.31, p = 0.764), but logistic regression showed that for each 10x increase in viral load, HC risk increased by 85% (OR = 1.85, 95% CI: 1.16–2.96, p = 0.010). APOBEC3 mutations were detected in the complete genome, as well as within VP1 and TAg regions. Signature patterns were detected with VESPA. This study contributed 31 individual genomes to the BK genome database and provided foundations for future studies of subtype-specific pathogenicity and therapeutic developments.

Conclusion: A nonsignificant association between BKPyV subtype and HC onset suggests a potential trend that warrants validation in larger cohorts.

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