CHARACTERIZATION OF CIRC-VIRF4 IN KAPOSI'S SARCOMA-ASSOCIATED HERPESVIRUS

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Circular RNA (circRNA) is a class of RNAs that are single-stranded and form a closed structure via backsplicing, a process that covalently joins the 5' and 3' ends of exons. CircRNAs are relatively stable, resistant to exonucleases, and believed to be implicated in gene regulation and diseases, including multiple types of cancers. Recent studies using RNase R-seq revealed the circRNAome of Epstein Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV), as well as interactions between KSHV and host circRNAs. The KSHV viral interferon regulatory factor 4 (vIRF4) region expresses a protein and two circRNA isoforms with high expression in KSHV tumors, suggesting that circ-vIRF4 may contribute to KSHV pathogenesis and/or tumorigenesis. To characterize the function of circ-vIRF4, a KSHV mutant lacking the splice donor site (Δcirc-vIRF4) was generated in the BAC16 bacmid. RT-PCR of Δcirc-vIRF4-infected iSLK cells shows that wild type isoforms are not detectable, but cloning of products from Δcirc-vIRF4 suggests that alternative backsplice sites are used to express novel vIRF4 circRNAs. RNA-seq analyses comparing WT- and Δcirc-vIRF4-infected iSLK cells during either latent or lytic replication demonstrated differential expression of both host and viral genes. KSHV gene expression was generally upregulated in the lytic libraries, with some of the top upregulated genes belonging to the immediate early category of lytic genes. Gene ontology (GO) analysis of the top 50 differentially expressed host genes indicated roles in signal transduction, cell cycle, and apoptosis. Preliminary analysis of latent libraries shows differential gene expression occurs primarily in host genes, with the GO analysis returning terms related to cell adhesion, cell differentiation, and cell development. These results suggest that a necessity exists for KSHV to express circRNA from the vIRF4 locus and numerous possibilities for circ-vIRF4 to regulate KSHV infection. Work is ongoing to validate and study genes of interest from both gene expression analyses. Lastly, a secondary structure analysis of circ-vIRF4 is currently in-progress via the use of self-splicing, T4 td permuted intron-exon (PIE) constructs that yield full-length circRNA of both circ-vIRF4 isoforms.