Targeting the Kaposi’s Sarcoma-associated Herpesvirus Genome With the CRISPR-Cas9 Platform in Latently Infected Cells

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Research

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Abstract

Background: Kaposi’s sarcoma-associated herpesvirus (KSHV) is a transforming gammaherpes. Like other herpesviruses, KSHV infection is for life long and there is no treatment that can cure of patients from the virus. In addition, there is urgent need to target viral genes to study their role during the infection cycle. The CRISPR-Cas9 technology offers a means to target viral genomes and thus may offer a novel strategy for viral cure as well for better understanding of the infection process. We evaluated the suitability of this platform for the targeting of KSHV.

Methods: We have used BAC16 genome, which contains an expression cassette encoding hygromycin-resistance and a GFP marker gene. Three genes were targeted: gfp which serves as a marker for infection; orf45 encoding a lytic viral protein; and orf73, encoding LANA which is crucial for latent infection. The fraction of cells expressing GFP as well as viral DNA levels and LANA expression were monitored and viral genomes were sequenced.

Results: We found that KSHV episomes can be targeted by CRISPR-Cas9. Interestingly, the quantity of KSHV DNA declined, even when target sites were not functionally important for latency. In addition, we show that antibiotic selection, used to maintain infection, interferes with the outcome of targeting.

Conclusions: Our study provides insights to the use of this fundamental approach for the study and manipulation of KSHV. It provides guidelines for the targeting CRISPR-Cas9 to the viral genome and for outcomes interpretation.

Full Text

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