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

This preprint is available for download as a PDF.

Figures

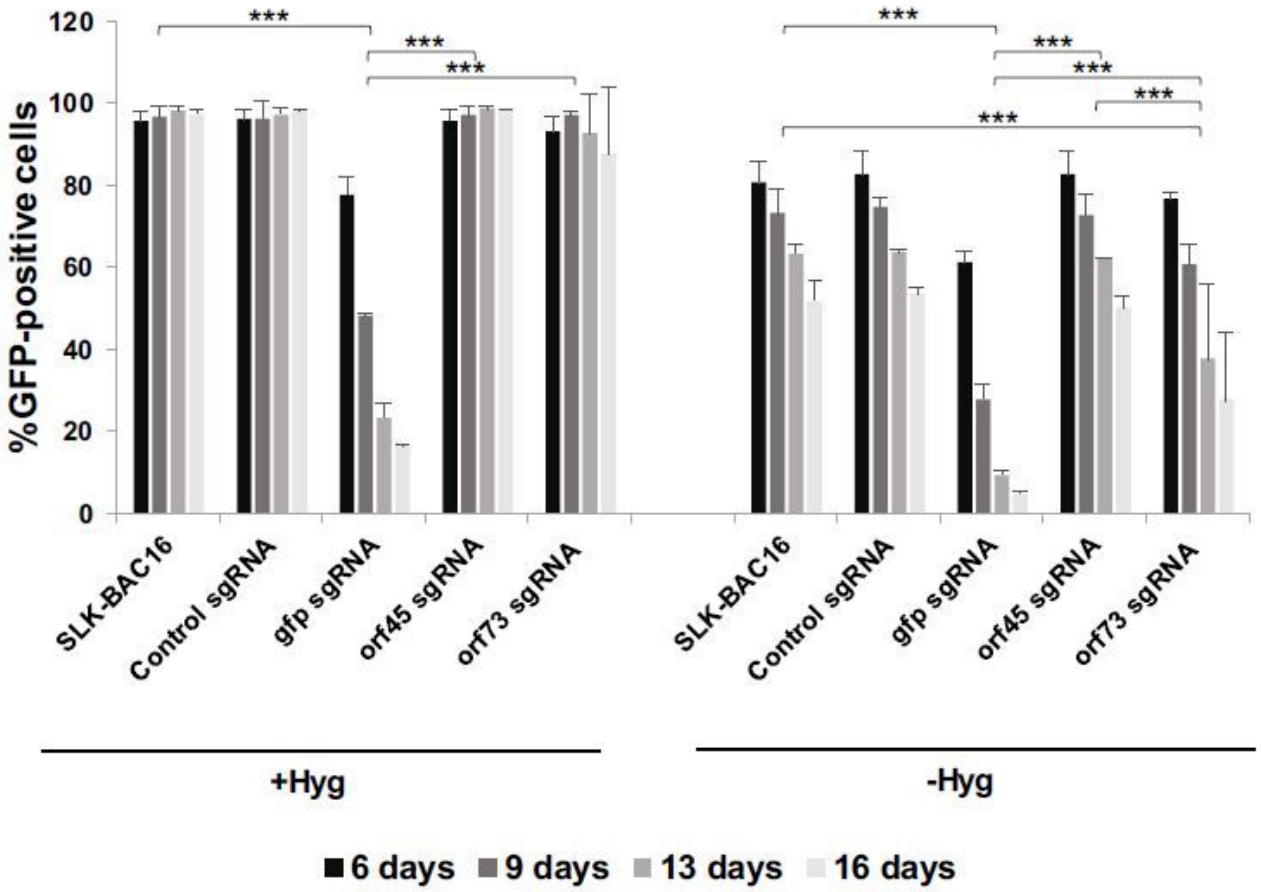

Figure 1

GFP expression in SLK-BAC16-mCherryORF45-infected cells following targeting of selected genes by CRISPR-Cas9. BAC16-mCherryORF45-infected SLK cells expressing Cas9 were transduced with recombinant lentiviruses encoding a random non-targeting sgRNA (control sgRNA) or sgRNAs targeting gfp, orf45 and orf73. Each gene was targeted with a combination of two guides. Transduced cells were selected with 1µg/ml puromycin and were grown in the absence or presence of 600 µg/ml hygromycin. Cells were harvested 6, 9, 13 and 16-hr post transduction, and GFP expression was monitored by FACS analysis. Mock treated SLK-BAC16 cells that were either maintained in the presence or absence of hygromycin were used as controls. *p<0.05, **p<0.01, ***p<0.001.

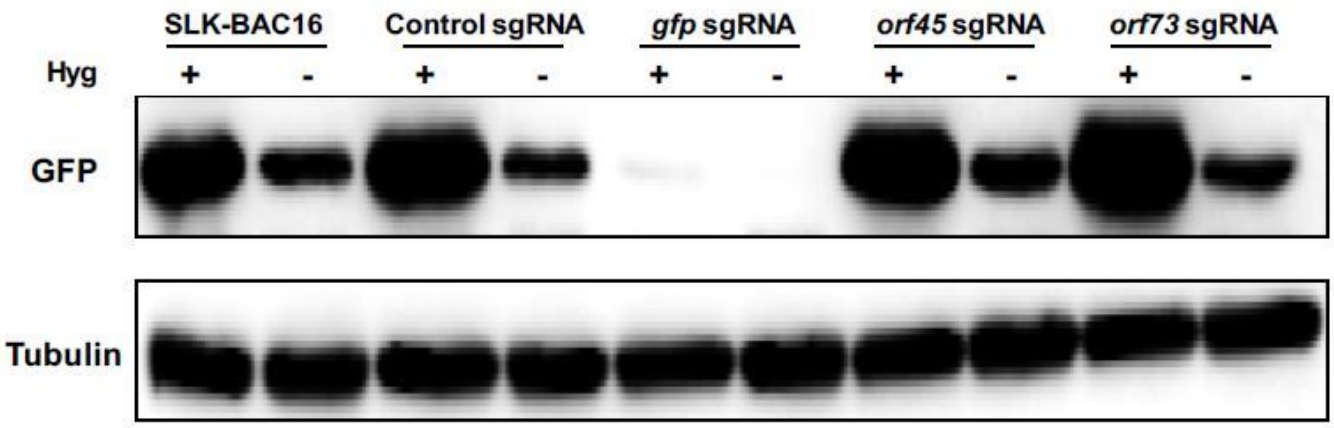

Figure 2

Western blot analysis of GFP in SLK-BAC16-mCherry-ORF45-infected cells 16 days following targeting of selected genes by CRISPR-Cas9. BAC16-mCherry-ORF45-infected SLK cells expressing Cas9 were treated as described in Fig. 1. On day 16 post transduction, cells were collected, and protein extracts were prepared. Samples containing 40 µg of protein were used to determine GFP protein expression; anti-Tubulin was used as a loading control.

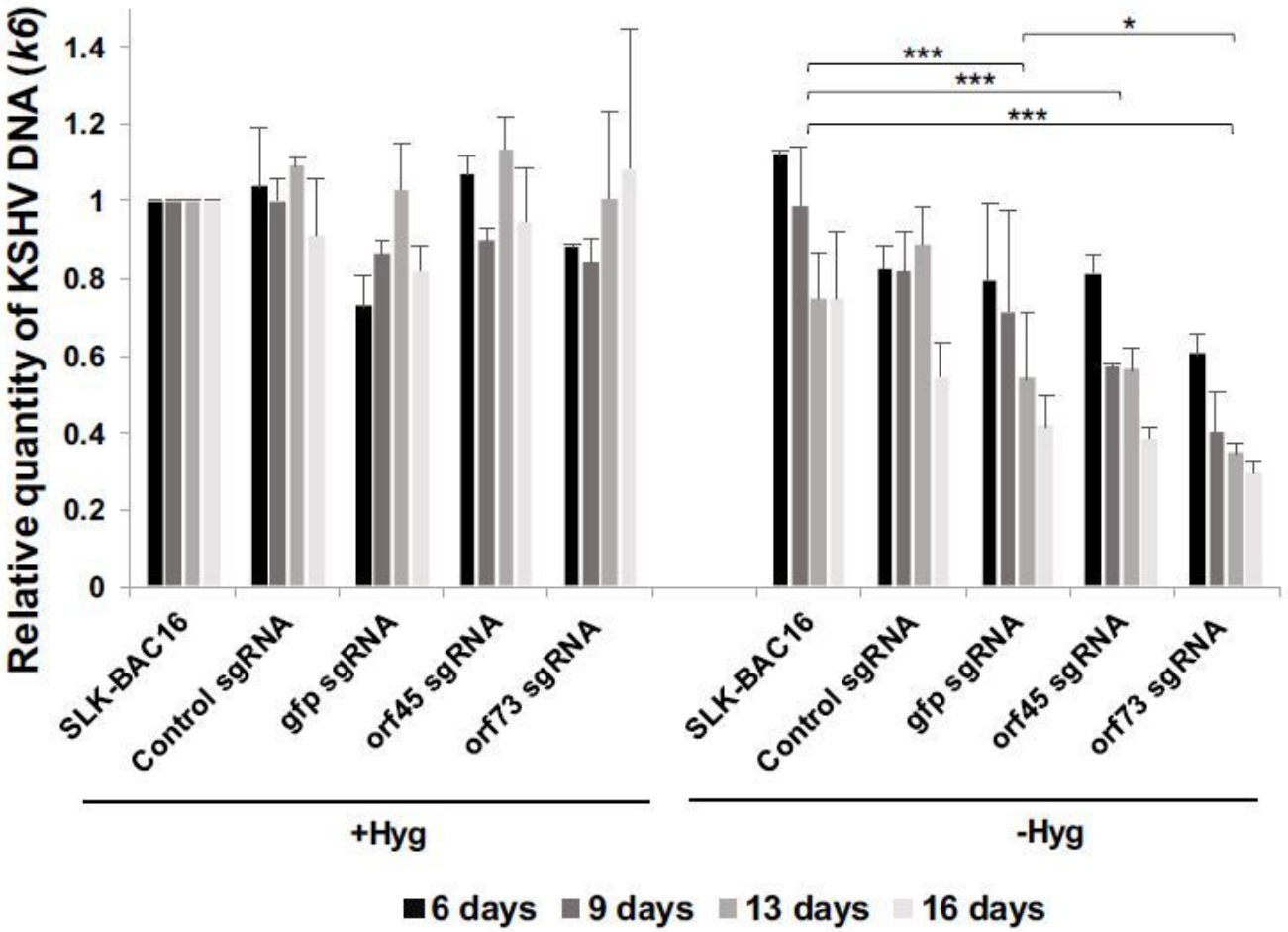

Figure 3

Viral DNA quantification following CRISPR-Cas9 targeting of gfp, orf45 or orf73. BAC16-mCherry-ORF45-infected SLK cells expressing Cas9 were treated as described in Fig. 1. DNA was extracted and subjected to TaqMan real-time PCR for viral DNA using primers that target the viral orfK6 gene along with the cellular erv-1 gene, which was used to normalize loading. *p<0.05, ***p<0.001.

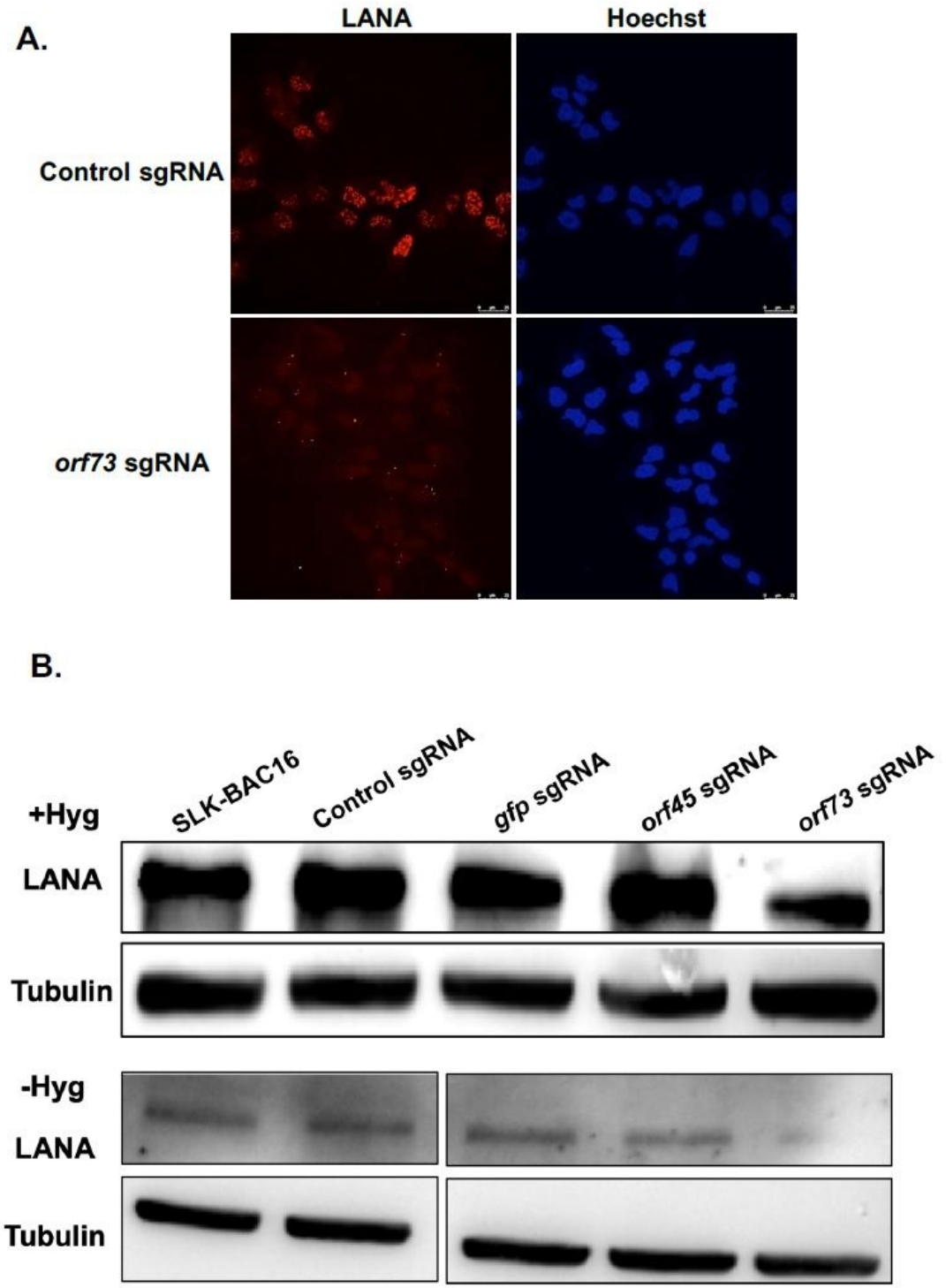

Figure 4

Visualization of LANA dots and Western blot analysis of LANA 16 days following targeting of orf73 by CRISPR-Cas9. Cas9-BAC16-mCherryORF45-infected SLK cells were transduced with recombinant lentiviruses expressing a random non-targeting sgRNA or orf73 sgRNAs. Cells were maintained with no hygromycin selection, fixed 16 days post transduction and stained with rat monoclonal anti-LANA followed by anti-rat Alexa Fluor 647-conjugated secondary antibodies. The corresponding staining of

nuclear DNA by Hoechst is also displayed (A). BAC16-mCherryORF45-infected SLK cells expressing Cas9 were treated as described in Fig. 1. Cells were collected 16 days post transduction, and protein extracts were prepared. Samples containing 60 µg of protein extracts from cells that were maintained with hygromycin and with no 17 hygromycin selection, respectively, were used to determine LANA protein expression while Tubulin was used as loading control (B).

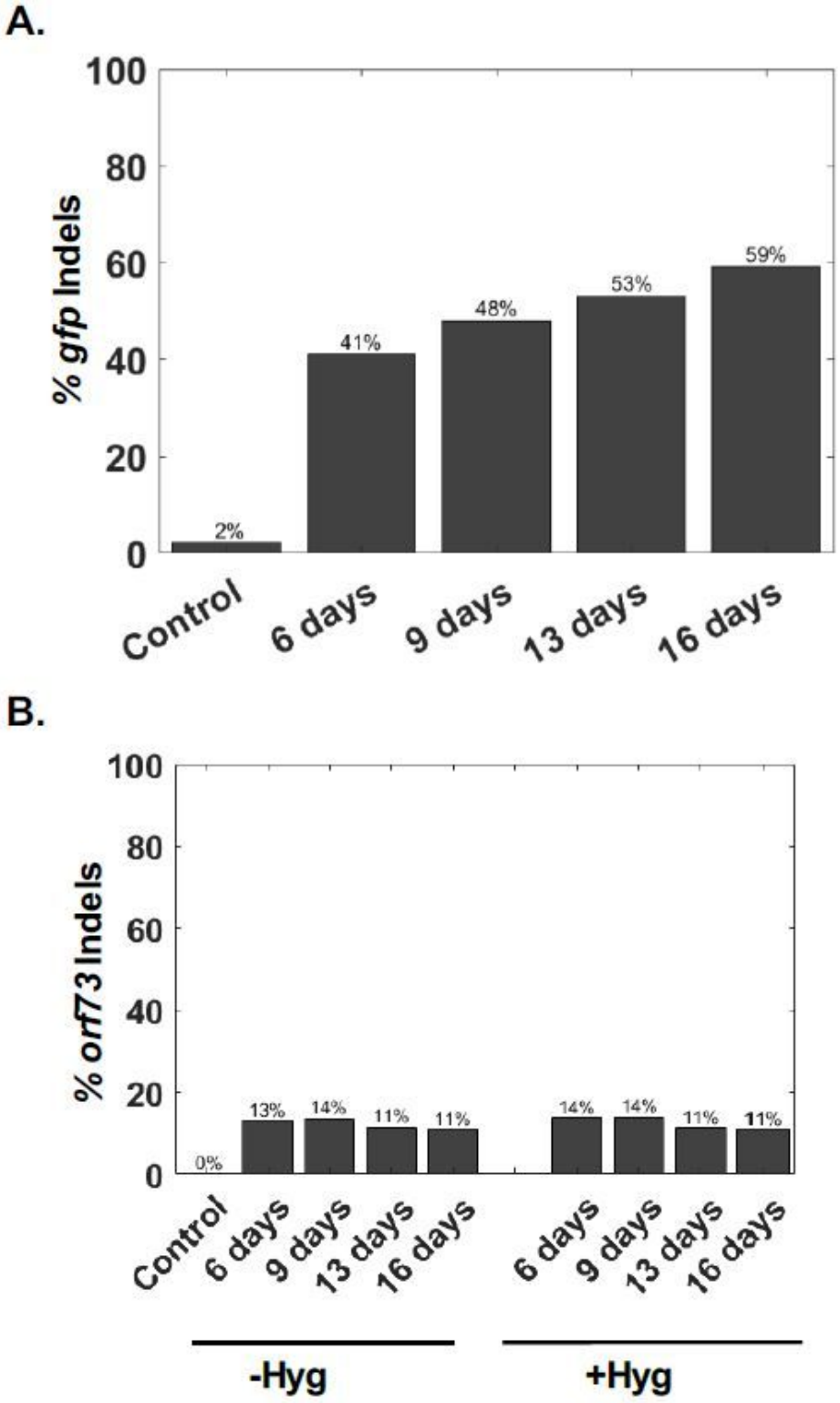

Figure 5

Sequencing the episome CRISPR-Cas9 cut sites. To map insertions and deletions (indls), we amplified genomic DNA and subjected it to deep sequencing. The percentage on the y axis represents the total number of indels divided by the total numbers of reads. The identities of the samples are indicated on the x axis, including the name of the gene and the number of days post BAC insertion. Percentage of indels around the cutting site in gfp (A). Percentage of indels around the cutting site in orf73 (B).