Cellular Requirements for KSHV Latent Infection

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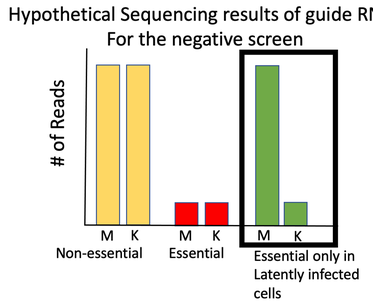
Abstract

Kaposi Sarcoma (KS) is one of the most common tumors of AIDS patients and in central Africa. Kaposi Sarcoma-associated herpes virus (KSHV) was discovered as the cause of KS in 1994. All herpesviruses have both lytic and latent phases. During latency, , the primary infection state of KSHV in KS tumor cells, only a few viral genes of the over 100 total are expressed. Due to this limited viral gene expression, we are targeting cellular requirements of latent infection. Previously, the Lagunoff Lab performed a genome wide CRISPR-Cas9 screen with 18,000 human genes, identifying cellular genes essential only to cells latently infected with KSHV. My project aims to validate the top scoring genes from the screen by single knockout of the target gene through cloning of guide RNAs (gRNA) into pRRL plasmid with CRISPR-Cas9 machinery. I hypothesize that many of the genes that were highly scored in the screen are essential for KSHV latently infected cells, but not mock infected and will be good therapeutic targets for KS tumors. Several gRNAs have been successfully cloned so far, including those targeting DUSP5, CMKLR1 and WWC3. These cloned plasmids will be used to make lentivirus, which will be transduced into Tert-Immortalized Microvascular Endothelial (TIME) cells. These TIME cells will then be either mock or KSHV infected, and differences in viability will be observed to validate the gene as essential to KSHV latency.

Introduction

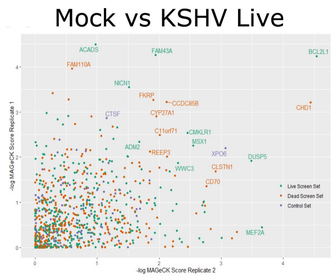
Kaposi Sarcoma (KS) is a cancer that causes lesions in soft tissue and was first reported in 1872 by Moritz Kaposi. It is organized into four types- classical, endemic, iatrogenic and AIDS-related. In 1994, through representational difference analysis, Kaposi Sarcoma-associated herpes virus (KSHV) was discovered to be the cause of KS. While all herpes virus have two states of infection- lytic and latent- the primary state of infection for KSHV is latent. During latency, viral gene expression is limited, there is no cytotoxicity and virus is not released. The low viral gene expression make targeting viral genes difficult, hence the focus on cellular requirements of KSHV latent infection.

CRISPR-Cas9 Screen



Tert-Immortalized Microvascular Endothelial (TIME) cells were transduced using lentivirus encoding Cas9 to knock out a pool of 18,000 human genes. The TIME cells were then either mock or KSHV infected, and both live and dead cells were sequenced after 8 days for gRNA counts. The genes essential only in latently infected cells were identified by low gRNA counts in KSHV infected cells but high gRNA counts in mock infected cells for the live cell screen, and vise versa for dead cell screen.

CRISPR-Cas9 Subpool Screen



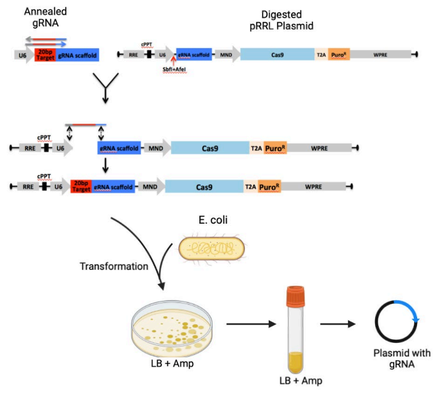
The top 350 hits from the live cell screen and the top 350 hits from the dead cell screen were then screened again in two replicates. The graph shows two different replicates independent of each other on each axis.

To validate the CRISPR-Cas9 screen identified genes, the gRNA to the genes were cloned into plasmids to be used to knock out the target gene in TIME cells.

Hypothesis

Genes that were highly scored in the CRISPR-Cas9 screen are essential for KSHV latently infected cells, but not mock infected and will be good therapeutic targets for KS tumors.

Method: Cloning



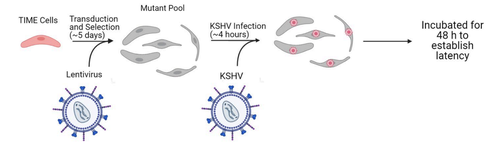
- Target gRNA from CRISPR-Cas9 screen annealed
- pRRL plasmids containing ampicillin resistance and Cas9 linearized through restriction digest
- Annealed gRNA inserted into plasmid through in-fusion cloning
- E. coli transformed with plasmid then grown on LB containing carbenicillin plates for selection
- Single colony isolated and grown overnight on LB containing ampicillin media
- Plasmid isolated from cells and sequenced to confirm gRNA presence

Genes Successfully Cloned

Gene	Function
DUSP5	Phosphatase that negatively regulates cell proliferation
CMKLR1	Receptor that negatively regulates cell survival, proliferation and differentiation
WWC3	Inhibits cell proliferation

Future Directions

To continue to validate the results from the CRISPR-Cas9 screen, the cloned plasmid will be used to make lentivirus, which will be used to transduce TIME cells to knock out the target gene. The mutant TIME cells can then be infected with either mock or KSHV to compare cell viability



Acknowledgements

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Cesarman E, Damania B, Krown SE, Martin J, Bower M, Whitby D. Kaposi sarcoma. Nat Rev Dis Primers. 2019 Jan 31;5(1):9. doi: 10.1038/s41572-019-0060-9. PMID: 30705286; PMCID: PMC6685213.

Holmes, D. L., Vogt, D. T., & Lagunoff, M. (2020). A CRISPR-Cas9 screen identifies mitochondrial translation as an essential process in latent KSHV infection of human endothelial cells. *Proceedings of the National Academy of Sciences*, *117*(45), 28384–28392. https://doi.org/10.1073/pnas.2011645117

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