CONSTRUCTION OF A KSHV INFECTED TELOMERASE IMMORTALIZED ENDOTHELIAL CELL LINE PERMISSIVE OF ROBUST LYTIC VIRUS REPLICATION AND VIRUS PRODUCTION

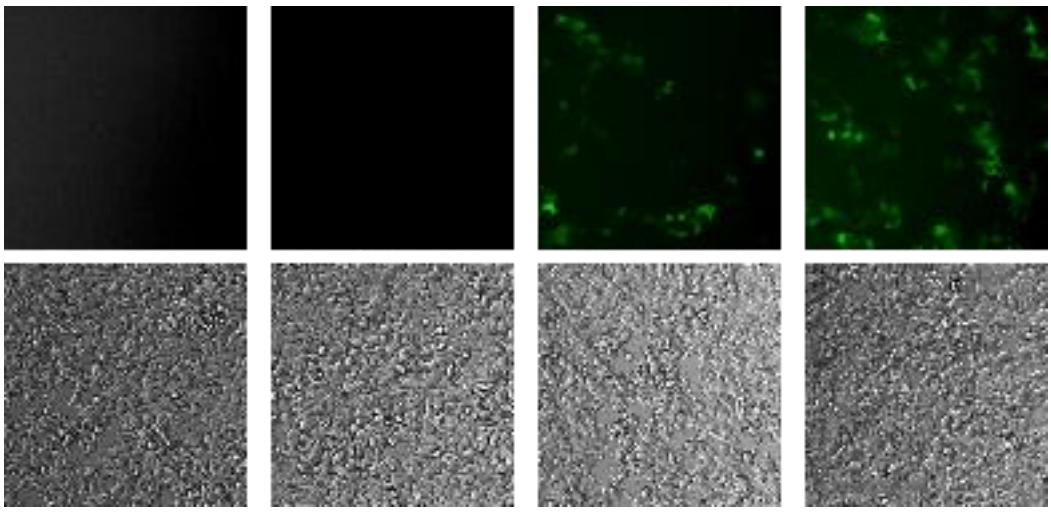
Zola Wagner[1], Dinesh Verma[1], Trenton Church[1], Rolf Renne[2] and Sankar Swaminathan[1]

[1]Division of Infectious Diseases, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT, 84132 USA, Department of Microbiology and Molecular Genetics, University of Florida, Gainesville, FL 32611

sankar.swaminathan@hsc.utah.edu

Endothelial cell lines stably infected with Kaposi's sarcoma associated herpesvirus (KSHV) are tightly latent in contrast to KSHV infected primary effusion lymphoma derived B lymphocyte lines. While an epithelial cell line transduced with an inducible KSHV transactivator gene, Rta (ORF50), is permissive of KSHV lytic reactivation, no endothelial cell systems to study KSHV lytic replication currently exist. Endothelial cells are the cell type naturally infected by KSHV and are the origin of the pathognomonic spindle cell of Kaposi's sarcoma (KS). KSHV lytic replication and expression of lytic gene products in endothelial cells is also thought to contribute to the malignant phenotype of KS.

We therefore set out to generate an endothelial cell line in which lytic KSHV replication could be studied in vitro. Cell lines of dermal microvascular endothelial cells (DMVEC) immortalized with HPV E6 and E7 or human umbilical vein endothelial cells immortalized with telomerase (TIVE) have previously been generated which were stably infected with recombinant KSHV. Although stable latent infection with KSHV was established, these cell lines become tightly latent, with only a small percentage of cells expressing lytic cycle genes or producing infectious virions. While KSHV in TIVE cells enters lytic cycle upon transduction of exogenous Rta shortly after KSHV infection, they lose the ability to enter lytic cycle with longer term culture in vitro. Here we describe generation of KSHV infected TIVE cells stably transduced with an inducible KSHV Rta gene, which robustly enter the lytic cycle and produce high titers of infectious KSHV upon induction of Rta but only in combination with histone deacetylase inhibitors. Neither Rta not HDAC inhibitors alone resulted in virus production. ChIP analyses demonstrated that histone deacetylation cannot be overcome by Rta in endothelial cells. This cell line provides a tractable method to study KSHV lytic replication in vitro and sheds light on the epigenetic mechanisms that repress lytic reactivation in endothelial cells.



Uninduced Induced Rta Induced Rta + NaB Induced Rta +NaB +VA