WT1 Upregulation by lytic induction of Kaposi Sarcoma herpesvirus

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BACKGROUND

Kaposi Sarcoma herpesvirus (KSHV or HHV8) is the etiologic agent of Kaposi sarcoma and is associated with Multicentric Castleman disease and Primary effusion lymphoma. KSHV demonstrates two different phases of infection in the host: latent and lytic which are both essential to KSHV pathogenesis. The viral protein regulator or transcription activator (RTA) is required for the latent to lytic switch. In previous work, we have demonstrated that de novo KSHV latent infection and in particular vFLIP upregulates the host WT1 expression along with its oncogenic isoforms known to have various functions contributing to tumorigenesis. The expression and functions of WT1 have not been explored during lytic KSHV infection.

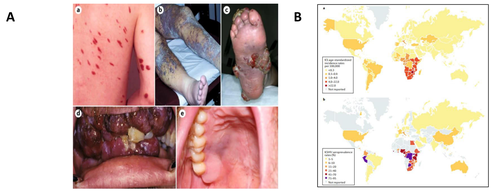

Figure 1. A. Clinical manifestations of KS as cutaneous skin lesions over torso, lower extremities, oral mucosa. **B.** KS global incidence and global seroprevalence.

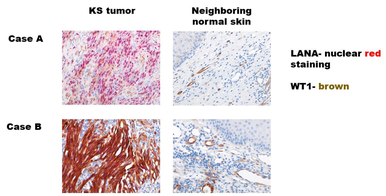

Figure 2. Immunohistochemistry, demonstrating overlap of LANA (nuclear, red) and WT1 (cytoplasmic and occasionally nuclear) expression in two KS lesions compared to neighboring normal skin.

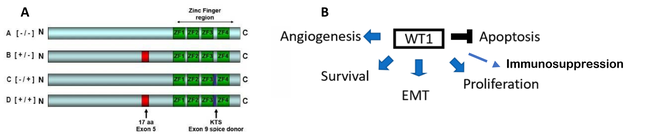

Figure 3. A. The four major WT1 isoforms, which differ by the presence or lack of a 17AA insert in exon 5 and a KTS insert between exon 9 and 10 (Ex5, KTS) as a result of alternative splicing (Modified from Morrison et. al 2008), can have distinct impacts on tumorigenesis, **B**.

METHODS

Cell culture models of latent and lytic KSHV infection were utilized, including iSLK-BAC16 cells latently infected with KSHV as well as HuARLT-1 and HUVEC E4 endothelial cells that were infected with KSHV. iSLK-BAC16 cells, which express RTA under a doxycycline-inducible promoter, were treated with sodium butyrate and doxycycline 2ug/ml for lytic reactivation and HUVEC E4 and HuARLT-1 with sodium butyrate or TPA to induce the lytic program. WT1 siRNA was transfected in the iSLK-BAC16 model systems using Lipofectamine RNAimax.

Both latent and lytic KSHV infection upregulate WT1 expression.

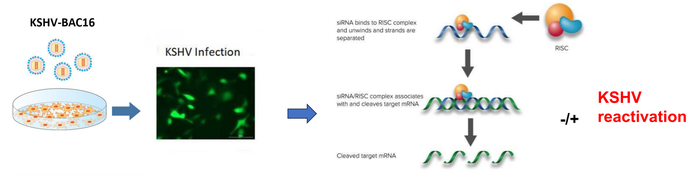

Figure 4. Method of knockdown of WT1 in cell culture models of KSHV infection using a siRNA approach followed by lytic reactivation compared to latent infection (adapted from scbt.com).

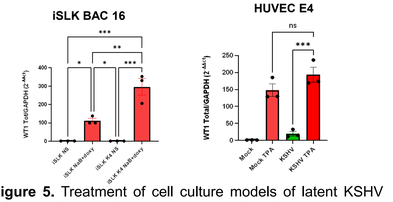

Figure 5. Treatment of cell culture models of latent KSHV infection, iSLK BAC 16 (left) and HUVEC E4 (right) with or without lytic reactivation, leading to upregulation of WT1 expression.

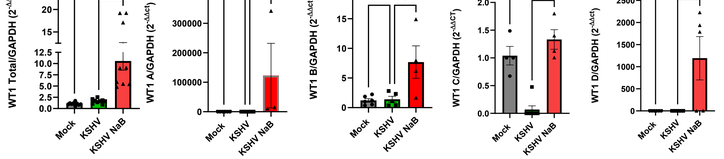

Figure 6. Upon treatment of KSHV infected HuARLT-1 endothelial cells with sodium butyrate, total WT1 and the major WT1 isoforms are upregulated.

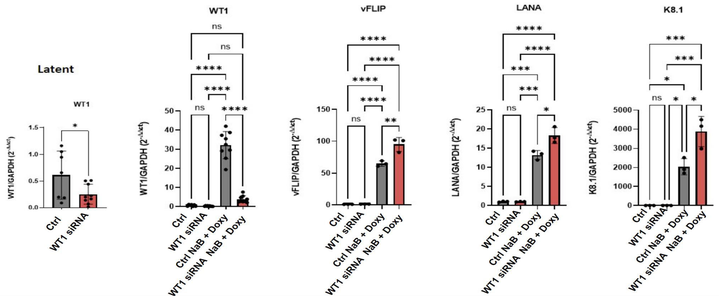

Figure 8. iSLK BAC16 cells which contain a doxycycline inducible RTA that triggers viral replication upon treatment. Upon doxycycline treatment we found a marked increase of WT1 after KSHV reactivation compared to latent infection. WT1 knockdown during lytic reactivation was much more robust than in latent infection (Morales et. al 2024) and results in upregulation of latent and lytic KSHV viral genes.

RESULTS

Here we have found that upon induction of lytic reactivation of KSHV-infected iSLK cells, WT1 is upregulated significantly and in particular oncogenic isoforms. Significant WT1 upregulation is also noted upon treatment with TPA or sodium butyrate of de novo KSHV-infected HUVEC E4 and HuARLT-1 endothelial cells respectively. Using the iSLK-BAC16, we also demonstrate that upon WT1 knockdown during lytic reactivation, there is noted further increase in vFLIP, LANA, and K8.1 viral gene expression.

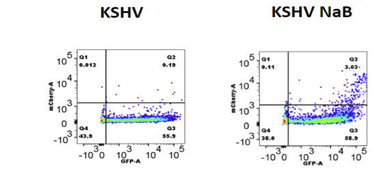

Figure 7. HuARLT-1 endothelial cells assessed by flow cytometry for mCherry (indicative of lytic reactivation) and GFP fluorescence (consistent with constitutive KSHV Infection) after de novo BAC-16 KSHV infection.

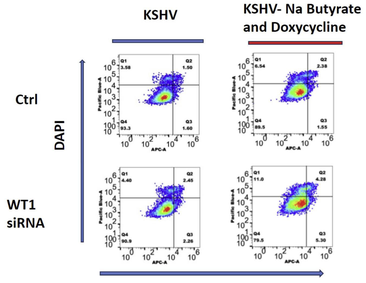

Figure 9. WT1 knockdown in ISLK BAC16 cells with and without treatment with sodium butyrate and doxycycline to induce lytic reactivation.

CONCLUSIONS

These findings suggest that both latent and lytic KSHV infection upregulate WT1 expression with increased upregulation of WT1 during lytic reactivation than latent infection. Furthermore, given the findings that WT1 knockdown during lytic reactivation leads to marked upregulation of viral gene expression, our data suggests that WT1 may play a complex role in regulating these two phases of infection that we are exploring further in ongoing studies.

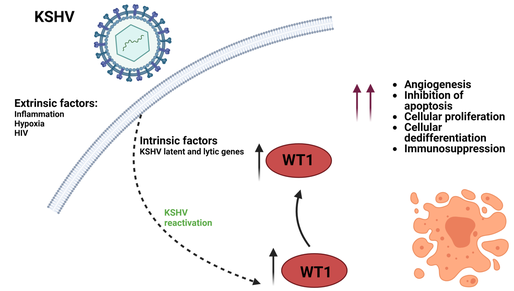

Figure 10. Model of the role of WT1 in KSHV Oncogenesis. Environmental factors such as hypoxia, viral co-infections as well as KSHV intrinsic factors may potentially upregulate WT1 expression during Kaposi sarcoma herpesvirus infection, increasing expression of the major oncogenic WT1 isoforms that contribute further to KS oncogenesis.

FUTURE DIRECTIONS

- Develop optimized cell culture models of KSHV reactivation with and without WT1 knockdown to characterize the role of WT1 in lytic KSHV infection.
- Identify extrinsic environmental and intrinsic viral factors that contribute to WT1 upregulation in KSHV lytic infection.
- Determine the mechanism by which WT1 is upregulated during KSHV lytic reactivation and the role of WT1 in the regulation of the KSHV latent to lytic switch.

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