Targeting HO-1 by SnPP Induces Necrosis in KSHV-Infected Cells and Suppresses Tumorigenesis in vivo

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INTRODUCTION

- Kaposi sarcoma-associated herpesvirus (KSHV), also known as Human Herpesvirus-8 (HHV-8), causes several types of cancers in immunocompromised patients, including Kaposi sarcoma (KS). KS affects endothelial cells lining the blood and lymphatic vessels resulting in purple, red, or brown clusters of skin lesions. There are four subtypes of KS: European (classic) KS, African (endemic) KS, AIDS-related (epidemic) KS, and transplant-related (iatrogenic) KS. The introduction of antiretroviral therapy has dramatically decreased the incidence of KS overall, but AIDS-KS still represents the most common AIDS-associated malignancy.
- An inducible enzyme, heme oxygenase-1 (HO-1), is highly expressed in AIDS-KS lesions and its enzymatic activity is upregulated within KSHV-infected cells. HO-1 metabolizes heme to generate free iron, carbon monoxide, and biliverdin which in turn facilitates the recruitment of chemokines and growth factors, particularly vascular endothelial growth factor (VEGF). The accumulation of VEGF-A increases angiogenesis, a crucial process required for KSHV-associated tumorigenesis. Tin protoporphyrin IX (SnPP), a potent HO-1 competitive inhibitor, is effective in downregulating HO-1 activity and suppressing VEGF-A expression in some cancer cells.

OBJECTIVES

- In this study, we sought to determine whether targeting HO-1 by SnPP induces KSHV-infected cell death and through which underlying mechanisms.
- We are also investigating whether targeting HO-1 can be developed as a novel therapeutic strategy to improve KS treatment.

METHODS

- Immunoblot and immunofluorescence were used to detect gene expression and cellular distribution
- Flow cytometry was used to quantify programed cell death
- ELISA was used to detect VEGF concentration in cultured cell supernatant
- An established KS-like nude mouse model was used to illustrate the in vivo effects of SnPP on KSHV-infected cell growth and tumorigenesis
- Immunohistochemistry was used to detect viral and/or host protein expression within AIDS-KS tumor tissue and KS-like tissues from vehicle- or SnPP-treated nude mice

HO-1 EXPRESSION

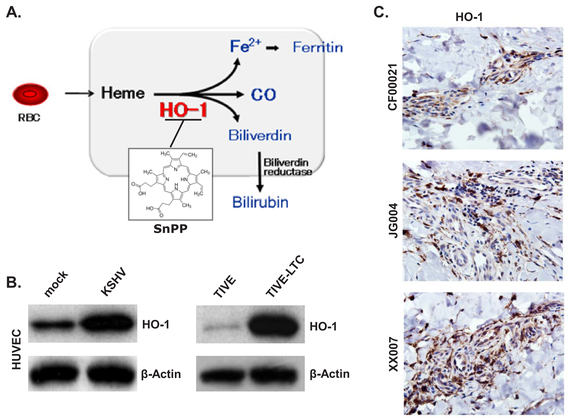

Figure 1. ***HO-1 is highly expressed in KSHV-infected cells and AIDS-KS tumor tissues.*** (A) A simple diagram showing the role of HO-1 in heme metabolism. (B-C) HO-1 protein expression was detected using immunoblots and immunohistochemistry (IHC), respectively.

FLOW CYTOMETRY

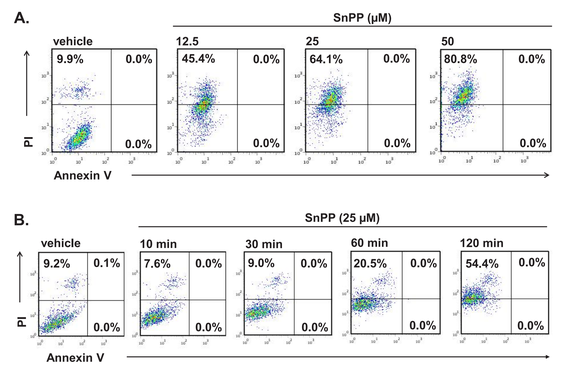

Figure 2. ***Targeting HO-1 by SnPP induced KSHV-infected cell death is independent of apoptosis.*** (A) TIVE-LTC were incubated with indicated concentrations of SnPP for 24 h, then cells were analyzed using Annexin-V/PI staining and flow cytometry. (B) TIVE-LTC were treated with 25 μM SnPP for indicated time.

DNA DAMAGE AND NECROSIS

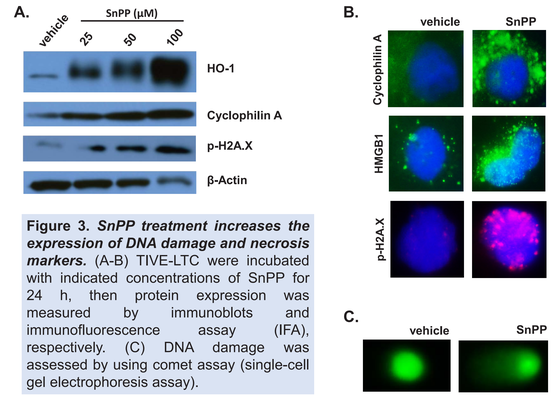

Figure 3. ***SnPP treatment increases the expression of DNA damage and necrosis markers.*** (A-B) TIVE-LTC were incubated with indicated concentrations of SnPP for 24 h, then protein expression was measured by immunoblots and immunofluorescence assay (IFA), respectively. (C) DNA damage was assessed by using comet assay (single-cell gel electrophoresis assay).

VEGF ELISA

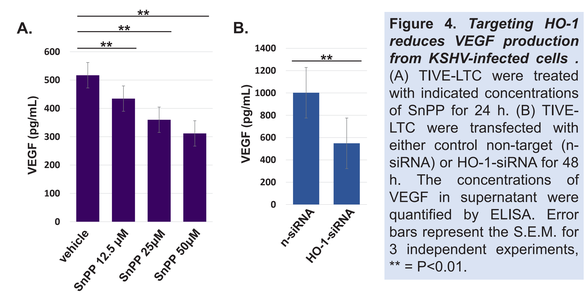

Figure 4. ***Targeting HO-1 reduces VEGF production from KSHV-infected cells*** . (A) TIVE-LTC were treated with indicated concentrations of SnPP for 24 h. (B) TIVE-LTC were transfected with either control non-target (n-siRNA) or HO-1-siRNA for 48 h. The concentrations of VEGF in supernatant were quantified by ELISA. Error bars represent the S.E.M. for 3 independent experiments, ** = P<0.01.

IN VIVO

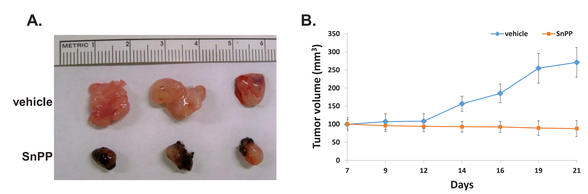

Figure 5. ***SnPP treatment suppresses KSHV-infected cell growth and tumorigenesis in vivo.*** (A-B) TIVE-LTC (5 x 10^5 cells 1:1 with growth factor-depleted Matrigel) were injected subcutaneously into the right and left flanks of nude mice (3 mice per group), respectively. After the tumor reached 10x10mm at about 1 week, the mice were in situ injected with vehicle or SnPP (10 μmol/kg) daily for 2 weeks. The mice were observed and measured every 2~3 d for the presence of palpable tumors.

CONCLUSION

- HO-1 is highly expressed in KSHV-infected cells and AIDS-KS tumor tissues
- SnPP induced KSHV-infected cell death occurs through DNA damage and necrosis
- Targeting HO-1 by RNAi or SnPP reduces VEGF production from KSHV-infected cells
- SnPP treatment successfully suppresses KSHV-infected cell growth and tumorigenesis in a KS-like nude mouse model

FUTURE WORKS

- Working on establishment of a biochemical assay to assess HO-1 activities with or without SnPP treatment.
- Determine whether SnPP treatment will affect viral gene expression (latency to lytic “switch”)
- Detect viral and host gene expression in tumor tissues from vehicle- and/or SnPP-treated mice, including LANA, K8.1, Cyclophilin A, p-H2A.X and Ki67 by using IHC

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