Herpes Simplex Oncolytic Viral Immunotherapy Against Colorectal Cancer

Ifeanyi K. Uche[1]; Vijay Kumar[1]; Vladimir N. Chouljenko[2,3]; Konstatin G. Kousoulas[2,3]; John H. Stewart IV[1]

[1]Department of Interdisciplinary Oncology, Stanley S. Scott Cancer Center, School of Medicine, LSUHSC
[2]Department of Pathobiological Sciences , Louisiana State University Veterinary School of Medicine
[3]Division of Biotechnology and Molecular Medicine, Louisiana State University Veterinary School of Medicine

Introduction

Oncolytic viral immunotherapy (OViT) is an immunotherapeutic approach whereby viruses are used to target and destroy cancer cells. In 2015, the Food and Drug Administration (FDA) approved the first oncolytic virus (OV) called Talimogene laherparepvec (T-VEC or Imlygic) for treating patients with melanoma. T-VEC is a herpes simplex type-1 derived OV that contain mutations in its infected cell protein 34.5 and ICP 47 genes [1]. T-VEC also expresses human granulocyte-macrophage colony-stimulating factor (GM-CSF). Although, T-VEC have shown great clinical success for melanoma patients, its anti-tumor effect against other cancer such as colorectal carcinoma is not fully understood. Several research groups are currently investigating the potential application of T-VEC as a more generalized cancer therapy [1].

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the second leading cause of cancer death in the United States. Current treatment options for colorectal cancer have limited efficacy. This shortcoming emphasizes the urgency of developing more efficacious therapies patients with colorectal cancer.

Objective: To use a mouse version of T-VEC to understand its antitumor effect against colorectal cancer CT26 cells.

Materials & Methods

Construction of HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus. The virus was constructed using the two-step double red recombination technology implemented on the HSV-1 (F) genome cloned into a bacterial artificial chromosome (BAC). The virus harbors mutations in its infected cell protein 34.5 and ICP47 genes. Mouse GM-CSF was inserted in place of the deleted genes. The virus was grown, and its titer determined in Vero cells.

Immunohistochemistry staining. Infected Vero cells incubated for 48 hours and fixed with 3.5% formalin overnight at room temperature. Cells were washed and incubated with a rabbit polyclonal anti-herpes simplex virus type 1 antibody for 1 hour. Subsequently, polyclonal goat anti-rabbit immunoglobulins conjugated to horseradish peroxidase were added for 30 min at room temperature. Vector© NovaRED peroxidase substrate kit was used for colorization.

***In vitro* cytotoxicity studies**. Colorectal cancer CT26 cells were plated in six-well plates at 2 x 10^5 cells/well. After a 24-hr incubation at 37°C, cells were infected with HSV-1 ΔICP34.5 mGM-CSF ΔICP47 or WT virus at MOI of 0.01, 0.1, or 1. The number of surviving cells was counted daily with a BioRad TC20 Automated Cell Counter.

Results

Construction of HSV-1 ΔICP34.5 mGM-CSF ΔICP47 Virus

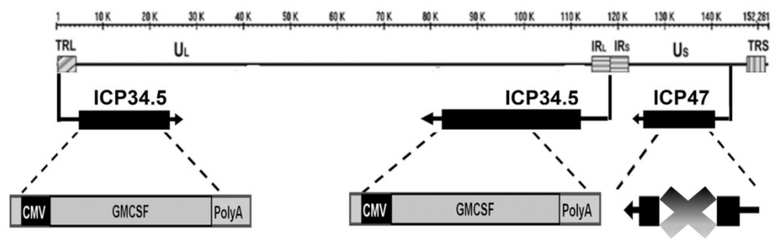
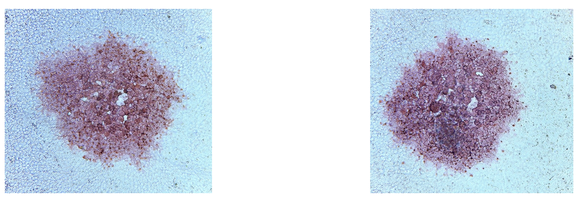


Figure 1. Design of the HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus. The virus was constructed using double red recombination technology. The virus was engineered to contain mutation in its ICP 34.5 and ICP47 genes. Mouse GM-CSF was inserted in place of the deleted ICP34.5 genes.

HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus plaque phenotype



F Wildtype Virus — HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus

Figure 2. Representative plaque morphology of HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus. Vero cells infected with HSV-1 ΔICP34.5 mGM-CSF ΔICP47 or wildtype virus for 48 hours were fixed, and immunohistochemistry staining was performed. The images were taken using an Echo-Revolve hybrid microscope.

Cytopathic effect of HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus *in vitro*

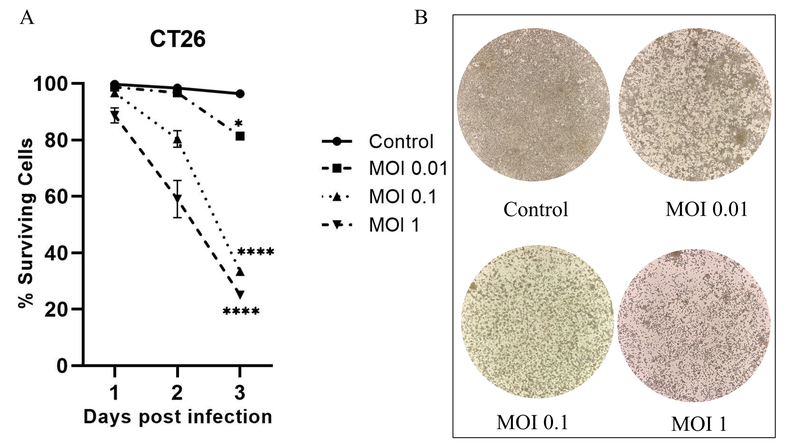


Figure 3. Cytopathic activity of HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus. A. CT26 cells seeded in a six-well plates at 2x 10^5 cells/well were infected with HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus at various MOIs, 0.01–1 or mock infected. The number and percentage of surviving cells was counted daily. B. Representative images of control or infected wells 48 hours post infection. The images were taken using an Echo-Revolve hybrid microscope.

Conclusion

- ❖ The HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus has similar plaque formation as the wildtype F virus.
- ❖ The HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus efficiently lysed CT26 cancer cells *in vitro*.

Future Work

- ❖ To investigate the antitumor efficacy of the HSV-1 ΔICP34.5 mGM-CSF ΔICP47 virus in immunocompetent mice engrafted with colorectal cancer CT26 cells.

References

1. Uche, I.K., K.G. Kousoulas, and P.J.F. Rider, *The Effect of Herpes Simplex Virus-Type-1 (HSV-1) Oncolytic Immunotherapy on the Tumor Microenvironment.* Viruses, 2021. **13**(7).

This research project was supported through the LSU Health Sciences Center, School of Medicine.