Impact of Human Cytomegalovirus Infection on Host Stress Response Genes

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Introduction

Human Cytomegalovirus (HCMV) is a widespread pathogen that causes lifelong latent infection, making it a significant pathogen of interest. HCMV rarely causes disease in healthy adults. However, immune-compromised individuals like transplant recipients and AIDS patients can suffer from life-threatening disease.

HCMV encodes US27, an orphan G-protein coupled receptor that is found in the virus and in the membrane of infected cells (1). US27 has been found to increase the signaling activity of CXCR4 (2,3), which is a host chemokine receptor important for development, hematopoiesis, and immune cell trafficking. Preliminary data suggests that US27 increases signal output by stimulating higher levels of CXCR4 gene expression in a phosphatidylinositol-3 kinase (P13K)-dependent manner.

Nuclear respiratory factor-1 (NRF-1) is the primary transcription factor regulating CXCR4 gene expression through a regulatory sequence called anti-oxidant response element (ARE). Since NRF-1 governs expression of many metabolic genes regulating cellular growth and respiration, we wondered whether these genes would also be upregulated upon HCMV infection (Figure 1).

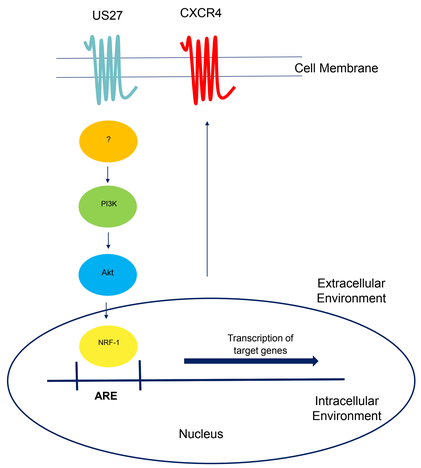


Figure 1. Schematic of US27 inducing CXCR4 Expression
Proposed mechanism of the signaling activity of CXCR4. PI3K activates Akt, which phosphorylates NRF-1. NRF-1 induces expression of ARE-containing genes, such as CXCR4 through the PI3K pathway. Two ARE containing gene targets, EIF2S1 and CD47 are investigated using polymerase chain reaction experiments.

In this project, metabolic gene targets of NRF-1 containing an ARE will be investigated by determining expression levels of HEK293 cells. A few genes have been selected for preliminary investigation. The elongation initiation factor 2 (EIF2S1) is essential for initiating the translation of proteins. One other gene, CD47 has been found to be overexpressed in many different tumor cells.

Polymerase chain reaction was used to investigate the expression of ARE-containing metabolic genes in HEK293 cells expressing US27. These results are expected to clarify the role of US27 during HCMV infection and could aid in the discovery of novel anti-viral drug targets.

Methods

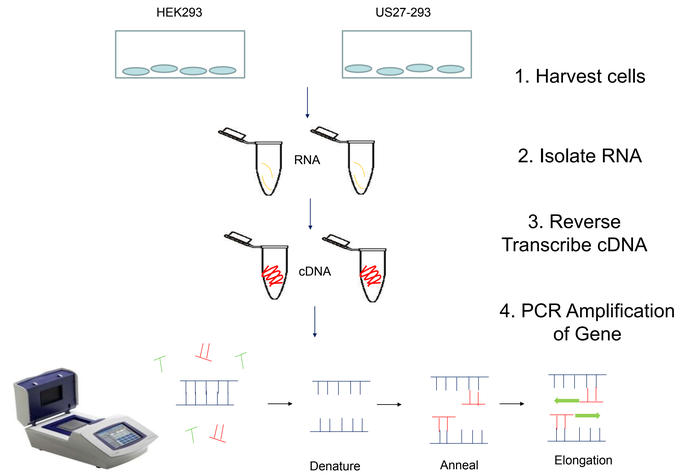
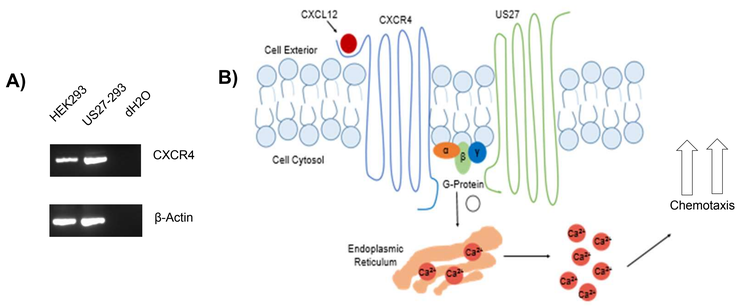


Figure 2. Polymerase Chain Reaction (PCR) with nucleic acid from HEK293 cells. Human embryonic kidney cells (HEK293) were cultured in standard growth medium in a 37 °C humidified incubator with 5% CO2. RNA was isolated from parent HEK293 cells and from a stable cell line expressing HCMV US27 (US27-293), HEK293 cells were seeded at a density of 2 x 10^6 cells/mL in media. The RNA was reverse transcribed into complementary DNA (cDNA) and then gene specific primers were used to amplify target genes using the polymerase chain reaction to investigate levels of gene expression between the HEK293 and US27-293 (HCMV-infected) cells.

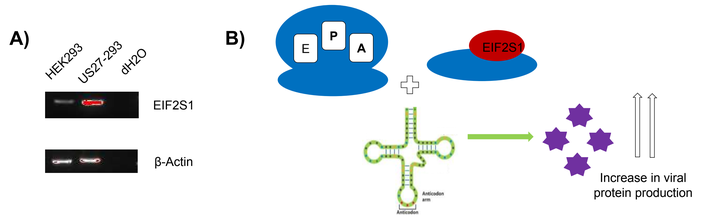
Results



CXCR4 gene functions to induce chemotaxis towards the bone marrow. CXCR4 is a receptor that guides cell movement and increased CXCR4 expression could help disperse infected cells.

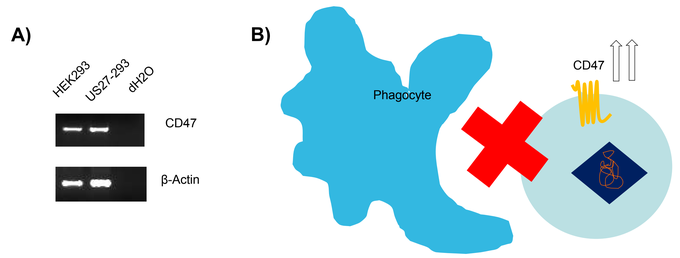
Figure 3. Increased CXCR4 expression when US27 is present. A)PCR of β-Actin and CXCR4 primers with HEK293, US27-293, and dH2O. dH2O served as a control sample as well as β-Actin serving as a control to ensure that both HEK293 and US27-293 samples were functioning. Resulting bands were visualized on a 1.2% agarose gel. CXCR4 bands expected around 400 bp, which both HEK293 and US27-293 bands showed with US27-293 band indicating greater amount of expression than HEK293. B) Cartoon depiction of proposed impact of increased gene expression of HCMV infected cells.

Results, continued



EIF2S1 gene functions to initiate translation of proteins by promoting initiator tRNA to the 40S ribosomal subunits. EIF2S1 regulates protein translocation and increased levels would aid in the productive of new virus particles.

Figure 4. Increased EIF2S1 expression when US27 is present. A) β-Actin and EIF2S1 primers utilized in PCR reactions with HEK293, US27-293, and dH2O. dH2O served as a control sample as well as β-Actin serving as a control to ensure that both uninfected and infected samples were functioning. Resulting bands were visualized on a 1.2% agarose gel. EIF2S1 bands expected around 224 bp, which both HEK293 and US27-293 bands displayed. US27-293 band is more concentrated when compared to the HEK293 band. B) Cartoon depiction of proposed impact of increased gene expression of HCMV infected cells. Note: Band appears red due to high signal intensity.



CD47 gene functions to prevent phagocytosis, aiding in immune evasion and clearance of virus.

Figure 5. Increased CD47 expression when US27 is present. A) β-Actin and CD47 primers utilized in PCR reactions with HEK293, US27-293, and dH2O. dH2O served as a control sample as well as β-Actin serving as a control to ensure that both uninfected and infected samples were functioning. Resulting bands were visualized on a 1.2% agarose gel. CD47 bands expected around 499 bp. B) Cartoon depiction of proposed impact of increased gene expression of HCMV infected cells.

Future Directions & Conclusions

- By using the polymerase chain reaction to amplify DNA, we were able to identify ARE-regulated genes that are expressed at higher levels when HCMV US27 is present.
- Future experiments would include quantifying amount of gene production by utilizing qPCR and measuring increased protein levels using western blot and flow cytometry techniques. In addition, incorporating cells expressing US28, a HCMV GPCR as another control.
- These results help clarify how HCMV alters host cellular functions and immune responses to persist successfully in the human population.

References and Acknowledgements

1. Margulies BJ, Gibson W. 2007. The chemokine receptor homologue encoded by US27 of human cytomegalovirus is heavily glycosylated and is present in infected human foreskin fibroblasts and enveloped virus particles. Virus Res 123:57-71.
2. Arnolds et. al,., 2013. The US27 gene product of human cytomegalovirus enhances signaling of host chemokine receptor CXCR4. Virology 439:122-131.
3. Boeck, J.M. and J.V. Spencer. 2017. Effect of Human Cytomegalovirus (HCMV) US27 on CXCR4 Receptor Internalization Measured by Fluorogen-activating Protein (FAP) Biosensors. PLoS One. 12(2):e0172042.

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