How Human Cytomegalovirus UL4 Controls the Proinflammatory Cytokine TNFSF10 to Control Cellular Functions

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Abstract

Human cytomegalovirus (HCMV), a human beta herpesvirus, establishes lifelong infection in the host. HCMV is a leading cause of complications during fetal development and causes significant morbidity and mortality during hematopoietic stem cell and solid organ transplants. Specifically, HCMV can alter cellular proliferation, differentiation cytokines, and transcription factors by controlling the cell cycle. TNFSF10 is a key cytokine involved in regulating the cell cycle, which may interact with a proposed viral latency factor, HCMV UL4. In this study, we will confirm this interaction in fibroblasts and look at the mechanism of how the virus regulates this cytokine. To accomplish this, we will introduce TNFSF10 into pcDNA 3.1 and perform a quantitative reverse transcription polymerase reaction (qRT-PCR) to determine mRNA levels of UL4 and TNFSF10, giving us how much of the gene is transcribed or expressed. Next, a linked immunosorbent assay (ELISA) will be performed to measure levels of TNFSF10 secreted. We will look at these effects both during infection and when UL4 is expressed alone. Once completed, our research will demonstrate the effects of HCMV UL4 on regulating TNFSF10 in the cell. Understanding how HCMV infection controls the cells is important for; the health of infected individuals, and the future production of antiviral medication and vaccines for HCMV, as well as other herpesviruses.

Human Cytomegalovirus (HCMV)

- Beta-herpesvirus
- Infects 60%-90% of the population worldwide
- lifecycle = acute infection, latency, and reactivation
- Manipulates hematopoiesis by controlling differentiation and proliferation

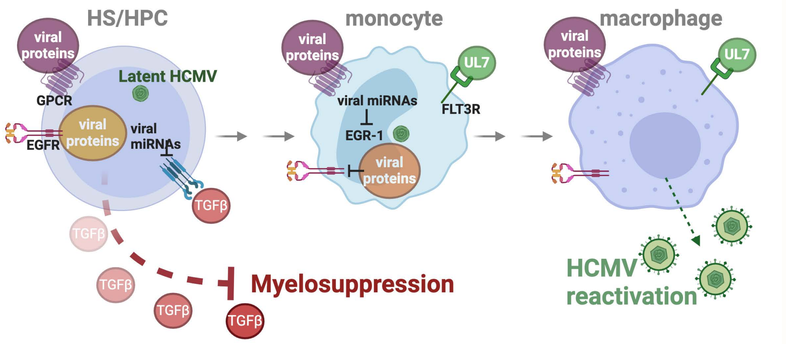

Figure 1. HCMV Manipulation of Hematopoietic Differentiation. Hematopoietic stem/progenitor cells (HS/HPCs) are a critical reservoir for HCMV latency. Differentiation provides a cellular environment appropriate for viral replication and reactivation. Both differentiation and latency are specifically regulated by a subset of viral gene products which in turn directly regulate the infected cell, and indirectly manipulate uninfected neighboring cells by altering the cytokine environment, such as via TGF-β mediated myelosuppression.

MCNAIR SCHOLARS PROGRAM

HCMV UL4

- Encodes a glycoprotein (gpUL4)
- gpUL4 is secreted from HCMV infected cells
- Part of the RLL11 region
  - Some genes associated with immunological functions
- Required for latency and reactivation

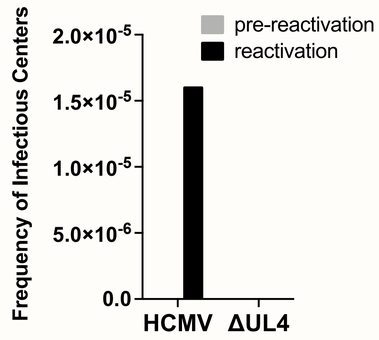

Figure 2. UL4 is Required for Latency
HPCs were infected with wild-type HCMV (TB40E-GFP) or HCMV lacking UL4 for 48hrs. Viable $CD34^+$ GFP^+ HPCs were sorted by FACS and plated on stromal cell support for 12 days. Viral reactivation was induced by co-culture on fibroblasts with cytokine stimulation for up to 28 days and reactivation measured by ELDA assay. Data is representative of 3 independent experiments.

- Nobre et. al., (2019) showed:
  - Used mass spectrometry to analyze protein: protein interactions between HCMV proteins and cellular proteins
  - We analyzed their data for which cellular proteins interact with UL4

Cellular Protein	Uniprot-I	Average PSMs	Entropy	z-score	NWD score
TNFSF10	P50591	1.5	0.95	12.33	53.54
STC2	O76061	2	0.88	11.02	22.98
FAT1	Q14517	2.5	0.81	1.08	3.37
FBXO2	Q9UK22	3.5	0.90	5.61	2.30
CNNM3	Q8NE01	2.5	0.98	1.37	2.27
SLC25A22	Q9H936	2.5	0.98	3.80	1.41
MOCS3	O95396	1.5	0.95	2.88	1.03

Table 1: Cellular Proteins that interact with HCMV UL4 from Nobre et al., (2019)

TNFSF10

- Also known as TRAIL
- Part of the Tumor neurosis factor (TNF) family
- Induces apoptosis when it binds to receptor TRAILR1 or 2
- Regulates the cell cycle

- Vlachava et al., (2023) showed:
  - UL4 binds to TNFSF10
    - Acts as a soluble decoy receptor
    - Binding is 8-300x stronger than its original receptor (TRAILR1)
  - UL4 acts on NK cells to inhibit their activation

→ HCMV-infected cells are protected from apoptosis

How does the UL4 and TNFSF10 interaction affect latency?

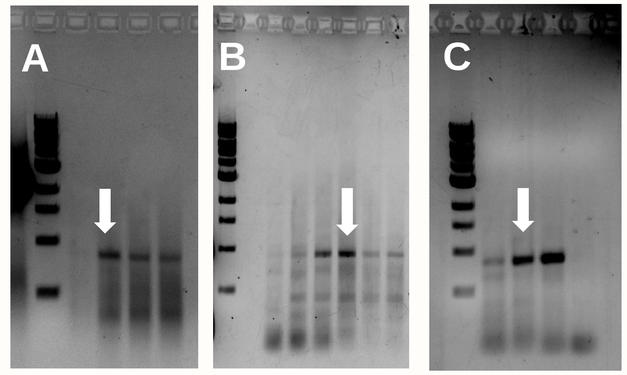

Figure 3: PCR optimization of TNFSF10
Electrophoresis gel (pre-stained with Ethidium bromide and run at 100V for 30 mins) of PCR products using standard Taq Polymerase (NEB) to optimize: **A)** different $MgCl_2$ concentrations; **B)** temperature gradient; **C)** different cDNA templates [NHDF, THP-1, Kasumi-3, 293T (left to right)].

Optimal PCR conditions to amplify TNFSF10 to clone into pCDNA3.1

- 3mmol $MgCl_2$
- Annealing temperature of 57.3°C
- Template cDNA from THP-1 (monocyte cell line)

In progress

- Clone TNFSF10 into pCDNA 3.1 → pCDNA3.1-TNFSF10
- Transfect UL4 into fibroblasts
- Collect samples from HCMV-infection timecourse

Future Directions

Does UL4 change TNFSF10 expression?

1) **RNA expression for UL4 and TNFSF10**
   1) Quantitative reverse transcription polymerase chain reaction (qRT-PCR)
   2) pCDNA3.1-TNFSF10 for copy number standard control
   3) Inform us how much of the gene is transcribed or expressed during infection or after UL4 expression alone using transfection
2) **Secretion of TNFSF10 cytokine**
   1) Perform linked immunosorbent assay (ELISA)
   2) Measure levels of TNFSF10 secreted

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