A Semester of Immunoregulation in Review

By J.F. Treml

The Biotechnology program at the University of Kansas places a strong emphasis on equipping its graduates with the ability to proficiently comprehend intricate scientific research articles. To achieve this, the program mandates students to participate in a Selected Topics class, wherein they are organized into small groups. Within these groups, students systematically break down and explain different research articles to the class using structured PowerPoint presentations. To enhance the interactive nature of this activity, articles are thoughtfully chosen to mirror the chronological evolution of exemplary publications on a specific subject. In the 2023 spring semester, students delved into various instances of host immune regulation by microorganisms. A listing of the primary articles can be found in "A Primary (Sourced) Education" on page 12 of this issue, laying the groundwork for students to subsequently formulate an insightful review of a recently published article on the same topic. Enclosed herein are notable examples of papers authored by students enrolled in the course, serving as exemplars of those written by students enrolled in the course.

Partners in Crime:

Synergistic Anti-apoptotic Effects of HCMV Gene Products in Infected Cells

By Kaitlyn Sy

The means by which human cytomegalovirus (HCMV) evades the immune system has been a hot research topic for the past three decades. While much of the research has focused on viral proteins that interfere with the host's immune response, recent investigations suggest that proteins are not the only culprits. New findings by Hancock et al. indicate that HCMV-encoded microRNAs (miRNAs) work alongside viral proteins to produce synergistic antiapoptotic effects, shedding light on the mechanisms by which HCMV establishes latent infections in host cells.

Human cytomegalovirus (HCMV) is a common herpesvirus, estimated to infect over half the global population. In immunocompetent hosts, acute infection is controlled by a vigorous immune response, resulting in mild symptoms or no symptoms at all. However, in immunocompromised hosts, overwhelming replication of HCMV could lead to organ damage. Regardless of host immune status, HCMV establishes lifelong latent infection in CD34+ hematopoietic progenitor cells (HPCs). Latent infection may reactivate when the immune system is weakened, making HCMV the most common opportunistic infection in organ transplant patients.[1] HCMV infection is known to activate proapoptotic pathways that normally thwart viral replication,[2] so elucidating the mechanisms that enable HCMV to establish latency has been of interest to scientists. Understanding these mechanisms could aid in the development of more effective prevention and treatment strategies for HCMV infections.

Early research focused on interference of HCMV proteins with antigen presentation on MHC class I to evade detection by CD8+ T cells. HCMV protein US3 associates with and retains MHC class I molecules in the endoplasmic reticulum (ER).[3] Retained MHC class I molecules are then dislocated by the HCMV-encoded ER-resident transmembrane glycoprotein US11 to the cytosol to be degraded.[4] Cells that fail to express MHC class I are usually destroyed by natural killer (NK) cells, but UL18, an HCMV homolog of MHC class I heavy chain, generates surrogate MHC class I molecules by associating with β2-microglobulin and by binding peptides, and in so doing avoids NK-cell-mediated cellular cytotoxicity.[5] More recently, research has focused on direct interference of HCMV gene products with proapoptotic pathways. For example, HCMV protein vMIA suppresses cell death by binding to and sequestering the proapoptotic protein Bax, preventing mitochondrial membrane permeabilization, a key step in the intrinsic apoptotic pathway.[6] Another HCMV protein, pUL38, also inhibits the intrinsic apoptotic pathway by blocking proteolytic activation of two key apoptotic enzymes, caspase 3 and poly(ADP-ribose) polymerase (PARP).[7] Research about proteins that enable HCMV to establish latency has enabled scientists to piece together parts of the picture, but much remains elusive. However, work recently published by Hancock et al.2 uncovered three HCMV gene products—pUL7, miR-US5-1, and miR-UL112-3p—that modulate FOXO3a, a key transcription factor that targets several genes involved in both the intrinsic and extrinsic apoptosis pathways.[8]

pUL7, a glycoprotein secreted by HCMV-infected cells, binds directly to Fms-like tyrosine kinase 3 (Flt-3R), inducing downstream signaling cascades. While unnecessary for lytic replication, it is necessary for reactivation of HCMV from latency.[9] In a preliminary experiment, telomerized human fibroblasts (THFs) transfected with Flt-3R were stimulated with either pUL7 or Flt-3 ligand (Flt-3L, to serve as a positive control) with and without Flt-3R inhibitor AC220. Immunoblots indicated rapid phosphorylation of FOXO3a in THFs treated with pUL7 or Flt-3L and inhibition of phosphorylation in THFs treated with AC220, indicating that pUL7 stimulates phosphorylation of FOXO3a via a Flt-3R-dependent mechanism. To identify which pathway downstream of Flt-3R is involved in FOXO3a phosphorylation, the authors stimulated THFs with UL7 in the presence of inhibitors that block either PI3K or MEK. Only inhibition of MEK

prevented pUL-7-induced FOXO3a phosphorylation, suggesting that it occurs via the MAPK pathway. Phosphorylation of FOXO3a by pUL-7 was found to have an inactivating effect, indicated by significantly lower levels of BCL2L11 mRNA and BIM protein (both downstream products of FOXO3a target genes) in THFs stimulated with pUL-7, determined by qRT-PCR and immunoblot, respectively. However, stimulation with pUL-7 did not affect mRNA or total protein levels of FOXO3a. The authors' next question was whether phosphorylation of FOXO3a affects its distribution between the cytosol and the nucleus. Immunoblots and immunofluorescence microscopy indicated that FOXO3a is translocated to the cytoplasm in cells stimulated with pUL-7, and this translocation is inhibited when MEK is blocked. The authors repeated these experiments in RS4;11 cells (a lymphoblast cell line) and primary CD34+ HPCs, confirming that pUL7 has the same effects on FOXO3a across all three cell lines. Their data aligned well with reports of viral proteins that inactivate FOXO3a in other herpesviruses, but Hancock et al. did not stop with this. There was growing evidence in the literature that many viral proteins do not function alone, but rather work in tandem with microRNAs (miRNAs).[10] Bioinformatic analysis suggested that FOXO3a was a target of HCMV-encoded miR-US5-1 and miR-UL112-3p. In a preliminary experiment, Hancock et al. transfected HEK293T cells with vectors encoding miR-US5-1 or miR-UL112-3p. When FOXO3a mRNA and protein levels were assessed, there was a clear downregulation of both. Hancock et al. successfully obtained similar results when they repeated this experiment in primary CD34+ HPCs, confirming that miR-US5-1 and miR-UL112-3p target FOXO3a. Finally, to determine the effects of pUL7, miR-US5-1, and miR-UL112-3p on apoptosis, Hancock et al. transduced primary CD34+ HPCs with adenoviral vectors carrying pUL7, miR-US5-1, or miR-UL112-3p. When apoptosis was assessed via flow cytometry, it was observed that all three HCMV gene products protected cells from apoptosis (Illustrated in **Figure 1**).

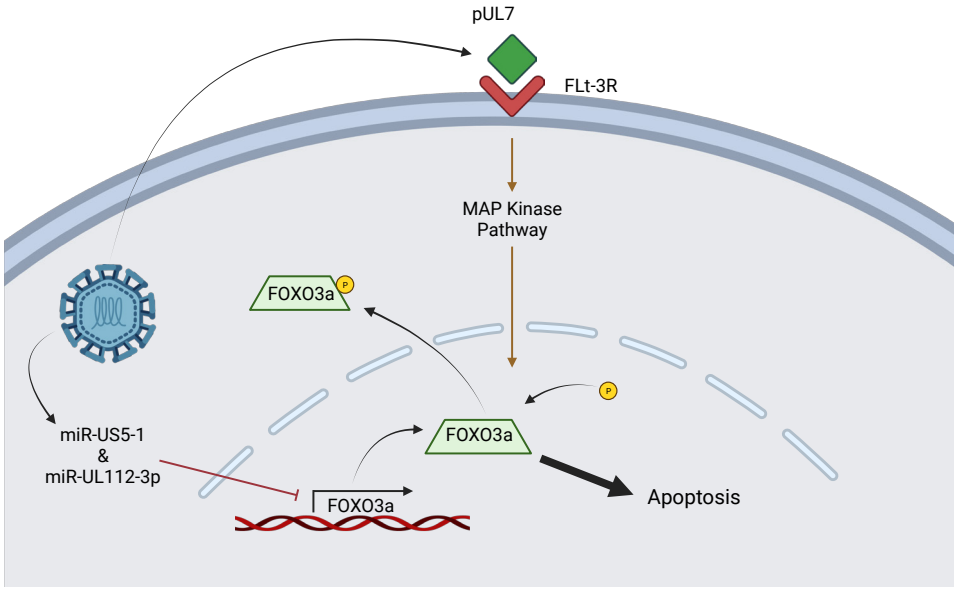

Figure 1 | Regulation of apoptosis.
The glycoprotein pUL7 and microRNAs collaborate to inhibit cellular apoptosis in HCMV-infected cells.

With this research in mind, several questions come to light. Are pUL7, miR-US5-1, and miR-UL112-3p expressed simultaneously in infected cells? If so, how is FOXO3a affected when the host cell expresses each of these products together, compared to separately? If not, what conditions warrant the expression of one (e.g., pUL7) versus another (e.g., miR-UL112-3p)? Even if these questions were not addressed by Hancock et al. in this study, their results shed light on an interesting synergy between antiapoptotic viral miRNAs and protein. miR-US5-1 and miR-UL112-3p target FOXO3a transcripts in HCMV-infected cells, significantly downregulating expression of FOXO3a. Further, any FOXO3a that host cells manage to produce is targeted by HCMV pUL7. Binding of pUL7 to Flt-3R triggers phosphorylation of FOXO3a via the MAPK pathway, resulting in translocation of FOXO3a from the nucleus to the cytoplasm. Consequently, the proapoptotic downstream products of FOXO3a gene targets are significantly downregulated, protecting host cells from apoptosis.

In conclusion, while understanding of the mechanisms by which HCMV establishes latency remains incomplete, this study by Hancock et al. adds valuable insights into the multifaceted approach by which HCMV lowers host defenses.

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