Human cytomegalovirus UL135 mediates myelosuppression of hematopoietic progenitor cells

Linh Tran, Jason Buehler, Michael Rak, and Felicia Goodrum

University of Arizona

COLLEGE OF MEDICINE TUCSON Immunobiology

BIO5 Institute

ABSTRACT

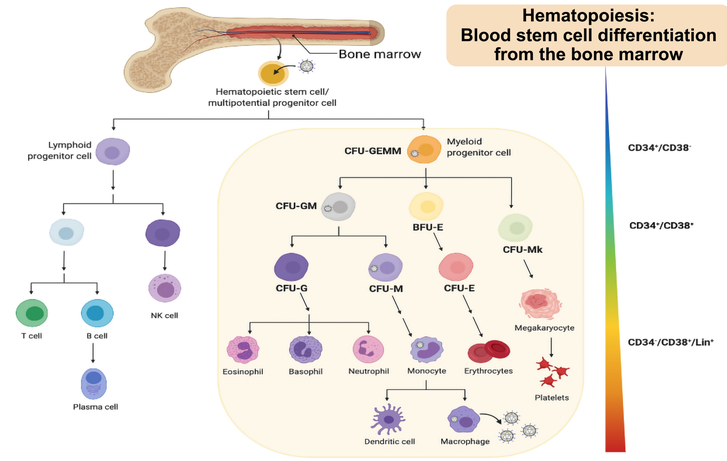
Human cytomegalovirus (HCMV) is a main cause of morbidity and mortality for patients undergoing solid organ transplantation and hematopoietic stem cell transplantation due to its suppression of hematopoiesis down the myeloid lineage (myelosuppression). The molecular mechanisms by which HCMV causes myelosuppression of infected hematopoietic progenitor cells (HPCs) are largely unknown. **We have identified a viral protein, UL135, that is required for HCMV-mediated myelosuppression of infected HPCs**. A mutant virus lacking UL135 fails to induce myelosuppression compared to wildtype virus in colony forming unit assay.

UL135 has many host and viral protein interactors, including **adaptor proteins CIN85 and Abi-1**, which regulate the cytoskeleton, endocytic trafficking and signaling. Both adaptor proteins have roles in pathways important for HPC maintenance and differentiation, namely EGFR signaling and trafficking. We are currently investigating the significance of the UL135-host interactions and their regulation of **EGFR downstream signaling** and other related pathways to identify the mechanism by which UL135 causes myelosuppression. These results will provide some of the first mechanistic insights into myelosuppression induced by HCMV.

HYPOTHESIS

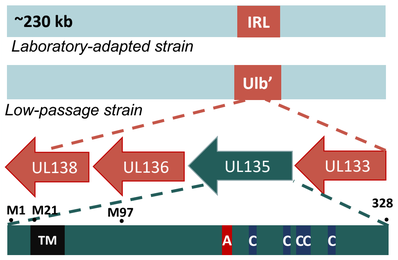
We hypothesize that UL135, through host interactor proteins, downregulates EGFR downstream signaling to drive HPC differentiation towards specific lineages permissive for viral replication.

BACKGROUND



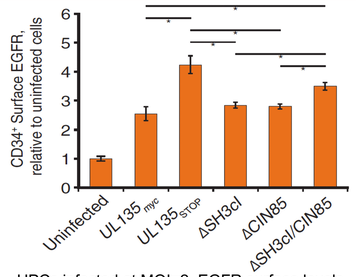
Hematopoiesis: Blood stem cell differentiation from the bone marrow

Schematics of the HCMV genome, the UL133-8 gene locus, and UL135



IRL: internal repeat long; TM: transmembrane domain; A: Abi-1 interaction motif; C: CIN85 interaction motif

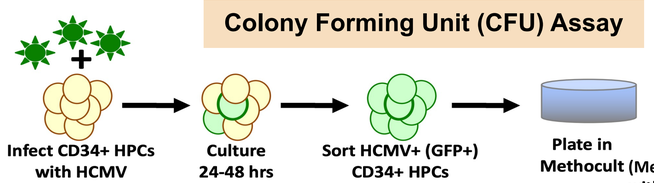
UL135 downregulates EGFR surface level in CD34+ HPCs by interacting with Abi1 & CIN85



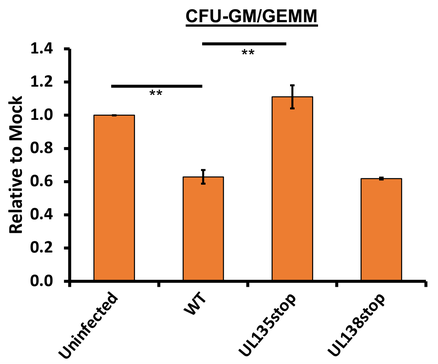
HPCs infected at MOI=2, EGFR surface levels measured at 24 hours post infection.

METHOD

Colony Forming Unit (CFU) Assay



CD34+ HPCs infected with wildtype (WT) HCMV has a reduced differentiation potential, while HPCs infected with ΔUL135 virus does not.



- Count Progenitor Colony Types: CFU-E, BFU-E, CFU-GM, CFU-GEMM
- Compare Mock vs Infected CD34+ HPCs

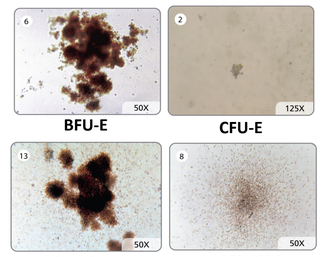
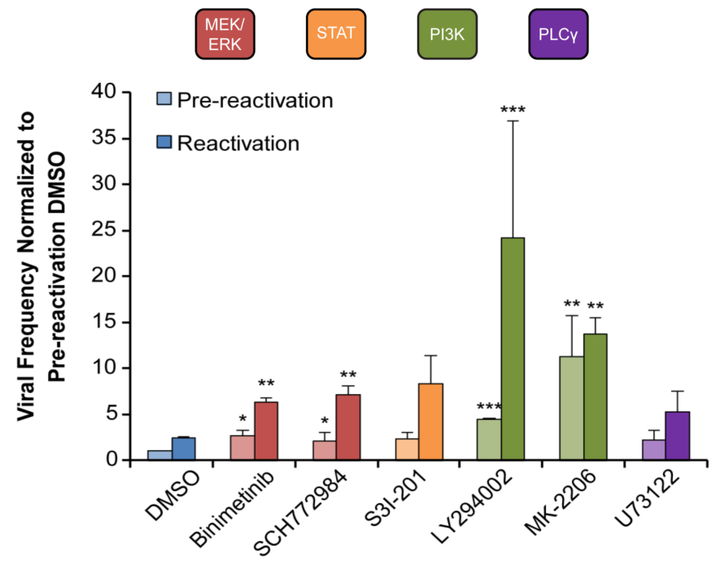


Image courtesy of STEMCELL Technologies

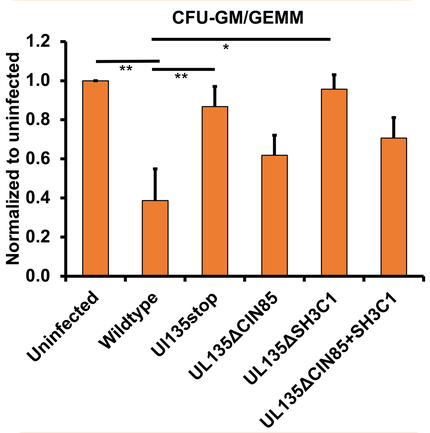
FUTURE DIRECTION

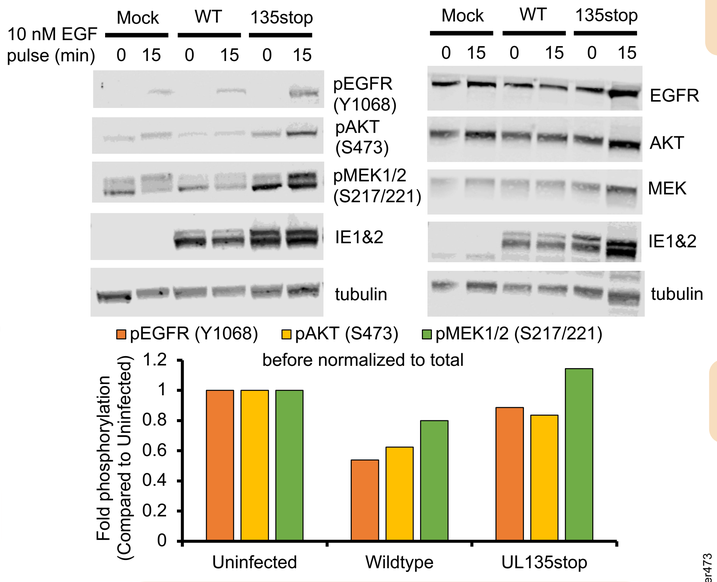


- **Identify downstream EGFR signaling pathway(s) targeted by HCMV during HPC infection causing myelosuppression**
- **Measure signaling through protein phosphorylation levels in HPCs during an infection time course**
- **Investigate other roles of CIN85 and Abi1 in HPC maintenance and differentiation outside the context of EGFR signaling**

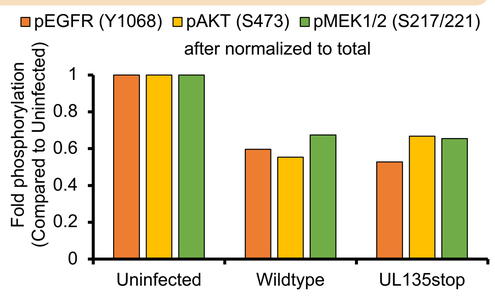
RESULTS & CONCLUSIONS

UL135 defective in interacting with CIN85/Abi1 fails to fully induce myelosuppression

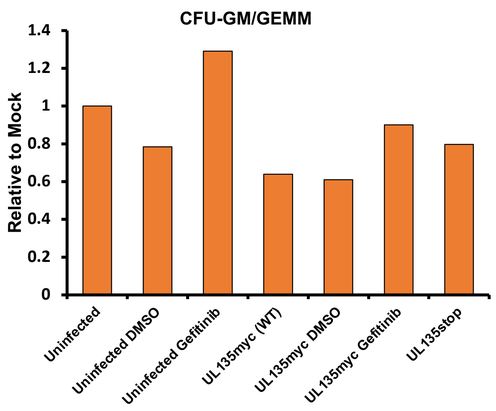




EGFR and downstream signaling during productive infection in fibroblasts at 24 hours post infection



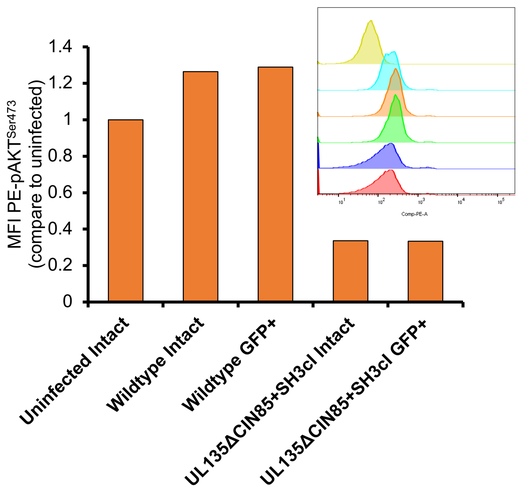
EGFR inhibition improves myelopoiesis of uninfected and infected HPCs



CONCLUSIONS

- **CFU assays:**
  - UL135 interactions with Abi1 and CIN85 are important for UL135-induced myelosuppression of infected HPCs.
  - EGFR signaling is inhibitory to myelopoiesis.
- **Western blot analysis of phospho-proteins in fibroblasts:**
  - Compared to uninfected, WT-infected cells have dampened EGFR, AKT, and MEK signaling independent of total protein expression level, while UL135stop-infected cells have increased total protein expression levels that partially restored signaling defect observed in WT-infected cells.
- **Flow cytometry analysis of phospho-AKT in monocytes**
  - Unexpectedly, AKT signaling is dampened instead of elevated in UL135ΔCIN85/Abi-1-infected monocytes at 24 hpi, suggesting a potentially different mechanism at play in this cell type.

UL135ΔCIN85/Abi-1-infected monocytes have altered AKT signaling compared to wildtype-infected cells



ACKNOWLEDGEMENT

This work was funded by the National Institute of Health (R01 AI079059 and P01 AI127335-01) to F.G, the American Cancer Society Post-Doctoral Research Fellowship (129842-PF-16-212-01-TBE) to J.B, and the Graduate Program in Molecular Medicine, Department of Immunobiology, and the Arizona Biological & Biomedical Sciences Program to L.T.

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