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Role of RIOK3 in Innate Immunity

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Implications of TRIM40 on Cellular Innate Immunity

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Introduction

Understanding how the cell's innate immune response is regulated can elucidate better means of preventing and ending viral infections. Rift Valley fever virus (RVFV), an RNA virus, is a pathogen of particular importance due to its deadly effects on domestic livestock and humans. Our lab investigates RVFV's effects on proteins involved in the cell's innate antiviral response. A key protein in the innate response is RIOK3. Although the precise roles of RIOK3 remain controversial, it has been determined to affect the type-1 interferon (IFN) response. Results from our lab showed that RIOK3 helps mount an IFN response in human epithelial cells,[1] but it was recently suggested by another group that RIOK3 plays a role in suppressing the IFN response in immune cells.[2]

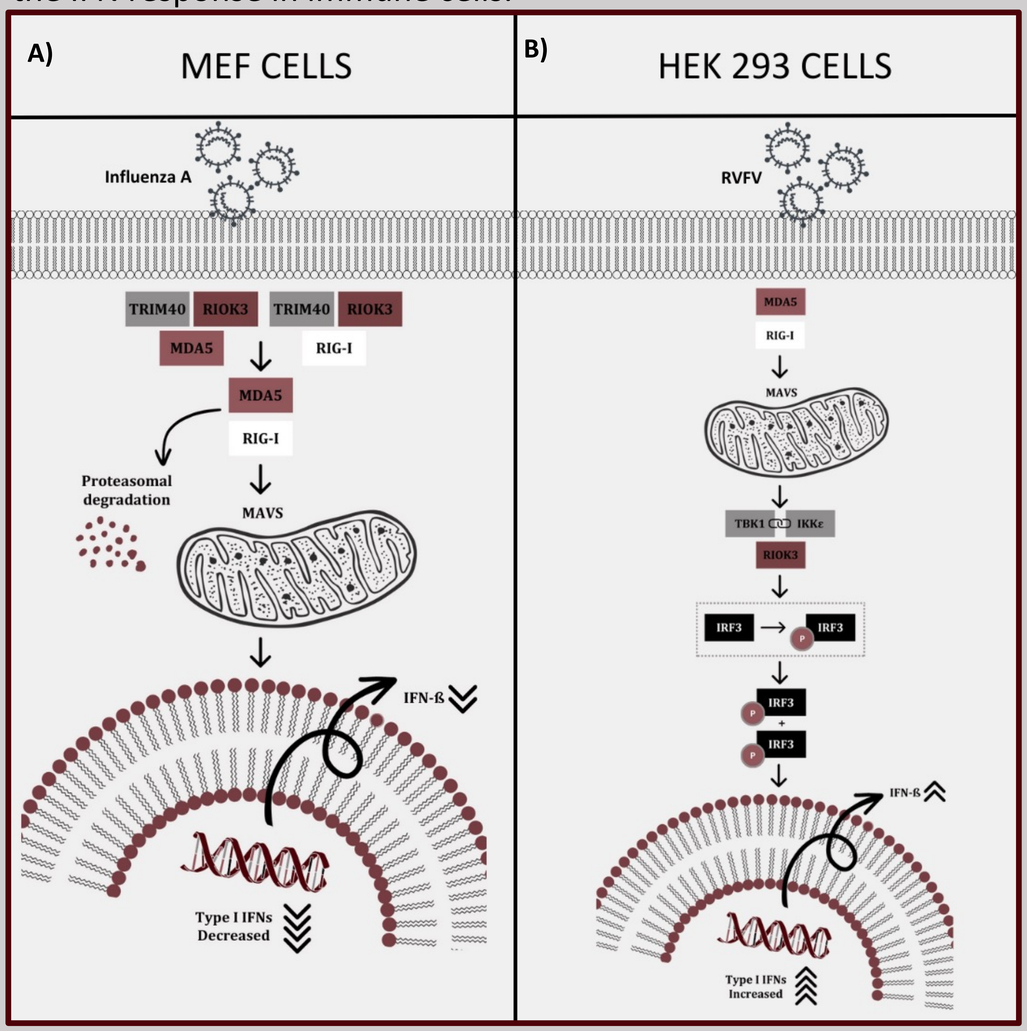

Figure 1: A) Proposed mechanism of TRIM40/RIOK3/MDA5 interaction and downregulation of IFN-β expression in MEF cells[2]. **B)** Proposed mechanism of upregulation of IFN-β expression due to in RIOK3 KO HEK 293 cells[1].

Methods

To test the hypothesis that, similar to mouse immune cells, TRIM40 decreases IFN expression in human epithelial cells, immortalized human embryonic kidney cells (WT HEK-293) were transfected with vectors expressing the protein TRIM40. Cells were immune stimulated using poly(I:C), a potent IFN inducer that mimics viral RNA. We compared expression levels of MDA5 in the cells expressing TRIM40 to control cells by immunoblotting. Additionally, HEK-293s (WT and and RIOK3 KO) were transfected with TRIM40 or GFP and then infected with RVFV (MP12), and expression of IFN-β was assayed by RT-qPCR.

Results

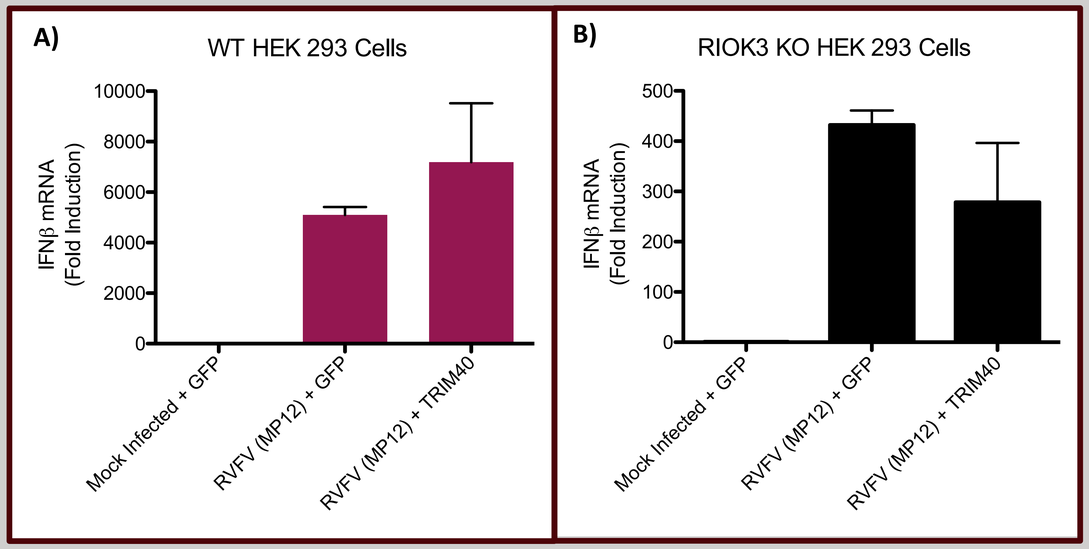

Figure 2: RT-qPCR analysis of relative IFN-β expression in HEK 293 cells transfected with TRIM40 or GFP and infected with MP-12 or mock infected.
A) WT HEK 293 cells transfected with TRIM40 or GFP and infected with MP-12 or mock infected. No statistically significant difference in levels exists.
B) RIOK3 KO HEK 293 cells. No statistically significant difference in levels exists.

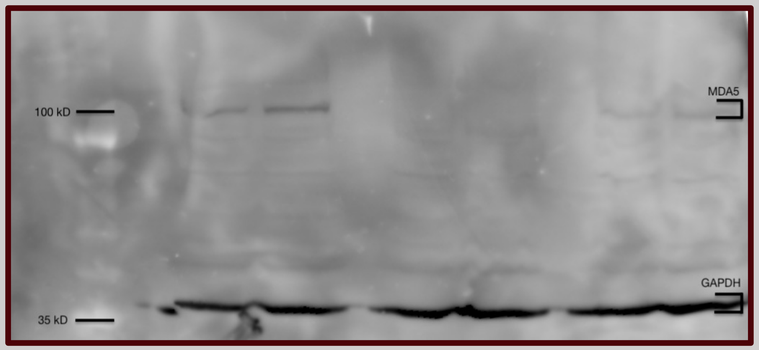

Figure 3: Western Blot analysis of MDA5 protein level expression in WT HEK-293s immune stimulated with Poly I:C or mock stimulated and harvested 30 hours post stimulation.

Discussion

The results of my experiments showed no comparative decrease in cells' expression of MDA5 when TRIM40 was present and also no difference in expression of IFN-β during infection when TRIM40 was overexpressed. Collectively, these results suggest some mechanism other than TRIM40 expression alone is responsible for the other group's observed suppression of the IFN response by RIOK3 in immune cells.

References: 1) Havranek, K. E., White, L. A., Bisom, T. C., Lanchy, M., & Lodmell, J. S. (2021). The Atypical Kinase RIOK3 Limits RVFV Propagation and Is Regulated by Alternative Splicing. *Viruses*, *13*(3). https://doi.org/10.3390/v13030367
2) Shen, Y., Tang, K., Chen, D., Hong, M., Sun, F., Wang, S., Ke, Y., Wu, T., Sun, R., Qian, J., & Du, Y. (2021). Riok3 inhibits the antiviral immune response by facilitating TRIM40-mediated RIG-I and MDA5 degradation. *Cell Reports*, *35*(12), 109272. https://doi.org/10.1016/j.celrep.2021.109272due. **Acknowledgements:** Special thanks to Tom Bisom and Stephen Lodmell for their assistance with this project.