Cellular Metabolism and its Impact on Viral Infection

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Abstract

During viral infection to a cell, the interferon (IFN) is produced and secreted, which will activate the JAK/STAT pathway, which will in turn upregulate the transcription of interferon-stimulated genes (ISGs). One such subset of ISGs is the OAS family genes whose primary function is to produce 2' to 5' linked oligoadenylates by utilizing cellular ATP (Fleming, 2016). In this experiment, we examine the relationship between the changes in cellular ATP levels in response to a viral transfection. Evidence suggests that the synthesis of 2-5As results in an inverse relationship in cellular ATP, due to the activation of OAS, providing an essential link of the metabolic processes during viral infection.

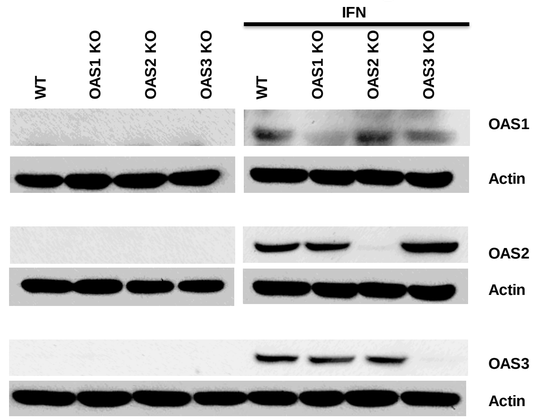
Introduction & Methods

The prevalence of viruses and the impact they can have, as seen by the COVID-19 pandemic, clearly demonstrates the need to identify the mechanisms of various cellular innate antiviral defense pathways. One specific pathway that will be focused on in this research is the 2′-5′ oligoadenylate synthetase (OAS) / RNase L pathway, which is dependent on the amounts of cellular ATP. This pathway is activated in the presence of interferons and the accumulation of the viral dsRNA in the cell.

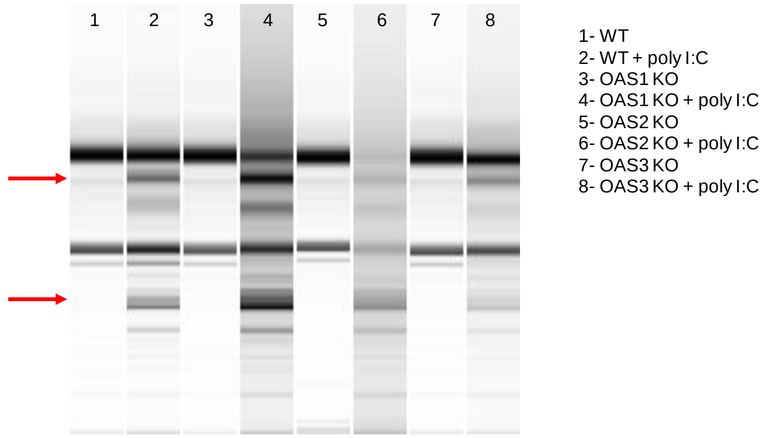
For this experiment, I will be utilizing cell culture, double-stranded RNA transfection, luminescent assay, RNA gel electrophoresis, Western blot, and fluorescence assay. These tools will be used to maintain stable cell lines expressing the proper knockout genes, introduce viral RNA to the cells, measure initial and final amounts of ATP, observe the degradation of RNA and inhibit other defense pathways from being activated.

Results

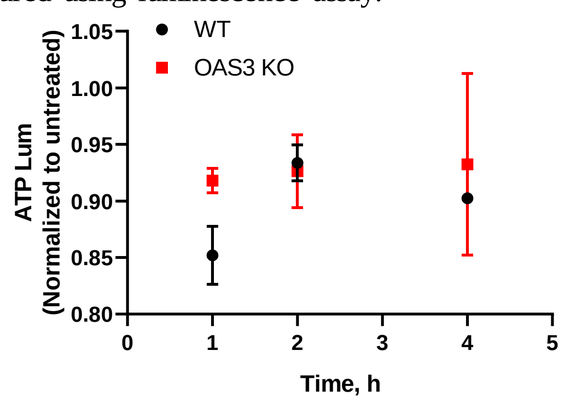
- Using CRISPR-Cas9 technique, OAS1, OAS2 and OAS3 KO cell lines were created and verified using western blotting.



- Poly I:C were treated to all cell lines and RNA cleavage (red arrows) was monitored using RNA chip.



- Cells were transfected with poly I:C and ATP levels were measured using luminescence assay.



Conclusions

- The accumulation of 2-5A in the cells triggers the dimerization of RNase L causing it to degrade all forms of cellular and viral RNA, thus inducing autophagy (Chakrabarti, Jha, & Silverman, 2011).
- Due to dsRNA transfection, cellular energy, ATP is lowered as it is being utilized by the family of OAS genes to synthesize 2-5A.

Future Directions

- The knowledge gained in this research can probe more questions into how varying levels of cellular ATP can impact the survivability of cells.
- Additionally, the data gathered can impact future anti-viral medications in pharmacology.

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THE HUMANITY FIRST RESEARCH SYMPOSIUM

Abstract Submission

Cellular Metabolism and its Impact on Viral Infection

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The prevalence of viruses and the impact they can have, as seen by the COVID-19 pandemic, clearly demonstrates the need to identify the mechanisms of various cellular innate antiviral defense pathways. One specific pathway that will be focused on in this research is the 2′-5′ oligoadenylate synthetase (OAS) / RNase L pathway, which is dependent on the amounts of cellular ATP for which I hypothesize that if cells are exposed to dsRNA or a viral infection, the activation of OAS will decrease cellular ATP level by converting ATP to 2-5A, providing an essential link of the metabolic processes during viral infection. In order to understand the relationship between the OAS/RNase L mechanism and cellular ATP, the procedures I will be utilizing for this experiment include cell culture, viral transfection using plasmid, luminescent assay, RNA gel electrophoresis, Western blot, and fluorescence assay. These tools will be used to maintain stable cell lines expressing the proper knockout genes, introduce viral RNA to the cells, measure initial and final amounts of ATP, observe the degradation of RNA and inhibit other defense pathways from being activated. Based on literature and prior research, it is expected that viral transfections will lower the ATP levels in the cells while also causing the degradation of dsRNA. The data collected from gel electrophoresis clearly indicates that upon viral transfection, the dsRNA in the cell is degraded due to the activation of the RNase L. Moreover, it is predicted that the three OAS gene knockouts will have varying impact on the response to viral RNA based on the amount of ATP consumed to create 2-5As. This project can yield a great deal of information regarding innate immunity that can be used in both clinical and pharmaceutical settings, drastically improving the quality of life for many individuals. Moreover, this research will pose new questions regarding how cells resist viral infections due to other stressors, various plasmids, and the introduction of different viral proteins.