



Introduction

- Epigenetics: The study of how gene expression is changed by environmental or behavioral factors.
- Long-COVID: Condition where COVID-19 symptoms persist much beyond the initial phase of SARS CoV-2 infection¹.
 - Hyperinflammation is noted as one of the cause of those problems¹.
- ATF7 is a stress-responsive transcription factor (TF) that represses the expression of certain genes.²

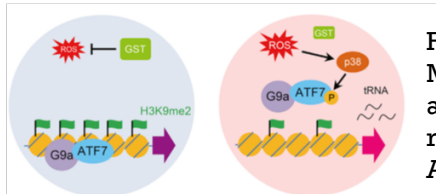


Figure 1: Mechanism of action and regulation of ATF-7³

- The link ATF-7-MAPK was found through bioinformatic analysis.
- Bioinformatics use computer science to get and interpret biological data.
- MAPK pathways are signaling pathways.⁴
 - In our case, between a virus and our cells to which the virus bind.
- **We hypothesize that ATF-7 is a critical epigenetic regulator of genes involved in the inflammatory response seen in long-COVID patients through the MAPK pathway.**

Methods

- We will use the system CRISPR-Cas9 to knockout ATF-7 gene in airway epithelial cells (Beas-2B).⁵
 - The “molecular scissor”
 - We will compare the transcription of certain genes with the knockout and the wild-type.

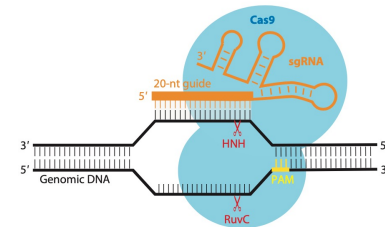


Figure 2: Overview of CRISPR-Cas9 machinery and function⁵

- How CRISPR-Cas9 knockout works⁵:
 - Contains 2 tools: (1) Cas9; (2) guide RNA.
 - Cas9 cuts DNA. Guide RNA guides Cas9 to the precise cut site.
 - It will cut in the coding region of the ATF7 gene.
- How I will actually create this CRISPR cell line:



- Then, look at the transcription and expression of key genes!

Expected results

- In ATF-7 Beas-2Bs knockouts, we should see an upregulation of genes regulated by the predicted MAPK pathway.
- Provide strong evidence that ATF-7 is involved in inflammation regulation through this pathway.

Further directions

- Use relevant cells from patients of long-COVID and look at the ATF-7 regulation in those cells.
- Look at ATF-7 as therapeutic target for long-COVID symptoms.

References

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- (2) Yoshida, K. & al., 2015, *Nat. Immunol.*, <https://doi.org/10.1038/ni.3257>.
- (3) Yoshida, K. & al., 2020, *Mol. Cell*, <https://doi.org/10.1016/j.molcel.2020.02.028>.
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- (5) Ran, F. A. & al., 2013, *Nat. Protoc.*, <https://doi.org/10.1038/nprot.2013.143>.

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