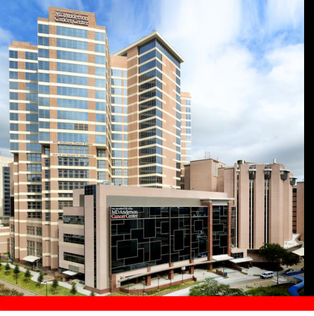

Generation of MLE-15 IRF9 knockdown cells for studying the antimicrobial role of TLRs agonist-induced lung epithelium's reactive oxygen species

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Introduction

- On the global scale, viral and bacterial pneumonia are prominent health threats.[1]
- Immunomodulation via inhalation of synergistic agents Pam2CSK4 ("Pam2," a TLR2/6 ligand) and ODN M362 ("ODN," a TLR9 ligand), together known as "Pam2ODN," broadly protects mice against lower respiratory tract infections by lung epithelial reactive oxygen species (ROS)-mediated pathogen killing.[2,3]
- Current efforts focus on understanding the molecular mechanism underlying this Pam2ODN-induced ROS. The Interferon-Stimulated Gene Factor 3 (ISGF3) transcription factor complex–composed of STAT1, STAT2, and IRF9–drives a *DUOX2*-induced ROS antiviral immune response through its non-canonical STAT2/IRF9-dependent activation pathway.[4]
- Previous microarray data reported the upregulation of IRF9 by Pam2ODN in the cell lines culture and highlighted the requirement of Pam2ODN-induced *Duox2* and ROS to protect mice against viral and bacterial infections.[2,3,5]

Early Findings

- Our examination of IRF9, STAT1, and STAT2 gene regulation using small interfering RNA (siRNA) showed a significant decrease in *Duox2* expression with the siRNA IRF9 and STAT2, but saw normal *Duox2* with siRNA STAT1, alluding to the hypothesis that IRF9 and STAT2 are more likely required for *DUOX2* expression.

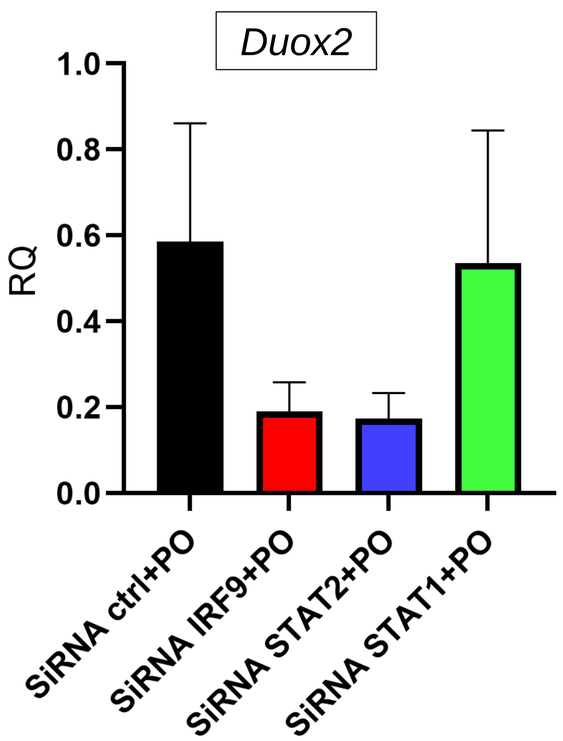

Fig 1. Compared to siRNA control, siRNA IRF9 and siRNA STAT2 show significantly lower *Duox2* expression. siRNA STAT1 *Duox2* expression is insignificantly affected.

Objective

- Evaluate the driving relationship between IRF9 and *DUOX2.*
- Apply molecular biology techniques to generate a stable IRF9 knockdown MLE-15 cell line for study.

Methods

- Bacterial transformation using competent *E. coli* cells was used to clone IRF9 and insert the DNA sequence into a vector backbone.
- Extracted DNA was subsequently used in lentiviral transfection with 293T cells; viral media was collected and used to infect MLE-15 cells to complete IRF9 knockdown.
- Green fluorescent protein (GFP) expression was measured following transfection and infection to confirm the knockdown of IRF9 in the MLE-15 cell line.

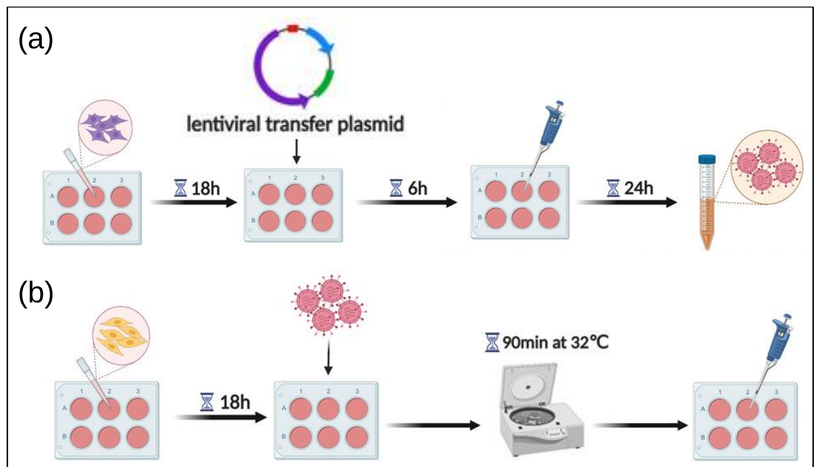

Fig 2. Diagram of steps for (a) lentivirus production from HEK 293T cells and (b) lentivirus transduction of MLE-15 target cells via centrifugation.[6]

Results

Expression of GFP in MLE-15 cells alludes to successful IRF9 lentivirus infection and creation of a stable IRF9 MLE-15 knockdown cell line. GFP-positive cells were sorted and expanded following infection.

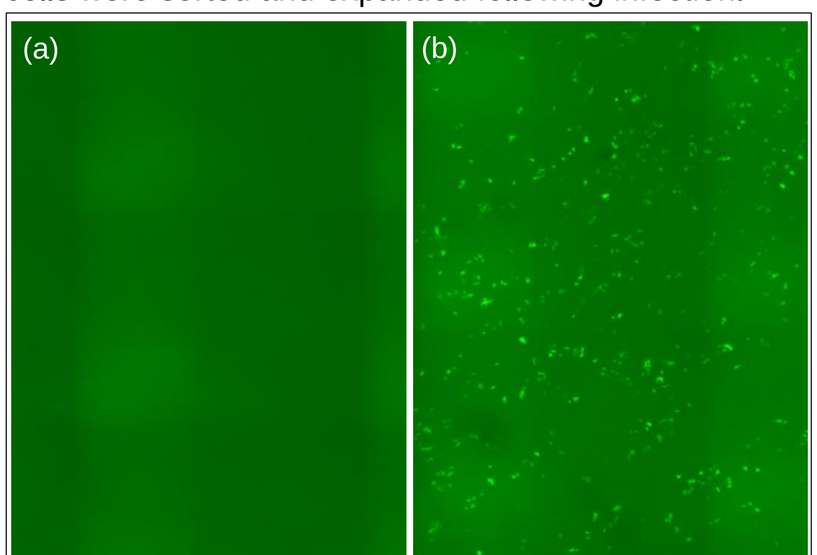

Fig 3. (a) Control MLE-15 cells without IRF9 lentivirus infection and (b) treated MLE-15 cells with IRF9 lentivirus infection.

Conclusion

- After transfection, positive cells inserting the target IRF9 knockdown can be clearly distinguished by GFP expression.
- GFP-positive MLE-15 cells indicate successful infection of the IRF9 lentivirus.
- Generation of a knockdown IRF9 MLE-15 cell line is critical for evaluating the relationship between IRF9 and *Duox2*, as well as other ISGF3-induced genes, as the line offers RNA stability not provided by the siRNA IRF9.

Future Directions

- Use of the MLE-15 IRF9 knockdown line will be used in further molecular biology and immunology experiments including qPCR, blotting, and microarrays to explore the causal relationship between IRF9 and *DUOX2* expression.

Acknowledgements

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