Dissecting the role of Muc5ac in K-RAS Mutant Lung Cancer Progression

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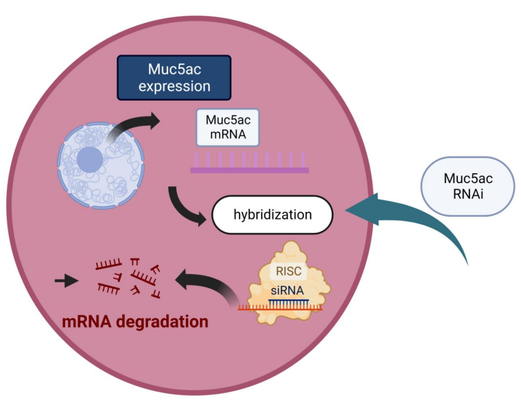
Introduction

- Of all cancer types, lung cancer accounts for the most deaths per year and K-ras mutations are a leading mutational driver. There are limited pharmacological treatment options for K-ras mutant lung cancer due to the resilient nature of the mutation, raising the need to find and target downstream or cooperating effectors of K-ras.
- The mucosal lining of the airways is integral for airway homeostasis and functions to clear pathogens and debris. In healthy tissue, lung epithelial cells secrete two main mucins: moderate levels of muc5b and low levels of muc5ac.
- Notably, patients with K-ras mutant lung adenocarcinoma (KM-LUAD) have been shown to commonly overexpress muc5ac. Overexpression of muc5ac can promote respiratory illnesses through its promotion of an inflammatory lung environment, which can lead to promotion of KM-LUAD.
- Through a genetic approach, we previously observed that knocking out the gene that encodes for MUC5AC (Muc5ac) in a K-ras mutated lung adenocarcinoma mouse model (CC-LR) leads to significantly decreased tumor burden.
- Therefore, Muc5ac might be a potential druggable target in KM-LUAD which we explored in this study.

Methodology

- We utilized CC-LR mice and a pharmacologic approach for inhibiting Muc5ac expression: RNA interference (RNAi). CC-LR mice had muc5ac RNAi treatment started at 6 weeks for a treatment duration of 8 weeks. Treatments are administered through intratracheal injections. MUC5AC RNAi treatment were given twice a week (5mg/kg biweekly).

Muc5ac RNAi Mechanism



Results

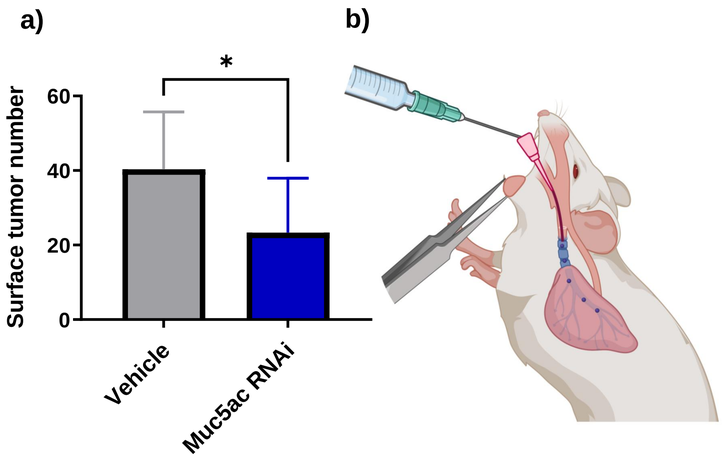


Figure 1. Muc5ac inhibition through RNAi treatment decreases tumorigenesis.
CC-LR mice were treated with saline vehicle or Muc5ac RNAi treatment. **A)** Surface tumor numbers depicted. **B)** Schematic of intratracheal injections. Mice are anesthetized, then injected with 50µL of either RNAi treatment or vehicle (saline).

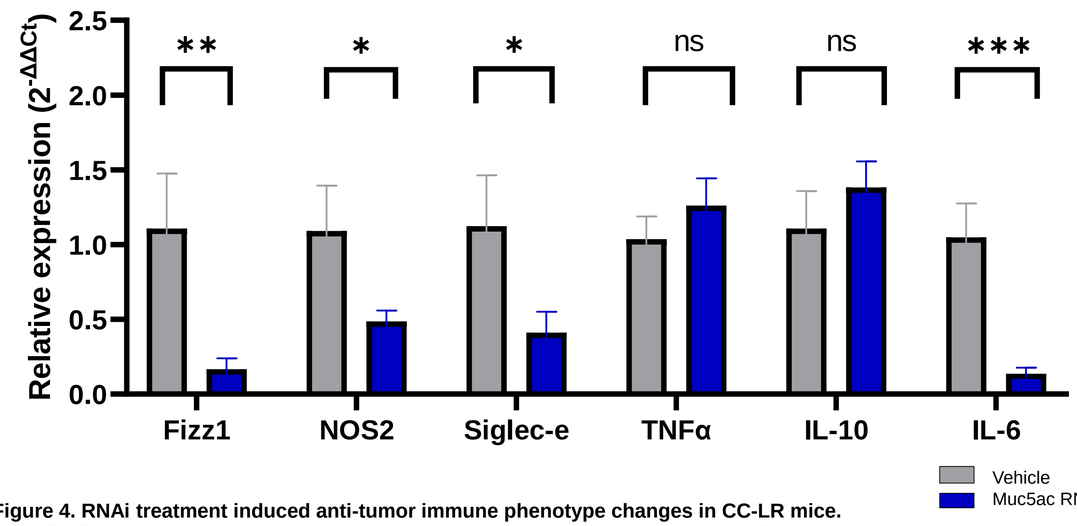


Figure 4. RNAi treatment induced anti-tumor immune phenotype changes in CC-LR mice.
a) Muc5ac RNAi reduced the expression of Fizz1, an M2 macrophage polarization maker. **b)** Muc5ac RNAi reduced the expression of NOS2, an M1 macrophage polarization maker. **c)**. There was a reduction in expression of Siglec-e, a cell surface protein primarily expressed on macrophages with treatment. **d)** TNF-α levels showed a trend of decrease. **e)**IL-10 levels showed a trend of decrease. **f)** IL-6, a pro-inflammatory cytokine, was reduced with RNAi tx.

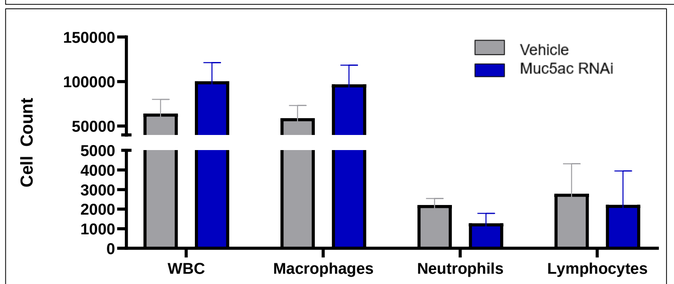


Figure 2. Muc5ac RNAi treatment led to a trend of increase in total WBC and macrophages and a decrease in lymphocytes and neutrophils. White blood count for CC-LR mice analyzed at 14 weeks old. Counts shown for both RNAi vehicle mice cohorts and treatment mice cohorts.

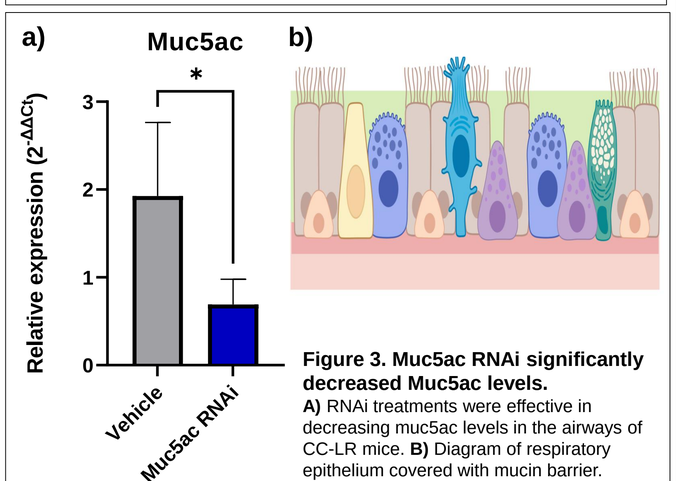


Figure 3. Muc5ac RNAi significantly decreased Muc5ac levels.
A) RNAi treatments were effective in decreasing muc5ac levels in the airways of CC-LR mice. **B)** Diagram of respiratory epithelium covered with mucin barrier.

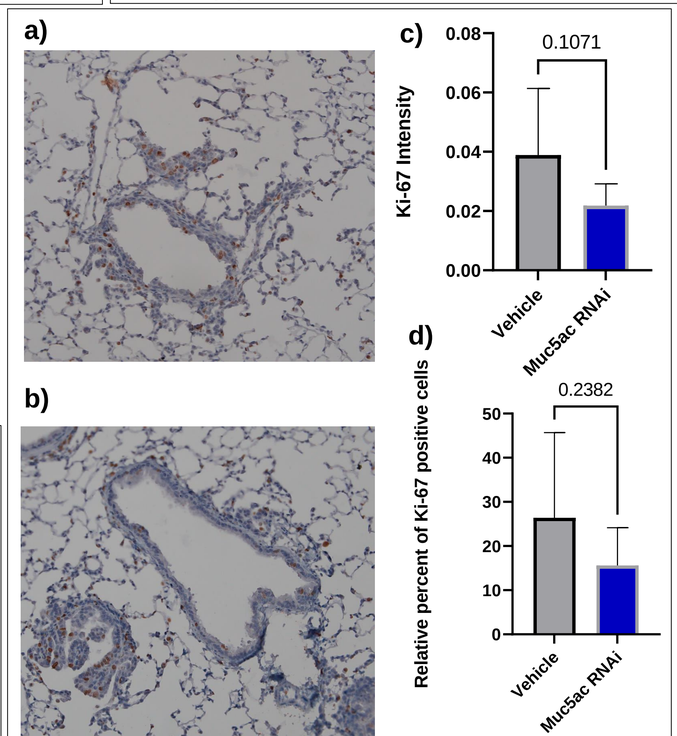


Figure 5. Muc5ac RNAi decreased the percent and intensity of Ki-67+ tumor cells.
A) Representative image of Ki-67 stained lung tissue for vehicle CC-LR mouse treated with saline. **B)** Representative image of Ki-67 stained lung tissue for CC-LR mouse treated with Muc5ac RNAi. **C)** Intensity of Ki-67 staining for vehicle and Muc5ac RNAi treated mice. **D)** Percent of Ki-67+ stained tumor cells for vehicle and Muc5ac RNAi treated mice.

Conclusion

Targeting Muc5ac using RNAi is a potential alternative approach for prevention and treatment of KM-LUAD via tumor cell intrinsic and immunomodulatory paracrine mechanisms.

Future Work

- Immunophenotype the lung TME using flow cytometry.
- Look at reactive oxygen species (ROS) levels
- Utilize CC-LR Siglec-e$^{-/-}$ mice
- Use immunofluorescence to stain for Siglec-e and macrophages/muc5ac to see co-localization
- Redo experiments with more mice cohorts.

Acknowledgements

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