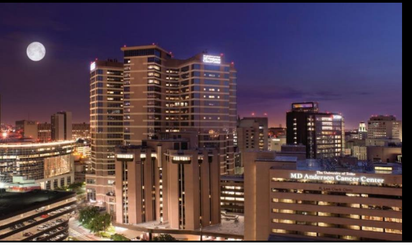

Iron dynamics and microbiome dysbiosis during tobacco-associated lung adenocarcinoma development

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Background

Our group has shown that loss of the airway lineage-specific G-protein-coupled receptor 5a (*Gprc5a*$^{-/-}$) leads to lung adenocarcinoma (LUAD) development particularly in animals exposed to the tobacco-specific carcinogen nicotine-derived nitrosamine ketone (NNK) (1). Lipocalin 2 (LCN2), an immunomodulatory protein that we showed to exert protective roles against LUAD development (2), is involved in microbiome homeostasis and prevents non-commensal bacterial overgrowth that causes inflammation and potential carcinogenesis (3). LCN2 was shown to bind to iron-laden siderophore proteins that are needed by non-commensal bacteria to thrive (4). However, interplay between LCN2, iron dynamics, and lung microbiome changes in relation to LUAD development is yet to be determined. Towards this, we probed iron and microbial changes during LUAD oncogenesis.

Hypothesis

Microbial dysbiosis and changes in iron levels are associated LUAD development. These may be further excoriated by loss of *Lcn2*.

Methods

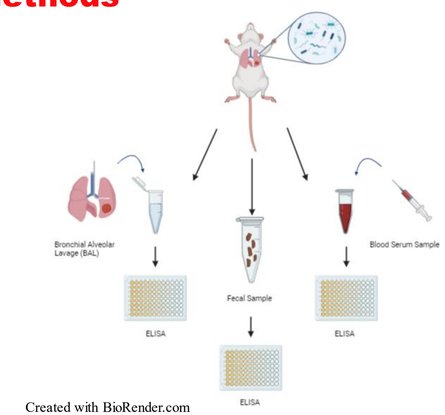
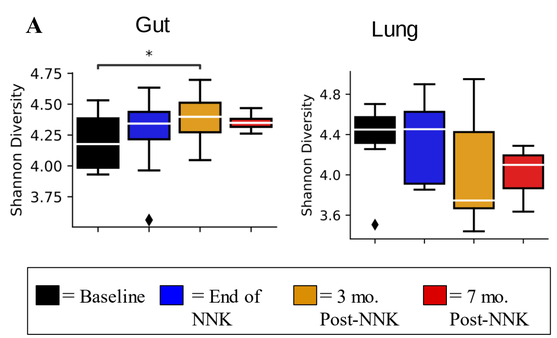


Figure 1. Analysis of microbial flora in the gut and lung as well as iron levels during LUAD development *in vivo*. Bacterial microbiome profiling (both gut and lung) was performed by sequencing the !6S v4 region using the Illumina platform. Analysis of changes in microbial alpha diversity were statistically examined using Shannon diversity index. Temporal analysis of ferritin, an iron storing protein, and free iron levels (Fe^{2+} and Fe^{3+}) in sera and bronchoalveolar lavage fluid (BALF) from *Gprc5a*$^{-/-}$ mice seven months post-NNK exposure were compared to baseline. Serum ferritin was also examined in wild type (WT), *Gprc5a*$^{-/-}$, and *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ littermates four hours post lipopolysaccharide (LPS) injection. Data were plotted and two-tailed paired t-tests were performed to determine significance.

Results

***Gprc5a*$^{-/-}$**



***Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$**

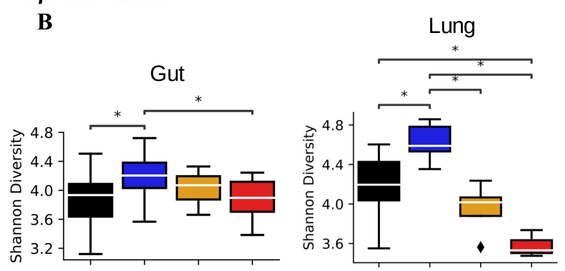


Figure 2. Changes in diversity of gut (left panels) and lung (right panels) microbiomes with time, *p<0.05.

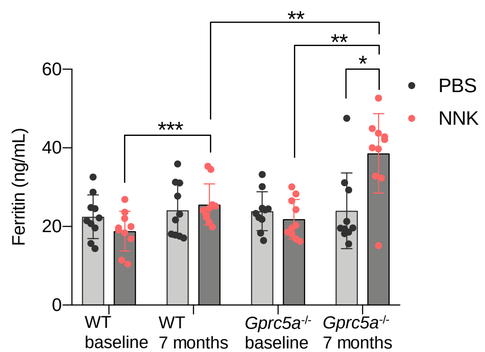


Figure 3. Changes in iron levels in BALF from WT and *Gprc5a*$^{-/-}$ mice. *p<0.05, **p<0.01, ***p<0.001

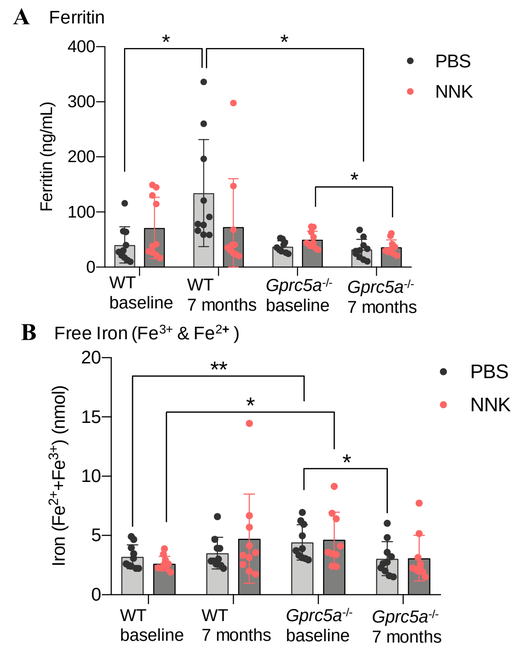


Figure 4. A.& B Changes in iron levels in serum from WT and *Gprc5a*$^{-/-}$ mice. *p<0.05, **p<0.01, ***p<0.001

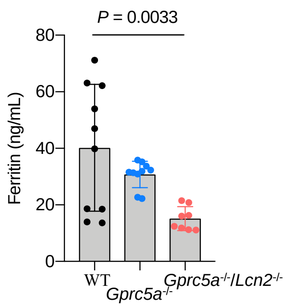


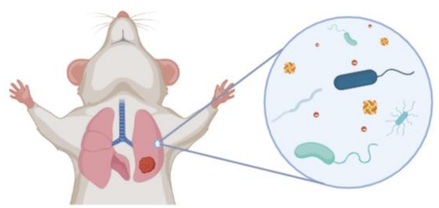
Figure 5. Changes in iron levels in serum four hours post LPS stimulation.. *p<0.05, **p<0.01, ***p<0.001

Discussion

- Iron levels increase in the lung late during oncogenesis.
- In *Gprc5a*$^{-/-}$ mice, free iron and ferritin circulating in the serum are less pronounced than iron levels onsite during lung oncogenesis.
- Loss of Lcn2 further attenuates microbial diversity and ferritin levels suggesting that this immunomodulator counteracts microbiome dysbiosis during LUAD development.

Future Directions

- Investigation of the interplay between iron levels and immune response during LUAD development



References

1. Fujimoto J et al, Development of Kras mutant lung adenocarcinoma in mice with knockout of the airway lineage-specific gene Gprc5a, International Journal of Cancer, 2017
2. Treekitkarnmongkol W et al, Augmented Lipocalin-2 Is Associated with Chronic Obstructive Pulmonary Disease and Counteracts Lung Adenocarcinoma Development, American Journal of Respiratory and Critical Care Medicine, 2021
3. Moschen AR, Lipocalin 2 Oritects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations, Cell Host & Microbe, 2016
4. Flo TH et al, Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron, Nature, 2004

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