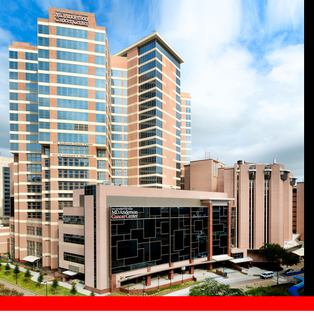

Lipocalin 2 protects from lung tumorigenesis associated with gut microbiota alterations

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Introduction

Lung adenocarcinoma (LUAD), the most common cancer diagnosed in smokers, frequently exhibits somatic mutations in the *KRAS* oncogene (1). *KRAS*-mutant LUAD (KM-LUAD) displays dismal prognosis warranting new strategies for early interception. Limiting advances is poor understanding of early events in pathogenesis. Growing evidence shows the microbiome is a key player in modulating host immune response, tumorigenesis, and response to therapy (2). Yet, our knowledge of the gut-lung axis is still in its infancy.

Our group has shown that loss of G protein coupled receptor, class C, group 5, member A (*Gprc5a*$^{-/-}$) leads to tumors after exposure to the carcinogen nicotine-specific nitrosamine ketone (NNK) that harbor the driver mutation *Kras*^{G12D} found in human KM-LUAD (3). We further found progressive lipocalin 2 (Lcn2) elevation during inflammation and LUAD development. Lcn2 is an anti-microbial protein that was shown to sequester iron-laden siderophores essential for bacterial metabolism and prevent the overgrowth of bacterial species associated with inflammation and carcinogenesis (2). Lcn2 loss increased LUAD tumorigenesis, supporting its tumor protective effects (1). Tumor burden after NNK exposure was associated with progressive and distinct changes in gut microbiome composition in *Gprc5a*$^{-/-}$ and *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ mice. This suggests that Lcn2 loss could be implicated in microbial dysbiosis and may explain the ensuing increased tumor burden. To address this hypothesis, we investigated the effects of gut microbiome modulation in a syngeneic lung cancer mouse model using fecal microbiota transfer (FMT).

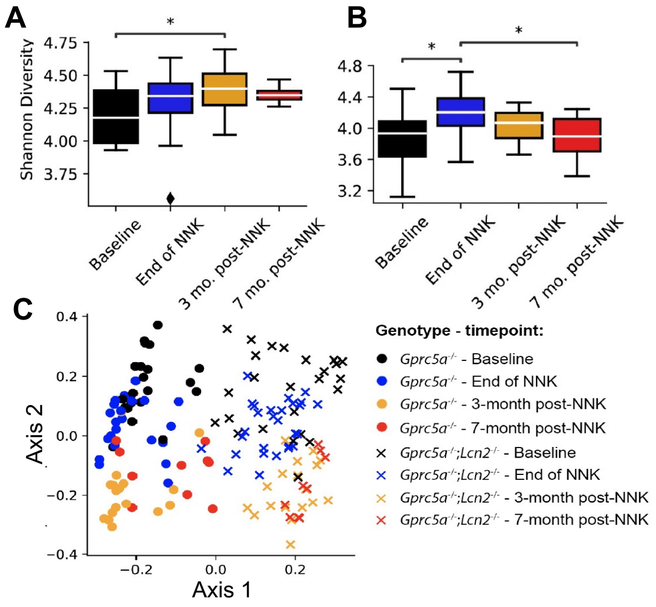
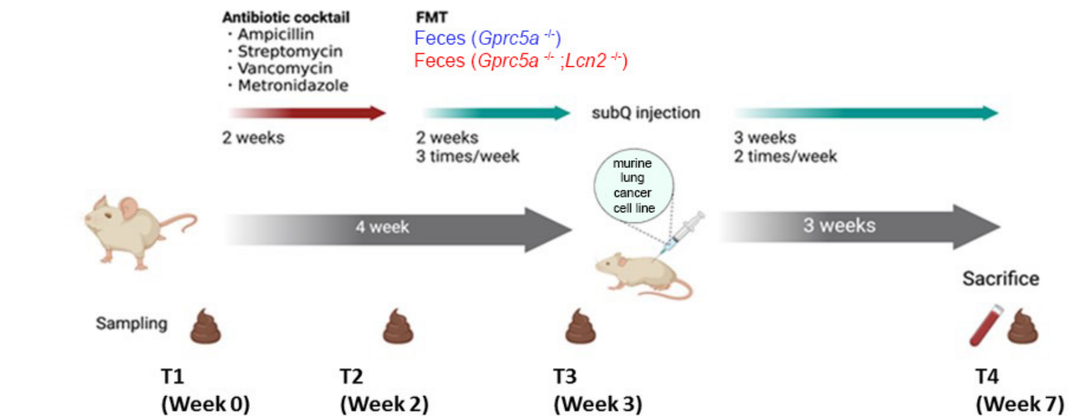


Figure 1. Gut microbiome changes during the phenotypic evolution of tobacco carcinogen-associated LUAD. **A, B.** Gut microbiome α-diversity evaluated using the Shannon index of *Gprc5a*$^{-/-}$ (**A**) and *Gprc5a*$^{-/-}$/ *Lcn2*$^{-/-}$ (**B**) fecal samples collected at Baseline, end of NNK, 3-, and 7-month post-NNK. **C.** Bray-Curtis and OTU-based clustering of fecal samples showing segregation based on genotype (axis 1) and timepoint (baseline, end of NNK, three and seven months post-NNK; axis 2).

Methods



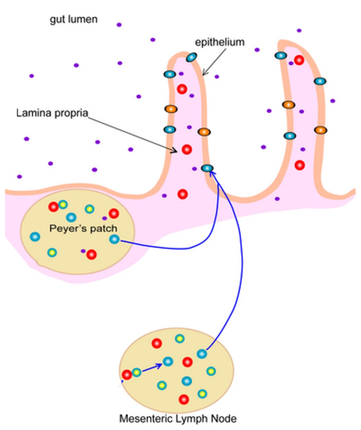
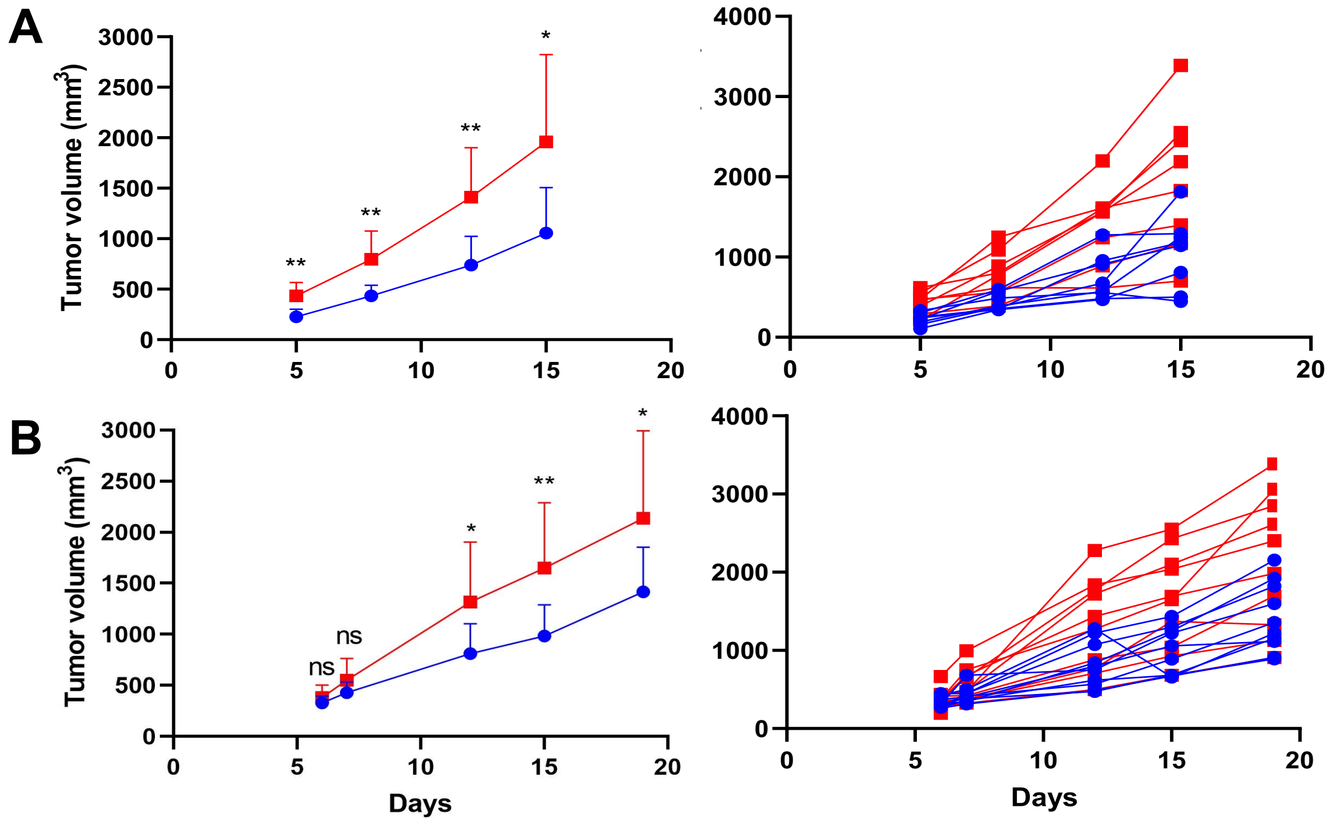


Figure 2. Upper panel. Antibiotic cocktail was continuously supplied to each of *Gprc5a*$^{-/-}$ or 129-Elite WT mice in water for two weeks. Recipients (from each genotype) received FMT derived from either *Gprc5a*$^{-/-}$ or *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ feces via oral gavage for two weeks. 5 million mF471 cells or 2 million LKR13 cells were then subcutaneously injected into *Gprc5a*$^{-/-}$ and 129-Elite WT mice, respectively. FMT continued for 3 more weeks following injection and tumors were measured twice weekly starting at day 5 post-injection using a digital caliper. At endpoint (tumor volume ≥ 2000mm^3), fecal pellets and blood were collected. **Left panel.** The lamina propria, Peyer's patches, and mesenteric lymph nodes were also collected for downstream analyses. Adapted from Moretto MM et *al., PLOS Pathogens,* 2012.

Results



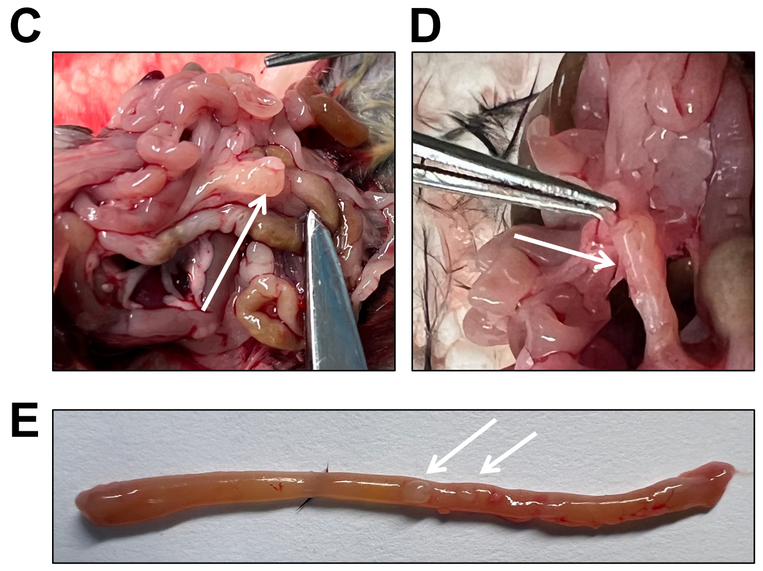


Figure 3. A, B. Average and individual tumor volumes in *Gprc5a*$^{-/-}$ (**A**) and 129-Elite (**B**) mice following subcutaneous tumor cell implantation. Red denotes mice receiving FMT derived from *Gprc5a*$^{-/-}$ animals and blue denotes mice receiving FMT derived from *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ mice. *, $P \leq 0.05$; **, $P \leq 0.01$. **C, D.** Mesenteric lymph nodes (indicated by white arrows) from *Gprc5a*$^{-/-}$ mice that received FMT from *Gprc5a*$^{-/-}$/ *Lcn2*$^{-/-}$ feces (**C**) compared to mice receiving FMT derived from *Gprc5a*$^{-/-}$ feces donors (**D**). **E.** Erythematous and inflamed Peyer's patches (white arrows) from *Gprc5a*$^{-/-}$ mouse receiving FMT from *Gprc5a*$^{-/-}$/ *Lcn2*$^{-/-}$ feces.

Conclusions & future directions

- Our FMT experiments conducted in two different syngeneic models highlight a novel protective role for gut-specific microbiome homeostasis in the development of LUAD. Our novel findings show that Lcn2 is critically important to control bacterial community makeup against bacteria that perpetuate LUAD tumorigenesis.
- We will perform 16S rRNA sequencing on fecal samples obtained during our FMT experiments. Future experiments will involve iron chelators and narrow-spectrum antibiotics to target specific tumor-promoting bacteria.
- We will further validate our findings in a lung carcinogenesis model; *Gprc5a*$^{-/-}$ and *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ mice exposed to NNK will receive FMT derived from *Gprc5a*$^{-/-}$ and *Gprc5a*$^{-/-}$/*Lcn2*$^{-/-}$ feces.
- Single-cell RNA sequencing and flow cytometry analyses will be done on tumor plugs and mesenteric lymph nodes to assess the immune contexture and trajectories taking place from the gut to the systemic circulation.

References

1. Treekitkarnmongkol W, et al. *American Journal of Respiratory and Critical Care Medicine*. 2021.
2. Moschen AR, et al. *Cell Host & Microbiome.* 2016.
3. Fujimoto J, et al. International Journal of Cancer. 2017.

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