Methods
microbiota transfer (FMT).

In our study, we demonstrated that G protein coupled receptor, class C, group 5, member A (Gprc5a-/-) leads to tumors after exposure to the carcinogenic nicotine-specific nitroamine ketone (NNK) that harbor the driver mutation Kras12D12 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). With the 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 the syngeneic lung cancer mouse model using fecal microbiota transfer (FMT).

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 carcinogenic nicotine-specific nitroamine ketone (NNK) that harbor the driver mutation Kras12D12 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).

Results

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-/- and Gprc5a-/-/Lcn2-/- by 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 time point (baseline, end of NNK, three and seven months post-NNK; axis 2).

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 ≥ 2000mm³), 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.

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 ≤ 0.05; **, P ≤ 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-/-/Lcn2-/- 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


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