Elucidating the Mechanistic Role of IL-1R in Late-Stage K-ras Mutant Lung Cancer: Uncovering Therapeutic Potential

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Background

- Lung cancer is the leading cause of cancer-related deaths worldwide in both men and women.
- K-ras mutant Lung Adenocarcinoma (KM-LUAD) is strongly linked with the activation of **pro-inflammatory pathways**
- The **interleukin-1 receptor (IL-1R)** has emerged as a critical mediator of inflammation and tumorigenesis due to its interactions with IL-1β, a potent activator of the NF-κB pathway.
- Conditionally knocking-out IL-1R in murine models that constitutively express $Kras^{G12D}$ ($CCSP^{Cre}$/LSL-$Kras^{G12D}$, CC-LR) at 14 weeks of age (early-stage KM-LUAD) has shown a significant decrease in tumor burden as well as an overall increase in inflammation.
- Previous studies administering IL-1β blockade to CC-LR mice showed therapeutic potential, however the **precise mechanistic role of IL-1R** in lung cancer progression within the lung epithelium remains poorly understood, especially in late-stage KM-LUAD.

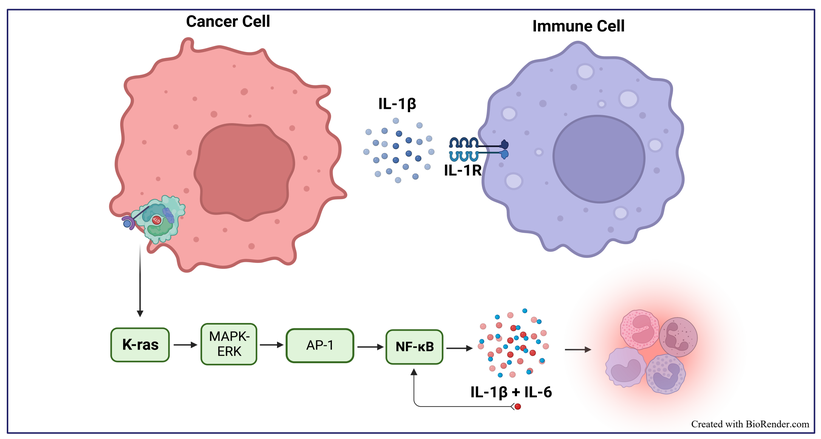
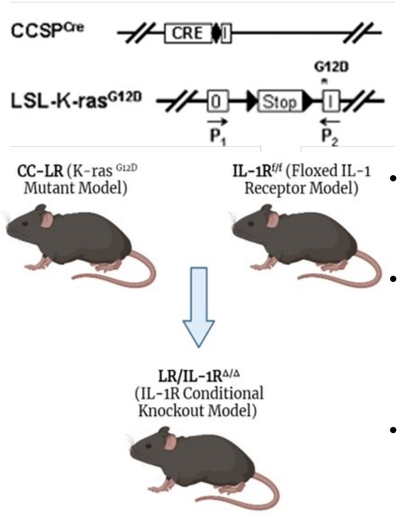


Figure 1. K-ras mutant lung cancer cell interaction with immune cell via IL-1R/IL-1β signaling in response to inflammation within the tumor microenvironment.

Methods



- H&E staining and analysis to visualize microanatomy and measure tumor area
- IHC staining and analysis to view immunoinflammatory response
  - Ki-67 (proliferation)
  - ERG (angiogenesis)
- Rt-qPCR to confirm activity of inflammatory pathways and view gene expression

Figure 2. Development of the CC-LR murine model, *Moghaddam et al.;* How the LR/IL-1R$^{Δ/Δ}$ model was created through crossing two distinct Cre-lox lines; List of methodological techniques used in study

What is the true role of IL-1R with regards to the tumor epithelium?

Is there a difference in results between what we see in early vs. late stage?

How does the tumor microenvironment change as a result of blocking the IL-1R receptor?

Results

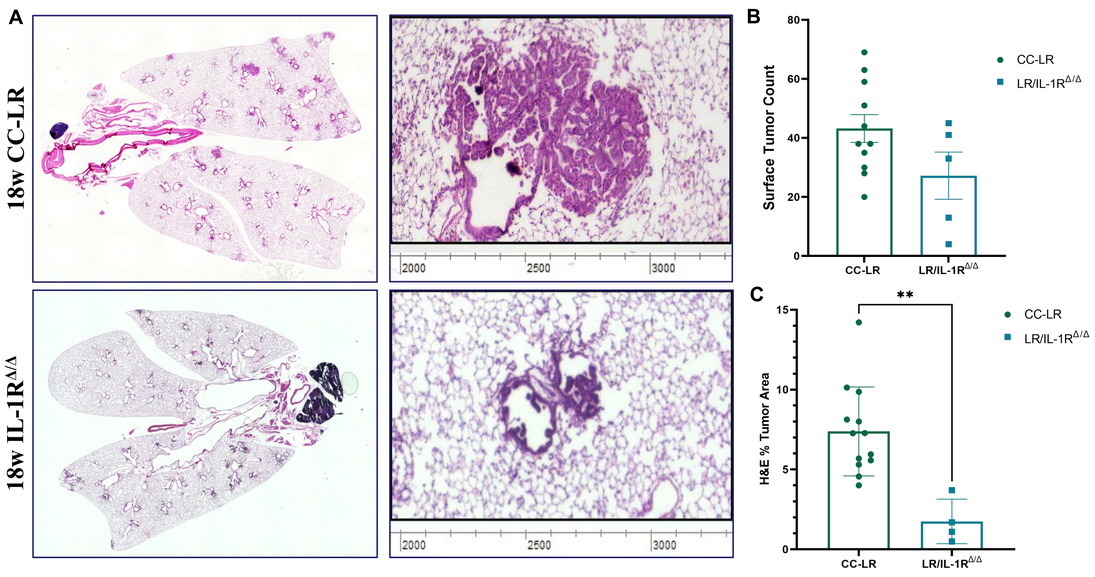
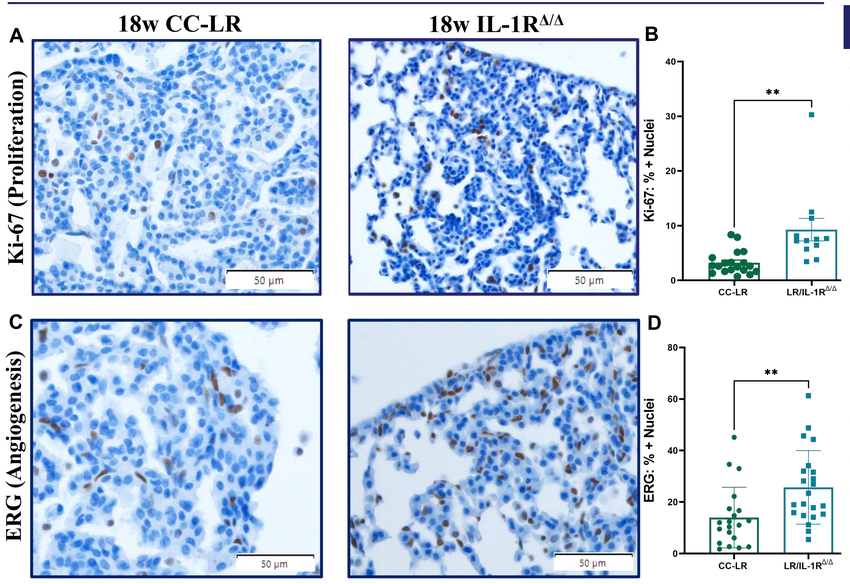


Figure 3. Conditional knockout of the IL-1 receptor within tumor epithelial cells led to a reduction in late-stage tumor burden as well as shift from adenocarcinoma (ADC) to an atypical adenomatous hyperplasic (AHH) tumor phenotype. Mice were dissected at 18 weeks of age, threshold where adenomas and adenocarcinomas begin forming. (A) Representative photomicrographs of H&E stained lung sections (4x) and corresponding representative images of tumor histology (20x) within 18-week CC-LR control mice and LR/IL-1R$^{Δ/Δ}$ mice respectively. (B) Total surface tumor number was obtained for each mouse upon dissection. (C) Quantification of lung tumor area. Data represents mean±SEM; unpaired t-test, *p<0.05.



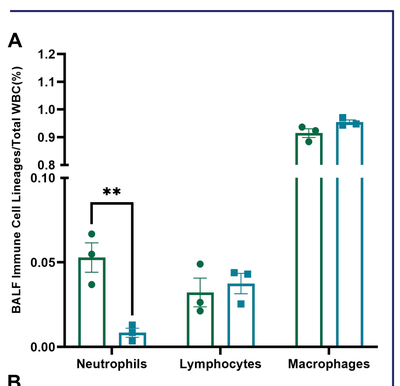


Figure 4. Conditional knockout of IL-1R in tumor epithelium increases tumor proliferation and angiogenesis in late-stage mice. (A&C) Representative photomicrographs of IHC stained whole lung sections (40x). (B&D) Percentage of intertumoral cells expressing Ki-67 proliferation marker and ERG angiogenesis marker respectively.

Figure 5. Conditional knockout of IL-1R in tumor epithelium potentially promotes an anti-tumor immune phenotype. Indicated via a significant decrease in neutrophils and decreased trend in M2 macrophages and NF-κB pathway associated genes. (A) Immune cells in broncho alveolar lavage fluid (BALF), taken as a percentage over total white blood cell count. (B) Quantitative polymerase chain reaction (qPCR), normalized to *Cd45.* Data represents mean±SEM; unpaired t-test, *p<0.05.

Discussion

We have found that at the late-stage timepoint, although there were insignificant effects on surface tumor number, there was a significant decrease in tumor area in the LR/IL-1R$^{Δ/Δ}$ group. Further analysis via H&E staining showed a shift from an ADC stage towards a AHH stage. Upon IHC analysis using Ki-67 and ERG markers the LR/IL-1R$^{Δ/Δ}$ group was seen to have increased angiogenesis and tumor proliferative cells, both of which are hallmarks of the hyperplasic stage. This supports the idea that IL-1R inhibition hindered tumor growth at the early-stage timepoint, prompting the existence of highly proliferative hyperplasic structures upon reaching the late-stage timepoint. Additional analysis of BALF showed a significant decrease in neutrophils among other immune cell types, providing evidence that the immunosuppressive phenotype of the tumor epithelium was being combatted. This was supported by qPCR analysis that showed a decreased trend in *Cxcl1*, *IL-17*, and IL-6, known neutrophil chemo-attractants, as well as a significant decrease in *Arg1* and other myeloid specific immunosuppressive markers. A decrease in *IL-6* also suggests inhibition of the NF-κB pathway via receptor knockout. This supports the potential mechanistic involvement of IL-1R in regulating tumor burden within the tumor microenvironment specifically in late-stage K-ras mutant lung cancer. These findings are mostly consistent with the knockout mechanism originally hypothesized in the early-stage study and suggest the IL-1 receptor to be a promising target for immuno-preventative therapy at the early rather than late-stage timepoint.

Conclusion & Future Directives

Our data indicates a shift in immunoinflammatory response upon knockout of the IL-1 receptor between early and late stage timepoints. This potentially supports targeting IL-1R for immuno-preventative therapy at the early rather than late-stage timepoint.

Going forward, we would like to confirm our findings by running a comparative study containing both 14- and 18-week-old LR/IL-1R$^{Δ/Δ}$ mice. Additional experiments such as p65 staining via IHC, flow cytometry, and qPCR using NF-κB associated markers such as IKBα would further characterize the TME and evaluate the potential adaptive response to the IL-1R knockout.

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