129 PLATFORM TO CONDITIONALLY INDUCE LUNG TUMORS IN GENETICALLY ENGINEERED MOUSE MODELS USING CRE MRNA CONTAINING NANOPARTICLES

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Background Pre-clinical models are crucial for better understanding and treating human diseases such as cancer. Autochthonous models of lung cancer are excellent tools to study tumor progression and evolution. However, current approaches rely on intra-tracheal, viral delivery of Cre recombinase to initiate tumorigenesis by activating oncogenes and deleting tumor suppressor genes.[1] Two caveats of this technique are the lack of cell-type specificity of viral vectors and the resulting immunogenicity of these vectors. We have demonstrated that lentiviral delivery of Cre recombinase (Cre-LV) to the lungs of mice results in infection of dendritic cells (DCs) and macrophages and innate immune activation. To study the immune response to naturally progressing lung cancer while controlling for the confounding immunogenicity of viral vectors, we have developed a virus-free platform to induce lung tumors in genetically engineered mice.

Methods We encapsulated Cre mRNA in polymeric nanoparticles (NPs) made from a Poly(Beta-Amino Ester) (PBAE). The resulting cationic NPs were layered with anionic poly-aspartic acid (PLD) to preferentially target epithelial cells and reduce immunogenicity by decreasing interactions with immune cells.[2] To optimize particle size and mRNA packaging for efficient *in-vivo* delivery, we screened different mRNA:PBAE ratios. Top candidates were then tested *in-vitro* on the Green-Go reporter cell line, which becomes GFP^+upon Cre expression, to determine the transfection efficiency of these NPs.[3] To induce lung tumors, we intra-tracheally injected the optimized PLD-layered Cre mRNA-NPs (PLD Cre-NPs) into $Kras^{LSL\text{-}G12D}$; $p53^{fl}$; $TdTomato^{fl}$ ($KP^{Tdtomato}$) reporter mice.[1] We compared tumor initiating capacity of these NPs to Cre-LV and analyzed tumor progression, innate immune activation and cell-type specific infection.

Results PLD Cre-NPs efficiently formed tumors in $KP^{Tdtomato}$ mice, with comparable histology to Cre-LV induced tumors. However, unlike Cre-LV induced tumors, PLD Cre-NP administration resulted in fewer lesions at late timepoints, which better recapitulates human disease. Further, PLD Cre-NPs primarily infect epithelial cells and minimally infect myeloid cells such as DCs and macrophages. Finally, compared to Cre-LV infection, PLD Cre-NP administration reduces innate immune activation.

Conclusions Current approaches to induce lung tumors in autochthonous mouse models rely on viral delivery of Cre which elicits an immune response. PLD Cre-NPs are efficient at forming lung tumors without activating and infecting innate immune cells, enabling a more controlled characterization of the immune landscape in lung cancer. This technology can also be extended to other conditional mouse models of cancer where delivery of the NPs can be localized to the tissue of origin.

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Ethics Approval All mouse experiments were approved by MIT's Committee on Animal Care (CAC) – DHHS Animal Welfare Assurance # D16–00078

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