Precision Cut Lung Slices as a model for Lung Chemoprevention Studies

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1 Background

Precision cut lung slices (PCLS) have become a popular method for studying multicellular interaction *in vitro,* as they maintain translational relevance while minimizing the cost and time burden of large *in vivo* mouse studies. While PCLS has been used to primarily study various diseases in the lung such as upper respiratory infections, fibrosis, COPD, etc, a cancer model using PCLS has not been explored.[1] Iloprost, a prostacyclin analog, exhibited anti-tumor properties by reducing the growth of endobronchial dysplasia a Phase II clinical trial.[2] In order to assess the validity of PCLS as a chemoprevention model, Iloprost was administered to PCLS from untreated, and *in vivo* urethane treated mice. The results were compared back to previous *in vivo* models in our lab to confirm the ability of PCLS to recapitulate *in vivo* observations.

Our goal is to validate PCLS as a translational *ex vivo* model for future lung cancer chemoprevention exploration

2 Experimental Design

Wildtype female FVB/N mice were used to collect 500uM precision cut lung slices (PCLS)

PCLS were dosed with 10µM Iloprost *ex vivo* every 48 hours

Chemoprevention effects of Iloprost in PCLS *ex vivo* were analyzed by Presto Blue, Immunoblot, PPRE Dual Luciferase Assay, and RTqPCR for chemoprevention signaling and compared back to *in vivo* studies with Iloprost.

3 Results

Wildtype Iloprost treatment

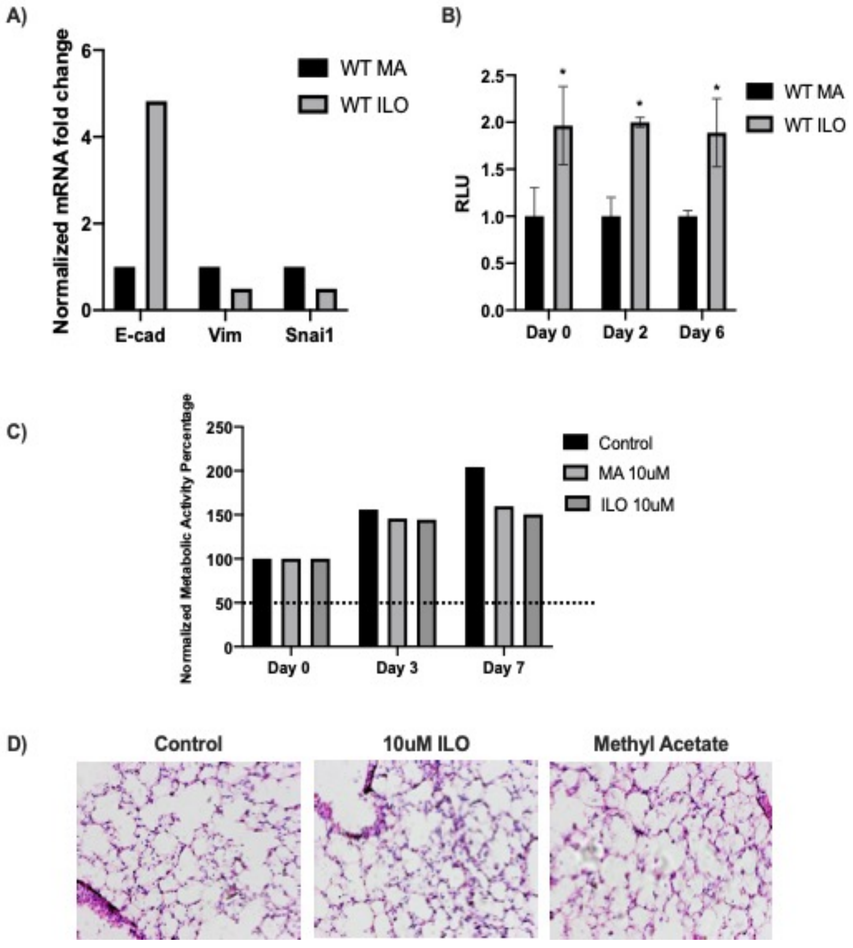

Figure 1. ***In vivo Iloprost signaling was recapitulated in wild-type PCLS treated with 10uM Iloprost.*** A) EMT genes were analyzed in wild-type PCLS treated with 10uM iloprost or the control to confirm congrinuity between *in vivo* and *ex vivo* Iloprost signaling. B) PPRE dual-luciferase assay confirmed PPARλ signaling was increased in wild-type PCLS following Iloprost treatment *ex vivo*, similarly observed *in vivo*. C) Presto Blue data confirmed the wild-type PCLS punches maintained viability throughout the duration of the experiment regardless of dosing group. D) H&E stains were performed on frozen wild-type PCLS slices following *ex vivo* Iloprost or control treatment for 7 days to confirm structural integrity of the tissue was maintained.

Urethane *in vivo* Iloprost *ex vivo* Treatment

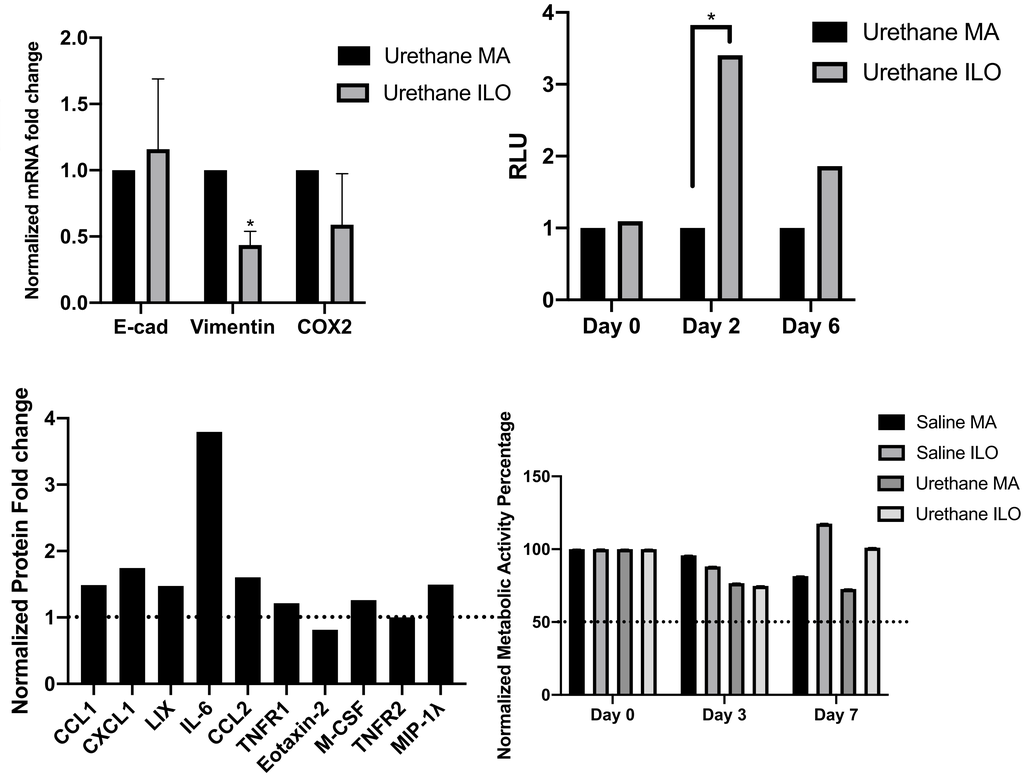

Figure 2. NTCU Ex Vivo PCLS exhibit changes in gene expression while maintaining viability. A) PCLS punches were dosed with NTCU or the corresponding vehicle for six weeks. The viability of the punches was analyzed weekly by a Presto Blue assay. B) Changes in E-cadherin and PTGS2 gene expression were analyzed in PCLS punches in hydrogels both hydrogel conditions by qPCR.

4 Conclusions And Future Directions

- **PCLS recapitulates *in vivo* findings as a NSCLC chemoprevention model *ex vivo* with Iloprost.**
- **In addition to the data provided, additional NSCLC chemoprevention agents undergoing clinical trials have been tested on PCLS, and recapitulated *in vivo* findings from previous studies.**
- **In an effort to test the anti-cancer properties of various chemoprevention agents, our lab and a bioengineering lab have collaborated to embed PCLS in hydrogels in order to extend viability ex vivo. By extending their viability, we plan to induce lesions with vinyl carbamate *ex vivo,* and test the ability of various chemoprevention agents to inhibit lesion development *ex vivo*.**

References

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