Loss of Frizzled 9 alters lung epithelial cell signaling pathways and interferes with the protective effect of iloprost chemoprevention

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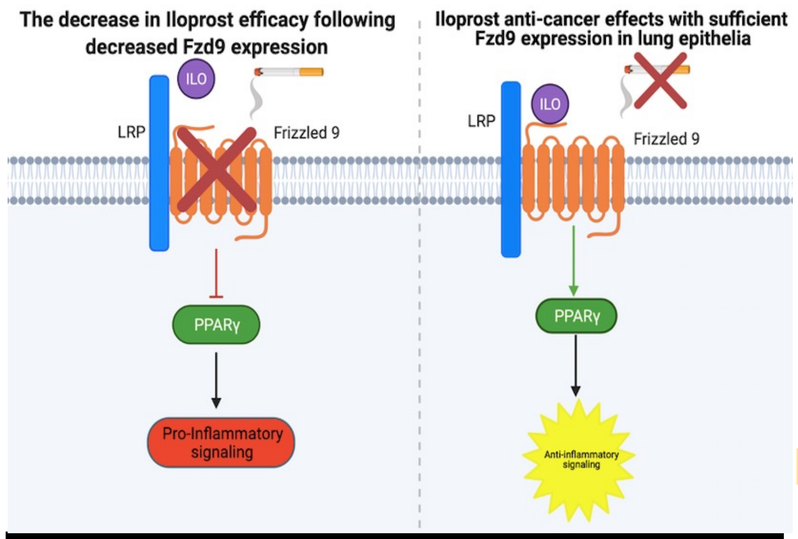
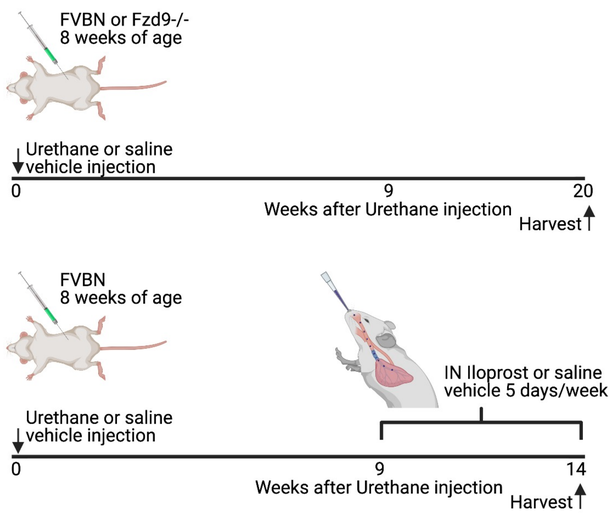
Background

- **Lung cancer** continues to be **the leading cause of cancer death worldwide** and accounts for about **25% of cancer mortality annually**[1].
- Chemoprevention in former smokers may be more effective than treatment of established lung cancers.
- Iloprost is a prostacyclin analog that acts as a PPARγ agonist to inhibit tumorigenesis in NSCLC tissues.
- A phase II trial found that iloprost improves endobronchial dysplasia in former smokers, but not in current smokers[2].
- **We hypothesize that Fzd9 expression is necessary for iloprost activity in NSCLC chemoprevention.**



Methods

In Vivo



In Vitro

- Human epithelial bronchial cells (HBECs) were transfected with optimem, trans-IT2X, and a Fzd9 siRNA or siRNA control.
- After 48 hours, cells were collected for RNA extraction and analyzed by RTqPCR.

Results

Figure 1: Fzd9 loss increases tumor multiplicity and decreases iloprost efficacy *in vivo*.

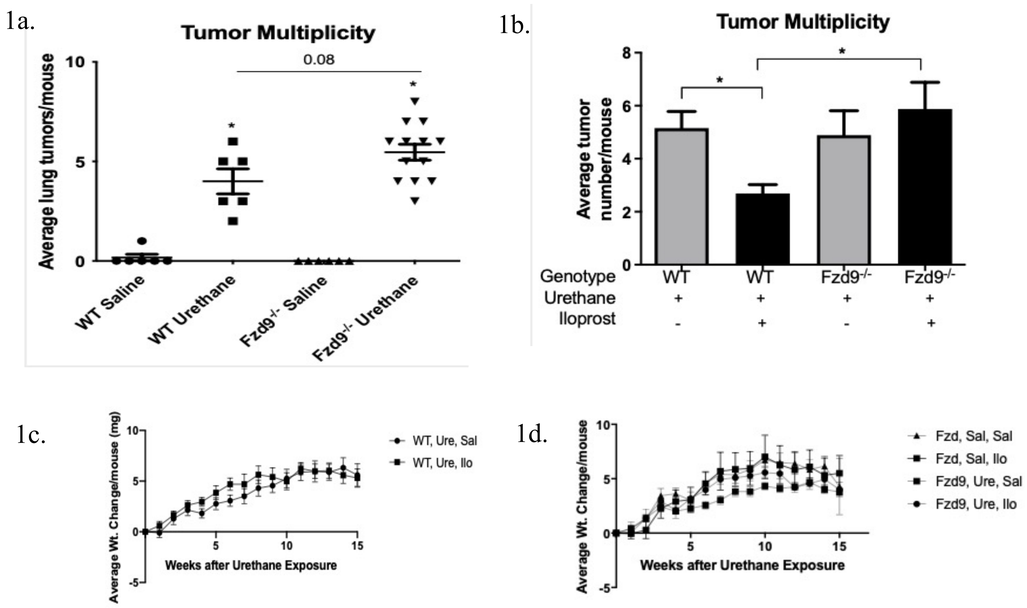


Figure 1. (a) Tumor multiplicity from wild type and Fzd9-/- mice treated with urethane or saline control for 20 weeks. (c) Weekly weights based on change per mouse. (b) Tumor multiplicity from wild type and Fzd9-/- mice treated with 1mg/ml urethane and 100ul/mouse iloprost. (d) Weekly weights based on change per mouse. WT, wild type. * indicates $p < 0.05$.

Figure 2. Loss of Fzd9 *in vivo* increases EMT, inflammation, and cancer signaling.

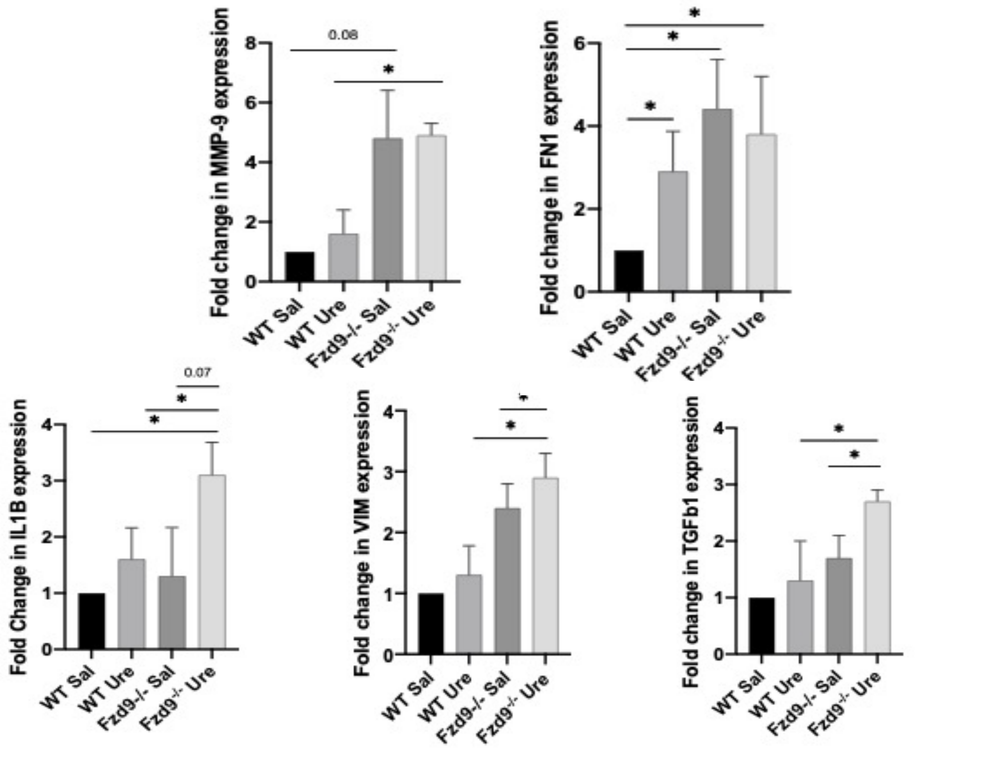


Figure 2: qPCR in wild type saline-treated (WT Sal), wild type urethane-treated (WT Ure), Fzd9-/- saline-treated (Fzd9-/- Sal) and Fzd9-/- urethane-treated (Fzd9-/- Ure) for MMP-9 (2a), Vimentin(2b), fibronectin (2c), Il1-β (2d), and TGFβ1 (2e). * $p < 0.05$.

Figure 3. Loss of Fzd9 *in vitro* increases EMT and cancer signaling

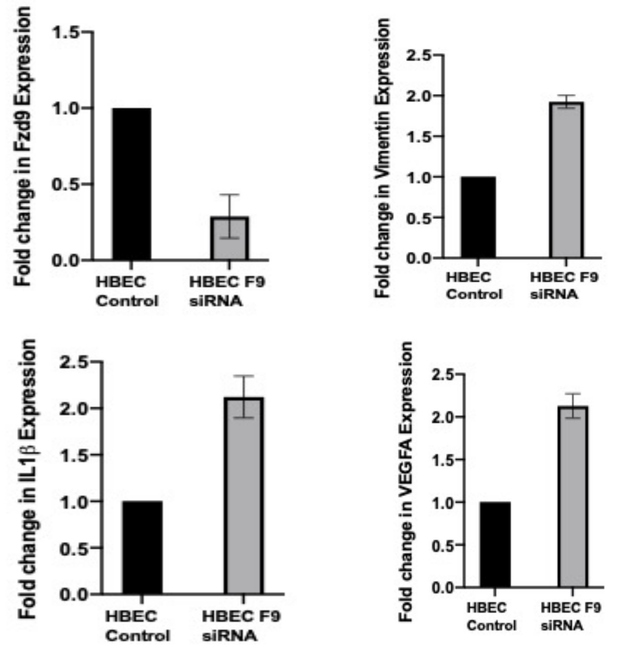


Figure 3. HBECs transfected with a Fzd9 siRNA or control siRNA were analyzed by RTqPCR for Fzd9(3a), vimentin (3b), IL1-β (3c), and VEGFA (3d) *$p < 0.05$.

Conclusion

- Loss of Fzd9 may contribute to increased tumor burden *in vivo.*
- Loss of Fzd9 reduces the chemopreventive effect of iloprost on premalignant lesions *in vivo.*
- Increased tumor-promoting inflammatory signaling is associated with Fzd9 loss.
- Gene expression changes resulting from loss of Fzd9 expression *in vivo* are similar to those observed with *in vitro.*

Future Directions

- Samples from the Fzd9-/- Inhaled Iloprost study will be analyzed for additional downstream effectors of Fzd9 loss.
- We will continue to study the effects of Fzd9 loss and iloprost treatment in HBECs *in vitro.*
- Observe various mutations paired with Fzd9 in organotypic culture that may lead to premalignant lesions

References

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4. Tennis MA, Van Scoyk M, Heasley LE, et al. Prostacyclin inhibits non-small cell lung cancer growth by a frizzled 9-dependent pathway that is blocked by secreted frizzled-related protein 1. Neoplasia. 2010 Mar;12(3):244-53.