The role of cigarette smoke and miR-520a in pulmonary Fzd9 expression

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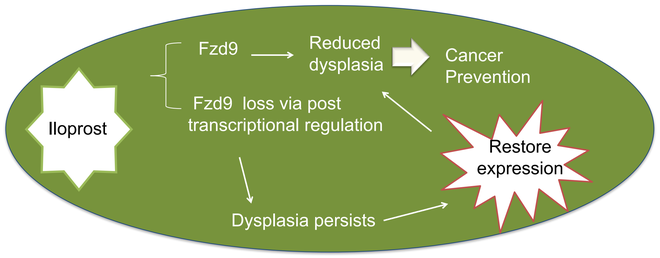
1 Background

Lung cancer remains the leading cause of cancer death in the United States and chemoprevention of lung cancer in high risk populations is a critical area of investigation in the face of limited therapeutic success.

- Endobronchial histology in former smokers improved after treatment with the **prostacyclin analogue iloprost** in a phase II trial in high risk subjects[1].

Iloprost + Fzd9 → PPARγ ⊣ EMT [2,3]

- Fzd9 expression is frequently lost in NSCLC, but mutations or LOH are rare, suggesting **either post-transcriptional or post-translational suppression** may play a role. Fzd9 expression is decreased with exposure to tobacco carcinogens and increased with prostacyclin *in vitro* and *in vivo*.
- A cigarette smoke condensate (CSC) extraction protocol has been established to mimic smoking *in vitro*. Iloprost has been shown to have similar effects to the clinical trial data on CSC treated cells [4].
- miRNA database analysis suggested miR-95, miR-106b, and miR-520a as potential post-transcriptional regulators of Fzd9.
- Increased understanding of Fzd9 loss and regulation will improve the application of prostacyclin chemoprevention (iloprost).



We hypothesize that miR-520a will regulate Fzd9 translation in lung epithelium, resulting in varying effects from cigarette smoke and Iloprost treatments.

2 Results

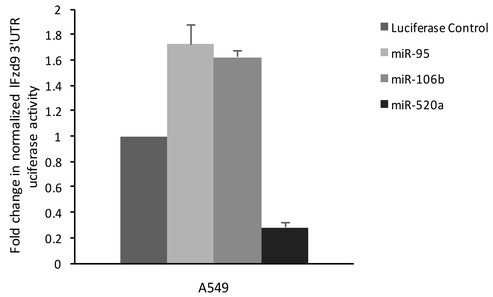


Fig. 1 Predicted Fzd9 miRNA transfection A) A549 cells were transfected with a Fzd9 3'UTR luciferase plasmid and a miR520a mimic. Normalized luciferase activity was compared to the luciferase control. * p=0.03 vs control

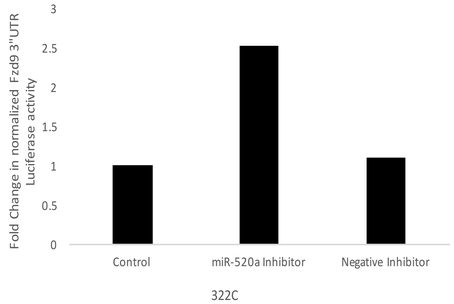


Fig. 2 miR520a inhibitor transfection 322C cells were transfected with a Fzd9 3'UTR luciferase plasmid and a miR520a mimic inhibitor. Normalized luciferase activity was compared to the luciferase control

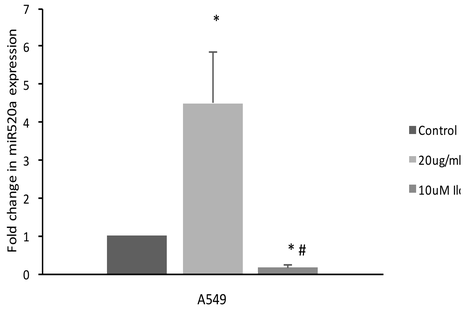


Fig. 3 miR520a Expression in CSC treated cells A549 were treated for 2 weeks with 20ug/ml CSC or 10uM iloprost and expression of miR250a was compared to

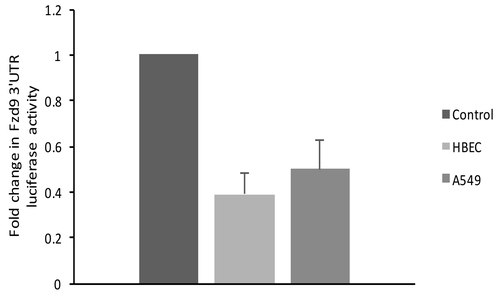


Fig. 4 Fzd9 3'UTR activity in CSC treated cells A549 and HBEC were transfected with a Fzd9 3'UTR luciferase plasmid and treated with 20ug/ml or 5ug/ml cigarette smoke condensate for 48 hours. 3'UTR luciferase activity was normalized and compared to control transfected cells.

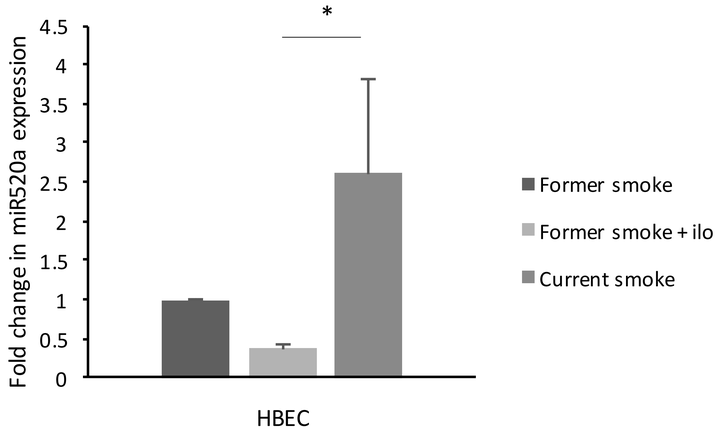


Fig. 5 *In vitro* clinical trial mimic experiment HBEC were treated with 5ug/ml cigarette smoke condensate for 4 weeks, then separated into three cultures for the next four weeks: no treatment (former smoke), 10uM iloprost (former smoke + ilo), and continued CSC exposure (current smoke). miR520a expression was measured by qPCR. * p=.001

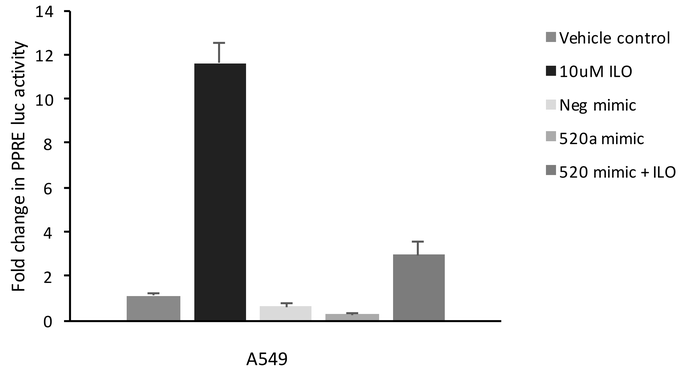


Fig. 6 Downstream activity after Fzd9 regulation A549 were transfected with a PPRE-luc plasmid and a miR520a mimic or mimic control and treated with 10uM iloprost or vehicle for 48 hours. Normalized luciferase activity was compared to PPRE-luc alone. p<0.03, * vs vehicle control and # vs 520a mimic. p=0.08, 520a mimic vs neg mimic

3 Conclusions

- miR-520a decreases the activity of the 3'UTR of Fzd9, while inhibition of miR-520a increases 3'UTR activity.
- Expression of miR-520a increases in both human bronchial and NSCLC cells after CSC treatment, suggesting a mechanism for post-transcriptional regulation of Fzd9 in high risk individuals.
- Iloprost can rescue Fzd9 expression by decreasing miR-520a after prolonged exposure of CSC
- Iloprost also shows potential for rescuing downstream activity of PPRE, a known regulator of EMT, further supporting the idea of a potential chemopreventative agent

4 Future Directions

- Characterize Iloprost and Fzd9 interaction mechanism
- Utilize stable cell lines for long term exposure studies.
- Utilize more miR-520a inhibitor experiments for rescue studies.
- Utilize both stable and parental cell lines for low adherence, transformative growth measurement assays
- Characterize miR-520a effects on Fzd9 protein expression

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