Prenatal Environment and Respiratory Disease

The Impact of Chronic Nicotine Exposure on Elastin and TGF-β Signalling

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INTRODUCTION

Prenatal exposure to cigarette smoke increases risk of respiratory disease

- ↑ Risk of asthma
- Possible predisposition to COPD (Chronic obstructive pulmonary disease) development

Chronic nicotine exposure decreases baseline elastin expression in lung fibroblasts

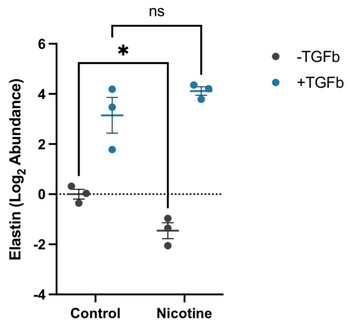

Figure 1: Pilot data indicating that in cells chronically exposed to nicotine, baseline elastin expression is significantly decreased (*=statistically significant; ns=not significant)

Elastin abundance is altered in chronic respiratory disease

- Elastin levels ↑ in severe asthma = disorganization
- Elastin levels ↓ in COPD = increased lung compliance

The mechanism of altered elastin expression in these diseases is currently unknown

TGF-β signalling may be altered by chronic nicotine exposure

TGF-β (Transforming Growth Factor Beta) is a profibrotic cytokine that promotes elastin expression in lung fibroblasts

AIM

To determine if chronic nicotine exposure causes alterations to the TGF-β signaling pathway which modulate changes in elastin expression

We hypothesize that chronic nicotine exposure reduces TGF-β signalling by increasing negative regulators of TGF-β

METHOD

Cell culture of human lung fibroblasts

- Human Lung Fibroblasts (HLF) from three female non-smokers were cultured in normal growth media with or without 10μM nicotine for five days
- Growth arrested for 24 hours
- RNA isolated, cDNA made

Quantification of gene expression (qPCR)

- Changes in abundance of 40 genes involved in TGF-β signalling measured using qPCR (quantitative polymerase chain reaction)
- Relative abundances calculated and normalized to three housekeeping genes

Statistical analysis

Genes with the greatest change in abundance are presented as mean fold change (Log2) ± SD, with significance defined as $p<0.05$ (n=3)

RESULTS

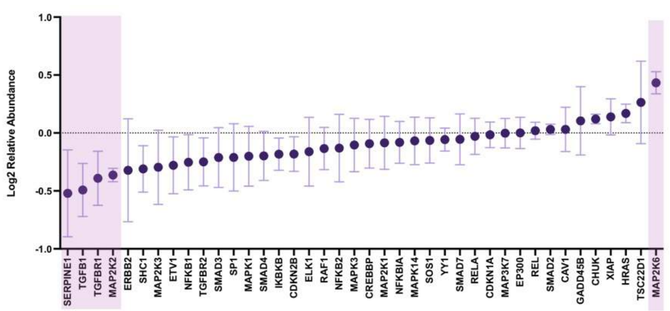

Figure 2: Relative abundance of 40 genes involved in TGF-β signalling plotted on a Log2 relative abundance scale in HLF treated with 10 μM nicotine for five days. The genes with the greatest change in abundance are highlighted

The most downregulated genes in cells chronically exposed to nicotine were TGFB1 (-0.492 ± 0.229, p=0.04*), SERPINE1 (-0.521 ± 0.378, p=0.14), TGFBR1 (-0.391 ± 0.234, p=0.08), and MAP2K2 (-0.363 ± 0.059, p=0.05). MAP2K6 was the most upregulated gene (0.433 ± 0.095, p=0.1). Negative regulators of TGF-β signalling, such as Smad7 (-0.055 ± 0.220), were not altered.

TGFB1=Transforming Growth Factor Beta 1 (gene), PAI-1=Plasminogen activator inhibitor 1, TGFBR1=Transforming Growth Factor Beta Receptor 1, MAP2K2=Mitogen Activated Protein Kinase Kinase 2, MAP2K6=Mitogen Activated Protein Kinase Kinase 6

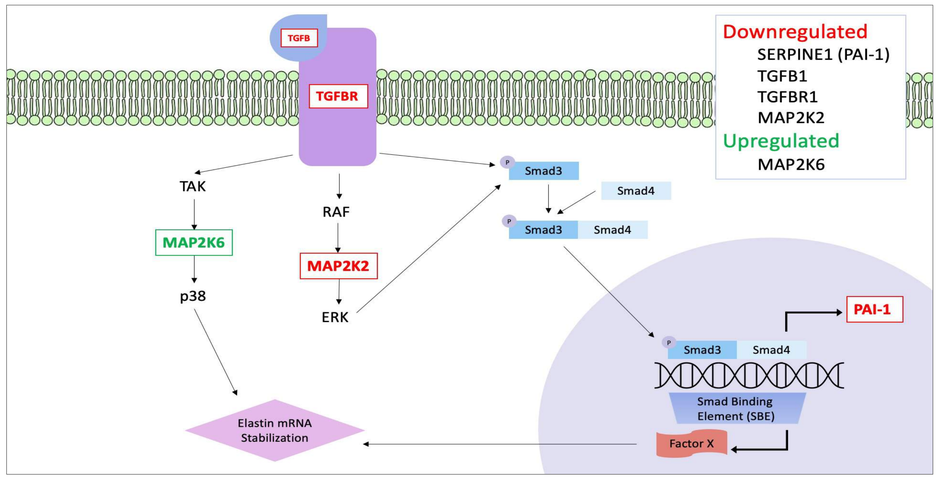

Figure 3: Partial diagram of the TGF-β signalling pathway demonstrating the impact of the observed changes in gene abundance on elastin mRNA stabilization. Decreased abundance of TGFB1, TGFBR1, and MAP2K2 all decrease stabilization of elastin mRNA, while increased abundance of MAP2K6 promotes elastin mRNA stabilization in response to stimulation with TGF-β.

Figure modified from:

Tzavlaki, K. (2020*)* TGF-β Signaling, *Biomolecules* 10(3) http://dx.doi.org/10.3390/biom10030487

Kucich, U. (2001) Transforming Growth Factor- β Stabilizes Elastin mRNA by a Pathway Requiring Active Smads, Protein Kinase C-δ, and p38, *American Journal of Respiratory Cell and Molecular Biology* 26(2) https://doi.org/10.1165/ajrcmb.26.2.4666

TGFB1 is the ligand that initiates signalling along the pathway

- ↓TGFB1 = ↓ Elastin

TGFBR is the receptor which is activated by TGFB1

- ↓TGFB1 = ↓ Elastin

MAP2K2 propagates the signal initiated by TGFB1

- ↓MAP2K2 = ↓ Elastin

SERPINE1 (PAI-1) production is regulated by the TGF-β signalling pathway and downregulation of this gene indicates that decreased signalling is occurring

MAP2K6 propagates the signal initiated by TGFB1

- ↑MAP2K6 = ↑ Elastin

CONCLUSION

A decrease in autocrine TGF-β signalling following nicotine exposure may mediate the loss of elastin in HLF. Additionally, increased MAP2K6 abundance, which stabilizes elastin mRNA, may increase elastin mRNA in response to inflammatory stimuli. This could align with airway remodelling seen in asthma.

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