Epigenetic Modulation of Small Airway Fibrosis in Chronic Obstructive Pulmonary Disease

A thesis submitted for the degree of
Doctor of Philosophy

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Razia Zakarya, declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Abstract

Chronic Obstructive Pulmonary Disease is commonly associated with cigarette smoke exposure in developed nations. However, research demonstrating that a minority proportion of smokers develop COPD alongside findings that show a stronger correlation between lung function and familial relation than smoke exposure demonstrates that the link between cigarette smoking and airway obstruction is not linear. Previous work from our lab has shown that airway mesenchymal cells of COPD patients produced more extracellular matrix (ECM), thereby contributing to small airway fibrosis. We hypothesise that the mechanisms underpinning increased ECM production in COPD was epigenetic.

Using primary human airway smooth muscle cells, we carried out a microarray gene analysis to determine which ECM genes were aberrantly upregulated in COPD. We determined that transforming growth factor β1 (TGF-β1) stimulation lead to significantly higher induction of *COL15A1* and *TNC* in COPD *in vitro*. Further, we carried out IHC analysis to show that collagen 15α1 and tenascin-C were deposed in the airway smooth muscle (ASM) layer in small airways of COPD patients. Upon quantifying amount of ECM protein deposed within the ASM layer, we determined that collagen 15α1 deposition was significantly higher in COPD airways; demonstrating that the ECM protein was aberrantly expressed *in vivo*.

Investigating epigenetic modulations directed us towards studying specific acetyl-lysine histone modifications at the target gene promoter regions. We, for the first time, demonstrated that increased ECM expression in COPD is modulated by histone H4 acetylation induced upon stimulation with TGF-β1. Further, we found that the epigenetic reader, Brd4, plays a role in propagating the epigenetic mark to sustain prolonged *COL15A1* and *TNC* expression.

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Presentations and Posters Arising from This Work

Small airway fibrosis is mediated by histone acetylation. R. Zakarya, H. Chen, C.A.A. Brandsma, I.M. Adcock, B.G.G. Oliver 2019 Poster discussion at the annual International Conference of the American Thoracic Society in 2019

Role of histone acetylation in fibrosis in chronic obstructive pulmonary disease. R. Zakarya, H. Chen, C.A.A. Brandsma, I.M. Adcock, B.G.G. Oliver 2018 Poster discussion at the annual International Conference of the American Thoracic Society in 2018

Investigating the effect of histone acetylation on TGF-β induced fibrosis in COPD. R. Zakarya, H. Chen, C.A.A. Brandsma, I.M. Adcock, B.G.G. Oliver 2018 Oral Presentation at the Thoracic Society of Australia and New Zealand National Annual Scientific Meeting 2018

Epigenetic Control of TGF-β Induced Fibrosis in COPD. R. Zakarya, H. Chen, C.A.A. Brandsma, I.M. Adcock, B.G.G. Oliver 2017 Oral presentation at the Thoracic Society of Australia and New Zealand NSW Annual Scientific Meeting 2017

Investigating the Role of Histone Acetylation in TGF-β Induced Fibrosis in COPD. R. Zakarya, H. Chen, C.A.A. Brandsma, I.M. Adcock, B.G.G. Oliver 2017 Poster presentation at the European Respiratory Society International Congress 2017

Publications Included as an Adjunct to This Work

The following publications do not pertain directly to this work but have been included as evidence of research productivity. Articles listed below can be found in Appendix A.

A Mitochondrial Specific Antioxidant Reverses Metabolic Dysfunction and Fatty Liver Induced by Maternal Cigarette Smoke in Mice. Gerard Li, Yik Lung Chan, Suporn Sukjamnong, Ayad G Anwer, Howard Vindin, Matthew Padula, **Razia Zakarya**, Jacob George, Brian G Oliver, Sonia Saad, Hui Chen *Nutrients* 2019

Dietary fatty acids amplify inflammatory responses to infection through p38 MAP kinase signalling. Sandra Rutting, **Razia Zakarya**, Jack Bozier, Dia Xenaki, Jay C Horvat, Lisa G Wood, Philip M Hansbro, Brian G Oliver *American Journal of Respiratory Cell and Molecular Biology* 2019

MitoQ supplementation prevent long-term impact of maternal smoking on renal development, oxidative stress and mitochondrial density in male mice offspring. Suporn Sukjamnong, Yik Lung Chan, **Razia Zakarya**, Long The Nguyen, Ayad G Anwer, Amgad A Zaky, Rachana Santiyanont, Brian G Oliver, Ewa Goldys, Carol A Pollock, Hui Chen, Sonia Saad *Scientific Reports* 2018

Biomass Smoke Exposure Enhances Rhinovirus-Induced Inflammation in Primary Lung Fibroblasts. Sarah J. Capistrano, **Razia Zakarya**, Hui Chen, Brian G. G. Oliver. *International Journal of Molecular Sciences* 2016

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List of Abbreviations

5mC	5-methylcytosine
ANOVA	Analysis of variance
ASM	Airway smooth muscle
BAL	Bronchoalveolar lavage
BALT	Bronchial associated lymphoid tissue
BCA	Bicinchoninic acid
BET	Bromodomain and extraterminal domain protein
BID	Basic residue-enriched interaction domain
BSA	Bovine serum albumin
CAT	COPD Assessment Test
CDK	Cyclin dependant kinase
cDNA	Complementary DNA
ChIP	Chromatin immunoprecipitation
COPD	Chronic Obstructive Pulmonary Disease
CSE	Cigarette smoke extract
DC	Dendritic cell
DEPC	Diethylpyrocarbonate
DMEM	Dulbecco's modified eagle medium
DNA	Deoxynucleic acid
DNMT	DNA methyltransferase
DSIF	DRB-sensitivity-inducing factor
DTT	Dithiothreitol
ECM	Extracellular matrix
EMA	European Medicines Agency
FBS	Foetal bovine serum
FEV_1	Forced expiratory volume in 1 second
FOT	forced oscillation technique
FOXP3	Forkhead box P3
FVC	Forced vital capacity
FVC	Forced vital capacity
GDV	Genome data viewer
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GSK-3β	Glycogen synthase kinase-β
GWAS	Genome wide association study
H3ac	Histone H3 acetylation
H3K27me3	Trimethylation of lysine 27 on histone 3
H4ac	Histone H4 acetylation
H4K9me2	Dimethylation of lysine 9 on histone 4
H4K9me3	Trimethylation of lysine 9 on histone 4
HAT	Histone acetyltransferase
HDAC	Histone deacetylase

HDM	House dust mite
IL	Interleukin
IL-5	Interleukin 5
IL-5Rα	Interleukin 5 receptor subunit alpha
IP	Immunoprecipitation
IPF	Idiopathic pulmonary fibrosis
K	Lysine
KAT	Lysine acetyltransferase
KDAC	Lysine deacetylase
KMT	Lysine methyltransferase
LPS	Lipopolysaccharide
Me-DIP	Methylated DNA precipitation
mMRC	modified MRC dyspnea scale
mRNA	Messenger ribonucleic acid
NAD+	Nicotinamide adenine dinucleotide
NCBI	National Center of Biotechnology Information
NELF	Negative elongation factor
NF-κB	Nuclear factor kappa B
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDID	Phosphorylation dependent interaction domain
PGP	Proline-glycine-proline matrikine
PIC	Protease inhibitor cocktail
PRMT	Arginine methyltransferase
pTEFb	Positive transcription elongation factor
PTM	Post-translational modification
R	Arginine
SAHA	Suberoylanilide hydroxamic acid
SAM	*S*-adenosyl-L-methionine
SNP	Single nucleotide polymorphism
TGF-β	Transforming growth factor beta
TNF-α	Tumour necrosis factor alpha
TSA	Trichostatin A
TSLP	Thymic stromal lymphopoeitin
TSS	Transcriptional start sites
VEGF	Vascular endothelial growth factor
WNT	Wingless/integrase 1

I hereby state that this submission is a thesis by compilation.