UTS UNIVERSITY OF TECHNOLOGY SYDNEY

Therapeutic targeting of mitochondrial dysfunction in chronic obstructive pulmonary disease (COPD)

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Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of
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December 2022

Certificate of Original Authorship

I, ***Shatarupa Das***, declare that this thesis is submitted in fulfilment of the requirements for the award of ***Doctor of Philosophy***, in the ***School of Life Sciences*** 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.

This research is supported by the Australian Government Research Training Program.

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Date: 23.12.2022

Acknowledgement

This thesis was a new and memorable experience for me. Words cannot express my gratitude to my supervisor and the director of Centenary UTS Centre for Inflammation, Prof. Philip Hansbro for giving me this opportunity as a PhD candidate. My gratitude extends to the Faculty of Science, for the IRS and president's scholarships to undertake my studies at the School of Life Sciences, UTS.

I also could not have undertaken this journey without my co-supervisor A/Prof. Andy Philp, who generously provided his knowledge and expertise. Additionally, this endeavour would not have been possible without the support of our Centre manager, Dr Nicole Hansbro.

I would like to express my profound gratitude to my co-supervisor Dr Matt Johansen, for his invaluable supervision, advice, continuous support, and patience during these last three years. His immense knowledge and plentiful experience have encouraged me and kept me motivated in academic research as well as in my daily life. He has been a wonderful mentor and my best friend throughout the most challenging phase of my life. His influence in shaping my experiment methods and critiquing my results has immensely helped to develop my critical thinking as a scientist. Thank you, for your kind words of encouragement that have made it possible to chase my dreams.

I would like to extend my gratitude to our past and current technical staff, Carol Devine, Simon Gao, Nimmy, and Bob Lu, for their immense hard work in the smoking facility. Special thanks to Solomon Odgers, who has put in a huge amount of work as a technical officer and now as a research assistant. In addition, I would also like to thank our other

research assistants, Dr Duc Nguyen, Dr Christina Nalkurthi, Nisha Panth, Linda Tong, and Caroline Wilson for their constant support.

My wholehearted thanks to all my peers at the Mitochondria metabolism and Ageing lab, Dr Ashleigh Philp, Dr Dean Campelj, and James Sligar with who I have greatly benefited and enjoyed working.

I am also grateful to my friends and colleagues, for being my family in Australia, thank you for all the extreme endpoint days, late-night feedback sessions and moral support. It is their kind help and support that have made my study and life in Sydney a wonderful time. Thank you, Dr Vrushali Chimankar, Dr Annalicia Vaughan, Dr Vyoma Patel, Dr Izabela Galvo, Jacqueline Marshall, Stefan Miemczyk, Hamidreza Sadegh, Tayyaba Sadaf, Angelica Katsifis, Karl Hegarty, and Vamshikrishna Malyla for all the cherished time spent together in the lab and social settings.

I would be remiss in not mentioning my family, especially my mom, and my siblings. Their belief in me has kept my spirits and motivation high during this process. Unforgotten, thank you to my late dad, whom I miss dearly. A special feeling of gratitude for my grandparents and lastly, I would also like to thank my cats for all the entertainment and emotional support.

Summary

Chronic obstructive pulmonary disease (COPD) is characterised by airway inflammation resulting in irreversible damage to the lung, which leads to airway remodelling, alveolar destruction or emphysema, and impaired lung function. Currently, there are no available treatments that inhibit the progression or reverse the disease. Cigarette smoking (CS) is a primary cause of COPD and is an instigator of airway inflammation which is driven by oxidative stress. This further induces the over-activation of nuclear enzyme poly (ADP-ribose) polymerase-1 (PARP-1). PARP-1 is a major consumer of nicotinamide adenine dinucleotide (NAD^+), a critical energy intermediate for the maintenance of all metabolic interactions.

Currently, there is limited knowledge as to how NAD^+ metabolism is altered in COPD and whether targeting NAD^+ pathways can have therapeutic benefits in COPD. Therefore, in Chapter 3, we examined the efficacy of NAD^+ targeted therapeutics, Nicotinamide Riboside (NR) and Pterostilbene (PT) in a prophylactic COPD model. Using our experimental CS-induced COPD mouse model, we found reduced NAD^+ levels associated with PARP hyperactivity in the lung. We also observed an increase in the gene expression of oxidative stress markers like NADPH-oxidase 2 (NOX2) and inflammatory markers like TNFα and CXCL1. Based on these findings, we hypothesised that reduced NAD^+ levels may instigate oxidative stress and inflammation in COPD. Thus, in Chapter 4 we investigated the therapeutic effect of NR and PT in halting the progression and reversing the disease features in COPD. Following daily dietary administration of NR and PT both prophylactically and therapeutically, we found significant reductions in inflammatory cell infiltration into the lung and reduced gene expression of inflammatory markers like TNFα and CXCL1. Further, NR and PT reduced airway remodelling,

emphysema, and improved lung function. NR and PT also restored NAD^+ levels and PARP activity in COPD. This resulted in the protection of mitochondrial structure and function via reduced oxidative stress. In conclusion, NR and PT reduced inflammation, and COPD features via increased mitochondrial function and reduced oxidative stress. Thus, NR and PT have significant potential as therapeutics for COPD.

NAD^+ is essential for cell survival and is tightly regulated by enzymes such as Nicotinamide mononucleotide adenyl transferases (NMNATs). NMNATs are involved in the final step of NAD^+ synthesis. Based on our findings from Chapter 3, imbalances in NAD^+ levels in COPD might be due to imbalances in NAD^+ synthesis. Furthermore, we determined that the administration of NR and PT in COPD increased gene expression of NMNAT1, NMNAT2 and NMNAT3. Therefore, in Chapter 5, we investigated the role of NMNATs in modulating NAD^+ levels in comparison to the treatment of NR and PT in COPD using transgenic NMNAT1 and NMNAT3 overexpressing mice. Additionally, we also administered NR and PT to the overexpressing NMNAT1 and NMNAT3 mice to elucidate if an additional increase in NAD^+ levels can potentially exhibit enhanced benefits in reducing COPD features. We found significant reductions in airway inflammation via reduced cellular infiltrates in the lung as well as a reduction in the gene expression of TNFα and CXCL1 with both the NMNAT1 and NMNAT3 overexpression as well as upon treatment of these mice with NR and PT. Additionally, we observed a reduction in airway remodelling and emphysema for both the NMNAT1 and NMNAT3 overexpression as well as upon treatment with NR and PT. However, there was no improvement in lung function with the overexpression of NMNAT1 and NMNAT3 mice. Additionally, treatment with NR and PT in overexpressed NMNAT1 and NMNAT3 mice further increased impaired lung function in COPD. Moreover, NMNAT1 overexpressing mice did not show an increase in the NAD^+/NADH levels in COPD which did not change

with the administration of NR and PT. On the other hand, NMNAT3 overexpressing mice exhibited an increase in the NAD^+ levels in COPD which did not change further upon NR and PT treatment. We further observed a reduction in PARP hyperactivity with both the NMNAT1 and NMNAT3 overexpression as well as upon treatment with NR and PT, and this further showed a reduction in oxidative stress and an increase in mitochondrial membrane potential. In conclusion, we hypothesise that NMNAT3 might potentially be driving NAD^+ modulation to protect from COPD features. Critically, NAD^+ levels might potentially be rate-limiting and thus treatment of NR and PT in overexpressing NMNAT1 and NMNAT3 mice did not show a further increase in NAD^+ levels in COPD.

The development and progression of COPD is largely driven by chronic inflammation, however, there is limited knowledge about the exact molecular and metabolic interactions in the immune cells involved during the progression of the disease. Therefore, the objective of the Chapter 6 study was to determine if chronic CS exposure alters immunometabolism in COPD and whether pharmacological interventions can restore imbalances and exert therapeutic benefit. Using our CS-induced COPD mouse model, we determined that chronic exposure to CS reduced gene expression of pyruvate kinase markers 2 (PKM2) and Hypoxia-inducible factor 1α (HIF1α) indicating reduced metabolism in the lung, while we also observed an increase in oxidative stress and inflammatory markers. Based on these findings, we hypothesised that the reduction in PKM2 may be an important driver of immunometabolism in COPD. Therefore, we next examined whether immunomodulator TEPP46, which is importantly a PKM2 activator, was able to therapeutically improve COPD disease features. Following administration of TEPP46 three times per week throughout the duration of the model, we observed a significant reduction in airway inflammation followed by reduced airway remodelling, emphysema, and improved partial lung function in COPD. TEPP46 also protected PKM2

and HIF1α protein content and promoted metabolic function, resulting in reduced oxidative stress and protection of mitochondrial structure and function. In conclusion, TEPP46 has significant preclinical potential as a therapeutic for the treatment of COPD.

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List of publications and conferences

Conference proceedings:

1. Das S., Johansen M. D., Marshall J, Sadega H., Sadaf T., Hansbro N., Thomas C., O'Neil L., Philp A., Hansbro P. M. (2022) **Investigating the therapeutic efficacy of immunomodulator TEPP46 in Chronic Obstructive Pulmonary Disease (COPD).** In: 50th Annual Scientific Meeting of Australia and New Zealand Society for Immunology, Melbourne, Australia: 29th November-2nd December 2022.

2. Das S., Johansen M. D., Marshall J, Sadega H., Sadaf T., Hansbro N, Thomas C., O'Neil L., Philp A., Hansbro P. M. (2022) **Mitochondrial dysfunction and its modulation in Chronic Obstructive Pulmonary Disease (COPD).** In: Australian Inflammation Centres 2022 Symposium-Virtual, Melbourne, Australia: 24th-25th November 2022.

3. Das S., Johansen M. D., Marshall J, Sadega H., Sadaf T., Hansbro N, Thomas C., O'Neil L., Philp A., Hansbro P. M. (2022) **Investigating mitochondrial dysfunction as a potential therapeutic target for Chronic Obstructive Pulmonary Disease (COPD).** In: 16th Annual New South Wales Asthma Meeting (NAMe), Sydney, Australia: 17th-18th of November 2022.

4. Das S., Johansen M. D., Marshall J, Sadega H., Sadaf T., Hansbro N, Thomas C., O'Neil L., Philp A., Hansbro P. M. (2022) **Targeting impaired immunometabolism to prevent the development of Chronic Obstructive Pulmonary Disease (COPD).**

In: 7th biennial Australian conference for mitochondrial research AussieMit 2022, Sydney, Australia: 16th-18th of November 2022.

5. Das S., Johansen M. D., Marshall J, Sadega H., Sadaf T., Hansbro N, Thomas C., O'Neil L., Philp A., Hansbro P. M. (2022) **Investigating the therapeutic efficacy of immunomodulator TEPP46 in Chronic Obstructive Pulmonary Disease (COPD).** In: 9th EMBL Australia Postgraduate Symposium, Melbourne, Australia: 9th-11th of November 2022.

Table of contents

List of Figures

List of tables

Abbreviations

°C	Degrees Celsius
β-HAD	β-hydroxyacyl CoA
ΔΨm	Mitochondria membrane potential
µg	Microgram
µl	Microlitre
µm	Micrometre
4-HNE	4-hydroxy-2-nonenal
3-NT	3-nitrotyrosine
A	estimation of IC
AATD	α-1-antitrypsin deficiency
AAt	α-1-antitrypsin
Acetyl-CoA	Acetyl coenzyme A
ADP	Adenosine diphosphate
ALS	amyotrophic lateral sclerosis
ARC	Animal Resources Centre
ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
BSA	Bovine serum albumin

CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CRS	Respiratory system compliance
CS	Cigarette smoke
Cst	Static compliance
CXCL1	Chemokine ligand 1
DBQ	Decyl ubiquinone
DC	Detergent Compatible
DMSO	Dimethyl sulfoxide
DPBS	Dulbecco's Phosphate-Buffered Saline
DTNB	5,5-dithio-bis-(2-nitrobenzoic acid)
EC	Endothelial cells
ETC	Electron transport chain
FADH2	Flavin adenine dinucleotide
FEV	Forced expiratory volume
FRC	Functional residual capacity
FVC	Forced vital capacity
FITC	Fluorescein Isothiocyanate
G	Tissue damping
GCN5	Gene control non-derepressible 5

GOLD	Global Initiative on Chronic Obstructive Lung Disease
GLUT1	Glucose transporter 1
H	Tissue elastance
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
HDAC2	Histone deacetylase 2
HIF1α	Hypoxia inducing factor-1α
HK1	Hexokinase-1
HO1	Heme oxygenase 1
HPRT	Hypoxanthine-guanine phosphoribosyl transferase
IC	Inspiratory capacity
IL-1β	Interleukin-1β
ICAM1	Intermolecular adhesion molecule
iNOS	Inducible nitric oxide synthase
KCN	Potassium cyanide
Kpi	Potassium phosphate
LDHA	Lactate dehydrogenase A
LPS	Lipopolysaccharide
LTB4	Leukotriene B4
MMP-12	matrix metalloproteinase-12

MLI	Mean linear intercepts
mtROS	Mitochondrial reactive oxygen species
mtRNS	Mitochondrial reactive nitrogen species
NA	Nicotinic acid
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide
NAAD	Nicotinic acid adenine dinucleotide
NADK1/2	NAD^+ kinase 1/2
NADH	Nicotinamide adenine dinucleotide
NADSYN	NAD^+ synthase
NAFLD	Non-alcoholic fatty liver disease
NAM	Nicotinamide
NAMN	Nicotinic acid mononucleotide
NAMPT	Nicotinamide phosphoribosyl transferase
NAPRT	Nicotinate phosphoribosyl transferase
NF-κβ	Nuclear factor-κβ
NHMRC	National Health and Medical Research Council of Australia
NMN	Nicotinamide mononucleotide
NMNT	Nicotinamide mononucleotide transferase

NMNATs	Nicotinamide mononucleotide adenylyl transferase
NK	Natural killer cells
NOX2	NADHP oxidase
Nrf2	Nuclear factor erythroid 2-related factor-2
NR	Nicotinamide riboside
NRKs	Nicotinamide ribose kinases
NTHi	Non-typeable Haemophilus influenzae
O^-	Superoxide
Oct-4	Octamer binding transcription factor-4
Oxphos	Oxidative phosphorylation
Oxstress	Oxidative stress
PARPs	Poly (ADP-ribose) polymerases
PBS	Phosphate buffer saline with tween
PDK1	3-Phosphoinositide-dependent kinase 1
PDH	Pyruvate dehydrogenase
PEP	Phosphoenol pyruvate
PGC1α	Peroxisome proliferator-activated receptor-gamma co-activator 1α
PK	Pyruvate Kinase
PKF	Phosphofructokinase

PNP	Purine Nucleoside Phosphorylase
qPCR	Quantitative PCR
RBC	Red blood cell
ROS	Reactive oxygen species
Rn	central airway resistance
RNS	Reactive nitrogen species
Rrs	Transpulmonary resistance
RV	Residual volume
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SEM	Standard error mean
SIRTs	Sirtuins
SLHD	Sydney Local Health District
STAT3	Signal transducer and activator of transcription 3
TBST	Tris buffer saline with tween
TCA	Tricarboxylic acid
TEM	Transmission emission microscopy
TMPRSS2	Transmembrane serine protease 2
TNFα	Tumour necrosis factor α
TLC	Total lung capacity
TRP	Tryptophan

UNSW	University of New South Wales
UPRmt	Mitochondrial unfolded protein responses
VC	Vital capacity
YM1	chitinase-like protein 3