Investigating autophagy as a target in asthma and understanding how autophagy can modulate allergen-induced airway remodelling

Thesis

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I Kielan Darcy McAlinden declare that this thesis, is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Graduate School of Health 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

Background:

Airway remodelling is an untreatable hallmark of asthma. Autophagy, the cellular homeostatic recycling mechanism has emerged as a factor playing a role in asthma and potentially airway remodelling.

Objectives:

To explore the involvement of autophagy in asthmatic airway remodelling and test autophagy inhibition as a novel therapeutic target in asthmatics.

Methodology:

Autophagy protein expression was measured in the airways of both human and mouse asthmatic tissue by immunohistochemistry. Autophagy inhibitors chloroquine (CQ) and bafilomycin A1 (BafA1) were tested in murine asthma models. Relevant lung function, cell counts, histological staining and protein expression were supported by *in vitro* experiments.

Results:

We have found increased autophagy protein expression involved in asthmatic airway remodelling in human and mice tissue. Transforming growth factor beta (TGF-β) concomitantly induces remodelling changes and the upregulation of autophagy. Autophagy inhibition reduced the pathophysiological symptoms of asthma.

Conclusion:

Autophagy contributes to airway remodelling in asthma and autophagy modulation is a promising approach in developing therapies that target remodelling.

Dissemination of Research

Peer reviewed publications

1. **KD McAlinden** et al. (2019). There can be smoke without fire. Warranted caution in promoting e-cigarettes and heat not burn devices as a safer alternative to cigarette smoking. European Respiratory Journal Open Research, Aug 12;5(3):00114

2. **KD McAlinden** et al. (2019). Pharmacologic Inhibition of Vacuolar H+ATPase Attenuates Features of Severe Asthma in Mice. American Journal of Respiratory Cell and Molecular Biology, Jan; 62(1):117-120.

3. **KD McAlinden** et al. (2019). Altered Calcium in Ciliary Dysfunction: Potential Role of ER stress and Ciliophagy. American Journal of Respiratory Cell and Molecular Biology, Dec; 61(6):794-795.

4. **KD McAlinden** et al. (2018). Autophagy Activation in Asthma Airways Remodelling. American Journal of Respiratory Cell and Molecular Biology, May; 60(5):541-553.

5. MS Eapen, A Kota, H Vindin, **KD McAlinden**, D Xenaki, BG Oliver, DA Deshpande, SS Sohal, P Sharma (2018). Apoptosis signal-regulating kinase 1 (ASK1) inhibition attenuates human airway smooth muscle growth and migration in chronic obstructive pulmonary disease (COPD). Clinical Science, July 13:1615-1627.

6. MS Eapen, **KD McAlinden**, PM Hansbro, RY Kim, C Ward, TL Hackett, EH Walters, SS Sohal (2017). Abnormal M1/M2 macrophage phenotype profiles in the small airway wall and lumen in smokers and chronic obstructive pulmonary disease (COPD). Scientific Reports Vol. 7 (1):13392.

7. MS Eapen, **KD McAlinden**, D Tan, S Weston, C Ward, Muller HK, EH Walters SS Sohal (2017). Profiling cellular and inflammatory changes in the airway wall of mild to moderate COPD. Respirology Vol. 22 (6):1125-1132.

Conference proceedings

1. **High fat diet in conjunction with electronic cigarette vaping worsens lung function and inflammation**

   American Thoracic Society Conference 2019 (Dallas, USA)

2. **Electronic-cigarette vaping in combination with a high fat diet augments lung function and inflammation**

   Thoracic Society of Australia and New Zealand Conference 2019 (Gold Coast, AUS)

3. **Autophagy is Selectively Activated and Correlated with Airway Remodelling in Asthma**

   American Thoracic Society Conference 2018 (San Diego, USA)

4. **Selective activation and targeting of autophagy in severe asthma**

   European Respiratory Society International Congress 2018 (Paris, FRA)

   (Unable to attend - work was presented by supervisor: Dr Pawan Sharma)

   * Awarded Best Abstract in Airway Diseases (supported by GlaxoSmithKline)

5. **Expression of Autophagy markers in the human asthmatic epithelium**

   Thoracic Society of Australia and New Zealand Conference 2018 (Adelaide, AUS)

6. **Maternal E-cigarette Vaping Enhances Development of Allergic Asthma in the Offspring**

   American Thoracic Society Conference 2017 (Washington, USA)

   (Unable to attend - work was presented by supervisor: Dr Pawan Sharma)

   * Awarded ATS Stuart J. Hirst Award (Top ranked scientific abstract in the RSF category submitted by a fellow or student that relates to airway biology and physiology).

7. **Maternal e-cigarette vaping enhances development of allergic asthma in the offspring**

Thoracic Society of Australia and New Zealand Conference 2017 (Canberra, AUS)

(Unable to attend - work was presented by colleague: Dr David Chapman)

* Awarded TSANZ Best Poster Prize (supported by Boehringer Ingelheim)

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Abbreviations

3-MA 3-methyladenine

A Adenine

AAD allergic airways disease

AHR airway hyperresponsiveness

ALS Amyotrophic lateral sclerosis

AMPK 5' adenosine monophosphate- activated protein kinase

ASM airway smooth muscle

ASM/LP, % proportion of ASM in the airway wall

ATG Autophagy-related gene

ATG3 Autophagy-related gene – 3

ATG4 Autophagy-related gene – 4

ATG5 Autophagy-related gene – 5

ATG7 Autophagy-related gene – 7

ATG8 Autophagy-related gene – 8

ATG12 Autophagy-related gene – 12

ATG13 Autophagy-related gene – 13

ATG14 Autophagy-related gene – 14

ATG16 Autophagy-related gene – 16

ATG101 Autophagy-related gene – 101

ATP Adenosine triphosphate

BCG (Bacillus Calmette–Guérin) vaccine

BafA1 Bafilomycin A1

β2 agonists β2-adrenergic receptor agonists

BAL Bronchoalveolar Lavage

Ca^{2+} Calcium

cAMP Cyclic adenosine monophosphate

CD11c cluster of differentiation molecule 11c

CFA complete Freund's adjuvant

CO_2 Carbon dioxide

COPD chronic obstructive pulmonary disease

CQ Chloroquine

CS cigarette smoke

CSE cigarette smoke extract

CysLT Cysteinyl leukotrienes

DAB 3,3'-Diaminobenzidine

DALYs disability-adjusted life years

DMEM Dulbecco's modified Eagle's medium

ECM extracellular matrix

EMT epithelial-mesenchymal transition

ER Endoplasmic reticulum

ERK extracellular signal–regulated kinases

FDA The United States Food and Drug Administration

FEV1 Forced expiratory volume in one second

FP fluticasone propionate

$FSTL_1$ Follistatin-related protein 1

G Guanine

GFP green fluorescent protein

GRE glucocorticoid responsive elements

GWAS genome wide association studies

H&E Haematoxylin and Eosin

H_20_2 hydrogen peroxide

H2 relaxin human gene-2 relaxin

HCQ Hydroxychloroquine

HDAC histone deacetylases

HDAC-6 Histone Deacetylase 6

HDM House dust mite

HIF-1 hypoxia inducible factor

HRP horse-radish peroxidase

ICS inhaled corticosteroids

IFN-γ interferon-gamma

IgE Immunoglobulin E

IHC Immunohistochemistry

IMPase Inositol monophosphatase

IP_3 1,4,5-inositol trisphosphate

IL-1b Interleukin-1b

IL-4 Interleukin-4

IL-5 Interleukin-5

IL-8 Interleukin-8

IL-10 Interleukin-10

IL-13 Interleukin-13

IL-25 Interleukin-25

IL-33 Interleukin-33

IκBα nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha

KC CXCL1

LABA long-acting β2 agonists

LC3 microtubule-associated protein light chain 3

LP Lamina propria

MAPK mitogen-activated protein kinase

MCC mucociliary clearance

MHC major histocompatibility complex

MIICs MHC class II-containing compartments

MYLK myosin light-chain kinase

MMP-2 matrix metallopeptidase-2

MMP-9 matrix metallopeptidase-9

mRNAs Messenger ribonucleic acids

MTECs mouse tracheal epithelial cells

mTOR mammalian (or mechanistic) target of rapamycin

mTORC1 mammalian target of rapamycin complex 1

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NIH National Institute of health

$NADP^+$ Nicotinamide adenine dinucleotide phosphate

NF-κB Nuclear factor-kappa B

NSCCa non-small cell carcinoma

NSCLC Non-small cell lung cancers

O_2 Oxygen

OCR oxygen consumption rate

ORAI3 ORAI calcium release-activated calcium modulator 3

OVA ovalbumin

PAS Periodic acid–Schiff

p62 Sequestosome-1

PB1 (Phox and Bem1) **domain**

PBCs peripheral blood cells

PDE phosphodiesterase

PE phosphatidylethanolamine

PI3K phosphatidylinositol 3-kinase

PI3P phosphatidylinositol-3-phosphate

PKA Protein kinase A

PKC Protein kinase C

PMN polymorphonuclear

RBM reticular basement membrane

ROS reactive oxygen species

SAECs small airway epithelial cells

SD Standard deviation

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

SABA short-acting β2 agonists

SQSTM1 sequestosome 1

SNP single-nucleotide polymorphism

TAS2R taste 2 receptor

TEM transmission electron microscopy

TGFβ Transforming growth factor-beta

T_H1 Type 1 Helper T cell

T_H2 Type 2 Helper T cell

TLSP thymic stromal lyphopoietin

TNF Tumour necrosis factor

ULK1 UNC-51-like kinase 1

UPR unfolded protein response

UPS ubiquitin-proteasome system

V-ATPase Vacuolar-type H+-ATPase

VEGF Vascular endothelial growth factor

WHO World Health Organisation

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