Endoplasmic Reticulum Stress and the Unfolded Protein Response in the Pathogenesis of Asthma

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Statement of Originality

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository **, subject to the provisions of the Copyright Act 1968 and any approved embargo.

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Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains published papers of which I am a joint author. I have included as part of the thesis a written declaration endorsed in writing by my supervisor, attesting to my contribution to the joint publications.

By signing below I confirm that Prabuddha Sanjeewa Pathinayake contributed by critical literature review, manuscript writing, and manuscript revision to the publications entitled;

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Conference Presentations:

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American Thoracic Society Conference 2018. Poster presentation by P.S. Pathinayake. San Diego, California, USA.

Pathinayake PS, Nichol KS, Baines KJ, Wood LG, Hsu AC-Y, Wark PAB. The Unfolded Protein Response Is Heightened in Eosinophilic Asthma. American Journal of Respiratory and Critical Care Medicine 2018;197: A1298, Poster presentation, San Diego, California, USA.

International Congress of Immunology 2016. Poster presentation by P.S. Pathinayake. 2019 August, Melbourne, Australia.

Pathinayake, P.S., Hsu, A.C.-Y., Parsons, K., Loo, S.-L., Fricker, M., Wood, L.G., Hansbro, P., Wark, P.A.B. Effect of oxidative stress and rhinovirus infection on mitochondrial/endoplasmic reticular function in human primary bronchial epithelial

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Wark PAB, Ramsahai JM, **Pathinayake P**, Malik B, Bartlett NW. Respiratory Viruses and Asthma. Semin Respir Crit Care Med (2018) 39(01):045-55. doi: 10.1055/s-0037-1617412.

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Synopsis

Endoplasmic reticulum (ER) serves as a protein folding organelle and translocates correctly folded proteins into secretory pathways. Defectively folded protein aggregates are fatal and therefore the ER degrades them via ER-associated degradation (ERAD). Failure to remove terminally misfolded proteins results in an unfolded protein response (UPR) which increases the protein folding capacity while reducing the nascent protein folding load and protein translation. Unlike acute ER stress, chronic ER stress activates apoptosis, growth arrest, and cell death.

In the first study, we extensively explored the evidence of ER stress in various asthma groups using a range of different airway clinical samples and techniques. We demonstrated that chronic ER stress is a feature of severe asthma but not in mild asthma. We also observed that UPR is upregulated in those with active eosinophilic and neutrophilic inflammation. In sputum samples of those patients, expression of protein folding chaperones and ERAD function was reduced while apoptosis, and cell death markers were significantly increased.

ER stress also triggered various inflammatory signaling pathways and modulated pathogen-induced innate immune responses. In the second study, using a cell culture model we demonstrated the impact of chronic-high dose ER stress on RV1B induced inflammatory and antiviral responses in primary bronchial epithelial cells (pBEC). ER stress increased RV1B induced antiviral gene expressions; IFN-β, IFN-λ, IP-10, Viperin, and ISG56. However, the secretory profile of inflammatory and anti-viral cytokines was considerably low with UPR activation. ER stress also upregulated TSLP gene expression via CHOP dependent signaling pathway in bronchial epithelial cells but not IL-33 and IL-25. However, secretory TSLP could not be detected in the culture

supernatant. Therefore, maladaptive ER stress greatly attenuated the protein synthesis process in airway epithelial cells regardless of gene expression.

Chronic type II inflammation in asthmatic airways may induce ER-UPR and contributes to epithelial remodeling however the mechanisms are unknown. Therefore, in the third study, we investigated if exposure of airway epithelial cells to IL-13 induces ER-UPR and evaluated the efficacy of known FDA approved ER-UPR inhibitors, 4-PBA, and TUDCA on IL-13 induced mucus hypersecretion and pro-fibrotic factors in a pBEC air-liquid interface cell culture model (ALI). IL-13 increased ER-UPR (XBP1s, BiP and EDEM1) in ALIs while 4-PBA markedly reduced IL-13 induced ER-UPR. IL-13 significantly induced MUC5AC gene expression in both pBEC ALIs. Treatment with TUDCA reduced MUC5AC gene expression approximately by 50% while 4-PBA reduced it by 99%. Muc5ac ELISA and IHC staining showed a significant decrease in secreted mucin with 4-PBA. None of the treatments reduced IL-13 induced STAT6 activation. However, 4-PBA significantly reduced IL-13 induced mucin transcription factors. Epithelial fibrotic factor periostin was reduced by TUDCA but not 4-PBA while SerpinB2 only reduced by 4-PBA. Therefore we demonstrate that Th2 cytokines upregulate ER-UPR in the airway epithelium and inhibiting ER-UPR reduces IL-13 induced mucus hypersecretion and epithelial remodeling.

Collectively these studies have identified potential novel pathways and molecules that are implicated in asthma. Importantly, these studies have expanded our understanding of disease pathogenesis and demonstrate that therapeutically targeting these pathways and molecules may be novel therapeutic avenues for severe asthma.

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List of commonly used Abbreviations

4-PBA	4-Phenylbutric Acid
ALI	Air-Liquid Interface Cell Culture Model
BIP	Binding Immunoglobulin Protein
BLF	Bronchial Lavage Fluid
cDNA	Complementary DNA
CHOP	C/EBP Homologous Protein
COPD	Chronic Obstructive Pulmonary Disease
EDEM1	ER Degradation Enhancing Alpha-Mannosidase Like Protein 1
eIF2α	Eukaryotic Initiation Factor 2α
ER	Endoplasmic Reticulum
ERAD	ER-Associated Degradation
FACs	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
HSP	Heat Shock Protein
ICS	Inhaled Corticosteroids
IFN	Interferons
IL	Interleukin
ILC2	Innate Lymphoid-2 Cells

IRE-1	Inositol Requiring Protein 1
ISGs	Interferon Stimulated Gene
LABA	Long-acting beta-agonists
mRNA	Messenger RNA
NF-κb	Nuclear Factor kappa-light-chain-enhancer of activated B cells
OVA	Ovalbumin
PAMPs	Pathogen Associated Molecular Patterns
pBEC	Primary Bronchial Epithelial Cells
PERK	Protein Kinase RNA-Like Endoplasmic Reticulum Kinase
PRRs	Pattern Recognition Receptors
qPCR	Quantitative Real-Time Polymerase Chain Reaction
ROS	Reactive Oxygen Species
RSV	Respiratory Syncytial Virus
RV	Rhinovirus
TLR	Toll Like Receptors
TNF	Tumour Necrosis Factor
TSLP	Thymic Stromal Lymphopoietin
TUDCA	Tauroursodeoxycholic Acid
UPR	Unfolded Protein Response
XBP1	Splicing of X-Box Binding Protein 1