Investigation of the proteins SPARC and HMGB1 in chronic airways disease

A thesis submitted for the degree of
Doctor of Philosophy

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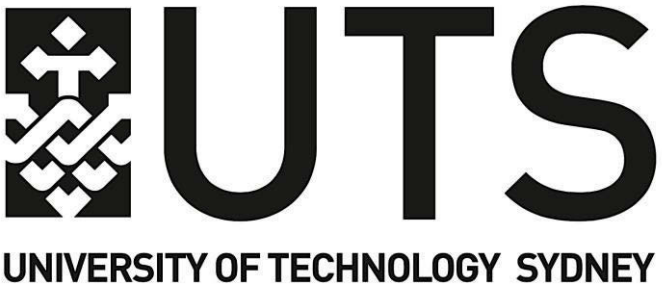

March 2017

CERTIFICATE OF ORIGINAL AUTHORSHIP

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as part of the collaborative doctoral degree and/or fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE

Published journal manuscripts

The SPARC protein: an overview of its role in lung cancer and pulmonary fibrosis and its potential role in chronic airways disease

Wong SL and Sukkar MB

British Journal of Pharmacology (2017) 174(1):3–14

Presentations

Potential role of SPARC, a downstream mediator of TGF-β in chronic airways disease

Wong SL, Ibrahim ZA, Wark PA, Sukkar MB

European Respiratory Journal 2015, Vol 46, Issue S59, PA904

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SPARC: a downstream mediator of TGF-β, which may potentially play a role in chronic airways disease

Wong SL, Ibrahim ZA, Wark PA, Sukkar MB

Respirology 2015, Vol 20, Issue S2, p. 144

Potential role of SPARC in chronic airways disease

2nd Annual Respiratory Epithelium Workshop 2014

Potential role of SPARC in inflammatory airways disease

Woolcock Institute of Medical Research Symposium 2014

University of Technology Sydney 3 Minute Thesis (3MT) 2014

Awards linked to abstracts

The Thoracic Society of Australia and New Zealand (TSANZ) Travel Award 2014

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LIST OF ABBREVIATIONS

4-PBA	4-phenylbutyric acid
AECs	Airway epithelial cells
AHR	Airway hyperresponsiveness
APP	Amyloid precursor protein
ASM	Airway smooth muscle
ATF6	Activating transcription factor 6
Ca^{2+}	Calcium
CAPZA1	F-actin-capping protein subunit alpha-1
COPD	Chronic obstructive pulmonary disease
CRT	Calreticulin
DAMP	Danger-associated molecular pattern
ECM	Extracellular matrix
EGTA	Ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid
eIF2α	Eukaryotic translation initiator factor 2α
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
GO	Gene Ontology
GRP78	Glucose-regulated protein 78
GSK-3β	Glycogen-synthase kinase-3 beta
HMGB1	High mobility group box 1
hrCNE	High resolution clear native electrophoresis
IFN-γ	Interferon gamma
ILC2s	Type 2 innate lymphoid cells
ILC3s	Type 3 innate lymphoid cells
IPF	Idiopathic pulmonary fibrosis
IRE1α	Inositol-requiring kinase 1 alpha
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
MEFs	Mouse embryonic fibroblasts
MMPs	Matrix metalloproteinases

MWCO	Molecular weight cut-off
NSCLC	Non-small cell lung cancer
ORMDL3	Orosomucoid like 3
PAI-1	Plasminogen activator inhibitor-1
PAMPs	Pathogen-associated molecular patterns
PBST	Phosphate Buffered Saline containing Tween
PDGF	Platelet-derived growth factor
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
RAGE	Receptor for advanced glycation end products
RIDD	IRE1-dependent decay
ROS	Reactive oxygen species
SASP	Senescence-associated secretory phenotype
SERCA	Sarco/endoplasmic reticulum Ca^{2+}-ATPase
Siglec	Sialic acid-binding immunoglobulin-like lectin
SPARC	Secreted protein acidic and rich in cysteine
TBST	Tris-buffered saline containing Tween
TGF-β	Transforming growth factor-beta
TGFβRII	TGF-β-receptor type II
TLRs	Toll-like receptors
TMAO	Trimethylamine N-oxide dehydrate
TNF-α	Tumor necrosis factor alpha
TSLP	Thymic stromal lymphopoietin
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor
XBP-1	X-box binding protein 1
XBP-1s	Spliced XBP-1
ZO-1	Zona occludin -1

ABSTRACT

The matricellular protein, secreted protein acidic and rich in cysteine (SPARC), mediates the interaction between cells and their surrounding extracellular matrix (ECM) but does not contribute structurally to the matrix. It regulates basic cellular functions such as cell adhesion and proliferation, as well as the processing and deposition of ECM proteins. SPARC is overexpressed in many fibrotic tissues including the lung. SPARC also serves as a down-stream mediator of transforming growth factor-beta (TGF-β), a key driver of airway remodeling in chronic airways disease, and demonstrates context-dependent immunoregulatory functions. Although airway inflammation and remodeling are prominent features of asthma and chronic obstructive pulmonary disease (COPD), the role of SPARC in these conditions has not been studied.

In this thesis, we investigated the expression of SPARC in airway structural cells including airway epithelial cells (AECs) and airway smooth muscle (ASM) cells, and also determined if its expression is altered in cells derived from subjects with asthma or COPD. We demonstrated that TGF-β increases SPARC expression and release in AECs and ASM cells, although to a lesser extent in the former. We observed that type 1 and type 2 cytokines tend to suppress basal and TGF-β-mediated SPARC expression in AECs, and showed that TGF-β-induced SPARC expression in ASM cells is regulated by the unfolded protein response (UPR). Notably, we observed distinct abnormalities in SPARC expression in asthma and COPD. Our preliminary studies suggest SPARC is overexpressed in AECs from subjects with asthma. In contrast, there

was a trend for reduced SPARC expression in ASM cells from COPD subjects, compared to those from non-COPD subjects. Functional studies indicate SPARC does not impart immunoregulatory functions or regulate changes in airway epithelial cell phenotype, although this requires further validation.

Our studies herein also explored the potential homeostatic role of extracellular high mobility group box 1 (HMGB1) in AECs. HMGB1 is a danger-associated molecular pattern (DAMP) that normally resides in the intracellular compartment, and is released into the extracellular space upon cellular injury, stress or death to orchestrate inflammatory responses. Although it is implicated as a mediator of the airway inflammatory response, its physiological role in lung homeostasis has received little attention. Interestingly, we detected HMGB1 in the culture supernatant of AECs under basal conditions, and found that it presents exclusively as a constituent of protein complexes. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomic approaches, we generated an unbiased profile of HMGB1-binding proteins in the extracellular space of unstimulated AECs. Protein network analysis of identified binding proteins indicates a role for extracellular HMGB1 in epithelial cell homeostasis and airway mucosal immunity.

In summary, findings in this thesis suggest aberrant regulation of SPARC expression in airway structural cells may be a contributing factor to the pathogenesis of chronic airways disease. Our studies also provide a new understanding of the extracellular functions of HMGB1 in AECs and opens new research directions for its use as a therapeutic target.