Inhibitor of Apoptosis Proteins as Regulatory Factors in the Normal and Inflamed Airways

Thesis submitted by

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For my Mother, Teresa Roscioli

Table of Contents

Abstract

Asthma is potentiated by complex gene-environment interactions, and characterised by inflammation and degenerative changes to the conducting airways. Current therapeutics targeting the inflammation and bronchoconstriction are restricted to prophylactic effects. The airway epithelium is known to participate in the pathogenesis of asthma, and represents a therapeutic target. Airway epithelial cells (AEC) from asthmatics exhibit apoptotic changes, which correlate with disease-associated factors presented by the epithelium. However, the mechanisms which cause the apoptosis are not well defined. In particular, there has been little study of the role of the family of Inhibitor of Apoptosis Proteins (IAPs) in models of AEC apoptosis. The over-arching hypothesis in this thesis is that anomalies in one or more of the IAPs contribute to inflammation-induced AE apoptosis in asthma. Experiments in this thesis explored the role of XIAP, cIAP1 and cIAP2 in asthma, models of asthma-related inflammation, and genetic susceptibility to asthma.

The major methods used in these experiments included cell culture of primary AEC from both asthmatics and controls with/without treatment with IFNγ and TNFα. siRNA knockdown, qPCR, western blotting, immunocytochemistry, functional caspase assays, and genotyping.

The major findings of this study are i) surprisingly, primary AECs do not undergo apoptosis after prolonged exposure to the proinflammatory cytokines IFNγ and TNFα *ex vivo*; ii) rather, IFNγ elicits a proapoptotic state in AECs, evidenced by, partial processing of procaspase-3, the absence of Poly (ADP-ribose) polymerase (PARP) cleavage, an increased Bax:Bcl2 transcript ratio, and the absence of morphological changes associated with apoptosis; iii) both XIAP and cIAP1 were constitutively expressed in AEC, and protein levels were unaffected by cytokine treatment. In contrast cIAP2, initially weakly expressed, was strongly inducible by cytokine treatment; iv) No differences were observed between AEC from asthmatics and controls in terms of either basal IAP gene expression

levels or their response to cytokine treatment; v) siRNA-mediated depletion of cIAP2-transcripts allows AEC to progress into apoptosis after extended culture, conditions which also resulted in a decrease in both cIAP1 and Bcl2; vi) genetic polymorphism in the genes encoding, XIAP cIAP1 and cIAP2 do not associate with susceptibility for asthma. However, cIAP1 polymorphism may modulate disease severity within asthmatics.

This thesis contributes to the knowledge of IAPs and apoptosis in asthma, and provides evidence that they are important for sustaining AEC survival, and participate within a cooperative of endogenous regulators of apoptosis. There is no evidence of intrinsic dysregulation of IAPs in asthma, yet cIAP1 polymorphism may modulate asthma severity, and IAPs are central in maintaining a proapoptotic state in AECs exposed to asthma related cytokines. Epithelial activation and damage, coupled with non-progression to apoptosis may contribute to fragility of the AE observed in asthma. Therapies targeting the IAPs may therefore provide a means of ameliorating the disease by allowing AECs to progress into apoptosis.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Publications, Presentations, and Achievements

Publications, presentations, and achievements made during this PhD are listed below.

Published Journal Articles

Roscioli E, Hamon R, Lester S, Murgia C, Grant J, Zalewski P. Zinc-rich inhibitor of apoptosis proteins (IAPs) as regulatory factors in the epithelium of normal and inflamed airways. Biometals 2013; 26(2):205-27. (Appendix 4).

Roscioli E, Hamon R, Ruffin R, Lester S, Zalewski P. Cellular inhibitor of apoptosis-2 is a critical regulator of apoptosis in airway epithelial cells treated with asthma-related inflammatory cytokines. Physiological Reports 2013: 1(5):e00123 (Chapter 3).

Roscioli E, Hamon R, Ruffin RE, Zalewski P, Grant J, Lester S. X-linked inhibitor of apoptosis single nucleotide polymorphisms and copy number variation are not risk factors for asthma. Respirology 2013; 18(4):697-703 (Chapter 4, Part A).

Lang C, Hansen M, Roscioli E, Jones J, Murgia C, Leigh Ackland M, *et al.* Dietary zinc mediates inflammation and protects against wasting and metabolic derangement caused by sustained cigarette smoke exposure in mice. Biometals 2011; 24(1):23-39 (Appendix 5).

Murgia C, Grosser D, Truong-Tran A, Roscioli E, Michalczyk A, Ackland M, *et al.* Apical localization of zinc transporter ZnT4 in human airway epithelial cells and its loss in a murine model of allergic airway inflammation. Nutrients 2011; 3(11):910-28. (Appendix 6).

Tan N, Tran H, Roscioli E, Wormald P, Vreugde S. Prevention of false positive binding during immunofluorescence of *Staphylococcus aureus* infected tissue biopsies. Journal of immunological methods 2012; 384(1-2):111-7 (Appendix 7).

Submitted Manuscripts

Roscioli E, Hamon R, Ruffin R, Zalewski P, Grant J, Lester S. *BIRC2* single nucleotide polymorphisms are protective of severe asthma in a Caucasian community-based cohort. International Journal of Immunogenetics 2013 (Chapter 4, Part B).

Manuscripts in Preparation

Tan N, Cooksley C, Roscioli E. Drilling A, Douglas R, Wormald P, Vreugde S. Small colony variants and phenotype switching of intracellular *Staphylococcus aureus* in chronic rhinosinusitis.

Tran H, Roscioli E, Tan N, Coocksley C, Zalewski P, Wormald P, Vreugde S. Increased expression and sub-cellular translocation of AIM2 protein in conjunction with caspase-1 activation in sinus epithelium of the asthmatic endotype of chronic rhinosinusitis.

Conference Presentations

“Expression and Function of Inhibitor of Apoptosis Proteins in Asthmatic and Non-Asthmatic Airway Epithelial Cells Treated with Inflammatory Cytokines.” Presented at The Queen Elizabeth Hospital Research Day; Adelaide, 2012. Accepted for the seminar series competition based on abstract submission.

“Transcript Levels of Inhibitor of Apoptosis Proteins in the Normal and Asthmatic Airway Epithelium.” Presented at the Thoracic Society of Australia and New Zealand conference in Brisbane; 2010.

“The Role of the Inhibitor of Apoptosis Proteins in Normal and Asthma Affected Airway Epithelium.” Presented at the Thoracic Society of Australia and New Zealand Young Investigator of the Year in Adelaide; 2010.

“The Role of the Inhibitor of Apoptosis Proteins in Normal and Asthma Affected Airway Epithelium.” Presented at The Queen Elizabeth Hospital Research Day seminar seriesin Adelaide; 2010.

Achievements

Merit-based entry and scholarship recipient ($24,000) for The University of Adelaide postgraduate certificate in Technology Innovation and Entrepreneurship. Awarded the postgraduate certificate in Technology Innovation and Entrepreneurship; 2012.

Runner-up in the Thoracic Society of Australia and New Zealand Young Investigator of the Year seminar series competition, South Australian branch, Adelaide; 2010.

Co-investigator for The Queen Elizabeth Hospital Research Foundation research grant. Awarded funding ($5,000) for genetics research presented in this thesis; 2010.

Awarded the Thoracic Society of Australia and New Zealand travel grant ($450), to participate in the Brisbane conference; 2010.

List of Figures

List of Tables

Commonly Used Abbreviations

°C - Degrees celcius

AE - airway epithelium

AEC - Airway epithelial cell

ALI - Air-liquid interface

Bax - Bcl-2-associated X protein

Bcl2 - B-cell lymphoma 2

BEGM - Bronchial epithelial growth media

BLAST - Basic local alignment search tool

bp - base pair

cDNA - Complementary deoxyribonucleic acid

cIAP - Cellular inhibitor of apoptosisbp - Base pairs

DAPI - 4',6-diamidino-2-phenylindole

DISC - death-inducing signalling complex

Dox - Doxorubicin

DTT - Dithiothreitol

EDTA - Ethylenediaminetetraacetic acid

g - Gravitational acceleration

gDNA - Genomic deoxyribonucleic acid

h - Hour

HPRT-1 - Hypoxanthine-guanine phosphoribosyltransferase

HRP - Horse radish peroxidase

IL - Interleukin

IFN-γ - Interferon- γ

IRF - Interferon regulatory factor

IL-1β - Interleukin-1β

IL-4 - Interleukin-4

IL-13 - Interleukin-4

kDa - Kilo Dalton

M - Molar

min - Minutes

ml - Millilitre

mm - Millimetre

mM - Millimolar

mRNA - Messenger ribonucleic acid

NF-κB - Nuclear factor kappa B

NHBE - Normal human bronchial epithelial cell

nm - Nanometre

PARP - Poly (ADP-ribose) polymerase

PBS - Phosphate buffered saline

PCR - polymerase chain reaction

PET - Polyethylene terephthalate

PI - Propidium Iodide

qRT-PCR - Quantitative reverse transcription real-time polymerase chain reaction

RT - Room temperature

s - Seconds

siRNA - Short interfering ribonucleic acid

SFB - Serum free blocker

Smac - Second mitochondrial activator of caspases

STAT - Signal transducer and activator of transcription

TBP - Tata box binding protein

TBST - Tris-buffered saline

TNFα - Tumour necrosis factor-α

TRADD - TNF receptor-associated death domain

µg - Microgram

µl - Microliters

XAF1 - XIAP associated factor-1

XIAP - X-linked inhibitor of apoptosis