The role of microRNAs in allergic airways disease and T cell biology

By

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THESIS STATEMENT

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 this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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Maximilian Plank

May 2014

CONTRIBUTION TO CO-AUTHORED PAPERS

Chapter 1

Sections of the introduction (specifically relating to microRNAs, their functions in the immune system and in inflammatory disorders) have previously been published in an invited review (Paper 1) and contributed to a review on the emerging role of microRNAs in regulating immune and inflammatory responses in the lung (Paper 2).

Paper 1:

> Plank M, Maltby S, Mattes J, Foster PS. Targeting translational control as a novel way to treat inflammatory disease: the emerging role of microRNAs. Clin Exp Allergy. 2013 Sep;43(9):981-99.

Paul Foster and Maximilian Plank initially conceptualised this manuscript. Maximilian Plank performed the literature search, generated figures and tables and wrote the initial complete draft. Paul Foster, Joerg Mattes and Steve Maltby contributed to the final version which was edited and submitted by Maximilian Plank.

Paper 2:

> Foster PS, Plank M, Collison A, Tay HL, Kaiko GE, Li J, et al. The emerging role of microRNAs in regulating immune and inflammatory responses in the lung. Immunological reviews. 2013 May;253(1):198-215.

Paul Foster, Hock Tay, Adam Collison and Maximilian Plank initially conceptualised and Hock Tay, Adam Collison and Maximilian Plank contributed sections to the paper, which was collated and written by Paul Foster.

Laureate Professor Paul Foster

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LIST OF ABBREVIATIONS

-/-	knockout mouse
AAD	allergic airways disease
AD	allergic dermatitis
AEC	airway epithelial cell
AHR	airways hyperreactivity
Ant	antagomir
APC	allophycocyanin, antigen presenting cell
bp	basepair
BSA	bovine serum albumin
c-Myb	transcriptional activator Myb
CCL	chemokine ligand
CFSE	carboxyfluorescein diacetate succinimidyl ester
Chr	chromosome
Chol	cholesterol
CIP	calf intestine alkaline phosphatase
COPD	chronic obstructive pulmonary disease
COX2	cyclooxygenase 2
CxCL	chemokine (c-X-c motif) ligand
Da	dalton
DC	dendritic cell
DEX	dexamethasone
DGCR8	DiGeorge syndrome critical region gene 8
DMEM	Dulbecco's modified eagle medium
DNA	desoxyribonucleic acid
dsDNA	double-stranded DNA
dNTP	desoxyribonucleic triphosphate
EAE	experimental autoimmune encephalomyelitis
EDTA	ethylenediaminetetraacetic acid
FCS	fetal calf serum
FITC	fluorescein
FoxO1	forkhead box O1
FoxP3	forkhead box P3
Gata3	GATA binding protein 3
GSK3B	glycogen synthase kinase-3β
HBSS	Hanks buffered saline solution
HDM	house dust mite
HPF	high power field
ICS	inhaled corticosteroids
IFN	interferon
Ig	immunoglobulin

IL	interleukin
i.n.	intranasal
IRAK1	IL-1 receptor associated kinase
iTreg	induced regulatory T cell
JNK	c-Jun NH(2)-terminal kinase
LCs	Langerhans cells
Let-7	lethal 7
LPS	lipopolysaccharide
miRNA	microRNA (micro ribonucleic acid)
MyD88	myeloid differentiation primary response gene (88)
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer
OBF.1/BOB.1	POU domain class 2 associating factor 1
OVA	ovalbumin
OVA/OVA	OVA sensitised/OVA challenged mice
OVA/OVA DEX	OVA/OVA mice treated with dexamethasone
PAMP	pathogen-associated molecular pattern
PDCD4	programmed cell death protein 4
PBLN	peribronchial lymph node cells
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PBS/PBS	PBS sensitised/PBS challenged mice
PBS/OVA	PBS sensitised/OVA challenged mice
PCR	polymerase chain reaction
PE	r-phycoerythrin
PE-Cy7	r-phycoerythrin-Cy7 conjugate
PerCP-Cy5.5	peridinin-chlorophyll-protein complex: Cy5.5 conjugate
PGE2	prostaglandin E2
PMNs	polymorphonuclear neutrophils
Pri-miRNA	primary microRNA transcript
PTEN	phosphatase and tensin homologue
qRT-PCR	quantitative real-time PCR
RA	rheumatoid arthritis
RANTES	regulated and normal T cell expressed and secreted
RBC	red blood cell
RISC	RNA-inducing signalling complex
RNA	ribonucleic acid
RORγt	retinoid orphan receptor γt
RPM	rounds per minute
Scramble	Scrambled control antagomir
SHIP1	SH2 domain-containing inositol phosphatase 1
siRNA	short interfering ribonucleic acid
SOCS1	suppressor of cytokine signalling 1
SPF	specific pathogen-free

ssDNA	single-stranded DNA
STAT	signal transducer and activator of transcription
TAB2	mitogen-activated protein kinase kinase kinase 7 binding protein 2
Tbet	T-box transcription factor expressed in T cells
TCR	T cell receptor
TGF-β	transforming growth factor beta
Th	T helper
TLR	toll like receptor
TNF	tumour necrosis factor
TOM1	target of Myb1
TRAF6	TNF receptor-associated factor 6
TRAIL	tumour necrosis factor – related apoptosis - inducing ligand
TRBP	TAR RNA-binding protein
Treg	regulatory T cell
UTR	untranslated region

SYNOPSIS

MicroRNAs (miRNAs) are post-transcriptional regulators of gene expression and their cellular expression is differentially regulated at various developmental and functional stages. The objective of my PhD research was to assess 1) whether miRNAs are differentially regulated in an ovalbumin-induced model of allergic airways disease, 2) whether corticosteroid (dexamethasone) treatment alters miRNA expression, 3) whether miRNAs play a functional role in disease development, 4) whether miRNAs are differentially regulated in Th cell differentiation and 5) whether miRNAs play a functional role in Th cell differentiation and function. To address these questions, we performed miRNA profiling on the lungs of allergic mice and compared these profiles to lung profiles from dexamethasone-treated mice and non-allergic controls, using miRNA microarray analysis and real-time PCR. We generated distinct miRNA signatures and identified 29 miRNAs that showed significantly altered expression in allergic lungs. Analysis of predicted miRNA targets revealed novel target genes with altered mRNA expression and demonstrated synergistic miRNA regulation within allergic lungs. Using antagomirs, we inhibited the function of two specific miRNAs (mmu-miR-155-5p and mmu-miR-449a-5p) in the airway wall and investigated the effect on hallmark features of allergic airways disease. While antagomir administration successfully reduced expression of targeted miRNAs, it failed to induce alterations to disease phenotype, suggesting multiple miRNAs regulate changes associated with allergic disease. We further show that antagomir delivery to the lung achieves only variable efficacy across different cell types. While antagomir delivery efficiently reduced specific miRNA expression in myeloid cells, lymphocytes are only partially targeted, suggesting that therapeutic targeting of miRNA function in lymphocytes may require a different approach. We further performed miRNA profiling in naïve and effector Th cells *in vitro* and identified a global up-regulation of miRNA expression in activated Th cells. Using antagomirs, we inhibited the function of several miRNAs and again found that antagomir delivery to Th cells proves inadequate for suppression of target miRNA expression.