Identification of novel therapeutics for chronic obstructive pulmonary disease/emphysema

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Statement of Originality

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Krishna Priya Sunkara

September 2016
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Table of Contents

Synopsis .................................................................................................................................................. 10

Publications arising from this thesis ................................................................................................. 12

List of figures ....................................................................................................................................... 13

List of tables ......................................................................................................................................... 16

Abbreviations ....................................................................................................................................... 17

Chapter 1: INTRODUCTION ............................................................................................................... 21

1.1 CHRONIC OBSTRUCTIVE PULMONARY DISEASE ................................................................. 21
   1.1.1 Epidemiology of COPD ........................................................................................................... 22
   1.1.2 Pathology of COPD ................................................................................................................ 22
   1.1.3 Diagnosis and disease severity ............................................................................................... 22

1.2 COPD pathogenesis ....................................................................................................................... 23
   1.2.1 Inflammation .......................................................................................................................... 23
   1.2.2 Oxidative stress ...................................................................................................................... 31
   1.2.3 Protease: Anti-protease imbalance ......................................................................................... 33
   1.2.4 Apoptosis ............................................................................................................................... 33
   1.2.5 Autophagy ............................................................................................................................. 37
   1.2.6 Autoimmunity ......................................................................................................................... 37

1.3 COPD EXACERBATIONS ............................................................................................................... 38

1.4 SYSTEMIC INFLAMMATION IN COPD ...................................................................................... 39
1.5 PATHOPHYSIOLOGY OF COPD ................................................................. 39
  1.5.1 Airflow obstruction ....................................................................... 39
  1.5.2 Emphysema ................................................................................. 40
  1.5.3 Mucous hypersecretion ............................................................... 40
  1.5.4 Fibrosis ....................................................................................... 40

1.6 CURRENT THERAPIES ........................................................................ 41

1.7 MICORNAS (miRs) ........................................................................... 41
  1.7.1 miR biogenesis and mechanism of action ..................................... 42
  1.7.2 miRs in immunity and COPD ....................................................... 44
  1.7.3 Potential miR therapeutics .......................................................... 47

1.8 STUDY RATIONALE .......................................................................... 50

Chapter 2: Methods .................................................................................. 52
  2.1 Ethics statement ............................................................................. 52
  2.2 Induction of experimental COPD .................................................... 52
  2.3 RNA isolation and quantification ................................................... 52
  2.4 Microarray-based miR expression profiling .................................... 53
  2.5 Quantification of miR and gene expression through real-time quantitative PCR (qPCR) .......................................................... 53
  2.6 Antagomir administration ............................................................... 56
  2.7 Ant-9 and TMC administration ....................................................... 57
  2.8 In situ hybridisation (ISH) ............................................................... 57
  2.9 Airway Inflammation ..................................................................... 58
  2.10 Protein Isolation ........................................................................... 58
  2.11 Enzyme linked immunosorbent assay (ELISA) ................................ 58
  2.12 Airway remodelling ...................................................................... 59
  2.13 Alveolar enlargement ................................................................... 59
  2.14 Lung function analysis .................................................................. 59
2.15 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis .......................................................... 60
2.16 Immunofluorescence (IF) ........................................................................................................ 60
2.17 NF-κB activity assays ............................................................................................................. 61
2.18 Combined antagonir administration ...................................................................................... 61
2.19 Statistics .................................................................................................................................. 62

CHAPTER 3: MICROARRAY-BASED MICRORNA (miR) EXPRESSION PROFILING IN EXPERIMENTAL COPD ................................................................. 63

3.1 INTRODUCTION .......................................................................................................................... 63

3.2 RESULTS ..................................................................................................................................... 64

3.2.1 miR expression profile in lungs in response to CS exposure in experimental COPD ................. 64

3.2.2 Validation of differentially regulated miR expression using qPCR .......................................... 66

3.3 DISCUSSION AND CONCEPTION OF STUDIES ...................................................................... 73

CHAPTER 4: ROLE OF miR-9 IN THE PATHOGENESIS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE ................................................................. 75

4.1 INTRODUCTION .......................................................................................................................... 75

4.2 RESULTS ..................................................................................................................................... 76

4.2.1 CS exposure induces a persistent increase in miR-9 expression in experimental COPD .............. 76

4.2.2 Inhibition of miR-9 suppresses pulmonary inflammation in CS-induced experimental COPD .................................................................................................................. 78

4.2.3 Depletion of CS-induced miR-9 expression inhibits airway fibrosis back to basal levels, causes a trend towards a decrease in emphysema and improves lung function in experimental COPD ................................................................. 80

4.2.4 NRF2 expression and protein levels are increased with Ant-9 mediated inhibition of CS-induced miR-9 in experimental COPD .................................................................................................................. 82
4.2.5 NRF2 expression and NFR2 induced anti-oxidant gene expression are increased in CS-exposed airways but not parenchyma experimental COPD. .................................................................................................................................................. 84

4.2.6 Treatment with 10mg/kg of 2-trifluoromethyl-2'-methoxychalone (TMC) suppresses CS-induced acute inflammation.................................................................................................................. 86

4.2.7 Ant-9 mediated inhibition of CS-induced miR-9 and TMC administration to activate NRF2 suppresses BALF inflammation in experimental COPD .... 88

4.2.8 Ant-9 mediated inhibition of CS-induced miR-9 and TMC administration to activate NRF2 does not improve emphysema ................................................. 91

4.2.9 CS-induced increases in miR-9 expression inhibits SOCS5 levels which are restored by inhibition of miR-9 in experimental COPD................................. 93

4.3 DISCUSSION .......................................................................................................................................................... 95

CHAPTER 5: miR-21 SUPPRESSION OF SATB1 INDUCES S100A9/NF-KB AND COPD PATHOGENESIS ................................................................................. 102

5.1 INTRODUCTION .................................................................................................................................................. 102

5.2 RESULTS .......................................................................................................................................................... 102

5.2.1 CS exposure increases miR-21 expression in experimental COPD...... Error! Bookmark not defined. 

5.2.2 Inhibition of CS-induced miR-21 suppresses airway inflammation in experimental COPD ................................................................................................................. 105

5.2.3 Inhibition of CS-induced miR-21 reduces small airway remodelling and improves lung function, but not emphysema, in experimental COPD ..... 108

5.2.4 Inhibition of CS-induced miR-21 selectively inhibits canonical targets in experimental COPD .................................................................................................................. 111

5.2.5 CS-induced lung miR-21 targets SATB1 in experimental COPD .......... 113

5.2.6 Airway epithelium expresses higher SATB1 mRNA than parenchyma in pulmonary tissue .............................................................................................................. 116

5.2.7 S100A9 expression and NF-κB activity are increased in experimental COPD, and are suppressed by inhibiting miR-21 expression ......................... 117

5.2.8 Inhibition of CS-induced miR-21 does not affect pAKT levels in experimental chronic obstructive pulmonary disease (COPD) ......................... 120
5.3 DISCUSSION........................................................................................................122

CHAPTER 6: ROLE OF miR-135b AND miR-146b IN THE PATHOGENESIS OF EXPERIMENTAL CHRONIC OBSTRUCTIVE PULMONARY DISEASE ......129

6.1 INTRODUCTION.....................................................................................................129

6.2 RESULTS................................................................................................................130

6.2.1 CS-exposures induces persistent increase in miR-135b and miR-146b expression in experimental COPD .................................................................130

6.2.2 CS-induced airway inflammation is reduced by inhibition of miR-135b, but not with miR-146b, in experimental COPD .......................................................132

6.2.3 CS-induced miR-135b and miR-146b expression promotes small airway remodelling and emphysema in experimental COPD ............................................134

6.2.4 Inhibition of CS-induced miR-135b and miR-146b selectively inhibits some canonical targets in experimental COPD ..............................................................137

6.3 DISCUSSION..........................................................................................................139

CHAPTER 7: COMBINED TARGETING OF CIGARETTE SMOKE-INDUCED miRS IN EXPERIMENTAL COPD .................................................................................144

7.1 INTRODUCTION.....................................................................................................144

7.2 RESULTS................................................................................................................146

7.2.1 Treatment with combinations of two miR-specific antagonirs simultaneously decreases the expression of target miRs in experimental COPD ............146

7.2.2 Treatment with combinations of two miR-specific antagonirs reduces CS-induced airway inflammation in experimental COPD .................................148

7.2.3 Combined targeting of CS-induced miR-21 and miR-146b expression suppresses small airway remodelling and emphysema-like alveolar enlargement in experimental COPD ..................................................152

7.3 DISCUSSION..........................................................................................................156

CHAPTER 8: GENERAL DISCUSSION AND CONCLUSION ......................................162

8.1 Significance of research .........................................................................................162
8.2 Microarray-based miR expression profiling in experimental COPD........163
8.3 Role of miR-9 in the pathogenesis of COPD ..................................164
8.4 miR-21-mediated suppression of SATB1 induces S100A9/NF-κB and COPD pathogenesis .................................................................167
8.5 Role of miR-135b and miR-146b in the pathogenesis of experimental COPD ............................................................................................169
8.6 Combined targeting of CS-induced miRs in experimental COPD ..........173
8.7 Future directions .................................................................173
8.8 Conclusion ..............................................................................174

8. REFERENCES..................................................................................176
Synopsis

Chronic obstructive pulmonary disease (COPD) is characterised by progressive decline in lung function that is caused by aberrant inflammatory responses, small airway remodelling and emphysema. The key risk factor of COPD is cigarette smoking. Current mainstay therapies of COPD only provide symptomatic relief and fail to limit the disease progression. Thus there is an urgent requirement for the development of new therapies. However this is hampered by the lack of understanding of the mechanisms that drive COPD pathogenesis. Therefore there is a need for the elucidation of the mechanisms that underpin the development of COPD.

MicroRNAs (miRs) are evolutionarily conserved small noncoding RNAs that regulate the expression of their target genes at the post-transcriptional level. More than 1,000 human miRNAs have been identified and are known to regulate numerous biological processes such as cell differentiation and proliferation, apoptosis, and immune responses. Importantly, altered expression of miRs are implicated in the development of several cancers and inflammatory diseases including asthma. However, their role in the pathogenesis of COPD is limited. Thus, our studies were aimed to understand the roles of CS-induced dysregulated miRs and interrogate their potential for therapeutic targeting in experimental COPD.

Using microarray-based miR profiling technique, we identified a range of dysregulated miRs in CS-induced experimental mouse model of COPD. Acute and chronic CS-exposure chronically upregulated the expression of four miRs (miR-9, -21, -135b and-146b) in the lungs. Using miR-specific antagomirs we inhibited the CS-induced miRs and demonstrated that targeting CS-induced miRs may be an effective therapy in COPD treatment. We showed that CS-induced miR-9 and miR-21 promote airway inflammation and small airway remodelling and worsened lung function in experimental COPD. Treatment with miR-9- and miR-21-specific antagomirs, Ant-9 and Ant-21 lead to reduced airway inflammation, suppressed small airway remodelling and improvement
in impaired lung function. Thus indicating a potential pathogenic role for the miRs in the development of COPD. Our studies identified increased levels of oxidative stress responsive transcription factor NRF2 and restored levels of cytokine signalling suppressor protein, SOCS5 to play important roles reducing the COPD pathologies. We also identified a novel miR-21-dependent pro-inflammatory pathway in COPD pathogenesis. We demonstrated that CS-exposure induces a miR-21/SATB1/S100A9/NF-κB axis in the lungs and thus advances our understanding of the pro-inflammatory role of miR-21 in COPD pathogenesis. Our studies also demonstrated that CS-miR-135b promotes neutrophilic airway inflammation and showed reduced BMPR2 expression, a potential mediator of macrophage recruitment and may play a role in small airway remodelling in experimental COPD. We also demonstrated that treatment with Ant-135b and Ant-146b suppresses airway remodelling and emphysema-like alveolar enlargement. This indicates that miR-135b and miR-146b may play potentially overlapping roles in mediating COPD pathogenesis. We also showed that miR-135b and miR-146b may promote emphysema through VEGF and IRAK1 and TRAF6-dependent mechanisms. Furthermore our studies demonstrated for the first time that inhibition of combinations of CS-induced miRs, may have beneficial effects in reducing some features of COPD. Further we also showed that combined inhibition of CS-induced miR-21 and miR-146b may be more effective in suppressing key features of COPD.

Collectively, these studies further extend our understanding of the pathogenesis of COPD and identifies CS-induced miRs as potential novel therapeutic strategies in the treatment of COPD. Therapeutic targeting of a CS-induced miR or in combination may be more beneficial as miRs regulate multiple pathogenic pathways. Further, exploration of the CS-induced miR-dependent mechanisms identified in our studies may assist in the development of miR-based therapeutic strategies for the treatment of COPD.
Publications arising from this thesis

KP Sunkara, AG Jarnicki, RY Kim, TJ Haw, PA Wark, JC Horvat, PS Foster, PM Hansbro
Role of miR-9 in the pathogenesis of chronic obstructive pulmonary disease. Prepared for submission to *European Respiratory Journal*.

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Future publications

Role of miR-135b and miR-146b in the pathogenesis of experimental chronic obstructive pulmonary disease

Combined targeting of cigarette smoke-induced miRs in experimental COPD
List of figures

**Figure 1.1:** Inflammatory and immune cells involved in mucous hyper secretion, alveolar wall destruction and fibrosis in COPD pathogenesis.

**Figure 1.2:** Lung epithelial cell apoptosis and development of emphysema mediated by decreased VEGF production.

**Figure 1.3:** microRNA (miR) biogenesis

**Figure 1.4:** microRNA (miR) silencing *in vivo* with antagomirs

**Figure 3.1:** Altered expression of microRNAs (miRs) during cigarette smoke (CS) exposure in experimental COPD.

**Figure 4.1:** Acute and chronic cigarette smoke (CS) exposure induces increases in miR-9 expression in experimental chronic obstructive pulmonary disease (COPD).

**Figure 4.2:** Cigarette smoke (CS)-induced increases in miR-9 expression are inhibited by antagomir-9 (Ant-9), which reduces pulmonary inflammation in experimental chronic obstructive pulmonary disease (COPD).

**Figure 4.3:** Cigarette smoke (CS)-induced increases in miR-9 expression are inhibited by antagomir-9 (Ant-9), suppressing small airway remodelling, decreasing emphysema and improving impaired lung function in experimental chronic obstructive pulmonary disease (COPD).

**Figure 4.4:** Inhibition of cigarette smoke (CS)-induced increases in miR-9 expression by antagomir-9 (Ant-9), increases nuclear factor-erythroid 2 related factor2 (NRF2) expression and protein levels in experimental chronic obstructive pulmonary disease (COPD).

**Figure 4.5:** Nuclear factor-erythroid-2 related factor2 (NRF2) and NFR2-induced antioxidant gene expression in airways and parenchymal tissue in cigarette smoke (CS)-induced experimental chronic obstructive pulmonary disease (COPD).
Figure 4.6: Optimisation of 2-trifluoromethyl-2'-methoxychalone (TMC) treatment doses in the suppression of inflammation induced by acute cigarette smoke (CS)-exposure.

Figure 4.7: Inhibition of CS-induced miR-9 with antagonir-9 (Ant-9) and administration of 2-trifluoromethyl-2'-methoxychalone (TMC) reduces airway inflammation in experimental chronic obstructive pulmonary disease (COPD).

Figure 4.8: Effects of inhibition of CS-induced miR-9 and administration of 2-trifluoromethyl-2'-methoxychalone (TMC) on airway remodelling and emphysema in experimental chronic obstructive pulmonary disease (COPD).

Figure 4.9: Cigarette smoke (CS)-induced increases in miR-9 expression inhibits SOCS5 levels and with the inhibition of miR-9 SOCS5 transcript and protein levels are increased in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.1: Cigarette smoke (CS) exposure increases lung miR-21 expression in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.2: Cigarette smoke (CS)-induced miR-21 expression induces pulmonary inflammation in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.3: Cigarette smoke (CS)-induced miR-21 expression induces small airway remodelling and impairs lung function, but not emphysema-like alveolar enlargement, in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.4: Cigarette smoke (CS)-induced miR-21 expression selectively affects the expression of canonical target genes in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.5: Cigarette smoke (CS)-induced miR-21 expression reduces SATB1 transcript and protein levels in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.6: SATB1 expression is higher in airway epithelium than parenchyma in both normal air and cigarette smoke (CS)-exposed groups.
Figure 5.7: Cigarette smoke (CS)-induced miR-21 expression attenuates S100A9 transcript and NF-κB (p65) activity in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.8: Cigarette smoke (CS) induced miR-21 reduces pAKT levels in experimental chronic obstructive pulmonary disease (COPD).

Figure 5.9: miR-21 mediated inflammatory pathways in chronic obstructive pulmonary disease (COPD).

Figure 6.1: Cigarette smoke (CS) exposure increases lung miR-135b and miR-146b expression in experimental chronic obstructive pulmonary disease (COPD).

Figure 6.2: Cigarette smoke (CS)-induced pulmonary inflammation is partially dependent on miR-135b in experimental chronic obstructive pulmonary disease (COPD).

Figure 6.3: Cigarette smoke (CS)-induced miR-146b promotes small airway remodelling, emphysema and impaired lung function in experimental chronic obstructive pulmonary disease (COPD).

Figure 6.4: Cigarette smoke (CS)-induced miR-135b and miR-146b selectively affect the expression of some putative target genes in experimental chronic obstructive pulmonary disease (COPD).

Figure 7.1: Treatment with combinations of two miR-specific antagomirs simultaneously decreases the expression of target miRs in experimental chronic obstructive pulmonary disease (COPD).

Figure 7.2: Treatment with combinations of two miR-specific antagomirs decreases cigarette smoke (CS)-induced airway inflammation in experimental chronic obstructive pulmonary disease (COPD).

Figure 7.3: Combined targeting of cigarette smoke (CS)-induced miR-21 and miR-146b expression suppresses small airway remodelling and emphysema-like alveolar enlargement in experimental chronic obstructive pulmonary disease (COPD).
**Figure 8.1** Summary diagram depicting the effects of Ants -9, -21, -135b and -146b on various pathways in chronic obstructive pulmonary disease

**List of tables**

**Table 1.1**: Gold Stages of COPD

**Table 2.1**: Primers used in qPCR primers sequences

**Table 2.2**: Antagomir treatment regimen in experimental COPD

**Table 3.1**: microRNAs (miRs) with altered expression in the lung during CS exposure in experimental COPD

**Table 3.2**: Custom-designed microRNA (miR) primers used to validate microarray miR data by qPCR

**Table 3.3**: Potential pathways, and associated diseases and functions linked with increased expression of microRNAs (miRs) and their known and predicted targets during the initiation and throughout CS-exposure in experimental COPD

**Table 7.1**: Summary table showing the effects of inhibiting CS-induced miR expression through miR-specific antagomirs

**Table 7.2**: Summary table showing the expected and detected effects of inhibiting CS-induced combined miR expression through miR-specific antagomirs
Abbreviations

Akt: Protein kinase B
AP-1: Activator protein
ATP: Adenosine triphosphate
BALF: Bronchoalveolar lavage fluid
BM: Basement membrane
BMPR: Bone morphogenetic protein receptor
BSA: Bovine serum albumin
CCL: Chemokine (C-C motif) ligand
CR: C-C chemokine receptor
CS: Cigarette smoke
COPD: Chronic obstructive
COX-2: Cyclooxygenase-2
CXCL: Chemokine (C-X-C motif)
CXCR: C-X-C chemokine receptor type
DAMP: Damage-associated molecular
DC: Dendritic cell
DMSO: Dimethyl sulfoxide
ECM: Extracellular matrix
EGFR: Pro-epidermal growth factor
ELISA: Enzyme linked immunosorbent assay protein
FEV1: Forced expiratory volume in
FOXO: Forkhead box protein
FVC: Functional vital capacity
GCLC: Glutamate--cysteine ligase catalytic subunit
GM-CSF: Granulocyte macrophage colony-stimulating factor
GPX: Glutathione peroxidase
GST: Glutathione S-transferase
GSTP: Glutathione S transferase
H&E: Hematoxylin and eosin
HDAC: Histone deacetylase
HO: Heme oxygenase
HPRT: Hypoxanthine-guanine
HRP: Horseradish peroxidase
i.n.: Intranasally
ICAM: Intercellular adhesion molecule
IFN: Interferon
IL: Interleukin
IL-1R: IL-1 receptor
IRAK: Interleukin-1 receptor associated kinase
JAK: Tyrosine-protein kinase
LPS: Lipopolysaccharide
MAPK: Mitogen-activated protein
miR: MicroRNA
MMP: molecular pattern monophosphate
MUC: Mucin
MyD88: Myeloid differentiation
NE: Neutrophil elastase
NF-κB: Nuclear factor κB
NOX: NADPH oxidase
NRF: Nuclear factor erythroid
nt: nucleotide
PAIS: Protein inhibitor of activated STAT
pAkt: Phosphorylated Akt
**PAMP**: Pathogen-associated pattern

**PBS**: Phosphate-buffered saline

**PBS-T**: PBS and Tween 20

**PDCD**: Programmed cell death protein

**PDGFRB**: Platelet-derived growth factor receptor beta

**PI3K**: Phosphoinositide-3-kinase

**pol II**: Polymerase II

**Pre-miRNA**: Precursor-miRNA

primary response gene 88

**Pri-miRNA**: Primary miRNA

**PRR**: Pattern recognition receptors

**PTEN**: Phosphatase and tensin

pulmonary disease

**PVDF**: Polyvinylidene difluoride

**qPCR**: Quantitative PCR

**RAGE**: Advanced glycosylation end product-specific receptor

**RECK**: Reversion-inducing cysteine-rich protein with Kazal motifs region gene 8

**RIN**: RNA integrity number

**RISC**: RNA-induced-silencingcomplex

**RNA**: ribonucleic acid

**RNAi**: RNA interference

**RNS**: Reactive nitrogen species

**ROS**: Reactive oxygen species

**RT**: Room temperature

**S100**: S100 calcium-binding protein

**SATB**: Special AT-rich sequence-binding protein

**Scram**: Scrambled antagomir

**SHIP**: SH2 domain containing inositol signalling
siRNA: Short-interfering RNA
SIRT: Sirtuin
SMAD: Mothers against decapentaplegic homolog
snoRNA: Small nucleolar RNA
snRNA: Small nuclear RNA
SOCS: Suppressor of cytokine
SPRY: Protein sprouty homolog
STAT: Signal transducer and activator
TBP: TATA binding protein
TBS: Tris-buffered saline
TBS-T: TBS and Tween 20
TGF: Transforming growth factor
TH: T helper lymphocyte
TIMP: Metalloproteinase inhibitor
TLR: Toll-like receptor
TNF: Tumour necrosis factor
TRAF: TNF receptor-associated factor
UTR: Untranslated region
VEGF: Vascular endothelial growth factor
WOB: Work of breathing
WT: Wild-type