Chronic mucus hypersecretion in COPD and asthma

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CHAPTER 9

Summary, general discussion and future perspectives

SUMMARY

Chronic mucus hypersecretion (CMH) is an important clinical feature in chronic obstructive pulmonary disease (COPD) and asthma. It is associated with lower quality of life, more severe symptoms, and an increased risk of exacerbations and mortality1–3. Important characteristics for CMH pathophysiology are exaggerated mucin secretion, increased goblet cell number, and impaired cilia function. In this thesis, we hypothesized that aberrant stromal cell-epithelium crosstalk contributes to CMH pathophysiology and that miRNAs are involved in CMH via this crosstalk or mechanisms underlying mucin secretion, mucociliary differentiation, and pro-inflammatory responses. We used bioinformatics approaches and *in vitro* models to investigate the involvement of miRNAs in CMH and the role of stromal cells in epithelial mucin secretion.

In **Chapter 2**, we discussed the recent findings on the role of miRNAs and exosomes in asthma pathogenesis. In relation to CMH, specifically, miR-34/449 family—which is less expressed in asthma than in controls—has been shown to regulate ciliated cell differentiation; while let-7 family—which improves airway hyperresponsiveness and alleviates mucus production—is less expressed in exosomes isolated from asthma-derived bronchoalveolar lavage fluid compared to from healthy controls. Other miRNAs, e.g. miR-19, miR-21, miR-455, may be involved in airway remodeling in asthma but their role in CMH has not been reported.

In **Chapter 3**, we identified 10 CMH-associated miRNAs in bronchial biopsies of COPD patients of which expression was significantly correlated with at least one mRNA. The miRNAs positively associated with CMH were let-7a-5p, let-7d-5p, let-5f-5p, miR-31-5p, and miR-708-5p; and miRNAs negatively associated with CMH were miR-134-5p, miR-146a-5p, miR-193a-5p, miR-500a-3p, and miR-1207-5p. We created miRNA–mRNA co-expression networks based on miRNA-mRNA correlations which highlighted miR-134-5p, miR-146a-5p and the let-7 family as well as their correlated targets (e.g. KRAS and EDN1) as key regulators of CMH in COPD. Gene Set Enrichment Analyses (GSEA) revealed that molecular mechanisms related to MUC5AC expression likely contribute to CMH in COPD. Of the key miRNAs, miR-134-5p expression was lower in the fibroblasts derived from COPD patients with CMH compared to those without CMH, supporting our hypothesis that fibroblasts are involved in CMH pathophysiology.

Our next aim was to determine whether miRNAs are also involved in CMH in asthma and whether CMH in asthma and COPD share common mechanisms mediated by miRNAs. In **Chapter 4**, we identified 17 miRNAs associated with CMH in bronchial biopsies from asthmatic patients. Among these miRNAs, miR-31-5p was the only miRNA associated with CMH in both asthma and COPD, so we propose that this miRNA may be involved in a shared mechanism underlying CMH in both

diseases. miR-31-5p expression was higher with CMH and negatively correlated with several predicted targets including ST3GAL2, PITPNM2, and ARHGEF15 which were also negatively associated with CMH in both asthma and COPD. Similar to our findings in COPD, GSEA revealed that molecular mechanisms related to MUC5AC expression likely contribute to CMH in asthma. Moreover, significant enrichment of the CMH-associated gene set in COPD among CMH-associated genes in asthma suggests that molecular mechanisms underlying CMH in asthma and COPD are overlapping.

In **Chapter 5**, we addressed the role of fibroblast-epithelium crosstalk in mucous cell differentiation and epithelial mucin secretion. We set up a long-term co-culture model of ALI-differentiated primary bronchial epithelial cells (PBECs) and primary airway fibroblasts (PAFs). We found more differentiation towards mucous cells and higher MUC5AC and MUC5B expression and secretion by PBECs upon co-culture with PAFs; and showed that the effect on MUC5B was (partly) mediated by fibroblast-derived IL-6. Overall, these findings support the hypothesis that stromal cells are involved in mucous cell differentiation and mucin secretion and indicate that MUC5B expression is modulated by fibroblast-derived IL-6, which may have implications for CMH in COPD.

Since our data showed that fibroblasts support mucous cell differentiation and mucin secretion by epithelial cells, we further assessed whether any of the CMH-associated miRNAs identified in bronchial biopsies from COPD patients are involved in this crosstalk. In **Chapter 6**, we identified let-7a-5p and miR-146a-5p as potential candidates regulating the crosstalk in CMH via their effects in PAFs.

In **Chapter 7**, we investigated the function of miR-146a-5p, which was positively associated with CMH in bronchial biopsies, in fibroblast-epithelium crosstalk in COPD. Since the focus of this study was on the role of fibroblasts in inflammatory response to epithelial damage, not mucociliary differentiation, we used 16HBE14o- immortalized bronchial epithelial cells as a model to keep the epithelial component stable. A submerged co-culture model of 16HBE14o- cells and primary human lung fibroblasts showed that miR-146a-5p expression was upregulated in the fibroblasts upon co-culture with the epithelial cells and this upregulation was less pronounced in COPD-derived fibroblasts compared to non-COPD controls. Furthermore, we showed that the upregulation of miR-146a-5p was mediated by epithelial-derived IL-1α and proposed miR-146a-5p as an anti-inflammatory miRNA. In **Chapter 8**, we investigated how airway smooth muscle cells (ASMCs) contribute to CMH pathophysiology in asthma. We observed that ASMCs derived from asthmatic patients respond more strongly to IL-1β by upregulating several pro-inflammatory genes, including CCL20. ASMCs derived from moderate asthmatic patients secreted more CCL20 than those from mild asthmatic patients and healthy controls, which was accompanied by lower expression of MIR146A (a precursor for miR-146a-3p

and miR-146a-5p). MiR-146a-5p overexpression suppressed IL-1β-induced CCL20 release, while CCL20 induced higher mucus production by Calu-3 and PBECs. Thus, miR-146a-5p may not only act as a negative regulator of pro-inflammatory signals but may also suppress mucus production upon crosstalk between stromal and epithelial cells.

GENERAL DISCUSSION

In this thesis, we first used the available miRNA and mRNA expression profiles of bronchial biopsies derived from COPD and asthma patients to identify miRNA-mRNA networks that underlie CMH in both diseases (**Chapter 3 and 4**). Using an *in vitro* model of a long-term co-culture at ALI, we were able to demonstrate the involvement of fibroblasts in mucous cell differentiation and mucin secretion (**Chapter 5**). Here, we found support for various CMH-associated miRNAs potentially involved in this crosstalk (**Chapter 6**). The role of a selected miRNA, miR-146a-5p, in regulating stromal cell-epithelium crosstalk was further supported by our finding that miR-146a-5p suppresses fibroblast pro-inflammatory responses upon epithelial-derived IL-1α and that this mechanism is impaired in COPD (**Chapter 7**). This suppression of IL-6 release by fibroblasts may have important consequences for mucus production, as IL-6 was shown to mediate epithelial MUC5B expression upon co-culture with fibroblasts (**Chapter 5**). Additionally, miR-146a-5p may regulate epithelial mucus production through suppression of IL-1β-induced CCL20 which can be suppressed by miR-146a-5p (**Chapter 8**). The overview of these findings is illustrated in figure 1.

It is well known that goblet cell hyperplasia contributes to exaggerated mucin secretion, one of the markers of CMH, in COPD and asthma patients[4–6]. In this thesis, our *in vitro* findings suggest that stromal cells—such as fibroblasts and ASMCs—are also involved in CMH, supporting both MUC5AC and MUC5B secretion by epithelial cells as well as their differentiation towards mucus-producing cells (**Chapter 5 and 8**). Our findings indicate the involvement of pro-inflammatory signals from stromal cells in exaggerated mucin secretion in COPD. Notably, mucus obstruction itself can also trigger airway inflammation even in the absence of infection[7]. Without effective clearance, the epithelium is continually exposed to harmful particles that are trapped in sticky mucus. This chronic condition induces cellular stress and hypoxia[7]. *In vitro* studies showed that hypoxia induced by mucus plugging leads to necrotic cell death and the release of IL-1α, similar to the damage induced by cigarette smoke[8], and triggers IL-1 receptor 1 (IL-1R1)/MyD88 signaling in neighboring cells. This subsequently leads to NF-κB activation and secretion of various cytokines and chemokines, resulting in infiltration of inflammatory cells[7,9,10]. As IL-1R1 signaling also increases a release of stromal pro-inflammatory cytokines, e.g. IL-6, CXCL8, and CCL20; in turn, mucus production may be enhanced in a vicious cycle resulting in chronic inflammation and CMH. miR-146a-5p may serve as regulatory mechanism to suppress this process, and this type of regulatory feedback may be impaired in CMH.

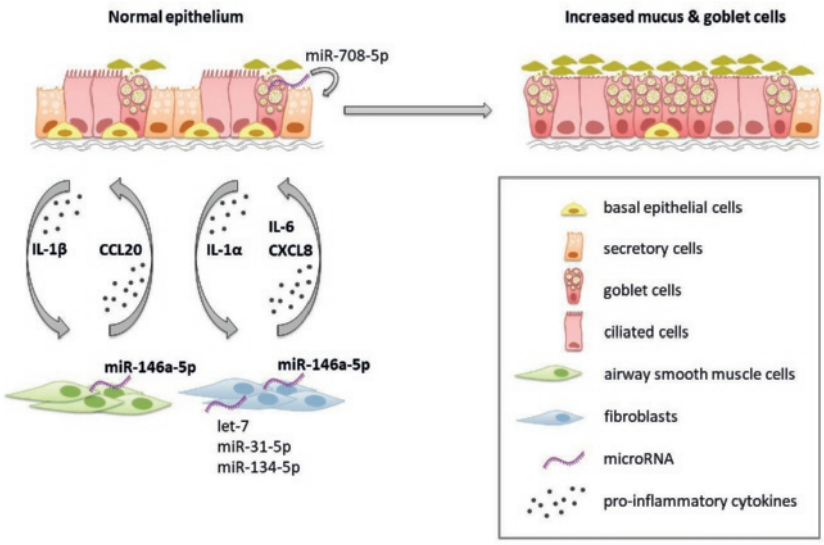

Figure 1. Proposed mechanisms underlying CMH pathophysiology. The main hypothesis addressed in this thesis is that miRNAs and stromal cell-epithelium crosstalk are involved in mucus production and goblet cell differentiation in CMH. miR-708-5p may regulate epithelial mucin secretion intracellularly or in autocrine fashion as we observed that miR-708-5p mimics suppressed mucin secretion of A549 adenocarcinoma cells. miR-146a-5p in fibroblasts or ASMCs mediates a negative feedback loop to suppress inflammatory response induced by damaged epithelium. Other CMH-associated miRNAs, i.e. let-7 family, miR-31-5p, and miR-134-5p may be involved in CMH development by mediating mechanisms within or from fibroblasts as the changes in their expression were observed in this cell type but not epithelial cells. Various pro-inflammatory cytokines (i.e. IL-1 family, IL-6, CXCL8, and CCL20) mediate stromal cell-epithelium crosstalk which may contribute to aberrant mucin production and secretion.

Stromal cell-epithelium crosstalk also exists in healthy physiological conditions, but we speculate that especially the lack of negative feedback mechanisms may result in abnormal inflammatory responses and mucus secretion in COPD and asthma. For instance, we previously observed that IL-1α is critically involved in the upregulation of IL-6 and CXCL8 secretion by fibroblasts upon co-culture with epithelial cells11. In this thesis, we demonstrated that fibroblasts from COPD patients as well as ASMCs from asthma patients were less able to upregulate the anti-inflammatory miR-146a-5p upon co-culture with epithelial cells or exposure to epithelial-derived cytokines (i.e. IL-1 family members) than control-derived cells, suggesting a hampered anti-inflammatory regulation in COPD and asthma resulting from aberrant crosstalk

(**Chapter 7**)12. We also observed that miR-146a-5p expression was lower in bronchial biopsies of COPD patients with CMH than those without CMH but did not observe differential expression of miR-146a-5p in fibroblasts from patients with and without CMH at baseline (**Chapter 3**). Future studies should compare the levels of miR-146a-5p upregulation in fibroblasts from both patient groups upon co-culture with epithelial cells or upon exposure to epithelium-derived signals. Notably, we observed that the expression of miR-134-5p, another miRNA negatively associated with CMH in bronchial biopsies, was lower in airway fibroblasts from the patients with CMH than those without CMH (**Chapter 3**). These findings suggest that a negative feedback loop—for instance, those mediated by miRNAs—may be impaired in the patients with CMH. Interestingly, we previously demonstrated that primary lung fibroblasts derived from COPD patients with CMH expressed higher IL-1R1 at baseline and higher FZD8 receptor upon stimulation with IL-1β compared to fibroblasts from COPD patients without CMH13. The increased expression of IL1R1 as well as FZD8 may lead to increased IL-6 and CXCL8 secretion by the fibroblasts, which in turn can promote mucin production in epithelial cells13. Together with the findings in this thesis, we propose that in COPD patients with CMH, airway fibroblasts express higher IL-1R1 leading to higher (FZD8-mediated) IL-6 secretion upon exposure to epithelial-derived stimuli, including IL-1α. Since miR-146a-5p expression was lower in CMH and less upregulated upon co-culture in COPD-derived fibroblasts, IL-6 may not be efficiently suppressed in COPD (and especially in CMH-derived fibroblasts) upon prolonged co-culture, leading to more mucin synthesis and release by epithelial cells (figure 2). In addition, airway epithelial cells from COPD patients with CMH may secrete more pro-inflammatory signals and alarmins, including IL-1 family members, to promote fibroblast responses. Future studies are needed to compare epithelial cells from COPD patients with and without CMH. Although our *in vitro* data suggest that miR-146a-5p also plays a role in abnormal ASMC-epithelium crosstalk in asthma patients, we did not identify miR-146a-5p as one of the CMH-associated miRNAs in asthma-derived bronchial biopsies (**Chapter 4**). It is possible that this effect is specific to ASMCs and that the lower miR-146a-5p expression in ASMCs derived from asthma patients with CMH was negated by other cell types that are more abundantly present in bronchial biopsies.

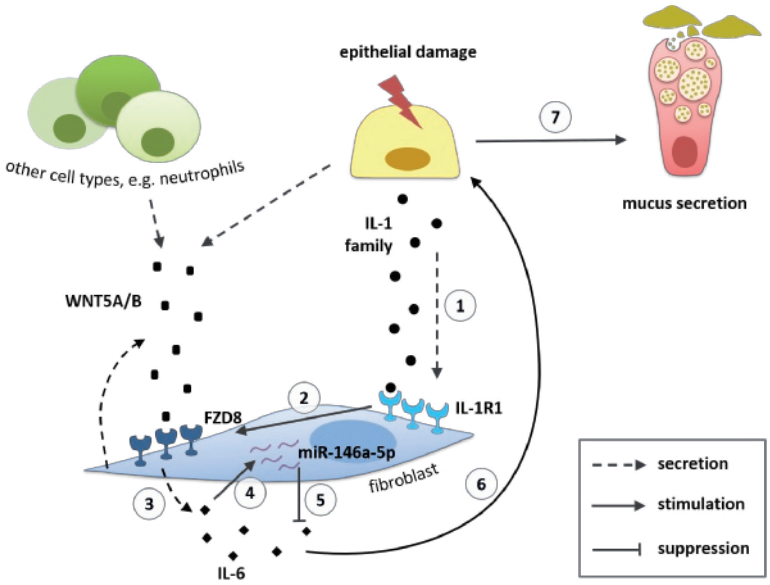

Figure 2. Proposed role of miR-146a-5p in stromal cell-epithelium crosstalk underlying chronic mucus hypersecretion. Upon damage, epithelial cells secrete alarmins (e.g. IL-1 family) which activates IL-1R1 signaling leading to upregulation of a WNT receptor, FZD8. This upregulation results in higher secretion of pro-inflammatory cytokines, e.g. IL-6, which upregulates anti-inflammatory miR-146a-5p as a negative feedback mechanism to suppress IL-6-induced inflammation. However, this negative feedback may be impaired in CMH patients, allowing IL-6 to be continually released and trigger epithelial cells to differentiate to mucous cells and secrete more mucins.

Although only the function of miR-146a-5p was studied in more detail in this thesis, other CMH-associated miRNAs may also be involved in CMH and/or stromal cell-epithelium crosstalk. The majority of the CMH-associated miRNAs in COPD are expressed in both COPD-derived PBECs and COPD-derived PAFs (**Chapter 3 and 6**), indicating the potential role of these miRNAs in modulation of CMH via their effects in these cell types. For instance, let-7a-5p, which was higher expressed in bronchial biopsies derived from CMH patients (**Chapter 3**), was upregulated in PAFs upon co-culture with PBECs (**Chapter 6**). We found that the other two miRNAs from the same cluster, let-7d-5p and let-7f-5p, were also positively associated with CMH in COPD. The expression of these miRNAs was negatively correlated with predicted targets such as NKD1, a gene involved in WNT/β-catenin signaling pathway14 which plays a crucial role in airway epithelial differentiation15 and is suggested to be involved in CMH13. The enrichment of MUC5AC-associated genes among genes positively correlated with the let-7 family also supports a role for these miRNAs in

MUC5AC expression (**Chapter 3**). Interestingly, intranasal delivery of let-7 family was reported to alleviate airway hyperresponsiveness and mucus production in an asthmatic mouse model16 (**Chapter 2**). let-7 miRNAs can inhibit the expression of IL-1316, an important T cell-derived mediator of type 2 inflammatory responses in allergic asthma17 which is also upregulated in COPD18 and is well known for its central role in regulating mucus production through induction of SPDEF19–21. Lower let-7 expression was found in exosomes isolated from asthma-derived bronchoalveolar lavage fluid than in healthy controls22 suggesting that they can act as mediators of cell-cell crosstalk (**Chapter 2**). Future studies should examine whether this miRNA family is secreted by stromal cells in order to act on epithelium.

Another miRNA that should not be overlooked is miR-708-5p, which was higher expressed with CMH in bronchial biopsies (**Chapter 3**) and tended to be upregulated in the epithelial cells upon co-culture with the fibroblasts (**Chapter 6**). Interestingly, this miRNA was previously shown to be downregulated upon mucociliary differentiation at ALI23, suggesting that it may have a negative effect on this differentiation. Our preliminary experiments showed that overexpression of miR-708-5p using miRNA mimics in MUC5AC-producing A549 alveolar adenocarcinoma epithelial cells resulted in lower MUC5AC secretion compared to treatment with negative control siRNA (data not shown). This finding needs to be validated in primary airway epithelial cells. We speculate that miR708-5p is a negative regulator of mucin secretion and is upregulated in COPD patients with CMH to compensate this feature.

Last but not least, miR-31-5p may serve as a modulator or biomarker of CMH in both COPD and asthma as its expression in bronchial biopsies was higher with CMH in both diseases (**Chapter 4**). Little is reported about the link between this miRNA and CMH or relevant biological processes, apart from cigarette smoke condensate upregulates miR-31-5p expression in both normal human airway epithelial cells and lung cancer cells24. This thesis provided further clues for future functional studies of this miRNA. First of all, it is expressed in both control- and COPD-derived PBECs and COPD-derived PAFs (**Chapter 3**) suggesting its potential role in both cell types. Secondly, its expression tends to be upregulated in PAFs upon co-culture with PBECs suggesting it may be involved in fibroblast-epithelium crosstalk (**Chapter 6**). Moreover, MUC5AC-associated genes are enriched among its positively correlated genes (**Chapter 3**) indicating its potential involvement in MUC5AC expression. Lastly, one of its negatively correlated targets is ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2 (ST3GAL2), a member of sialyltransferases, which may play a role in post-translational modification of mucins and consequently affects mucus viscoelasticity (**Chapter 4**).

FUTURE PERSPECTIVES

To the best of our knowledge, this is the first time an unbiased approach has been used to identify miRNAs associated with CMH in COPD (**Chapter 3**) and asthma (**Chapter 4**) which revealed several novel candidate miRNAs that could be involved in CMH pathophysiology. Since this is a cross-sectional study that identified associations, not causal effects, miRNAs of which expression was altered with CMH could be a propagator promoting CMH (i.e. their up- or down-regulation leads to higher mucin secretion or impaired mucus clearance), a suppressor of CMH (i.e. they are up- or down-regulated as a negative feedback loop to lower mucin secretion or increase clearance), or just a downstream molecule being affected by increased mucus levels in the airways. Therefore, whether these miRNA changes are a cause, an effect, or a bystander of CMH feature remains to be studied in the future. Even if it is just a bystander effect, this could be of relevance as these miRNAs could serve as a biomarker for CMH.

Since CMH-associated miRNAs in this thesis were identified in bronchial biopsies, it raises at least two important questions: in which cell types are these miRNAs expressed and where do these miRNAs function? In this thesis, we determined the expression of CMH-associated miRNAs in COPD-derived PBECs and PAFs; whereas the expression of CMH-associated miRNAs in asthma, except miR-31-5p, remains to be studied in asthma-derived cells. Apart from stromal and epithelial cells, other cell types, particularly inflammatory cells (e.g. eosinophils, T-cells, neutrophils, and macrophages) which play a crucial role in both COPD and asthma25–27, could also be the sources and targets of these miRNAs and therefore should also be studied. In addition, future studies should consider unbiased screening for CMH-associated miRNAs in other patient materials, e.g. bronchial brushings of which more than 90% are epithelial cells28, or bronchoalveolar lavage fluid or sputum which contain extracellularly secreted miRNAs29,22 and inflammatory cells28,30. The latter are of interest to evaluate whether these are suitable to identify potential mediators of cell-cell crosstalk as these mediators including miRNAs (e.g. let-7 family) can be secreted into extra-vesicular exosomes (**Chapter 2**). However, as different lung compartments may show essential differences in this respect, it should be realized that also studies in the bronchial wall tissues are needed as that is the actual site of the cellular interplay and crosstalk. Furthermore, other techniques beyond qPCR could be used to assess miRNA expression. For example, in situ hybridization or single-cell sequencing can determine in which specific cell types the miRNAs of interest are expressed in the tissues. Apart from miRNAs, this thesis also reports mRNAs that are correlated with CMH-associated miRNAs and are associated with CMH itself (**Chapter 3 and 4**). As a future approach, it would be informative to also study proteomic data as miRNAs can alter protein translation without inducing mRNA degradation31 and the

expression levels of coding mRNAs do not always represent the production levels of corresponding proteins.

Various experimental approaches can be taken in order to investigate the functions of miRNAs and the involvement of stromal cell-epithelium crosstalk in CMH. In this thesis, it is shown that fibroblasts can promote epithelial mucus secretion and differentiation towards mucus-producing cells and thus proposed that aberrant crosstalk between the two cell types may occur in COPD patients with CMH, thus playing a role in CMH pathogenesis. Therefore, it would be important for the future co-culture models to compare the effects of airway fibroblasts derived from patients with and without CMH on airway epithelial cells, ideally of the same patients. Vice versa, it would also be informative to compare the epithelial cells derived from the patients with and without CMH since they signal differently to the fibroblasts. These studies will help to further validate the hypothesis that the aberrant fibroblast-epithelial cell crosstalk indeed is a main component in CMH pathophysiology in COPD. In asthma, we used mono-culture models to demonstrate potential mechanisms regulating epithelial mucus production via CCL20, a pro-inflammatory cytokine secreted by ASMCs which could also be modulated by miR-146a-5p. An *in vitro* model including co-culture between the two cell types would help to confirm this mechanism. Apart from ASMCs, future experiments should consider studying the crosstalk between epithelium and fibroblasts, as they are an important player in airway remodeling in asthma26 and another cell type expressing miR-146a-6p (**Chapter 4 and 7**), as well as inflammatory cells as they are drivers of allergic inflammation. Moreover, since we are looking at a specific clinical feature (i.e. CMH) in these diseases, it would be informative to compare the expression of candidate miRNAs in the cells derived from the patients with CMH and those from the patients without CMH both at baseline and upon relevant stimulators such as the IL-1 family members. Apart from *in vitro* models, animal models are also available for studying CMH features both in COPD32 and asthma33–35 and could be suitable for confirming functional roles of candidate signaling molecules in a more systemic manner in which different cell types and components can interact *in vivo*. Overall, these studies will shed light into how abnormalities in the complex interplay between structural cells in the airways contribute to CMH and which gene networks are involved.

This thesis focused on mucus secretion and mucous cell differentiation, but there are also other markers of CMH that were not studied. For instance, mucus clearance is known to be impaired in COPD and asthma36–38. Ciliated cells are the major cell type that helps facilitating mucus clearance and cilia dysfunction was observed in both diseases36,38. Besides, biological processes related to cilia development and functions are also enriched among CMH-associated mRNAs (**Chapter 3**). In this thesis, we cultured PBECs at ALI for 2 weeks which is the time point where mucin production could be observed but this could be too early for cilia

development. Therefore, future experiments aiming to study cilia development should allow epithelial cells to differentiate for a longer time, i.e. 3-4 weeks39. Notably, it has been reported that miRNAs are involved in ciliated cell differentiation. miR-449, for instance, has been shown to regulate this process via promoting centriole multiplication and multiciliogenesis partly part by targeting NOTCH140 (**Chapter 2**). Apart from cilia development and function, the changes in mucin composition41, mucus dehydration42 and/or higher viscoelasticity resulting from more mucin cross-linking or impaired mucin degradation43–45 could also contribute to less effective mucus clearance. In this respect, it would also be of interest to examine if a selected miRNA induces changes in mucin composition by comparing MUC5B:MUC5AC ratio produced by epithelial cells upon miRNA overexpression. Other markers such as mucus viscoelasticity can be attributed to higher secretion of extracellular DNA43,44 or impaired cleavage of mucins which can be caused by impaired or lower neutrophil elastase—both of which are the results of a complex interplay between different cell types and components and thus would require *in vivo* models to study them. For example, neutrophil elastase activity in bronchoalveolar lavage fluid collected from mice or sputum derived from patients can be assessed by Foerster-resonance energy transfer (FRET)–based neutrophil elastase reporter assays42. Including all above features in future studies would improve the understanding of CMH pathogenesis and pathophysiology from a more thorough perspective.

At present, there are various drugs being developed to treat CMH but their efficacy remains debatable46. As the findings in this thesis suggests that mechanisms regulating CMH in COPD and asthma are overlapping and could involve miR-31-5p (**Chapter 4**), it would be worthwhile to look further into these mechanisms to identify promising therapeutic targets which may be effective for both groups of patients. Interestingly, a previous study on *in vitro* and *in vivo* models proposed pendrin—a transmembrane protein responsible for anion exchange—as a mediator of mucus production in both COPD and asthma47. Although we did not observe a correlation between SLC26A4 mRNA (a gene encoding for pendrin) and miR-31-5p in bronchial biopsies, this might be due to various cell types present in the samples or miR-31-5p only affects its protein production. Future studies should investigate whether miR-31-5p is involved in molecular pathways related to pendrin protein production and activity. Moreover, it would be of interest to investigate whether post-translational modification of mucin by ST3GAL2 sialyltransferases leads to changes in mucus viscoelasticity and thus consequently contributes to CMH. If miR-31-5p is shown to be a possible regulator of CMH in both diseases, it might serve as a new therapeutic target for CMH treatment to which the use of an antagomir can be applied. To be successful, certain challenges related to safety and specific delivery as also described in **Chapter 2** still require to be overcome.

Overall, this thesis started from identifying candidate miRNAs and mRNAs

that may be involved in CMH using clinical data (i.e. **bed**) and investigated the role of stromal cell-epithelium crosstalk as well as selected miRNAs in *in vitro* models (i.e. **bench**). We hypothesized that aberrant stromal cell-epithelium crosstalk contributes to CMH pathogenesis and pathophysiology and that miRNAs are involved in CMH via this crosstalk or molecular mechanisms underlying mucin secretion, mucociliary differentiation, and pro-inflammatory responses. We identified CMH-associated miRNAs in COPD and asthma, as well as demonstrated the involvement of fibroblast- and ASMC-epithelium crosstalk in inflammatory responses contributing to mucin expression and/or secretion and mucous cell differentiation by epithelial cells. With these findings, we improved our understanding of the molecular mechanisms involving CMH in both COPD and asthma. Ultimately, it would be a great perspective if in the next steps we could translate these discoveries back to the clinic (i.e. **the bed**) and provide insights that lead to the development of a new therapeutic strategy that could help the patients better than currently available options (figure 3).

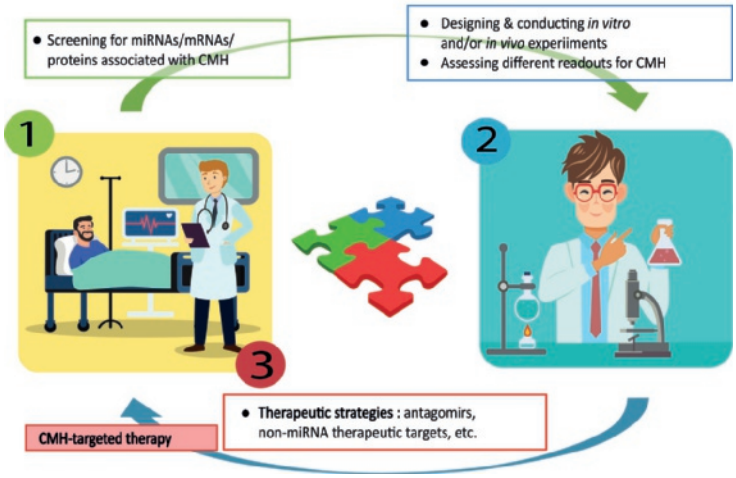

Figure 3. Steps from bed to bench and back to bed leading towards CMH-targeted therapy.

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