Chapter 6

Summary, discussion and future perspectives
Chronic obstructive pulmonary disease (COPD) is a complex and incurable lung disease that is mainly caused by cigarette smoke, and other exposures like indoor cooking and air pollution (1). Because of its slow, long-term development COPD is mainly prevalent from middle age. Given the overlap between COPD and certain features of ageing it has been proposed as an ageing lung disease (2). With the age-shift in the population and high prevalence of smoking, the burden of COPD continues to increase (3).

During normal ageing, lung function will decline in part due to extracellular matrix (ECM) changes which can contribute to thickening of the airway walls and loss of elasticity of alveolar septa (4, 5). Loss of proper ECM homeostasis is one of the ageing hallmarks that plays a role in COPD (6). However, in COPD ECM changes in general occur at an earlier age compared to normal ageing and result in the presence of emphysema in the peripheral parts of the lung with lack of repair and/or remodelling of the (small) airway walls (7). This abnormal repair and remodelling response in COPD is driven by the exposure to smoke resulting in chronic inflammation, extracellular matrix damage and hampered tissue repair (8). In the lung, fibroblasts are the principal cells that play an essential role in lung connective tissue homeostasis and their activity is mainly regulated by TGF-β as an external factor. As miRNAs are important intracellular regulators of cell function, these can be expected to also play a main regulatory role in fibroblasts in maintaining the homeostasis.

In this thesis, we hypothesized that miRNAs with deregulated expression in lung fibroblasts, are crucial players in the impaired lung tissue repair and remodelling as observed in COPD. To explore this, we focused on miRNA expression changes in the lung. The effects of TGF-β as a main regulator and current smoking as a main pathogenetic factor were studied in fibroblasts. Comparisons were made between COPD derived fibroblasts and those from subjects with normal lung function. For these studies, we used primary parenchymal lung fibroblasts obtained from stage II, III and IV COPD patients and from non-COPD control subjects with a normal lung function. The non-COPD control subjects had a lung tumour but no other lung diseases. Stage IV COPD lung fibroblasts were derived from stage IV COPD patients undergoing lung transplantation surgery. The primary fibroblasts from stage II and III COPD patients, and non-COPD control subjects were isolated from parenchymal lung tissue located far away from the tumour. The non-COPD control lung fibroblasts are referred to as control lung fibroblasts.

Furthermore, the association of ageing with gene and miRNA expression and their interactions were investigated in bronchial biopsies from healthy individuals.

**TGF-β-regulated miRNA expression changes in lung fibroblasts**

TGF-β is a cytokine with multiple functions, which is involved in lung homeostasis, tissue repair and remodelling (9). It regulates the functionality of lung fibroblasts, the major
extracellular matrix (ECM) producers in the lung (10). We investigated the effect of TGF-β on miRNA expression profiles in peripheral lung fibroblasts.

In chapters 2 and 3, we identified 21 and 49 miRNAs upregulated in control lung fibroblasts, using microarray (chapter 2) and small RNA sequencing (chapter 3), respectively. Sixteen of these miRNAs were upregulated in both studies (Figure 1A). The overlap between the miRNAs downregulated upon TGF-β treatment in control lung fibroblasts was limited to three miRNAs (Figure 1B). It is noticeable that we identified more TGF-β-regulated miRNAs using small RNA sequencing than in the microarray study. This difference might be explained by the larger group size for the small RNA sequencing (n=15) as compared to the microarray (n=9), which means more power in the small RNA sequencing analyses. Furthermore, the TGF-β stimulation was stronger in terms of concentration (3.1 ng/ml in chapter 2 versus 7.5 ng/ml in chapter 3). Another possible explanation might be that the microarray data are less quantitative as compared to small RNA sequencing to detect these differences (11). Finally, the microarray contained probe sets for 847 human miRNAs, whereas with small RNA sequencing we analysed the expression of all 2,588 human miRNAs. After filtering, we ended up with more miRNAs in small RNA sequencing compared to microarray that were included in the analyses (349 miRNAs from the small RNA sequencing versus 205 miRNAs from the microarray).

Subsequently, we have focussed on miR-455-3p (chapter 2), miR-21-3p (chapter 2) and miR-27a-5p (chapter 3), which were all three significantly upregulated upon TGF-β stimulation in both studies (Figure 1A). We performed Ago2-IP to identify the genes targeted by miRNAs (miRNA-targetomes) in TGF-β stimulated and unstimulated control lung fibroblasts. Ago2-RIP-Chip is an unbiased genome-wide approach for capturing Ago2-miRNA-mRNA complexes and thereby identifying all miRNA target genes in a specific cell population. For miR-455-3p and miR-21-3p we identified TGF-β and Wnt signalling pathway related target genes (i.e. NGF, DLD, HHEX) to be more enriched in the Ago2-IP after TGF-β stimulation. This is of interest as both signalling pathways are involved in tissue repair and remodelling by regulating ECM production amongst others (12-14). Our findings suggest that these two miRNAs might fine tune tissue homeostasis, repair and remodelling in the lung. To provide further evidence on how these three target genes are involved in these signalling pathways in lung fibroblasts, we should establish the functional consequences induced by modulation of the miRNAs and their target genes. Such experiments may include inhibition of the miRNAs and/or overexpressing of the target genes and assessing potential effects on well-known TGF-β-induced genes, e.g. FNI, COL1A1 and α-SMA (15), and Wnt target genes, e.g. GREMLIN2, HOXB3 and AXIN2 (16).

The IP-enriched miR-27a-5p target genes (i.e. NR6A1 and PRDM1) are involved in negative regulation of transcription. Future studies should include confirmation of direct targeting of miR-27a-5p to their transcripts in lung fibroblasts. As a next step, we could
establish which genes are repressed by NR6A1 and PRDM1 in lung fibroblasts and subsequently elucidate their role in tissue repair and remodelling.

Another TGF-β-induced miRNA that was identified in both studies was miR-503-5p. In chapter 2, we were not able to validate the TGF-β-induced upregulation of miR-503-5p using RT-qPCR, which may be explained by lack of specificity of the specific assay for this miRNA. The upregulation of this miRNA was confirmed in chapter 3 by small RNA sequencing. A previous study showed that miR-503-5p regulates VEGF protein expression in lung fibroblasts by targeting the VEGF transcript (17). Seemingly contradictory, TGF-β was previously shown to upregulate VEGF gene expression in MRC-5 fibroblasts (18). An explanation may be that miR-503 induction by TGF-β provides a negative feedback mechanism to control VEGF production in lung fibroblasts.

Figure 1. Overlap of TGF-β-regulated miRNAs in lung fibroblasts between chapter 2 and 3. The TGF-β-regulated miRNAs in lung fibroblasts were identified using microarray (chapter 2) and small RNA sequencing (chapter 3). A) Overlap of upregulated miRNAs upon TGF-β stimulation. B) Overlap of downregulated miRNAs upon TGF-β stimulation.

Differential regulation of miRNA expression by TGF-β in COPD compared to control lung fibroblasts

In chapter 3, we have also identified TGF-β-regulated miRNAs (33 up- and 13 downregulated) in COPD lung fibroblasts. The TGF-β effect in COPD lung fibroblasts was less pronounced than in control lung fibroblasts (46 versus 86 differentially expressed miRNAs, respectively). Based on this difference, we speculate that COPD lung fibroblasts are less or differently responsive to TGF-β stimulation, possibly due to continuous exposure to high TGF-β levels (19).

We next set out to identify miRNAs differentially regulated by TGF-β in COPD compared to control lung fibroblasts in chapter 3. We identified three miRNAs, miR-148b-3p, miR-589-5p and miR-376b-3p, which responded significantly different to TGF-β in...
COPD compared to control lung fibroblasts. Of these miRNAs, only miR-148b-3p was expressed at reasonable levels (>1,000 standardized reads) and therefore selected for further validation. MiR-148b-3p expression levels were decreased in control lung fibroblasts upon TGF-β stimulation, whereas no change was observed in COPD lung fibroblasts. This is in line with the speculation that COPD lung fibroblasts may be less responsive to TGF-β compared to control lung fibroblasts.

The most IP-enriched predicted target gene of miR-148b-3p was HMGA2. MiR-148b-3p may indirectly influence α-SMA expression via targeting this gene. HMGA2 is required for transcription of GATA6, which mediates TGF-β-induced upregulation of α-SMA expression (20, 21). Other targets of miR-148b-3p that were enriched in the miRNA-targetome of control lung fibroblasts were mainly involved in the regulation of gene transcription. Further functional analysis of miR-148b-3p target genes should focus on possible associations with aberrant tissue repair and remodelling in COPD. It would for instance be interesting to directly investigate whether miR-148b-3p can influence the ECM production by overexpressing miR-148b-3p in lung fibroblasts. In addition, it is of interest to see how and whether this miRNA influences the activity of lung fibroblasts by measuring its effects in a collagen gel contraction assays. Lung fibroblasts contracting three-dimensional collagen gels is a commonly used model of tissue repair and remodelling (22). As miR-148b-3p regulates several genes related to regulation of gene transcription, further experiments should also focus on the identification of genes and pathways that are regulated by miR-148b-3p target genes.

To explain the less pronounced TGF-β effect in COPD lung fibroblasts, it is of interest to check basal levels of TGF-β and the TGF-β receptors type I and type II in lung fibroblasts from COPD patients and non-COPD control subjects. In addition, it might be of interest to study the expression of factors required for the processing of the latent form of TGF-β into its active form, e.g. proteolysis and reactive oxygen species (9).

A previous study showed that MMPs, including MMP-9, can activate TGF-β (23). Although it has been shown that MMP-9 is increased in emphysema (24), it is yet to be investigated whether MMP-9 expression and activity is different in COPD compared to control lung fibroblasts and its role in TGF-β activation. MiRNAs may also be involved in activating TGF-β by targeting factors that are involved in this process. MMP-9 for example is a direct target of let-7e (25). In addition, reactive oxygen species can also activate TGF-β; a previous study showed that superoxide dismutase and catalase reduced the TGF-β activation (26).

It would also be interesting to investigate which other TGF-β regulating mechanisms/factors are different in COPD compared to control fibroblasts. One of the important mechanisms is the regulation by binding to and release of TGF-β by decorin, an important proteoglycan in the lung, also involved in collagen fiber stability (27, 28). In our
previous studies we found reduced peribronchial decorin in COPD in lung tissue (29) and also less decorin production by COPD lung fibroblasts (30, 31).

**Differential miRNA expression in COPD lung fibroblasts**

Different from the marked effect of TGF-β on miRNA expression, we found only one miRNA differentially expressed between COPD and control lung fibroblasts: higher miR-660-5p levels in COPD (chapter 3). One of the reasons for not finding more differentially expressed miRNAs could be lack of power due to limited group sizes and high donor variability in primary cells. We may therefore need to expand the number of donors to study differentially expressed miRNAs in COPD. Another reason could be that differences are lost upon cell isolation and cell culture, which will be further discussed below.

An interesting consequence of the increased miR-660-5p levels might be that this miRNA indirectly increases p53 protein levels, by targeting MDM2 (32, 33). Increased p53 levels have been associated with emphysema (34, 35). The predicted targets of miR-660-5p that were enriched in the miRNA-targetome of lung fibroblasts were mainly involved in the regulation of gene transcription. Similar further studies as described above for miR-148b-3p, should clarify whether and how this miRNA is involved in aberrant tissue repair and remodelling in COPD.

**Downregulation of miR-335-5p in lung fibroblasts from current smokers**

Apart from the effects of TGF-β on miRNA expression in fibroblasts, exposure to cigarette smoking may also have impact on miRNA expression. In chapter 4 we studied miRNA expression in control lung fibroblasts from current and ex-smokers. We found a lower miR-335-5p expression in control lung fibroblasts from current smokers compared to ex-smokers. This finding was replicated in lung tissue. We also found a lower miR-335-5p expression in bronchial biopsies from current smokers compared to never-smokers. It is remarkable that we do find differences in *ex-vivo* cultured fibroblasts, in which smoke exposure is not continued. One of the explanations of finding a difference in miRNA expression in control lung fibroblasts between current and ex-smokers could be smoking-induced epigenetic modifications that remain even after stopping exposure to smoke. A previous study showed that tobacco-smoking can change the methylation pattern (36) and that these changes in DNA methylation may be reversible or permanent (36, 37). As miR-335-5p expression was shown to be associated with aberrant hypermethylation in hepatocellular carcinoma (38), we investigated DNA methylation in the enhancer region of the miR-335 host gene in our cigarette smoke extract-treated control lung fibroblasts and in the replication set of lung tissues. In both cases, no differences in methylation of the tested enhancer were observed. Nevertheless, we cannot rule out that aberrant methylation has
occurred at other loci in the promoter region and impacted on miR-335-5p expression. Treatment of control lung fibroblasts from current smokers with 5-aza-2’-deoxycytidine revealed increased miR-335-5p levels (Figure 2). However, DNA hypomethylation by 5-aza-2’-deoxycytidine is not specific for the miR-335-5p locus and might be an indirect effect of demethylation at other genomic loci. Thus, it is possible that there is an indirect (trans-)effect on miR-335-5p expression. The mechanism of regulation of miRNA expression by DNA methylation is supported by the presence of CpG rich sequences in the promoter regions in almost half of all miRNA genes (39, 40). Therefore, it would be interesting for future studies aiming to identify the effect of current smoking on miRNA expression and to analyze aberrant methylation. In addition, it would be worthwhile to correlate miRNA expression changes with aberrant methylation patterns.

![Figure 2. 5-aza-2’-deoxycytidine increased miR-335-5p expression in control lung fibroblasts from current smokers](image)

In chapter 4, we only focused on DNA methylation. However, other epigenetic factors, including histone deacetylase (HDAC) and polycomb group proteins (PcG), have been reported to regulate miRNA expression as well (40, 41). Moreover, DNA methylation and histone modifications often cooperate to regulate miRNA expression (41). Thus, it would be of interest to study other epigenetic mechanisms, in addition to DNA methylation, in regulating miR-335-5p expression in lung fibroblasts from current and ex-smokers.

Of note, information about the time since smoking cessation of ex-smoking donors in our study is not complete. Of the nine ex-smoking donors of lung fibroblasts, the time since smoking cessation of four donors is known (range: 0.5-20 years). This information is crucial as previously published epigenome-wide association studies in blood samples identified CpG sites whose methylation level reverts to the same level as never-smokers after smoking cessation (42). This reversibility of the methylation patterns is depended on the dose, the duration of smoking and of smoking cessation (42-44). It is plausible that the time since smoking cessation also influences the methylation pattern in lung fibroblasts, which subsequently may affect the expression of certain genes and miRNAs. The large
range of time since smoking cessation (minimally 20 years) may lead to a dispersion of the expression of certain miRNAs in lung fibroblasts from ex-smokers, which could also be the case for miR-335-5p. Moreover, the number of years after smoking cessation may also be a reason for the fact that we only observed a limited smoking status-related effect in our small RNA sequencing data. In addition to current smoking donors, it will be worthwhile to include never-smokers and multiple groups of ex-smoking donors of control lung fibroblasts based on years of smoking cessation and assess the correlation between miRNA expression profiles and DNA methylation patterns in these lung fibroblasts.

We identified three miR-335-5p target genes (i.e. Rb1, CARF and SGK3) in our miRNA-targetome that were previously proven as direct targets using luciferase reporter assays. In bronchial biopsies, we observed a lower miR-335-5p and a slightly higher SGK3 expression in current smokers compared to never-smokers. Additional studies are required to assess the function of miR-335-5p in lung fibroblasts through targeting these three and potentially other target genes.

**Studying COPD in primary parenchymal lung fibroblasts**

*Chapters 2, 3 and 4* focused on miRNA expression changes in relatively large groups of well-characterized primary parenchymal lung fibroblasts from control subjects and COPD patients. The control subjects included in our studies were patients with a normal lung function undergoing lung surgery for a lung tumour. Left-over lung tissues located far away from the tumour were used for fibroblast isolation. Whether these lung tissues indeed represent normal tissue is to be questioned, but currently this is the best source of control lung fibroblasts that we have available. A recent study showed that the transcriptomic profile of histologically normal tissue located >2 cm away from the tumour was different from non-tumour-bearing tissue (45). However, these non-tumour-bearing tissues were derived from autopsies in subjects who may have passed away due to other diseases than cancer. Alternative options are left-over cells from lungs that are rejected for lung transplant or cells derived from post-mortem analysis. In both cases limited information is present regarding lung function and smoking history and it can be doubted whether these would be more “normal”. Another alternative is to use transbronchial biopsies that also contain parenchymal lung tissue to isolate lung fibroblasts (46), although the yield would be low and this method may lead to ethical discussions.

In the studies presented in this thesis, we used parenchymal lung fibroblasts. Previous studies showed that the phenotype and function of parenchymal lung fibroblasts are different from airway fibroblasts (31, 47, 48). Therefore, our results only apply for the lung parenchyma. Given that COPD is characterized by a lack of repair in the lung parenchyma and excessive ECM deposition in the airway walls (27), it would be of interest to compare
miRNA expression profiles in parenchymal lung fibroblasts with those of airway fibroblasts from control subjects and COPD patients as well.

The lung fibroblasts used in this thesis were isolated, cultured, stored in liquid nitrogen until further use (31), replenished and cultured until passage 5. It is possible that during this whole culturing process, expression levels of miRNAs might have changed and differences in expression levels might have disappeared. A previous study showed that culture conditions (i.e. cell density and passage) affect miRNA expression in primary lung fibroblasts (49). In our studies, lung fibroblasts were cultured till passage 5 to obtain enough number of cells for our experiments. MiRNA expression profiles in lung fibroblasts measured directly after isolation may be more representative of the actual expression profile. However, larger pieces of lung tissue will be needed to isolate enough cells and over time differences in cell culture conditions may influence the results.

In our studies, the lung fibroblasts were cultured as monolayer. There are three-dimensional (3D) culturing models, including decellularized lung scaffolds, available that may give complementary information as these better reflect the functional microenvironment in vivo (50-54). It will thus be worthwhile to investigate the miRNA expression changes and the role of differentially expressed miRNAs in lung fibroblasts in 3D culturing models.

Using Ago2-RIP-Chip to identify miRNA target genes relevant in lung fibroblasts

For each miRNA of interest, we used Ago2-IP data and predicted targets in TargetScan (55) to identify their target genes relevant in lung fibroblasts. For the Ago2-IP, we used TGF-β stimulated and unstimulated control lung fibroblasts from two control subjects. Initially, we planned to compare the miRNA-targetomes between all lung fibroblast groups (i.e. TGF-β vs no TGF-β, COPD vs controls, current vs ex-smokers). We started with Ago2-IP in lung fibroblasts from two control subjects and two COPD patients with and without TGF-β stimulation. In this experiment, we observed differences in the efficiency of the Ago2 enrichment between the lung fibroblasts, in particular between the two COPD patients where COPD patient 1 showed an overall lower enrichment compared to COPD patient 2 (Figure 3). With this experiment we learned that due to the heterogeneity between primary fibroblasts from different donors, the resulting miRNA-targetomes of COPD and control donors might lack consistency due to a main effect of donor heterogeneity and Ago2-IP efficiency. Therefore, we decided not to compare Ago2-IP on multiple COPD and control donors, but to use the Ago2-IP data from the two control subjects to define the top-1,500 most enriched probes as the miRNA-targetome of parenchymal lung fibroblasts.

Our Ago2-IP approach of identifying miRNA target genes is not specific for the miRNAs of interest. By overexpressing the miRNA of interest in lung fibroblasts and then...
performing Ago2-IP (56), we could experimentally identify the target genes of a specific miRNA without partly relying on target gene prediction program. Other techniques that can be considered for target gene identification are high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) and crosslinking, ligation, and sequencing of hybrids (CLASH) (57, 58). HITS-CLIP provides more detailed information on the miRNA binding site region and thus a more accurate prediction of the most likely miRNA that was bound to the target gene. By direct ligation of the miRNA binding site to the miRNA, CLASH provides exact information on which miRNA binds to which region of a target gene (58).

Figure 3. IP/T ratio of the top-1,500 probes in COPD and control lung fibroblasts. Lung fibroblasts from two controls and two COPD patients +/- TGF-β stimulation were used for the Ago2-IP. IP = immunoprecipitated fraction, T = total fraction. The median and interquartile range is shown (red).

Potential use of miRNAs in the clinic

Currently, there is no cure for COPD; available treatments only help to slow down progression of the disease and relieve the symptoms. MiRNAs are involved in the vast majority of signalling pathways and cellular processes and several miRNAs were shown to be involved in COPD (59), including miR-660-5p identified in chapter 3. Therefore, miRNAs may serve as novel biomarkers or therapeutic targets for COPD. MiRNAs identified in serum (miR-7, miR-20a, miR-28-3p, miR-34c-5p and miR-100), sputum (let-7c and miR-125b), and in exhaled breath condensates (let-7a, miR-21 and miR-328) were suggested to be potential biomarkers for COPD (60).

Possible miRNA-based pharmacotherapy for miRNAs overexpressed in COPD may include anti-miRNAs, locked-nucleic acids (LNA) and antagomirs (61). A well-known and successful miRNA-based therapeutic strategy used in clinical trials is the locked-
nucleic acid modified miR-122 inhibitor for treating hepatitis C virus infection (62, 63). For miRNAs downregulated in COPD, miRNA mimics may be a putative therapeutic strategy (61). However, whether miRNA-based therapeutic strategies can be used for treating COPD in the future has yet to be determined. One factor complicating the selection of miRNA candidates is the poor overlap between differentially expressed miRNAs between studies (59, 64). So far, miR-660-5p has not been reported as differentially expressed in COPD other than in lung fibroblasts as discussed in chapter 3. Another limiting factor is that the function of candidate miRNAs and the functional consequences of aberrant miRNA expression in COPD are frequently not well characterized. This might lead to undesired side effects in lung fibroblasts or other cell types. Delivering the miRNA-based therapeutics to the correct cell type in the lung, for example to parenchymal lung fibroblasts, without being degraded or excreted before reaching their target cells, is also a big challenge (65). Moreover, as a miRNA can target multiple target genes, the use of miRNA-based therapeutics may induce many side effects. Studies investigating the functions of the miRNA candidates using in vitro as well as in vivo models are therefore crucial. Altogether, there is still a long road to go before reaching the stage of using miRNA-based therapeutics in the clinic for COPD.

**Effect of age on gene and miRNA expression changes and their interactions**

In chapter 5, we identified age-related genes and miRNAs in bronchial biopsies from healthy individuals. Genes with higher expression levels with age were shown to be involved in synapse-related processes. It would be of interest to investigate how the nervous system of the respiratory tract changes during ageing, as that is still poorly understood (66). Genes with lower expression levels with age were mainly involved in three ageing hallmarks, i.e. genomic instability, cellular senescence and altered intercellular communication. Of the identified age-related miRNAs, miR-146a-5p, miR-146b-5p and miR-142-5p showed lower expression levels with age. Moreover, predicted target genes of these miRNAs were enriched amongst genes with higher expression levels with age. Our further analyses showed that RIMS2, a gene expressed at higher levels with age, was negatively correlated with miR-146a-5p and miR-146b-5p.

Our study was a cross-sectional study. When investigating ageing-related topics, such as age-related gene and miRNA expression changes, it would be worthwhile to perform a longitudinal study to obtain a more accurate picture of the age effect. An obvious downside of performing a longitudinal study for this goal and a disease like COPD is that the study probably would take up to decades to finish. In addition, the participants included in the study would need to undergo bronchoscopy multiple times. Considering such limitations, our study provides a good starting point for hypothesis generation which should be
confirmed in further studies. One of the strengths of our study is that we assessed the effect of age in respiratory healthy individuals. Thus, no other lung-related diseases interfered with the observed age-related gene and miRNA expression profiles.

CONCLUSIONS

In conclusion, in this thesis we identified miRNAs in primary parenchymal lung fibroblasts of which the expression levels were affected by TGF-β stimulation, presence of COPD and smoking status. New light has been shed on the possible role of these miRNAs in the function of lung fibroblasts, which play an important role in lung tissue homeostasis and tissue repair. Furthermore, we identified miRNA expression changes related to ageing in bronchial biopsies from healthy individuals. Linking of these data to gene expression changes associated with age was used to identify potential functionally relevant interaction. An overview of the most important miRNAs identified in our studies is shown in Figure 4.

We found a strong TGF-β effect on miRNA expression in primary parenchymal lung fibroblasts from control subjects and COPD patients. There was a remarkably more pronounced TGF-β effect in the lung fibroblasts from control subjects compared to COPD patients, which may suggest that the TGF-β response is different in COPD lung fibroblasts. Next, we identified miR-148b-3p, miR-589-5p and miR-376b-3p to be differentially regulated by TGF-β in COPD compared to control lung fibroblasts. Furthermore, we identified miR-660-5p to be upregulated in COPD compared to control lung fibroblasts. In addition to TGF-β- and COPD-related miRNA expression changes, smoking status of subjects also affected expression levels of miRNAs. We identified miR-335-5p to be expressed at lower levels in current smokers compared to ex-smokers. Our studies suggest that the identified differentially expressed miRNAs may affect the function of lung fibroblasts, including their function in tissue repair and remodelling.

In the airways of healthy individuals, we identified genes and miRNAs that were related to ageing. Genes with lower expression with ageing were involved in hallmarks of ageing. Genes with higher expression with ageing were involved in synapse-related processes, of which RIMS2 may be targeted by miR-146a-5p and miR-146b-5p, two miRNAs with lower expression with age.

Altogether, these studies provide a good stepping stone for further studies aiming to clarify the complex role of these miRNAs in relation to abnormal tissue repair in COPD and ageing.
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Summary, discussion and future perspectives

Figure 4. Overview of miRNA expression changes induced by different for COPD relevant factors identified in this thesis.
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