Sputum exosomes: promising biomarkers for idiopathic pulmonary fibrosis

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing interstitial lung disease of unknown aetiology which leads rapidly to death. As diagnosis of IPF is complex, we aimed to characterise microRNA (miRNA) content of exosomes from sputum of patients with IPF. Using miRNA quantitative PCR array, we found a substantial dysregulation of sputum exosomal miRNA levels between patients with IPF and healthy subjects and identified a unique signature of three miRNAs. Interestingly, we found a negative correlation between miR-142-3p and diffusing capacity of the lungs for carbon monoxide/alveolar volume. This is the first characterisation of miRNA content of sputum-derived exosomes in IPF that identified promising biomarkers for diagnosis and disease severity.

BACKGROUND

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrosing interstitial lung disease of unknown aetiology which leads rapidly to death. As diagnosis of IPF is complex, we aimed to characterise microRNA (miRNA) content of exosomes from sputum of patients with IPF. Using miRNA quantitative PCR array, we found a substantial dysregulation of sputum exosomal miRNA levels between patients with IPF and healthy subjects and identified a unique signature of three miRNAs. Interestingly, we found a negative correlation between miR-142-3p and diffusing capacity of the lungs for carbon monoxide/alveolar volume. This is the first characterisation of miRNA content of sputum-derived exosomes in IPF that identified promising biomarkers for diagnosis and disease severity.
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Figure 2 Diagnostic value of sputum-derived exosomal miR-142-3p, miR-33a-5p and let-7d-5p for idiopathic pulmonary fibrosis (IPF) disease. (A) Quantitative real-time PCR analysis of differentially expressed microRNAs (miRNAs) in sputum-derived exosomes from patients with IPF (n=16) compared with healthy subjects (HS; n=14). Cohort 1 was used to identify exosomal miRNAs dysregulated in the sputum of patients with IPF; cohort 2 is the validation cohort. (B) Receiver operator characteristic (ROC) curves with (C) corresponding area under the curves (AUCs) for comparing the ability of miR-142-3p, miR-33a-5p and let-7d-5p to discriminate patients with IPF versus HS. ROC curves were constructed by combining the two cohorts (cohort 1 and cohort 2). Five logistic regression models were generated: (1) model 1: age and sex; (2) model 2: model 1+miR-33a-5p; (3) model 3: model 1+let-7d-5p; (4) model 4: model 1+miR-142-3p and (5) model 5: model 1+miR-33a-5p+let-7d-5p+miR-142-3p.
Correlation analysis of lung function and sputum-derived exosomal microRNAs (miRNAs) in patients with idiopathic pulmonary fibrosis (IPF).

Correlation between diffusing capacity of the lungs for carbon monoxide (DLCO)/alveolar volume (VA) (%pred) and miRNA levels in patients with IPF.

Their predicted targets revealed that these regulators play key roles in several pathophysiological processes involved in the initiation and progression of IPF disease, such as deposition of extracellular matrix, collagen secretion and epithelial to mesenchymal transition (online Supplementary figure S1).

We further validated three promising biomarker candidates (miR-142-3p, miR-33a-5p, let-7d-5p) by quantitative real-time-PCR in an independent cohort (cohort 2) consisting of 10 patients with IPF and eight HS (online Supplementary figure S2). By combining the two cohorts, we observed significantly increased levels of miR-142-3p and miR-33a-5p (9.4 and 3.13 fold, respectively) and decreased levels of let-7d-5p (0.49-fold) in sputum-derived exosomes from patients with IPF (n=16) compared with HS (n=14) (figure 2A). The results were not altered after adjusting for age or gender distribution (online Supplementary tables S3 and S4).

To investigate their diagnostic value, we constructed receiver operator characteristic (ROC) curves using logistic regression models with adjustment for age and sex for each altered miRNAs (figure 2B). The area under the ROC curve (AUC) of the model 1, comprising age and sex, was 0.864 (95% CI 0.735 to 0.993, p<0.001) (figure 2C). The addition of miR-33a-5p, let-7d-5p or miR-142-3p to the model increased the AUC to 0.933 (95% CI 0.848 to 1.000, p<0.0001), 0.942 (95% CI 0.865 to 1.000, p<0.0001) and 0.964 (95% CI 0.893 to 1.000, p<0.0001), respectively (figure 2C). When combining the three miRNAs, the AUC reaches 0.978 (95% CI 0.930 to 1.000, p<0.0001) suggesting that this miRNA signature may be useful for IPF detection and diagnosis.

Then, the correlation between the expression level of sputum exosomal miRNAs and lung function was studied in the group of patients with IPF. Correlation studies revealed a negative correlation between diffusing capacity of the lungs for carbon monoxide/alveolar volume (DLCO/VA) and miR-142-3p (r=−0.68, p=0.010) and a positive one with let-7d-5p (r=0.55, p=0.048) (figure 3). All these observations suggest that sputum exosomal miRNAs are associated with the severity of lung disease in the particular case of patients with IPF.

DISCUSSION

We identified for the first time miRNAs modifications from sputum-derived exosomes in patients with IPF as potential biomarkers correlated with disease severity. We propose a novel signature composed of three miRNAs (miR-142-3p, miR-33a-5p, let-7d-5p) to discriminate with a high specificity and sensibility patients with IPF of HS.

One of the main finding of our research is the negative correlation observed for miR-142-3p with lung function assessed by DLCO/VA, a marker of alveolo-capillar function, whereas let-7d-5p goes in the opposite direction. This observation suggests that those miRNAs are not only promising biomarkers but also putative players in the disease.

Interestingly, miR-142-3p was shown to contribute to the proper proliferation of mesenchymal progenitors by controlling WNT signalling. Elevated levels of miR-142-3p might thus lead to an altered balance between mesenchymal cell proliferation and differentiation, a feature observed during IPF progression. Furthermore, let-7d-5p downregulation has been widely reported as primordial miRNA involved IPF pathophysiology, by contributing to epithelial mesenchymal transition in lung epithelial cells. Of note, miR-33a-5p has been recently implicated in liver fibrosis.

This observation deserves further investigations on the role of this miRNA in lung fibrosis.

Our study identified miRNAs previously identified in plasma as well as new miRNAs with potential functional role in IPF progression. Our findings may thus lead to a better understanding about the roles of identified sputum exosomal miRNAs.
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in IPF pathogenesis and thus open new avenues for therapeutic approaches. Unfortunately, the cross-sectional design of our study does not allow the identification of predictive and/or prospective biomarkers. Further longitudinal studies will be performed to validate the potential of sputum exosomal miRNAs as biomarkers in IPF to predict disease severity and evolution.

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Contributors MSN and JG designed and performed experiments, analysed the data and wrote the manuscript. MAH collected and processed sputum samples. ON performed experiments. FD, JLC and REL contributed to experiments analysis and interpretation of the results. IS design, analysed, supervised the experiment and wrote the manuscript.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval The protocol was approved by the ethics committee of CHU of Liège (Belgian number: B707201422832; ref: 2014/302).

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