ELSEVIER
Journal of Cystic Fibrosis xx (2017) xxx – xxx
Journal of Cystic Fibrosis
www.elsevier.com/locate/jcf

The expression of *Mirc1/Mir17–92* cluster in sputum samples correlates with pulmonary exacerbations in cystic fibrosis patients

Kathrin Krause [a,g,i,1], Benjamin T. Kopp [b,f,i,1], Mia F. Tazi [a,i], Kyle Caution [a,g,i], Kaitlin Hamilton [a,g,i], Asmaa Badr [a,g,i], Chandra Shrestha [b,f,i], Dmitry Tumin [d,f,i], Don Hayes Jr. [b,f,i], Frank Robledo [b,f,i], Luanne Hall-Stoodley [a,i], Brett G. Klamer [e,i], Xiaoli Zhang [e,i], Santiago Partida-Sanchez [b,f,i], Narasimham L. Parinandi [g,i], Stephen E. Kirkby [b,f,i], Duaa Dakhlallah [i], Karen S. McCoy [b,f,i], Estelle Cormet-Boyaka [c,g], Amal O. Amer [a,g,i,*,1]

[a] *Department of Microbial Infection and Immunity, Columbus, OH, USA*
[b] *Department of Pediatrics, Columbus, OH, USA*
[c] *Department of Veterinary Biosciences, Columbus, OH, USA*
[d] *Department of Anesthesiology & Pain Medicine, Columbus, OH, USA*
[e] *Center for Biostatistics, Columbus, OH, USA*
[f] *Nationwide Children's Hospital, Columbus, OH, USA*
[g] *Dorothy M. Davis Heart and Lung Research Institute, Columbus, OH, USA*
[h] *Microbiology, Immunology and Cell Biology Department, West Virginia University, Morgantown, WV, USA*
[i] *The Ohio State University College of Medicine, Columbus, OH, USA*

Received 1 August 2017; revised 27 October 2017; accepted 16 November 2017

Abstract

Introduction: Cystic fibrosis (CF) is a multi-organ disorder characterized by chronic sino-pulmonary infections and inflammation. Many patients with CF suffer from repeated pulmonary exacerbations that are predictors of worsened long-term morbidity and mortality. There are no reliable markers that associate with the onset or progression of an exacerbation or pulmonary deterioration. Previously, we found that the *Mirc1/Mir17–92*a cluster which is comprised of 6 microRNAs (Mirs) is highly expressed in CF mice and negatively regulates autophagy which in turn improves CF transmembrane conductance regulator (CFTR) function. Therefore, here we sought to examine the expression of individual Mirs within the *Mirc1/Mir17–92* cluster in human cells and biological fluids and determine their role as biomarkers of pulmonary exacerbations and response to treatment.
Methods: Mirc1/Mir17–92 cluster expression was measured in human CF and non-CF plasma, blood-derived neutrophils, and sputum samples. Values were correlated with pulmonary function, exacerbations and use of CFTR modulators.
Results: Mirc1/Mir17–92 cluster expression was not significantly elevated in CF neutrophils nor plasma when compared to the non-CF cohort. Cluster expression in CF sputum was significantly higher than its expression in plasma. Elevated CF sputum *Mirc1/Mir17–92* cluster expression positively correlated with pulmonary exacerbations and negatively correlated with lung function. Patients with CF undergoing treatment with the CFTR modulator Ivacaftor/Lumacaftor did not demonstrate significant change in the expression *Mirc1/Mir17–92* cluster after six months of treatment.
Conclusions: Mirc1/Mir17–92 cluster expression is a promising biomarker of respiratory status in patients with CF including pulmonary exacerbation. Published by Elsevier B.V. on behalf of European Cystic Fibrosis Society.

Keywords: Cystic fibrosis; MicroRNA; Mir17–92a; Biomarker; Correlation; Pulmonary exacerbation

* Corresponding author at: Department of Microbial Infection and Immunity, Center for Microbial Interface Biology and the Department of Internal Medicine, The Ohio State University, Biomedical Research Tower, 460 W 12th Ave, Room 706, Columbus, OH 43210, USA.
E-mail address: amal.amer@osumc.edu (A.O. Amer).
[1] Equal contribution.

https://doi.org/10.1016/j.jcf.2017.11.005
1569-1993Published by Elsevier B.V. on behalf of European Cystic Fibrosis Society.

1. Introduction

Cystic fibrosis (CF) is a multi-organ disease caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel. Patients with CF characteristically suffer from repeated episodes of acute worsening in respiratory symptoms and decline in lung function termed a pulmonary exacerbation (PEx) [1]. Many definitions of PEx have been proposed [2], but regardless of the designation used, these clinical events are associated with progressive long-term deterioration of lung function and heightened morbidity and mortality [3,4]. While a number of biomarkers based on inflammatory markers in CF have been suggested, relevant biomarkers associated with exacerbation or decline in lung function have not been developed for clinical use. Current efforts are attempting to predict and identify PEx earlier to be able to more promptly provide treatment with antimicrobials and anti-inflammatory agents [5]. Yet, there are no reliable diagnostic or prognostic risk-related markers associated with PEx. Therefore, identifying biomarkers that can reflect the existence of an exacerbation at an early stage would provide an invaluable opportunity for early detect PEx, to avoid further physiologic and immunologic injury, and prevent the need for costly medical care, such as hospitalization.

Biomarkers are anatomical, physiological, biochemical or imaging features that can be used for diagnosis, monitoring disease progression, or response to treatment. An ideal biomarker should be stable and easy to measure, cost efficient and consistent across gender and ethnic groups. To date, none of the available biomarkers in CF satisfy all of these criteria. The major limitations of markers are low specificity, sensitivity, and false positive results.

MicroRNAs (Mirs) are endogenous, evolutionarily conserved small non-coding RNA that have been shown to be effective tools to study the biology of diseases and to have great potential as novel diagnostic and prognostic biomarkers with high specificity and sensitivity [6]. Circulating Mirs are short non-coding RNAs involved in biological and pathological processes of every cell type. Mirs have many necessary features of ideal biomarkers. Mirs are stable in various biological fluids, such as plasma, serum, saliva, milk, cerebrospinal fluid. Particularly, the expression of serum Mirs is firmly linked to various diseases. Mirs are considered potential biomarkers for several chronic disorders due to their stability in the circulation, and are both disease- and tissue-specific, which makes them attractive circulatory biomarkers [7,8]. In addition, the quantity of Mirs can be easily estimated by various methods, such as qRT-PCR, microarray, hybridization and deep-sequencing. qRT-PCR (quantitative real time polymerase chain reaction) is the most common, reliable and available, inexpensive method used for quantifying the small amount of miRNAs with the highest sensitivity and specificity [9,10].

Mirs act primarily through degradation of target mRNA with subsequent decrease or loss of expression of encoded proteins. The *Mir17–92* family maps to human chromosome 13 and encodes for the *Mirc1/Mir17–92* cluster (*Mir17*, *Mir18a*, *Mir19a*, *Mir20a*, *Mir19b-1*, *Mir92a*) and two paralogs (*Mir106a*, *Mir106b*) [11]. We have recently found that the expression of *Mir17* and *Mir20a* within the *Mirc1/Mir17–92* cluster is elevated in CF macrophages from F508del mice and humans. These two Mirs target several essential autophagy proteins such as Atg5, Atg12, Atg7 and Atg16 [12,13]. We have confirmed that the upregulation of *Mir17* and *Mir20a* is associated with down-regulation of their predicted autophagy targets contributing to weak autophagy activity in murine macrophages. Restoring the level of *Mir17* and *Mir20a* to normal levels in vivo by intratracheal administration of specific antagomirs to live CF mice improves autophagy, controls infection, and reduces pneumonia [13]. Notably, in vitro, *Mir17* and *Mir20a* antagomirs ameliorate CFTR function via the activation of autophagy [13]. Our published data suggest that targeting *Mir17* and *Mir20a* improves several clinical symptoms in patients with CF. However, it is not clear if these *Mirs* are elevated in human samples and if their level correlates with clinical symptoms and change in response to treatment. Hence, in the current study, we determined that the *Mirc1/Mir17–92* cluster is expressed broadly in human cells including neutrophils and in biological fluids including plasma and sputum. In addition, the most striking finding is that Mir cluster expression in sputum positively correlated with clinical symptoms such as PEx.

2. Methods

2.1. Ethics statement

All human subjects were recruited as approved by the Institutional Review Board (IRB) of Nationwide Children's Hospital (IRB15-00611 and IRB12-00405). All subjects underwent written consent for the procedures including all adult subjects provided informed consent, and a parent or guardian of any child participant provided informed consent on their behalf along with written assent from children.

2.2. Subjects

Male and female children older than 12 years and adult patients with CF were recruited from the CF clinic in either a state of baseline health or during the onset of a PEx. The diagnosis of CF was confirmed by the presence of two CF causing mutations on genetic blood testing and/or an elevated sweat chloride test. Data including patient demographics, medications, hospitalizations for PEx, and relevant clinical factors were collected in a database upon recruitment. Age and gender-matched healthy controls were recruited through Clinical Research Services. The demographics of subjects enrolled in this study are listed in Table 1. The control population was older and had more females, but subjects undergoing Ivacaftor/Lumacaftor treatment were not matched to healthy controls, and tended to be younger skewing the overall cohort. The CF subjects had moderate lung disease as shown by forced expiratory volume in 1 s (FEV_1)% predicted based on American Thoracic Society criteria, [14] and the majority were pancreatic insufficient. The majority of the CF cohort had at least one copy of the F508del mutation.

Please cite this article as: Krause K, et al, The expression of *Mirc1/Mir17–92* cluster in sputum samples correlates with pulmonary exacerbations in cystic fibrosis patients..., J Cyst Fibros (2017), https://doi.org/10.1016/j.jcf.2017.11.005