Role of microRNAs and exosomes in asthma
van den Berge, Maarten; Tasena, Hataitip

Published in:
Current Opinion in Pulmonary Medicine

DOI:
10.1097/MCP.0000000000000532

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 16-09-2023
Role of microRNAs and exosomes in asthma

Maarten van den Berge and Hataitip Tasena

INTRODUCTION

Asthma is a commonly occurring inflammatory airways disease characterized by variable airflow obstruction in association with symptoms of wheeze and dyspnea. The asthmatic inflammatory process is characterized by a complex interplay of resident cells (i.e., epithelial and dendritic cells, fibroblasts, nerves, endothelial cells) and inflammatory cells (eosinophils, mast cells, neutrophils, macrophages, T-lymphocytes).

At present, physicians have limited choice in anti-inflammatory and bronchodilator treatments for asthma and there is no cure available for the disease. Better insight into the underlying mechanisms that drive asthma is needed as a first step toward discovering new druggable targets. MicroRNAs (miRNAs) are recognized to play an important role in asthma. MiRNAs are small noncoding RNA transcripts (18–25 nucleotides) that are highly conserved across species. They are involved in the post-transcriptional regulation of gene expression. Once formed, miRNAs bind to argonaute-2 (AGO2) proteins, which are part of the RNA-induced silencing complex (RISC). In the RISC complex, miRNAs induce cleavage of their target mRNAs or inhibit their translation. A single miRNA can target hundreds of genes and it is estimated that as many as 60% of mRNAs are controlled by miRNAs [1]. It is now known that numerous cellular responses are under miRNA control enabling miRNAs to regulate signaling pathways and inflammatory responses in tissues. Some miRNAs are secreted into exosomes, cell-derived nano-sized vesicles reported to be involved in various human diseases including asthma [2,3]. In the present review, the role of miRNAs and exosomes in the pathogenesis of asthma will be discussed.
KEY POINTS

- Obesity is associated with more severe disease and differential response to asthma therapies.
- The obese asthma phenotype is heterogeneous and there are overlapping inflammatory phenotypes in obese patients.
- Surrogate markers of inflammation may be unreliable in obese asthma, particularly blood eosinophils therefore must be used with caution and interpreted in the context of the clinical presentation of the patient.
- Novel inflammatory mechanisms that are unique to obese asthma that may result in specific endotypes have been discovered making the possibility of future targeted therapies more likely.
- Weight loss improves outcomes in obese asthma but only if more than 10% of the baseline weight loss is achieved.

MICRON RNAS AND ASTHMA

Several studies investigated miRNA expression levels both in vitro and in vivo in human and experimental models of asthma, a table with an overview can be found in reference [4]. Although many studies have been performed in vitro and in animal models of asthma, studies on the role of miRNAs in humans are scarce. Williams et al. [5] compared the expression of 227 miRNAs in bronchial biopsies between eight corticosteroid-naive patients with mild allergic asthma and eight healthy controls. No differences were found which may have been due to the fact that mild asthma patients were included or to the cellular heterogeneity within bronchial biopsies which may have masked differences in miRNA expression within specific cell types. Another possible explanation may simply be that the study was underpowered for an unbiased approach analyzing 227 miRNAs as only 16 individuals were included. Solberg et al. [6] compared the miRNA expression profile in isolated epithelial cells derived from bronchial brushings between 16 steroid-naive patients with asthma and 12 healthy controls using microarrays. They identified 22 differentially expressed miRNAs that could be validated with PCR and showed that members of the miRNA-34/449 family are downregulated in asthma. They validated their findings in air-liquid interface cultured epithelial cells demonstrating that exposure to IL-13 represses miRNA-34/449 levels. This effect persisted after treating the cells with corticosteroids. These findings are of interest as miRNA-449 has previously been found to regulate differentiation of airway ciliated cells promoting centriole multiplication and multiciliogenesis, in part by targeting NOTCH1 mRNA [7]. It has been shown in animal models that an increase in NOTCH contributes to the airway mucous metaplasia frequently observed in asthma [8]. Taken together, the IL13-induced reduction in miRNA-34/449 in the bronchial epithelium, as observed by Solberg et al. may contribute to the alterations in epithelial differentiation that are often seen in asthma.

Other miRNAs found to be downregulated in epithelial cells derived from steroid-naive patients with asthma were members of the let-7 family (let-7a-5p, let-7c, let-7f-5p, let-7g-5p, and let-7i-5p) [6]. Several studies have shown the let-7 family to be important in asthma. Polikepahad et al. demonstrated that IL13 is a direct target of let-7 using a luciferase reporter system and inhibition of let-7a significantly upregulated IL13 expression in T cells [9,10]. In ovalbumin (OVA)-challenged murine model, expression of several let-7 family members decreased in allergic inflammatory lungs compared with healthy lungs [11]. Intranasal delivery of a let-7 mimic improved airway hyperresponsiveness and mucus production and decreased inflammatory cell infiltration in this experimental model of asthma. These findings suggest that decreased levels of let-7 increases type 2 inflammation thus contributing to a more severe asthma. However, this could not be confirmed in a subsequent study by Polikepahad et al. [9] where an antagomir of let-7 did not worsen the asthmatic inflammatory process in their OVA-challenged murine model, but contrastingly reduced inflammatory cell counts in bronchoalveolar lavage fluid and decreased levels of IL4, IL5, and IL13. Possible explanations for the discrepant findings between the studies could be different dose regimens or route of administration of the antagomir used or the fact that the antagomir used by Polikepahad et al. only inhibited four members of the let-7 family. In a recent study by Kim et al. [12] exposure of human airway smooth muscle cells to β2-agonists increased let-7f by two to three-fold together with a ~90% decrease in β2-receptors, while inhibition of let-7f reduced this downregulation by 50%. These findings suggest that inhibition of let-7 may render airway smooth muscle more sensitive to bronchodilator effects of β2-agonists. In a study in Chronic Obstructive Pulmonary Disease (COPD), we recently found increased let-7 in bronchial biopsies to be associated with chronic mucus hypersecretion (CMH) [13]. Whether this is also the case in asthma is currently under investigation. One miRNA can target multiple miRNAs, validation of its target genes is challenging since many miRNA–mRNA interactions are still unknown [14]. Several algorithms have...
been proposed to predict which genes are targeted by a specific miRNA, but there is large variability across different algorithms and the false positive rates are high [15]. Importantly, we analyzed both miRNA and mRNA data available from the same bronchial biopsies derived from COPD patients [13], which allowed us to create miRNA–mRNA coexpression networks to directly evaluate the miRNA–mRNA interactions. This approach enabled us to identify the let-7 family with its CMH-associated targets including EN1, NKD1, PDGFB, COL4A1, and COL4A2 as key important regulators of CMH in COPD.

In a recent study, Martinez-Nunez et al. performed miRNA sequencing (miRNA-seq) in cultured bronchial epithelial cells derived from eight severe asthma patients and five healthy controls. To determine the genome-wide relationship between differentially expressed miRNAs and their mRNA targets, they performed subcellular fractionation and RNA-seq (frac-seq) in the same samples allowing detection of both cytoplasmic mRNA as well as polyribosome-bound mRNA transcripts [16]. A total of 21 miRNAs were differentially expressed between severe asthma and healthy controls. Importantly, these miRNAs were found to preferentially target polyribosome-bound rather than cytoplasmic mRNA transcripts, suggesting a higher impact on translating mRNAs. Amongst the most differentially expressed miRNAs in the study by Martinez-Nunez et al. was miRNA-19. This is in agreement with the findings of Haj-Salem et al. [17] who showed miRNA-19a to be specifically upregulated in cultured bronchial epithelial cells derived from patients with severe asthma compared with those with mild asthma and healthy controls. Follow-up functional studies revealed that upregulation of miRNA-19a leads to an increased epithelial cell proliferation rate by targeting TGFβ2 mRNA, thus possibly contributing to airway remodeling in severe asthma. In another study, miRNA-19, part of the 17–92 cluster (also including miRNA-17, miRNA-18, and miRNA-92), was found to play an important role in T cells regulating Th2-cell differentiation [18]. The miRNA was upregulated in CD3+ CD4+ T cells sorted from bronchoalveolar lavage fluid (BAL) fluid from asthma patients compared with healthy controls, independent of the use of steroids. In follow-up experiments, it was demonstrated that CD4 cells lacking the miRNA-17–92 cluster produced fewer Th2 cytokines like IL4 and IL13, whereas this cytokine defect could be completely restored after transfection with a miRNA-19a or miRNA-19b mimic, but not after transfection with a mimic for other members of the 17–92 cluster. In a recent study, miRNAs of the miRNA-17–92 cluster were also found to be critically important for normal type 2 innate lymphoid cell survival, another source of type 2 cytokines; and similar as in T cells, miRNA-19 was particularly involved in IL13 production [19].

Little is known about how miRNAs contribute to airway hyperresponsiveness and remodeling which are important features of asthma and COPD. Chiba et al. [20] reported that miR-133a inhibition in in-vitro cultured human bronchial smooth muscle cells leads to an upregulation of RhoA, a key protein regulating contractility of smooth muscles. Recently, we investigated how miRNAs contribute to airway remodeling, an important feature in asthma and COPD. To this end, we investigated how TGF-β influences miRNA expression in fibroblasts and how this affects their function [21]. After exposure to TGF-β, miRNA-21 and miRNA-455 expression increased in cultured human lung fibroblasts. We then used the AGO2 Immunoprecipitation-gene Chip approach to identify miRNA targets on a large scale. With this approach the AGO2 protein is directly immunoprecipitated. Subsequently, the AGO2-associated mRNA transcripts were analyzed by microarray, to investigate the miRNA targetome (the whole miRNA-regulated gene set) in fibroblasts either or not exposed to TGF-β. We found that the predicted miRNA-455 and miRNA-21 targets present in the miRNA targetome of unstimulated and TGF-β-stimulated human lung fibroblasts are significantly enriched for TGF-β associated signaling processes. These findings show that there exists a cross-talk between the TGF-β pathway and the miRNAs, that is miRNA-445 and miRNA-21. In animal models of asthma, miRNA-21 has been shown to be upregulated in the airway wall [22,23]. Using the OVA-model, Lu et al. found that miRNA-21-deficient mice had reduced levels of eosinophilic inflammation and IL4 in their lungs and BAL fluid [22,23]. A plausible implication of miRNA-455 was reported in a study by Garbacki et al. [24] who observed miR-455 upregulation in the lungs of allergen-exposed mice. Taken together, accumulating evidence points towards a potentially important role of several miRNAs in airway inflammation and remodeling in asthma, including miRNA-34/449, let-7, miRNA-19, miRNA-21, and miRNA-455, as summarized in Fig. 1.

**EXOSOMES**

Exosomes are small (10–150 nm) membrane-bound vesicles. They can be released by for example, inflammatory cells upon activation and are thought to play a crucial role in intercellular signaling and communication [25]. Genetic material, proteins, and lipid mediators can be packaged in exosomes.
Exosomes are found in a large variety of body fluids including blood, urine, BAL fluid, exhaled breath condensate, saliva and nasal lavage fluid. Exosomes also contain miRNAs and it has been shown that miRNAs packaged in exosomes can contribute to inflammation in many diseases including asthma. Sinha et al. [26] were able to demonstrate that exosomes are present in exhaled breath condensate from patients with asthma and healthy controls and contain the majority of the 634 miRNAs detectable in exhaled breath condensate. Levänen et al. [3] demonstrated the presence of miRNAs in exosomes isolated from BAL fluid from 10 asthmatics and 10 healthy controls. Despite the fact that patients with mild intermittent asthma were included, as many as 24 exosomal miRNAs were found to be differentially expressed, including let-7 which was downregulated in asthma. A total of 22 out of the 24 exosomal miRNAs in BAL fluid were previously also found to be altered in the airway epithelium of patients with asthma in the study by Solberg et al. [6] with the same direction of effect. Thus, exosomes can carry and transport miRNAs making intercellular communication possible and may play important roles in asthma.

**MICRORNA-BASED TREATMENT OF ASTHMA**

There is now increasing evidence that specific miRNAs play potentially important roles in asthma and
they can be either inhibited or overexpressed. Therefore, miRNA-based therapeutics are an attractive area of investigation.

Although there are several successful examples of treatments with miRNA inhibitors or mimics in animal models of asthma, safe and effective delivery to target tissues or cells in humans remains a challenge. The latter is important as miRNA targeting can affect multiple genes and therefore ‘off-target’ side-effects should be prevented as much as possible. A commonly used method to inhibit the function of a specific miRNA is to deliver oligonucleotides with a complementary sequence that bind and inactivate the miRNA. To prevent degradation of these oligonucleotides by RNases, chemical modifications can be used, including LNAs and addition of 2′-O-methyl groups [27]. Further, addition of cholesterol tags may help to facilitate their uptake into target cells [28]. An alternative to chemically modified antisense oligonucleotides may be the introduction of a so-called miRNA sponge-encoding transgene into the cell, the expressed transcript being an engineered RNA molecule with multiple complementary binding sites to the target miRNA. Conversely, overexpression of miRNAs can be achieved with synthetic miRNAs that mimic natural miRNAs. These miRNA mimics often require liposomes, lipoprotein-based carriers, or nanoparticles as vehicles for their delivery [29,30]. Currently, miRNA-based therapeutics are not (yet) in development for asthma, but there are successful examples that modulation of miRNAs can be treatment strategy. The best example so far is miravirsen, the world’s first miRNA therapeutic, which is a short LNA for miR-122 currently in phase II clinical trials for the treatment of hepatitis C virus infection [31]. Other examples of miRNA therapeutics in clinical trials include anti-miR-103/107, antimiR-155, miR-29 mimic, miR-16

FIGURE 2. MicroRNA–mRNA coexpression network showing the key regulatory microRNA and their correlated predicted target genes associated with chronic mucus hypersecretion in COPD. This analysis identified amongst other, the let-7 family with its chronic mucus hypersecretion-associated targets including EDN1, NKD1, PDGFB, COL4A1, and COL4A2 as key important regulators. Red diamonds represent microRNAs higher expressed with chronic mucus hypersecretion; blue diamonds represent microRNAs lower expressed with chronic mucus hypersecretion; green circles represent predicted target genes negatively correlated with that microRNA; black circles represent predicted target genes negatively correlated with that microRNA whose expression was also associated with chronic mucus hypersecretion. Line width correlates to degree of significance of the microRNA–mRNA correlation. COPD = Chronic Obstructive Pulmonary Disease Reproduced with permission of the ERS 2018 from [13].
mimic, and miR-34 mimic [29]. There are also several other miRNA therapeutics currently being investigated in preclinical models [29].

CONCLUSION

There is now increasing evidence that miRNAs play potentially important roles in asthma. Several miRNAs have already been identified including miRNA-34/449, let-7, miRNA-19, miRNA-21, and miRNA-455. A limitation of studies performed so far is that they were mostly done in *in vitro* and in animal models of asthma and evidence in humans remains limited. Adequately powered studies leading to a better insight in the role of miRNAs in relevant human cells and tissues are now needed. MiRNAs can play a central role in the regulation of inflammatory responses as one miRNA can target multiple genes. However, once a relevant miRNA has been identified, it is often difficult to predict which genes/pathways are actually regulated by that specific miRNA as target prediction software is far from perfect. Therefore, we have previously combined genome-wide miRNA analyses with transcriptome data from the same sample, in this case bronchial biopsies, in relation to CMH. Using this approach, we examined direct and indirect miRNA–mRNA associations responsible for CMH. In addition, we were able to perform a genome-wide miRNA–mRNA network analysis to identify the key miRNA regulators (Fig. 2). Another approach to identify miRNA target genes is to perform AGO2-Immunoprecipitation in cultured cells, that is, with and without overexpression or inhibition of a miRNA of interest. With this technique, all genes regulated by miRNAs are captured and can then further be investigated using microarray or RNA-sequencing to identify the targetome of the miRNA of interest. The central role of miRNAs in regulation of inflammatory processes makes them attractive targets for therapeutic intervention and there have been several successful examples of treatments with miRNA inhibitors or mimics in animal models of asthma. Although the safe and effective delivery to target tissues and cells in humans remains a challenge, improved technologies are emerging to facilitate the development of potential miRNA-based treatments in several indications including asthma.

Acknowledgements

None.

Financial support and sponsorship

None.

Asthma

Confl icts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Describes discovery of first microRNA-based treatment for a human disease.