

Distinct MicroRNA Expression in Human Airway Cells of Asthmatic Donors Identifies a Novel Asthma-Associated Gene

Melanie J. Jardim¹, Lisa Dailey¹, Robert Silbajoris¹, and David Diaz-Sanchez¹

¹National Health and Environmental Effects Research Laboratory, Environmental Public Health Division, United States Environmental Protection Agency, Chapel Hill, North Carolina

Airway inflammation is a hallmark of asthma, and suggests a dysregulation of homeostatic mechanisms. MicroRNAs (miRNAs) are key regulators of gene expression necessary for the proper function of cellular processes. We tested the hypothesis that differences between healthy and asthmatic subjects may be a result of distinct miRNA cellular profiles that lead to differential regulation of inflammatory genes. We collected human bronchial epithelial cells from seven healthy donors and seven patients with asthma, and profiled miRNA expression, using the Affymetrix (Santa Clara, CA) miRNA array platform. Results were confirmed according to quantitative RT-PCR on RNA isolated from 16 healthy and 16 asthmatic donors. We identified 66 miRNAs that were significantly different (≥ 1.5 -fold; $P \leq 0.05$) between the two groups, and validated three of them in epithelial cells from 16 asthmatic and 16 healthy subjects. Molecular network analysis indicated that putative targets were principally involved in regulating the expression of inflammatory pathway genes ($P \leq 10^{-4}$). Our analysis confirmed the prediction that the expression of IL-8, Cox2, and TNF- α was up-regulated in asthmatic cells, whereas the expression of IL-6 was lower compared with that in healthy control subjects. Network analysis was also used to identify a novel asthma-associated gene. The top-ranked predicted target of the highly down-regulated miRNA-203 in asthmatic cells was the aquaporin gene *AQP4*. Its expression was confirmed to be significantly higher in cells from patients with asthma. Overall, these data suggest that the heightened inflammatory pathway activation observed in patients with asthma may be attributed to underlying aberrant miRNA expression.

Keywords: asthma; inflammation; microRNA; epigenetic; gene expression

Asthma is a complex disorder characterized by recurring episodes of airway hyperresponsiveness, shortness of breath, coughing, and wheezing (1). Gene expression profiling and genome-wide association studies have attempted to identify signatures that characterize allergic asthma and implicate genes involved in its pathogenesis, but have yet to be successful (2). These studies have the potential to identify new therapeutic targets, but have

CLINICAL RELEVANCE

Our current understanding of how epigenetic mechanisms regulate asthma pathogenesis is quite limited. This study is the first to document differential microRNA (miRNA) expression profiles in bronchial epithelial cells between healthy donors and donors with asthma, suggesting that miRNAs may regulate underlying disease processes. Further insight into these mechanisms will enhance our understanding of disease phenotypes and ultimately lead to the development of novel therapies.

been hampered by the presence of different asthma subtypes and the large number of genes involved (2, 3). Pathologically, the central feature of asthma common to all clinical subtypes is the presence of underlying airway inflammation (4). We hypothesize that the difference between patients with asthma and healthy individuals involves alterations in the regulatory processes that control gene expression in patients with asthma. This disturbance may lead to a disruption of homeostasis and the potential for enhanced airway inflammation.

MicroRNAs (miRNAs) are single-stranded RNA species that constitute a class of noncoding RNAs emerging as key regulators of gene expression (5, 6). They are generally thought to inhibit protein synthesis by either the translational repression or degradation of mRNA targets (7–9). Over the last several years, miRNAs have been shown to regulate a wide spectrum of biological functions, including apoptosis, proliferation, and differentiation, suggesting that they play a pivotal role in protecting organ tissue from disease (10). Aberrant miRNA expression has been implicated in the pathogenesis of several human diseases, including malignancies, heart disease, congenital organ defects, and neurodegenerative diseases (6, 10). Recently, miRNAs were also shown also to mediate inflammatory disorders, such as eczema and psoriasis, in addition to autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (11–13).

Evidence is emerging that miRNAs are critical for lung development and for maintaining the lung in a disease-free state (11, 13). For example, loss-of-function of the miRNA-17–92 (miR-17–92) cluster leads to the development of hypoplastic lung tissue in murine models (14). Comparisons of normal lung tissue and tissue from different lung cancers reveal significant differences in miRNA profiles, and have shown that different miRNAs can act as both tumor suppressors and oncogenes (15, 16). Indeed, miRNA profiles have been proposed as diagnostic tools to predict survival and relapse in patients with lung cancer (17).

Here we report data suggesting that bronchial epithelial cells from mild asthmatic and healthy subjects harbor differing miRNA profiles. To our knowledge, only one previous study attempted to profile asthmatic human airways in this way. Williams

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Correspondence and requests for reprints should be addressed to Melanie J. Jardim, Ph.D., National Health and Environmental Effects Research Laboratory, Environmental Public Health Division, United States Environmental Protection Agency, Chapel Hill, NC 27514. E-mail: melaniejardim@gmail.com

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and colleagues found no difference between the miRNA expression profiles of healthy and mild asthmatic donors (18). However, in that study, airway biopsies were used that included a mixed cell population, whereas we chose to focus on only one cell type, which may account for the differences in the findings of these two studies. Using primary bronchial epithelial cells (BECs) from mild asthmatic donors and healthy subjects, we demonstrate that patients with asthma exhibit a distinct profile, characterized by alterations in miRNAs that may be linked to dysregulated inflammatory pathways. In addition, we use the profiles generated to predict the expression of a gene previously not associated with asthma (*AQP4*), and confirm that it is up-regulated in BECs from asthmatic donors.

MATERIALS AND METHODS

Characteristics of Subjects

The characteristics of the 32 donors used in this study are summarized in Table 1 and Table E1 in the online supplement. All donors were non-smoking individuals. Subjects were examined by a physician, and mild asthma was diagnosed as defined by American Thoracic Society guidelines, with a forced expiratory volume in 1 second (FEV₁) at $\geq 85\%$ of predicted. Our protocol and consent form were approved by the Committee on the Protection of the Rights of Human Subjects at the School of Medicine of the University of North Carolina.

Cell Culture

BECs were obtained from adult donors. Cells were cytologically brushed at bronchoscopy, and expanded in bronchial epithelial growth media (BEGM; Clonetics, Allendale, NJ). Cells were plated onto collagen-coated filters with a 0.4- μ m pore size (Costar, Cambridge, MA), and cultured in a 1:1 mixture of BEGM and Dulbecco's Modified Eagle's Medium-H with SingleQuot supplements (Cambrex, East Rutherford, NJ), bovine pituitary extracts (13 mg/ml), BSA (1.5 μ g/ml), and nystatin (20 U). Upon confluence, *all-trans*-retinoic acid was added to the medium, and air-liquid interface culture conditions were created.

miRNA Microarray Analysis

Total RNA was isolated with Trizol (Invitrogen, Carlsbad, CA). Seven samples from each group were randomly chosen for miRNA microarray expression profiling. miRNA arrays were manufactured by Affymetrix, and sequences were obtained from the Sanger miRBase, release 11.0. Labeling and hybridization were performed according to the manufacturer's protocol with 800 ng RNA. Raw data files have been deposited in the Gene Expression Omnibus (accession number GSE25230). Data were processed and normalized using the Partek Genomics Suite (St. Louis, MO). The ComparativeMarkerSelection suite in GenePattern software (<http://www.broadinstitute.org/cancer/software/genepattern>) was used to compare miRNA expression between groups (19–21). Hierarchical clustering was performed by GenePattern software according to several parameters: Column distance measure, Pearson correlation; Row distance measure, No row clustering; Clustering method, Pairwise complete-linkage; Log transform, no; Row center, no; Row normalize, no; Column center, no; Column normalize, no.

Molecular network analysis was performed using the Ingenuity Database (Ingenuity Systems, Redwood City, CA). Genes are overlaid onto a global molecular network developed from information contained

in the Ingenuity Pathways Knowledge Base and algorithmically generated, based on their connectivity. The functional analysis of a network identifies the biological functions and/or diseases that are most significant to the genes in the network. The Fisher exact test was used to calculate a *P* value determining the probability that each biological function and/or disease assigned to that network was attributable to chance alone.

Quantitative RT-PCR

Complementary DNA was synthesized using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Pre-developed primer/probe sets were purchased from Applied Biosystems (TaqMan miRNA assays). The quantitative fluorogenic amplification of cDNA was performed using the ABI Prism 7500 Sequence Detection System (Applied Biosystems). The relative abundance of miRNAs or mRNAs was calculated using the $\Delta\Delta$ Ct method (22). The miRNA results were normalized to U6 concentrations, and mRNA results were normalized to 18s (23, 24). Independent samples were run in triplicate.

Statistical Analysis

P values for array data were generated using the ComparativeMarker-Selection module, which uses a *t* test to assess the differential expression between groups (21). Unpaired *t* tests were used to determine the significance between groups for all quantitative RT-PCR data.

RESULTS

Bronchial Epithelial Cells from Asthmatic and Healthy Donors Exhibit Differences in miRNA Expression Profiles

Our miRNA microarray analysis of primary BECs isolated via bronchoscopy from our groups of seven healthy and seven asthmatic donors detected 165 miRNAs (Figure 1A) in these cells. Furthermore, 66 of these miRNAs were differentially expressed between healthy and asthmatic donors, with a fold-difference of greater than or equal to 1.5 and *P* ≤ 0.10 (see Figure E1 in the online supplement). Consistent with the heterogeneity observed in asthma phenotypes, we were unable to identify an individual miRNA that was up-regulated or down-regulated in the BECs of all asthmatic donors. Remarkably, however, as seen in Figure 1B, hierarchical clustering analysis on the entire profile of all detectable miRNAs successfully distinguished donors according to disease group, with the exception of two individuals (one patient with asthma and one healthy donor). This result strongly suggests that the miRNA expression profile of BECs from asthmatic donors may be inherently different from that of healthy donors. We validated the microarray data by selecting three miRNAs, based on their overall expression intensities, and determined their expression by quantitative real-time PCR in a larger set of BECs from 16 asthmatic and 16 healthy subjects. These results confirmed that the expression of miRNA (miR)–let7f, miR-487b, and miR-181c* was significantly higher in BECs from asthmatic donors (Figure 1C).

Molecular Network Analysis Suggests that BECs from Asthmatic Donors Have Dysregulated Inflammatory Signaling

To further understand the intracellular processes that may be affected by differentially expressed miRNAs, we focused on miRNAs demonstrating the most significant difference between asthmatic and healthy donors. We identified 24 miRNAs whose expression was greater than or equal to 2-fold between groups, with *P* ≤ 0.1 (Figure 2A). We then used a bioinformatics-based approach to create lists of putative targets to predict the pathways affected by differential miRNA expression in asthmatic cells. These lists were created by using publicly available

TABLE 1. SUMMARY OF DONOR CHARACTERISTICS

Characteristic	Healthy Donors	Asthmatic Donors
Number of donors	16	16
Sex		
Female	6	7
Male	10	9
Median age, in years (range)	23 (19–38)	25.5 (19–40)
Median body mass index (range)	26.8 (19.8–43.3)	24.2 (21.5–33.1)

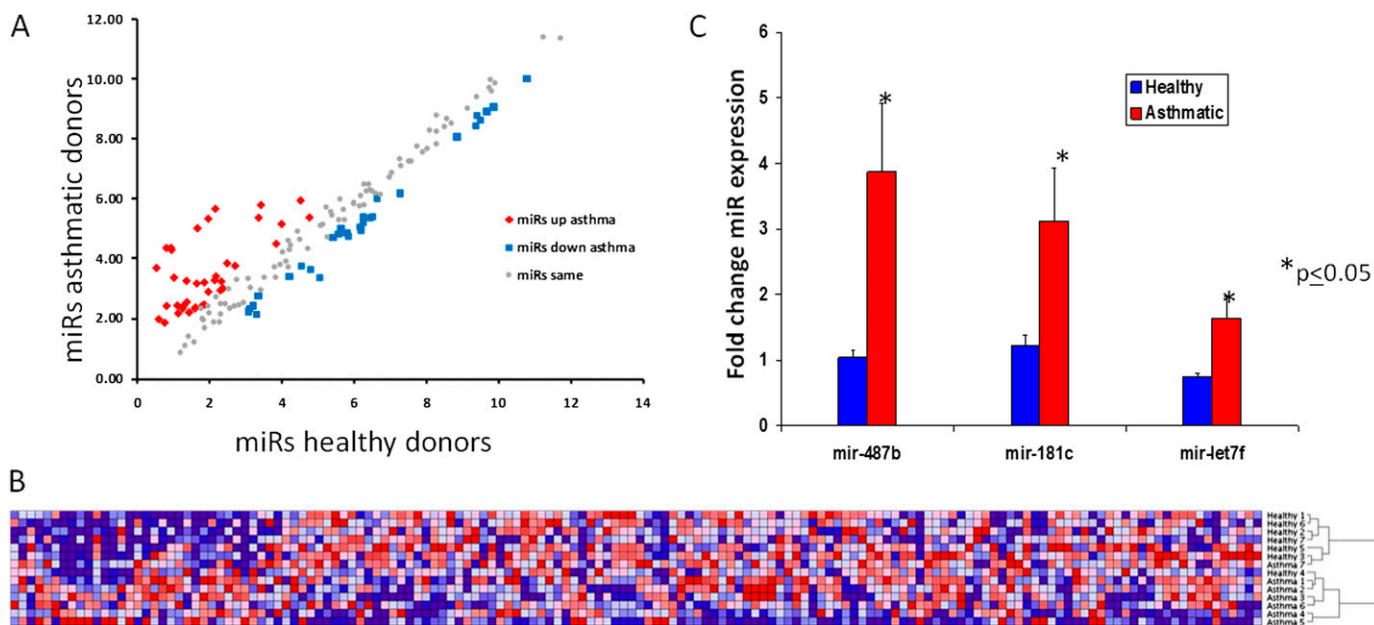


Figure 1. Differences in microRNA (miRNA) expression profiles in bronchial epithelial cells (BECs) from asthmatic donors. (A) Mean intensity of miRNA expression in BECs from seven patients with asthma versus seven healthy donors. One hundred and sixty-five miRNAs were identified among the donors. A miRNA was considered detectable if it was present in five out of seven donors in each group. Of these, 66 presented a 1.5-fold difference or greater in expression level, with $P \leq 0.1$ between healthy and asthmatic donors. Red dots indicate miRNAs that were elevated in asthmatic cells. Blue dots indicate miRNAs that were reduced in asthmatic cells. Gray dots represent miRNAs whose expression was not different between the two groups. (B) Hierarchical clustering was performed on all 165 miRNAs identified in our samples. Six of the seven asthmatic donors clustered together as one group, and six of the seven healthy donors comprised another cluster. Blue indicates down-regulation, whereas red indicates up-regulation. (C) Validation of miRNA (miR) array findings by quantitative RT-PCR. We confirmed that three representative miRNAs (miR-487b, miR-181c, and miR-let7f) were significantly elevated in BECs of a larger set of asthmatic versus healthy donors ($n = 16$ for each group). U6 was used as an internal control for miRNA quantitative RT-PCR reactions. * $P \leq 0.05$.

target-identifying databases, including Targetscan and miRDB. We then used ingenuity pathway analysis to ascertain whether enriched molecular networks could be identified from the lists of putative targets of differentially expressed miRNAs. Consistent with the concept that aberrant inflammatory signaling is an underlying feature of asthma (25–27), putative miRNA targets were enriched for genes involved in mediating inflammatory signaling and biologically linked to dermatological, immunological, musculoskeletal, and respiratory disorders (Figures 2B and 2C). We confirmed the accuracy of our bioinformatics predictions by measuring gene expression levels of the proinflammatory mediators, Cox2, IL-8, TNF- α , and IL-6 in the BECs from our 16 asthmatic and 16 healthy donors. As predicted by our models, Cox2, IL-8, and TNF- α concentrations were significantly elevated in BECs from asthmatic donors, whereas IL-6 was reduced, compared with concentrations from healthy subjects (Figure 2D). We also queried the KEGG database to see if a similar biological interpretation of our target gene dataset could be achieved. We observed a similar phenomenon wherein the gene dataset contained genes enriched for inflammatory mediators (Figures E2 and E3). These results demonstrate the validity of using target prediction databases to identify important pathways in disease pathogenesis.

Molecular Network Analysis Identifies *AQP4* as a Novel Asthma-Associated Gene

In addition to identifying pathways, molecular network analysis can be used to identify genes not previously associated with the disease studied. Previous studies suggested that miR-203 may be important in immunological disorders (12). Our miRNA array

analysis indicated that concentrations of miR-203 were significantly lower in BECs isolated from subjects with asthma. When considering the 16 donors in each group, quantitative RT-PCR results confirmed that miR-203 concentrations were significantly lower in BECs from patients with asthma than from healthy donors (Figure 3A). Using online databases of miRNA target predictions, including Targetscan and miRDB, we identified the putative targets of this miRNA, and observed that this list of genes was enriched for genes that regulate cellular development (Figure 3B). This approach identified *AQP4*, a gene encoding a member of the aquaporin family, as a predicted target of miR-203. Therefore, quantitative RT-PCR for the *AQP4* transcript was performed, and revealed that transcript levels were indeed significantly higher in BECs from asthmatic donors than from healthy subjects (Figure 3C). To determine if our observations were specific to *AQP4*, we also performed quantitative RT-PCR analysis on another predicted target, *AHR*. We did not observe any significant difference in the concentrations of this gene between healthy and asthmatic donors, suggesting that our findings are indeed specific to *AQP4* (Figure E4). This novel finding is consistent with results using bioinformatics prediction tools, and ultimately shows that *in silico* models can accurately predict *in vivo* results. To further assess the relationship between miR-203 and *AQP4* expression in asthmatic donors, we plotted the relative expression levels of each transcript, and observed that higher concentrations of miR-203 were correlated with lower concentrations of *AQP4* (Figure E5). Although lists of putative targets for differentially expressed miRNAs can be quite long, ultimately leading to the identification of several hundred targets for any given miRNA, the data

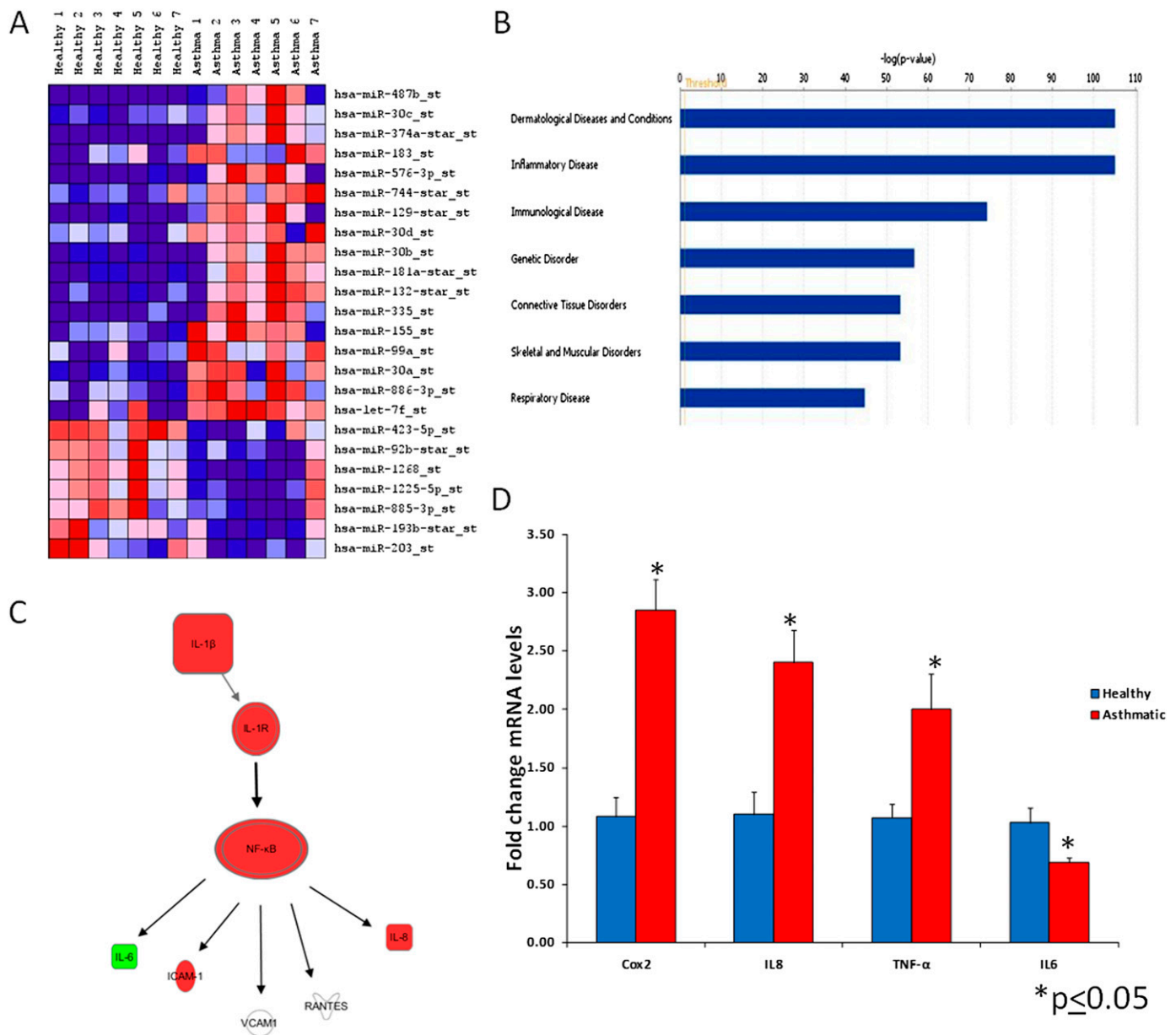


Figure 2. Molecular network analysis suggests dysregulation of inflammatory signaling pathways in asthmatic cells. (A) Heat map of expression values for the 24 miRNAs identified as being expressed differentially between the two disease groups. Blue indicates low expression. Red indicates high expression. (B) Graph illustrates biological functions of genes putatively affected by the observed differential miRNA profile identified by ingenuity pathway analysis. (C) Network analysis of the putative targets for miRNAs in A revealed enrichment for inflammatory pathways, and predicted the up-regulation of gene expression among proinflammatory mediators. Red indicates up-regulation. Green indicates down-regulation. (D) Quantitative RT-PCR confirms bioinformatics predictions. Proinflammatory mediators (IL-8, Cox2, and TNF- α) were up-regulated, whereas IL-6 transcript levels were lower in asthmatic versus healthy donors ($n = 16$ for each group). 18S was used as an internal control for quantitative RT-PCR reactions. $*P \leq 0.05$.

presented here suggest that these tools can still be used to identify novel genes in disease etiology.

DISCUSSION

This is the first demonstration, to the best of our knowledge, of inherent differences in the expression of miRNAs in BECs isolated from healthy and asthmatic donors. We believe that the observed changes in the expression of miRNAs may be linked to differences in gene expression, which in turn may explain the underlying pathological differences between these two groups. The molecular network analysis of putative targets of

significantly different miRNAs suggested that BECs from asthmatic donors have a higher baseline level of inflammation, a finding characteristic of asthma as a disease. Furthermore, we were able to use the same bioinformatics tools to identify a novel gene, *AQP4*, whose expression was up-regulated in BECs from asthmatic donors.

The data presented here are in contrast to those of Williams and colleagues, who detected no differences in miRNA expression in a similar population (18). Although this discrepancy may reflect differences in experimental approaches between studies, we cannot rule out that this phenomenon may be attributable to inherent differences in study populations. Most of our asthmatic

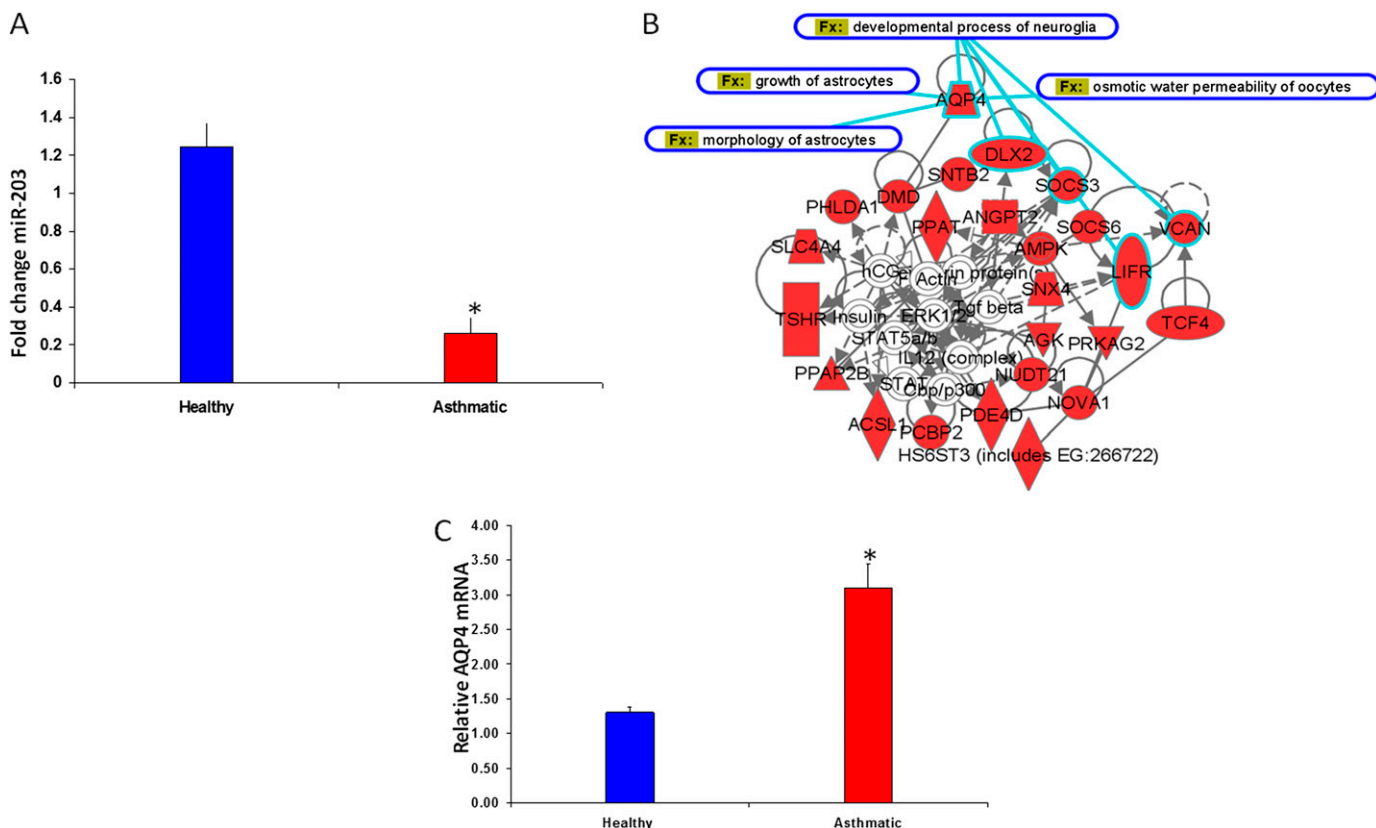


Figure 3. Bioinformatics tools may identify genes important in asthma. (A) Quantitative RT-PCR confirms that one miRNA identified in the array, miR-203, is significantly down-regulated in BECs from asthmatic versus healthy donors. U6 was used as an internal control for miRNA quantitative RT-PCR reactions. $*P \leq 0.05$. (B) Network of predicted targets of miR-203 ($P < 10^{-5}$). Red indicates the up-regulation of putative target genes. The top-ranked target was AQP4. (C) Quantitative RT-PCR results confirm that cells from asthmatic donors contain higher levels of AQP4, a finding consistent with prediction algorithms ($n = 16$ for each group). 18S was used as an internal control for miRNA quantitative RT-PCR reactions. $*P \leq 0.05$.

donors were receiving daily medication, and this alone may have altered the expression of some miRNAs. However, the results presented in this study suggest that several miRNAs are associated with the pathology of asthma. Enrolled subjects were on several different treatment protocols, which could presumably result in the differential expression of miRNAs among subjects with asthma. Nonetheless, we were able to identify a set of miRNAs that were differentially expressed regardless of treatment protocol. Furthermore, although Williams and colleagues did not see any differences in miRNA expression between healthy and asthmatic donors, they also reported that 1 month of treatment with inhaled corticosteroid did not affect miRNA expression, thus providing a direct comparison within the same population (18). Additional studies in larger cohorts are necessary to better document the effects of different asthma medications on the expression of miRNAs in bronchial epithelial cells. Although it is not currently understood whether these differences drive the disease or are a result of disease pathologies, findings suggest that miRNAs may be intricately involved in underlying disease mechanisms.

To assess the biological significance of the observed changes in miRNA expression patterns between healthy and asthmatic donors, we used bioinformatics tools to identify putatively important gene networks that may be affected by these differences. When considering the putative mRNA targets of the miRNAs whose expression was most different between these two groups, an enrichment for genes associated with inflammatory mechanisms was observed. Although we generally used a single program

to assess this network, we also queried the KEGG database, and observed that the results were biologically similar. We were not surprised when either database identified that some inherent inflammatory mechanisms are altered between asthmatic and healthy individuals, because that is the primary feature of this disease. Although the overall protein expression of predicted genes may be regulated by posttranscriptional modifications and protein levels were not assessed in this study, we believe our data provide preliminary evidence that miRNAs may regulate transcripts of inflammatory genes involved in the pathogenesis of asthma. Future studies are needed to understand fully the relationship between miRNA and mRNA expression, and how temporal and structural factors can affect protein expression.

The lists of putative targets for differentially expressed miRNAs were generated using databases that calculate the likelihood of a miRNA targeting an mRNA transcript, and vice versa, based on sequence algorithms alone. Ultimately, this can lead to the identification of several hundred targets for any given miRNA, making it difficult to decipher the most important targets in any given sample. Indeed, *AHR* is a predicted target of miR-203, but no significant differences were evident in the expression levels of this gene between healthy and asthmatic donors, suggesting that it is not a target of miR-203 in bronchial epithelial cells. However, generated molecular network analyses based on *in silico* models were able to predict that transcript levels of *AQP4*, a predicted target of miR-203, were more down-regulated in BECs from asthmatic donors, and were up-regulated in BECs of asthmatic donors. The regulation of miR-203 is not

currently well understood, although miR-203 is speculated to exert some tumor suppressive functions, because it is up-regulated in several cancers, including bladder, blood, and ovarian (28–32). It has also been found to promote the differentiation of skin cells by suppressing markers of differentiation such as p63 (33). Indeed, lung tissue undergoes the active differentiation of cells, and miR-203 may be acting in this capacity in the lung. Given the known dysregulation of tissue homeostasis in asthma and the active fibrosis occurring in the asthmatic lung, we are not surprised that some pathologies of asthma may be associated with the inability of lung epithelial cells to properly maintain the process of differentiation. Furthermore, miR-203 has been associated with several inflammatory diseases, such as psoriasis of the skin and gingivitis (12, 34, 35). Although the role of miR-203 with respect to inflammatory mechanisms remains unclear, the aberrant expression of this miRNA can lead to several consequences. To acquire further mechanistic insights into the regulation and action of miR-203 in BECs, and to see how miR-203 correlates with the varying pathologies associated with asthma, will be interesting.

Data mining identified the aryl hydrocarbon receptor (AHR) as a putative target of miR-203. Although we did not confirm this in our samples, the aberrant expression of AHR was previously documented in inflammatory disease and asthma (36–38). The highest ranking target for miR-203, according to prediction algorithms used by www.miRDB.org, was *AQP4*. We were able to confirm this *in silico* finding using quantitative RT-PCR for *AQP4* transcript levels in our samples, and we observed that *AQP4* transcript levels were indeed higher in BECs isolated from asthmatic donors. Although a role for *AQP4* was not previously described in patients with asthma, it has been strongly implicated in tissue swelling, a pathological characteristic observed in asthmatic lungs (39–41).

Pulmonary edema and swelling are observed in the lungs of patients with asthma. However, the underlying mechanism that regulates this disease feature remains unclear (1, 4, 25). *AQP4* is a member of the aquaporin family of water-selective channels, which collectively ensure the proper transport of water across cell membranes in response to osmotic gradients (40, 41). This is particularly important in the lung, where fluid movement must occur between multiple sections within the lung, including the airspace and cellular and vascular compartments, to maintain homeostatic conditions. Pulmonary edema was documented to contribute to airflow obstruction and bronchial reactivity in patients with asthma, implying that fluid transport in the lung is compromised in these individuals (1, 4, 25, 26). It is not unreasonable to hypothesize that the up-regulation of *AQP4* in the BECs of patients with asthma may represent a mechanism by which the lung is attempting to clear excessive fluid. Krane and colleagues reported that the up-regulation of *AQP4* was observed on the basolateral membranes of epithelial cells in allergen-induced and IL-13-induced murine models of asthma (42). More recently, the loss of *AQP4* expression was observed in non-small cell lung cancers (43). Data mining performed by that group revealed that *AQP4* expression may be linked to several cellular processes important in lung tissue homeostasis, such as the maintenance of pulmonary surfactant and surface tension (43). Ultimately, that study showed that *AQP4* expression was elevated in well-differentiated adenocarcinomas, and was consistent with a better overall prognosis (43).

Several recent studies using models of brain injury showed that *AQP4* expression may be regulated by the transcriptional regulator NF- κ B (44, 45). Indeed, NF- κ B is well documented to regulate the expression of several proinflammatory cytokines, including IL-8, which was shown to be up-regulated in BECs from asthmatic individuals (46). These data may be interpreted to suggest that the

up-regulation of IL-8 in asthmatic donors is attributable to the activation of NF- κ B, which may also be driving *AQP4* expression in these cells. Whether the observed increase of *AQP4* transcript levels in BECs from asthmatic donors is causal or merely a response to environmental factors remains unclear. However, it is not unfathomable that *AQP4* may be up-regulated in these cells, given that edema is associated with the pathogenesis of asthma, and that these cells may be trying to reestablish homeostasis through the clearance of water. These findings may ultimately provide great insights into the nature of swelling and inflammation in the asthmatic lung.

Asthma is a heterogeneous disorder involving several cell types, making it difficult to discern underlying molecular mechanisms of the disease (1). Similarly, heterogeneity is observed in asthma phenotypes. The present data are consistent in terms of highlighting the degree of heterogeneity of the disease when considering that the generated expression profiles were unable to identify a unique miRNA that was up-regulated or down-regulated in all donors of each disease group. This may have been the case because the subjects enrolled in this study were identified as manifesting only mild asthma, and many had normal or near normal FEV₁ values. It will be interesting to characterize potential miRNA expression pattern differences in patients with increasingly severe asthma, to determine whether each disease phenotype harbors a distinct miRNA expression pattern.

Although our subjects did not undergo a detailed phenotypic analysis, and we were unable to identify a unique expression profile of miRNAs across all asthmatic donors, we observed significant differences between patients with mild asthma and healthy individuals, demonstrating the power of this approach. A single miRNA may regulate several gene targets, quite possibly in a network-specific manner, suggesting that miRNA expression profiling may provide a significantly more accurate and comprehensive approach to understanding and identifying disease processes (5, 9, 11). Indeed, miRNA profiling was already demonstrated to provide a more sensitive method in diagnosing certain cancers, and it may serve as a better predictor of disease and therapeutic outcomes (17, 47). Further study into the role of miRNAs in regulating airway inflammation and asthma may lead not only to better diagnostic technologies, but also to the subcategorization of asthma phenotypes, and ultimately to more targeted therapeutic strategies in the clinic.

Overall, these studies comprise the first demonstration that miRNA expression profiles differ between asthmatic and healthy individuals, and suggest that miRNAs serve as a layer of regulation in the pathogenesis of asthma. Furthermore, our studies suggest that *AQP4* may be involved in the pathogenesis of asthma, and demonstrate the utility of using prediction algorithms to identify novel factors within disease processes. Ultimately, we believe our data represent a novel method to identify genetic sources of susceptibility that may contribute to the heightened responses to environmental factors observed in individuals with asthma.

Author disclosures are available with the text of this article at www.atsjournals.org.

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