A Smoking-Associated miRNA-mRNA Coexpression Network

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Adverse effects of cigarette smoking on our health are well documented. On the other hand, we do not fully understand the mechanisms by which these adverse effects occur. In this issue of Circulation: Cardiovascular Genetics, Willinger et al1 identified a smoking-related microRNA (miRNA)/messenger RNA (mRNA) coexpressed gene network in the whole blood of previous and current smokers. They performed the study as part of the extensive phenotypic characterization performed in the participants of the Framingham Heart Study.2 miRNAs, which are small noncoding RNAs that regulate gene expression by binding to their target mRNAs causing translational repression or target degradation,3 have been recognized for their role in cardiovascular pathology.4–6 The results of the current study add another dimension to previous studies by pointing to dysregulated inflammatory responses in leukocytes by miRNA-specific gene regulatory networks in cigarette smokers.

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The authors arrived at this conclusion via a series of well-designed experiments. They used total RNA isolated from peripheral whole blood obtained from 5023 nonfasting participants of the Framingham Study. These participants were part of the Offspring and Third Generation cohorts. The mean age of the participants was 55 years old, and 54% of them were female. Ten percent of them were current smokers, 41% were former smokers, and 48% had never smoked. In this study, former smokers were defined as those participants who had not smoked a cigarette in the past year before data collection. As expected, current smokers had increased systemic inflammation and airflow obstruction.

The authors first measured the expression level of 754 miRNAs in a subset of their samples using a high-throughput reverse transcription quantitative polymerase chain reaction platform using Taqman miRNA assays. This number represents ≈30% of all known human miRNAs.3 After extensive quality control, they determined that they could reliably measure the expression level of 289 miRNAs in their samples. Using linear regression, they identified 6 miRNAs which were associated with smoking status; only one was positively correlated (Figure). Five of these miRNAs were associated with current smokers, whereas one was associated with former smokers compared with those who never smoked. These miRNAs could be biomarkers of smoking or smoking-related complications of pulmonary function.

The authors then tested the association of 6 smoking-related miRNAs for inflammatory markers in the serum and measures of pulmonary function derived from spirometry tests. miR-1180, which had lower expression in current smokers, was negatively associated with CRP (C-reactive protein) levels in the serum and positively associated with the prevalence of airway obstruction. Unexpectedly, miR-1285-3p, which also had lower expression in current smokers, was positively associated with serum IL-6 (interleukin-6) levels, a proinflammatory cytokine. It is possible that the miR-1285-3p association is because of a false-positive finding as many statistical tests were performed in this large-scale study. If true, however, additional studies will be needed to identify the discrepancy between the miRNA associations with CRP and IL-6.

The authors further examined the coexpression pattern of mRNAs with the 6 smoking-related miRNAs. They measured the expression level of 17873 genes using Affymetrix Human Exon 1.0 ST microarrays. More than 2000 genes were correlated with these miRNAs; however, only 116 of the miRNA-mRNA pairs in which the miRNA was the predicted or validated target of the correlated miRNA, suggesting that the coexpression analyses captured the downstream impact of perturbed miRNAs. Because a single miRNA can potentially target hundreds of transcripts, even minor differences in miRNA abundance in a population can have significant impact; therefore, it will be important to understand the primary targets of dysregulated miRNAs. The majority of the enriched Gene Ontology pathways for the coexpressed genes were in inflammatory pathways, pointing to genes involved in monocyte differentiation, viral response, and T-cell homeostasis. Overexpression of 2 of the miRNAs, miR-1180 and miR-1285-3p, in lung epithelial cells resulted in changes in the release of GM-CSF (granulocyte macrophage colony-stimulating factor), CXCL5 (chemokine C-X-C motif ligand 5), and CXCL6 (chemokine C-X-C motif ligand 6) under basal and tumor necrosis factor-α-stimulated cells. Interestingly, IL-6 production did not change.

Smoking-related systemic inflammation leads to changes in the cellular composition of the blood, with prominent influences on the proportion of leukocytes; therefore, it is plausible that the miRNA differences may be a consequence of this change. To mitigate this, the authors first estimated the counts of multiple blood cell types using the whole-genome gene expression data and then used these counts as covariates in their linear regression model to remove the bias. However, this is an imperfect method because imputed complete blood
counts had a prediction accuracy ranging from 0.25 to 0.89 depending on the cell type. In the future, understanding the mechanistic role of cigarette smoking on our health will require the identification of impacted miRNAs’ primary targets in subsets of leukocytes.

Overall, this comprehensive study identified a set of smoking-associated miRNAs some of which were also correlated with inflammatory markers and pulmonary function in a well-characterized cohort. These miRNAs may be biomarkers of exposure to cigarette smoke or may act mechanistically to regulate target gene expression. Two previous studies of subcutaneous adipose tissue miRNA expression in moderately sized cohorts found very little overlap of their results; therefore, validation of the results of the current study in an independent cohort will be needed to establish the role of these miRNAs as biomarkers. This will certainly be challenging as various technical difficulties in reliably measuring miRNAs using different methods, such as reverse transcription quantitative polymerase chain reaction, microarrays, or small RNA sequencing, have been extensively documented. Further mechanistic studies and mediation analysis will also determine whether the differences in miRNA levels are the cause or consequence of inflammation or smoking-related diseases. Some of the authors of the current study previously published the results of their analyses on the genetic loci which are associated with the abundance of miRNAs in the same study participants. On the basis of those results, it seems that genetic variants do not regulate the expression levels of 6 smoking-related miRNAs, suggesting that environmental factors lead to differences in miRNA expression among smokers and nonsmokers.

### Figure
Smoking-related microRNAs (miRNAs) in the Framingham cohort. miRNA expression was measured in the peripheral whole blood isolated from 5023 participants of the Framingham Heart Study. “−” and “+” represent negative and positive, associations with smoking, inflammation, and pulmonary function, respectively. n.s. indicates nonsignificant results.

| miRNA      | Smoking | Inflammation | Pulmonary Function |
|------------|---------|--------------|--------------------|
| miR-1180   | −       | −            | −                  |
| miR-181a-2-3p| −      | −            | n.s.               |
| miR-423-5p | −       | −            | n.s.               |
| miR-25-5p  | −       | −            | n.s.               |
| miR-1285-3p| −       | n.s.         | −                  |
| miR-342-5p | +       | +            | n.s.               |

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### Disclosures
None.

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