Andrew A. Monte, M.D.*
University of Colorado
Aurora, Colorado
and
Rocky Mountain Poison & Drug Center
Denver, Colorado

Hao Sun, M.D.*
University of Colorado
Aurora, Colorado

Anna Malin Rapp-Olsson, M.S.
Rocky Mountain Poison & Drug Center
Denver, Colorado

Fahim Mohamed, Ph.D.
University of Peradeniya
Peradeniya, Sri Lanka
and
University of Sydney
Sydney, Australia

Nicholas A. Buckley, M.D.
University of Peradeniya
Peradeniya, Sri Lanka
and
University of Colorado
Aurora, Colorado

Christopher M Evans, Ph.D.
University of Colorado
Aurora, Colorado

*A.A.M. and H.S. share co-first authorship on this work.

References


To the Editor:

Increased DNA damage response (DDR) contributes to the pathophysiology of aging disorders, including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and cancer (1, 2). DNA damage resulting from cigarette smoke–induced oxidative stress activates the DDR signaling pathway, controlled mainly by the ATM (ataxia-telangiectasia mutated) protein kinase. Accumulation of DNA damage promotes cellular senescence and disrupts tissue homeostasis (2). Using circulating endothelial precursors called blood outgrowth endothelial cells (BOEC) or endothelial colony–forming cells, we have previously demonstrated increased DDR, endothelial senescence, and dysfunction in smokers and patients with COPD (3), supporting the concept of accelerated aging of the endothelium as contributing to CVD in these groups.

MicroRNAs are important regulators of almost every cellular process, including DNA repair. MicroRNA-126 (miR-126; also known as miR-126-3p), a microRNA enriched in endothelial cells, plays a key role in angiogenesis and vascular homeostasis (4). miR-126 is reduced in patients susceptible to develop or with CVD (5–7) and in several types of cancer, including lung cancer (8). The link between mir-126 and DDR signaling in COPD is currently unknown. Therefore, we investigated whether miR-126 is reduced by cigarette smoke in vivo and in endothelial and lung epithelial cells from patients with COPD, as well as its link to DDR activation.

Methods

To investigate DDR resulting from cigarette smoke in vivo, male C57BL/6 mice were challenged for 28 days with cigarette smoke or ambient air, as previously described (9). BOEC were isolated from human blood samples, and human primary bronchial epithelial cells were isolated from lung tissue as described (3, 10). Informed consent was obtained from all individuals (Table 1). Data have been derived from subjects and animals used in previous reports (3, 9, 10). Human umbilical vein endothelial cells were transfected with miRCURY


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Downregulation of MicroRNA-126 Augments DNA Damage Response in Cigarette Smokers and Patients with Chronic Obstructive Pulmonary Disease

To the Editor:

Increased DNA damage response (DDR) contributes to the pathophysiology of aging disorders, including chronic obstructive pulmonary disease (COPD), cardiovascular disease (CVD), and cancer (1, 2). DNA damage resulting from cigarette smoke–induced oxidative stress activates the DDR signaling pathway, controlled mainly by the ATM (ataxia-telangiectasia mutated) protein kinase. Accumulation of DNA damage promotes cellular senescence and disrupts tissue homeostasis (2). Using circulating endothelial precursors called blood outgrowth endothelial cells (BOEC) or endothelial colony–forming cells, we have previously demonstrated increased DDR, endothelial senescence, and dysfunction in smokers and patients with COPD (3), supporting the concept of accelerated aging of the endothelium as contributing to CVD in these groups.

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with Spearman correlation coefficient expressed as mean ± SEM. Comparisons between groups were made using Student’s t test, or Mann-Whitney U test, according to normality of the data. Correlations were estimated using Spearman correlation coefficient (r).

Table 1. Clinical Characteristics of Volunteers

<table>
<thead>
<tr>
<th>Blood outgrowth endothelial cells</th>
<th>Healthy Nonsmokers</th>
<th>Healthy Smokers</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number, n</td>
<td>7</td>
<td>6</td>
<td>5 (4 moderate; 1 severe)*</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>4/3</td>
<td>4/2</td>
<td>5/0</td>
</tr>
<tr>
<td>Age, yr</td>
<td>54 ± 4</td>
<td>57 ± 4</td>
<td>69 ± 3†</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>0</td>
<td>37 ± 10</td>
<td>68 ± 38</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>99.1 ± 6.4</td>
<td>93.6 ± 3.8</td>
<td>61.4 ± 5.8‡,‡</td>
</tr>
<tr>
<td>FEV₁/FVC, % predicted</td>
<td>77.9 ± 0.8</td>
<td>71.6 ± 3.6</td>
<td>53 ± 5.2§,§</td>
</tr>
</tbody>
</table>

Lung epithelial cells

<table>
<thead>
<tr>
<th>Number, n</th>
<th>6</th>
<th>6 (3 moderate; 1 severe; 2 very severe)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M/F</td>
<td>0/6</td>
<td>5/1</td>
</tr>
<tr>
<td>Age, yr</td>
<td>63 ± 6</td>
<td>66 ± 2</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>0</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>97.2 ± 5.6</td>
<td>47.2 ± 10.5§‡</td>
</tr>
<tr>
<td>FEV₁/FVC, % predicted</td>
<td>75.2 ± 3.4</td>
<td>42.02 ± 9.2‡‡</td>
</tr>
</tbody>
</table>

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.

Values are expressed as means ± SEM unless otherwise indicated. FEV₁ and FEV₁/FVC ratio are post-bronchodilator for subjects with COPD, smokers, or nonsmokers.

*Staging of COPD is according to the Global Initiative for Chronic Obstructive Lung Disease criteria.

†P < 0.05 (comparison between nonsmokers and COPD).

‡P < 0.001 (comparison between smokers and nonsmokers).

§P < 0.01 (comparison between smokers and COPD).

Results

The ATM protein kinase is rapidly phosphorylated and activated in response to DNA double-strand breaks and regulates the DDR (2). ATM phosphorylation was significantly increased in BOEC from patients with COPD compared with nonsmokers (Figure 1A), suggesting activation of ATM signaling pathway in COPD. We next investigated whether ATM activation is linked to miR-126 dysregulation. miR-126 levels were reduced in BOEC from smokers and patients with COPD compared with nonsmokers (Figure 1B), suggesting cigarette smoke downregulates miR-126 expression in the endothelium and could contribute to the aberrant activation of DDR in these groups.

Subsequently, we examined whether miR-126 regulates ATM protein kinase and DDR. As previously shown (4), inhibition of miR-126 function in human umbilical vein endothelial cells increased the expression of p85α and SPRED1 (Figure 1C). Under the same conditions, ATM mRNA and protein levels were increased (Figure 1C), suggesting miR-126 controls DDR by repressing ATM expression..

We then used a mouse model of subchronic exposure to cigarette smoke to confirm our findings in vivo. We found that miR-126 levels were reduced in lungs from mice exposed to cigarette smoke (Figure 1D). Within the same samples, there was also increased ATM expression and activity (Figure 1E-F), in line with our ex vivo findings in patients’ endothelial cells.

As miR-126 was dysregulated in whole-lung tissue in our in vivo model, we investigated whether miR-126 expression is affected in lung epithelial cells in COPD. Interestingly, we found reduced miR-126 levels in lung epithelial cells from patients with COPD (all current or ex-smokers) compared with nonsmokers (Figure 1G). In addition, miR-126 levels in lung epithelial cells negatively correlated with smoking history assessed as pack-years (Spearman’s r = −0.73; P < 0.01) and positively correlated with disease severity measured as FEV₁% predicted (Spearman’s r = 0.57; P < 0.05), suggesting miR-126 expression is downregulated with extended exposure to cigarette smoke and increased severity of lung disease.

These results identify a novel miR-126–dependent pathway controlling DDR caused by cigarette smoke, where downregulation of miR-126 enhances ATM activation and thereby promotes tissue aging and dysfunction.

Discussion

In this study, we demonstrate that chronic exposure to cigarette smoke causes reduced expression of miR-126 and increases the DDR. Using a combined in vivo and ex vivo approach, namely, mice exposed to cigarette smoke and endothelial and lung epithelial cells from patients with COPD, we show that exposure to cigarette smoke downregulates miR-126 expression.

miR-126 is critical for endothelial function, and miR-126 supplementation has been proposed therapeutically in in vivo models of vascular injury and pulmonary hypertension (5, 6). We have previously shown that BOEC from both smokers and patients with COPD exhibit increased DDR, senescence, and dysfunction (3); these molecular defects could contribute to CVD, a major cause of mortality in these individuals. Here we demonstrate that miR-126 is downregulated in BOEC and in lung epithelial cells from patients with COPD, highlighting miR-126 as a possible contributor to endothelial and lung tissue dysfunction, and thus potential therapeutic target for COPD.
Figure 1. MicroRNA-126 (miR-126) is downregulated and enhances ATM (ataxia-telangiectasia-mutated) activation in smokers and patients with chronic obstructive pulmonary disease (COPD) and in an in vivo model of subchronic cigarette smoke exposure. (A) Activation of ATM was assessed by measuring phosphorylation of the ATM/ATR substrate by immunofluorescence staining (n = 3). Deep Red Anthraquinone 5 (DRAQ5) was used for nuclear staining. The number of nuclear foci per nucleus was quantified with Volocity software in at least five optic fields (z-stack images). An increased number of cells with focal nuclear staining of phosphorylated ATM/ATR (p-ATM/ATR) was observed in patients with COPD compared with nonsmokers. Arrows show nuclei with increased number of nuclear foci (scale bars, 20 μm). (B) We measured miR-126 by TaqMan real-time PCR in blood outgrowth endothelial cells from nonsmokers (n = 7), healthy smokers (n = 6), and patients with COPD (n = 5). RNU44 was used for normalization. (C) Human umbilical vein endothelial cells were treated with specific anti–miR-126 inhibitor versus control for 72 hours (baseline conditions, no growth factors). mRNA levels for PIK3R2 (phosphoinositol-3 kinase regulatory subunit 2; also known as p85-β) and SPRED1 (Sprouty-related EVH1 domain containing 1) (positive controls for miR-126 inhibition) and for ATM were measured by real-time PCR. ATM protein levels were quantified by Western blotting. GAPDH and α-tubulin were measured for normalization. (D–F) Male C57BL/6 mice were challenged for 28 days with cigarette smoke (for 1 hour twice daily, 4 hours apart) or ambient air. (D) miR-126 was measured by TaqMan real-time PCR in lung tissue from mice exposed to cigarette smoke (n = 6) versus control mice lung tissue (n = 5). Small nucleolar RNA135 (snoRNA135) was measured for normalization. (E and F) ATM mRNA levels were measured by real-time PCR and activation of ATM (phospho-ATM), and ATM protein levels were measured by Western blotting in lung tissue from mice exposed to cigarette smoke (n = 6) versus control mice (n = 6). GAPDH and α-tubulin were measured for normalization. (G) miR-126 was measured by TaqMan real-time PCR in lung epithelial cells from nonsmokers (n = 6) and patients with COPD (n = 6). RNU44 was used for normalization. Data presented are means ± SEM. *P < 0.05; **P < 0.01. ATR = ataxia telangiectasia and Rad3-related protein.
We also show that miR-126 controls the DDR by repressing the ATM protein kinase activity in endothelial cells. This is important, as it highlights a novel pathogenetic pathway of miR-126 in age-related diseases, including cancer. Further studies will show whether restoration of miR-126 has a beneficial effect on lung and vascular function in COPD by regulating the aberrant activation of ATM and downstream molecular pathways that promote accelerated lung and vascular aging.

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Koralia E. Paschalaki, M.D., Ph.D.
Imperial College London
London, United Kingdom

Anna Zampetaki, Ph.D.
King’s College London British Heart Foundation Centre of Research Excellence
London, United Kingdom

Jonathan R. Baker, Ph.D.
Mark A. Birrell, Ph.D.
Richard D. Starke, Ph.D.
Maria G. Belvisi, Ph.D.
Louise E. Donnelly, Ph.D.
Imperial College London
London, United Kingdom

Manuel Mayr, M.D., Ph.D.
King’s College London British Heart Foundation Centre of Research Excellence
London, United Kingdom

Anna M. Randi, M.D., Ph.D.*
Peter J. Barnes, M.D., D.Sc.*
Imperial College London
London, United Kingdom

*These authors share senior authorship.

ORCID ID: 0000-0002-4114-6140 (K.E.P.)

References


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X Chromosome-encoded MicroRNAs Are Functionally Increased in Cystic Fibrosis Monocytes

To the Editor:

Cystic fibrosis (CF) is an autosomal recessive disease. In addition to primary CFTR (CF conductance regulator) gene defects, other factors contribute to CF lung disease, including altered microRNA (miRNA) expression. miRNAs are short regulatory RNAs responsible for posttranscriptional inhibition of protein expression. Various miRNA expression profiling studies have demonstrated altered miRNA expression profiles in people with CF and in vitro CF cell models (reviewed in [1]). Researchers in functional studies have assessed the biological consequences of some of the altered miRNAs. Whether the expression of miRNA in peripheral blood monocytes, and specifically miRNAs encoded on the X chromosome, is altered is p.Phe508del homozygous individuals is not known.

The human genome comprises 22 pairs of autosomes and a pair of sex chromosomes, XX in females and XY in males. X chromosome inactivation achieves dosage compensation between the sexes, whereas X upregulation achieves a balanced expression between the X chromosome and autosomes. These mechanisms can be inconsistent, leading to variability in gene expression between sexes, individuals, cell types, and developmental stages (2, 3). The X chromosome has a high density of miRNAs compared with autosomes, encoding approximately 7% of the total miRNAs in the genome (miRBase, release 21). The contribution of sex chromosomes to CF is virtually unknown.

In the present study, we examined the expression patterns of 86 X-linked miRNAs in monocytes of males and females with and without CF using PCR arrays. Twenty-four individuals were recruited into this study; 12 were stable p.Phe508del CF homozygotes confirmed by genotyping, and 12 were non-CF control subjects (6 females and 6 males per cohort) with mean ± SD ages of 23.5 ± 5.1 years and 27.3 ± 3.6 years, respectively. FEV1 percent predicted and exacerbation rate did not differ between the

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