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Altered long non-coding RNAs expression in normal and diseased primary human airway epithelial cells exposed to diesel exhaust particles

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Abstract

Background: Exposure to diesel exhaust particles (DEP) has been linked to a variety of adverse health effects, including increased morbidity and mortality from cardiovascular diseases, chronic obstructive pulmonary disease (COPD), metabolic syndrome, and lung cancer. The epigenetic changes caused by air pollution have been associated with increased health risks. However, the exact molecular mechanisms underlying the lncRNA-mediated pathogenesis induced by DEP exposure have not been revealed.

Methods: Through RNA-sequencing and integrative analysis of both mRNA and lncRNA profiles, this study investigated the role of lncRNAs in altered gene expression in healthy and diseased human primary epithelial cells (NHBE and DHBE-COPD) exposed to DEP at a dose of 30 μg/cm^2.

Results: We identified 503 and 563 differentially expressed (DE) mRNAs and a total of 10 and 14 DE lncRNAs in NHBE and DHBE-COPD cells exposed to DEP, respectively. In both NHBE and DHBE-COPD cells, enriched cancer-related pathways were identified at mRNA level, and 3 common lncRNAs *OLMALINC, AC069234.2,* and *LINC00665* were found to be associated with cancer progression. In addition, we identified two *cis*-acting (*TMEM51-AS1* and *TTN-AS1*) and several *trans*-acting lncRNAs (e.g., *LINC01278, SNHG29, AC006064.4, TMEM51-AS1*) only

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Disclosure statement

The authors report no conflict of interest.

Supplementary material

Supplementary material is available for: (1) certified mass fraction values for PAHs in SRM 1650b, (2) list of differentially expressed mRNAs in NHBE cells, (3) list of differentially expressed mRNAs in DHBE-COPD cells, (4) differentially expressed known lncRNAs in NHBE cells, (5) differentially expressed known lncRNAs in DHBE-COPD cells, (6) list of the enriched pathways from unique DEGs only in NHBE cells exposed to diesel exhaust particles, (7) list of the enriched pathways from common DEGs in both NHBE and DHBE-COPD cells exposed to diesel exhaust particles, (8) list of the enriched pathways from unique DEGs only in DHBE-COPD cells exposed to diesel exhaust particles, (9) *cis*-targeted mRNAs of DE-lncRNAs in DHBE-COPD cells, (10) *trans*-targeted mRNAs of DE-lncRNAs in NHBE cells, and (11) *trans*-targeted mRNAs of DE-lncRNAs in DHBE-COPD cells.

differentially expressed in COPD cells, which could potentially play a role in carcinogenesis and determine their susceptibility to DEP exposure.

Conclusions: Overall, our work highlights the potential importance of lncRNAs in regulating DEP-induced gene expression changes associated with carcinogenesis, and individuals suffering from COPD are likely to be more vulnerable to these environmental triggers.

Keywords

diesel exhaust particle; COPD; lncRNAs; lncRNA-mRNA interaction; carcinogenesis; susceptibility

1. Introduction

As the usage of diesel-powered engines in private transportation has increased, diesel exhaust particles (DEP) have become one of the most prominent anthropogenic pollutants globally, particularly in densely populated metropolitan areas. DEP is one major class of traffic-related particles comprised of numerous toxic constituents, including polycyclic aromatic hydrocarbons (PAHs). Several molecular mechanisms linking excessive exposure to PAHs and the development of lung cancer have been identified. For example, it is known that the metabolism of PAHs in the biological system by cytochrome P450 (CYP) and other metabolic enzymes results in the formation of reactive metabolites such as diol-epoxides and o-quinones, which then contribute to the formation of DNA adducts, changes in gene expression, DNA mutations, and, eventually, carcinogenesis (Moorthy et al. 2015). DNA adducts caused by PAHs have been widely used as a marker of possible cancer risk and have been linked to cancer risk in both experimental and epidemiologic studies (Kriek et al. 1993). In addition, DEP exposure has been reported to induce pulmonary oxidative stress and inflammation, both of which have been implicated in the onset or exacerbation of respiratory diseases such as COPD, and genotoxicity is a significant outcome that is associated with oxidative stress and inflammation (Ahmed et al. 2018; Schwarze et al. 2013; Steiner et al. 2016). Furthermore, DEP exposure may contribute to the pathogenesis of COPD by releasing inflammatory mediators, possibly via NF-κB, MAPK and PI3K signaling pathways (Wang et al. 2020). While multiple metabolic pathways, cellular signaling, and genetic susceptibility all contribute to the development of lung cancer, the causal relationship and underlying molecular processes linking DEP and lung carcinogenesis remain to be elucidated.

A number of long noncoding RNAs (lncRNAs) have been implicated in the etiology of lung cancer (Jiang et al. 2019). LncRNAs are noncoding transcripts that exceed 200 nucleotides in length, and they have recently been identified as one of the biggest and most diverse RNA families. LncRNAs are categorized as intergenic (between genes), intragenic/intronic (within genes), or antisense depending on their proximity to protein-coding genes (Derrien et al. 2012). While the biological functions of most lncRNAs are unclear, several lncRNAs have been identified as regulators of cancer initiation and progression at the transcriptional and post-transcriptional levels, including cell proliferation, apoptosis, metastasis, and differentiation (Sun et al. 2018). The expression of both lncRNA and mRNA profiles has been shown to be significantly affected by traffic-related PM exposure, which is thought to

be linked to a variety of disorders. In particular, lncRNAs have a vital regulatory function in the metabolic reprogramming associated with human cancer (Sellitto et al. 2021). The expression levels of lncRNAs are tightly controlled in the healthy state and can be disrupted by a variety of mechanisms during the development of disease.

Increasing evidence suggests that COPD and lung cancer may be distinct manifestations of the same disease (Durham and Adcock 2015). Furthermore, COPD has also been considered as a significant risk factor for lung cancer, with COPD patients having a twofold increased risk of developing lung cancer (Papi et al. 2004). This high prevalence of lung cancer in COPD patients suggests that there may be common mechanisms or common pathogenic factors for either disease (e.g., genetic susceptibility, activation of intracellular pathways, or epigenetics) (Barnes and Adcock 2011). As an epigenetic factor, lncRNAs are critical for regulating gene expression at the transcriptional and post-transcriptional levels (Dykes and Emanueli 2017), ultimately contributing to lung carcinogenesis and associated consequences (He et al. 2017; Reddy et al. 2015; Schones et al. 2015). However, the lncRNA-mediated pathogenic changes after DEP exposure, as well as their potential roles in susceptibility and disease progression, are not fully understood.

In this study, we investigated the role of lncRNAs in lung carcinogenesis induced by DEP using primary human bronchial epithelial cells from healthy (NHBE) and diseased (DHBE-COPD) donors. We hypothesize that through activation of oncogenes or loss-of-function of tumor suppressor genes, lncRNAs mediate the regulatory pathways in lung cancer initiation. Due to the fact that lung cancer does not exhibit obvious symptoms in its early stages, the majority of lung cancers are identified in their late stages, complicating therapy, and considerably lowering the overall lung cancer survival rate (Knight et al. 2017). Therefore, identification of lncRNAs as novel biomarkers involved in lung cancer development due to DEP exposure and elucidation of lncRNAs regulatory networks will contribute to the current scientific understanding of source-specific adverse outcome pathways, which is critical for protecting vulnerable populations from increased risks of traffic-related PM induced adverse health outcomes.

2. Materials and Methods

2.1. Diesel Exhaust Particles (DEP) and Particle Extraction.

The DEP standard reference material (SRM 1650b, with certified 26 PAHs and 7 nitro-PAHs) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). The DEP extracts were dissolved in cell media and used for subsequent cell exposures.

2.2. NHBE and DHBE-COPD cell culture and differentiation.

Primary normal human bronchial epithelial (NHBE) cells and diseased human bronchial epithelial (DHBE) cells from donors with COPD were purchased from Lonza (NHBE, Catalog # CC-2540s; DHBE-COPD, Catalog # 00195275; Walkersville, MD). BEGM bronchial epithelial cell growth media (Lonza, Catalog # CC-3170) and Human Bronchial/Tracheal Epithelial Cells (HBTEC) Air-liquid Interface (ALI) differentiation media were

purchased from LONZA (Catalog # 00193514, Walkersville, MD) and Lifeline Cell Technology (Frederick, MD), respectively. A type I human atelocollagen solution VitroCol® (Advanced BioMatrix, Catalog #5007, San Diego, CA) was used for coating the culture flasks and plates. Briefly, the BEGM growth medium was used to revive NHBE and DHBE-COPD cells from cryopreservation, seeded as passage one (P1) into T75 cell culture flasks, and incubated at 37°C, 5% CO_2. Upon 70–80% confluency, cells were then sub-cultured at ALI to allow differentiation on 24 mm Transwell® inserts with 0.4 μm pore size (Corning; Fisher Scientific) at a density of 1×10^5 cells/insert housed in 6 well cell culture plates. The cells were cultured at ALI for 28 days to facilitate differentiation into ciliated, mucus-producing cells (Ghio et al. 2013). 2.0 mL of fresh medium was supplied in the basal chamber every 48 hours.

2.3. Particle Exposure.

After 4 weeks, the apical chambers of the grown cells were rinsed with 1 mL of Hanks' Balanced Salt Solution (HBSS) buffer before being exposed to particles, and the buffer was immediately removed. DEP particles were suspended in 250 μL of BEGM medium to prepare the desired dosages (30 μg/cm^2, 20 μg/cm^2, and 10 μg/cm^2) before being applied on the cells in the apical chamber of the Transwell® inserts and gently stirred for at least 2 minutes. All treatments were performed in triplicate. A schematic diagram for the timeline of cell culture, ALI differentiation, and the subsequent DEP exposure is shown in Figure 1.

2.4. Cytotoxicity Assay.

As a prescreen for the downstream transcriptome analysis, the lactate dehydrogenase (LDH) test was performed according to the manufacturer's protocol (Roche) to detect the cytotoxicity of cells exposed to DEP at dosages of 10 μg/cm^2, 20 μg/cm^2, and 30 μg/cm^2. The supernatants were collected 24 hours after the exposure. To induce 100% cell death, Triton X-100 (0.1%) was used as a positive control. Absorbance was measured at 490 nm with a reference wavelength of 620 nm (TECAN SpectraFluor Plus).

2.5. RNA isolation, library construction, and RNA sequencing.

NHBE and DHBE (COPD) cells exposed to 30 μg/cm^2 of DEP were selected for transcriptomic analysis. The TRI Reagent and the spin column-based Direct-zol RNA MiniPrep kit were used to extract and purify total RNA (Zymo Research). All RNA integrity numbers (RINs) exceeded 8.0. The RNA-Seq libraries for both mRNA and lncRNA were prepared with 150–300 ng of total RNA and the NEBNext ultra II Directional RNA Library Prep Kit. Then, the rRNA was removed using the NEBNext rRNA Depletion Kit. RNA-Seq analysis (Illumina NextSeq 500 high throughput 75bp pair end) was carried out at the University of California, Riverside's Institute for Integrative Genome Biology (IIGB) (UCR).

2.6. RNA-Seq Data Analysis for both mRNA and lncRNA.

FastQC (version 0.11.7) (Andrews 2010) was used to assess read quality after RNA sequencing. Trimming was accomplished using the Trimmomatic (version 0.35) program (Bolger et al. 2014). MINLEN:50 was used to keep reads that were at least 50 bases

long. After that, we used kallisto (version 0.46.1) to quantify the abundances of transcripts from our RNA-Seq data (Bray et al. 2016) and annotated transcripts with GENCODE version 37 (Frankish et al. 2021). Kallisto is based on pseudoalignment, which allows rapid determination of read compatibility with targets without alignment (Bray et al. 2016). DESeq2 (version 1.18.1) (Love et al. 2014) was used for normalization and differential mRNA and lncRNA expression analyses in R (version 3.6.3). For mRNA and lncRNAs between exposed and unexposed samples, significance was defined as the adjusted *p* value (i.e., false discovery rate (FDR)) ≤ 0.05 and the absolute log 2-fold change (log2FC) ≥ |±1|.

2.7. Prediction of lncRNA Target Genes.

To investigate how DE lncRNAs interact with nearby or distant target genes to regulate gene expression, we divided target genes of DE lncRNAs into two groups: *cis* and *trans* targets. Based on prior research, *cis* target genes were initially defined as genes located within 10 kb upstream or downstream of the differentially expressed lncRNA (Ahmed et al. 2021). However, using bedtools (version 2.29.2) (Quinlan 2014), we were unable to locate any nearby target genes within 10 kb upstream and downstream. Thus, we performed further analysis to identify *cis*-targeted genes at longer distances (up to 50 kb upstream and downstream) to locate potential long-range *cis*-regulatory elements (Laverré et al. 2022). The *trans*-regulated genes of DE lncRNAs were then predicted using rtools (http://rtools.cbrc.jp/cgi-bin/RNARNA/index.pl) (Iwakiri et al. 2017). To construct the interaction network for NHBE and DHBE-COPD cells based on the minimum energy of lncRNA and mRNA interaction, the top 200 *trans*-targeted genes were chosen from a total of 1,000 genes in NHBE and 1,400 genes in DHBE-COPD cells, respectively, using the network (version 1.16.0) and ggnet2 (version 0.1.0) packages in R (version 3.6.3).

2.8. Pathway Enrichment Analysis for Susceptibility.

DE mRNAs from both NHBE and DHBE-COPD cells were used for pathway enrichment analysis using the ConsensusPathDB database (Kamburov et al. 2009) to compare and contrast the gene expression changes in healthy and diseased cellular models. Overrepresentation analysis was performed. The significance (p-value) of the observed overlap between the DE mRNAs and members of established biological pathways was calculated using the hypergeometric distribution (Zhavoronkov et al. 2014). The results were obtained using the following criteria: (1) the input list and pathways must share at least 5 genes, and (2) a *p*-value cut-off of 0.01 is required.

2.9. Code availability.

Data analysis and codes are available at https://github.com/biplabua/DEP_lncRNA_mRNA_Co-expression_2021.

3. Results

3.1 Cytotoxicity Assay

Following a 24-h exposure to DEP, the cytotoxicity of NHBE and DHBE-COPD was assessed using the LDH assay. As shown in Figure 2, there was no significant cytotoxicity (≤30%) observed in cells exposed to DEP at levels of 30 μg/cm^2, 20 μg/cm^2, and 10 μg/cm^2,

indicating that the cells were stressed during exposure, but the exposure dosage was not too toxic to preclude further transcriptomic evaluation (ISO 2009). Thus, we used a dose of 30 μg/cm^2 for the subsequent transcriptomic analysis in this study. Notably, doses of 30 μg/cm^2 and 20 μg/cm^2 induced relatively higher cytotoxicity in DHBE-COPD cells than in the healthy NHBE cells, which might be connected to the pre-existing COPD condition.

3.2 DE mRNAs

DESeq2 identified 503 and 563 DE mRNAs in NHBE and DHBE-COPD cells, respectively, using the of log2FC > |±1| and FDR < 0.05 criteria (Figure 3). A total of 142 mRNAs were common between NHBE and DHBE-COPD cells exposed to DEP (Figure 3c). As the exposed cells did not exhibit substantial cytotoxicity (Figure 2), these results reflected the real transcriptional changes in NHBE and DHBE-COPD cells under the given exposure condition (i.e., a dosage of 30 μg/cm^2 following 24 hr exposure).

3.3 DE lncRNAs

DESeq2 identified 10 and 14 DE lncRNAs in NHBE and DHBE-COPD cells, respectively, using the log2FC > |±1| and the FDR < 0.05 criteria (Figure 4). In addition, we found a total of 4 lncRNAs were common between NHBE and DHBE (COPD) cells exposed to DEP (Figure 4c). Some lncRNAs may co-express with mRNAs and regulate gene expression. Integrative investigation of DE lncRNAs and DE mRNAs may uncover epigenetic regulation of gene expression via lncRNAs.

3.4. Prediction of *cis*-targeted Genes of the DE lncRNAs

Within the 50 kb window, we did not find any differentially expressed *cis*-targeted mRNA in NHBE cells under the given exposure condition. However, two *cis*-targeted mRNAs were identified in DHBE-COPD cells, including *KAZN* (kazrin, periplakin interacting protein), and *TTN* (titin). *KAZN* is targeted by lncRNA *TMEM51-AS1*, and *TTN* is targeted by lncRNA *TTN-AS1*, respectively. Notably, *TMEM51-AS1* is also identified as one of the *trans*-acting lncRNAs only found in DHBE-COPD cells (discussed in detail in Section 4.2.3). Additionally, *TTN-AS1* has been reported as a potential diagnostic and prognostic biomarker for multiple cancers, including lung cancer (Qi and Li 2020; Zheng et al. 2021b).

3.5. Prediction of *trans*-targeted Genes of the DE lncRNAs

A total of 5 out of 10 DE lncRNAs in NHBE cells and a total 7 out of 14 DE lncRNAs in DHBE-COPD cells were identified to construct regulatory networks with *trans*-target genes from rtools. Among the 1,000 predicted *trans*-acting genes in NHBE cells, 8 target genes were found to be differentially expressed (log2FC > |±1| and FDR < 0.05) in our analysis and to have the same expression pattern (for up and down regulation), whereas 13 DE target genes had the opposite expression trend as the *trans*-acting lncRNAs (Figure 5). In contrast, among the 1,400 *trans*-acting genes in DHBE-COPD cells, 28 DE target genes (log2FC > |±1| and FDR < 0.05) showed the same expression trend (for up and down regulation) as the *trans*-acting lncRNAs, while 16 genes showed the opposite expression trend (Figure 6). Most coding genes are regulated by different lncRNAs, but some, like *BMP1* in NHBE cells

and *PEX5, TMEM63B,* and *IGF2R* in DHBE-COPD cells, are controlled by more than one lncRNA (Figures 5–6).

4. Discussion

4.1. DEP exposure and biological pathway perturbation at mRNA levels

The airway epithelium is the initial defensive barrier that protects the airway from external stimuli. When epithelial functions are compromised, cellular signaling pathways involved in homeostasis maintenance, such as inflammation, repair, and differentiation, may be disturbed. The reduced airway epithelial function is a common pathological alteration in COPD, suggesting that populations with such pre-existing health issues may be more vulnerable to pollutant exposure.

In this study, the most significantly altered pathways from unique DEGs only found in NHBE cells are associated with control mechanisms of cell cycles to maintain the genomic stability, including RND GTPase cycles (FDR value 1.96E-02) and G2/M transition (FDR value 2.36E-02). As the DEP consists of PAHs and their derivatives, DEP-induced ROS generation, oxidative stress and DNA damage may occur via the xenobiotic metabolism (Nemmar et al. 2013). Following pathway analysis, we found that pathways associated with chromatin modifying enzymes (FDR value 3.27E-04) and direct p53 effectors (FDR value 8.79E-03) were enriched in both NHBE and DHBE-COPD cells. Furthermore, we found several pathways such as TNF alpha signaling pathway (FDR value 4.12E-03), p53 signaling pathway (FDR value 9.50E-03), and PIP3 activates AKT signaling pathway (FDR value 5.91E-03) enriched only in DHBE-COPD cells. All these pathways are linked to carcinogenesis (Altomare and Testa 2005; Muller and Vousden 2013; Wajant 2009).

4.2. *Trans*-acting gene regulation by lncRNAs in DEP exposed NHBE and DHBE-COPD cells

At the transcriptional and post-transcriptional levels, lncRNAs play a major role in pathogenesis by regulating gene expression (Ahadi 2021; Jandura and Krause 2017). The expression of these non-coding lncRNA transcripts has been found to be associated with the expression of target genes via *cis* and *trans* mechanisms (Ahmed et al. 2021; Li et al. 2019). Due to the fact that most lncRNAs in the human genome are still uncharacterized, functional annotations of lncRNAs remain challenging and are currently under extensive research (Li et al. 2019; Ramakrishnaiah et al. 2020; Zhang et al. 2021). In this study, as we identified very few differentially expressed *cis*-targeted genes within the 50 kb window in our search (discussed in detail in Section 3.4), we focused on the discussion of potential regulatory function of DE lncRNAs by the *trans* mechanism.

4.2.1. Common lncRNAs differentially expressed in both NHBE and DHBE-COPD cells—In this study, 3 common lncRNAs were found differentially expressed in both NHBE and DHBE-COPD cells, including *LINC00665*, *OLMALINC* (Oligodendrocyte Maturation-Associated Long Intergenic Non-Coding RNA) and *AC069234.2* (Figures 5–6). Among identified DE lncRNAs, prior research has revealed that *LINC00665* is involved in tumor progression, DNA damage repair, and is upregulated in a variety of cancers (Dai

et al. 2021; Ding et al. 2020), such as the lung adenocarcinoma (LUAD) (Cong et al. 2019). Interesting correlations were found for these 3 common lncRNAs in both NHBE and DHBE-COPD cells: (1) *LINC00665* was upregulated in both NHBE (log2FC = 21.98, FDR value =1.23E-06) and DHBE-COPD (log2FC = 20.00, FDR value =2.51E-05) cells; (2) *OLMALINC* was upregulated in NHBE cells (log2FC = 24.2, FDR value =6.36E-07), whereas downregulated in DHBE-COPD cells (log2FC = −22.12, FDR value =1.02E-05); (3) *AC069234.2* were downregulated in NHBE cells (log2FC = −30.92, FDR value = 2.74E-12), whereas upregulated in DHBE-COPD cells (log2FC =19.94, FDR value = 5.67E-05). The different expression patterns in NHBE and DHBE-COPD cells underscore the function of lncRNAs in regulating gene expression.

Through integrative analysis of our lncRNA and mRNA profiling data, we found that lncRNAs *OLMALINC* and *AC069234.2* are associated with *ZMIZ2* (Zinc Finger MIZ-Type Containing 2) and *ATXN2L* (Ataxin 2 like) genes, respectively (Figures 5–6). It has been reported that the depletion of *ZMIZ2* gene plays a significant role in attenuation of the colorectal tumorigenesis, which is also considered as a potential therapeutic target (Zhu et al. 2020). Our study shows that the *ZMIZ2* gene was downregulated in both NHBE and DHBE-COPD cells, whereas expression pattern was opposite for the lncRNA *OLMALINC* in NHBE (upregulated) and DHBE-COPD cells (downregulated), respectively (Figures 5–6). In addition, the *ATXN2L* genes has been suggested to promote cell invasiveness and oxaliplatin resistance via EGF via PI3K/Akt signaling (Lin et al. 2019a). In our study, the *ATXN2L* gene was shown to be elevated in both NHBE and DHBE-COPD cells, but the lncRNA *AC069234.2* was found to be downregulated in NHBE cells but upregulated in DHBE-COPD cells (Figures 5–6). These findings suggest that altered epithelial functions in DHBE-COPD cells may result in lung disease aggravation in response to DEP exposure, while identified lncRNAs may act as early indicators for disease development.

4.2.2. DE lncRNAs only found in NHBE cells—In NHBE cells, lncRNAs *NUTM2B-AS1* (NUTM2B antisense RNA 1) (log2FC = 6.15, FDR value = 3.38E-02) and *OTUD6B-AS1* (OTUD6B antisense RNA 1) were both found to be upregulated (log2FC = 5.66, FDR value = 1.53E-03). In our analyses, lncRNA *NUTM2B-AS1* was found to be associated with four genes, including *ASH1L* (ASH1 Like Histone Lysine Methyltransferase), *IFT172* (Intraflagellar Transport 172), *BMP1* (Bone Morphogenetic Protein 1), *PBXIP1* (PBX Homeobox Interacting Protein 1) (Figure 5). Downregulation of *IFT172* has been linked to dysfunction of intraflagellar transport machinery and maintenance of cilia length seen in COPD (Hessel et al. 2014), suggesting that DEP exposure may be a risk factor for COPD development. In addition, lncRNA *AC069234.2* has the opposite interaction with *MYO5A* (Myosin VA), *MAP4K4,* and *MLEC* (Malectin) genes (Figure 5). Previous studies reported that downregulation of *MAP4K4* results in induction of apoptosis (Liu et al. 2011). *MYO5A* has been reported to be remarkably upregulated in esophageal squamous cell carcinoma tissues and cells (Liang et al. 2020), while upregulation of *MLEC* (Malectin) has been linked to colorectal cancer (Mao et al. 2020). On the other hand, the *ACVR1* (Activin A Receptor Type 1) gene was downregulated together with lncRNA *AC069234.2* in our study (Figure 5). Increased expression of *ACVR1* has been linked to decreased survival in a large cohort of 227 hepatocellular carcinoma cases (Li et

al. 2015). Furthermore, *CANX* (Calnexin) is detected in high abundance in breast cancer patients at both primary and metastatic stages (Moradpoor et al. 2020), and high *CANX* expression has been linked to a worse survival rate for breast cancer patients (Geng et al. 2019). Our study found that lncRNA *OTUD6B-AS1* was upregulated whereas *CANX* gene was downregulated (Figure 5). Thus, overexpression of lncRNA *OTUD6B-AS1* in NHBE cells may provide protective functions for maintaining cellular homeostasis.

4.2.3. DE lncRNAs only found in DHBE-COPD cells—Previous studies suggested that pre-exiting COPD conditions may play as an independent risk factor for cancer progression (Durham and Adcock 2015; Papi et al. 2004). Mounting evidence suggest that altered expression of lncRNAs can lead to carcinogenesis by acting like the oncogene and interacting with the enhancer of a gene (Ahadi 2021; Martínez-Terroba and Dimitrova 2020; Zhang et al. 2013). Therefore, the associations between lncRNAs and mRNAs and their expression patterns are important to decipher their molecular mechanisms. For example, lncRNA *FGD5-AS1* that was found to be upregulated in DHBE-COPD cells in this study (log2FC = 19.43, FDR value = 2.08E-04) has been reported as an oncogene and promoted tumorigenesis (Fan et al. 2020). Elevated expression of *FGD5-AS1* upregulates the *FGFRL1* (fibroblast growth factor receptor like 1) gene by sponging miRNA has-miR-107 and induces non-small cell lung carcinoma cell proliferation (Fan et al. 2020). Another upregulated lncRNA *LINC01278* (log2FC = 17.24, FDR value = 1.01E-03) was reported to accelerate cancer progression via miRNAs (e.g., miR-134-5p/Lysine demethylase 2A (KDM2A) axis) (Liu et al. 2021) and upregulate the *KDM2A* expression (associated with the cyclin D1 expression) to induce cancer progression and proliferation. By forming axis with miR-559/*TCF12*, miR-376c-3p/*DNM3*, miR-133a-3p/*PTHR1*, *LINC01278* is known to induce hepatocellular carcinoma (Song et al. 2020), papillary thyroid carcinoma (Lin et al. 2019b), and osteosarcoma (Qu and Li 2020), respectively. We found that lncRNA *LINC01278* regulates 6 target genes with same direction (all upregulated) (Figure 6). Upregulation of *DYNC1H1* (Dynein Cytoplasmic 1 Heavy Chain 1) and *ANP32E* (Acidic Nuclear Phosphoprotein 32 Family Member E) genes is known to promote cell proliferation and migration (Gong et al. 2019; Huang et al. 2020).

On the other hand, lncRNA *SNHG29* (also named as *LRRC75A-AS1*) that activates the p53/p21 signaling and results in cell senescence (Jiang et al. 2021) was found downregulated in DHBE-COPD cells (log2FC = −6.42, FDR value = 1.82E-05), while its *trans*-targeted gene *COL5A2* (Collagen Type V Alpha 2 Chain) was found to be upregulated (Figure 6). Recent studies reported that the gene expression of *COL5A2* was significantly upregulated in colorectal cancer (Wang et al. 2021b), gastric cancer (Tan et al. 2021), bladder cancer (Zeng et al. 2018), and prostate cancer (Ren et al. 2021). The expression pattern indicates the potential risk of DHBE-COPD cells in cancer development (Figure 6).

Another lncRNA *TMEM51-AS1* was found to be upregulated in our study (log2FC = 20.30, FDR value = 1.64E-07), which induced the cell proliferation by sponging miRNA response elements in laryngeal squamous cell carcinoma (Lyu et al. 2020). Furthermore, lncRNA *TMEM51-AS1* is considered as a competitive endogenous RNA (ceRNA) of *ARID4A* (AT-Rich Interaction Domain 4A) gene, which targets hsa-miR-1254. In addition, *TRAPPC10* (Trafficking Protein Particle Complex Subunit 10) gene targets hsa-miR-106B

(Hui et al. 2019) to induce cell proliferation in laryngeal squamous cell carcinoma (Lyu et al. 2020). Downregulation of *TMEM51-AS1* (strong relevance with p53-R273H) was found preventing the tumorigenic capacity of colorectal cancer stem cells *in vitro* and *in vivo* (Zhao et al. 2019). This result indicates the upregulation of lncRNA *TMEM51-AS1* in DHBE-COPD may be involved in carcinogenesis.

Furthermore, we identified downregulated lncRNA *AC006064.4* (log2FC = −10.85, FDR value = 2.5E-02) coexpressed with upregulated *RAC2* and *MECOM* (MDS1 And EVI1 Complex Locus) genes (Figure 6). Elevated expression of the *RAC2* gene has been associated with the cell migration and cell proliferation of osteosarcoma by regulating the Wnt signaling pathway (Xia et al. 2019). It has been reported that the mRNA level of *MECOM* gene in glioblastoma multiforme (GBM) tissues was significantly higher than that in the adjacent tissues, which suggests that *MECOM* may play a driving role in GBM oncogenesis (Hou et al. 2016). In addition, lncRNA *AC006064.4* has recently been linked to SARS-Cov-2 infection (Vishnubalaji et al. 2020), indicating that it might be an important modulator of several biological pathways in severe lung damage.

4.2.4. *Trans*-acting mRNAs only found in DHBE-COPD cells—A few unique mRNAs that are associated with multiple lncRNAs were identified in DHBE-COPD cells, including *TMEM63B* (Transmembrane Protein 63B), *PEX5* (Peroxisomal Biogenesis Factor 5), and *IGF2R* (Insulin Like Growth Factor 2 Receptor) (Figure 6). The *TMEM63B* gene was linked to lncRNAs *LINC01278* (log2FC =17.24, FDR value =1.01E-03), and *AC069234.2* (log2FC =19.94, FDR value =5.67E-05), while *PEX5* gene was connected with lncRNAs *LINC00665* (log2FC =20.00, FDR value =2.51E-05) and *LINC01278* (Figure 6). Genetic variants of PEX5 in the peroxisome pathway have been linked to cutaneous melanoma, and these variants may serve as novel biomarkers for predicting survival in patients with cutaneous melanoma (Wang et al. 2021a). Furthermore, both *OLMALINC* (log2FC = −22.12, FDR value =1.02E-05) and *AC069234.2* were found to regulate the *IGF2R* gene. Prior research found that the M6P/*IGF2R* posttranscriptional dysregulation is a contributing mechanism in breast cancer development and breast cancer response to therapy (Iwamoto and Barber 2007), and *IGF2R* is also a poor prognostic factor for early-stage cervical cancer (Takeda et al. 2019). In the current study, the upregulated *IGF2R* is linked to downregulated lncRNA *OLMALINC* and upregulated lncRNA *AC069234.2*. For lncRNA *AC069234.2* that is connected to multiple genes (Figure 6), a study by Zheng et al. (2021a) reported that upregulation of *PIP5K1A* promotes the progression of glioma, while downregulation of *IFNGR2* (Interferon Gamma Receptor 2) gene may impair the interferon-γ (IFN-γ) signaling that is related to tumor progression (Castro et al. 2018). The same expression patterns observed in DHBE-COPD cells (Figure 6) indicates that these lncRNA-mRNA pairs may function in concert in cancer progression.

4.4. Potential Limitations and Future Research Needs

Some potential limitations of this study should be noted. First, while the Encyclopedia of DNA Elements (ENCODE) project classified over 9,640 human genome loci as lncRNAs, only ~100 have been thoroughly characterized to determine their cellular functions (Bánfai et al. 2012), and since lncRNA expression is tissue specific, we were only able to annotate

a limited number of known lncRNAs in human airway epithelial cells in the current study. In addition, the expression of mRNAs and lncRNAs is highly dynamic and is influenced by many internal and external factors. The results shown in the current study only represent a snapshot of the complex system. Lastly, the relationships that have been identified do not prove causality. Further validation studies are necessary to confirm the effects of lncRNA alterations on DEP-induced health outcomes. Experiments using RNA interference (RNAi), antisense oligonucleotides (ASOs), or CRISPR/Cas9 genome editing methods, in particular, will be useful to validate the regulatory function of identified lncRNAs in controlling gene expression.

5. Conclusions

Overall, this study highlights the vulnerability of people with pre-existing lung diseases to DEP exposure. Dysfunctional DHBE-COPD cells are shown to be more sensitive to the same level of DEP, with the altered expression of mRNAs (e.g., *IGF2R, IFNGR2, PEX5, TMEM63B*) and lncRNAs (e.g., *LINC01278, SNHG29, AC006064.4, TMEM51-AS1*) clearly linked to carcinogenesis (Figure 7). These findings help understand lncRNA-mRNA interactions and the relevance of air pollution in lung cancer. As a result, the lncRNAs identified in this work could provide an experimental basis for the potential use of lncRNAs as biomarkers for diagnosis and prognosis to assist with the early detection of lung cancer and protect vulnerable populations from increased risks of traffic-related PM induced adverse health outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Abbreviations

COPD	chronic obstructive pulmonary disease
DE	differentially expressed
DEP	diesel exhaust particles
DHBE	diseased human bronchial epithelial cells
FDR	false discovery rate

lncRNA	long non-coding RNA
log2FC	log2 fold change
NHBE	normal human bronchial epithelial cells
PAHs	polycyclic aromatic hydrocarbons

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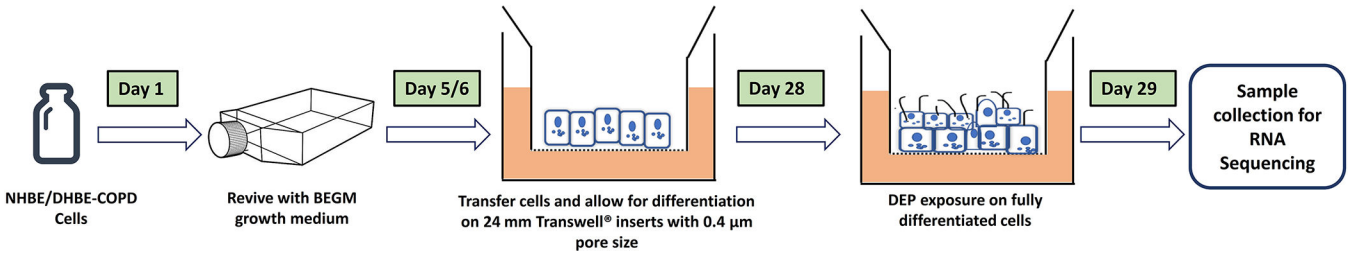

Figure 1:

A schematic diagram for the timeline of cell culture, ALI differentiation, and the subsequent DEP exposure.

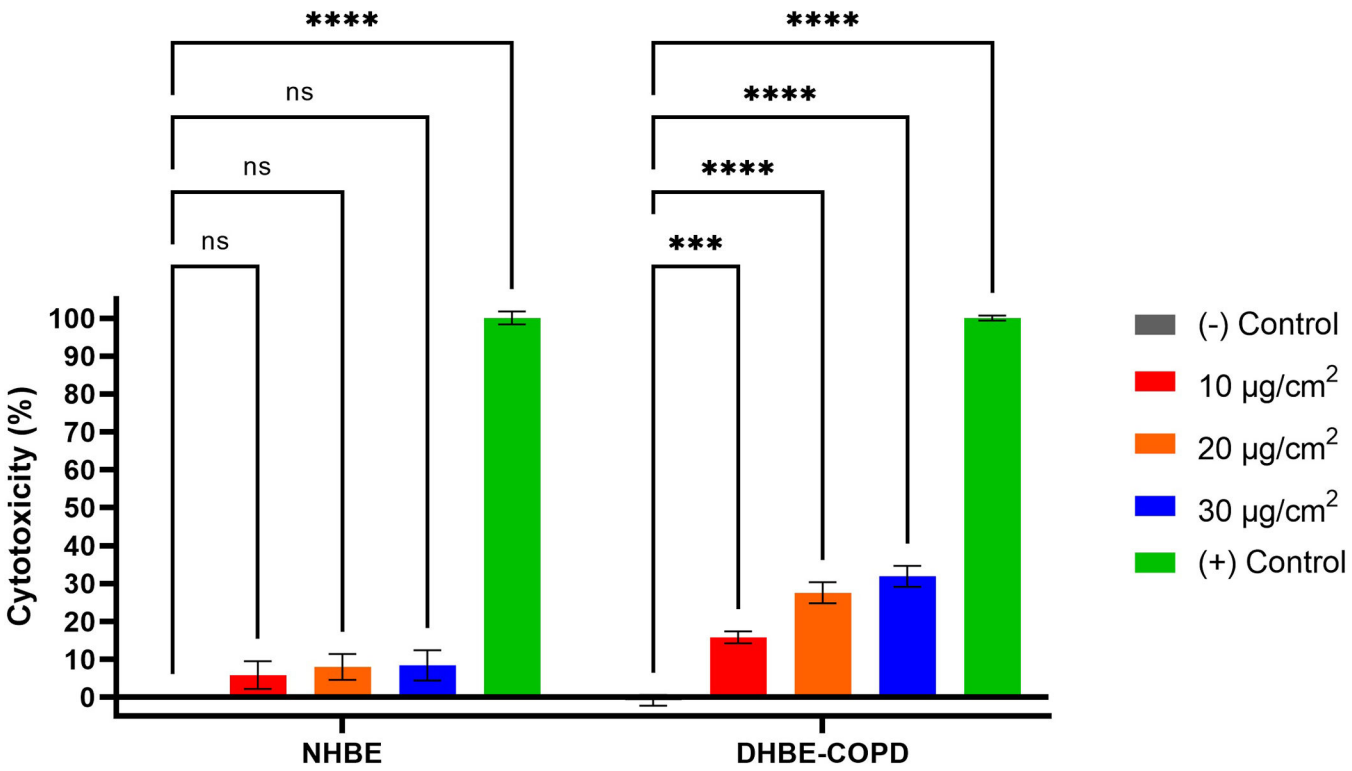

Figure 2: Cytotoxicity in NHBE and DHBE-COPD cells following DEP exposure.

Cells were exposed to DEP at concentrations of 10 µg/cm^2, 20 µg/cm^2, and 30 µg/cm^2 for 24 h. LDH release was used to calculate the percentage of cytotoxicity compared to negative controls of unexposed cells maintained in cell media and positive controls treated with Triton X-100 (0.1% v/v). To determine statistical significance in comparison to the negative controls, two-way ANOVA was used; ns: not significant ($p > 0.05$), *** ($p < 0.001$), and **** ($p < 0.0001$).

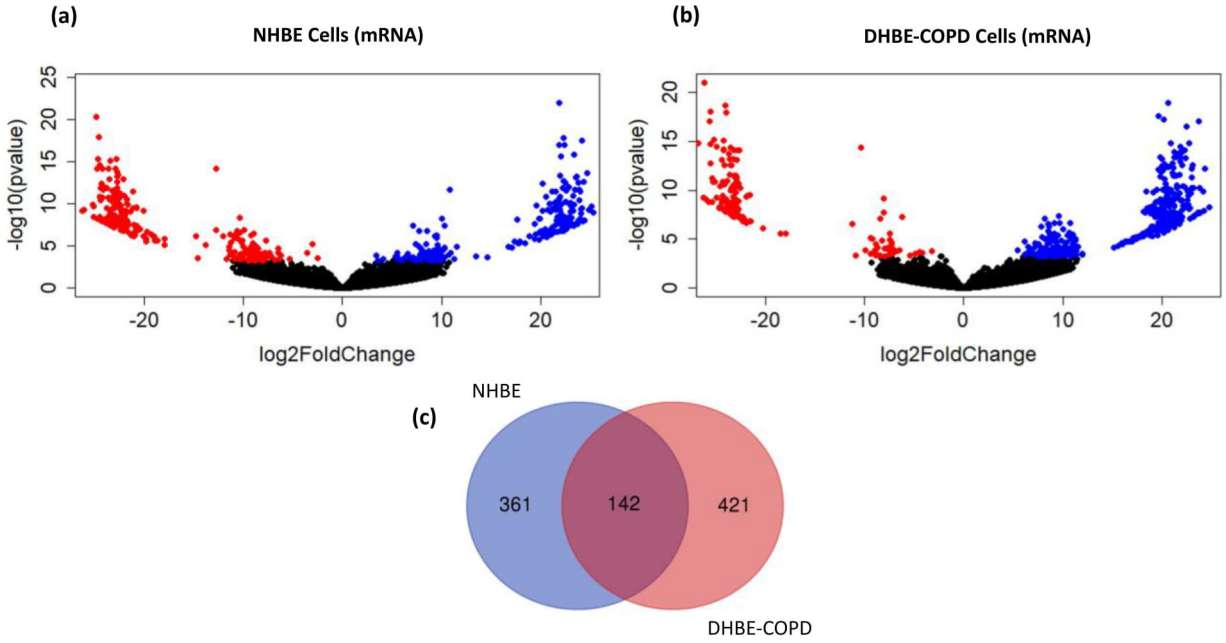

Figure 3: Differential expression of mRNAs (DE mRNAs) in NHBE and DHBE-COPD cells.

DE mRNA volcano plots in (a) NHBE and (b) DHBE-COPD cells after DEP exposure. The *X*-axis represents the log 2-fold change, while the *Y*-axis represents the adjusted *p* values: −log 10 (padj). The blue dots represent significantly upregulated mRNAs, while red dots represent significantly downregulated mRNAs. Non-differentially expressed mRNAs are represented by black dots. (c) A Venn diagram depicts the number of distinct and common (overlapping) DE mRNAs in NHBE and DHBE-COPD cells.

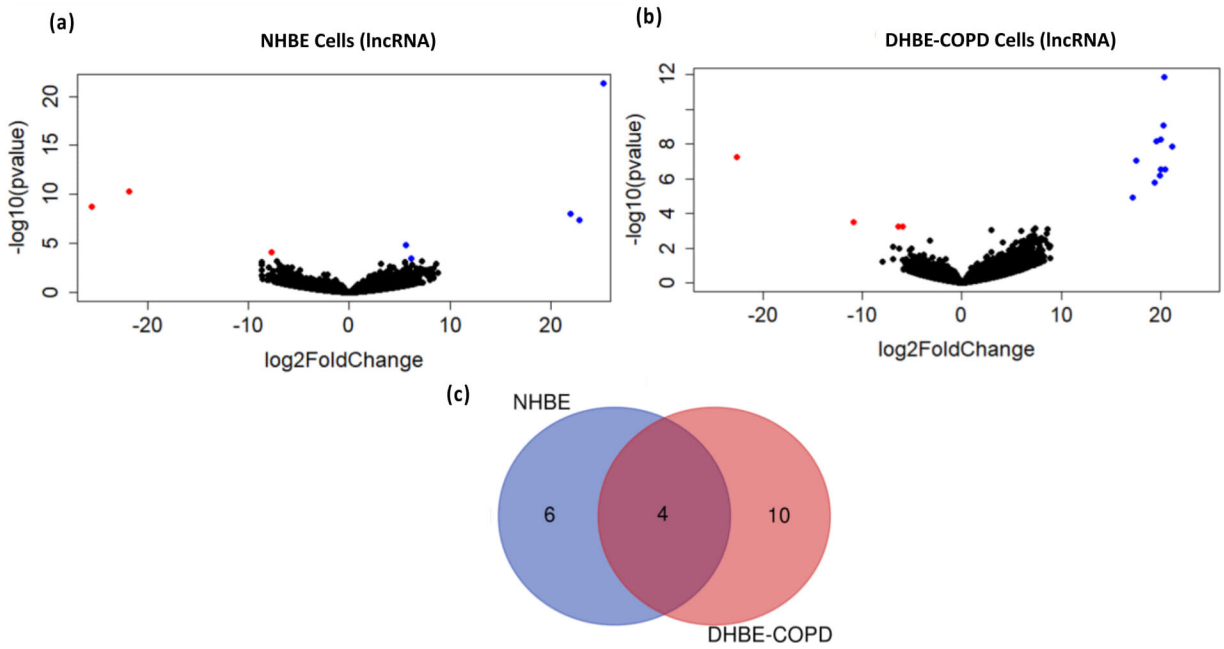

Figure 4: Differential expression of lncRNAs (DE lncRNAs) in NHBE and DHBE-COPD cells.

DE lncRNA volcano plots of in (a) NHBE and (b) DHBE-COPD cells after DEP exposure. The *X*-axis represents log 2-fold change, while the *Y*-axis represents the adjusted *p* values: −log 10 (padj). Blue dots represent significantly upregulated lncRNAs, while red dots represent significantly downregulated lncRNAs. Non-differentially expressed lncRNAs are represented by black dots. (c) A Venn diagram shows the number of distinct and common (overlapping) DE lncRNAs in NHBE and DHBE-COPD cells.

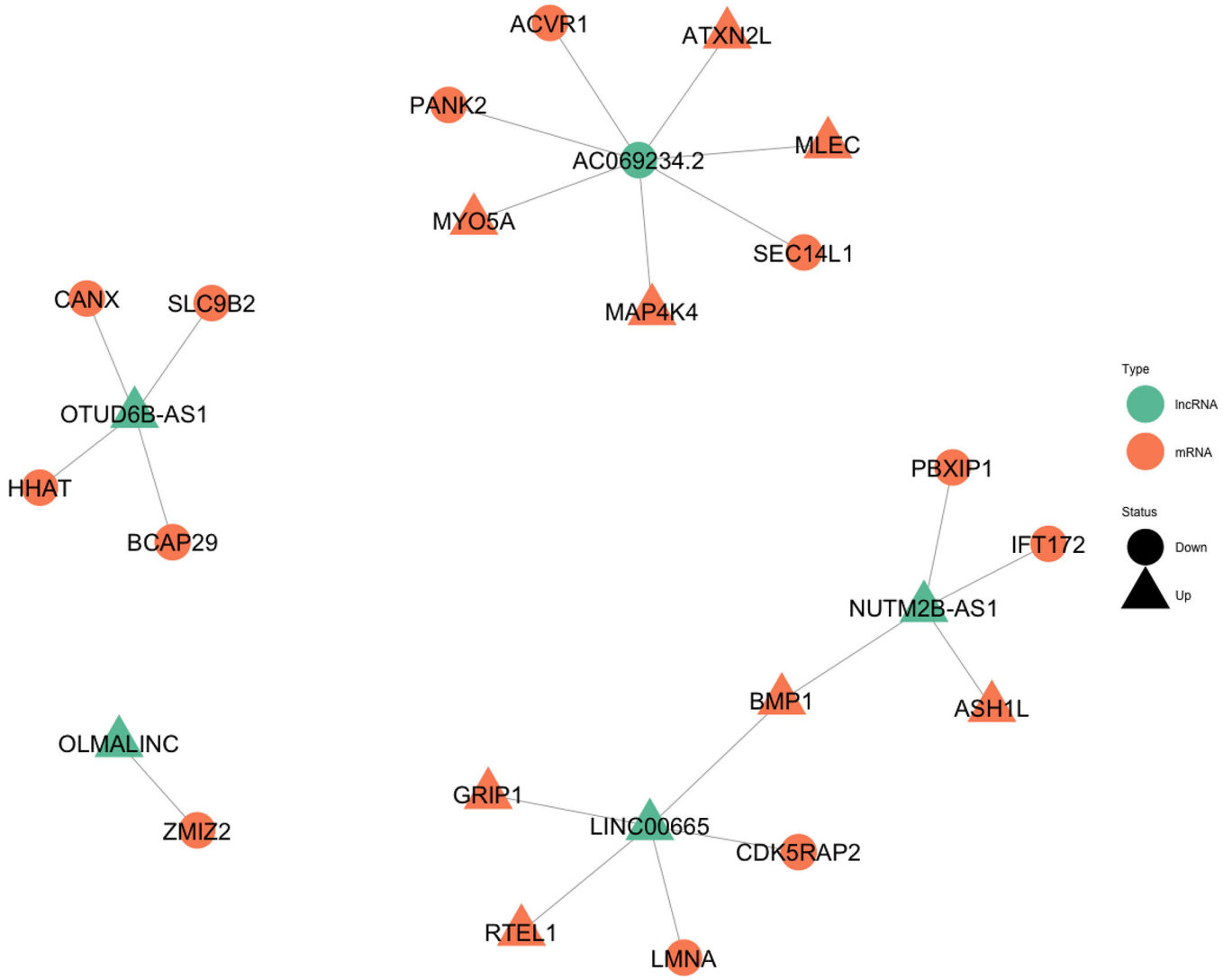

Figure 5: Predicted *trans*-targeted genes (log2FC>|±1|) and regulatory network of DE lncRNAs in NHBE cells.

The DE lncRNAs regulatory network was built for the NHBE cells using the R package (version 3.6.3). The colors represent the different types of RNAs; orange: mRNA, and green: lncRNA. The triangles represent upregulation, while the dots represent downregulation.

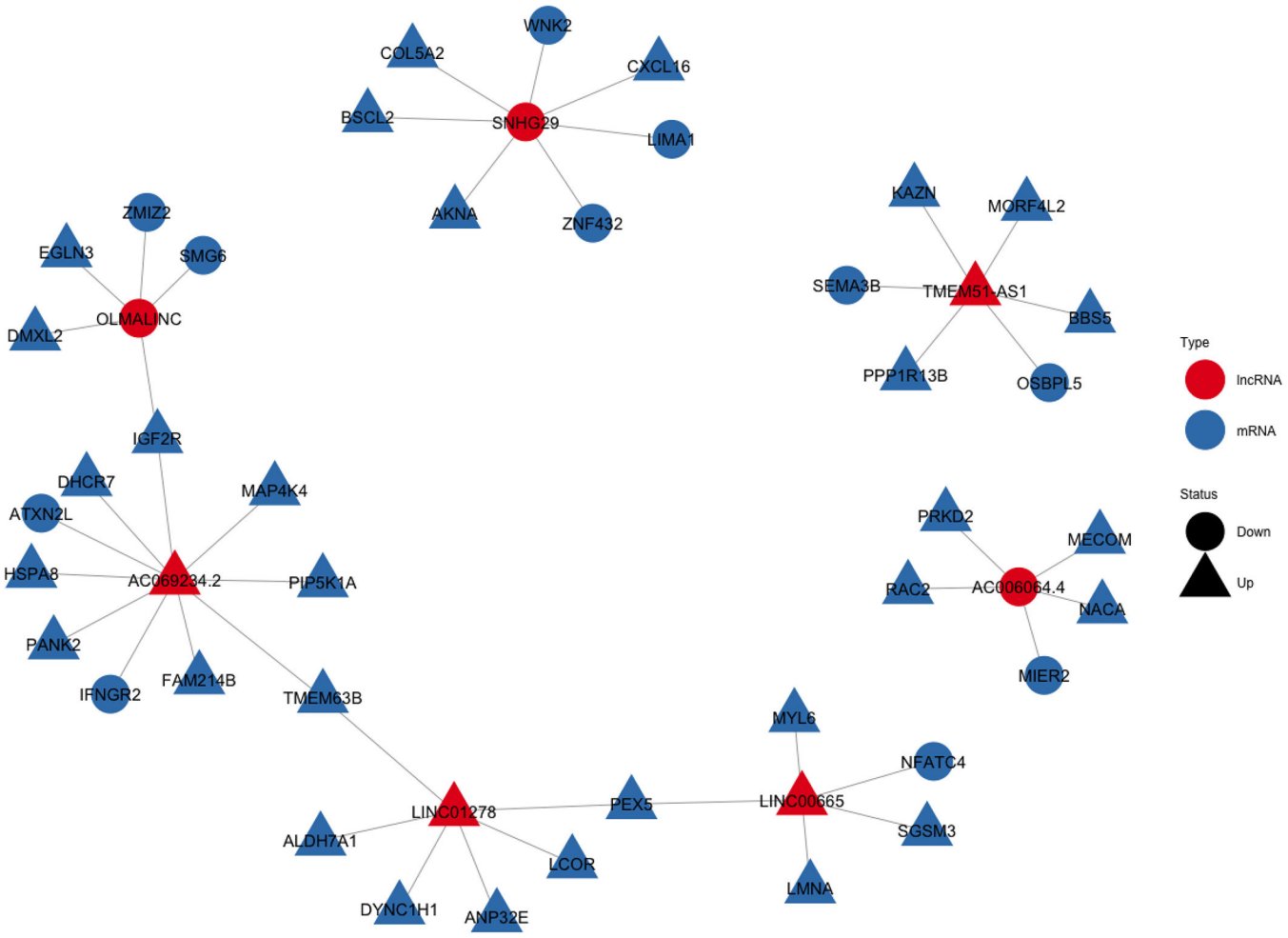

Figure 6: Predicted *trans*-targeted genes (log2FC>|±1|) and regulatory network of DE lncRNAs in DHBE-COPD cells.

The DE lncRNA regulatory network was constructed for DHBE-COPD cells using the R package (version 3.6.3). The colors represent the different types of RNAs; blue: mRNA, and red: lncRNA. The triangles represent up-regulation, while the dots represent downregulation.

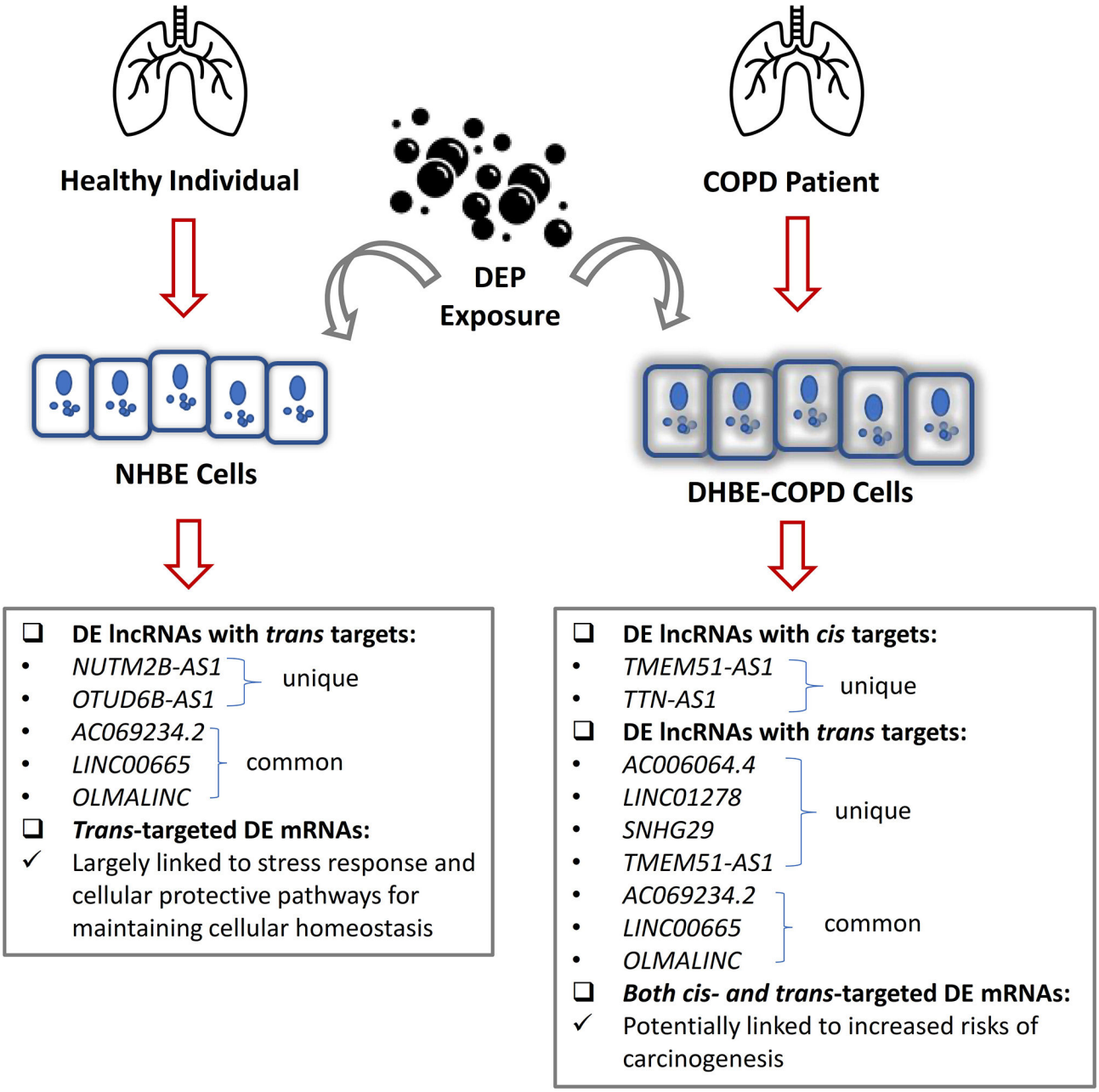

Figure 7:
A schematic diagram of DEP exposure-mediated health outcomes in the NHBE and DHBE-COPD cells.