Respiratory Research

RESEARCH Open Access

The different response of $PM_{2.5}$ stimulated nasal epithelial spheroids in control, asthma and COPD groups

Paulina Misiukiewicz-Stępień[1], Elwira Zajusz-Zubek[2], Katarzyna Górska[1], Rafał Krenke[1] and Magdalena Paplińska-Goryca[1*]

Abstract

Background Pathobiology of asthma and chronic obstructive pulmonary disease (COPD) is associated with changes among respiratory epithelium structure and function. Increased levels of $PM_{2.5}$ from urban particulate matter (UPM) are correlated with enlarged rate of asthma and COPD morbidity as well as acute disease exacerbation. It has been suggested that pre-existing pulmonary obstructive diseases predispose epithelium for different biological response than in healthy airways. The aim of this study was to assess the impact of $PM_{2.5}$ on the biological response of healthy as well as asthma and COPD respiratory epithelium using 3D/spheroid culture model.

Methods The spheroids from 5 healthy controls, 8 asthma patients, and 8 COPD patients were exposed to 100 µg/ml of $PM_{2.5}$ for 24 h.

Results The common pattern for healthy asthma and COPD epithelium inflammatory response to $PM_{2.5}$ stimulation include the increase in IL-1β, IL-6, IL-8 mRNA expression, and secretion of IL-6. Asthmatic spheroids produced higher amount of TNF-α and IL-8, whereas COPD spheroids expressed increased mRNA level of MUC5AC and decreased level of MMP7. $PM_{2.5}$ treatment induced changes in AHR and TLR4 expression on secretory epithelium in COPD.

Conclusion The response of airway epithelium to air pollution is different in healthy people than in obstructive lung disease patients. The impairment of airway epithelium in asthma and COPD changes their response to toxic environmental stimuli. This physiological dysfunction might be associated with diseases exacerbation of obstructive lung diseases.

Keywords Air pollution, Epithelium, Spheroids, Inflammation, Asthma, COPD

*Correspondence:
Magdalena Paplińska-Goryca
magdalena.paplinska@wum.edu.pl
[1]Department of Internal Medicine, Pulmonary Diseases and Allergy, Medical University of Warsaw, Banacha 1a, Warsaw 02-097, Poland
[2]Faculty of Energy and Environmental Engineering, Department of Air Protection, Silesian University of Technology, Gliwice, Poland

Introduction

Ambient air pollution, encompassing elevated concentrations of particulate matter (PM), constitutes a significant environmental threat to public health, contributing to 4.2 million deaths worldwide each year [1]. Although a discernible decrease in ambient air $PM_{2.5}$ concentrations has been observed across many European nations over the past thirty years, these reductions remain inadequate [2, 3]. The primary origins of $PM_{2.5}$ stem predominantly from anthropogenic sources such as fossil fuel combustion and vehicular traffic [4]. The deleterious impact of these pollutants on human health is associated their chemical composition and particle size.

Short-term exposure to $PM_{2.5}$ triggers acute mucus hypersecretion in airways enhancing particle entrapment efficiency. Prolonged exposure induces additional changes with significant shifts in epithelial cell types and its phenotype alterations [5]. A significant correlation between increased levels of air pollution and heightened morbidity and mortality risk, especially among people with obstructive pulmonary diseases has been shown [6, 7]. This is closely related to the pathogenesis of chronic obstructive pulmonary disease (COPD) and the fact that exposure to airborne dust intensifies oxidative stress in the airways culminating in cellular DNA damage [8]. What is more, smokers are more susceptible to airborne particulate matter in comparison to non-smokers [9]. Long-term exposure to harmful particles, including $PM_{2.5}$, contributes to a progressive decline in lung function and the development of emphysematous lesions that significantly compromise the quality of life [10].

The effect of air pollution on the airway epithelium is an extensively studied topic. The most commonly employed experimental model include submerged monocultures of epithelial cells derived from immortalised cell lines as well as primary monocultures obtained from study subjects. In vitro studies have demonstrated that the primary mechanisms triggered by the exposure to airborne particles encompass the heightened inflammatory response, oxidative stress and the disruption of intercellular junctions [11]. In recent years, a multitude of studies have embraced an advanced epithelial culture model known as the air-liquid interface (ALI) for investigation of intercellular interactions involving immune or structural cells [12]. In our recent study we harnessed ALI-cultured nasal epithelium with monocyte derived cells (dendritic cells and macrophages) revealing the activation of several novel mediators (*TIPARP*, *BPIFA2*, *ENPEP*) involved in epithelial response to airborne particles [13]. The latest frontier in cell culture, continually evolving and gaining traction in toxicological and disease studies, involves 3D spatial cultures like as spheroids [14, 15]. Those 3D cultures provide an avenue to delve into structural and functional changes, as well as cell-to-cell signalling that closely mimics tissue-level interactions. They broaden the horizons of in vitro cultures for airborne pollution research.

The objective of the current study was to evaluate the impact of $PM_{2.5}$ on the biological response of healthy as well as asthma and COPD afflicted respiratory epithelium using 3D/spheroid culture model. The morphology, expression of inflammatory mediators, structural proteins as well as ROS production were evaluated in control, asthma and COPD epithelial spheroid after 24 h $PM_{2.5}$ treatment. Gaining insights into the pathophysiological alterations within the airway epithelium during asthma and COPD holds the potential to pinpoint therapeutic targets and better anticipate exacerbations of obstructive diseases triggered by exposure to air pollutants. We suggest that pre-existing pulmonary obstructive diseases predispose epithelium for different biological response than in healthy airways.

Materials and methods

General study design

This was an experimental study based on cell cultures of epithelial cells obtained from asthma, COPD patients, and healthy subjects. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/58/A2021) and informed written consent was obtained from all the participants.

Patient characteristics

The study involved 5 controls, 8 asthma patients, and 8 COPD patients. In all patients, the diagnosis of asthma or COPD was previously established according to the current recommendations of the Global Initiative for Asthma (GINA) and the Global Initiative for Chronic Obstructive Lung Disease (GOLD), respectively. Inclusion criteria for COPD group were as follows: (1) the age of 40 years and over, (2) diagnosis of COPD based on the past medical history, history of smoking ≥ 10 packyears, typical signs and symptoms and irreversible airway obstruction found in spirometry (z-score of the post-bronchodilator FEV1/FVC below − 1.645). Inclusion criteria for asthma were as follows: (1) the age of 18 years and over, (2) diagnosis of asthma based on past medical history, typical signs and symptoms and demonstration of variable expiratory airflow limitation. The exclusion criteria included: (1) severe asthma or severe COPD, (2) unstable or uncontrolled disease (3) a concomitant COPD and asthma diagnosis, or any other chronic or acute lung disease, autoimmune and haematological diseases, malignancies, severe cardiovascular diseases (4) treatment with systemic or nasal corticosteroids within 4 weeks before the study enrolment (5) asthma, or COPD exacerbation within 3 months from mucosal sampling as well as symptoms of respiratory tract infection in the preceding 3 months. The control

group consisted of non-smoking participants, with normal spirometry, chronic diseses and without symptoms of respiratory tract infection in the preceding 3 months.

Cell culture

Epithelial cells were obtained by nasal brushing (Cytobrush Plus GT, CooperSurgical), cultured to 80% of confluency in Airway Epithelial Growth Medium (PromoCell, Germany) with antibiotics (Thermo Fisher Scientific) and then seeded into sterile plastic T25 bottles (Thermo Fisher Scientific).

The spheroid culture was performed according to methodology described before [16]. Matrigel Basement Membrane Matrix (354234, Corning) and Airway Epithelial Growth Medium (PromoCell) were mixed to obtain protein concentration at least 5 mg/ml, according to manufacturer protocol. 1.5-1.9 × 10^4 cells per single well were added and seeded into 24-well plate (Greiner Bio-One). The spheroids were cultivated in Matrigel on basal side of the well covered with 500 µl of Pneumacult Airway Seeding Medium (StemCell) with antibiotics. Seeding stage lasts for the first 4–6 days and afterwards the spheroids were differentiated for 21 days in Pneumacult Airway Differentiating Medium (StemCell). After full differentiation, the spheroids were exposed for 24 h to 50 µg $PM_{2.5}$/well (100 µg/ml) added directly into culture medium (apical side).

Spheroids were recovered from Matrigel according to the adapted manufacturer's protocol. Culture medium was removed, and 1 ml of cold gentle cell dissociation reagent (StemCell) was added into every single well. Plates were put on tilting platform with agitation 30 rpm for 30 min on ice. Matrigel domes were triturated with 1 ml pipette tip pre-rinsed in anti-adherence rinsing solution (StemCell) and transferred into pre-rinsed 15 ml tube (Corning). Tubes were put on ice and tilted for 30 min, 30 rpm. Tubes were placed in stand and left for 10 min to settle by gravity, supernatants were aspirated, and spheroids were washed with 5 ml of PBS and gently centrifuged (60 rpm, 5 min, 4 °C). The diameter of 20 spheroids of each group were measured three times based on the spheroid width on the image (using ImageJ software). The scale was set according to the microscope image scale bar.

Particle preparation

Urban particulate matter was obtained from the Silesian University of Technology. The samples were collected with a low-volume PM sampler type PNS-15 (Atmoservice) 1.5 m above the ground, at a flow rate of 2.3m^3/ h in Gliwice during heating season. Daily $PM_{2.5}$ samples were collected continuously on high-purity quartz (SiO2) microfiber filters (QM-A Whatman) with 47 mm diameter. The filters were weighed, cut into small pieces, suspended in PBS; the particles were detached from filters by sonication, filtrated through strainers with 70 µm pores (Corning). The sediment of the particles was dried at 96 °C to dry mass, weighted, resuspended in PBS into stock solution containing 5 µg/µL $PM_{2.5}$, and autoclaved. Quartz microfiber filters without particles were processed the same way and constituted a control suspension for cell treatment.

$PM_{2.5}$ trace elements analysis

The QM-A filter was mineralized under high pressure and high temperature in a system for microwave mineralization in 8 mL HNO_3 and 2 mL H_2O_2 (Merck). Elemental concentrations of nine elements (Zn, Fe, Mn, Pb, Cd, Cu, Cr, Ni and Co) were analysed for each sample by atomic absorption spectrometry (Avanta PM, GBC Scientific Equipment Pty Ltd) [17, 18].

Cell viability

To evaluate viability of nasal epithelial cells treated with $PM_{2.5}$ lactate dehydrogenase (LDH) release was measured with Cytotox96 assay (Promega).

Immunofluorescence staining

Immunofluorescence staining was performed on 3D spheroid cell cultures. Spheroids were fixed with 4% paraformaldehyde at room temperature. Antigen retrieval was performed in 98 °C using citrate buffer pH6 for 20 min. Spheroids were then washed in 0.3 M glycine, permeabilized and blocked with 5% goat serum with 1% triton X-100 for 24 h (ThermoFisher Scientific). The blocking solution was aspired, spheroids were divided into two separate tubes. For half of the tubes, mouse monoclonal pan-cytokeratin antibody (cocktail which recognizes acidic (Type I or LMW) and basic (Type II or HMW) cytokeratins) (1:100) (Novus Biologicals) was added, while the other half received mouse monoclonal MUC5AC antibody (1:100) (Novus Biologicals). The addition took place overnight at room temperature with gentle agitation at 20 rpm. After washing, second primary antibodies rabbit polyclonal anti-alpha tubulin (1:150) (Abbexa) and rabbit polyclonal anti-tight junctions' protein 1 (1:150) (Novus Biologicals) were added respectively, overnight at room temperature with agitation 20 rpm. After triple washing, secondary antibody anti-mouse Alexa Fluor 488 (1:500, ThermoFisher Scientific) was added to all tubes for overnight at room temperature with agitation 20 rpm. Further, after washing, additional incubation of all tubes with secondary antibody anti-rabbit Alexa Fluor 555 (1:500, ThermoFisher Scientific) was carried out overnight at room temperature with agitation 20 rpm. Spheroids were then washed with PBS and transferred onto slides (Surgipath Snowcoar Pear Slides, Leica Biosystems). Slides were mounted

with Fluoroshield mounting medium with DAPI (Abcam) and images were captured using fluorescence microscope Nikon ECLIPSE Ts2R equipped with Hamamatsu ORCA-Flash4.0 camera and analysed with Nikon NIS-Elements and ImageJ software.

RT-qPCR

Total RNA was isolated from the cells using Tri reagent (Sigma Aldrich). The concentration and purity of isolated RNA was assessed by DU650 spectrophotometer (Beckman Coulter). cDNA synthesis was carried out with 1 µg of total RNA using cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher Scientific). cDNA amplification was performed in ABI-Prism 7500 Sequence Detector System (Applied Biosystems, Thermo Fisher Scientific). For quantitative PCR (qPCR) reaction 0.7 µl of cDNA was amplified in 14 µl PCR volume, containing a TaqMan master mix (Thermo Fisher Scientific). By real-time qPCR the expression of IL-1β, IL-6, IL-8, TNF-α, MMP7, MMP9, MUC5AC, BPIFA2, ENPEP TIPARP, TGF-β and EGF was quantified. 18 S rRNA served as a reference gene. Assay ID of primers or their sequence used for real-time qPCR are shown in Table S1. Relative quantification values were calculated by the $2^{-\Delta\Delta CT}$ method (7500 Software, Thermo Fisher Scientific), the unstimulated spheroids of each individual were used as a calibrator.

Protein concentration measurements

The levels of IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12p70 were measured in cell supernatants by BD Cytometric Bead Array (CBA) Human Inflammatory Cytokines Kit (BD Biosciences, Catalog No: 551811) according to manufacturer's procedure. As concentrations of IL-8 exceeded the kit's detection threshold, additional measurements for the cytokine concentrations were performed using ELISA (Thermo Fisher). The MMP-9 level was measured by Human MMP9 ELISA (Biorbyt) according to manufacturers' instruction. The sensitivity of kits was 20 pg/ml for CBA and 2 pg/ml for ELISA.

Flow cytometry analysis

After spheroid recovering from matrigel cells were dissociated into single cell suspension by Accutase (BD Biosciences) treatment at 37 °C, shaking at 1400 RPM for 15 min and twice PBS rinsing and centrifugation at 1800 RPM. The cell suspension was filtrated through strainers with 70 µm pores (Corning). Human TruStain FcX (Biolegend) (5 µl per 100 µl of sample) was added to block non-specific bindings. Cells were stained with antibodies against the surface binding molecules, for epithelial cells: Tetraspanin-8 (TSPAN8) (BV786, rat anti-human cat. no. 748226), CD49f (BV650, rat anti-human, cat. no. 563706), epidermal growth factor receptor (EGFR) (BV421, mouse anti-human, cat no. 566254), CD151 (BB700, mouse anti-huma, cat. no. 746219), Toll-like receptor 4 (TLR4) (BV605, mouse anti-human, cat. no. 743692), Aryl Hydrocarbon Receptor (AHR) (PE-CF594, mouse anti-AhR cat. no. 565790) (BD Biosciences), in BD Horizon Brilliant Stain Buffer (BD Biosciences), and incubated for 20 min in the dark at room temperature. After washing away, the reagents, the cells were fixed and permeabilizated using lysis buffer and permeabilizations solution 2 (BD Biosciences) then stained with intracellular marker: β-tubulin (Alexa Fluor 488 Mouse anti-β-Tubulin, Class III, cat. no. 560381) and Transforming Growth Factor beta-1 (TGF-β) (PE, mouse anti-huma, cat. no. 562339, BD Biosciences,) for 20 min in the dark.

Cells were analysed by flow cytometry using a FACSCelesta instrument (BD Biosciences) equipped with blue (488-nm), violet (405-nm), and red (640-nm) lasers. Unstained cells and compensation beads (BD Biosciences) were used to set voltages and create single stain negative and positive controls. Compensation was set to account for spectral overlap between the seven fluorescent channels used in the study. Samples were examined by side scatter area (SSC-A) versus forward scatter area (FSC-A), then using forward scatter height (FSC-H) versus FSC-A to select single cells, eliminating debris and clumped cells from the analysis.

The basal, secretory and ciliated epithelial cells were identified using markers CD49f, β-Tubulin, TSPAN described previously [19]. The epithelial cell subpopulations were defined as follows:

- Epithelial cells with basal phenotype: CD49f + β-Tubulin- TSPAN-.
- Epithelial cells with secretory phenotype: TSPAN + CD49f- β-Tubulin-.
- Epithelial cells with ciliated phenotype: β-Tubulin + TSPAN-CD49f-.

Reactive oxygen species (ROS) analysis

The ROS identification was performed using the total ROS assay kit (Invitrogen, Thermo Fisher) according to manufacturer's instruction. The cells were analyzed on FACSCelesta instrument (BD Biosciences) at 520 nm (FITC channel).

Statistical analysis

Statistical analyses were performed using Statistica 13.3 software (StatSoft Inc.) and GraphPad Prism software (version 9.3.1) (GraphPad Software). Data are presented as median and interquartile range (IQR) or number and percentage. Differences between continuous variables in 3 groups were tested using Kruskal-Wallis test and between 2 groups using nonparametric Mann–Whitney U test. Fisher's exact test was used to test the differences

Table 1 The study subjects' characteristics

	control (*n* = 5)	asthma (*n* = 8)	COPD (*n* = 8)	*p*-value
Age (years)	44.0 (27.0-51.5) #	55.0 (38.0–74.0)	68.0 (64.0–74.0) #	0.008
Gender (F/M)	4 F/1 M	6 F/2 M	3 F/5 M	0.192
BMI (kg/m^2)	23.0 (20.8–24.7)	26.9 (23.0-28.7)	28.0 (23.8–31.7)	0.0668
Atopy (n)	2	5	0	0.014
Smoking exposure (pack-years)	0.0 (0.0–0.0) #	0.0 (0.0-0.8) &	35.5(24.8–50.0) #, &	< 0.0001
FEV_1 (% predicted)	103.0 (94.0-124.5) #	82.0 (75.0–87.0)	65.5 (60.3–82.0) #	0.002
FEV_1/VC (%)	87.1 (79.7–102.0) #	85.0 (73.0–91.0) &	57.2 (43.9–67.0) #, &	0.0004
FeNO (ppb)	15.9 (13.9–20.9)	29.7 (26.8–42.2)	26.1 (18.7–48.6)	0.05
ACT (points)	n.a.	23.0 (19.0–23.0)	n.a.	n.a.
ICS treatment (n)	0	4	0.0 (0.0–0.0)	n.a.
CAT (points)	n.a.	n.a.	6.5 (5.8–10.5)	n.a.
mMRC (points)	n.a.	n.a.	1.0 (1.0-2.5)	n.a.

Data are presented as median (IQR) or n. ACT - asthma control test, BMI – body mass index, FeNO - fractional exhaled nitric oxide FEV_1 - forced expiratory volume at first second, ICS- inhaled corticosteroids, mMRC - modified Medical Research Council, N.A. - not applicable, VC - vital capacity. Kruskal Wallis or Chi square test. *Control vs. asthma; # control vs. COPD; &asthma vs. COPD

Table 2 Characteristic of epithelial subpopulation in control, asthma and CODP spheroids

	basal	ciliated	secretory	rest
control	58.2 (54-61.7)	8.5 (8-9.6)	20.2 (18.1–23.3)	24.4 (22.8–26.3)#
asthma	58.1 (41-66.9)	8.4 (6.1–12.8)	23.8 (16.5–24.8)	27.8 (17.6–37.1)
COPD	43.6 (43.1–50.9)	9.54 (7.3–10.2)	24.6 (19.2029.50	35.9 (29.6–37.7)#

Data are presented as median (IQR), # control vs. COPD;

between nominal variables. A p-value of less than 0.05 was taken as the threshold of statistical significance.

Results

The characteristic of study participants

The basic clinical characteristics of patients and controls are summarized in Table 1. The study participants did not differ according to gender distribution and BMI. The COPD patients were older and had the worse spirometry test results.

PM sample characterization

The concentrations of heavy metals in PM are shown in Table S2. Of all analyzed heavy metals UPM used in experiments had the highest concentration of Fe and Zn.

The spheroid morphology analysis and immunostaining characteristics

The median diameter of epithelial spheroids after 21 days of cultivation in control group was 117.4 μm (100–132.6 μm), 126.1 μm (108.7–150 μm) in asthma and 130.4 μm (106.4–144.7 μm) in COPD group. The example morphology of 21-day cultured spheroids is shown in Figure S1. The characteristic of epithelial subpopulations of control, asthma and COPD spheroids is presented in Table 2. The major spheroid subpopulation were basal epithelial cells followed by secretory epithelial cells. Ciliated cells were the less frequent spheroid epithelial cell subpopulation. We did not observe any changes in spheroid size after 24 h of $PM_{2.5}$ exposition.

The expression of cytokeratin, α-tubulin, MUC5AC, tight junction protein-1 (ZO-1) was confirmed in immunostaining in all evaluated groups, its distribution in spheroids with and without $PM_{2.5}$ treatment is presented on Figs. 1 and 2. The presence of UPM after 24-hour treatment was visualized in immunostained epithelial spheroids.

Cytotoxic analysis in control, asthma and COPD epithelial spheroids after $PM_{2.5}$ treatment

24 h 100 μg/ml stimulation of $PM_{2.5}$ did not induce cytotoxicity in spheroid cultures (Fig. 3).

The effect of $PM_{2.5}$ on cytokines' and mediators' mRNA expression in control, asthma and COPD epithelial spheroids

$PM_{2.5}$ stimulation increased IL-1β, IL-6, and IL-8 mRNA expression in all evaluated groups (Fig. 4). Interestingly, the expression of IL-1β, IL-6, and TNF-α in $PM_{2.5}$ treated spheroids was higher in control group than in asthmatics. $PM_{2.5}$ stimulation upgraded MMP9, TNF-α, BPIFA, ENPEP, TIPARP mRNA expression in control and COPD groups. Additionally, we found the decreased MMP7 and enhanced MUC5AC mRNA expression after $PM_{2.5}$ treatment in COPD spheroids only. We found the increase of EGF and TGF-β mRNA expression after $PM_{2.5}$ treatment in control epithelial spheroid only. Fold change of mRNA expression of EGF in $PM_{2.5}$ treated spheroids was higher in control comparted to asthma and COPD, whereas TGF-β mRNA changes were higher in control group compared to COPD.

The effect of $PM_{2.5}$ on cytokine proteins' secretion by control, asthma and COPD epithelial spheroids

Similarly, to mRNA level, the IL-6 secretion by $PM_{2.5}$ treated spheroids was increased compared to unstimulated cultures in all evaluated groups (Fig. 5). IL-1β concentration was higher in control and asthma group after $PM_{2.5}$ exposure whereas the higher level of IL-8 and TNF-α was found for $PM_{2.5}$ exposed asthmatic

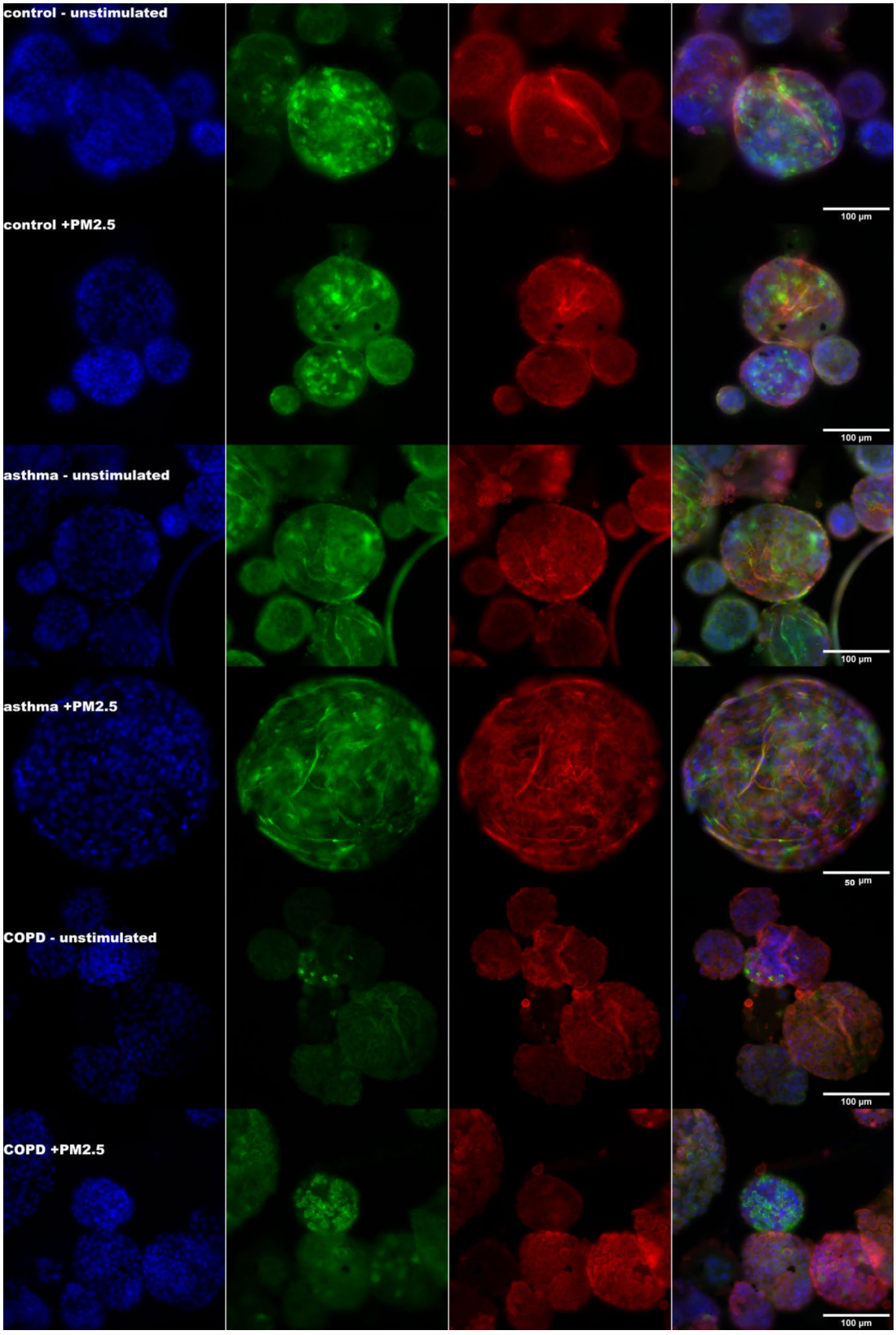

Fig. 1 DAPI (blue), α-tubulin (green), pan-cytokeratin (red) expression in epithelial spheroids of control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation

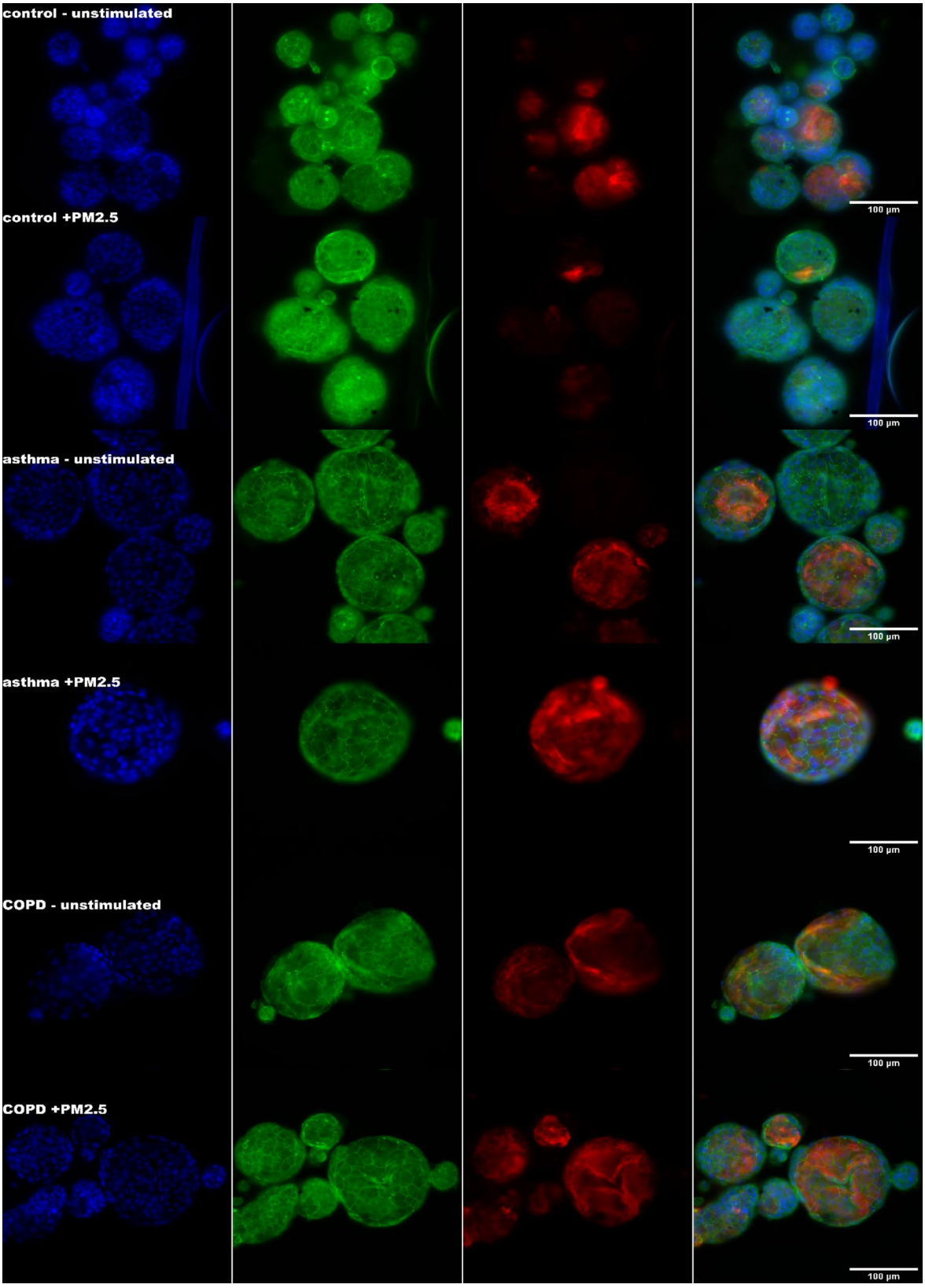

Fig. 2 DAPI (blue), tight junction protein-1 (ZO-1) (green), MUC5AC (red) expression in epithelial spheroids of control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation

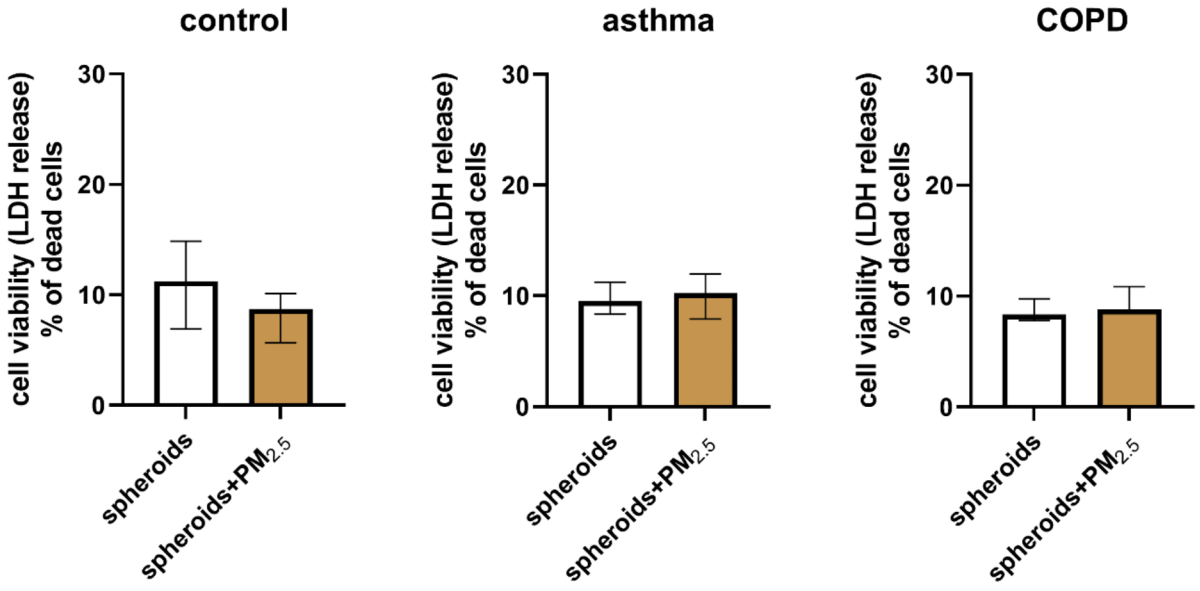

Fig. 3 Cell viability of epithelial cells spheroids from control, asthma and COPD groups. The data are presented as median (column) and IQR (whiskers)

spheroids only. We did not find the significant differences in MMP-9 protein level between $PM_{2.5}$ treated and untreated spheroids in any of investigated groups, however the concentration of MMP9 tended to be higher in $PM_{2.5}$ stimulated spheroids of asthma (136723 pg/ml (124520–184224 pg/ml) vs. 158296 pg/ml (138452–178088 pg/ml) and COPD (88406 pg/ml (58292–157926 pg/ml) vs. 102394 pg/ml (68063–158367 pg/ml).

The impact of $PM_{2.5}$ on AHR, CD151, TLR4, EGFR, TGF-β expression in epithelial spheroids of control, asthma and COPD groups

The cytometric analysis revealed the highest expression of AHR (compared to controls), TLR4, EGFR (compared to both control and asthma group) on unstimulated spheroids from COPD patients (Fig. 6). $PM_{2.5}$ treated spheroids from COPD patients expressed more TLR4 (than controls) and EGFR (than asthmatics). The 24 h $PM_{2.5}$ stimulation decreased AHR expression on epithelial cells in COPD group only.

The detailed analysis of the expression of AHR, CD151, TLR4, EGFR, TGF-β on different epithelial cells phenotypes is shown on Figure S2 and Table S3. AHR expression was found mainly on basal and secretory epithelial cells in all groups but not on ciliated epithelial cells from controls. We observed the decreased AHR expression on secretory epithelial cells in COPD group after $PM_{2.5}$ exposure. From all evaluated epithelial phenotypes, secretory epithelial cells were characterized by the highest amount of CD151 + cells in all groups, however no significant changes were found for CD151 expression with or without $PM_{2.5}$ stimulation. Interestingly, TLR4 was expressed exclusively on secretory epithelial cells in control, asthma, and COPD groups in similar level. $PM_{2.5}$ treatment decreased the number of TLR4 + secretory epithelial cells in COPD. The number of EGFR + ciliated epithelial cells tended to rise after $PM_{2.5}$ stimulation in control group, but this observation did not reach the statistical significance. The highest level of TGF-β + cells was observed for unstimulated basal, secretory, and ciliated epithelial cells of COPD patients.

ROS analysis in control, asthma and COPD epithelial spheroids after $PM_{2.5}$ stimulation

We showed the elevated level of ROS (expressed as % of control) after $PM_{2.5}$ exposure in asthma and COPD groups in contrast to controls where such increase was not found (Fig. 7).

Discussion

Ambient air pollution is ranked as the seventh leading factor contributing to worldwide burden of disease (in 2019). Numerous toxicological and epidemiological studies have been conducted to investigate the link between ambient air pollution and its impact on human health, in particular on respiratory system [20, 21]. Here, we performed the complex analysis of epithelial response of healthy, asthmatic and COPD spheroids, 3D cell culture

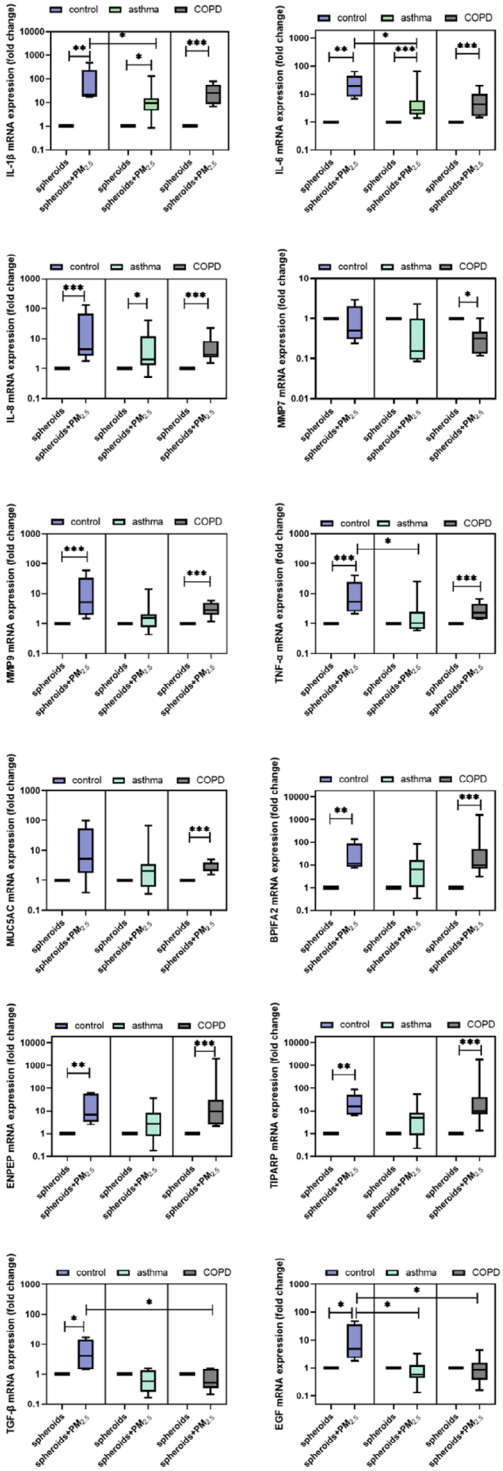

Fig. 4 IL-1β, IL-6, IL-8, MMP7, MMP9, TNF-α, MUC5AC, BPIFA2, ENPEP, TIPARP, TGF-β and EGF mRNA expression in epithelial spheroids of control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation. The unstimulated spheroids were used as a calibrator individually in control, asthma and COPD groups. The data are presented as median (line), IQR (box) and 5–95 percentile (whiskers). The significant changes are marked as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

system after the exposure to air pollution. We found that common (demonstrated in healthy and diseased epithelium) inflammatory response to $PM_{2.5}$ stimulation include the increase in IL-1β, IL-6, IL-8 mRNA expression, and secretion of IL-6. Asthmatic spheroids produced a higher amount of TNF-α and IL-8, whereas COPD spheroids expressed increased mRNA level of MUC5AC and decreased level of MMP7. COPD epithelium was characterized by higher expression of AHR, TLR4 and EGFR on cells. We found that $PM_{2.5}$ treatment induced changes in AHR and TLR4 expression on secretory epithelium in COPD. The response of asthmatic and COPD but not control spheroids to $PM_{2.5}$ exposition was associated with ROS accumulation in epithelial cells. The results of our study might help to understand the substantial processes associated with air pollution toxicity and its impact on healthy and impaired epithelial function.

The results of our study showed that the most unique inflammatory marker of epithelial response to $PM_{2.5}$ exposition was IL-6. This finding is in line with other studies [22, 23]. The classic interpretation of epithelial derived IL-6 involvement in response to air pollution includes its role as immune system activator. This cytokine induces alveolar macrophages for pro-inflammatory response and attracts neutrophils as well as monocytes into the airways, which might cause local tissue injury. Interestingly, we found the highest amount of pro-inflammatory cytokines (especially IL-6) released from control spheroids after $PM_{2.5}$ treatment. Our previous study where ALI epithelial cells co-cultivated with macrophages and dendritic cells were used showed different results: the strongest inflammatory response was attributed to asthma and COPD groups. The discrepancy in observations is probably due to different in vitro models used in these studies. It seems that epithelial interactions with immune cells encourage these cells for stronger inflammatory response to environmental stimuli in obstructive lung diseases. It is worth to notice that IL-6 as a pleiotropic cytokine may also protect lungs exposed to toxic air pollutants from local injury. It has been shown that IL-6 inhibit the TGF-β-mediated differentiation of naïve CD4 + T-cells into regulatory T-cells (T_{reg}), which inhibit autoimmunity and protect against tissue injury [24]. Moreover, also IL-1 and TNF-α signaling was reported to be involved in regeneration processes after tissue disruption caused by various injuries [25]. The balance between pro- and anti-inflammatory properties of cytokines depends on multiple factors such as dose and duration of stimuli, cytokine level and cell-cell co-interactions.

The results of our study clearly show the differential response of asthmatic and COPD epithelium to $PM_{2.5}$ exposure than in healthy subjects. The dysfunction of

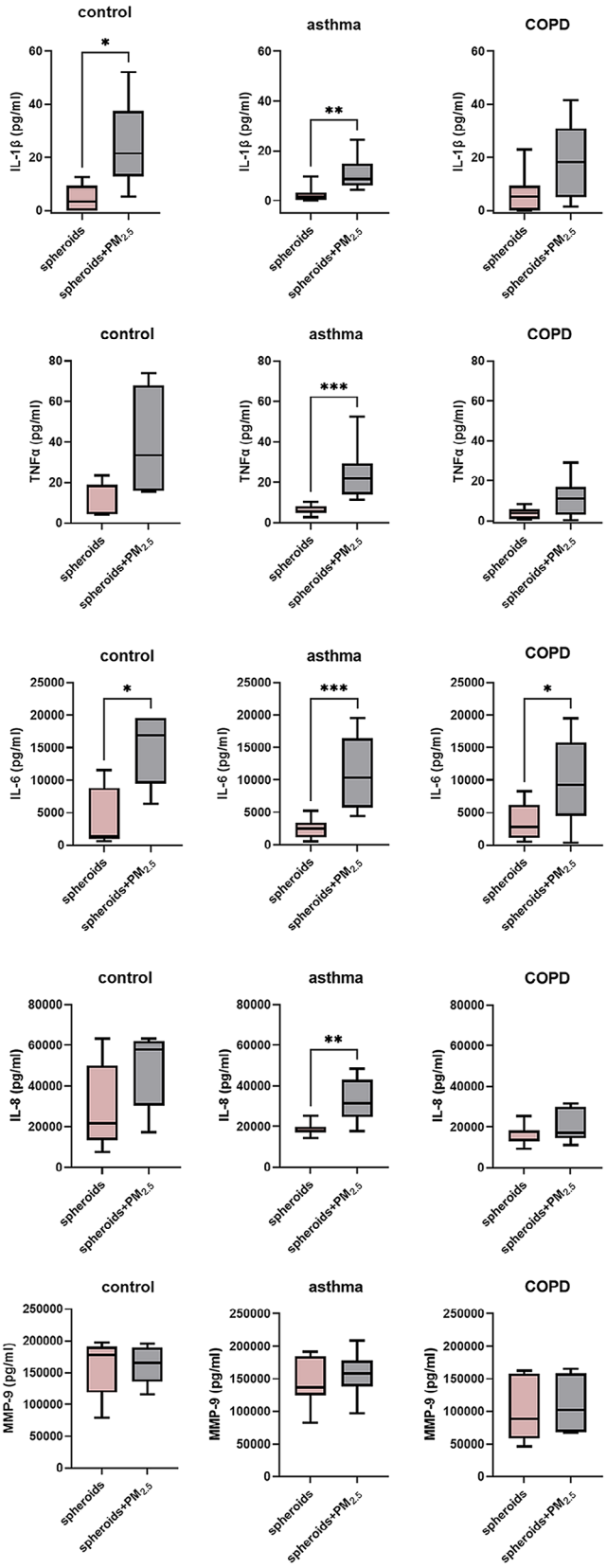

Fig. 5 IL-1β, TNF-α, IL-6, IL-8 and MMP-9 secretion by epithelial spheroids of control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation. The data are presented as median (line), IQR (box) and 5–95 percentile (whiskers). The significant changes are marked as * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

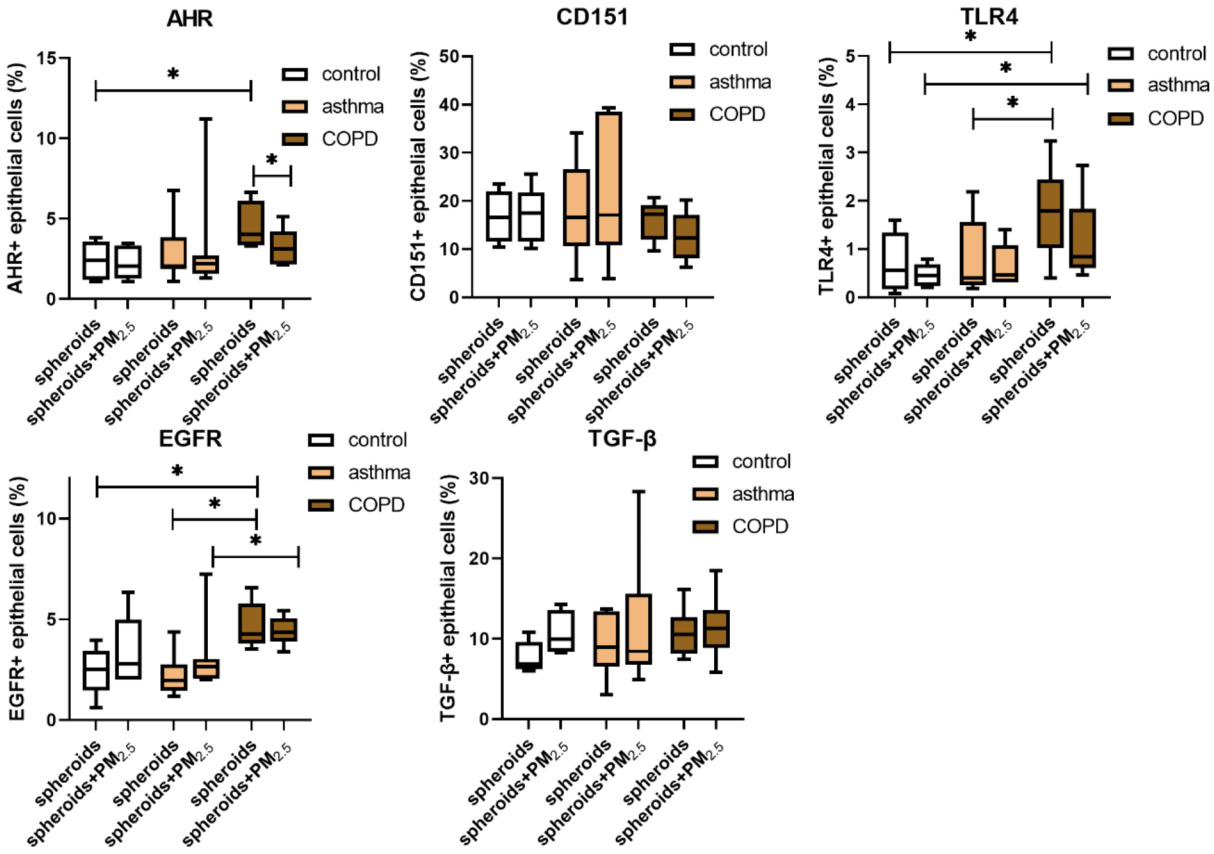

Fig. 6 AHR, CD151, TLR4, EGFR, TGF-β expression in epithelial spheroids of control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation. The data are presented as median (line), IQR (box) and 5–95 percentile (whiskers). The significant changes are marked as * $p \leq 0.05$

epithelial cells is an important feature of asthma and COPD pathogenesis. We found two diverse patterns of asthma and COPD epithelium response to air pollution stimuli than in healthy group. Firstly, asthmatic and COPD spheroids expressed and produced lower levels of inflammatory mediators than spheroids from healthy subjects. Secondly, distinct pattern of response which included changes in expression of different mediators of inflammation. These reactions might reflect the impaired function of airway epithelium in obstructive lung diseases. On particular note is the increased expression of MUC5AC in COPD spheroids following $PM_{2.5}$ stimulation. It is well known that air pollution exposition leads to loss of barrier integrity of the epithelium causing disruption of tight junctions and increased epithelial permeability [26]. This phenomenon was observed by other authors as downregulation of occludin, E-cadherin, claudin-1, and ZO-1 pathways [27, 28]. In our study, the fastest effect of short-term $PM_{2.5}$ stimulation was the increase in secretory cell MUC5AC expression, especially in COPD spheroids. This is consistent with the results of one recent study which showed that MUC5AC expression is strongly upregulated by $PM_{2.5}$ [29] as well as with observations that airway remodeling after the exposure to air pollution largely refers to metaplasia of mucus secretory cells and impaired function of ciliated cells. These functional changes might lead to decreased mucociliary clearance and increased mucus accumulation in the airways. The impaired mucus secretion from mucociliary epithelium upon PM exposure is probably disrupted by ROS independent mucus granule secretory pathway [30]. We also confirmed our previous results showing the increased expression of BPIFA2 (which encode antimicrobial defense proteins produced by secretory epithelial cells) after $PM_{2.5}$ stimulation [13] which in turn initiates a mucus secretory program in the airway epithelium. On the other hand, the observed phenomena might be associated with short term $PM_{2.5}$ exposure and should be evaluated using longer period of $PM_{2.5}$ treatment. It was shown that BEAS-2B produced various levels of e.g. IL-6 after 1 day or 16 days of $PM_{2.5}$ exposure [31]. As epithelial cells of patients with obstructive lung diseases

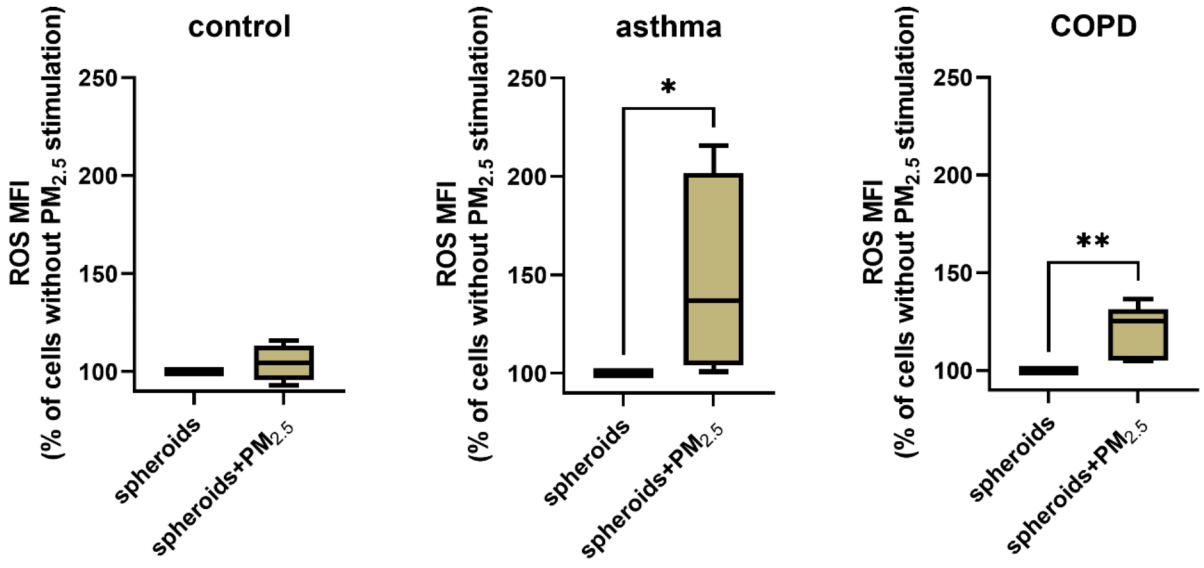

Fig. 7 Reactive Oxygen Species (ROS) level in epithelial spheroids from control, asthma, and COPD groups with or without $PM_{2.5}$ stimulation presented as % of unstimulated spheroids within each group. The data are presented as median (line), IQR (box) and 5–95 percentile (whiskers). The significant changes are marked as * $p \leq 0.05$; ** $p \leq 0.01$

are impaired their response might be considerably different after longer time of $PM_{2.5}$ stimulation. The results of our study suggest that COPD patients are more susceptible to epithelial remodeling changes after air pollution exposure.

The different phenotype of epithelium in obstructive airway diseases was confirmed by the results of the cytometric analysis. Of note is higher baseline expression of AHR, TLR4, EGFR on COPD epithelium. Moreover, COPD epithelium was characterized by increased TGF-β expression on basal, ciliated, and secretory cells compared to other groups. It seems that epithelium of COPD patients due to the advanced remodeling process after prolonged cigarette smoke exposure is linked to TGF-β and EGF signaling activation which are the important inducer of epithelial–mesenchymal transition (EMT) [32, 33]. The PCR results revealed that fold changes of TGF-β and EGF mRNA expression was higher in controls compared to asthma and COPD after $PM_{2.5}$ treatment, what suggest the impaired mRNA expression of these remodelling mediators in asthma and COPD after $PM_{2.5}$ stimulation. It is worth to notice that the results of PCR used in this study (analysed as $2^{-\Delta\Delta CT}$) did not reflect the exact quantity of mRNA level but its fold changes. Due to this discrepancy, it is difficult to compare the absolute number of mRNA copies of analysed mediators with e.g. flow cytometry results.

We believe that different profile of airway epithelium may impact the epithelial response to toxic substances such as $PM_{2.5}$ in COPD patients. In our study the number of AHR + epithelial cells was decreased after $PM_{2.5}$ stimulation in COPD group. Polycyclic aromatic hydrocarbons (PAHs) contained mainly in organic fraction of $PM_{2.5}$ functioned through AHR receptor. AHR is an important mediator of the toxicity of organic chemicals which activation involves the mitogen-activated protein kinase (MAPK), nuclear factor kappa b (NF-κB) pathways, oxidative stress and upregulates expression of inflammatory markers (IL-1β, TGF-β, IL-6 or IL-22, recruitment of neutrophils and eosinophils). It has been reported that $PM_{2.5}$ upregulated MMPs, IL-33 or VEGF production through AHR in airway epithelial cells [34–36]. The downregulation of AHR expression together with decreased level of inflammatory mediators after $PM_{2.5}$ exposure might represent the impaired function of airway epithelium in COPD patients. A similar surprising effect was described by Liu et al. The authors of this study used a murine COPD model and demonstrated a decreased degree of airway inflammation and emphysema as well as lower level of inflammatory cytokines (TNF-α, IFN-γ, IL-1β, IL-6) in BALf of $PM_{2.5}$ treated animals [36]. On the other hand, it should be kept in mind that in our experiments the water-soluble fraction of $PM_{2.5}$ which has low PAH content were applied. As we did not measure the PAHs concentration in PM suspension used for cell stimulation, the AHR expression changes after $PM_{2.5}$ exposition should be interpreted with caution.

Data suggest that air pollution may contribute to increased susceptibility of asthma and COPD patients to infectious and non-infectious disease exacerbations [37, 38]. Our study showed a lower number of TLR4 + secretory epithelial cells after $PM_{2.5}$ exposition in COPD spheroids. The prolonged exposure to air pollution including cigarette smoke, can lead to immune stimulation of the airway epithelium. Consequently, this trained immunity may result in a reduced response to subsequent PM stimulation. The reduction of pro-inflammatory TLRs on epithelial cells of COPD spheroids after $PM_{2.5}$ treatment might reflect its adaptive state to environmental stimuli in these patients. However, the immune response of epithelial cells to long-term $PM_{2.5}$ exposure could include different profile of biological reactions. To evaluate these responses a duration of $PM_{2.5}$ stimulation much longer than 24h is required.

We would like to acknowledge the limitations of this study, which are outlined as follows: (1) The use of nasal epithelial cells as easy to obtain surrogate of bronchial epithelial cells [39] may not reflect all biological phenomena occurring in lower airways after air pollution exposition. Nasal epithelial cells are not identical proxy for bronchial epithelial cells, it was shown that 619 genes were similarly and 1692 genes differently expressed in comparison of nasal and bronchial epithelial brushes [40]. On the other hand, nasal and bronchial epithelial cells presented the same pattern of response for toxic stimuli such as cigarette smoke. The gene expression profile of nasal epithelial cells identified inflammatory processes in the lower airways what was confirmed in asthma and might be used as non-invasive tool of asthma endotype characterization [41]. The nasal epithelial cells cultured in spheroids (contained different types of specialized epithelium) are functional equivalent for epithelial cells from lower parts of the respiratory tract. This study focused on comparison of healthy and diseased epithelium in response to $PM_{2.5}$ exposure, and evaluation of similar and different patterns of response to the stimuli. (2) 24-hour $PM_{2.5}$ stimulation reflects the short-time exposure to air pollution. It should be kept in mind that short and long term $PM_{2.5}$ exposure might differ in e.g. level of produced cytokines and cumulative effect of noxious and oxidative stress stimulation in epithelial cells. The observed biological response after 24-hour stimulation with high but still not-cytotoxic $PM_{2.5}$ dose illustrates the profile of early changes in airway epithelium. The prolonged air pollution exposure in in vitro model needs to be explored in the future. (3) The use of aqueous suspension of $PM_{2.5}$ did not fully reflect negative effect of air pollution on airway epithelium. For complex evaluation of this issue, the organic fraction of $PM_{2.5}$ (which contains higher amounts of PAHs and other water insoluble toxic substances) should be used. However, this fraction must be suspended in DMSO, which increases the cytotoxicity of stimulators and impacts the dose of $PM_{2.5}$ used for cell treatment.

Conclusions

The response of airway epithelium to air pollution is different in healthy people and obstructive lung disease patients, however some elements such as upregulation of IL-1β, IL-6, IL-8 mRNA expression, IL-6 secretion are common. The short term $PM_{2.5}$ stimulation impacted epithelial secretory cell function in COPD patients. The impairment of airway epithelium in asthma and COPD weakens their anti-oxidative and anti-inflammatory protection which might be associated with tissue damage. The effect of long term $PM_{2.5}$ exposure on epithelial cell remodeling and possible induction of asthma and COPD exacerbation should be investigated in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12931-025-03097-w.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
Concept and design: MPG, PMS, experiments, measurements, and data acquisition: PMS, MPG, EZZ, KG, data analysis: MPG, PMS, MPG, manuscript writing - review and editing, drafting the manuscript for important intellectual content: MPG, PMS, RK.

Funding
This study was funded by internal grant (1WU/1/M/MBM/N/21/21) of the Medical University of Warsaw. This work was supported by the Faculty of Energy and Environmental Engineering, Silesian University of Technology (statutory research).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

Statement of Ethics
All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This work has received approval for research ethics from Medical University of Warsaw Review Board (KB/58/A2021) and a proof/certificate of approval is available upon request. Informed consent was obtained from all individual participants included in the study.

Conflict of interest
RK reports personal fees and other from Boehringer Ingelheim, personal fees and other from Chiesi, personal fees and other from AstraZeneca, personal fees from Polpharma, outside the submitted work. KG reports personal fees for lectures and travel expenses from Boehringer Ingelheim, Chiesi, AstraZeneca, Polpharma and Roche, outside the submitted work. The other authors declare no conflict of interest.

Consent for publication
Not Applicable.

Received: 6 September 2024 / Accepted: 1 January 2025
Published online: 08 January 2025

References

1. Burnett R, Chen H, Szyszkowicz M, Fann N, Hubbell B, Pope CA, et al. Global estimates of mortality associated with long-term exposure to outdoor fine particulate matter. Proc Natl Acad Sci U S A. 2018;115:9592–7.
2. Juginović A, Vuković M, Aranza I, Biloš V. Health impacts of air pollution exposure from 1990 to 2019 in 43 European countries. Sci Rep. 2021;11:22516.
3. Commission Staff Working Document — Impact Assessment. European Commission. 2013; Available from: https://ec.europa.eu/environment/archives/air/pdf/Impact_assessment_en.pdf
4. Majewski G, Rogula-Kozłowska W, Rozbicka K, Rogula-Kopiec P, Mathews B, Brandyk A, Concentration. Chemical composition and origin of PM1: results from the First Long-Term Measurement Campaign in Warsaw (Poland). Aerosol Air Qual Res. 2018;18:636–654.
5. Aghapour M, Ubags ND, Bruder D, Hiemstra PS, Sidhaye V, Rezaee F et al. Role of air pollutants in airway epithelial barrier dysfunction in asthma and COPD. European Respiratory Review [Internet]. 2022 [cited 2022 Apr 28];31. Available from: https://err.ersjournals.com/content/31/163/210112
6. Jiang X-Q, Mei X-D, Feng D. Air pollution and chronic airway diseases: what should people know and do? J Thorac Dis. 2016;8:E31–40.
7. Orellano P, Quaranta N, Reynoso J, Balbi B, Vasquez J. Effect of outdoor air pollution on asthma exacerbations in children and adults: systematic review and multilevel meta-analysis. PLoS ONE. 2017;12:e0174050.
8. Barnes PJ. Oxidative stress-based therapeutics in COPD. Redox Biol. 2020;33:101544.
9. O'Beirne SL, Shenoy SA, Salit J, Strulovici-Barel Y, Kaner RJ, Visvanathan S, et al. Ambient Pollution-related reprogramming of the human small Airway Epithelial Transcriptome. Am J Respir Crit Care Med. 2018;198:1413–22.
10. Zhao J, Li M, Wang Z, Chen J, Zhao J, Xu Y, et al. Role of PM2.5 in the development and progression of COPD and its mechanisms. Respir Res. 2019;20:120.
11. Al-Rekabi Z, Dondi C, Faruqui N, Siddiqui NS, Elowsson L, Rissler J, et al. Uncovering the cytotoxic effects of air pollution with multi-modal imaging of in vitro respiratory models. R Soc Open Sci. 2023;10:221426.
12. Mertens TCJ, Karmouty-Quintana H, Taube C, Hiemstra PS. Use of airway epithelial cell culture to unravel the pathogenesis and study treatment in obstructive airway diseases. Pulm Pharmacol Ther. 2017;45:101–13.
13. Misiukiewicz-Stępien P, Mierzejewski M, Zajusz-Zubek E, Goryca K, Adamska D, Szeląg M, et al. RNA-Seq analysis of UPM-Exposed epithelium co-cultivated with macrophages and dendritic cells in Obstructive Lung diseases. Int J Mol Sci. 2022;23:9125.
14. Wang Q, Li Y, Hu P, Zhang Y, Liu Y, Yang Q, et al. Impact of enniatins and beauvericin on lipid metabolism: insights from a 3D HepaRG spheroid model. Environ Int. 2024;191:108969.
15. Aloisio GM, Nagaraj D, Murray AM, Schultz EM, McBride T, Aideyan L, et al. Infant-derived human nasal organoids exhibit relatively increased susceptibility, epithelial responses, and cytotoxicity during RSV infection. J Infect. 2024;89:106305.
16. Lee GY, Kenny PA, Lee EH, Bissell MJ. Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods. 2007;4:359–65.
17. Stacey B, Mathe F, Amodeo T. Ambient air - Standard gravimetric measurement method for the determination of the PM10 or PM2,5 mass concentration of suspended particulate matter: Norme européenne EN 12341:2014-07.
18. PN-EN 14902. 2010. Ambient air quality e standard method for the measurement of Pb, Pb, Cd, as and Ni in the PM10 fraction of suspended particulate matter.
19. Bonser LR, Koh KD, Johansson K, Choksi SP, Cheng D, Liu L, et al. Flow-Cytometric analysis and purification of Airway Epithelial-Cell subsets. Am J Respir Cell Mol Biol. 2021;64:308–17.
20. Manisalidis I, Stavropoulou E, Stavropoulos A, Bezirtzoglou E. Environmental and Health impacts of Air Pollution: a review. Front Public Health. 2020;8:14.
21. Juda-Rezler K, Zajusz-Zubek E, Reizer M, Maciejewska K, Kurek E, Bulska E, et al. Bioavailability of elements in atmospheric PM2.5 during winter episodes at Central Eastern European urban background site. Atmos Environ. 2021;245:117993.
22. Xie W, Ling M, Xiao T, Fan Z, Chen D, Tang M, et al. Tanshinone IIA-regulation of IL-6 antagonizes PM2.5 -induced proliferation of human bronchial epithelial cells via a STAT3/miR-21 reciprocal loop. Environ Toxicol. 2022;37:1686–96.
23. Yu M, Zheng X, Witschi H, Pinkerton KE. The role of interleukin-6 in pulmonary inflammation and injury induced by exposure to environmental air pollutants. Toxicol Sci. 2002;68:488–97.
24. Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, et al. Foxp3 inhibits RORgammat-mediated IL-17A mRNA transcription through direct interaction with RORgammat. J Biol Chem. 2008;283:17003–8.
25. Katsura H, Kobayashi Y, Tata PR, Hogan BLM. IL-1 and TNFα contribute to the Inflammatory Niche to enhance alveolar regeneration. Stem Cell Rep. 2019;12:657–66.
26. Zhao R, Guo Z, Zhang R, Deng C, Xu J, Dong W, et al. Nasal epithelial barrier disruption by particulate matter ≤ 2.5 μm via tight junction protein degradation. J Appl Toxicol. 2018;38:678–87.
27. Tung NT, Ho K-F, Niu X, Sun J, Shen Z, Wu F, et al. Loss of E-cadherin due to road dust PM2.5 activates the EGFR in human pharyngeal epithelial cells. Environ Sci Pollut Res Int. 2021;28:53872–87.
28. Xian M, Ma S, Wang K, Lou H, Wang Y, Zhang L, et al. Particulate matter 2.5 causes Deficiency in Barrier Integrity in Human nasal epithelial cells. Allergy Asthma Immunol Res. 2020;12:56–71.
29. Montgomery MT, Sajuthi SP, Cho S-H, Everman JL, Rios CL, Goldfarbmuren KC, et al. Genome-wide analysis reveals Mucociliary Remodeling of the nasal Airway Epithelium Induced by Urban PM2.5. Am J Respir Cell Mol Biol. 2020;63:172–84.
30. Yang H-S, Sim HJ, Cho H, Bang WY, Kim HE, Kwon TK, et al. Alpha-tocopherol exerts protective function against the mucotoxicity of particulate matter in amphibian and human goblet cells. Sci Rep. 2020;10:6224.
31. Chang-Chien J, Kuo M-L, Tseng Y-L, Huang H-Y, Tsai H-J, Yao T-C. Differential effects of long- and short-term exposure to PM2.5 on accelerating telomere shortening: from in vitro to epidemiological studies. Ecotoxicol Environ Saf. 2024;281:116650.
32. Park GB, Kim D. Cigarette smoke-induced EGFR activation promotes epithelial mesenchymal migration of human retinal pigment epithelial cells through regulation of the FAK-mediated Syk/Src pathway. Mol Med Rep. 2018;17:3563–74.
33. Unwalla HJ, Ivonnet P, Dennis JS, Conner GE, Salathe M. Transforming growth factor-β1 and cigarette smoke inhibit the ability of β2-agonists to enhance epithelial permeability. Am J Respir Cell Mol Biol. 2015;52:65–74.
34. Tsai M-J, Hsu Y-L, Wang T-N, Wu L-Y, Lien C-T, Hung C-H, et al. Aryl hydrocarbon receptor (AhR) agonists increase airway epithelial matrix metalloproteinase activity. J Mol Med (Berl). 2014;92:615–28.
35. Tsai M-J, Wang T-N, Lin Y-S, Kuo P-L, Hsu Y-L, Huang M-S. Aryl hydrocarbon receptor agonists upregulate VEGF secretion from bronchial epithelial cells. J Mol Med (Berl). 2015;93:1257–69.
36. Liu Y, Yuan Q, Zhang X, Chen Z, Jia X, Wang M, et al. Fine particulate matter (PM2.5) induces inhibitory memory alveolar macrophages through the AhR/IL-33 pathway. Cell Immunol. 2023;386:104694.
37. Choi J, Shim JJ, Lee MG, Rhee CK, Joo H, Lee JH, et al. Association between Air Pollution and viral infection in severe Acute Exacerbation of Chronic Obstructive Pulmonary Disease. J Korean Med Sci. 2023;38:e68.
38. Altman MC, Kattan M, O'Connor GT, Murphy RC, Whalen E, LeBeau P, et al. Associations between outdoor air pollutants and non-viral asthma exacerbations and airway inflammatory responses in children and adolescents living in urban areas in the USA: a retrospective secondary analysis. Lancet Planet Health. 2023;7:e33–44.
39. Roberts N, Al Mubarak R, Francisco D, Kraft M, Chu HW. Comparison of paired human nasal and bronchial airway epithelial cell responses to rhinovirus infection and IL-13 treatment. Clin Transl Med. 2018;7:13.
40. Imkamp K, Bernal V, Grzegorzcyk M, Horvatovich P, Vermeulen CJ, Heijink IH, et al. Gene network approach reveals co-expression patterns in nasal and bronchial epithelium. Sci Rep. 2019;9:15835.
41. Karp T, Faiz A, van Nijnatten J, Kerstjens HAM, Boudewijn I, Kraft M, et al. Nasal epithelial gene expression identifies relevant asthma endotypes in the ATLANTIS study. Thorax. 2024;79:905–14.

Publisher's note