High Content Screening (HCS) Approach to Characterize Phenotypic Changes Occurring During Long-term Treatment of Human Bronchial Epithelial Cells With Cigarette Smoke Total Particulate Matter

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Introduction

Chronic cigarette smoke exposure is responsible for airway epithelial changes which ultimately may cause lung cancer, although the current knowledge about the underlying molecular events is still not very detailed. Our aim was to establish an in vitro model which mimics chronic exposure conditions found in the airways of smokers, and utilizing BEAS-2B cells and an HCS approach, to more comprehensively characterize the chronological changes that occur in bronchial epithelial cells under those conditions. For this purpose, immortalized human bronchial epithelial BEAS-2B cells were continuously exposed to total particulate matter (TPM) from the reference cigarettes 3R4F for 4 weeks. Cell cycle, DNA damage, and oxidative stress were assessed weekly by high-content analysis. In addition, staining for epithelial-mesenchymal transition (EMT) markers was performed after 4 weeks of treatment.

Assay Development

Generation of TPM - 3R4F reference cigarettes were purchased from the University of Kentucky (http://www2.ca.uky.edu/mfrp) and stored in a cooling chamber at 63°C with uncontrolled humidity in the original packaging. The cigarettes were conditioned according to ISO standard 34021, i.e., for at least 48 h at 24°C and a relative humidity of 90% before total particulate matter (TPM) generation. TPM was collected on a 20-port Bergwaldt (Germany) RM00 smoking machine according to the ISO smoking regime. Five ml TPM were used to extract TPM from the filter packs (Fig. 1). After extraction, the TPM was prepared and stored at -80°C until further use.

Cell Culture and Treatment - The human bronchial epithelial cell line BEAS-2B (LOC Standards GmbH, Wiesbaden, Germany) was grown in complete BEGM (Lonza, Basel, Switzerland) on collagen A-coated plates. After two passages of the frozen stock, cells were seeded at an appropriate density and treated with TPM at a final concentration of 10 mg/ml. At a confluence of ca. 75%, cells were trypanized and passed in fresh treatment medium for a total treatment duration of 4 weeks. Cells cultured without TPM (mock control) and cells cultured in the presence of 0.2% DMSO (vehicle; Sigma Aldrich, Buchs, Switzerland) were used as controls. Additionally, treated and control cells were collected once a week for assessment of viability and high-content screening (HCS) as described above. This experiment was repeated three times, giving a total sample size of 3 per time point.

High-Content Screening (HCS) - CellaVision ArrayScan® VTi HCS reader (Thermo Fisher Scientific Inc., Berkshire, UK) was employed to assess oxidative stress (ROS and GSH), genotoxicity (DNA damage), cell cycle alterations and epithelial-mesenchymal transition (EMT). Briefly, BEAS-2B cells were seeded in black collagen A-coated, clear-bottom 96-well tissue culture plates (BD, Allschwil, Switzerland) at a density of 12,000 cells/well for all endpoints except the cell cycle assay, for which 4,000 cells/well were seeded. 24 h after the seeding, the cells were incubated for 24 or 72 h for the EMT assay. ROS was detected using the nuclear area (green circles in panel c and d), values were then normalized to DMSO control. DNA damage, ROS and GSH data were normalized to DMSO control of the respective strains.

Figure 1. Experimental design (a) to study the effect of long-term exposure to TPM. At the designated time points cells were collected for further analysis for a panel of different endpoints (b).

Results

• Chronic treatment of BEAS-2B cells with 3R4F TPM significantly increases phosphorylation of histone H2AX by 30% in the first 2 weeks of treatment while no difference were found after 4 weeks of treatment.

• Exposure to 3R4F TPM causes a significant increase of ROS only within the first week of treatment when compared to control (Fig. 8) and 4 weeks after the second cycle of treatment. The oxidative stress levels were similar in BEAS-2B cells exposed to TPM for 1, 2 and 4 weeks.

• Epithelial mesenchymal transition (EMT), is one of the first critical phenotypic alteration of cells that triggers invasion and metastasis. In this study, we show (Figures 8a-e) that expression of E-cadherin (epithelial marker) is decreased while Vimentin (mesenchymal marker) becomes upregulated in BEAS-2B cells exposed to TPM.

Conclusion

• Repeated exposure of bronchial epithelial cells to TPM induces ongoing phenotypic alterations related to tumorigenesis. In particular, cellular oxidative stress and DNA damage are increased causing cell cycle alterations and evidence of epithelial-mesenchymal transition is detected.

• This data here may lead to a better mechanistic understanding of the stepwise transformation of normal airway epithelial cells to full malignancy. The High Content Imaging platform represents a powerful tool with sufficient throughput capacity and precision to allow detection of phenotypic changes in toxicological studies.

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