Comparison of the Biological Effects of Long-Term Exposure of Human Bronchial Epithelial Cells to Total Particulate Matter from 3R4F Cigarette Smoke or Aerosol from the Candidate Modified Risk Tobacco Product (cMRTPT) THS2.2

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Abstract

Chronic cigarette smoke exposure leads to multiple epigenetic changes underlying lung tumorigenesis, although the elucidation of these mechanisms remains complex. To address this, we have employed BEAS-2B cells, a well-established model for chronic cigarette smoke exposure, and THS2.2 TPM, a lower concentration of THS2.2 TPM, to further investigate the biological effects of long-term exposure.

Materials and Methods

Cell Culture and Treatment

BEAS-2B cells were grown in DMEM/F12 serum-free media containing 10% FBS and 1% PenStrep. THS2.2 TPM and 3R4F TPM were purchased from the University of Kentucky and stored in a -80°C freezer.

Figure 1. Treatment of BEAS-2B cells with 3R4F TPM induced DNA damage early and a crisis around week 4 after which cells die out. Early, dose-dependent DNA damage was also seen with THS2.2 TPM treatment. However, crisis occurred only in cells treated with 150 μg/mL THS2.2 TPM.

Results

Long-term 3R4F TPM treatment of BEAS-2B cells results in rapid and sustained elevation of secreted pro-inflammatory mediators. In most cases, a 20 times higher THS2.2 TPM concentration is required to elicit a similar effect on mediator secretion.

Figure 2. Normal range of fold changes in the levels of soluble mediators (based on a) secreted by 3R4F TPM treated BEAS-2B cells, (b) secreted by THS2.2 TPM treated BEAS-2B cells and (c) measured in biological media from THS2.2 TPM treated BEAS-2B cells. Similar effects were observed only for treatment with 5 and 20 times higher THS2.2 TPM concentrations.

Figure 3. Comparison of the Biological Effects of Long-Term Exposure of Human Bronchial Epithelial Cells to Total Particulate Matter from 3R4F Cigarette Smoke or Aerosol from the Candidate Modified Risk Tobacco Product (cMRTPT) THS2.2

Table 1. Effect of long-term TPM treatment on the number of differentially expressed genes

Conclusions

Twelve-week treatment of BEAS-2B cells with a low dose of 3R4F TPM resulted in:

- Increased DNA damage and crisis on week 4
- Decrease in intracellular ROS abundance, but increased GSH levels
- Loss of adherens junctions and epithelial-mesenchymal transition
- Increased invasiveness

Similar effects were also noted in BEAS-2B cells treated with THS2.2 TPM at 20 times higher concentration than 3R4F, but not at comparable or 5 times higher concentration (no changes in cell viability, no significant oxidative stress, unaltered adhesive properties and loss of anchorage independence).

Follow-up studies will include the evaluation of global methylation patterns and alterations in the DNA sequence of selected genes known to be involved in lung tumorigenesis in these chronically treated cells. In addition, the potential of the subdomains to form tumors in immunocompromised mice will be investigated.

Figure 4. Changes in expression of differentially expressed genes. The heatmap represents relative increases in transcriptional activity on the x-axis relative to corresponding DMSO controls at 3R4F (TPM) and THS2.2 (TPM) concentrations. Significantly upregulated transcripts are indicated by a 2 or 3. Statistical significance was calculated with a FDR adjusted p-value of 0.05 compared to vehicle control (DMSO).

Figure 5. Endometrial expression of differentially expressed genes. The heatmap represents relative increases in transcriptional activity on the x-axis relative to corresponding DMSO controls at 3R4F (TPM) and THS2.2 (TPM) concentrations. Significantly upregulated transcripts are indicated by a 2 or 3. Statistical significance was calculated with a FDR adjusted p-value of 0.05 compared to vehicle control (DMSO).