Implications of miR-9 in cigarette smoke effects in naïve and desialylated human alveolar epithelial cells (A549)

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INTRODUCTION

Long-term exposure to cigarette smoke (CS) increases the risk for lung cancer but the mechanisms of CS-induced airway inflammation and biochemical pathways related to malignant transformation of respiratory system cells are unknown. In recent years, the discovery of microRNAs has made it evident that these molecules have widespread control of the expression of proteins involved in inflammatory signaling and play an essential role in the initiation and development of cancer. Cell surface sialoproteins are relevant not only to metastatic potential of cancer cells but are also important to the host resistance to viral and bacterial infections and to immune response.

METHODS

A549 cells were grown in CS-conditioned medium prepared using full- strength Red Marlboro cigarettes containing about 8.0 mg of tar, 0.6 mg of nicotine, and 9.0 mg of carbon monox- ide per cigarette. Naive or desialylated, preincubated with neuraminidase (100 U/ml) for 24 h, A549 cells were grown in CS-conditioned medium for 24 h. In some experiments cells were additionally treated with LPS (1 μg/mL) or DEX (10^{-5} M) for 24 h.

To quantify miR-9, reverse transcription and quantitative PCR (qPCR) were performed using the TaqManTM microRNA assay kit following the producer's protocol. U6 small RNA was used as a reference. MiR-9 expression was calculated with ΔΔCt method and was shown as fold changes.

Cell proliferation was quantified in flow cytometry using propidium iodide DNA staining and cell cycle analysis. Results were expressed as means SD of 3–5 assays run in triplicate. The relationship between mir-9 expression and cell proliferation was examined using a linear regression analysis. A p-value < 0.05 defined statistically significant differences.

CONCLUSIONS

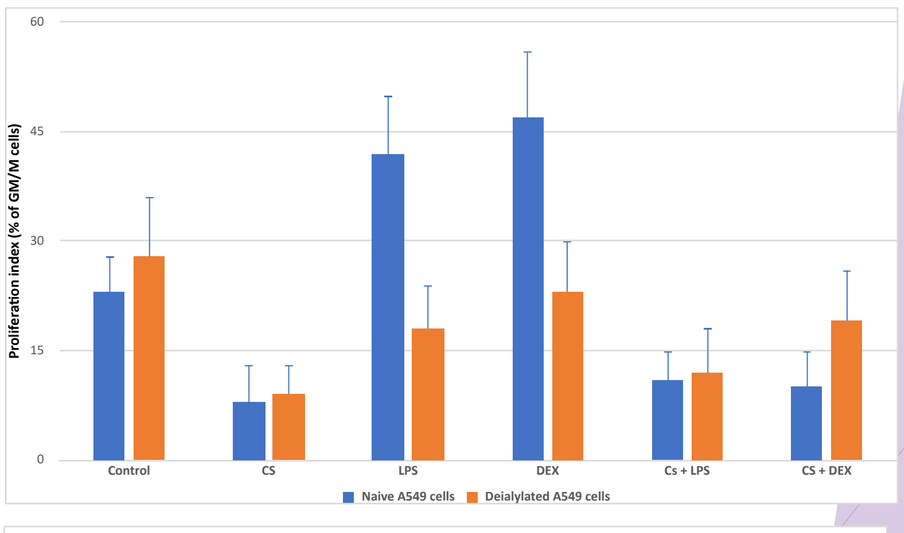
Herein we identify miR-9 as the cigarette smoke (decrease) and LPS-responsive but dexamethasone-unresponsive microRNA. Increased miR-9 levels in naïve A549 cells treated with LPS may be related to the activation of Toll-like 4 receptors. Moreover, differences in cell response (both miR-9 and proliferation) to dexamethasone in naïve and desialylated cells may point to non-genomic dexamethasone effects.

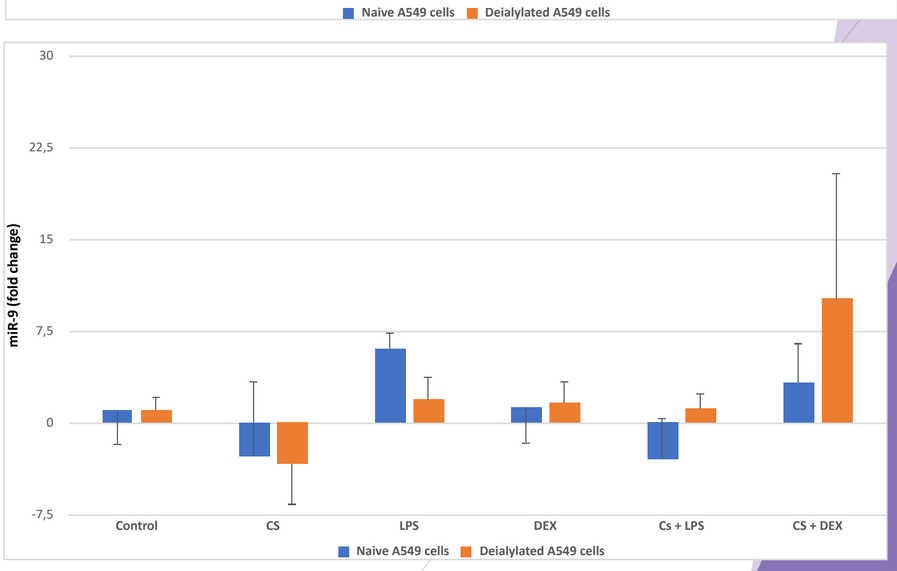
AIM

The aim of the study was to quantify miR-9 in naïve and desialylated A549 cells grown in CS-conditioned medium under pro-inflammatory (LPS) and anti-inflammatory (DEX) stimuli and to analyze changes in miR-9 in relation to cell proliferation. MiR-9 expression is especially interesting since apart from its possible suppressive role in cancer, miR-9 may inversely affect DEX sensitivity in an experimental model of steroid-resistant airway hyperresponsiveness.

RESULTS

Proliferation positively correlated with miR-9 levels in both naïve and desialylated cells. Cigarette smoke decreased miR-9 levels in both cell types by about three-fold but there was no significant correlation between both parameters. Dexamethasone was without substantial effect on cigarette smoke-induced changes in proliferation of naïve cells, but some normalization was observed in desialylated cells. Dexamethasone increased miR-9 levels in both cell types grown in cigarette smoke-medium but the effect was stronger in desialylated cells. LPS increased cell proliferation and miR-9 by more than six-fold only in naïve cells, while the correlation coefficient for both parameters in the cigarette smoke-LPS group was 0.41.





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