Smoking and smoking cessation

0036

THE EFFECT OF CIGARETTE SMOKE AND/OR LPS ON miR-9 LEVELS IN NAÏVE AND DESIALYLATED A549 CELLS; POSSIBLE ROLE OF NONGENOMIC DEXAMETHASONE EFFECTS

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MicroRNAs (miRNAs), a class of small noncoding RNAs, contribute to the regulation of inflammation and cell proliferation by adjusting gene expression at the posttranscriptional level. In this study, we assessed miR-9 levels in naïve and desialylated human alveolar epithelial cell line (A549), grown for 24 hours in cigarette smoke (CS)-conditioned medium. Cells were additionally treated with LPS (1 μg/mL) and/or dexamethasone (Dex). Naïve cells or cells grown for 24 hours with neuraminidase (100U/ml: desialylation) were grown for 24 hours in CS-conditioned medium and/or in medium supplemented with LPS or LPS and Dex [10(-5)M]. To quantify miR-9 a real-time PCR was performed on an ABI 7500HT System by using TaqMan miRNA reagents and primers provided by Thermo Fisher Scientific. All reactions were run 3 times in triplicate. Samples were normalized to internal control, U6 small nuclear RNA, and fold changes were calculated through relative quantification ($2^{-\Delta\Delta Ct}$). Cigarette smoke decreased miR-9 levels in both naive and desialylated cells by about 3 fold. Dex was without substantial effect on CS-induced changes in naïve cells, while some normalization was observed in desialylated cells. LPS increased miR-9 in naïve cells by more than 6 fold in naïve cells only, but not in desialylated cells while Dex decreased LPS-induced changes in naïve cells but significantly (by about 10 fold) increased miR-9 levels in desialylated cells treated with LPS.Herein we report the changes induced by CS and/or LPS in human epithelial cell line and identify miR-9 as the LPS-responsive miRNA most probably related to the activation of Toll-like receptor 4 (TLR4) in cell membrane. Since the effect of Dex is different in naïve and desialylated cells it seems that the non-genomic drug effects may be involved.