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Characterization of mainstream cigarette smoke (MCS): Induced phenotype in human primary bronchial epithelial cells (PBEC)

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Cigarette smoke triggers alterations of both physical and immunological barrier functions of the airway mucosa, leading to inflammatory and pro-neoplastic changes. We used cultures of polarized human PBEC exposed to controlled MCS to characterize a miRNA gene expression signature associated with smoke exposure.PBEC (n=3) isolated from cadaveric lungs of donors (history: negative for respiratory disease, positive for smoking tobacco) were cultured under air-liquid interface conditions and exposed to MCS (undiluted smoke) or filtered air (FA) in a smoke exposure chamber for 16 min (equivalent to two reference cigarettes), cultured for additional 18 hrs, then harvested. MiRNA expression was performed by next generation sequencing with sequencing-by-synthesis technology. Library preparation utilized an Illumina TruSeq small RNA sample preparation protocol. Gene expression was profiled using HT12 v.4.0 Illumina Array platform. MiRNA/Gene expression was assessed in cells harvested 18 hr. after MCS/FA exposure since initial experiments indicated a 42% increase in 7 kD FITC-dextran, a measure of macromolecular permeability, collected from the basolateral supernatant with <20% loss of viability (n=2, WST-1 assay). Twenty-eight miRNAs (as fold change ≥1.2, $p<0.05$) were regulated by MCS treatment, among which 12 were downregulated. miR1246, a p53-regulated miRNA implicated in cell cycle control, was downregulated 8±5-fold. Global gene expression identified MCS-induced increased expression of serpinB4, a gene implicated in neoplastic transformation of airway epithelial cells. The characterization of the miRNA signature and its targets can identify mechanisms of global posttranscriptional gene control with high therapeutic value that can function as disease biomarkers.

Biography

Becky M Vonakis is currently Assistant Professor of Medicine at The Johns Hopkins University (JHU) School of Medicine in Baltimore, Maryland. She received her PhD in Biochemistry from the George Washington University and completed Postdoctoral training at NIAMS/NIH and JHU. She is a Fellow of the American Academy of Allergy, Asthma and Immunology. She reviews grants for the National Medical Research Council of Singapore, European COST and the Qatar National Research Fund. She has over 30 publications, has been awarded 17 grants/contracts to date (form both the NIH and industry) and mentored more than two dozenstudents and fellows.

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