Online Data Supplement

Histone acetylation regulates cell-specific and interferon-gamma inducible EC-SOD expression in human pulmonary arteries

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Material and Methods

Cell Culture and exposures to cytokines and chemicals

HPAEC (human pulmonary artery endothelial cells) and HPASMC (human pulmonary artery smooth muscle cells) were obtained from Clonetics (Lonza, USA). HPAEC and HPASMC were cultured in EGM-2 Endothelial Cell Growth Medium-2 and SmGM-2 Smooth Muscle Cell Growth medium, respectively. MRC-5 (human embryonic lung fibroblast cell line) cells were obtained from ATCC (Manassas, VA). MRC-5 cells were cultured in Eagle's Minimal Essential medium with Earle's BSS and 2 mM L-glutamine supplemented with 10% FBS. Cells were maintained at 37 ^{o}C and 5% CO_2 in a humidified incubator.

HPAEC and HPASMC cells were plated onto 48 well plates at a density of 3.75 x 10^4 cells per well and grown overnight. The next day, cells were washed once with opti-MEM, and then incubated in the same medium containing 5,000 U/ml of recombinant human IFN-γ or 30 ng/ml of TNF-α dissolved in 0.1% human serum albumin (HSA) in PBS for the times indicated. As a control, cells were exposed to medium with only HSA. Media, with indicated chemicals, was replaced every 24 hours. Following exposure, cells were washed with PBS and total RNA purified using RNAqueous-Micro Kit (Applied Biosystems, Foster City, CA). To analyze the effects of JAK/STAT inhibitors on IFN-γ-inducible EC-SOD expression, JAK1, STAT3 and AG490 inhibitors were added to the culturing medium with or without cytokine and incubated for 48 hours. For some experiments HPAEC and HPASMC cells were treated with the indicated concentrations of 5-azacytidine, TSA or DMSO for 48 and 96 hours. Media with indicated chemicals was replaced every 24 hours.

McrBC Digestion and Analysis

Genomic DNA from various cell lines was prepared using a DNeasy Tissue kit (Qiagen, Chatsworth, CA) according to the manufacturer's protocol. Two hundred nanograms of genomic DNA were cleaved with McrBC (New England Biolabs, Beverly, MA) in final reaction volume of 50 µl at 37 ^{o}C for 3 h. The cleaved genomic DNA was purified using QIAquick PCR Purification kit (Qiagen, Chatsworth, CA) and eluted in 50 µl of water. One microliter was used as template for PCR to amplify specific regions of EC-SOD 5'-flanking sequence. PCR products were separated on a 1.2% agarose gel and visualized using ethidium bromide. PCR primer sequences are available on request.