cular remodeling. To study the mechanism of up-regulation of VEGF-D by hypoxia, two VEGF-D promoter fragments, a 3,448-base pair (bp) fragment and a 523-bp fragment, were isolated from a mouse genomic library by genome walking. The fragments were sequenced (GenBank AF345635) and cloned into a pGL3 luciferase reporter vector. The reporter constructs were transfected into rat pulmonary microvascular smooth-muscle cells. Transfection efficiency was normalized by cotransfected pRL-TK renilla luciferase activity. Dual luciferase assays were performed after transfected rat pulmonary microvascular smooth-muscle cells were exposed to either 1% oxygen or 21% oxygen for 24 h. The luciferase activity was expressed as fold of the pGL3 basic activity. Hypoxia significantly increased the VEGF-D promoter activity. The activity of the 3,448-bp fragment was increased from 3.3 ± 0.08-fold to 7.5 ± 0.59-fold. The activity of the two colonies of the 523-bp fragment was increased from 2.9 ± 0.12-fold to 6.3 ± 0.36-fold and from 3.3 ± 0.08-fold to 7.5 ± 1.0-fold, respectively. Sequencing confirmed that the 523-bp fragment was located at 3’ end of the 3,448-bp fragment. The results suggest that hypoxia-induced VEGF-D expression is regulated in a region within the 523-bp fragment.

Specific Bone Morphogenic Protein Receptor II Mutations Found in Primary Pulmonary Hypertension Cause Different Biochemical Phenotypes In Vitro*

Alan Q. Thomas, MD; Jennifer Carneal, MS; Cheryl Markin, BS; Kirk B. Lane, PhD; John A. Phillips III, MD; James E. Loyd, MD; and Radhika Gaddipati, MBBS

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Abbreviations: PPH = primary pulmonary hypertension; BMP = bone morphogenic protein; BMPRII = bone morphogenic protein receptor II

Primary pulmonary hypertension (PPH) is a progressive and fatal disease caused by proliferative occlusion of the smallest pulmonary arteries. It can occur at any age in either gender. PPH has also been recognized as a genetic disease, transmitted in an autosomal dominant manner. Mutations in the gene encoding the bone morphogenic protein receptor II (BMPRII) have been shown to be the molecular basis of familial PPH. BMPRII is a member of the transforming growth factor-β–receptor II family. Mutations in 12 of the 13 exons of BMPRII have been recognized but all produce a similar outcome, PPH. Using stable transfection of normal mouse mammary gland epithelium cells, we show that mutations in the different functional domains of BMPRII are capable of eliciting different biochemical phenotypes. Stable cell lines expressing wild-type or mutant BMPRII constructs were assayed for luciferase expression driven from a SMAD binding element-responsive promoter. The mutations constructed were those detected in PPH kindreds. Native cells express 11 times more luciferase after stimulation with the ligand bone morphogenic protein 4. Kinase domain mutations produced twice as much luciferase as the controls at baseline and expressed four times more than the baseline levels with stimulation with bone morphogenic protein 4. The cytoplasmic tail mutation expressed similar amounts of luciferase at baseline as the wild type but increased more than six times with stimulation. These data suggest that mutations in specific BMPRII domains affect bone morphogenic protein signaling differently in the presence or absence of ligand.

Pulmonary Gene Expression Profiles of Spontaneously Hypertensive Rats Exposed to Environmental Tobacco Smoke*

Srikanth S. Nadadur, PhD; Kent E. Pinkerton, PhD; and Urmila P. Kodavanti, PhD

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Abbreviations: ETS = environmental tobacco smoke; SH = spontaneously hypertensive

Global gene expression profile analysis can be utilized to derive molecular footprints to understand biochemical pathways implicated in the origin and progression of disease. Functional genomics efforts with tissue-specific focused gene array appears to be the most reasonable approach to derive relevant and meaningful information. In our efforts to understand the

*From the Pulmonary Toxicology Branch (Drs. Nadadur and Kodavanti), Environmental Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, NC; and Department of Anatomy (Dr. Pinkerton), Physiology and Cell Biology, School of Veterinary Medicine, University of California, Davis, CA. This article does not reflect US Environmental Protection Agency policy.

Correspondence to: Srikanth S. Nadadur, PhD, Pulmonary Toxicology Branch, Mail Drop 82, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC 27711; e-mail: nadadur.srikanth@epa.gov.
molecular basis for the toxicity and exacerbations in susceptible populations to environmental exposures, we are developing a gene expression database for a rat cardiopulmonary disease model exposed to air pollutants. The spontaneously hypertensive (SH) rat with underlying cardiovascular and pulmonary disease risk is being utilized toward these efforts. SH rats with polygenic traits toward hypertension exhibit similar heritable risk factors that are found in patients with COPD. We have shown that SH rats are more susceptible to lung injury/inflammation, and oxidative stress from exposure to combustion source particles, and from experimental induction of pulmonary disease compared to healthy normotensive Wistar rats. SH rats also elicited an inflammatory response to ETS exposure, which is less remarkable in other conventional rat strains used in laboratory studies. To understand the molecular basis for the inflammatory response, we screened for a pulmonary gene expression profile in SH rats exposed to ETS. Male SH rats (12 weeks old) were exposed either to filtered air or ETS at 90 mg/m³ for 6 h/d for 2 consecutive days; on the third day, total RNA was isolated from right lung lobes. Pulmonary gene expression was assayed by hybridizing 32P-labeled complementary DNA generated from total RNA to Atlas Rat complementary DNA expression array filter containing 588 genes (Clontech; Palo Alto, CA). Gene expression profile data indicated strong hybridization signals for 40 genes, including N-myc, p53, transforming growth factor-β, p21, bax-α and cytokine macrophage inflammatory protein-2. Pairwise comparison indicated ETS exposure-associated differential expression in 16 genes: increased expression of CYPIA1, calcium pump, RhoGAP, p122, and decreased expression of bile salt-activated lipase precursor, a fatty acid binding protein and fatty acid amide hydrolase. ETS induced a twofold to threefold increase in macrophage inflammatory protein-2 expression, suggesting lung injury and inflammation. Overexpression of matrix metalloproteinase-7 suggests a possible release of proteases from ETS-induced infiltration of neutrophils into the airways, consistent with our earlier observations. Increased expression of p27(Kip1), caspase-1, and Stat3 observed in the present study further supports apoptosis of infiltrating neutrophils in the lungs following exposure to ETS. Efforts are underway to explore whether long-term ETS exposure of SH rats will lead to a chronic disease state. A comprehensive expression database indicating ETS-induced changes in susceptible animal models will provide identification and the role of risk factors in understanding health effects of ETS and other environmental pollutant-induced cardiopulmonary disease.

Interleukin-18 Expression in Cystic Fibrosis Lungs*

Edward D. Chan, MD; Hyung-Seok Choi, MD; Carlyne Cool, MD; Frank J. Accurso, MD; and Gianila Fantuzzi, PhD

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Abbreviations: CF = cystic fibrosis; IFN = interferon; IL = interleukin

A primary dysregulation of pulmonary inflammation has been proposed as a mechanism leading to airways disease in patients with cystic fibrosis (CF). Proinflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-8, are elevated in the BAL fluid and sputum of CF patients. Although only a few reports have examined T-helper 1 vs T-helper 2 cytokine expression in CF patients, they suggest a relative defect in T-helper 1 cytokine response. For example, peripheral blood lymphocytes from CF patients produced significantly lower levels of interferon (IFN)-γ compared to lymphocytes from a control group when stimulated in vitro with anti-CD3. Furthermore, IFN-γ has been shown to inhibit the production of IL-8 and other proinflammatory cytokines. IL-18, a cytokine constitutively produced by macrophages and epithelial cells, induces IFN-γ in T cells. We undertook a study to examine the expression of IL-18 in children with CF. IL-18 levels were measured in the BAL fluid of 17 CF patients (median age, 12 years; age range, 4 months to 25 years) and compared to the levels observed in a control population comprised of 11 subjects with various pathologic conditions. Of the 17 CF subjects, only 3 subjects had detectable IL-18 levels (23 pg/mL, 26 pg/mL, and 37 pg/mL). However, IL-18 was above the detection limit in each subject of the control group (median, 78 pg/mL; mean ± SEM, 261 ± 118 pg/mL) [Fig. 1]. As expected, both IL-8 and IL-1β levels were significantly elevated in the same BAL fluids of CF patients compared to the control group. The IL-1β levels were 124 ± 37 pg/mL in the CF patients and 11 ± 1 pg/mL in the non-CF control subjects (p < 0.001); the IL-8 levels were 645 ± 149 pg/mL in the CF group and 167 ± 101 pg/mL in the non-CF control subjects (p < 0.01). The addition of protease inhibitors to the BAL fluid at the time of collection did

*From the Divisions of Infectious Diseases (Dr. Fantuzzi) and Pulmonary Sciences and Critical Care Medicine (Dr. Chan), Department of Pathology (Dr. Cool), University of Colorado Health Sciences Center; Cystic Fibrosis Center (Dr. Accurso), Children’s Hospital of Denver; and National Jewish Medical and Research Center (Dr. Choi), Denver, CO.

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Correspondence to: Gianila Fantuzzi, PhD, Box B168, Division of Infectious Diseases, Department of Medicine, University of Colorado Health Sciences Center, 4200 East Ninth Ave, Denver, CO 80262; e-mail: gianila.fantuzzi@uchsc.edu

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