Epidemiologic Study of the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy*

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The Epidemiologic Study on the Genetics and Environment of Asthma (EGEA) was planned to assess genetic and environmental risk factors and their interactions in patients with asthma and for the two related traits of bronchial hyperresponsiveness and atopy. The study was performed from 1991 to 1995 and combined a case control study and a study of the families of the asthmatic patients. A synthesis of the results already obtained is presented. Smoking was related to IgE levels, even in asthma patients. Smoking was clearly related to the clinical severity of the asthma, which is clinically insufficiently taken into account. The relationships of occupational exposures to asthma have been clinically insufficiently taken into account. The relations related to the clinical severity of the asthma, which is shown between asthma and the ΔF508 mutation of the cystic fibrosis gene. The analysis is still in progress with studies on the heterogeneity of asthma, with refined genetic studies, and by searching to integrate the results regarding environmental and genetic factors and studying their interactions.

Molecular and Physiologic Evidence for 5′CpG Island Methylation of the Endothelin B Receptor Gene in Lung Cancer*

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Abbreviation: ETBR = endothelin-B receptor

We have previously reported molecular and physiologic evidence for an endothelin-1 autocrine loop in lung cancer. The endothelin-B receptor (ETBR), which is believed to counter-regulate endothelin-1, is not expressed in the majority of lung cancer cell lines. We hypothesized that the inactivation of the ETBR is due to epigenetic silencing, specifically the hypermethylation of cytosine nucleotides of the 5′CpG “island” surrounding the transcriptional start region of the ETBR gene. This process has been associated with the silencing of a number of tumor-suppressor genes.

Bisulfite sequencing of the ETBR promoter region revealed that the 5′CpG island of the A549 cell line was methylated. Using a novel methylation-specific polymerase chain reaction assay, ETBR was determined to be methylated in six of eight small cell lung carcinoma cell

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lines, five of six adenocarcinoma cell lines, zero of two large cell carcinoma cell lines, and two of two squamous cell lines. Methylation was not detected in two bronchial epithelial cell lines. In the lung tumors tested, seven of nine adenocarcinomas, one of one large cell carcinoma, two of three squamous cell cancers, and zero of one carcinoid tumors were positive for ETBR methylation. Treatment with 5-Aza-2′ deoxycytidine (the inhibitor of DNA methylation) and trichostatin A (the inhibitor of histone deacetylase) induced the re-expression of ETBR by reverse transcriptase-polymerase chain reaction in several lung cancer cell lines. Hypermethylation of the 5′CpG island "encompassing" the transcriptional regulatory region of the ETBR gene may be an important gene-inactivating event of ETBR in lung cancer. We are currently testing the feasibility of using the ETBR methylation assay in the early detection of lung cancer in high-risk patients.

Proteomic Analysis of Mouse Lung Neoplasia*

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Abbreviations: 2DE = two-dimensional gel electrophoresis; MALDI-TOFMS = matrix-assisted laser desorption ionization time of flight mass spectrometry

Proteomics has the potential to significantly advance the understanding of biological systems. In this study, we applied classic and more novel proteomics techniques to in vitro and in vivo models of lung cancer. The traditional comparison of protein expression entails the separation of proteins by two-dimensional gel electrophoresis (2DE) and subsequent mass fingerprinting by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOFMS). Separations based on 2DE revealed at least 20 spots that differed between a nonneoplastic mouse lung epithelial cell line (E10) and its spontaneous transformant (E9). Differentially expressed proteins identified by this technique include lipocortin 1, which is of great interest because of the role of inflammation in lung tumorigenesis. Since 2DE is a time-consuming process and detects only a small fraction of the proteins expressed in cells or tissue, we also employed a combination of MALDI-TOFMS of whole-tissue homogenates and electrospray ionization liquid chromatography coupled to tandem mass spectrometry. This was applied to total pools of trypsinized proteins to compare protein expression in urethane-induced mouse lung adenomas with healthy lung tissue. Thus far, we have identified > 400 proteins from both the 10,000g fraction and the nuclear fraction of the tumor sample, and > 200 proteins from healthy tissue. By utilizing both of these techniques, we have attempted to provide a thorough description of the changes in protein expression that occur during lung carcinogenesis.

Partial Pneumonectomy Enhances Melanoma Metastasis to Mouse Lungs*

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Abbreviation: PNX = pneumonectomy

Tumor cell metastasis is the leading cause of morbidity and mortality associated with cancer. An improved understanding of the physiologic events that modulate metastases is needed to identify and employ effective treatments. Tissue growth can stimulate metastasis, markedly increasing both tumor incidence and multiplicity. In small mammals, left pneumonectomy (PNX) initiates rapid compensatory hyperplasia of the remnant lung lobes, which restores normal tissue mass, structure, and function. Our previous work demonstrated that PNX promotes pulmonary adenoma formation in carcinogen-treated mice. The present study tests the hypothesis that PNX enhances experimental metastasis to the lung. A left PNX or sham surgery was performed on syngeneic C57BL/6 mice at intervals prior to the IV injection of 5 × 10⁶ B16F10 melanoma cells. Two weeks after injection, the animals were killed and the number of pulmonary melanoma metastases was enumerated. In control animals, the mean (± SEM) number of tumors ranged from 56 ± 8 to 105 ± 14. In mice that had been subjected to PNX 1 to 7 days prior to B16F10 cell injection, the mean tumor number ranged from 150 ± 22 to 235 ± 27, an increase of 77 to 260% over controls (p < 0.01). The largest difference was observed between day-5 groups, in which PNX mice had 3.6-fold more metastatic tumors than controls. Moreover, measurements of the tumor area revealed that PNX mice harbored a substantially larger lung tumor burden than did control animals. PNX had no effect on the growth of subcutaneous B16F10 melanoma tumors, suggesting that experimental melanoma metastasis was en...