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 carcinomas, and zero of one carcinoïd 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 nontumorigenic 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 x 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-
hanced by local conditions associated with rapid lung growth. Because the lung is a frequent site for metastatic cancers, these results suggest that PNX is a relevant model in which to investigate the mechanisms that underlie the growth of metastases at a secondary site.

REFERENCE


Microarray Identifies Cyclooxygenase-2–Dependent Modulation of Insulin-like Growth Factor Binding Protein-3 in Non-small Cell Lung Cancer Cells*

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Abbreviations: COX = cyclo-oxygenase; IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; NSCLC = non-small cell lung cancer

Cyclooxygenase (COX)-2 is frequently overexpressed in non-small cell lung cancer (NSCLC). Previous studies have implicated a number of cytokines and growth factors in the regulation of COX-2 expression, and COX-2 itself modulates the expression of a variety of genes.1–5 Our previous studies show that prostaglandin E2, the major product of COX-2 activity, suppresses the T-cell–mediated immunity in lung cancer.6,7 In addition to its effects on cell-mediated immunity, tumor overexpression of COX-2 appears to be involved in enhanced invasion, promotion of angiogenesis, and resistance to apoptosis.8–11 Thus, there is mounting evidence indicating the importance of COX-2 in NSCLC. However, the knowledge about the changes in gene expression caused by the overexpression of COX-2 in NSCLC is fragmentary. In order to elucidate the COX-2–induced changes in NSCLC, we have conducted a microarray study using the Human Cancer Specific GeneChip (Affymetrix; Santa Clara, CA). In our study, we compared the expression of approximately 1,700 genes in the human lung adenocarcinoma cell line A549, transduced with COX-2, to A549 cells with either no or very little COX-2 expression. As a result, we have determined that the A549 cells of high COX-2 content express lower levels of insulin-like growth factor binding protein (IGFBP)-3 messenger RNA and, as determined by IGFBP-3 enzyme-linked immunosorbent assay, lower levels of free soluble IGFBP-3 protein. Thus, the amount of free soluble A549 inversely correlated with the expression level of COX-2. Previous studies have shown that IGFBP-3 antagonizes the mitogenicity of insulin-like growth factor (IGF)-1 and IGF-2.12,13 In addition, IGFBP-3 also has an IGF-independent tumor suppressor activity.14–16 Therefore, we hypothesize that COX-2–induced suppression of IGFBP-3 in lung cancer cells is one of the elements leading to increased tumorigenicity. This is the first study to implicate IGFBP-3 as a gene modulated by COX-2 overexpression in cancer cells.

REFERENCES


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