resulting multiplicative effect on cell proliferation in individuals simultaneously exposed to stimulators of both pathways is likely to result in a significant promoting effect, which in turn greatly enhances the lung cancer risk of smokers with chronic lung disease. Accordingly, our findings supply a mechanistic explanation for the well-documented high lung cancer risk of individuals with chronic lung disease. However, our data suggest that stimulation or inhibition of any components of these two pathways is likely to modulate the susceptibility of individuals for the development of neuroendocrine lung cancer, and may greatly change the progression of this cancer category. In light of these findings, Ca\(^{2+}\)-channel blockers that are widely used for the treatment of cardiovascular disease\(^5\) may inhibit the development and progression of such tumors, while cholinergic and serotonin stimulators used for the therapy of certain mental\(^6\) and neuromuscular disorders\(^10\) may promote this cancer type. Moreover, our findings support the hypothesis that PKC inhibitors may be of value for the prevention and therapy of SCLC.\(^11\)\(^12\)

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Angiogenesis in Mesotheliomas*

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Interleukin-8 (IL-8) has been described as an important angiogenic factor causing proliferation and chemotaxis of endothelial cells in vitro and the development of new capillaries in vivo. Importantly, several tumors have been shown to produce IL-8, which allows for local tumor growth and invasion. Pleural mesothelioma is characterized by local tumor extension and invasion of surrounding tissue, with distant metastasis being rare. We hypothesized that pleural mesotheliomas produce IL-8 as a mechanism for angiogenesis, with subsequent extension and propagation of the tumor.

Eighteen patients with malignant pleural effusions were seen over a 3-year period. Six patients had pleural effusions secondary to mesothelioma and 12 had pleural effusions secondary to other tumor metastases. Pleural fluid and pleural biopsies were collected from all patients during thoracoscopy. Pleural fluid from patients with mesothelioma contained significantly higher immunoreactive IL-8 levels than did fluid from patients with other malignant effusions (9.4±0.9 ng/mL compared to 0.7±0.3 ng/mL) as measured

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by enzyme-linked immnosorbent assay (ELISA). Immunohistochemistry of pleural biopsy specimens showed that IL-8 was localized to malignant mesothelioma cells, but not to other types of malignant metastatic deposits.

Normal human mesothelial cells were obtained from patients with pleural effusions secondary to congestive heart failure. Primary cultures were established in serum-free media. Mesothelioma cell lines were obtained from American Type Culture Collection. In vitro mesothelioma cell lines were demonstrated to constitutively express IL-8 messenger RNA while normal resting human mesothelial cells did not constitutively express IL-8. Supernatants of mesothelioma cell lines contained significantly greater IL-8 (5.4±0.6 ng/mL) than supernatants of unstimulated normal human mesothelial cells (0.4±0.1 ng/mL). We conclude that mesothelioma cells constitutively express IL-8, an angiogenic factor that induces neovascularization and local tumor propagation.

The Molecular Epidemiology of Oncoproteins*

Serum p53 Protein in Patients With Asbestosis

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One of the most common somatic genetic alterations in human cancers involves the loss of inhibitory function of the p53 tumor suppressor gene product. In many cases, a common mechanism is the loss of one normal p53 allele and/or point mutation of the other p53 allele. The resultant effect is a paradox increase in intracellular levels of the p53 protein, since many mutant forms of p53 have a much longer half-life than wild-type p53 and mutant forms of p53 can bind to the wild-type stabilizing its normally rapid degradation and extending its half-life. This accumulation of p53 protein has been observed in many human tumors, including lung cancers.

The intracellular accumulation of mutant p53 protein in cells in culture results in a corresponding increase in p53 protein in the extracellular supernatant. Similarly, individuals with tumors that have increased levels of mutant p53 protein can have correspondingly high levels of p53 in extracellular fluids, such as serum, which can be quantified using antibodies against p53 in enzyme-linked immnosorbent assays (ELISA). For example, we have previously reported the detection of elevated levels of mutant p53 protein in the serum of asbestos-exposed patients with lung cancers with known intracellular alterations in p53 as well as in the serum of asbestos-exposed patients who subsequently developed lung cancers. The current study is an extension of this work in which we have used two different ELISAs for p53 (a pantropic assay and a mutant-specific assay) to examine the serum levels of p53 protein in the banked serum samples of asbestos patients with and without lung cancers or mesotheliomas and nonasbestosis noncancer control subjects.

Materials and Methods

A previously described cohort of 111 patients with compensable asbestosis was followed at the Institute of Occupational Health in Helsinki from 1978 to 1987 with regular examinations every 1 to 3 years and, between 1981 and 1987, with the periodic collection of serum samples that were stored frozen at −70°C. Cancer incidence for this cohort was ascertained through the fall of 1994 from the Finnish Cancer Registry. At that time, 38 of 111 patients had developed malignant tumors, including 27 lung cancers (6 adenocarcinomas, 5 squamous cell carcinomas, 4 small cell carcinomas, and 12 lung cancers that were not further specified), 3 malignant pleural mesotheliomas, and 8 other assorted cancers. The cancer cases were primarily middle-aged to elderly, white, male current or ex-smokers with an average of 20 years of asbestos exposure. The noncancer asbestosis patients were virtually identical to the cancer cases in terms of age, sex, race, smoking status, and asbestos exposure. In addition, single serum samples collected in 1983 (and stored frozen at −70°C until the time of analysis) were available from nine normal control subjects seen at the Clinic of the Institute of Occupational Health in Helsinki for noncancer-, nonasbestosis-related problems. These control subjects were slightly younger than the asbestosis cohort (mean age, 53 years compared with 65 years for cancer cases and 68 years for asbestosis control subjects) but were otherwise similar in terms of sex and race (all white men).

The serum samples from these groups were assayed for the levels of p53 protein using a pantropic and a mutant-specific p53 ELISA. The pantropic p53 ELISA is a sandwich assay that utilizes a mouse monoclonal capture antibody (PAB 1801) and a rabbit polyclonal reporter antibody that will detect wild-type and mutant human p53 proteins and was performed according to the protocol of the manufacturer (Oncogene Science, Uniondale, NY).

For the assay, the equivalent of 100 μL of sample containing 1.5% normal mouse serum (added to minimize the potentially interfering effect of heterophilic antibodies) is added to microtiter wells that are precoated with the mouse monoclonal capture antibody and incubated at 37°C for 3 h. After washing with phosphate-buffered saline solution, 100 μL of the rabbit polyclonal reporter antibody diluted 1:20 is added to each well and incubated at room temperature for 2 h. After washing, 100 μL of horseradish peroxidase-conjugated goat antirabbit IgG is added to each well and incubated at room temperature for 1 h. After washing again, the wells are incubated with 100 μL of o-phenylenediamine substrate solution in the dark at room temperature for 30 min, then 100 μL of the stop solution (4N sulfuric acid) is added to each well, and the color is measured at 490 and 630 nm using a microplate reader.

A standard curve is generated from the average absorbances at 490 and 630 nm of solutions of known concentrations of purified, recombinant human p53 (0, 100, 300, 600, 1,000, and 1,500 pg/mL; in duplicate). This assay will detect 100 pg/mL of p53 at a signal level that is approximately twice background and generates a linear standard curve up to 1,500 pg/mL, and the assay does not cross-react with other cellular components of p53-negative cell lines. With serum samples spiked with known quantities of purified, recombinant