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Smooth Muscle Actin Is Expressed by Air Space Fibroblast-like Cells in Idiopathic Pulmonary Fibrosis and Hypersensitivity Pneumonitis

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Phenotypic classification of subpopulations of myofibroblasts in reorganizing tissues on the basis of smooth muscle cytoskeletal and contractile proteins has proved useful to understanding their biology. A hallmark of many inflammatory lung diseases is the proliferation of fibroblast-like cells within terminal airways and alveoli, a condition variably termed bronchiolitis obliterans (when the lesions predominantly involve terminal airways) or organizing pneumonia (when the lesions primarily involve the air spaces). The cells of these lesions have been shown to be active in the synthesis of matrix proteins, but their role in lung fibrosis is unclear. We studied 28 patients with inflammatory lung disease representing a wide spectrum of clinical outcomes: hypersensitivity pneumonitis (HP, n = 18), a reversible disease with a favorable prognosis; idiopathic bronchiolitis obliterans with organizing pneumonia (BOOP, n = 5), a variably reversible disease with an intermediate prognosis; and idiopathic pulmonary fibrosis (IPF, n = 5), a progressive disease, unresponsive to medical therapy, with a dismal prognosis. These 3 diseases were chosen because all are variably associated with air space fibroblast proliferation and we hypothesized that important differences might exist with regard to the distribution and number of such lesions and clinical outcome.

All patients in this study had undergone open lung biopsies. A minimum of 2 lung sections from each patient was examined for the presence of α-smooth muscle actin (αSMA) by immunohistochemistry. This actin isoform has been previously described in most recognized forms of nonstriated mammalian contractile/traction cells and serves as an excellent marker of certain wound repair fibroblasts. Immunoreactivity was quantified on a scale of 1 to 4. Four types of lesions were evaluated; the data are presented in Figure 1. Air space fibroblasts immunoreactive for α-SMA were identified in all 3 diseases studied, though to variable degree. In severely involved cases, a transition from air space to interstitial reactivity was noted. Unexpectedly, the presence of α-SMA reactive air space fibro-
blasts correlated directly with mortality in our small series of patients. Interstitial cells immunoreactive for ASMA were seen in all 3 disorders, and their presence did not correlate with survival. Patchy fibrosis with vascular prominence was noted primarily in IFP and the presence of this lesion correlated negatively with survival. Finally, a peculiar smooth muscle proliferation was identified around terminal airways in HP, in association with inflammation and foreign body reactions to inhaled material.

We conclude that the development of air space fibroblasts immunoreactive for ASMA may be a more common event in noninfectious lung disease than previously realized. Based on our findings we would speculate that contractile air space fibroplasia may be a precursor lesion to interstitial fibrosis in inflammatory lung diseases.

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Tropoelastin Pre-mRNA Is Alternately Spliced at Different Frequencies During Rat Lung Development

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Elastin, a major component of lung connective tissue, is important for alveolar development and maintenance of normal lung structure. The elastin precursor, tropoelastin, is encoded by a single multi-exon gene and its pre-mRNA is alternatively spliced in several species. We found that the rat tropoelastin pre-mRNA is subject to alternative splicing in 2 regions: 1 corresponding either to exons 13-15 or to exons 14 + 15 (by analogy to the bovine gene), and the other mapping to sequences beginning at the equivalent of bovine exon 33. The objective of this study was to examine potential developmental or tissue-specific regulation of splice site usage.

Total RNA was extracted from pooled aorta, lung, or skin tissues obtained from neonatal (10-d) and adult (6-wk) rats. An overlapping set of rat tropoelastin cDNA clones was used as probes in S1 nuclease protection assays. Autoradiographs were scanned by densitometry to determine the frequency of alternative splice site usage. Messenger RNAs lacking exons 14 + 15 were observed at low frequency in all tissues. Within a given tissue in neonatal or adult rats, the 3 sites of alternative splicing were used at different frequencies. In general, the frequency of alternative splicing, and particularly the frequency of alternative splicing at exon 33, decreased in adult tissues. Some differences in use of alternative splice sites were also observed between tissues at each developmental stage, with the greatest differences observed in skin vs aorta and lung at 10 d and 6 wk.

We conclude that the frequency of alternative splicing is developmentally regulated and tissue specific. The regulation of tropoelastin pre-mRNA by alternative splicing during the neonatal period could have significant implications for the role of elastin in lung development and in resynthesis of elastin after lung injury.

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Supported by NIH grant HL24264 and the Foundation of UMDNJ.