Collagens in Scar Carcinoma of the Lung

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Immunohistopathologic and biochemical studies of different collagen types extracted from human scar carcinoma of the lungs have been carried out for definition and evaluation of which types of collagen are involved in the scarring mechanism of such tumors. Tumor homogenates treated with 0.5 M acetic acid and followed by limited proteolysis with pepsin and then by fractional salt precipitation, demonstrated that Type I collagen consitutes the major collagenous component in addition to a significant increase in Type V collagen extracted from human scar carcinoma of the lung. However, when normal membranoalveolar peripheral lung tissues were processed under the same experimental conditions, Type III and IV collagens were relatively higher. Immunohistochemical studies were carried out, and the results confirmed the data above. Furthermore, these studies demonstrated a relative localized increase in Type III collagen in the area surrounding the tumor acini, which suggested that these areas are of active and recent scar formation. This supports the current concept of the scar origin as a desmoplastic reaction of the host tissues toward the neoplastic cell growth. (Am J Pathol 1985, 121:322–326)

CICATRICIAL, or "scar," cancers were first described by Friedrich in 1939[1] and Rossle in 1943[2] as a group of lung cancers that were characteristically originated around peripheral scars in the lung. Since then, there have been numerous attempts to study the relationship between pulmonary scarring and the development of specific types of pulmonary carcinoma. Some of the current concepts suggest that peripheral lung carcinoma can characteristically arise in association with pulmonary scars.[1,3,4] Others, however, have reported that the carcinoma-related scar is an example of desmoplastic reaction such as is seen in breast, stomach, pancreas, and colon cancers.[5–7]

It is generally agreed the pulmonary adenocarcinoma has a lower survival rate than squamous cell carcinoma, because the tumor often invades both lymphatic channels and blood vessels at a very early stage in its development[7,8] and also produces symptoms later in the course of the disease. Therefore, it becomes worthwhile to investigate the origin and significance of the fibrosing process associated with pulmonary scar carcinoma. The objective of this work is to investigate the different collagen types involved in the scarring process both biochemically ("quantitatively") and immunohistochemically ("qualitatively") for determination of the basic relationship between the scar and the neoplasm.

Materials and Methods

Histopathologic Studies

The tumors were evaluated as peripheral adenocarcinoma with associated scar from 4 cases of surgically resected lung tissue containing tumor. The criteria of evaluation was based upon microscopic examination of multiple slides stained with hematoxylin and eosin, (H&E), Masson's trichrome, and reticulum stains.

Collagen Preparation

Calf skin acid soluble Type I collagen was prepared from bovine by the method described by Glimcher et al.[9] The cross-reactivity of rabbit antibody to Type I calf collagen with human Type I collagen was confirmed by Furthmayer.[10] Type III, IV, and V collagens were prepared from human chorioamniotic membrane, according to the method described essentially by Dixit,[11] ex-

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cept that Type III was precipitated with the use of the heat gelation technique as described by Trelstad and Lawly,[12] followed by further purification with a modified differential denaturation and renaturation method as described by Chandra Rajun.[13] The purity of each collagen type was assessed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and amino acid analysis.

Preparation of Antisera

Four New Zealand white rabbits were immunized each with Type I, III, IV, or V collagen. Initial injections were given intracutaneously with 5 mg of the antigen previously dissolved in 5 ml 0.01 M acetic acid and emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich). The presence of antibodies was monitored by enzyme-linked immunosorbent assay (ELISA) as described by Gosslau.[14] Booster injections of 0.5 mg of the antigens without adjuvant were given to the rabbits every 3 weeks; the rabbits were bled and sacrificed 3 months after the first injection. Antisera to each type of collagen were purified by ammonium sulfate precipitation followed by passing through a DEAE-cellulose column equilibrated with 0.02 M potassium phosphate buffer, pH 7.8, as described by Fahey et al.[15] The titer and specificity were determined by immunofluorescence and ELISA.

Immunofluorescent Staining

Fresh normal or tumor-bearing lung specimens were snap-frozen in OCT compound (Miles Laboratories, Inc., Naperville, Ill) and cooled by freon aerosol. Four- to five-micron sections sliced in a cryostat were mounted on glass slides, air-dried, and pretreated with acetone for 10 minutes. Indirect immunofluorescent staining was carried out by incubating the sections with antibodies to Type I, III, IV, or V collagen for 60 minutes; after several washings by phosphate-buffered saline (PBS), pH 7.2, the sections were subsequently stained with fluorescein isothiocyanate (FITC)-labeled anti-rabbit IgG goat (Sigma Chemical Co., St. Louis, Mo). As a control, another set of sections were incubated with nonimmunized rabbit serum in place of anti-collagen antibody.

Extraction of Collagens From the Scar Carcinoma

Four specimens from resected peripheral adenocarcinoma associated with scar treated by lobectomy were selected and obtained at surgery. The tumor tissues and the associated scars were carefully dissected and processed for collagen extraction. As a control, four *peripheral* normal lung specimens were recovered at the time of forensic autopsy from patients who had died of unrelated causes. Only lungs without gross and microscopic evidence of disease were used. The larger tracheobronchial tissues and major blood vessels were removed, and the remaining peripheral lung was used.

The different types of collagens were isolated from the lung specimen by limited pepsin digestion, followed by fractional salt precipitation according to the procedure described by Chiang et al.[16] The purity of each collagen type was assessed by SDS polyacrylamide gel electrophoresis and amino acid analysis.

Results

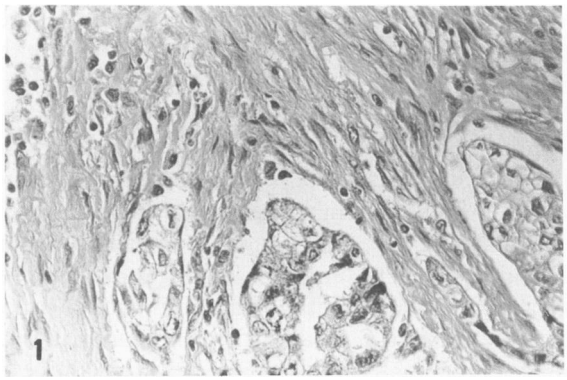
The clinical charts were reviewed retrospectively for relevant lung diseases as well as occupational and smoking histories. Table 1 reveals that heavy smoking was consistent in the 4 cases studied. In only 1 case had there been a history of chronic bronchitis, which may have been due to the heavy smoking. Lymph nodes metastases were negative in all 4 cases. One case was histopathologically diagnosed as primary well-differentiated adenocarcinoma, and the other 3 were poorly differentiated adenocarcinomas. All tumors were peripherally located and associated with an area of dense fibrosis by gross examination.

Histopathologic Examination

Microscopic examination of multiple histologic slides with H&E, Masson's trichrome, and reticulum stains revealed 1 case of well-differentiated adenocarcinoma and 3 cases of poorly differentiated adenocarcinoma. (The latter type is demonstrated by H&E stain in Figure 1). All tumor sections also demonstrated an area of associated dense collagenous matrix surrounding and adjacent to the tumor mass (Figures 1 and 2). Meanwhile, reticulum stain showed positive silver staining close to and surrounding the tumor nests and acini, as demonstrated in Figures 3 and 4.

Table 1—Smoking and History of Lung Disease

Cases	Sex/Age	Smoking history	Occupation	History of lung diseases
1	M/59	2–3 PPD/40 years	Salesman	Negative
2	M/73	3 PPD/50 years	"Various labor jobs"	Chronic bronchitis
3	F/63	2 PPD/40 years	Housewife	Negative
4	M/52	1 PPD/35 years	Truck driver	Negative



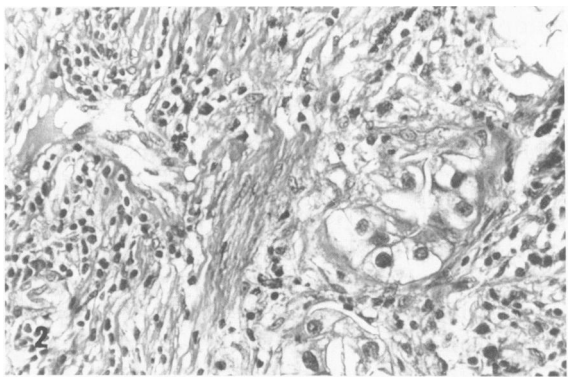
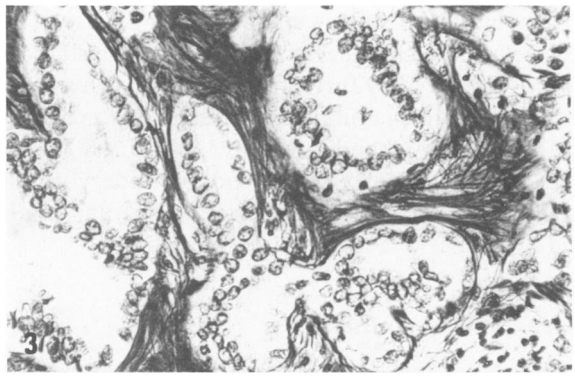


Figure 1—A well-differentiated adenocarcinoma of the lung arranged in an acinar pattern and surrounded by dense collagenous stroma with some chronic inflammatory cells. (H&E, × 100) **Figure 2**—The same tumor acini adjacent to a deep blue staining collagenous matrix which is moderately infiltrated by chronic inflammatory cells. (Masson's trichrome, × 200)



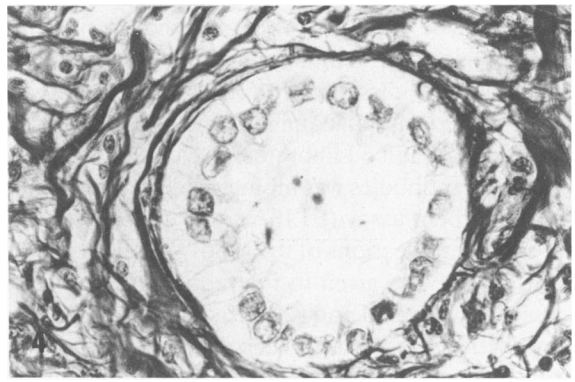


Figure 3—The tumor acini surrounded by recently formed collagen fibers as demonstrated by reticulum stain. (× 200) **Figure 4**—High-magnification photomicrograph of one of the tumor acini surrounded by recently formed collagen bundles, as evidenced by the reticulum stain. (× 400)

Immunofluorescence Examination

Immunofluorescent staining of 4 pulmonary adenocarcinomas reveals similar staining patterns for each collagen type. Staining of the fresh-frozen sections with labeled antibodies against Type I or V collagen demonstrated a significant increase in the immunofluorescent staining of these collagen types corresponding to the area of dense fibrosis surrounding the tumor mass (Figure 5) when compared with a specimen from the normal membranoalveolar peripheral lung tissues.

Immunofluorescent staining of Type III, however, revealed a localized increased labeling in the area surrounding the tumor nests as well as in the periacinar areas (Figure 6).

Biochemical Extraction of Collagen

Four types of collagens were extracted from about 1 g of washed lyophilized tumor or normal lung tissue as described above by the method of Chiang et al.[16]

Types I, III, IV, and V collagens were weighed after partial pepsin digestion, fractional salt precipitation, desalting, and lyophilization. The purity of each collagen type was assessed by gel electrophoresis on 7%

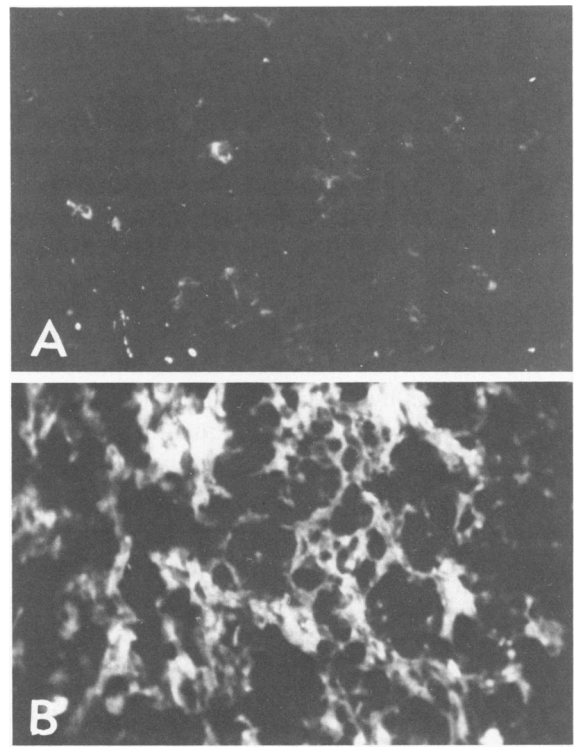
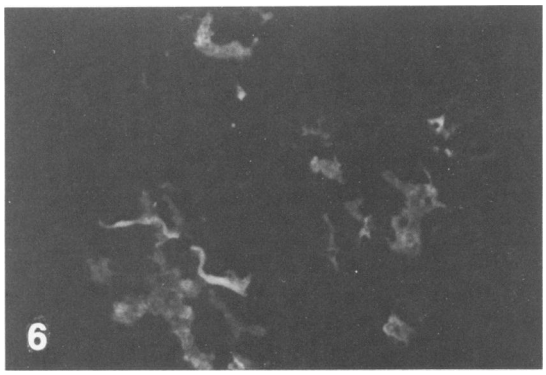
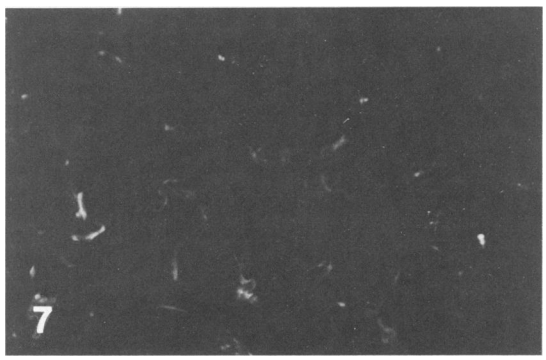


Figure 5—Indirect immunofluorescence stains of scar carcinoma specimen with antibodies to Type I collagen, demonstrating scanty irregular distribution in periacinar and area adjacent to tumor nests (**A**) and intense fluorescence in the area of dense fibrosis at the tumor periphery (**B**). (× 100)





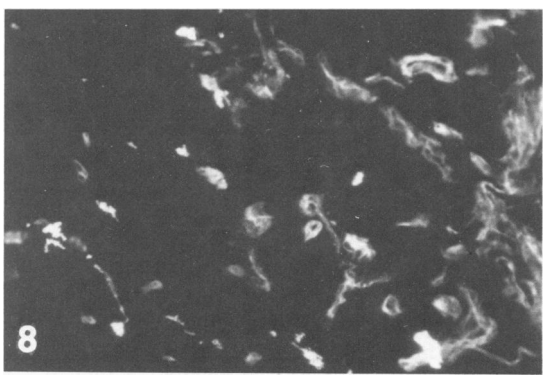


Figure 6—Indirect immunofluorescence staining pattern of scar carcinoma specimen with antibodies to Type III collagen showing a moderately intense fluorescence adjacent to and surrounding the tumor nests and acini. (×100) **Figure 7**—Indirect immunofluorescence stain of the same specimen with antibodies to Type IV collagen revealing severely disrupted linear fluorescence in the area of tumor acini. (×100) **Figure 8**—Indirect immunofluorescence stain of the same specimen with antibodies to Type V collagen demonstrating a relative increase in the fluorescence in the area of fibrosis adjacent to the tumor acini. (×100)

acrylamide gel (Figure 7) and amino acid (data not shown).

The results indicate that the Type I/Type III/Type IV/Type V average ratio is 32:44:14:9 by weight, respectively, for peripheral normal lung specimens; the ratio is 52:26:7:15 by weight, respectively, for the scar-associated tumor specimens. These results suggest a significant increase in Type I and V and a corresponding decrease in Type III and IV collagens extracted from the tumor specimens, compared with peripheral normal lung specimens (Table 2).

Table 2—Biochemical Extraction of Collagen From Normal and Scar Carcinoma of the Lung

	Lung tissue dry weight (mg)	Percentage collagen "dry weight"	Percentage collagen types			
			I	III	IV	V
Normal lung parenchma	1223	13	32	44	14	9
Scar carcinoma	1019	9	52	26	7	15

The values given for normal lung and scar carcinoma specimens are the averages of four case studies.

Discussion

There have been numerous attempts to study the relationship between pulmonary scarring and development of pulmonary adenocarcinoma. Most of the previous studies on scar carcinoma have been based on nonspecific research data from surgery or autopsy[4,5,17-19] case reports, which suggest that peripheral adenocarcinoma of the lung can characteristically arise in association with pulmonary scars.[1,3,20,21] However, our biochemical and immunohistopathologic studies on 4 surgically resected cases of pulmonary scar carcinoma suggest that the scar could be the result of a desmoplastic reaction of the host toward the growth of the tumor mass. Meanwhile, there was no clear evidence in our investigations to support the current concept of the scar as a source of origin of the tumor.

The selected tumor specimens for this study were carefully examined and histopathologically diagnosed by frozen section and confirmed later by H&E-stained permanent sections. All were associated with appreciable fibrosis.

Qualitative evaluation of collagen types associated with that tumor by the immunofluorescent technique demonstrated a localized increase in immunofluorescent labeling of Type III collagen in the periacinar area as well as the area adjacent to the tumor cell nests. These results suggest that these areas are of a recent, active, ongoing, immature, fibrosing process, a phenomenon which has been reported and well documented for an early stage of pulmonary[22] as well as nonpulmonary fibrosis.[22-24] Meanwhile, immunofluorescent labeling of Type I and V collagen (Figures 5 and 9) demonstrated a significant increase in both collagen types at the periphery of the tumor mass; this is consistent with an old fibrosis.[23] Moreover, the scanty and interrupted distribution of Type IV collagen could be due to Type IV collagen (Figure 8) breakdown as a result of tumor growth and invasion.[25]

Biochemical and enzymatic extraction of different collagen types support the immunofluorescent qualitative findings (Table 2). The decrease in the total collagen amount extracted from the tumor specimens, when

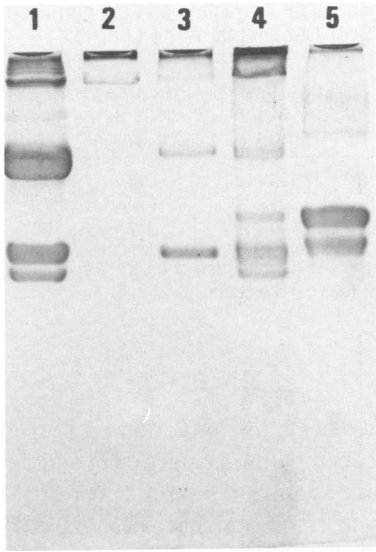


Figure 9—SDS polyacrylamide gel electrophoresis of collagen extracted from human scar carcinoma of the lung in 0.05M Tris borate buffer, pH 8.5, 8% gel. *1*, Type I collagen. *2*, Type III collagen. *3*, Type III collagen after reduction with dithiothreitol. *4*, Type IV collagen. *5*, type V collagen.

compared with that extracted from normal lung specimens may be due to marked increase in collagen cross-linking, making it less susceptible to pepsin digestion.[26,27] These findings support the concept of the scar origin as a host desmoplastic reaction to the tumor growth such as is seen in breast, stomach, pancreas, and colon carcinoma. Moreover, our data are in agreement with the investigations reported by Shimosato et al[7] and Madri et al[6] in that fibrosis associated with the tumor is the result, rather than the source of origin, of the neoplastic growth.

The basic mechanism for scar formation associated with peripheral lung cancer has remained unclear. However, some suggest that the scar could be due to repeated episodes of tumor necrosis and healing.[5]

Data from these studies should contribute to future knowledge of the nature and mechanism of the scarring process associated with this tumor and, in addition, promote better understanding of the role of host-tumor cell-cell interactions in the scarring process during tumorigenesis.

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