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Distribution of collagen types I and III and basal lamina in human gastric carcinoma: an immunohistochemical and electron microscopic study

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Summary. Collagen types I and III were examined immunohistochemically in 32 cases of gastric carcinoma classified as poorly differentiated adenocarcinoma with scirrhous stroma, well differentiated adenocarcinoma with intermediate stroma, or poorly differentiated adenocarcinoma with medullary stroma. In the stroma of scirrhous carcinoma, types I and III collagens were distributed abundantly in fibrillar or granular patterns with little difference in the intensity of staining. In well differentiated adenocarcinoma, type I collagen was diffusely distributed in the stroma with type III collagen distributed sparsely. In poorly differentiated adenocarcinoma with medullary stroma, the two types of collagen were only found around capillaries, constituting the tumor interstitium. Electron microscopic examination of scirrhous carcinoma showed tumor cells partially covered with fibroblasts, and discontinuous basal lamina, collagen fibers and microfibrils present between tumor cells and fibroblasts. In well differentiated carcinoma, tumor cells were surrounded by fibroblasts, and well developed basal lamina was observed beneath the tumor cells. In poorly differentiated carcinoma with medullary stroma, the stroma consisted of capillaries and very few fibroblasts with discontinuous basal lamina occasionally being present between tumor cells and fibroblasts.

Key words: Gastric carcinoma – Scirrhous carcinoma – Collagen – Immunohistochemistry – Electron microscopy

Five types of collagen differing both genetically and in their antigenic determinant have been reported in human tissue (Timpl et al. 1977). Using an immunohistochemical procedure, the distribution of these types of collagens has been investigated in several organs (Remberger et al. 1975; Gay et al. 1975; McCullagh et al. 1979; Madri et al. 1980; Voss et al. 1980; Hahn

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et al. 1980; Yamamoto et al. 1984). However, few studies have reported the collagen distribution in the stroma of gastric carcinoma. The scirrhous type of gastric carcinoma is a special kind of tumor characterized morphologically by extensive fibrosis and clinically by having a poor prognosis. The precise mechanism and significance of marked fibrosis in gastric scirrhous carcinoma are unknown.

The purpose of this study is to investigate the stroma of scirrhous and non-scirrhous gastric carcinoma using immunohistochemistry for collagen types I and III and electron microscopy. It is clearly important to obtain basic information on the stroma of gastric scirrhous carcinoma in the study of its pathogenesis.

Materials and methods

Tumor tissues were obtained from 32 patients having undergone surgical removal of gastric carcinoma. Tumor tissue was quick-frozen and stored at −70° C for immunohistochemical study of collagens or fixed in 10% buffered formalin for histological examination. Sections for histology were stained with H & E and periodic-acid-Schiff solution. According to the histological classification of the Japanese Research Society for Gastric Cancer (Nagayo 1974), 17 cases had well differentiated adenocarcinoma with intermediate type stroma, 13 cases had poorly differentiated adenocarcinoma with scirrhous type stroma, and 2 cases had poorly differentiated adenocarcinoma with medullary type stroma.

Immunohistochemistry of collagen. Type specific rabbit antibodies against type I and III collagens from human placenta were used. Preparation and purification of specific antibodies has been described in detail elsewhere (Yamamoto et al. 1984). Unfixed tumor tissues stored at −70° C were fixed in Zamboni's solution for 2 h. They were then washed overnight in 0.05 M phosphate buffered saline (PBS) with 10% sucrose, PBS with 15% sucrose for 6 h, PBS with 20% sucrose for 6 h and PBS with 20% sucrose and 5% glycerin for 1 h. The tissues were embedded in OCT compounds and quick-frozen. Frozen sections (6 μ in thickness) of tumor tissue were air-dried for 30 min and then rehydrated in PBS. The sections were incubated with rabbit antibody to collagen types I and III at room temperature in a humidified chamber for 3 h. Collagen antibodies were used at dilution of 10:1 for type I and 5:1 for type III collagen. The sections were then washed twice with PBS and incubated with a 1/20 dilution of FITC-labelled goat antirabbit IgG (MBL Co., Ltd., Japan) for 45 min. After the non-reactive conjugate was removed by repeated washing in PBS, the sections were embedded in glycerin. Specificity of the reaction was tested in two ways: (1) specific antisera to each collagen was absorbed at 4° C for 24 h with excess antigen, (2) non-immune rabbit sera was used in place of anticollagen antibody. Reactions were always negative. Moreover, the immunoreactivity of fixed materials in Zamboni's solution was well preserved as unfixed controls and fixed specimens were better in preservation of tissue structure than unfixed ones.

Electron microscopy. Small pieces of tumor tissue were immersed in 2% glutaraldehyde buffered with sodium cacodylate, postfixed in 2% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined using a JEM 100S electron microscope.

Results

Scirrhous gastric carcinoma

Histologically, all cases of scirrhous carcinoma were classified as poorly differentiated adenocarcinoma (Fig. 1a). Immunohistochemically, both types I and III collagens were diffusely deposited in the stroma. Type I colla-

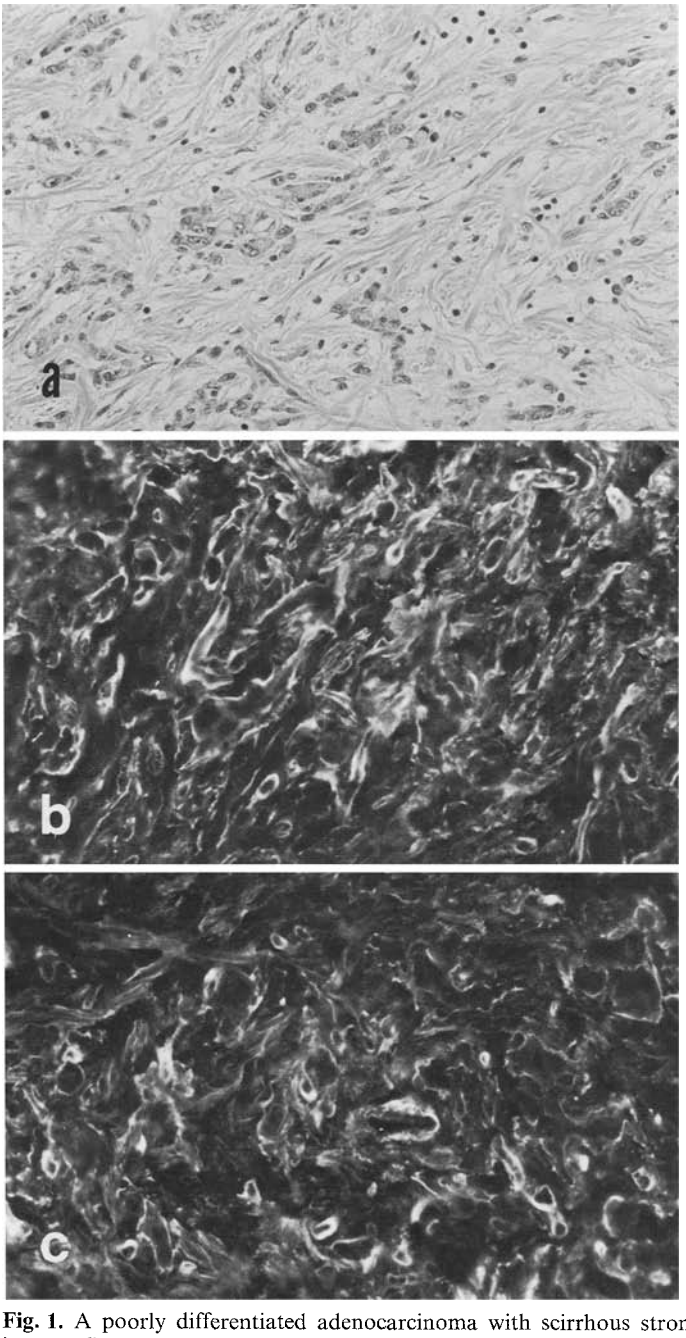

Fig. 1. A poorly differentiated adenocarcinoma with scirrhous stroma. Histological (**a**) and immunofluorescence appearances for type I (**b**) and III (**c**) collagens. Type I and III collagen are distributed diffusely in the stroma as fibrillar structures. × 180

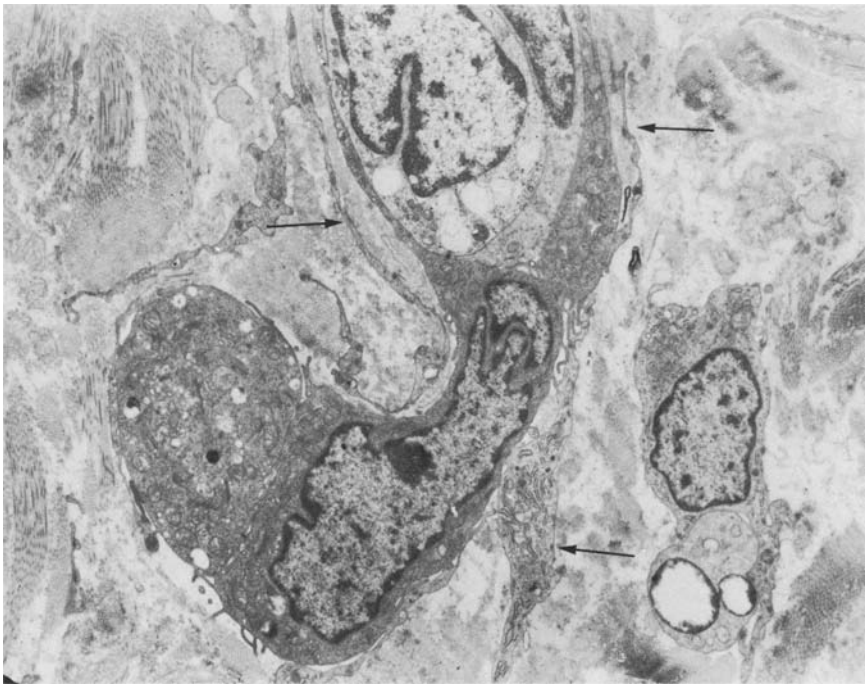

Fig. 2. Electron micrograph of the same case as in Fig. 1. Cancer cells are surrounded by cytoplasmic processes of the fibroblasts (*arrows*). × 3,800

gen was seen as thick fibrillar bundles (Fig. 1b). Type III collagen was arranged in either thin fibrillar bundles or in a granular form in the stroma (Fig. 1c). Electron microscopically, a dense array of mature collagen fibers with diameters of 300–500 Å and a periodicity of 640 Å were observed in the stroma. Among the collagen bundles, microfibrils with 100 Å diameter were distributed sparsely. A number of fibroblasts having long cytoplasmic processes and well developed rough endoplasmic reticulum were found among the collagen bundles or near tumor cells. Occasionally, tumor cells, either singly or in groups, were surrounded by fibroblasts (Fig. 2). In these areas, basal lamina structures were found discontinuously beneath the tumor cells (Fig. 3). Moreover, the intercellular space between tumor cells and fibroblasts had a few collagen fibers and microfibrils. However, the intercellular space between tumor cells without associated fibroblasts had no basal lamina.

Non-scirrhous gastric carcinoma

Nineteen cases of non-scirrhous carcinoma were classified histologically into 17 cases of well differentiated adenocarcinoma with intermediate type stroma (Fig. 4a) and 2 cases of poorly differentiated adenocarcinoma with medullary type stroma (Fig. 6a). In cases of well differentiated adenocarci-

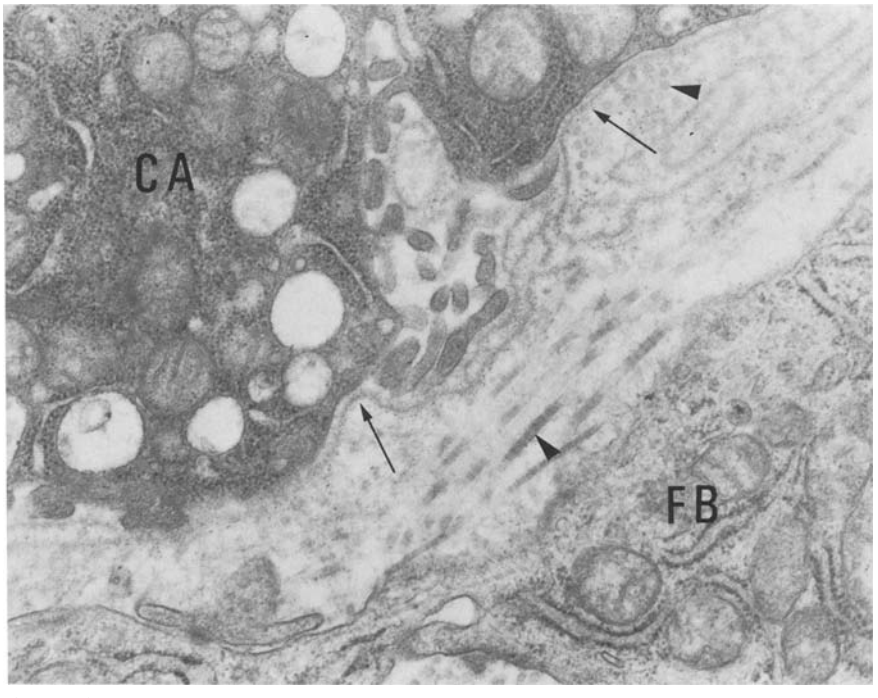

Fig. 3. Electron micrograph of scirrhous carcinoma. Between cancer cell (*CA*) and fibroblast (*FB*), discontinuous basal lamina (arrows) and collagen fibers (*arrow heads*) are noted. × 29,000

noma with intermediate stroma, type I collagen was distributed diffusely in thick bundles (Fig. 4b). Thin fibrillar type III collagen was distributed around tumor cells and sparsely in the stroma (Fig. 4c). Electron microscopically, most tumor cells were surrounded by fibroblasts. A continuous basal lamina structure was observed beneath the tumor cells. Some collagen fibers and microfibrils were also seen in the spaces between tumor cells and fibroblasts (Fig. 5).

In 2 cases of poorly differentiated adenocarcinoma with a small amount of stroma of the medullary type, an abundance of immunofluorescence for two types of collagen was seen around capillaries in the tumor tissue (Fig. 6b, c). However, fluorescence was rarely observed with only granular deposits of each collagen being detected around the individual tumor cells. Electron microscopically, the stroma consisted mainly of capillaries and a few fibroblasts. A continuous basement membrane, microfibrils and collagen fibers were observed around capillaries. Occasionally, a discontinuous basal lamina was present between tumor cells and fibroblasts.

Discussion

This study examined the distribution of two collagenous proteins, type I and III collagen, in the stroma of human gastric carcinoma immunohisto-

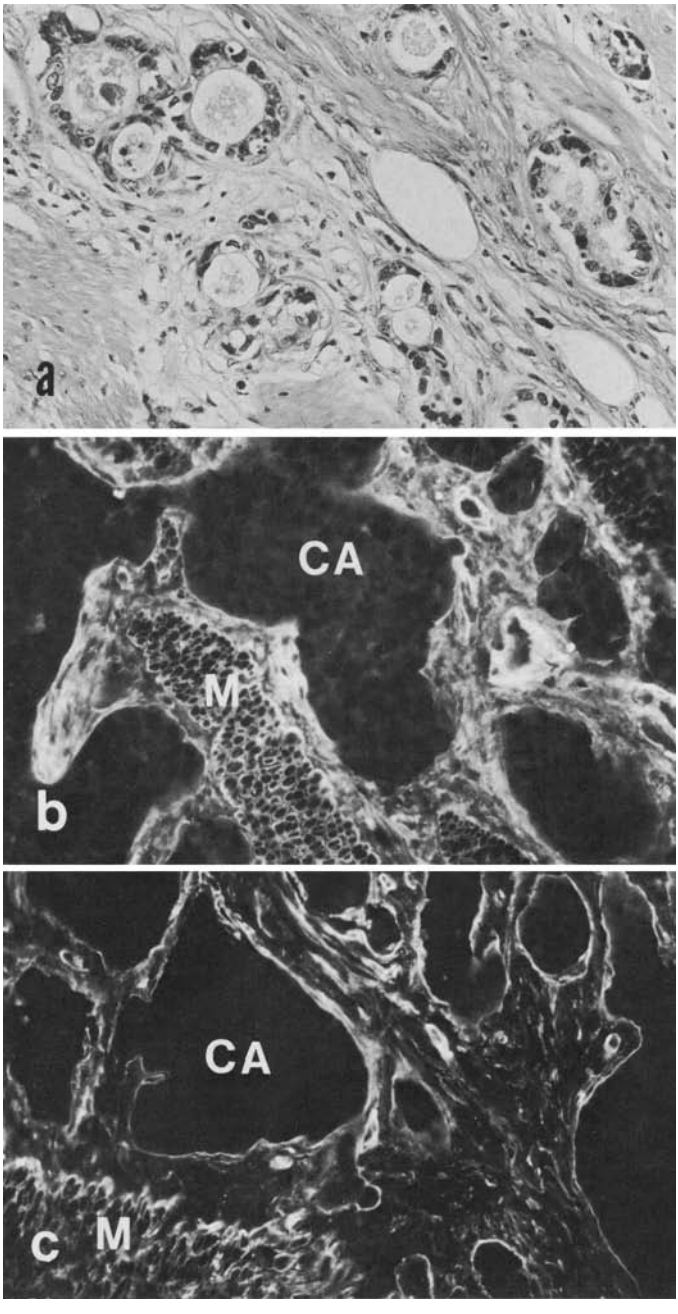

Fig. 4. A well differentiated adenocarcinoma with intermediate stroma. Histological (**a**) and immunofluorescence appearances for type I (**b**) and III (**c**) collagens. Type I collagen is densely stained in the stroma, and type III collagen is distributed sparsely as thin fibrillar fluorescence in the stroma. Each type of collagen is shown as a meshwork arrangement in the muscle layer. *CA*, cancer cell; M, muscle layer. ×180

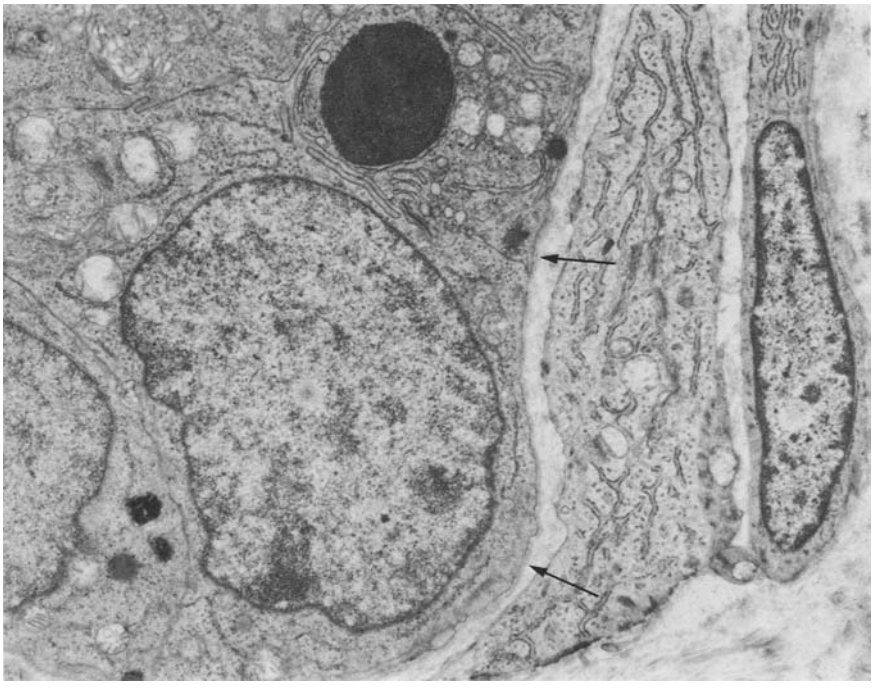

Fig. 5. Electron micrograph of the same case as shown in Fig. 4. Tumor cells are surrounded by fibroblasts. A continuous basal lamina (*arrows*) and fibrillar elements are observed between these cells. × 5,800

chemically. In scirrhous carcinoma, the stroma was composed of abundant type I and III collagens. However, the stroma of non-scirrhous carcinoma was mainly composed of type I collagen with less amount of type III collagen. These findings indicate that one of the characteristics of the stroma of scirrhous gastric carcinoma is the increase of type III collagen. In general, type III collagen is synthesized from fibroblasts and is found in most connective tissues. As immature collagen, it is abundant in the connective tissue of the fetus (Epstein 1974) and in the granulation tissue of the inflammatory process (Gabbiani et al. 1976). Moreover, an increase of type III collagen has been reported in the active stage of liver cirrhosis (Rojkind et al. 1979; Yamamoto et al. 1984). These findings suggest that increase of type III collagen is a marker, indicating an active fibrotic process.

With regard to collagen synthesizing cells in gastric tumor tissue, some investigators have recently reported that gastric cancer cells can produce interstitial collagen in culture (Sakakibara et al. 1982). In this study, however, scirrhous carcinoma with abundant fibroblastic proliferation was characterized by a prominent collagenous stroma, whereas medullary carcinoma having few fibroblasts in the stroma showed marked reduction in the deposition of collagens. Therefore, it is reasonable to assume that fibroblast is the main cell type producing collagens in tumor tissue in vivo.

There was another difference between scirrhous and non-scirrhous carci-

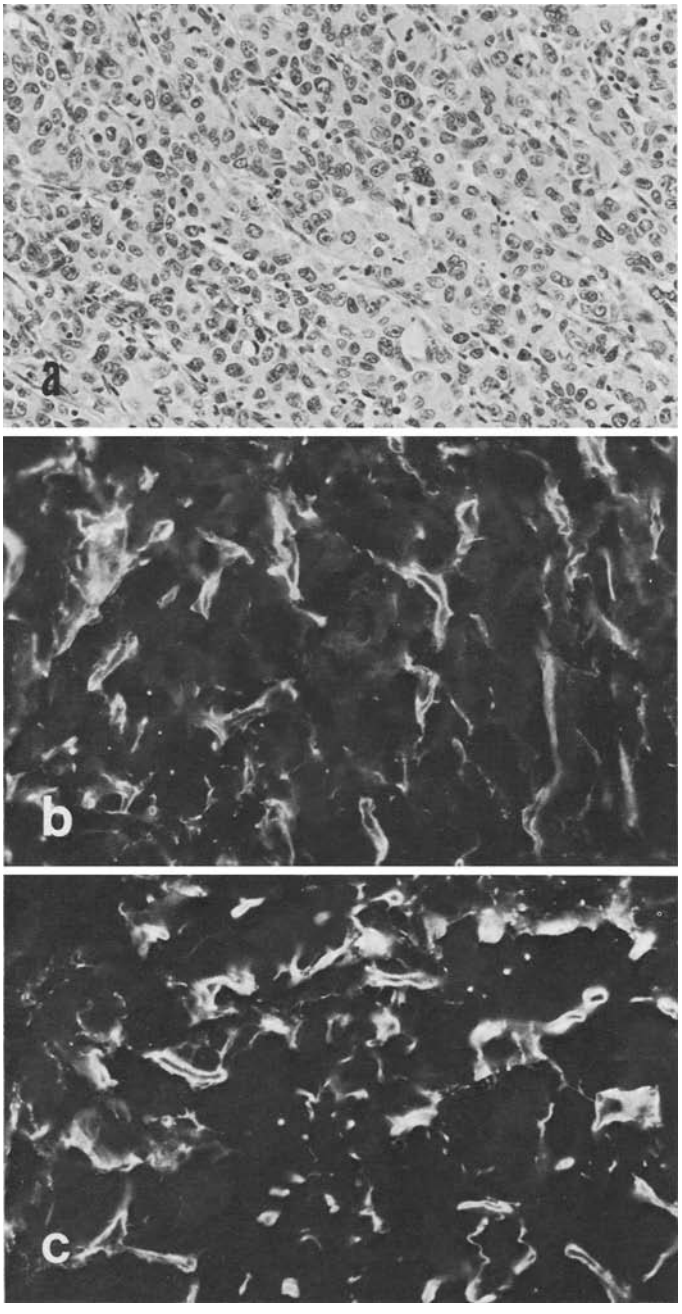

Fig. 6. A poorly differentiated adenocarcinoma with medullary stroma. Histological (**a**) and immunofluorescence appearances for type I (**b**) and III (**c**) collagens. Both types of collagens are found around the capillaries. However, only granular deposits for each type of collagen are distributed sparsely around tumor cells. × 180

noma in the formation of the basal lamina of the cancer cells. The cells of scirrhous carcinoma had an absent or discontinuous basal lamina, although tumor cells of non-scirrhous well differentiated carcinoma had a well developed and continuous basal lamina. From these findings, it is assumed that poor development of basal lamina in scirrhous carcinoma may be related to its markedly invasive growth. However, it is not known what factors control the formation of basal lamina in tumor tissue, although it was assumed from this study that the basal lamina was elaborated by co-operation between cancer cells and fibroblasts.

The precise mechanism of marked fibrosis in scirrhous gastric carcinoma is also uncertain. It is thought, however, that co-operation between cancer cells and fibroblasts is important in forming the tumor interstitium. In previous reports, we have shown that gastric scirrhous carcinomas frequently contain argyrophil cells that can produce multi-functional proteins such as peptide hormones, amines and enzymes (Tahara et al. 1982). Moreover, other investigators have reported that not only mesenchymal cells, but also some epithelial cells such as hepatocytes and gastric cancer cells produce collagenase (Nagai et al. 1982; Maruyama et al. 1982; Ueda et al. 1982). These reports suggest the possibility that tumor cells can produce some substances which are able to either directly stimulate fibroblast proliferation or to digest interstitial collagens. This would then be followed by the synthesis of collagens by fibroblasts as a response to tumor cell action.

References

Epstein EH (1974) $[\alpha 1(III)]_3$ human skin collagen. Release by pepsin digestion and preponderance in fetal life. J Biol Chem 249:3225–3231

Gabbiani G, Le Lous M, Bailey AJ, Bazin S, Delaunay A (1976) Collagen and myofibroblasts of granulation tissue – A chemical, ultrastructural and immunologic study. Virchows Arch [Cell Pathol] 21:133–145

Gay S, Fietzek PP, Remberger K, Eder M, Kühn K (1975) Liver cirrhosis: Immunofluorescence and biochemical studies demonstrate two types of collagen. Klin Wochenschr 53:205–208

Hahn E, Wick G, Pencev D, Timpl R (1980) Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin, and fibronectin. Gut 21:63–71

Madri JA, Furthmayr H (1980) Collagen polymorphism in the lung. An immunochemical study of pulmonary fibrosis. Human Pathol 11:353–366

Maruyama K, Okazaki I, Kobayashi T, Suzuki H (1982) Mammalian collagenase production by hepatic parenchymal and mesenchymal cells. J UOEH 4: [Suppl] 183–186

McCullagh KG, Duance VC, Bishop KA (1980) The distribution of collagen types I, III and V (AB) in normal and atherosclerotic aorta. J Pathol 130:45–55

Nagai Y, Hori H, Hata R, Konomi H, Sunada H (1982) Collagenase production by adult rat hepatocytes in primary culture. Biochem Res 3:345–349

Nagayo T (1974) Histological classification of gastric cancer. In: Nagayo T (ed) The general rules for the gastric cancer study in surgery and pathology. Japanese Research Society for Gastric Cancer. Kanehara

Remberger K, Gay S, Fietzek PP (1975) Immunohistochemische Untersuchungen zur Kollagencharakterisierung in Lebercirrhosen. Virchows Arch [Pathol Anat] Histol 367:231–240

Rojkind M, Giambrone M-A, Biempica L (1979) Collagen types in normal and cirrhotic liver. Gastroenterol 76:710–719

Sakakibara K, Suzuki T, Motoyama T, Watanabe H, Nagai Y (1982) Biosynthesis of an

interstitial type of collagen by cloned human gastric carcinoma cells. Cancer Res 42:2019–2027

Tahara E, Ito H, Nakagami K, Shimamoto F, Yamamoto M, Sumii K (1982) Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme, and HCG. Cancer 49:1904–1915

Timpl R, Wick G, Gay S (1977) Antibodies to distinct types of collagens and procollagens and their application in immunohistology. J Immunol Meth 18:165–182

Ueda N, Isozaki H, Miyamoto M, Okajima K (1982) Studies on localization of collagenase in gastric cancer tissue – by immunoperoxidase method. Proceedings of the Japanese Cancer Association, The 41th Annual Meeting, Osaka

Voss B, Rauterberg J, Allam S, Pott G (1980) Distribution of collagen type I and type III and of two collagenous components of basement membranes in the human liver. Pathol Res Pract 170:50–60

Yamamoto M, Sumiyoshi H, Nakagami K, Tahara E (1984) Distribution of collagen types I, III, and V in fibrotic and neoplastic human liver. Acta Pathol Jpn 34:77–86

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