Pericryptal Fibroblast Sheath in Intestinal Metaplasia, Dysplasia and Carcinoma of the Stomach

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ABSTRACT

Objective: To investigate the existence of pericryptal fibroblasts sheath (PCFS) in normal gastric mucosa, intestinal metaplasia (IM), indefinite for dysplasia (I-Dys), low grade dysplasia (L-Dys), high grade dysplasia (H-Dys) and gastric cancer (GC), and its association with gastric carcinogenesis.

Methods: In this study, we examined the existence of PCFS in normal gastric mucosa (N=10), IM (N=26), I-Dys (N=16), L-Dys (N=13), H-Dys (N=21) and GC (N=145) using immunohistochemical staining for two smooth muscle markers, alpha smooth muscle actin(α-SMA) and high molecular weight caldesmon (h-CD). The significance of PCFS was discussed, especially in association with gastric carcinogenesis.

Results: The PCFS was recognized in 65.4%(17/26) of IM, 62.5%(10/16) of I-Dys and 23.1% (3/13) of L-Dys respectively. No PCFS was detected in H-Dys and GC. The PCFS was gradually reduced in IM, Dys and GC in sequence (P<0.0001).

Conclusion: The PCFS is associated with the differentiation of epithelium and involved in gastric carcinogenesis via epithelial-mesenchymal interaction.

Key words: Pericryptal fibroblasts sheath (PCFS); Gastric cancer (GC); Carcinogenesis; Intestinal metaplasia (IM); Dysplasia

INTRODUCTION

The epithelial-mesenchymal interaction plays a essential role in normal tissue to maintain normal structure and function and in pathologic process including development and progression of preneoplastic and neoplastic lesions[1–4].

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The pericryptal fibroblast sheath (PCFS) consisting of a network of fibroblast and extracellular matrix, expressing alpha smooth muscle actin (α-SMA) and high molecular weight caldesmon (h-CD), is a part of mesenchyme of lamina propria in normal intestinal mucosa[1,5]. The PCFS locates at the epithelial-mesenchymal interface and tightly surrounds the epithelium. The PCFS has been shown to play a fundamental role in the differentiation of epithelium via epithelial-mesenchymal interaction[6,7]. In addition, the PCFS is reduced in adenoma, carcinoma in sequence and disappeared in invasive carcinoma of intestine, suggesting that it is involved in the development and progression of intestinal carcinoma[3,8]. Gastric carcinogenesis is a multistep complex process[9]. The intestinal metaplasia (IM) is an important

lesion in the course of development of gastric cancer (GC)[10]. The PCFS is not existent in normal mucosa of the stomach. Mutoh and colleagues reported that PCFS was present in IM, but absent in GC[11].

To investigate the association of PCFS with gastric carcinogenesis, we examined the PCFS in IM, indefinite for dysplasia(I-Dys), low grade dysplasia (L-Dys), high grade dysplasia(H-Dys) and GC.

PATIENTS AND METHODS

Specimens

Biopsy specimens from 26 cases with IM, 16 I-Dys, 13 L-Dys and 21 H-Dys taken from patients including 57 male and 19 female with a mean age of 64 years (range 45−78 years) were enrolled in this study. The endoscopic examination and biopsy were performed at Endoscopy Department in Beijing Cancer Hospital. In addition, 145 GC specimens from patients including 99 male and 46 female, mean age 59 years ranging from 29 to 78 years were included in this study. These patients received surgical treatment at Surgical Department in Beijing cancer Hospital. All of the specimens were diagnosed pathologically at Department of Pathology in Beijing Cancer Hospital.

All the specimens were fixed in 10% formalin and embedded in paraffin. Four μm thick sections were prepared for immunohistochemical stain.

Immunohistochemistry

The sections were deparaffinized in xylene and rehydrated in graded ethanol and phosphate buffered saline (PBS), and endogenous peroxidase activity was blocked by incubation for 10 min in 3% H_2O_2. In the immunohistochemical study for h-CD, deparaffinized tissue sections were heated in 10 mmol/L citrate buffer (pH 6.0) in an autoclave for 40 min before the primary antibody reaction. And antigen retrieval in the immunohistochemical staining for α-SMA was preformed in 1 mmol/L EDTA (pH 9.0) in an autoclave for 3 min. The monoclonal antibodies used were clone h-CD (Dakopatts, 1:50) which recognizes h-CD, and clone 1A4 (Dakopatts, 1:100) which reacts with α-SMA. The sections were incubated overnight at 4°C with the primary antibodies. After washing with PBS, the sections were incubated with Peroxidase-labeled goat anti-mouse immuno-globulin (PV-6002, DAKO, Glostrop, Denmark) for 1 h at room temperature (RT). The slides were colorized with 3,3'-diaminobenzidine tetrachloride (DAB), counterstained with haematoxylin and viewed under a light microscope. Positive and negative immunohistochemistry controls were routinely used.

Statistical Analysis

All statistical analyses were carried out using Chi-square test and Cochran-Armitage Trend Test (SAS 8.1 software). All statistical tests were two-sided. *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

PCFS in Normal Mucosa of Intestine and Stomach

The PCFS was observed in all of the 10 normal intestinal mucosa specimens. PCFS is immediately subjacent to the crypt epithelial cells and distinct from the rest of the mesenchymal elements of the lamina propria. Immunohistochemically, PCFS expressed both α-SMA and h-CD in cytoplasm of fibroblasts and formed a single cell layers (Figure 1A-D). PCFS was not seen in all of the 10 normal mucosa specimens of stomach (Figure 1E, F).

PCFS in IM, I-Dys, L-Dys, H-Dys and GC

PCFS was recognized in 65.4% (17/26) of IM, 62.5% (10/16) of I-Dys and 23.1% (3/13) of L-Dys respectively. No PCFS was detected in H-Dys and GC (Figure 2, Table 1). Compared with PCFS around the normal intestinal crypt of Lieberkühn, the PCFS detected in intestinal metaplasia, indefinite for dysplasia and low-grade dysplasia was less mature and in most area it did not form entire sheath around glands.

The development of PCFS in intestinal metaplasia had no significant difference compared with indefinite for dysplasia (P=0.85). Indefinite for dysplasia included foveolar hyperproliferation and hyper- proliferative intestinal metaplasia[12]. In our study, we combined intestinal metaplasia group and indefinite for dysplasia group. In low-grade dysplasia group, PCFS was significantly less developed than in intestinal metaplasia and indefinite for dysplasia group (P=0.009).

Cochran-Armitage Trend Test was done and we found that the development of PCFS was decreased in sequence of intestinal metaplasia and indefinite for dysplasia, low-grade dysplasia, high-grade