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Desmoplasia and Angiogenesis of Submucosa Invasive Carcinoma of the Stomach

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ABSTRACT

The prognosis of stomach cancer (gastric cancer) in advanced stage is generally poor, because the tumor has often metastasized. Desmoplasia (reactive fibrosis) and angiogenesis in the cancer microenvironment are important processes of cancer invasion/metastasis. We focused on the desmoplasia and angiogenesis using surgically/endoscopically resected submucosa-invasive carcinomas of the stomach. Desmoplasia is recognized as increase of cancer-associated fibroblasts, and irregular collagen bundles. Desmoplasia-positive cases significantly showed high-grade lymphatic/venous angiogenesis, compared with desmoplasia-negative cases (angiogenesis score: lymphatic 2.34 vs 1.27, $p<0.001$; venous 2.21 vs 1.69, $p<0.005$). Lymphatic/venous invasion of cancer cells was frequently found in the desmoplasia-positive cases, compared with desmoplasia-negative cases (lymphatic 89.5% vs 7.7%, $p<0.001$; venous 76.3% vs 34.6%, $p<0.001$). In conclusion, the desmoplasia is thought to play important roles of angiogenesis, and lymphatic/venous invasion, i.e., metastatic potentials of stomach cancer.

Keywords: Gastric cancer, Desmoplasia, Angiogenesis, Cancer invasion

INTRODUCTION

Worldwide, stomach cancer (gastric cancer) is the fifth most-common cancer, and becomes the third-leading cause of cancer-related death [1,2]. The most cases of stomach cancers are adenocarcinoma, and one of the most common causes is persistent infection of *Helicobacter pylori*. The patient's prognosis in advanced stage is generally poor, because the tumor has often metastasized. Presence of lymphatic/venous invasion of cancer cells becomes risk factors for lymph node/distant metastasis of the stomach cancer. The analysis of lymphatic/venous angiogenesis is important to determine the invasive progression and metastasis in patients.

In this study, we focus on the desmoplasia (reactive fibrosis) in the cancer stroma, and describe lymphatic/venous invasion and angiogenesis, using endoscopically/surgically resected submucosa-invasive carcinomas of the stomach.

MATERIALS AND METHODS

Stomach cancer specimens

This study performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Hirosaki University Graduate School of Medicine (organization number #1149). The pathological features of each cancer lesion were evaluated using paraffin-embedded tissue specimens from surgically/endoscopically resected 64 cases with submucosa-invasive carcinoma (pT1b tumor by TNM classification) [3]. The 64 cases included 17 well differentiated adenocarcinomas, 34 moderately differentiated adenocarcinomas, 13 poorly differentiated adenocarcinomas with/without signet-ring cell carcinoma components.

The area of deepest invasion was selected as a representative histological specimen of each cancer lesion. The selected sections were stained with hematoxylin & eosin (H&E),

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Masson trichrome (collagen fibers, blue), Verhoeff-van Gieson (elastic fibers, black), and immunohistochemical staining of D2-40 (podoplanin: marker of lymphatic endothelial cells), and α-smooth muscle actin (marker of cancer-associated fibroblast) [4,5].

Histological evaluation of desmoplasia, angiogenesis, and lymphatic/venous invasion of cancer cells

We divided the 64 cases into the two groups: desmoplasia-positive, and desmoplasia-negative **(Figures 1A & 1B)**. The desmoplasia-positive cases were defined as cancers in which more than 10% of area showed stromal desmoplastic reaction (H&E stain), increased collagen bundles (Masson trichrome stain) **(Figures 1C & 1D)** and presence of cancer-associated fibroblasts (α-smooth muscle immunostaining). Degrees of lymphatic/venous angiogenesis were classified into four groups: score 0 (no apparent angiogenesis in cancer area), score 1 (mild angiogenesis: one or two vessels over 200 μm in diameter), score 2 (moderate angiogenesis: three or four vessels), and score 3 (severe angiogenesis: more than five vessels). We divided the cases into the two groups: lymphatic/venous invasion-positive, and lymphatic/venous invasion-negative. Lymphatic and venous invasions were evaluated using D2-40 immunostaining **(Figures 2A & 2B)** and Verhoeff-van Gieson elastic staining **(Figures 2C & 2D)** respectively.

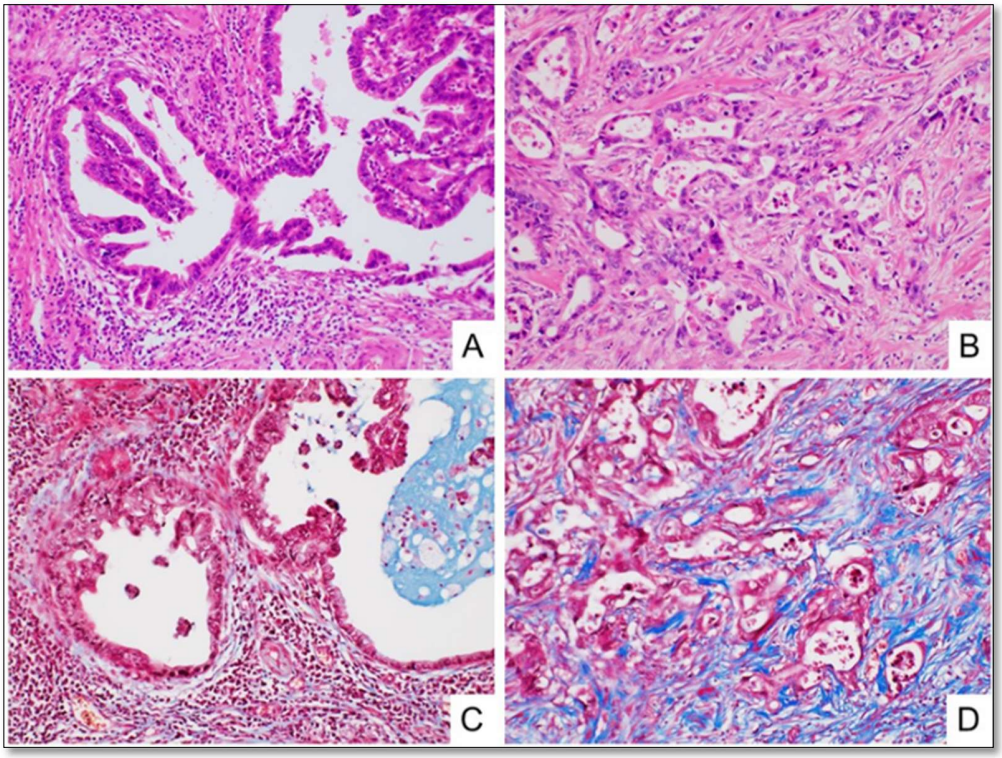

Figure 1. Histopathology of desmoplasia. Desmoplasia-negative case: no apparent fibrosis in the cancer stroma (A: top left, H&E staining). Desmoplasia-positive case: reactive fibrosis in the cancer stroma (B: top right, H&E staining). Small amounts of loose collagen bundles in the desmoplasia-negative cancer stroma (C: bottom left, Masson trichrome staining). Increase of dense collagen bundles in the desmoplasia-positive cancer stroma (D: bottom right, Masson trichrome staining).

Statistical analysis

Statistical comparisons between two groups were analyzed using the Student's *t*-test (angiogenesis score) or the Pearson's chi-square test (lymphatic/venous invasion) for categorical data. Differences were considered to be statistically significant if the p-value was <0.05.

RESULTS

Desmoplasia of sub mucosa-invasive carcinomas

Thirty-eight of the 64 submucosa-invasive carcinomas (59.4%) were desmoplasia-positive cases, in which more than 10% of area showed stromal desmoplastic reaction with increased collagen bundles and cancer-associated fibroblasts, while 26 (40.6%) were desmoplasia-negative.

Lymphatic/venous angiogenesis of sub mucosa-invasive carcinomas

According to the scoring of lymphatic angiogenesis (lymphangiogenesis), we classified the 64 cases into four groups as follows: one case (1.6%) of score 0, 24 cases (37.5%) of score 1, 19 cases (29.7%) of score 2, and 20 cases (31.3%) of score 3.

According to the scoring of venous angiogenesis, we classified the 64 cases into four groups as follows: none of score 0, 18 cases (28.1%) of score 1, 28 cases (43.8%) of score 2, and 18 cases (28.1%) of score 3.

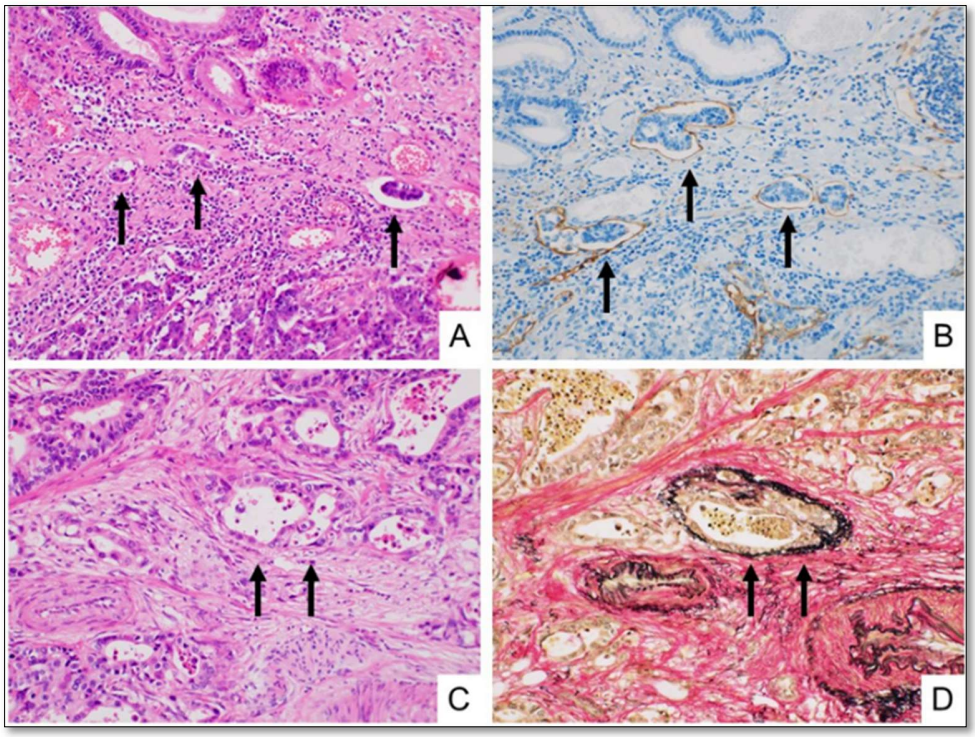

Figure 2. Lymphatic and venous invasion. Lymphatic invasion (arrows) is evaluated using H&E staining (A: top left), and D2-40 immunostaining (B: top right). Venous invasion (arrows) is evaluated using H&E staining (C: bottom left), and Verhoeff-van Gieson elastic staining (D: bottom right).

Correlation between desmoplasia and angiogenesis of submucosa-invasive carcinomas

Desmoplasia-positive cases significantly showed high-grade lymphatic/venous angiogenesis, compared with desmoplasia-negative cases (lymphatic angiogenesis score: 2.34 ± 0.75 vs 1.27 ± 0.60, p<0.001; venous angiogenesis score: 2.21 ± 0.74 vs 1.69 ± 0.68, p<0.005) **(Figures 3)**.

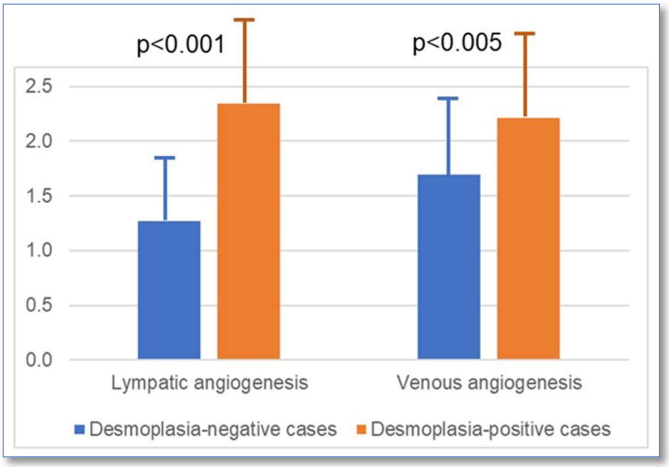

Figure 3. Correlation between desmoplasia and angiogenesis. Left: Desmoplasia-positive cases showed high-grade lymphatic angiogenesis, compared with desmoplasia-negative cases (2.34 ± 0.75 vs 1.27 ± 0.60, p<0.001). Right: Desmoplasia-positive cases showed high-grade venous angiogenesis, compared with desmoplasia-negative cases (2.21 ± 0.74 vs 1.69 ± 0.68, p<0.005).

Correlation between desmoplasia and lymphatic/venous invasion of sub mucosa-invasive carcinomas

Lymphatic/venous invasion of cancer cells was frequently found in the desmoplasia-positive cases, compared with desmoplasia-negative cases (lymphatic invasion: 89.5% (34/38) vs 7.7% (2/26), p<0.001; venous 76.3% (29/38) vs 34.6% (9/26), p<0.001) **(Figures 4)**.

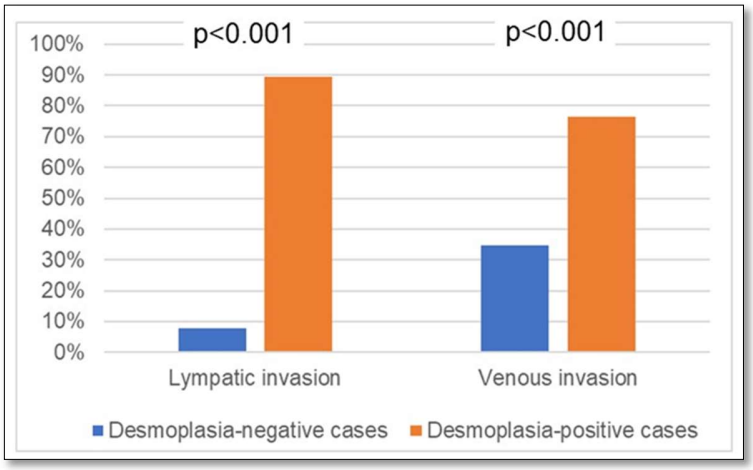

Figure 4. Correlation between desmoplasia and lymphatic/venous invasion. Left: Desmoplasia-positive cases showed frequent lymphatic invasion, compared with desmoplasia-negative cases [89.5% (34/38) vs 7.7% (2/26), p<0.001]. Right: Desmoplasia-positive cases showed frequent venous invasion, compared with desmoplasia-negative cases [76.3% (29/38) vs 34.6% (9/26), p<0.001].

DISCUSSION

In this study, we examined desmoplasia (reactive fibrosis) in the cancer stroma, using submucosa-invasive carcinomas of the stomach. The desmoplasia was statistically correlated with angiogenesis, and lymphatic/venous invasion, i.e., metastatic potentials stomach cancer.

Stomach cancer (gastric cancer) is the fifth most-common cancer, and becomes the third-leading cause of cancer-related death [1,2]. More than one million new cases were estimated worldwide in 2018 [6]. The stomach cancer is a multifactorial disease, and about 90% of cases are sporadic. The patient's prognosis in advanced stage is generally poor, because the tumor has often metastasized. Presence of lymphatic/venous invasion of cancer cells becomes risk factors for lymph node/distant metastasis of the stomach cancer [7].

Cancer microenvironment is important for the cancer cells growth in their native tissues/organs [8]. Invasion and metastasis are recognized as malignant phenotypes, and closely associated with interaction between cancer cells and non-cancerous stroma [9,10]. The non-cancerous stroma includes several cancer-associated cells, such as inflammatory cells, fibroblasts, and endothelial cells. The cancer-associated fibroblasts induce desmoplasia (reactive fibrosis with irregular collagen bundles) in the cancer stroma, and are associated with invasive growth and malignant potentials [11-16]. In addition, the cancer cells stimulate endothelial cells, and induce the angiogenesis, closely associated with cancer metastasis.

Several studies have reported that the intratumoral vascularization is correlated with metastatic potentials in the stomach cancer cases [17,18]. Our histopathological study defined the desmoplasia-positive cases, in which more than 10% of area showed stromal desmoplastic reaction with increased collagen bundles and cancer-associated fibroblasts. Desmoplasia-positive cases significantly showed high-grade lymphatic/venous angiogenesis, and frequent lymphatic/venous invasion of cancer cells.

In conclusion, the desmoplasia is thought to play important roles of angiogenesis, and lymphatic/venous invasion, i.e., metastatic potentials stomach cancer.

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