CD34 positive stromal cells in gastric adenocarcinomas

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Abstract

***Aims*—To investigate the role of CD34 positive stromal cells, namely dendritic interstitial cells, in gastric carcinomas, the distribution of CD34 positive stromal cells in gastric adenocarcinomas (GCs), with special reference to two histological types (diffuse (D-type) and intestinal (I-type)), was examined.**

***Methods*—In total, 55 surgically resected GCs (15 D-type and 40 I-type) and their normal tissues were examined. To distinguish CD34 positive stromal cells from vascular endothelial cells and to recognise the tumour border, immunostaining for CD34, CD31, and low molecular weight cytokeratins was performed.**

***Results*—In the 15 D-type GCs, eight of the nine D-type GCs invading the muscularis propria and subserosa had a large number of CD34 positive stromal cells in the tumour stroma, whereas all six D-type GCs confined to the submucosa had no CD34 positive stromal cells in the tumour stroma. All of the 40 I-type GCs had no CD34 positive stromal cells, regardless of tumour depth.**

***Conclusions*—These results suggest that CD34 expression in stromal cells is associated with progression of D-type GCs, and that absence of expression is also seen in I-type GCs that are progressing.**

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Keywords: CD34; stromal cell; diffuse type; intestinal type; gastric adenocarcinoma

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The CD34 molecule is a 110 kDa transmembrane cell surface glycoprotein,[1] which was originally described as a marker for human haematopoietic stem cells.[2] CD34 has no significant sequence homology to any known proteins.[2] CD34 positive stromal cells, namely dendritic interstitial cells,[3] are distributed throughout the human body including the gastrointestinal tract.[3–12] In the gastrointestinal tract, CD34 positive stromal cells are distinct from interstitial cells of Cajal.[10 11] An immunohistochemical study using confocal laser microscopy revealed that interstitial cells of Cajal are positive for c-kit and negative for CD34, whereas CD34 positive stromal cells are negative for c-kit.[10 11] It has been suggested that CD34 positive stromal cells throughout the human body play a supportive role, not only in the maturation or proliferation of adjacent mesenchymal and epithelial stem cells, but also in immune mediated responses.[4–6]

Recently, we reported that a lack of CD34 expression in colorectal tumour stromal cells is associated with desmoplastic stroma formation in well and moderately differentiated adenocarcinomas.[12] However, the relation between CD34 positive stromal cell distribution and histological types of gastric cancer has not been studied.

In our present study, to elucidate the importance of CD34 positive stromal cells in tumour stroma formation and progression of gastric adenocarcinomas with special reference to histological types, we examined the distribution of CD34 positive stromal cells in diffuse-type (D-type) and intestinal-type (I-type) gastric adenocarcinomas and their normal tissues.

Materials and methods

We examined 55 surgically resected invasive gastric adenocarcinomas that were confined to the gastric wall (not invading the adjacent organs) and their normal tissues from the surgical pathology files of the first department of pathology, Kochi Medical School and its affiliated hospitals from 1994 to 1999. The definitions used for histological classification were based on the criteria of Lauren[13]; 15 D-type and 40 I-type tumours were identified. Depth of tumour invasion was classified as submucosa (27 tumours; six D-type, 21 I-type), muscularis propria (13 tumours confined to the muscularis propria; five D-type, eight I-type), and subserosa (15 tumours; four D-type, 11 I-type). We classified the tumours confined to the submucosa as early cancers, and those invading the muscularis propria and subserosa as advanced cancers.

Immunohistochemical studies were performed by the streptavidin–biotin method using the Histofine SAB-PO(M) kit (Nichirei, Tokyo, Japan). Three monoclonal antibodies against CD34, CD31, and low molecular weight cytokeratins (LMW-CKs; cytokeratins 8 and 18) were used. Table 1 lists the monoclonal antibodies and staining procedures. We examined immunoreactivity for CD31 in all of the tumours and their normal tissues, to distinguish CD34 positive stromal cells from vascular endothelial cells, which are positive for both CD34 and CD31.[14] Vascular endothelial cells were used as the internal positive control of immunostaining for CD34 and CD31. (We did not apply digital subtraction.) Similar to our recent study regarding gastric cancer, immunostaining for LMW-CKs was

Table 1 Monoclonal antibodies used for immunohistochemical analysis

Antibody (clone)	*Specificity*	*Sources*	*Working dilution*	*Antigen retrieval*
MY10	CD34	Becton-Dickinson	1/20	—
JC/70A	CD31	Dakopatts	1/20	Pronase
CAM5.2	Cytokeratins	Becton-Dickinson	1/1 (prediluted)	Pronase

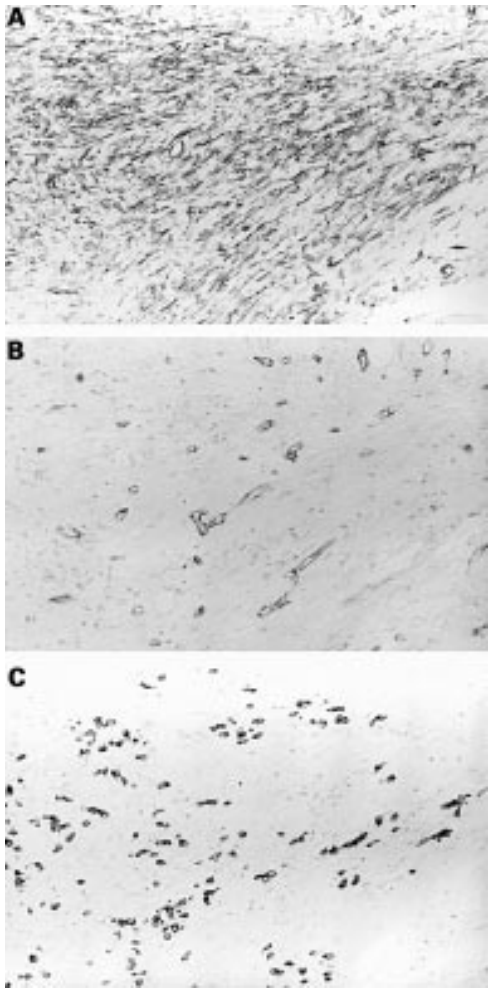

Figure 1 CD34 positive stromal cells in diffuse-type advanced gastric adenocarcinoma tissue. Staining for (A) CD34, (B) CD31, and (C) low molecular weight cytokeratins (LMW-CKs). Large numbers of CD34 positive stromal cells are detected in the tumour stroma. Immunoreactivity for CD31 is shown to distinguish CD34 positive stromal cells from vascular endothelial cells. Immunoreactivity for LMW-CKs is shown to recognise the diffuse-type gastric adenocarcinoma cells.

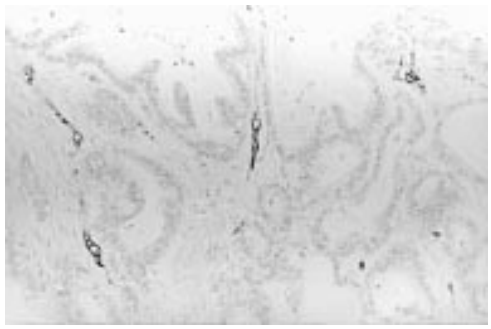

Figure 2 CD34 immunostaining in intestinal-type advanced gastric adenocarcinoma tissue. CD34 is positive in vascular endothelial cells; no CD34 positive stromal cells are seen.

also performed, to recognise the tumour border in every specimen examined.[15]

After immunostaining, we examined CD34 positive stromal cell distribution in gastric adenocarcinomas and normal tissues of the 55 cases. Regarding the tumours, the number of CD34 positive stromal cells was classified into two groups, namely: (+), tumours having CD34 positive stromal cells in the tumour stroma (fig 1A; note: fig 1B,C shows the expression of CD31 and LMW-CKs, respectively, at the same site as fig 1A); and (−), tumours having no CD34 positive stromal cells in the tumour stroma (fig 2). Statistical analysis was carried out using Fisher's exact probability test and p values < 0.05 were considered to be significant.

Results

Table 2 summarises the results.

In all of the 55 cases examined, CD34 positive stromal cells were mainly in the perivascular area of the normal submucosa, muscularis propria, and subserosa. CD34 positive stromal cells were not detected in the lamina propria but they were distributed in the areas adjacent to the muscularis mucosa. Results concerning the normal gastric wall were the same as the recent report by Kim *et al*,[16] who found that CD34 was expressed in stromal cells around vessels and muscle bundles in the gastric wall.[16]

In the 15 D-type gastric adenocarcinomas examined, eight tumours had CD34 positive stromal cells in the tumour stroma, whereas the remaining seven tumours had no CD34 positive stromal cells in the tumour stroma (table 2). All of the eight tumours were advanced cancers, and had a large number of CD34 positive stromal cells (fig 1A; note: fig 1B,C shows the expression of CD31 and LMW-CKs, respectively, at the same site as fig 1A). Eight of the nine advanced cancers had CD34 positive stromal cells in the tumour stroma classified as (+), whereas all of the six early cancers had no CD34 positive stromal cells and were classified as (−) (p = 0.0014).

In contrast, all 40 I-type gastric adenocarcinomas examined had no CD34 positive stromal cells classified as (−), regardless of tumour depth (fig 2).

Regarding the 28 advanced cancers, eight of the nine D-type adenocarcinomas were classified as (+), whereas all of the 19 I-type adenocarcinomas were classified as (−) (p = 0.0000029).

Table 2 The relation between tumour depth and CD34 positive stromal cell number in the tumour stroma of the 55 gastric adenocarcinomas

Tumour depth	*Number of cases*	*CD34 positive stromal cells*	
		(−)	*(+)*
Diffuse-type			
Early cancers			
SM	6	6*	0*
Advanced cancers			
MP + SS	9	1*†	8*†
MP	5	1	4
SS	4	0	4
Total	15	7	8
Intestinal-type			
Early cancers			
SM	21	21	0
Advanced cancers			
MP + SS	19	19†	0†
MP	8	8	0
SS	11	11	0
Total	40	40	0

SM, submucosa; MP, muscularis propria; SS, subserosa; (−), no CD34 positive stromal cells in the tumour stroma; (+), CD34 positive stromal cells in the tumour stroma.
*, p = 0.0014; †, p = 0.0000029.

Discussion

CD34 positive stromal cells were absent in the tumour stroma of well and moderately differentiated adenocarcinomas of the colorectum.[12] However, in our present study, we detected CD34 positive stromal cells in the D-type advanced gastric adenocarcinomas. To our knowledge, this is the first report to detect large numbers of CD34 positive stromal cells in malignant epithelial tumour stroma.

CD34 positive stromal cells, namely dendritic interstitial cells, are distributed in the connective tissue surrounding mammary acini,[4] salivary gland acini,[5] thyroid follicles,[6] hair follicles,[7] sweat glands,[8] and endocervical and deep endometrial glands of the uterus,[9] whereas no CD34 positive stromal cells are detected in the lamina propria of the colorectum[12] and stomach. In the colorectum[12] and stomach, CD34 positive stromal cells are found only in the submucosa, subserosa, muscularis propria, and muscularis mucosa. These results suggest that CD34 positive stromal cells are essential for the maintenance of gastrointestinal mesenchymal elements including smooth muscles and vessels, but not for normal gastrointestinal epithelial cell maturation and proliferation.

During haematopoietic differentiation, the expression of CD34 decreases and terminally differentiated cells do not express CD34.[17] Accordingly, in view of the distribution of CD34 positive stromal cells, the stroma of D-type gastric adenocarcinoma invading the muscularis propria and subserosa may be immature, whereas that of I-type gastric adenocarcinoma may be mature. Further studies including molecular and cell biological analyses are needed to confirm the biological meaning of CD34 expression.

D-type gastric carcinoma-like carcinomas are also reported in the colorectum, although at a very low frequency.[18] Further examination of CD34 expression in tumour stromal cells should be performed in malignant neoplasms of other organs showing a D-type gastric carcinoma-like growth.

In conclusion, D-type advanced gastric adenocarcinomas had large numbers of CD34 positive stromal cells in the tumour stroma, whereas D-type early gastric adenocarcinomas had no CD34 positive stromal cells in the tumour stroma. In contrast, regardless of tumour depth, I-type gastric adenocarcinomas had no CD34 positive stromal cells in the tumour stroma. These results suggest that CD34 expression is associated with progression of diffuse carcinomas, and that absence of this expression is also seen in intestinal carcinomas that are progressing. To elucidate the clinical relevance of these immunohistochemical findings, further clinicopathological investigations are needed.

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1 Greaves MF, Brown J, Molgaard HV, *et al*. Molecular features of CD34: a hematopoietic progenitor cell-associated molecule. *Leukemia* 1992;**1**:31–6.
2 Simmons DL, Satterthwaite AB, Tenen DG, *et al*. Molecular cloning of a cDNA encoding CD34, a sialomucin of human hematopoietic stem cells. *J Immunol* 1992;**148**:267–71.
3 von de Rijn M, Rouse RV. CD34. A review. *Applied Immunohistochemistry* 1994;**2**:71–80.
4 Yamazaki K, Eyden BP. Ultrastructural and immunohistochemical observations on intralobular fibroblasts of human breast, with observations on the CD34 antigen. *J Submicrosc Cytol Pathol* 1995;**27**:309–23.
5 Yamazaki K, Eyden BP. Ultrastructural and immunohistochemical studies of intralobular fibroblasts in human submandibular gland: the recognition of a "CD34-positive stromal cell network" communicated by gap junctions. *J Submicrosc Cytol Pathol* 1996;**28**:471–83.
6 Yamazaki K, Eyden BP. Ultrastructural and immunohistochemical studies of intralobular fibroblasts in human thyroid gland: recognition of a CD34-positive stromal cell network communicated by gap junctions and terminated by autonomic nerve endings. *J Submicrosc Cytol Pathol* 1997;**29**:461–76.
7 Nickoloff BJ. The human progenitor cell antigen (CD34) is localized on endothelial cells, dermal dendritic cells, and perifollicular cells in formalin-fixed normal skin, and on proliferating endothelial cells and stromal spindle-shaped cells in Kaposi's sarcoma. *Arch Dermatol* 1991;**127**:523–9.
8 Narvaez D, Kanitakis J, Faure M, *et al*. Immunohistochemical study of CD34-positive dendritic cells of human dermis. *Am J Dermatopathol* 1996;**18**:283–8.
9 Lindenmayer AE, Miettinen M. Immunophenotypic features of uterine stromal cells: CD34 expression in endocervical stroma. *Virchows Arch* 1995;**426**:457–60.
10 Vanderwinden JM, Rumessen JJ, De Laet MH, *et al*. CD34+ cells in human intestine are fibroblasts adjacent to, but distinct from, interstitial cells of Cajal. *Lab Invest* 1999;**79**:59–65.
11 Vanderwinden JM, Rumessen JJ, De Laet MH, *et al*. CD34 immunoreactivity and interstitial cells of Cajal in the human and mouse gastrointestinal tract. *Cell Tissue Res* 2000;**302**:145–53.
12 Nakayama H, Enzan H, Miyazaki E, *et al*. Differential expression of CD34 in colorectal normal tissue, peritumoral inflammatory tissue, and tumour stroma. *J Clin Pathol* 2000;**53**;626–9.
13 Lauren P. The two main types of gastric carcinoma. Diffuse and so-called intestinal type carcinomas. *Acta Pathol Microbiol Scand* 1965;**64**:31–49.
14 Miettinen M, Lindenmayer AE, Chaubal A. Endothelial cell markers CD31, CD34, and BNH9 antibody to H- and Y-antigens: evaluation of their specificity and sensitivity in the diagnosis of vascular tumors and comparison with von Willebrand factor. *Mod Pathol* 1994;**7**:82–90.
15 Nakayama H, Enzan H, Miyazaki E, *et al*. Myofibroblasts at the tumor border of invasive gastric adenocarcinomas: with reference to tumor depth and histological type. *Oncol Rep* 2000;**7**:1011–15.
16 Kim M, Higgins J, Cho E, *et al*. Expression of CD34, bcl-2, and kit in inflammatory fibroid polyps of the gastrointestinal tract. *Applied Immunohistochemistry and Molecular Morphology* 2000;**8**:147–53.
17 Civin CI, Strauss LC, Brovall C, *et al*. Antigenic analysis of hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J Immunol* 1984;**133**:157–65.
18 Sizer JS, Frederick PL, Osborne MP. Primary linitis plastica of the colon: report of a case and review of the literature. *Dis Colon Rectum* 1967;**10**:339–43.