Jpn. J. Cancer Res. 86, 934–940, October 1995

Expression of Integrin $\alpha 3$ in Gastric and Colorectal Cancers: Its Relation to Wall Contraction and Mode of Invasion

Narikazu Boku,[1] Shigeaki Yoshida,[1] Atsushi Ohtsu,[1] Takahiro Fujii,[1] Ikuro Koba,[1] Yasushi Oda,[1] Munemasa Ryu,[2] Takeo Matsumoto,[3] Takahiro Hasebe,[4] Koichi Hosokawa,[5] Takekazu Yamao,[5] Daizo Saito,[5] Nobuhiro Moriya[6] and Kaoru Abe[7]

[1]Department of Medicine, [2]Department of Surgery and [3]Division of Clinical Laboratory, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa-shi, Chiba 277, [4]Pathology Division, National Cancer Center Research Institute East, 6-5-1 Kashiwanoha, Kashiwa-shi, Chiba 277, [5]Department of Medicine and [6]Department of Surgery, National Cancer Center Hospital, and [7]National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104

We macroscopically classified 25 gastric and 23 colorectal advanced cancers into "contracted" and "uncontracted" types, and found immunohistochemically that integrin subunit $\alpha 3$ was more frequently expressed in the extracellular matrix (ECM) in the former than in the latter (75%:9/12 vs. 38%: 5/13 in gastric and 86%:6/7 vs. 25%:4/16 in colorectal cancers, respectively). Integrin subunit $\alpha 3$ was also expressed more frequently in cancers producing transforming growth factor-beta (TGF-β), which is related to ECM deposition, integrin expression and cell mobility, than in those which did not produce TGF-β (67%:10/15 vs. 40%:4/10 in gastric and 57%:4/7 vs. 38%:6/16 in colorectal cancers, respectively). In addition, integrin subunit $\alpha 3$ was not expressed in 2 benign gastric ulcers combined with gastric cancer elsewhere in the stomach. On the other hand, a retrospective analysis of 107 cases of rectal cancer which recurred after a curative operation revealed that local recurrence was more frequent in "contracted" than "uncontracted" types (44%:11/25 vs. 26%:21/82). These results may suggest that the abundant interstitial fibrosis which leads to remarkable gastric or colorectal wall contraction is a result of the interaction between cancer cells and ECM, along with the expression of integrin and/or the production of TGF-β. This fibrosis may also be closely related to the mode of gastric and colorectal cancer invasion.

Key words: Gastric and colorectal wall contraction — ECM — Integrin — TGF-β

Considerable attention has recently been focused on the interaction between cancer cells and extracellular matrix (ECM), i.e., growth promotion of fibroblasts by basic fibroblast growth factor[1] released from cancer cells, or induction of metalloproteinase (MMP-1) by cancer cells.[2,3] Adhesion molecules which target the ECM also play an important role in cancer invasion and metastasis.[4–6]

On the other hand, some adenocarcinomas are abundant in ECM with massive fibrosis, which leads to significant gastric or colorectal wall contraction, and this is thought to result from the interaction between cancer cells and ECM.

In this study, we attempted to determine the relationship of the macroscopic appearance of wall contraction in gastric and colorectal advanced cancers to their expression of integrin, transforming growth factor-beta (TGF-β), and tumor behavior.

MATERIALS AND METHODS

Criteria for macroscopical classification of "contracted" and "uncontracted" types Observing the macroscopic appearance of the fresh resected specimen, we classified the cancers which are accompanied with fold convergency and/or stricture of the gastrointestinal tract which looks like a bow tie into "contracted" type (Fig. 1, A and C) and the other cancers without such appearance into "uncontracted" type (Fig. 1, B and D).

Immunohistochemical analysis of integrin subunit $\alpha 3$ and TGF-β Twenty-five gastric and 23 colorectal advanced cancer cases which were operated upon at the National Cancer Center Hospital East from July 1992 to September 1994 were immunohistochemically examined for their expression of integrin subunit $\alpha 3$ and production of TGF-β. Two of the 25 gastric cancer cases were combined with benign gastric ulcer scar elsewhere in the stomach. These two lesions were also investigated immunohistochemically. All of the cancerous lesions were classified as either "contracted" or "uncontracted" type. The surgical specimens were embedded in OCT compound and stored at $-80^{\circ}C$ immediately after resection until they were sliced into 4 μm-thick sections.

The primary antibodies used were rabbit polyclonal anti-human integrin subunit $\alpha 3$ antibody (Chemicon, CA, USA) and rabbit polyclonal anti-human TGF-β antibody (R & D Systems, MN, USA). The sections were fixed for 5 min with a 1:1 mixture of acetone/chloroform, and treated for 30 min with 10% normal swine

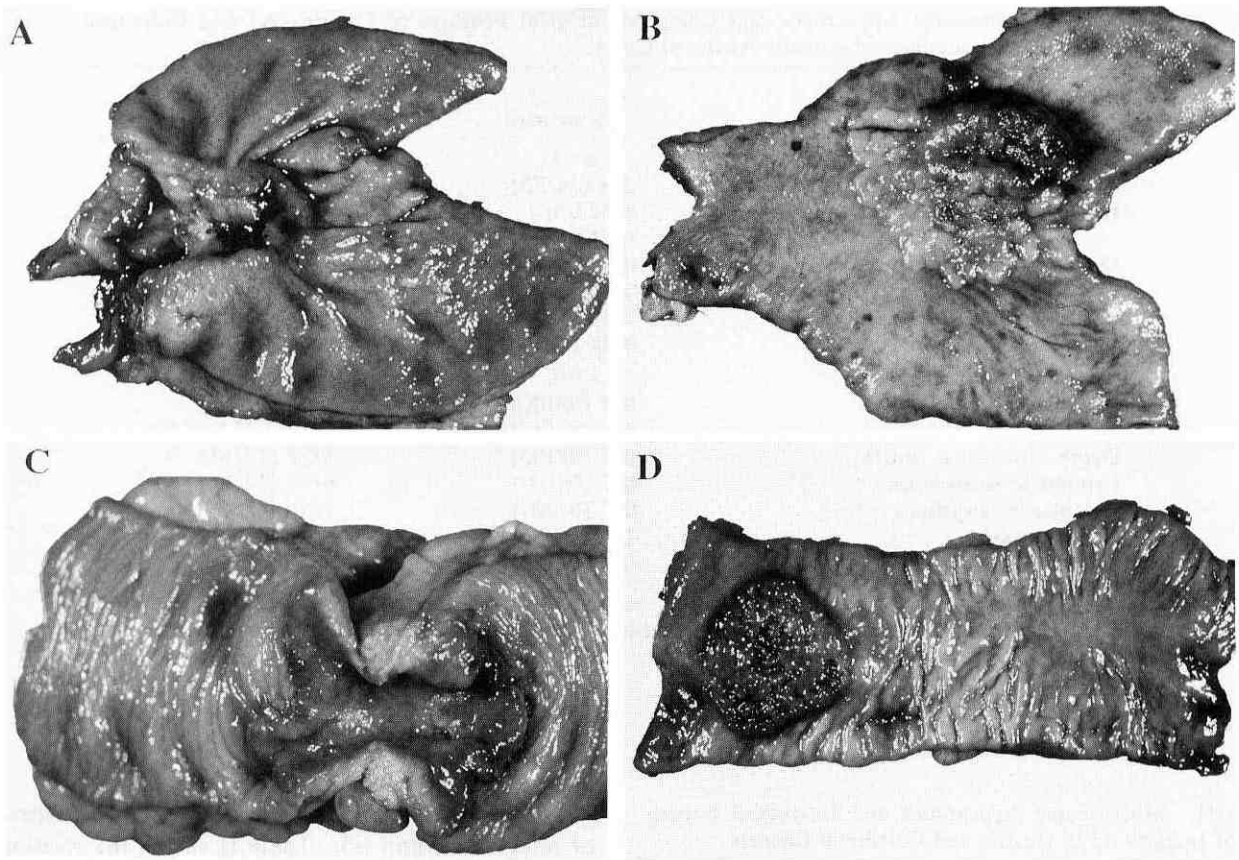

Fig. 1. Macroscopic appearance of fresh resected specimens of "contracted" (A and C) and "uncontracted" (B and D) types of gastric and colorectal cancers. The advanced gastric cancer is accompanied with fold convergency (A). The colon tract with stricture at the cancer looks like a bow tie (C).

serum in phosphate-buffered saline (PBS) (blocking buffer). The internal peroxidase was blocked with 0.3% H_2O_2 in methanol for 20 min. The sections were incubated overnight with the primary antibody diluted to a working concentration in blocking buffer at 4°C, and then rinsed three times with PBS and incubated for 1 h at room temperature with 1:100 diluted biotinylated anti-rabbit IgG antibody. The standard avidin-biotin-horseradish peroxidase method was used (Vectastain ABC kit, CA, USA). The sections were counterstained and examined microscopically.

To describe the clinicopathological features of gastric and colorectal cancers, we adopted the General Rules for Gastric Cancer Study of the Japanese Research Society for Gastric Cancer (1994) and the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus of the Japanese Research Society for the Colon and Rectum (1994), respectively. These features were compared between "contracted" and "uncontracted" types.

Macroscopic appearance and mode of recurrence To investigate the clinical significance of macroscopic wall contraction, 170 cases of colorectal cancer which recurred after curative operation performed at the National Cancer Center Hospital from 1975 to 1980 were examined. We classified all the cancers into "contracted" or "uncontracted" type based on pictures of the fresh resected specimens. The clinicopathological features and mode of recurrence were compared beteen "contracted" and "uncontracted" types. The mode of recurrence was based on the site at which recurrence was first detected.

RESULTS

Macroscopic appearance, expression of integrin subunit $\alpha 3$ and production of TGF-β

1. Clinicopathological features of the materials examined immunohistochemically: Table I shows the macroscopic appearance and clinicopathological features of the gastric and colorectal cancers. Of the 25 gastric

Table I. Macroscopic Appearance and Clinicopathological Features of Gastric (A) and Colorectal (B) Cancers: Immunohistochemically Analyzed Cases

Clinicopathological features	Macroscopic appearance	
	Contracted	Uncontracted
A. Gastric cancer	n=12	n=13
Macroscopic type (1,2/3,4)	3/9 (25/75)	8/5 (62/38)
Histology[a] (W/M/P/S/U)	0/4/5/1/2 (0/33/42/8/17)	3/6/1/3/0 (23/46/8/23/0)
Depth of invasion[b] (mp/s)	0/12 (0/100)	3/10 (23/77)
Lymphatic permeation (−/+)	0/12 (0/100)	2/11 (15/85)
Vascular permeation (−/+)	2/10 (17/83)	3/10 (23/77)
LN metastasis (−,+)	0/12 (0/100)	0/12 (0/100)
B. Colorectal cancer	n=7	n=16
Macroscopic type (1/2)	0/7 (0/100)	2/14 (13/87)
Histology (W/M/muc)	1/6/0 (14/86/0)	5/10/1 (31/63/6)
Depth of invasion (mp/s)	0/7 (0/100)	3/13 (19/81)
Lymphatic permeation (−/+)*	0/7 (0/100)	6/10 (38/62)
Vascular permeation (−/+)	0/7 (0/100)	7/9 (44/56)
LN metastasis (−,+)	1/6 (14/86)	8/8 (50/50)
Dukes (B/C)	0/7 (0/100)	8/8 (50/50)

a) W, well differentiated adenocarcinoma; M, moderately differentiated adenocarcinoma; P, poorly differentiated adenocarcinoma; S, signet ring cell carcinoma; U, undifferentiated carcinoma; muc, mucinous carcinoma.
b) mp, muscularis propria; s, serosa.
* $P<0.05$.

Table II. Macroscopic Appearance and Interstitial Expression of Integrin $\alpha 3$ in Gastric and Colorectal Cancers

Macroscopic appearance	Integrin $\alpha 3$ (+)	Integrin $\alpha 3$ (−)	Total
Gastric cancer			
Contracted	9 (75)	3 (25)	12 (100)
Uncontracted	5 (38)	8 (62)	13 (100)
Colorectal cancer			
Contracted	6 (86) *	1 (14)	7 (100)
Uncontracted	4 (25) *	12 (75)	16 (100)

* $P<0.05$.
( ): Percent.

cancer cases, 12 (48%) were "contracted" type and the remaining 13 (52%) were "uncontracted" type. According to the general rules for macroscopic typing, 75% (9/12) of the former, but only 38% (5/13) of the latter were type 3 or 4 ($P=0.0749$). Histologically, 67% (8/12) of the former, but only 31% (4/13) of the latter were diffuse type ($P=0.0812$).

Of the 23 colorectal cancer cases, 7 (30%) were "contracted" type and the remaining 16 (70%) were "uncontracted" type. The incidence of vascular (lymphatic and venous) permeation and lymph node metastasis was higher in the former group than in the latter ($P=0.0793$, 0.0467 and 0.1242, respectively).

2. Macroscopic appearance and interstitial expression of integrin subunit $\alpha 3$: Table II shows the relationship between macroscopic appearance and interstitial expression of integrin subunit $\alpha 3$. In gastric cancer cases, interstitial expression of integrin subunit $\alpha 3$ (Fig. 2A) was seen in 75% (9/12) of the "contracted" type, but in only 38% (5/13) of the "uncontracted" type ($P=0.111$). In colorectal cancers, this expression of integrin subunit $\alpha 3$ (Fig. 2B) was more frequently seen in "contracted" type (6/7:86%) than in "uncontracted" type (4/16:25%) ($P<0.05$). Interstitial expression of integrin subunit $\alpha 3$ was not observed in the benign ulcer scar present elsewhere in the stomach of two cases (Fig. 2C).

3. Macroscopic appearance and production of TGF-β: Table III shows the relationship between macroscopic appearance and production of TGF-β by cancer cells. In gastric cancers, production of TGF-β (Fig. 3A) was more frequently seen in "contracted" (9/12:75%) than in "uncontracted" (6/13:46%) cancers ($P=0.226$). In colorectal cancers, there was no difference in the frequency of TGF-β production (Fig. 3B) between "contracted" and "uncontracted" types (4/7:57%, 10/16:63%, respectively, $P=1.000$).

4. Interstitial expression of integrin subunit $\alpha 3$ and the production of TGF-β: Table IV shows the relationship between the interstitial expression of integrin subunit $\alpha 3$ and the production of TGF-β. Interstitial expression of integrin subunit $\alpha 3$ was observed in 10 (67%) of 15

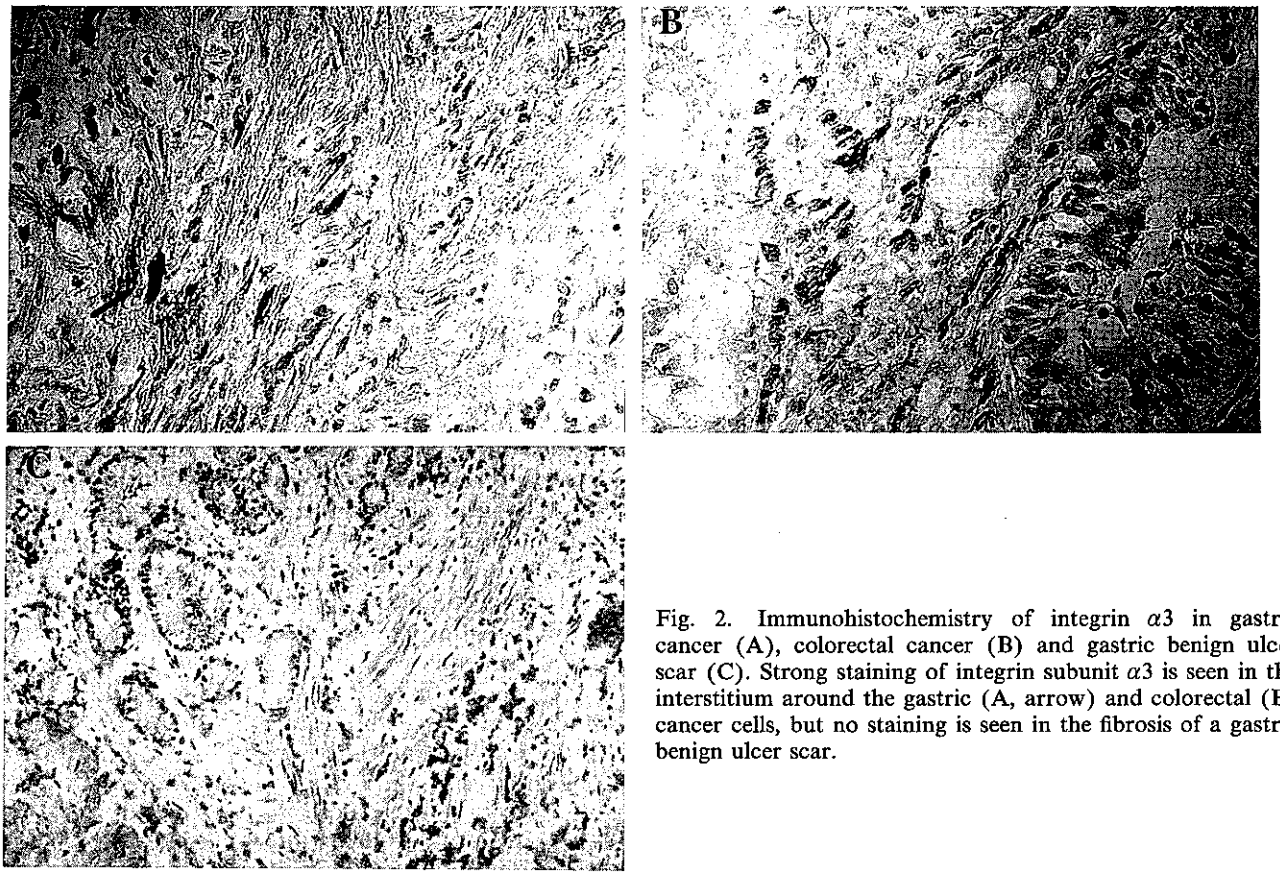

Fig. 2. Immunohistochemistry of integrin $\alpha 3$ in gastric cancer (A), colorectal cancer (B) and gastric benign ulcer scar (C). Strong staining of integrin subunit $\alpha 3$ is seen in the interstitium around the gastric (A, arrow) and colorectal (B) cancer cells, but no staining is seen in the fibrosis of a gastric benign ulcer scar.

Table III. Macroscopic Appearance and Production TGF-β in Gastric and Colorectal Cancers

Macroscopic appearance	TGF-β (+)	TGF-β (−)	Total
Gastric cancer			
Contracted	9 (75)	3 (25)	12 (100)
Uncontracted	6 (46)	7 (54)	13 (100)
Colorectal cancer			
Contracted	4 (57)	3 (43)	7 (100)
Uncontracted	10 (63)	6 (37)	16 (100)

( ): Percent.

TGF-β-producing gastric cancers, whereas it was seen in 4 (40%) of the remaining 10 cancers ($P=0.241$). In colorectal cancers this expression was seen in 4 (57%) of the 7 TGF-β-producing colon cancers, and in 6 (38%) of the remaining 16 cancers ($P=0.650$).

Macroscopic appearance and mode of recurrence

1. Clinicopathological features of recurrent colorectal cancer cases examined retrospectively: Table V shows the clinicopathological features of 170 recurrent cases. Of these 170 cases, 53 (31%) were classified as "contracted" and the remaining 117 (69%) were "uncontracted." Among the former, 47% (25/53) were located in the rectum, whereas 70% (82/117) of the latter were in the rectum ($P<0.01$). Serosa invasion is found in 98% (52/53) of the "contracted" type, but in 86% (101/117) of the "uncontracted" type ($P<0.05$). Except for the location and depth of invasion there were no significant differences in clinicopathological features between the two groups, such as sex, size, macroscopic and histological types, vascular permeation, lymph node metastasis or Dukes classification.

2. Mode of recurrence in "contracted" and "uncontracted" types: Overall, there was no significant difference in the mode of recurrence between the "contracted" and "uncontracted" types. However, among the rectal cancer cases, local recurrence was found more frequently in the "contracted" type (11/25:44%) than in "uncontracted" (21/82:26%) ($P=0.0787$), as shown in Table VI, although there were no significant differences in the clinicopathological features between the two types.

DISCUSSION

Several recent studies have indicated an interaction between cancer cells and ECM.[1-3] Integrin, an adhesion molecule to ECM, participates in invasion and metastasis.[4-6] Integrin $\alpha 3\beta 1$ (VLA-3) is a receptor for laminin, fibronectin and collagen.[7-9] Integrin subunit $\alpha 3$ is expressed after transformation by various oncogenes. Galactoprotein b3 (Gap b3), whose expression is enhanced on the surface of fibroblasts of guinea pigs after transformation, shares 89% homology with human integrin subunit $\alpha 3$.[10, 11] These findings strongly suggest that the expression of integrin subunit $\alpha 3$ is a characteristic resulting from malignant transformation.

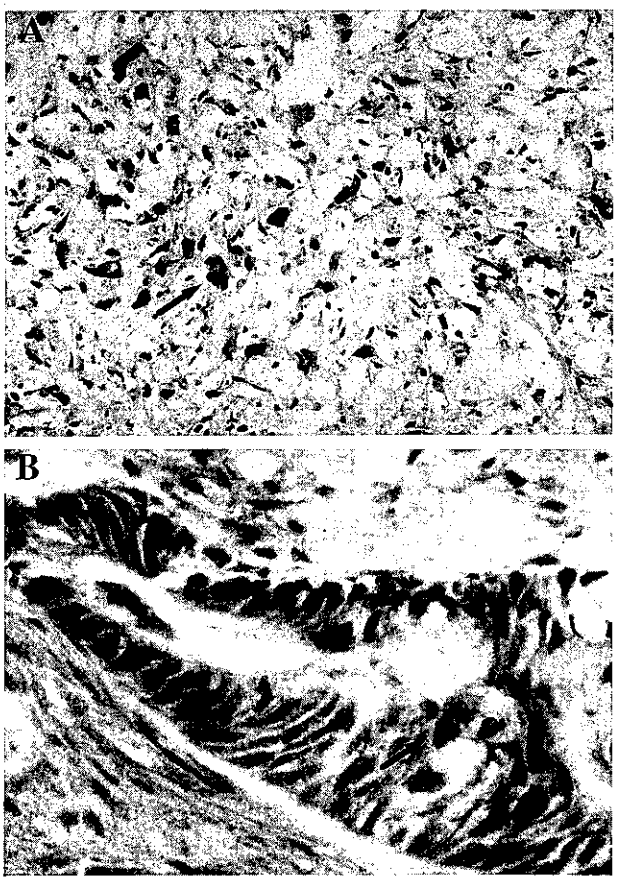

Fig. 3. Immunohistochemistry of TGF-β in gastric (A) and colorectal (B) cancer. Cytoplasmic staining is seen in signet ring carcinoma cells of a gastric cancer (A, arrow) and in well differentiated tubular adenocarcinoma cells of the colon (B, arrow).

Table IV. Interstitial Expression of Integrin Subunit $\alpha 3$ and Production of TGF-β in Gastric and Colorectal Cancers

	TGF-β	Integrin $\alpha 3$ (+)	Integrin $\alpha 3$ (−)	Total
Gastric cancer	(+)	10 (67)	5 (33)	15 (100)
	(−)	4 (40)	6 (60)	10 (100)
Colorectal cancer	(+)	4 (57)	3 (43)	7 (100)
	(−)	6 (38)	10 (62)	16 (100)

( ): Percent.

Integrin subunit $\alpha 3$ was expressed in ECM more frequently in the "contracted" type of gastric and colorectal cancers than in the "uncontracted" type. In normal organs, its expression is limited to a few cell types, including kidney glomeruli, and the basal cells of epidermis and other epithelia.[12-15] In addition, it was not expressed in a benign gastric ulcer scar which showed marked fibrosis due to ulceration, with many inflammatory changes. These findings also indicate that its expression is induced not by inflammation, but by cancer cells, especially of the "contracted" type, although it is unclear which factors are involved.

Interstitial expression of integrin subunit $\alpha 3$ was seen more frequently in TGF-β-producing gastric and colorectal cancer cases than in other cases, but was not found in all TGF-β-producing cancers. TGF-β increases the levels of all known $\beta 1$ integrin families ($\alpha 1$ to $\alpha 6$) in appropriate cells, as well as vitronectin receptor ($\alpha v\beta 3$) and LFA-1 ($\alpha 1\beta 2$), but does not induce the *de novo* synthesis of integrin subunits which are not apparently expressed yet.[16-18] Therefore, it is believed that some other factor(s) from cancer cells may induce the interstitial expression of integrin subunit $\alpha 3$, and TGF-β then increases the level of its expression.

On the other hand, TGF-β increases the production of interstitial fibronectin and collagens by mesenchymal,[19-21] epithelial,[22] endothelial[23] and cancer cells.[24, 25] Clinically, the expression of TGF-β has been detected in more than 90% of scirrhous gastric cancers by Northern blot analysis,[26] suggesting that TGF-β stimulates interstitial cells such as fibroblasts and increases the deposition of ECM in the "contracted" type cancers, particularly gastric cancers. Immunohistochemically, TGF-β is expressed in cancer cells more frequently in "contracted" than in "uncontracted" gastric cancers, but not colorectal cancers. Therefore, some mechanism, such as the expression of integrin subunit $\alpha 3$, may contribute to wall contraction in addition to TGF-β production.

Clinically, it is well known that scirrhous gastric cancer shows remarkable contraction of the gastric wall and a poor prognosis due to diffuse lymphatic permeation.[27] In the present retrospective analysis, local recurrence was more frequently seen in "contracted" than in "uncontracted" recurrent rectal cancers after curative resection, although no difference was seen considering all types of colorectal cancers. Since there was no difference

Table V. Macroscopic Appearance and Clinicopathological Features of Colorectal Cancers: Retrospectively Analyzed Cases

Clinicopathological features	Macroscopic appearance	
	Contracted n=53	Uncontracted n=117
Male/Female	37/16 (70/30)	65/52 (56/44)
Location (colon/rectum)	28/25 (53/47)*	35/82 (30/70)
Macroscopic type (1/2/3/4)	3/40/9/1 (6/75/17/2)**	9/98/10/0 (8/83/9/0)
Size (cm)	4.8	4.5
Histological type[a] (W/M/P)	22/24/7 (42/45/13)	51/49/17 (43/42/15)
Depth of invasion[b] (sm/mp/s)	0/1/52 (0/2/98)	1/15/101 (1/13/86)
Lymphatic permeation (−,+)	18/35 (34/66)	40/77 (34/66)
Vascular permeation (−,+)	39/14 (75/25)	79/38 (68/32)
LN metastasis (−,+)	21/32 (40/60)	43/74 (37/63)
Dukes classification (A, B, C)	1/17/35 (2/32/66)	7/33/77 (6/28/66)

* $P<0.01$, ** $P<0.05$.
( ): Percent.
a) W, well differentiated adenocarcinoma; M, moderately differentiated adenocarcinoma; P, poorly differentiated adenocarcinoma.
b) sm, submucosa; mp, muscularis propria; s, serosa.

Table VI. Macroscopic Appearance and Mode of Recurrence of Rectal Cancer Cases: Retrospectively Analyzed Cases

Macroscopic appearance	Mode of recurrence					
	Local	Peritoneum	LN	Liver	Lung	Other
Contracted	11 (44)	0 (0)	0 (0)	6 (24)	7 (28)	1 (4)
Uncontracted	21 (26)	4 (5)	1 (1)	31 (38)	24 (29)	1 (1)

( ): Percent.

in the pathological staging between the two, our findings suggest that a few cancer cells of "contracted" type which permeate beyond the surgical cut end may cause local recurrence, especially in rectal cancers which are anatomically difficult to resect with a wide margin. In fact, the incidence of vascular permeation and lymph node metastasis was higher in "contracted" than in "uncontracted" colorectal cancers of immunohistochemically analyzed cases, suggesting that "contracted" colorectal cancers, particularly rectal cancers, may be more invasive than "uncontracted" cancers. However, the number of examined cases was too small to draw any definite conclusion on this point.

In conclusion, gastric and colorectal wall contraction due to cancerous invasion can be considered an important tumor behavior which is based on the interaction between cancer cells and ECM, and involves various mechanisms such as interstitial expression of integrin subunit $\alpha 3$ and production of TGF-β by cancer cells.

(Received May 2, 1995/Accepted July 19, 1995)

REFERENCES

1) Schulze-Osthoff, K., Risau, W., Vollmer, E. and Sorg, C. *In situ* detection of basic fibroblast growth factor by highly specific antibodies. *Am. J. Pathol.*, **137**, 85–92 (1990).
2) Nakajima, M. and Chop, A. M. Tumor invasion and extracellular matrix degradative enzymes: regulation of activity by organ factors. *Semin. Cancer Biol.*, **2**, 115–127 (1991).
3) Wooly, D. E. Collagenolytic mechanisms in tumor cell invasion. *Cancer Metastasis Rev.*, **3**, 361–372 (1984).
4) Mortaniri, R., Anichini, A. and Parmiani, G. Heterogeneity for integrin expression and cytokine-mediated VLA modulation can influence the adhesion of human melanoma cells to extracellular matrix proteins. *Int. J. Cancer*, **47**, 551–559 (1991).
5) Chan, B. M. C., Matsuura, N., Takada, Y., Zetter, B. R. and Hemler, M. E. *In vitro* and *in vivo* consequences of

VLA-2 expression in rhabdomyosarcoma cells. *Science,* **251,** 1600–1602 (1991).
6) Humphries, M. J., Olden, K. and Yamada, K. M. A synthetic peptide from fibronectin inhibits experimental metastasis of murine melanoma cells. *Science,* **233,** 467–470 (1986).
7) Wayner, E. A. and Carter, W. G. Identification of multiple cell adhesion receptors for collagen and fibronectin in human fibrosarcoma cells possessing unique α and β subunits. *J. Cell Biol.,* **105,** 1873–1884 (1987).
8) Takada, Y., Wayner, E. A., Carter, W. G. and Hemler, M. E. Extracellular matrix receptors, ECMR II and ECMR I, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. *J. Cell. Biochem.,* **37,** 385–393 (1988).
9) Wayner, E. A., Carter, W. G., Piotrowicz, R. S. and Kikuchi, T. J. The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J. Cell Biol.,* **107,** 1881–1891 (1988).
10) Tsuji, T., Yamamoto, F., Miura, Y., Takio, K. and Tinani, K. Characterization through cDNA cloning of galactoprotein b3 (Gap b3), a cell surface membrane glycoprotein showing enhanced expression on oncogenic transformation. *J. Biol. Chem.,* **265,** 7016–7021 (1990).
11) Takada, Y., Murphy, E., Pil, P., Chen, C., Ginsberg, M. H. and Hemler, M. E. Molecular cloning and expression of the cDNA for α3 subunit of human $\alpha 3\beta 1$ (VLA-3), an integrin receptor for fibronectin, laminin, and collagen. *J. Cell Biol.,* **115,** 257–266 (1991).
12) Carter, W. G., Wayner, E. A., Bouchard, T. S. and Kaur, P. The role of integrin $\alpha 2\beta 1$ and $\alpha 3\beta 1$ in cell-cell and cell-substrate adhesion of human epidermal cells. *J. Cell Biol.,* **110,** 1387–1404 (1990).
13) Fradet, Y., Cordon-Card, C., Thompson, T., Daly, M. E., Whitmore, W. F., Jr., Lloyd, K. O., Melamed, M. R. and Old, L. J. Cell surface antigens of human bladder cancer defined by mouse monoclonal antibodies. *Proc. Natl. Acad. Sci. USA,* **81,** 224–228 (1984).
14) Morhenn, V. B., Schreiber, A. B., Soriero, O., McMillian, W. and Allison, A. C. A monoclonal antibody against basal cells of human epidermis. *J. Clin. Invest.,* **76,** 1978–1983 (1985).
15) Peltonen, J., Larjava, S., Jaakkola, S., Gralnick, H., Akiyama, S. K., Yamada, K. M. and Uitto, J. Localization of integrin receptors for fibronectin, collagen and laminin in human skin. *J. Clin. Invest.,* **84,** 1916–1923 (1989).
16) Ignotz, R. A. and Massague, J. Cell adhesion protein receptors as targets for transforming growth factor-beta action. *Cell,* **51,** 189–197 (1987).
17) Heino, J., Ignotz, R. A., Hemler, M. E., Crouse, C. and Massague, J. Regulation of cell adhesion receptors by transforming growth factor-beta. Concomitant regulation of integrins that share a common beta1 subunit. *J. Biol. Chem.,* **264,** 380–388 (1989).
18) Ignotz, R. A., Heino, J. and Massague, J. Regulation of cell adhesion receptors by transforming growth factor-beta. Regulation of vitronectin receptor and LFA-1. *J. Biol. Chem.,* **264,** 389–392 (1989).
19) Sporn, M. B., Roberts, A. B., Wakefield, L. M. and de Crombrugghe, B. Some recent advances in the chemistry and biology of transforming growth factor-beta. *J. Cell Biol.,* **105,** 1039–1045 (1987).
20) Massague, J. The TGF-beta family of growth and differentiation factors. *Cell,* **49,** 437–438 (1987).
21) Rizzino, A. Transforming growth factor-beta: multiple effects on cell differentiation and extracellular matrices. *Dev. Biol.,* **130,** 1375 (1988).
22) Nickoloff, B. J., Mitra, R. S., Riser, B. L., Dixit, V. M. and Varani, J. Modulation of keratinocyte mobility. Correlation with production of extracellular matrix molecules in response to growth promoting and antiproliferative factors. *Am. J. Pathol.,* **132,** 543–551 (1988).
23) Madri, J. A., Pratt, B. M. and Tucker, A. M. Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of extracellular matrix. *J. Cell Biol.,* **106,** 1375–1384 (1988).
24) Chakrabarty, S., Brattain, M. G., Ochs, R. L. and Varani, J. Modulation of fibronectin, laminin, and cellular adhesion in the transformation and differentiation of murine AKR fibroblasts. *J. Cell. Physiol.,* **133,** 415–425 (1987).
25) Chakrabarty, S., Tobon, A., Varani, J. and Brattain, M. G. Induction of carcinoembryonic antigen secretion and modulation of protein secretion/expression and fibronectin/laminin expression in human colon carcinoma cells by transforming growth factor-beta. *Cancer Res.,* **48,** 4059–4064 (1988).
26) Yoshida, K., Yokozaki, H., Niimoto, M., Ito, H. and Tahara, E. Expression of TGF-beta and procollagen type I and type III in human gastric carcinomas. *Int. J. Cancer,* **44,** 394–398 (1989).
27) MacDonald, J. S., Hill, M. C. and Roberts, I. M. Gastric cancer: epidemiology, pathology, detection and staging. *In* "Gastrointestinal Oncology," ed. J. D. Ahlgren and J. S. MacDonald, pp. 151–158 (1992). J. B. Lippincott Company, Philadelphia.