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Distribution of Transforming Growth Factor-β and Its Receptors in Gastric Carcinoma Tissue

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The distribution of the three mammalian isoforms of transforming growth factor (TGF)-β (TGF-β1, -β2, and -β3) as well as their signaling receptors, TGF-β type I and type II receptors (TβR-I and TβR-II, respectively), in gastric carcinoma tissue was examined by immunohistochemistry using specific antibodies. Tissue specimens were obtained from 25 cases of gastric carcinoma, which were classified into two groups according to Lauren's classification, i.e. 15 cases of diffuse carcinoma and 10 cases of intestinal carcinoma. In normal gastric mucosa apart from carcinoma nests, all of TGF-β1, -β2, -β3, TβR-I and TβR-II were clearly demonstrated in fundic glands. In sharp contrast, none of them was detectable in surface mucous cells. In carcinoma cells, strong staining for TGF-β1, -β2 and -β3 was obtained only in diffuse-type carcinoma. In particular, carcinoma cells scattered as single cells or small nests had a tendency to show strong staining for TGF-βs. The receptors tended to be distributed concomitantly with the ligands, and diffuse-type carcinoma showed stronger receptor staining than intestinal-type carcinoma. In cancer stroma, TGF-βs and receptors were detected in both diffuse and intestinal types, but the area with positive staining was wider and more dispersed in diffuse-type carcinoma than in intestinal carcinoma. These results suggest that TGF-β may contribute in part to the variety of histogenesis and mode of progression of gastric carcinoma.

Key words: TGF-β — TGF-β receptor — Immunohistochemistry — Gastric carcinoma

Most cases of gastric carcinoma are classified as adenocarcinoma, which can be roughly divided into two groups, i.e., intestinal carcinoma and diffuse carcinoma.[1] It is generally accepted that the difference between these two types is related to their degree of de-differentiation, the former being more differentiated than the latter. However, there could be another mode of variation of gastric carcinoma. For example, different directions of differentiation of the carcinoma cells may contribute to the difference between intestinal carcinoma and diffuse carcinoma.[2]

Recent progress in molecular cell biology has identified a variety of cytokines involved in cell growth regulation and differentiation. Transforming growth factor-β (TGF-β) is one such cytokine, which has multifunctional activity on cell growth, differentiation, accumulation of extracellular matrix and immune function.[3-5] A possible role of TGF-β in the accumulation of extracellular matrix to form scirrhous cancer stroma has been proposed.[6,7] Considering the multiple functions of TGF-β, such as the effect on cellular differentiation, it is possible that TGF-β is involved in the establishment of different properties of the carcinoma cells. Therefore, comparison of the distribution of TGF-β and its receptors between intestinal and diffuse types of gastric carcinoma may be very important.

TGF-β has several isoforms.[3,5] In mammals, three isoforms of TGF-β, i.e., TGF-β1, -β2 and -β3, have been identified. These isoforms are very similar in their amino acid sequences, and their biological activities on many cell types are almost identical. However, certain cell types, such as hematopoietic cells and endothelial cells, respond to TGF-β1 and -β3 more efficiently than to TGF-β2.[8,9] Moreover, expression profiles of the three TGF-β isoforms in embryos are quite different, suggesting different roles of the TGF-β isoforms during morphogenesis.[10,11] It is not known which isoforms of TGF-β are expressed in gastric mucosa. Therefore, we used isoform-specific antibodies raised against synthetic peptides corresponding to the N-terminal precursor portions of TGF-β1, -β2 and -β3.[12]

TGF-βs exert their effects through binding to specific receptors on the cell surface.[13] At least three different receptors for TGF-β, i.e., type I (TβR-I), type II (TβR-II), and type III, have been identified on many mammalian cells.[14-17] TβR-I and TβR-II have serine-threonine kinase domains in their cytoplasmic regions and both of them are required for the signal transduction of TGF-β.[18] TβR-I and TβR-II form heteromeric receptor complexes after the ligand binding. Then, the serine-threonine

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kinase of TβR-II transphosphorylates TβR-I, which eventually transduces various signals in the cells. On the other hand, the TGF-β type III receptor is considered to have no signaling activity by itself, but it presents ligands to TβR-II and TβR-I, and thus the type III receptor is indirectly involved in the signal transduction.[19]

In the present study, we have investigated the distribution of the three isoforms of TGF-β and two types of the TGF-β receptors essential for signal transduction, TβR-I and TβR-II, in the tissues of two different types of gastric carcinomas. We have found different patterns of distribution of the TGF-βs and their receptors with reference to the histological type of the carcinoma, suggesting that TGF-βs may contribute to the difference of the histological patterns and the mode of progression of gastric carcinoma.

MATERIALS AND METHODS

Samples and histological classification Twenty-five cases of advanced gastric carcinoma were investigated. The ages of the patients varied from 47 to 78 years, and the male-to-female ratio was 2:1. Tissue specimens of gastric carcinoma obtained during therapeutic surgical operations were subjected to routine pathological examination, and classified into 10 cases of intestinal carcinoma and 15 cases of diffuse carcinoma, according to Lauren's classification.[1] The degree of invasiveness into lymphatic vessels and small veins in the gastric wall was evaluated and assigned to one of four groups (0 to 3) according to the Japanese classification of gastric carcinoma.[20]

Tissue preparation for immunohistochemistry Fingertip-sized parts of carcinoma tissue and normal gastric wall apart from carcinoma tissue were embedded in OCT compound (Miles, Elkhart, IN), frozen in dry ice-acetone and stored at −70°C until use. Sections of 5 μm thickness were placed on slides coated with chromium sulfate and gelatin, fixed with 100% acetone and stored at −20°C.

Antibodies The polyclonal rabbit antisera, Ab96, Ab94 and Ab95, directed against the N-terminal precursor portions of TGF-β1, -β2 and -β3, respectively, were raised against synthetic peptides corresponding to specific amino acid sequences as previously described.[12,21] The polyclonal rabbit antiserum against platelet-derived latent TGF-β binding protein (LTBP), Ab39, was prepared as described.[22] The polyclonal rabbit antisera, VPN and DRL, against TβR-I and TβR-II, respectively, were made against synthetic peptides corresponding to the amino acid sequences of each receptor.[23] All antisera, except for Ab39, were affinity-purified by using CNBr-activated Sepharose CL-4B (Pharmacia, Uppsala, Sweden) coupled with the corresponding peptides used for immunizing rabbits, as described.[21]

Immunohistochemistry The tissue sections for staining with Ab96, Ab94, Ab95, VPN and DRL were treated with phosphate-buffered saline (PBS) containing 0.03% H_2O_2 for 30 min in order to exhaust intrinsic peroxidase activity. For immunostaining with Ab39, the sections were treated with methanol containing 0.03% H_2O_2 for 30 min. After pre-incubation with PBS containing 10% (w/v) bovine serum albumin and 0.02% (w/v) NaN_3 to block nonspecific binding of the antibodies, sections were incubated with one of the primary antibodies in a humidified chamber at 4°C overnight. The first antibodies were used at concentrations of 3 or 1 μg/ml. Then the sections were washed thoroughly with PBS. Incubation with biotinylated goat anti-rabbit IgG antibodies (Vector Laboratories, Burlingame, CA) at room temperature for 45 min was followed by further incubation with ABC Elite Complex (Vector) for 45 min. The bound immunocomplex was visualized by incubation with 0.04% (w/v) 3,3′-deaminobenzidine tetrahydrochloride in 0.05 *M* Tris-HCl buffer (pH 7.6) for 2 min in the presence of 0.024% (v/v) H_2O_2 and 0.01% (w/v) NaN_3. Finally, the sections were counterstained with hematoxylin.

False-positive staining due to nonspecific binding of the secondary antibody or the detection complex was excluded by comparison with sections processed in parallel without the primary antibody in each assay. The specificity of binding of the primary antibodies was previously established,[24-27] and further confirmed by another set of stainings carried out in the presence of excess amounts of the corresponding synthetic peptides. Purified LTBP from platelets was used to examine the specificity of Ab39 staining.

RESULTS

Histopathological classification of gastric carcinoma Among 25 patients of advanced gastric carcinoma, 10 cases were classified as intestinal type and 15 cases as diffuse type (Table I), according to Lauren's classification.[1] The ages of patients in the intestinal-type group varied from 59 to 78 years old (mean 67.5), while the diffuse-type group was a little younger (47 to 84 years old; mean 62). Highly invasive cases with metastasis into distant lymph nodes in group 3 or 4[20] or with frequent invasion figures into lymph vessels were observed only in diffuse type of carcinoma. On the other hand, 3 cases out of 10 intestinal-type and 3 cases out of 15 diffuse-type carcinoma exhibited frequent invasion into small veins (Table I). Other aspects of the clinical background had no obvious difference between the two groups.

Distribution of TGF-βs and receptors in normal gastric mucosa Normal gastric mucosa was obtained from the margin of the surgically resected stomach of patient No. 8 (Table I). Clear positive stainings for all of TGF-

Table I. Summary of Pathological Examinations and the Expression of TGF-βs and Their Receptors

Classification	Patient No.	Age	Sex	Pathological findings		ly	v	n	Carcinoma cells						Fibroblast-like cells in cancer stroma					
									β1	β2	β3	LTBP	TβR-I	TβR-II	β1	β2	β3	LTBP	TβR-I	TβR-II
Diffuse	1	62	M	se	sig	2	1	1	−	−	++	−	++	++	+	+	++	++	++	++
	2	65	M	se	por	3	2	4	+++	+++	+++	+	++	++	+	++	++	+++	+	+
	3	52	M	se	sig>por	2	1	1	−	+	+	−	+	+	+++	++	++	++	+	+
	4	61	M	se	por	2	0	2	−	+	+	−	+	+	+++	++	+++	++	++	++
	5	52	M	se	muc>sig	3	1	3	++	++	+++	−	++	++	++	+	+++	++	+	+
	6	59	M	se	sig	2	0	2	−	+	++	ND	+	+	+	+	+	ND	+	+
	7	52	F	sei	por	2	2	1	+++	++	+	ND	ND	ND	++	++	+	ND	ND	ND
	8	64	F	ss	sig>por	1	1	1	+++	++	++	+	ND	ND	+	+	+	+++	ND	ND
	9	54	M	se	por, tub1	3	3	2	+	−	+	−	+	+	++	+	+	++	+	+
	10	47	M	ss	por				+	++	+	ND	+	+	++	++	+	ND	+	+
	11	67	F	se	por>sig	2	3	1	+	+	+	ND	++	++	++	++	++	ND	+	+
	12	84	F	pm	por>tub2	3	1	1	+	+	+	ND	+	+	++	++	+	ND	++	+
	13	70	M	pm	por>pap	1	3	0	+	+	+	ND	+	+	+++	+++	+++	ND	++	++
	14	72	M	se	por	3	2	1	+	−	+	−	+	++	+++	++	++	++	++	++
	15	69	M	ss	por				++	++	+	−	++	+	+	+	+	+++	+	+
Intestinal	16	59	M	se	tub1				−	−	+	−	−	+	+++	+++	++	+	++	++
	17	72	F	se	tub1	2	2	0	−	−	+	−	−	+	+++	++	++	++	+	++
	18	75	M	se	tub1	2	1	1	−	−	+	−	−	+	++	++	++	++	+	+
	19	65	M	se	tub1	2	3	1	−	+	+	−	+	+	+++	++	++	+++	+	+
	20	60	M	se	pap	2	2	1	−	−	+	−	+	+	+++	+	++	++	+	+
	21	71	F	ss	pap≧por	1	3	1	−	−	−	−	−	−	++	++	+	++	++	+
	22	52	F	ss	pap				−	+	+	−	−	−	++	+	+	+	+	+
	23	74	M	se	tub1	2	2	1	−	−	+	ND	−	+	++	++	++	ND	++	++
	24	78	F	ss	pap	1	1	0	−	−	−	ND	−	−	++	++	+	ND	++	++
	25	69	F	ss	pap	1	3	(+)	−	−	−	ND	+	+	+++	+++	+++	ND	++	++

Pathological findings were determined with formalin-fixed specimens according to "Japanese Classification of Gastric Carcinoma." Abbreviations: se, serosa-exposed; sei, serosa-exposed and infiltrating; ss, subserosa; pm, muscularis propria; sig, signet-ring cell carcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; tub1, well differentiated type tubular adenocarcinoma; tub2, moderately differentiated type tubular adenocarcimona; pap, papillary adenocarcinoma; ly, degree of lymphatic invasion; v, degree of venous invasion; n, lymph nodes metastases. +++, strong positive stainings on most cells; ++, moderate stainings on most cells; +, weak stainings on a few cells; (−), below detectable level; ND, not determined.

Table II. Comparison of Two Histological Groups of Gastric Carcinoma as Regards Positivity for TGF-βs and Their Receptors (%)

Type	TGF-β1	TGF-β2	TGF-β3	TβR-I	TβR-II
Diffuse	73.3 (11/15) [a)]	80.0 (12/15) [b)]	100 (15/15)	100 (13/13)	100 (13/13)
Intestinal	0 (0/10)	20.0 (2/10)	70.0 (7/10)	30.0 (3/10)	70.0 (7/10)
Total	44.0 (11/25)	56.0 (14/25)	84.0 (22/25)	69.5 (16/23)	87.0 (20/23)

a) $P=0.0003$.
b) $P=0.0031$.

β1, -β2, -β3, TβR-I and TβR-II were detected on chief and parietal cells of the fundic glands. Parietal cells gave the strongest reaction for all of them. On the other hand, all stainings were negative or very weak in isthmus, which is composed of generating cells, and in surface mucous cells (Fig. 1). TGF-βs and their receptors were shown to be induced only on cells with specific differentiation into fundic glands, but not on surface mucous cells in normal gastric mucosa.

Expression of TGF-βs and receptors on carcinoma cells

Twenty-five cases of gastric carcinoma were stained for TGF-β1, -β2, -β3, TβR-I or TβR-II. Positive stainings were evaluated by comparison with the stainings on smooth muscle cells which served as intrinsic positive

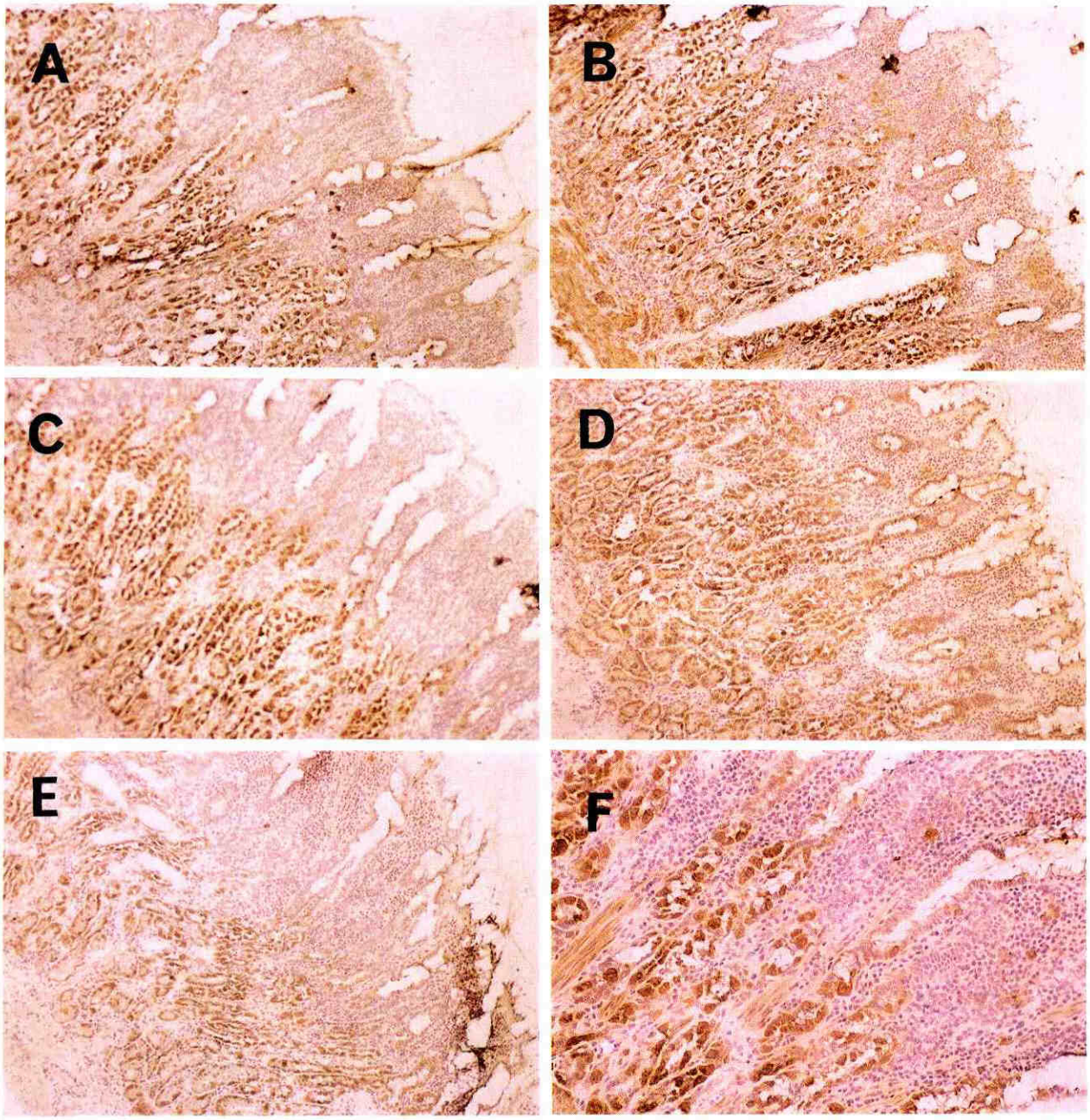

Fig. 1. Distribution of TGF-β1, -β2, -β3, TGF-β type I receptor (TβR-I) and TGF-β type II receptor (TβR-II) in normal gastric mucosa. Normal gastric mucosa shows positive staining with specific antisera against TGF-β1 (A), -β2 (B, F), -β3 (C), TβR-I (D) and TβR-II (E) with matching distributions. Note the clear positive staining on fundic glands, but weak staining on surface mucous cells.

controls. In general, carcinoma cells tended to show similar strengths of stainings for TGF-βs and their receptors (Table I). Carcinoma cells strongly positive for TGF-βs and their receptors belonged exclusively to the diffuse carcinoma group, while intestinal-type carcinomas were negative or exhibited only weak stainings on a few cells (Table I, Figs. 2 and 3). A few cases showed distinct degrees of stainings among TGF-β isoforms, or between ligand and receptor stainings, such as patients No. 1, 6 and 11 (Table I). Staining patterns of represen-

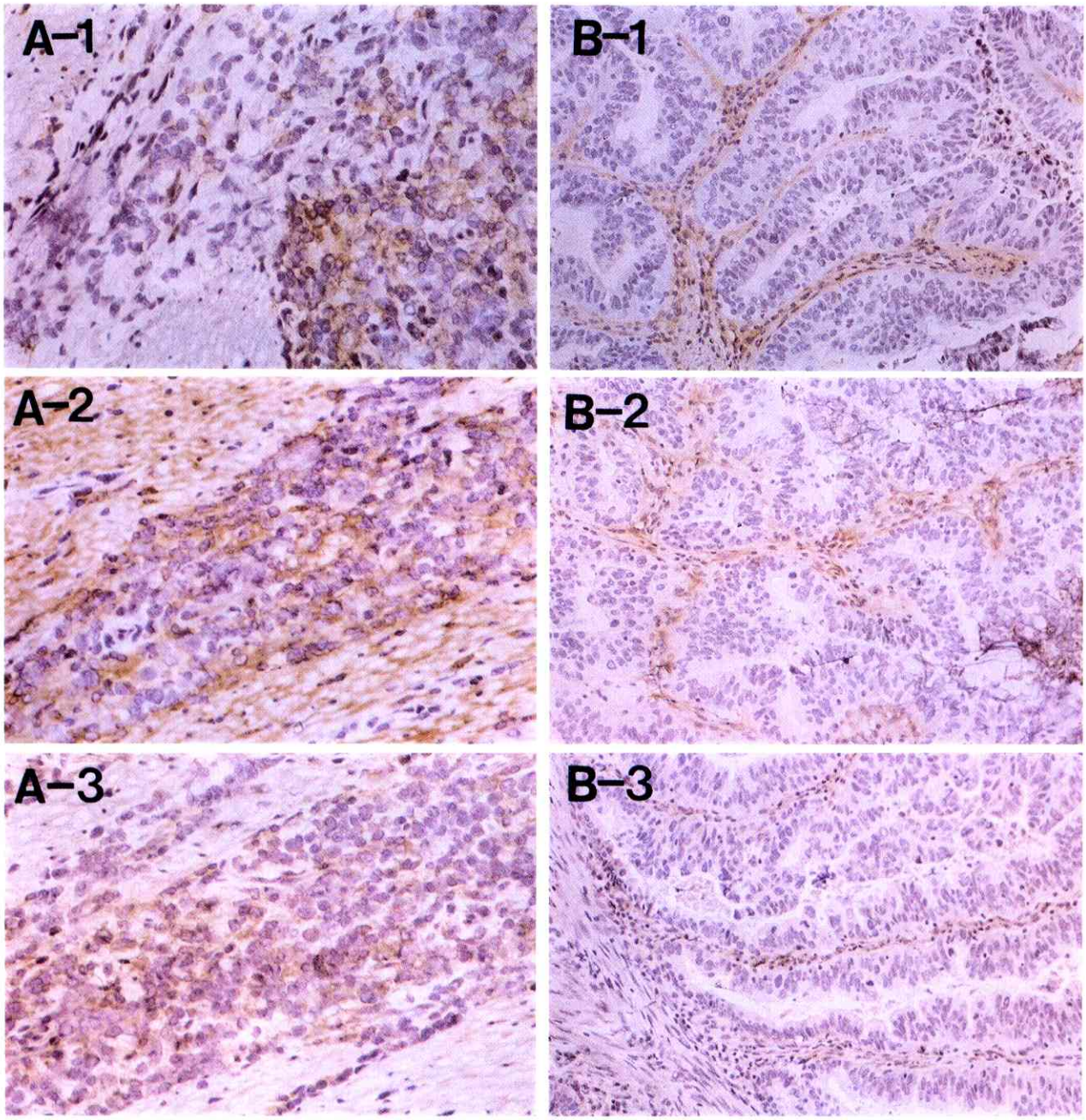

Fig. 2. Expression of three isoforms of TGF-β in the typical cases of diffuse type (A) and intestinal type (B) of gastric carcinomas. Serial sections were stained with specific antisera against TGF-β1 (A-1, B-1), TGF-β2 (A-2, B-2), or TGF-β3 (A-3, B-3).

tative cases are shown in Figs. 2 and 3. In the diffuse-type carcinoma of patient No. 10 (Fig. 2, A-1, 2, and 3, Fig. 3, A-1 and 2), all of the TGF-β isoforms and receptors were detected on cancer cells, while they were not detected on the intestinal-type cancer cells of patient No. 24 (Fig. 2, B-1, 2, and 3, Fig. 3, B-1 and 2). Statistical analysis of the expression of TGF-β1 and TGF-β2 in the diffuse-type and intestinal-type carcinomas showed that the two groups were significantly different (TGF-β1: $\chi^2 = 13.095$, $P = 0.0003$, TGF-β2: $\chi^2 = 8.766$, $P = 0.0031$).

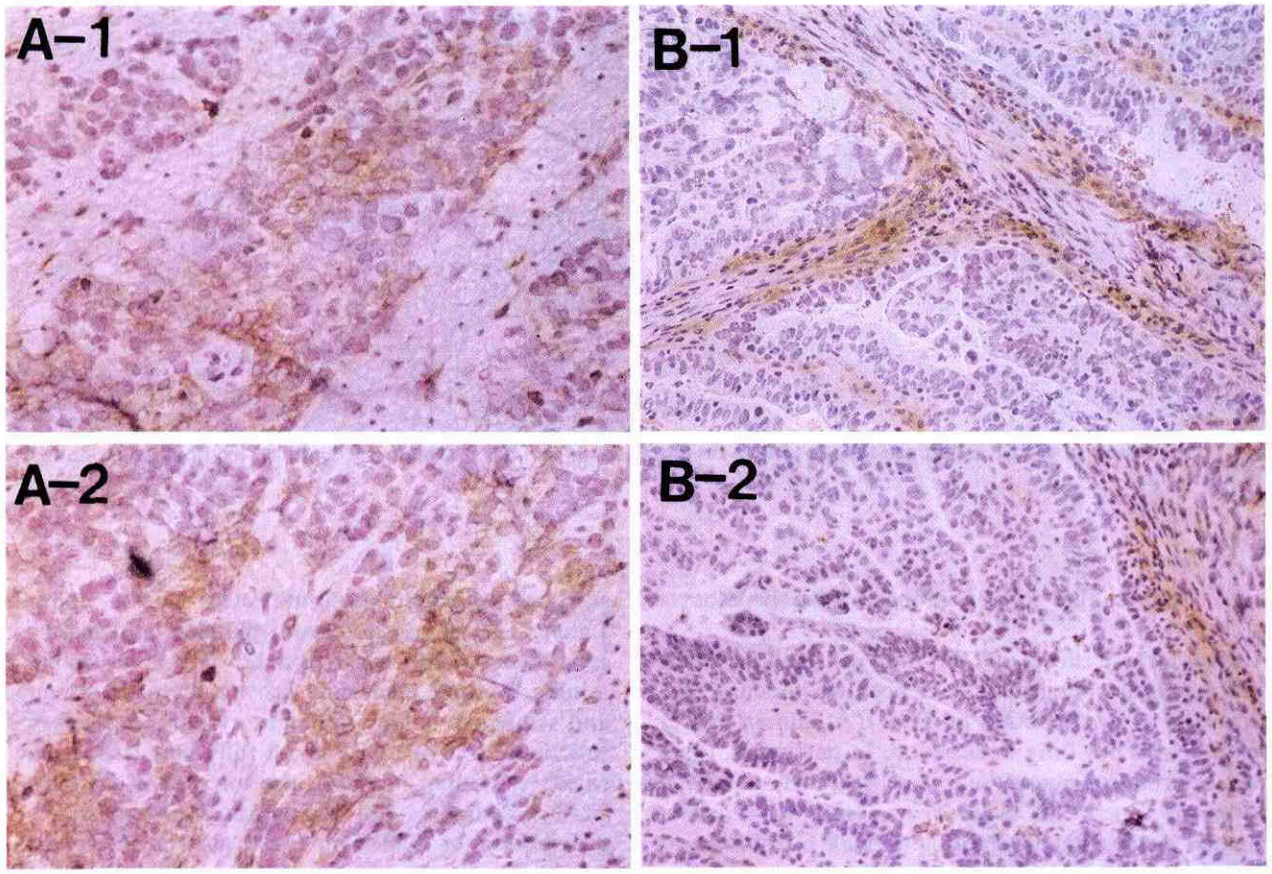

Fig. 3. Expression of two types of TGF-β receptors, TβR-I and TβR-II in diffuse type (A) and the intestinal type (B) of gastric carcinomas. Serial sections were stained with specific antisera against TβR-I (A-1, B-1), or TβR-II (A-2, B-2).

Distribution of TGF-β1, -β2, -β3, TβR-I and TβR-II in cancer stroma In cancer stroma, TGF-β and receptors were detected in both diffuse and intestinal types (Table I). The area with positive stainings was wide and dispersed in diffuse-type carcinoma. On the other hand, the strongly stained area in the case of intestinal carcinoma was narrow and sharply demarcated the cancer nests (Figs. 2 and 3).

Localization of LTBP in fibrous matrices TGF-βs are produced as latent high-molecular-weight complexes from producer cells.[28] Latent TGF-β complexes secreted from platelets as well as some cultured cells contain a 125–210 kDa protein known as LTBP, which is bound to the TGF-β precursor by disulfide bridges.[22] Mizoi *et al.*[7] have shown that LTBP is not found in cancer cells, but in the cells and matrices surrounding them. Among the 25 cases, 16 randomly selected cases of gastric carcinomas were examined for LTBP. The distribution pattern of this protein was consistent with the report by Mizoi *et al.*[7] LTBP was not observed in the cancer cells except for obscure stainings in patients No. 2 and 8, but in sharp contrast, it was clearly detected on the fibrous matrices in cancer stroma (Fig. 4).

DISCUSSION

Several lines of evidence indicate that a number of cytokines, including interleukin-1, interleukin-8, tumor necrosis factor and TGF-β, are involved in several processes of cancer development, i.e., transformation, progression and metastasis. Manipulation of the activities of those cytokines, supported by a better understanding of the roles of these molecules in cancer development, are expected to lead to effective methods for cancer therapy. Among the many cytokines involved in cell growth and differentiation, TGF-β is noteworthy because of its multifunctional activities on a variety of cells. TGF-β has also been reported to affect tissue morphogenesis, including tubular formation. Santos and Nigam[29] observed that TGF-β inhibits the tubular formation of a cell line

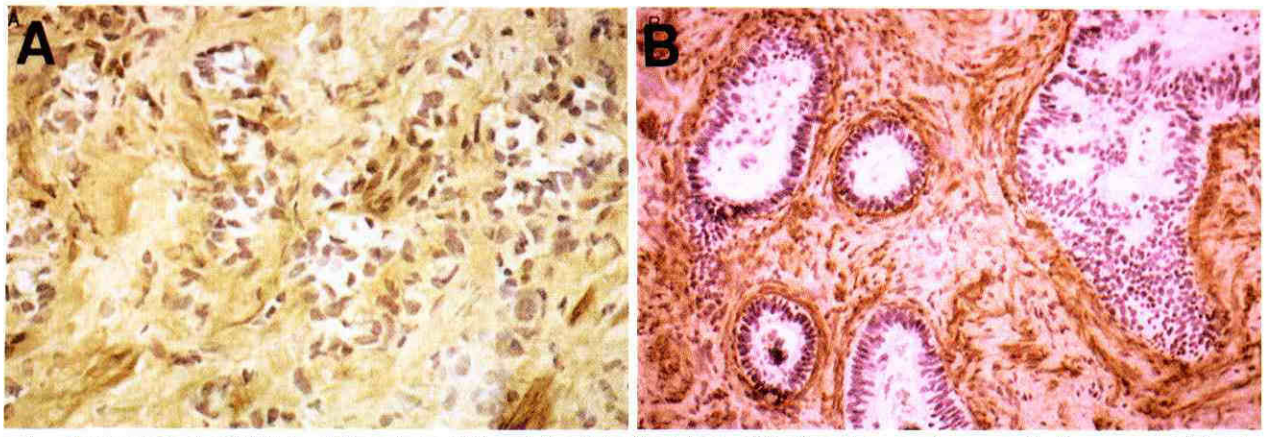

Fig. 4. Expression of LTBP in diffuse type (A) and the intestinal type (B) of gastric carcinomas. Sections were stained with Ab39 antiserum.

derived from canine kidney induced with hepatocyte growth factor.

Although these effects of TGF-β on cell growth, differentiation and morphogenesis have been well documented *in vitro*, the *in vivo* function of TGF-β in human cancer development is not fully understood. In particular, there is little information about the role of the different TGF-β isoforms in cancer. In the present study, we have investigated the specific distribution of TGF-β isoforms, as well as their receptors, in human gastric cancers. Gastric cancers are particularly interesting as materials to study the roles of TGF-β, since they can be divided into two types, the intestinal type, which exhibits tubular formation, and the diffuse type, which does not. They are different not only morphologically, but also in their rate of progression.

Expression of TGF-β1 and TGF-β2 was clearly tissue type-dependent. Strong staining for them could be detected in about one-third of diffuse-type carcinoma cells, but not in the intestinal-type cells. Carcinoma cells scattering as a single cells showed very strong stainings for TGF-βs and their receptors. Some of them may be simply poorly differentiated, but others could be dissociated by the function of TGF-β. Otherwise, those cells may have characteristics of, for example, parietal cells, which have large amounts of TGF-βs and their receptors from the first. The histological type-dependent expression of the TGF-β isoforms may be specifically regulated, because it is known that expression of each of the three TGF-β isoforms is regulated by distinct promoters with unique structures,[30] and probably contributes to the scirrhous matrix formation often observed in diffuse-type carcinoma.

Secretion of TGF-β is further modified post-translationally through a quite complex mechanism. TGF-βs are secreted as latent high-molecular-weight complexes. Latent TGF-β secreted from platelets and many other cells binds LTBP, which is a 125–210 kDa protein containing multiple epidermal growth factor-like sequences and sequences containing eight cysteine residues.[22] The functional role of LTBP is not fully understood, but, for example, it may facilitate the interaction of the latent TGF-β complex with the extracellular matrix.[31] The distribution of LTBP in these gastric carcinomas revealed that it was not detected in carcinoma cells, even though these cells expressed some types of TGF-β. In contrast, LTBP was strongly expressed in the tissue surrounding the cancerous lesion, consistent with a previous report.[7] Recently, new isoforms of LTBP (LTBP-2 and LTBP-3) have been cloned. TGF-βs from carcinoma cells mostly negative for LTBP could be associated with these new isoforms of LTBP.

TGF-βs exert their effects through binding to specific cell surface receptors. Several different molecules have been shown to bind TGF-βs. Among them, TβR-I and TβR-II play a major role in signal transduction of TGF-βs. Receptors for TGF-β in gastric carcinoma cell lines have been studied,[32, 33] but their distribution in human gastric carcinoma tissue has not been reported. In the present study, both TβR-I and TβR-II were expressed simultaneously on certain gastric cancer cells. One of the interesting findings is the differential expression of these receptors between the two types of gastric carcinomas. A significantly higher frequency of receptor staining was observed in diffuse-type carcinoma than in the intestinal type. Thus, in the diffuse type of gastric carcinoma, both TGF-βs and their receptors were expressed more frequently than in the intestinal type. In addition, when the tumors were divided into two groups according to the strength of TGF-βs and their receptor expression, carci-

noma with high TGF-β expression was associated with a higher incidence of lymphatic invasion than the group with low TGF-β expression, suggesting a possible role for TGF-βs in tumor invasion as well.

Generally, the intestinal type of gastric carcinoma is considered to be more differentiated than the diffuse type. Considering the high frequency of TGF-β expression in highly invasive tumors as well, our data suggest that TGF-β1 and TGF-β2 may play important roles in the progression of gastric cancers. However, it has been shown that certain malignant tumors become resistant to the growth inhibitory effect of TGF-β.[4] Some TGF-β-resistant cells have intact TGF-β receptors, and thus the intracellular signaling pathways are presumed to be perturbed in these cells.[34] In addition, recent data showed that some colon carcinomas with DNA repair defects lack TβR-II, but have intact TβR-I.[35] However, in the present study, expressions of TβR-II and TβR-I ware concomitant in all the gastric carcinoma tissues examined. Large amounts of TGF-β secreted by these TGF-β-insensitive carcinoma cells may suppress immune function or modulate the adhesion molecules on endothelial cells to contribute to the higher incidence of lymphatic invasion. Further studies, including epidemiological analysis, as well as *in vitro* studies on the receptors and signal transduction mechanism, may be needed before conclusions can be drawn regarding this postulated relationship between TGF-β and malignancy.

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REFERENCES

1) Lauren, P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol. Microbiol. Scand.*, **64**, 31–49 (1965).
2) Fujita, S. Biology of early gastric carcinoma. *Pathol. Res. Pract.*, **163**, 297–309 (1978).
3) Roberts, A. B. and Sporn, M. B. The transforming growth factor-βs. *In* "Peptide Growth Factors and Their Receptors, part I," ed. M. B. Sporn and A. B. Roberts, pp. 419–472 (1990). Springer-Verlag, Berlin.
4) Lyons, R. M. and Moses, H. L. Transforming growth factors and the regulation of cell proliferation. *Eur. J. Biochem.*, **187**, 467–473 (1990).
5) Massagué, J. The transforming growth factor-β family. *Annu. Rev. Cell Biol.*, **6**, 597–641 (1990).
6) Ura, H., Obara, T., Yokota, K., Shibata, Y., Okamura, K. and Namiki, M. Effects of transforming growth factor-β released from gastric carcinoma cells on contraction of collagen-matrix gels containing fibroblasts. *Cancer Res.*, **51**, 3550–3554 (1991).
7) Mizoi, T., Ohtani, H., Miyazono, K., Miyazawa, M., Matsuno, S. and Nagura, H. Immunoelectron microscopic localization of transforming growth factor β1 and latent transforming growth factor β1 binding protein in human gastrointestinal carcinomas: qualitative difference between cancer cells and stromal cells. *Cancer Res.*, **53**, 183–190 (1993).
8) Ohta, M., Greenberger, J. S., Anklesaria, P., Bassols, A. and Massagué, J. Two forms of transforming growth factor-β distinguished by multipotential haematopoietic progenitor cells. *Nature*, **329**, 539–541 (1987).
9) Cheifetz, S., Hernandez, H., Laiho, M., ten Dijke, P., Iwata, K. K. and Massagué, J. Distinct transforming growth factor-β (TGF-β) receptor subsets as determinants of cellular responsiveness to three TGF-β isoforms. *J. Biol. Chem.*, **265**, 20533–20538 (1990).
10) Millan, F. A., Denhez, F., Kondaiah, P. and Akhurst, R. J. Embryonic gene expression patterns of TGF β1, β2 and β3 suggest different developmental functions *in vivo*. *Development*, **111**, 131–144 (1991).
11) Pelton, R. W., Saxena, B., Jones, M., Moses, H. L. and Gold, L. I. Immunohistochemical localization of TGFβ1, TGFβ2, and TGFβ3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. *J. Cell Biol.*, **115**, 1091–1105 (1991).
12) Olofsson, A., Miyazono, K., Kanzaki, T., Colosetti, P., Engström, U. and Heldin, C.-H. Transforming growth factor-β1, -β2, and -β3 secreted by a human glioblastoma cell line: identification of small and different forms of large latent complexes. *J. Biol. Chem.*, **267**, 19482–19488 (1992).
13) Miyazono, K., ten Dijke, P., Ichijo, H. and Heldin, C.-H. Receptors for transforming growth factor-β. *Adv. Immunol.*, **55**, 181–220 (1994).
14) Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H. and Miyazono, K. Cloning of TGF-β type I receptor that forms a heteromeric complex with the TGF-β type II receptor. *Cell*, **75**, 781–792 (1993).
15) Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A. and Lodish, H. F. Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. *Cell*, **68**, 775–785 (1992).
16) López-Casillas, F., Cheifetz, S., Doody, J., Andres, J. L., Lane, W. S. and Massagué, J. Structure and expression of the membrane proteoglycan betaglycan, a component of the TGF-β receptor system. *Cell*, **67**, 785–795 (1991).
17) Wang, X.-F., Lin, H. Y., Ng-Eaton, E., Downward, J., Lodish, H. F. and Weinberg, R. A. Expression cloning and characterization of the TGF-β type III receptor. *Cell*, **67**, 797–805 (1991).
18) Wrana, J. L., Attisano, L., Wieser, R., Ventura, F. and Massagué, J. Mechanism of activation of the TGF-β receptor. *Nature*, **370**, 341–347 (1994).
19) López-Casillas, F., Wrana, J. L. and Massagué, J. Betaglycan presents ligand to the TGF-β signaling recep-

tor. *Cell,* **73,** 1435–1444 (1993).
20) Japanese Research Society for Gastric Cancer. "Japanese Classification of Gastric Carcinoma," First English Ed. (1995). Kanehara Co., Tokyo.
21) Waltenberger, J., Lundin, L., Öberg, K., Wilander, E., Miyazono, K., Heldin, C.-H. and Funa, K. Involvement of transforming growth factor-β in the formation of fibrotic lesions in carcinoid heart disease. *Am. J. Pathol.,* **142,** 71–78 (1993).
22) Kanzaki, T., Olofsson, A., Morén, A., Wernstedt, C., Hellman, U., Miyazono, K., Claesson-Welsh, L. and Heldin, C.-H. TGF-β1 binding protein: a component of the large latent complex of TGF-β1 with multiple repeat sequences. *Cell,* **61,** 1051–1061 (1990).
23) Yamashita, H., ten Dijke, P., Franzén, P., Miyazono, K. and Heldin, C.-H. Formation of hetero-oligomeric complexes of type I and type II receptors for transforming growth factor-β. *J. Biol. Chem.,* **269,** 20172–20178 (1994).
24) Taketazu, F., Kato, M., Gobl, A., Ichijo, H., ten Dijke, P., Itoh, J., Kyogoku, M., Rönnelid, J., Miyazono, K., Heldin, C.-H. and Funa, K. Enhanced expression of transforming growth factor-βs and transforming growth factor-β type II receptor in the synovial tissues of patients with rheumatoid arthritis. *Lab. Invest.,* **70,** 620–630 (1994).
25) Wataya-Kaneda, M., Hashimono, K., Kato, M., Miyazono, K. and Yoshikawa, K. Differential localization of TGF-β-precursor isotypes in normal human skin. *J. Dermatol. Sci.,* **8,** 38–44 (1994).
26) Yamada, N., Kato, M., Yamashita, H., Nistér, M., Heldin, C.-H., Miyazono, K. and Funa, K. Enhanced expression of transforming growth factor-β (TGF-β) and its type I and type II receptors in human glioblastoma. *Int. J. Cancer,* **62,** 386–392 (1995).
27) Iseki, S., Osumi-Yamashita, N., Miyazono, K., Franzén, P., Ichijo, H., Ohtani, H., Hayashi, Y. and Eto, K. Localization of transforming growth factor-β type I and type II receptors in mouse development. *Exp. Cell Res.,* **219,** 339–347 (1995).
28) Miyazono, K., Ichijo, H. and Heldin, C.-H. Transforming growth factor-β: latent forms, binding proteins and receptors. *Growth Factors,* **8,** 11–22 (1993).
29) Santos, O. F. P. and Nigam, S. K. HGF-induced tubulogenesis and branching of epithelial cells is modulated by extracellular matrix and TGF-β. *Dev. Biol.,* **160,** 293–302 (1993).
30) Roberts, A. B., Kim, S.-J., Noma, T., Glick, A. B., Lafyatis, R., Lechleider, R., Jakowlew, S. B., Geiser, A., O'Reilly, M. A., Danielpour, D. and Sporn, M. B. Multiple forms of TGF-β: distinct promoters and differential expression. *Ciba Found. Symp.,* **157,** 7–28 (1991).
31) Taipale, J., Miyazono, K., Heldin, C.-H. and Keski-Oja, J. Latent transforming growth factor-β1 associates to fibroblast extracellular matrix via latent TGF-β binding protein. *J. Cell Biol.,* **124,** 171–181 (1994).
32) Ito, M., Yasui, W., Nakayama, H., Yokozaki, H., Ito, H. and Tahara, H. Reduced levels of transforming growth factor-β type I receptor in human gastric carcinomas. *Jpn. J. Cancer Res.,* **83,** 86–92 (1992).
33) Park, K., Kim, S.-J., Bang, Y.-J., Park, J.-G., Kim, N.-K., Roberts, A. B. and Sporn, M. B. Genetic changes in the transforming growth factor-β (TGF-β) type II receptor gene in human gastric cancer cells: correlation with sensitivity to TGFβ. *Proc. Natl. Acad. Sci. USA,* **91,** 8772–8776 (1994).
34) Franzén, P., Ichijo, H. and Miyazono, K. Different signals mediate transforming growth factor-β1-induced growth inhibition and extracellular matrix production in prostatic carcinoma cells. *Exp. Cell Res.,* **207,** 1–7 (1993).
35) Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Volgelstein, B., Brattain, M. and Willson, J. K. V. Inactivation of the type II TGF-β receptor in colon cancer cells with microsatellite instability. *Science,* **268,** 1336–1338 (1995).