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Coexpression of Platelet-derived Growth Factor (PDGF) A-Chain and PDGF Receptor Genes in Human Gastric Carcinomas

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In this study we examined the expression of platelet-derived growth factor (PDGF) A-chain and PDGF receptor genes in seven human gastric carcinoma cell lines and 15 gastric carcinoma tissues. Expression of mRNA for PDGF A-chain was found in all gastric cell lines and all gastric carcinoma tissues. Two of the seven gastric carcinoma cell lines expressed PDGF receptor mRNA. Out of the 15 gastric carcinoma tissues, eight showed enhanced expression of PDGF receptor mRNA and all of them demonstrated prominent fibrous stroma. Moreover, the incidence of enhanced expression of PDGF receptor mRNA was higher in scirrhous carcinoma than in well differentiated adenocarcinoma. These results strongly suggest that PDGF produced by tumor cells acts as a paracrine growth factor for production of fibrous stroma in gastric carcinomas.

Key words: PDGF — PDGF receptor — Gastric carcinoma

PDGF[4] a polypeptide released from human platelets during clotting, is a potent mitogen for fibroblasts and smooth muscle cells and stimulates the proliferation of mesenchymal cells.[1,2] PDGF is composed of two homologous polypeptide chains, PDGF A-chain and PDGF B-chain,[3] and three types of isoform, PDGF AA, AB and BB, are known.[1] The gene encoding PDGF A-chain is localized on chromosome 7.[4] The PDGF B-chain is encoded by *SIS* protooncogene, the cellular counterpart of the simian sarcoma virus oncogene (v-*sis*),[5,6] and this gene is localized on chromosome 22.[7,8] The effect of PDGF on cells is mediated by a cell surface receptor showing tyrosine kinase activity.[9] The gene encoding PDGF receptor, which was recently identified,[10,11] is localized on chromosome 5.[11] This gene and colony stimulating factor-1 receptor gene (*FMS*) have similarities in chromosomal localization, organization and encoded amino acid sequence.[12]

Expression of PDGF A-chain and/or B-chain/*SIS* gene by malignant tumors was found in osteosarcoma, rabdomyosarcoma, synovial sarcoma,[4] mesothelioma,[13] glioma,[10,14] lung carcinoma,[15] mammary carcinoma[16,17] and myeloid cells.[18] Expression of mRNA for PDGF receptor was also detected in glioma[10,14] and an anaplastic thyroid cell line.[19] Moreover, PDGF has been suggested to be an autocrine or a paracrine growth factor for these tumors. However, there has been no study of PDGF and PDGF receptor mRNA expression by human gastric carcinomas.

In this study, we examined the expression of PDGF A-chain, *SIS*/PDGF B-chain and PDGF receptor genes in human gastric carcinomas.

Seven cell lines established from human gastric carcinomas were maintained in RPMI-1640 with fetal bovine serum. Cell lines used were TMK-1 established from a poorly differentiated adenocarcinoma; KATO-III, signet ring cell carcinoma; MKN-1, adenosquamous cell carcinoma; MKN-7, well differentiated adenocarcinoma; MKN-28, well differentiated adenocarcinoma; MKN-45, poorly differentiated adenocarcinoma and MKN-74, well differentiated adenocarcinoma. KATO-III was kindly provided by Dr. M. Sekiguchi, Tokyo University, and MKN series by Dr. T. Suzuki, Fukushima Medical College.

A total of 15 cases of gastric carcinoma were employed. Tumor tissues and corresponding normal tissues were frozen in liquid nitrogen immediately after their removal at surgery, and stored at −70°C until use. Clinicopathological data are summarized in Table I.

RNAs were extracted by the guanidium isothiocyanate/cesium chloride method. Fifteen μg of poly(A)$^+$-selected RNA from cell lines and 10 μg of total RNA from tissues were electrophoresed on agarose/formaldehyde gel and blotted onto nylon filters. The filters were hybridized with ^{32}P-labeled probe. Probes used were 1.3 kbp *Eco*RI-*Eco*RI fragment of PDGF A-chain cDNA[4]; pc-*sis*, 0.6 kbp *Pst*I-*Pst*I fragment of human *SIS* oncogene exon III[20]; 1.2 kbp *Hinc*II-*Hinc*II fragment of mouse

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[4] Abbreviations used in this paper: PDGF, platelet-derived growth factor; kb, kilobase; kbp, kilobase pair.

Table I. PDGF A-Chain and PDGF Receptor mRNA Expression in Gastric Carcinoma Tissues

Case Number	Age	Sex	Histological type[a]	Relative expression T/N[b]		
				PDGF A-chain	PDGF receptor	β-actin
1	39	F	sci	1.1[c]	4.0[c]	1.0[c]
2	63	F	sci	1.1	4.0	1.0
3	58	M	poorly	0.9	2.7	1.1
4	65	F	well	1.1	1.1	1.0
5	73	F	poorly	1.0	6.0	1.2
6	70	M	well	1.1	4.2	1.0
7	65	F	poorly	1.0	1.2	1.0
8	53	M	sci	1.1	3.7	1.1
9	56	M	well	1.0	1.0	1.0
10	50	M	well	1.0	1.1	1.0
11	67	M	well	0.9	1.0	1.0
12	69	M	well	1.1	4.0	1.0
13	70	F	well	1.1	1.3	1.2
14	47	M	well	1.0	4.4	1.0
15	75	F	sci	1.0	1.0	1.0

a) Based on the criteria of the Japanese Research Society for Gastric Cancer. Well, well differentiated adenocarcinoma including papillary and tubular adenocarcinoma; poorly, poorly differentiated adenocarcinoma including signet ring cell carcinoma and mucinous adenocarcinoma; sci, scirrhous carcinoma.
b) The T/N ratio of direct densitometric measurement of autoradiographic signals in gastric carcinoma tissues (T) and normal tissues (N).
c) As no normal tissue was obtained in Case 1, autoradiographic signals of the tumor tissue were compared with normal tissue of Case 2.

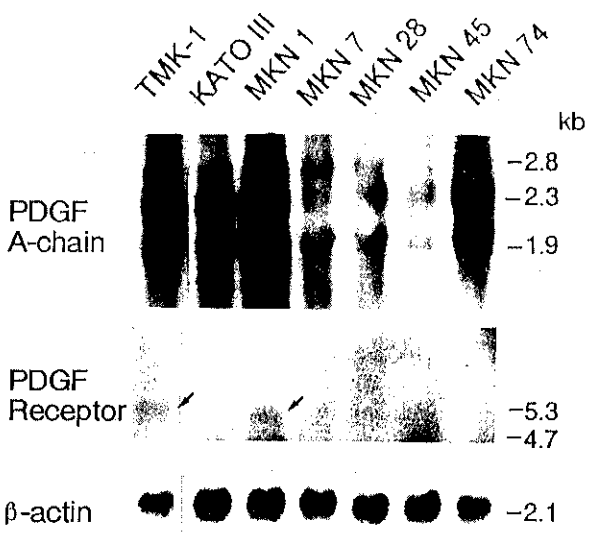

Fig. 1. Expression of mRNA for PDGF A-chain, PDGF receptor, and β-actin by gastric carcinoma cell lines. Each lane contains 15 μg of poly(A)$^+$ selected RNA. Ribosomal RNAs were used as size markers. Almost equal intensity of β-actin mRNA indicated that each lane contained almost equal amounts of RNAs. Arrows show the bands of PDGF receptor gene.

PDGF receptor cDNA[11] which has 86% homology with human cDNA[10]; and β-actin gene (Oncor Inc.). PDGF A-chain cDNA was kindly provided by Dr. C. Betsholotz, and mouse PDGF receptor by Dr. Y. Yarden. pc-*sis* was obtained from the Japanese Cancer Research Resources Bank.

The results of Northern blot analyses in human gastric carcinoma cell lines are shown in Fig. 1. The size of the PDGF A-chain mRNA was 2.8 kb, 2.3 kb and 1.9 kb, and that of PDGF receptor mRNA was 5.3 kb. PDGF A-chain gene was expressed by all gastric carcinoma cell lines, of which MKN-1 expressed the highest level of PDGF A-chain mRNA. PDGF receptor gene was expressed by TMK-1 and MKN-1. As the signal of the transcript of its gene was very weak, we performed autoradiography for a long time in this experiment. The signal of 4.7 kb was assumed to be the result of cross hybridization with 28S ribosomal RNA. Hybridization with *SIS*/PDGF B-chain gene was attempted, but *SIS*/PDGF B-chain mRNA band could not be detected (data not shown).

The results of Northern blot analyses in human gastric carcinoma tissues are shown in Fig. 2. The expression of mRNA for PDGF A-chain was detected in all gastric carcinoma tissues and in corresponding normal gastric

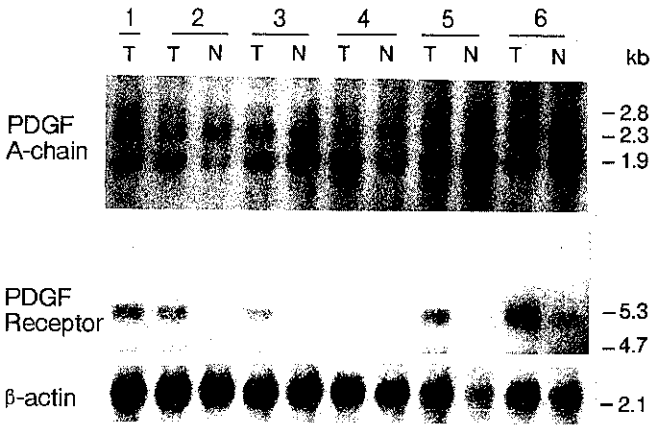

Fig. 2. Expression of mRNA for PDGF A-chain, PDGF receptor, and β-actin in gastric carcinoma tissues. Each lane contains 10 μg of total RNA. The number at the top is the patient number. T stands for tumor tissue and N, corresponding normal mucosa. No normal tissue was obtained in Case 1.

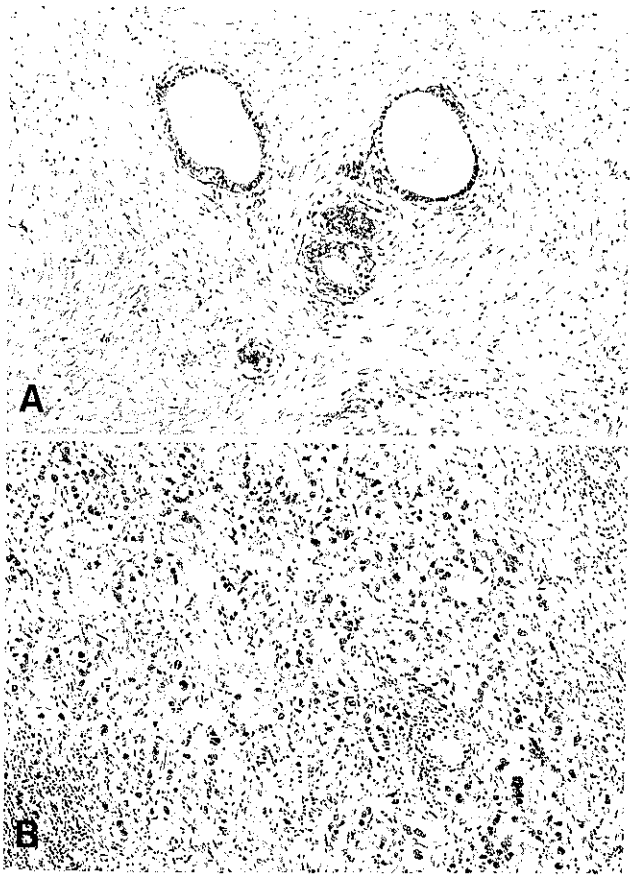

Fig. 3. Well differentiated adenocarcinoma with abundant stroma, Case 6 (A). Scirrhous carcinoma, Case 2 (B). HE stain, ×80.

mucosas. The level of PDGF A-chain mRNA in tumor tissues was almost the same as that in normal mucosas. In Cases 2, 3, 5 and 6, the level of PDGF receptor mRNA in carcinoma tissues was significantly higher than that in normal mucosas. Transcript of PDGF receptor mRNA was weak in all normal tissues. The expression of mRNA for PDGF receptor is summarized in Table I. Eight of the 15 cases (53%) showed enhanced expression of PDGF receptor mRNA in carcinoma tissues and had prominent fibrous stroma. Three of four scirrhous carcinomas (75%), two of three poorly differentiated adenocarcinomas (67%) and three of eight well differentiated adenocarcinomas (38%) showed enhanced expression of PDGF mRNA. Its incidence in scirrhous carcinomas was higher than that in well differentiated adenocarcinomas. Well differentiated adenocarcinomas with enhanced expression of PDGF receptor mRNA had abundant stroma, as did scirrhous carcinomas (Fig. 3). However, we could not detect the *SIS*/PDGF B-chain mRNA band (data not shown).

In this study we found the expression of PDGF A-chain and PDGF receptor genes in gastric carcinomas not only *in vitro* but also *in vivo*. The level of PDGF A-chain mRNA expression in tumor tissues was not remarkably different from that in normal mucosas. Evidence for PDGF A-chain gene expression by gastric carcinoma cell lines indicated that PDGF A-chain mRNA was expressed not only by cancer cells but also by stromal cells. *SIS*/PDGF B-chain mRNA expression was not detected. As we could not use a cDNA probe, the expression of PDGF B-chain/*SIS* gene remains to be determined. Therefore, PDGF produced by gastric carcinoma might be AA type or AB type. Osteosarcoma[21] and glioma[22] have been shown to secrete AA type of PDGF.

There are two conflicting reports on PDGF receptor gene. First, two populations of PDGF receptor exist, AB receptor and B receptor, and only PDGF BB type binds to B receptor, which is encoded by PDGF receptor gene[23]; second, any of AA type, BB type and AB type of PDGF binds to a single type of PDGF receptor which is encoded by PDGF receptor gene.[24] The second report implies that PDGF produced by gastric carcinomas can be a ligand for PDGF receptor.

The transcript level of PDGF receptor gene was found to be higher in gastric carcinoma tissues than in cell lines. Moreover, gastric carcinomas which expressed high levels of PDGF receptor mRNA demonstrated abundant stromas. This seems consistent with the expression of PDGF receptor mRNA in tumor tissues not only by tumor cells but also by stromal cells including fibroblasts and smooth muscle cells. It is likely that PDGF produced

by gastric carcinoma cells stimulates the growth of the fibroblasts and smooth muscle cells around tumor cells. In fact, non-small cell lung carcinoma cell lines which expressed PDGF A-chain mRNA demonstrated prominent fibrous stroma in nude mice, while small cell lung carcinoma without PDGF expression had no fibrosis.[25] Further, PDGF might enhance the level of PDGF receptor mRNA in tumor cells or stromal cells, as it has already been pointed out that EGF increased EGF receptor mRNA in gastric carcinoma cell lines.[26] Therefore, the present study indicates that PDGF and its receptor play an important role in production of stroma in gastric carcinomas.

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