

tie-1 Protein Tyrosine Kinase: A Novel Independent Prognostic Marker for Gastric Cancer¹

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ABSTRACT

Protein tyrosine kinases (PTKs) are a major class of proto-oncogenes that are involved in tumor progression. The purpose of this study was to establish a comprehensive PTK expression profile in gastric cancers, with the objective of identifying possible biomarkers for gastric cancer progression. We have designed degenerate primers according to the consensus catalytic motifs to amplify PTK molecules from gastric cancers by reverse transcriptase-PCR methods. The PTK expression profile was established by sequencing analysis of the cloned PCR products. We have identified 17 PTKs from a gastric adenocarcinoma. Two receptor PTKs, tie-1 and axl, were selected for *in situ* immunohistochemistry studies because of their higher expression level and their described roles in adhesion, invasion, and angiogenesis. Among the 97 gastric adenocarcinoma tissues examined, we observed positive immunohistochemical staining of tie-1 PTK in 69 and positive staining of axl kinase in 71 tissues. Statistical analysis with clinicopathological features indicates that tie-1 kinase expression is inversely correlated with patients' survival, whereas axl fails to show similar clinical significance. Our results illustrate the utility of tyrosine kinase gene family profiling in human gastric cancers and show that tie-1 tyrosine kinase may serve as a novel independent prognostic marker for gastric adenocarcinoma patients.

INTRODUCTION

Cancer of the stomach is the second-most common cancer in the world. A recent official report on mortality in Taiwan showed that gastric cancer ranks as the third leading cause of cancer-related deaths (1). The 5-year survival rate remains poor for this type of cancer, and only two survival-influencing factors, the depth of invasion and the presence of regional lymph node involvement, are commonly used in prognosis (2, 3). Compared to other more extensively investigated cancers such as breast and colon carcinomas, our present understanding of the molecular mechanisms involved in the transformation and progression of gastric cancer lags significantly behind. To improve the understanding of gastric cancer progression as well as to identify possible molecular prognosis factors, here we examine the expression profile of PTKs³ in human gastric cancers.

PTKs are a major class of oncogenes that are involved in growth signaling (4-6) and may serve as useful biomarkers for human cancers. Overexpression of the c-erbB2/neu oncoprotein in gastric carcinoma is clinically associated with poor prognosis (7-10). The expression of other specific tyrosine kinases such as c-met and k-sam in human gastric cancers has also been reported (11-17). Amplification of certain PTK genes has been found in human gastric cancers (18). However, these reports examined only a selected few kinases; a comprehensive study of the overall expression PTK expression profile in gastric cancer has not yet been performed. Given the extensive cross-talk among tyrosine kinases, we were interested in defining other tyrosine kinases that might also be involved in the development of gastric cancer. We have recently developed an improved RT-PCR approach that permits the identification of the majority, if not all, of the expressed PTK genes in a single screening effort (19, 20). Application of this approach to gastric cancer tissues revealed the presence of 17 PTK genes, among which two receptor PTK genes (*tie-1* and *axl/ufo*) were found to be expressed at high level and were selected for further evaluations of their clinical significance.

Members of the axl/ufo receptor PTK family were first isolated from various tumor cell lines, including axl/ufo, mer/nyk, and rse/sky (21-23). Their structures contain two immunoglobulin-like domains and two fibronectin type III domains in the extracellular region, a transmembrane region, and the kinase domain in the cytoplasmic region. Because of the similarity of extracellular domains with cell adhesion molecules, it has been suggested that axl/ufo family PTKs could be involved in the cell recognition process (24). Here, we demonstrated the common expression of axl/ufo receptor PTKs in human gastric carcinoma tissues by immunohistochemistry. *tie-1* was first isolated from a human erythroleukemia cell line (25), and it was subsequently

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³ The abbreviations used are: PTK, protein tyrosine kinase; RT-PCR, reverse transcriptase-PCR; ABC, avidin-biotin-peroxidase complex.

found that the *tie-1* gene was predominantly expressed in blood vessel endothelial cells. This receptor PTK family also contains two immunoglobulin-like domains and three fibronectin type III domains, in addition to three epidermal growth factor-like repeats in their extracellular region. Expression of *tie-1* provides antigenic marker for assessment of breast cancer angiogenesis (26). In melanoma progression studies, expression of *tie-1* and *eck* kinases are enhanced (27). There is no previous report of *tie-1* PTK expression in human epithelial carcinoma cells, including gastric cancer cells. Our report based on examination at the single-cell level provides the first evidence of the expression of *tie-1* PTK in human epithelial carcinoma cells, not due to contaminating vascular endothelial cells. More significantly, we show here that *tie-1* is a potentially useful independent prognostic marker.

MATERIALS AND METHODS

Surgical Specimens. A specimen from a 66-year-old male with stage IIIB gastric cancer (28) was used for the PTK profile. The tumor was an ulcerated and infiltrating type measuring 5.3×4.5 cm in size, located at midbody of the stomach. Microscopically, it showed an intestinal-type (29) adenocarcinoma. Stroma reaction was abundant. Cancer cells had infiltrated whole layers of stomach wall and metastasized to adjacent perigastric lymph nodes. No *Helicobacter pylori* was identified by modified Giemsa stain. Nine months after a total gastrectomy, the patient had liver metastasis and received chemotherapy but ultimately died 18 months later.

Ninety-seven gastric cancer tissues were used for immunohistochemical study. The maximum time between specimen removal and quenching of tissue was 1 h after a sketch and record. Tissue blocks were fixed overnight at 4°C with 4% neutral buffered paraformaldehyde, dehydrated, cleared with Hemo-De (Fisherbrand; ingredients: D-limonene and butylated hydroxyanisole, Fisher Scientific, Pittsburgh, PA), and then embedded in wax. Five- μ m-thick sections were used for staining. All specimens were obtained from patients who underwent surgical resection for gastric adenocarcinoma at Department of Surgery, Veterans General Hospital-Taipei, Taiwan. None of these patients had been subjected previously to any other form of therapy, such as chemotherapy or radiation. Informed consent was obtained from all patients.

RNA Purification from Surgical Specimens. Gastric cancer tissues used for RNA isolation were immediately frozen in liquid nitrogen after resection and stored at -70°C until use. The direct guanidine isothiocyanate lysis and cesium chloride gradient separation method was used for extracting total RNA from gastric cancer tissues (30). Briefly, gastric cancer tissues were lysed in a lysis solution containing 4 M guanidine isothiocyanate (BDH, Poole, England). The lysate was overlaid onto a layer of 5.7 M cesium chloride (BDH) and then spun at 90,000 rpm for 5.5 h with a Beckman TLX-100 ultracentrifuge. The RNA pellets were washed with 70% ethanol several times, dried, and resuspended with RNase-free water. Reverse transcription was done with 2 μ g of total RNA, oligo(dT)₁₅, and Moloney murine leukemia virus reverse transcriptase from Promega (Madison, WI). The quality of reverse transcription products was examined by agarose gel electrophoresis and by PCR

with specific primers for *glyceraldehyde-3-phosphate-dehydrogenase*, a housekeeping gene.

RT-PCR Amplification of PTK. The PCR primers are derived from the conserved motifs DFG and DVW of tyrosine kinase catalytic domain. Several pairs of degenerated PCR primers were designed from the following amino acid sequences, which would predict a PCR product of 150–170 bp (19). For the 5' primers (F1, F2, and F3), three degenerate oligonucleotide primers were designed from two amino acid sequences: 5'-(*Bam*HI)-K[V/I][S/C/G]DFG-3' and 5'-(*Bam*HI)-K[V/I][A/S/T]DFG-3'. For the 3' primer (R1), one primer was designed: 5'-DVW[S/A][F/Y]G-[*Eco*RI]-3'.

The PCRs were conducted at 42°C annealing temperature for 5 cycles and then at 55°C for 25 cycles with a Accugen 9600 PCR thermocycler and Takara Taq polymerase (Shiga, Japan). Four separate PCR experiments were performed for each set of primers to reduce PCR-related errors. The final PCR products were analyzed with 8% polyacrylamide gels. The 150–170-bp bands were eluted from gels and purified with a nuclear trap kit from Marherrey-Nagel (Duren, Germany). Following ligation with T-vector plasmid from Promega, resulting recombinants were screened for the proper 150–170-bp inserts by *Bam*HI and *Eco*RI digestion of plasmid DNA.

Sequence Analysis of PTK Genes. Following the identification of recombinants, plasmid DNA were isolated with Jet-Star kits (Genomed Inc., Research Triangle Park, NC). Sequence analysis was done with the dideoxy method with ³⁵S-dATP labels and an autosequencer (Applied Biosciences). The sequences were then analyzed and translated into amino acid sequences with a Macintosh SeqAPP program for identification of the DFG and DVW motif. For comparison with known PTK genes, computer alignment/comparison with GenBank and EMBL databases was performed with the FASTA⁴ and BLAST⁵ programs.

Immunohistochemical Staining of tie-1 and axl/ufo PTKs. *tie-1* and *axl/ufo* proteins in gastric tissues were localized with the ABC technique, as described previously (31). Antibodies and blocking peptides were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and all other histochemistry reagents were obtained from Vector Laboratories (Burlingame, CA). *tie-1* (C-18) is an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to amino acids 1121–1138, mapping to the COOH terminus of *tie-1* of human origin. *axl/ufo* (C-20) is an affinity-purified goat polyclonal antibody raised against a peptide corresponding to amino acids 868–887, mapping to the COOH terminus of the *axl* precursor of human origin. Briefly, the rehydrated tissue sections were first treated with microwave in a sodium citrate buffer (0.06 M, pH 6), then with normal rabbit or goat serum to remove endogenous peroxidase activity and to reduce nonspecific background staining. The tissue sections were incubated with rabbit antihuman *tie-1* or goat antihuman *axl/ufo* antiserum at 1:100 dilution at room temperature overnight in a moist chamber. The tissue sections were then treated with biotin-

⁴ <http://www.ncbi.nlm.nih.gov>.

⁵ <http://www.ebi.ac.uk>.

Table 1 Protein-tyrosine kinases identified in a gastric adenocarcinoma tyrosine kinase profiling study

By a modified RT-PCR approach in a single screen, we have identified 17 known different kinases in a given gastric adenocarcinoma from 110 clones analyzed. This profile includes 11 receptor PTKs, 4 nonreceptor PTKs, and 2 dual kinases

Identity	Sequence of PTK identified
Receptor PTK	
tie-1	DFGLSRG - - -EEVYVKTMGRLPVRWMAIESLNYSVYT- - TKSDVW
axl/ufo	DFGLSKKIY - SGDYRQRIAKMPVKWIAIESLADRVT- - SKSDVW
neu	DFGLARLLDI -DETEYHA-DGGKVPKWMALLESILRRRFT- - HQSDVW
brk	DFGLARLIK - -EDVYLS-HDHNIPIYKWTAPEALSRGHYS- - TKSDVW
c-fms	DFGLARDIM - NDSNYIVKGNARLPVKWMAPEISIFDCVYT- - VQSDVW
c-kit	DFGLARDIR - NDSNYVVKGNARLPVKWMAPEISIFSCVYT- - FESDVW
cak	DFGMSRNLY - AGDYRVRVQGRAVLPKIRWMAWECILMGKTT - - ASDVW
cek-5	DFGLSRFLEDDTSDPTYTSALGGKIPKIRWTAPEAIQYRKFT- - SASDVW
eph	DFGLTRLL -DDFDGTYETO-GGKIPKIRWTAPEAIAHRIFT- - TASDVW
PDGF-R β	DFGLARDIM - RDSNYISKGSTFLPLKWMAPESIFNSLYT- - TLDVW
VEGF-R	DFGLARDI - YKNPDYVRKGDTRLPLKWMAPESIFDKIYS- - TKSDVW
Nonreceptor PTK	
csk	DFGLTKEA- - - - SSTQDTGKLPVKWTAPEALREKKFS- - TKSDVW
fyn	DFGLARLI - -EDNEYTARQGAKFPKWTAPEAALYGRFT- - IKSDVW
itk	DFGMTRFV- - LDDQYTSSTGTKFPVKWASPEVFSFYS- - SKSDVW
tyk2	DFGLAKAV -PEGHEYYRVEDGDSVPVFWYAPECLKEYKFY- - YASDVW
Dual kinases	
kkiamre	DFGFARTL - - AAPGEVYTDYVATRWRAPPELLVGDVKYG - KAVDVW
mkk4	DFGISGQL - - -VDSIAKTRDAGCRPYMAPERIDPSASRQGYDVRSDVW

labeled goat antirabbit or anti-goat IgG antibodies (50 μ l) in 10 ml of PBS, followed by ABC. Fresh ABC was made by incubating 10 μ l of avidin and 10 μ l of biotin-peroxidase in PBS for 30 min before use. ABC stain for negative controls was carried out by omission of primary antiserum or replacement of primary antiserum by nonimmune rabbit normal serum. The section adjacent to that for ABC staining was stained with H&E for comparison. Because tie-1 protein eventually found to be a significant parameter in this study, several tumor sections were further incubated with tie-1- or tie-2-blocking peptide to demonstrate its specificity.

Statistical Analysis. The survival rate was calculated by the Kaplan-Meier method. Statistical comparisons were made with the general Wilcoxon test. Cox's multivariate regression analysis with forward stepwise procedure was performed to select a set of significant prognostic factors. $P < 0.05$ was considered significant with a two-tailed test.

RESULTS

PTK Profile in a Gastric Adenocarcinoma Tissue. For analysis of PTKs, our approach takes advantage of the highly conserved nature of DFG and DVW motifs, which are located in subdomains VII and IX in the catalytic domain among all tyrosine kinases. F1, F2, and F3 were the degenerate forward primers for the DFG region, and R1 was the reverse primer for the DVW region. They were chosen to accommodate the different codons used in the DFG forward primers (F1, F2, and F3) and the DVW reverse primer (R1). All these combinations were used in individual reactions to identify expressed kinases. Purified amplification products were subcloned into a plasmid vector (T-vector) for DNA sequencing. Most of the clones analyzed carried the expected size of inserts (~170 bp). We then sequenced 110 positive clones from the gastric cancer tissue. More than 95% of the clones sequenced belong to the PTK gene family (106 clones). After comparison of the sequencing data

with various database, a total of 17 different known PTK genes were identified (Table 1). These PTK genes include receptor PTK genes (*tie-1*, *axl/ufo*, *c-fms*, *cak*, *cek-5*, *eph*, *neu*, *PDGF-R β* , *brk*, *c-kit*, and *cek-5*) and nonreceptor PTK genes (*csk*, *fyn*, *itk*, and *tyk2*), as well as dual and other kinases (*mkk4* and *kkiamre*). In addition, one novel PTK gene was also identified and is currently under investigation (data not shown). To determine whether the above profile is representative of gastric adenocarcinoma in general, we initially used the RT-PCR method to study the expression of selected kinases in several gastric cancer tissues. Gene-specific primers were used to obtain individual RT-PCR products. Several PTKs (*e.g.*, *tie-1*, *axl/ufo*, *cak*, *neu*, *brk*, *fyn*, and *mkk4*) examined, indeed, confirmed their general expression pattern in human gastric cancer tissues (data not shown). Because of the limitations of RT-PCR approach in identifying the specific cell type that expressed the particular tyrosine kinase and in screening clinical tissue samples retrospectively, we used immunohistochemistry approach for further examination of the clinical relevance of these tyrosine kinases.

Clinical Relevance of tie-1 and axl/ufo Protein Kinases in Gastric Adenocarcinomas. We chose *tie-1* and *axl/ufo* kinases for this study because they are expressed at a relatively higher level based on the large number of clones identified from the PTK profile, and there have been no reports regarding their roles in gastric adenocarcinoma tissues. Their cell adhesion molecule-related motif in the extracellular region could implicate their possible involvement in the tumor progression processes. To examine whether these two kinases relate to clinical features of gastric adenocarcinoma patients, we used immunohistochemical staining to localize these kinases. As illustrated in Fig. 1A, immunoreactivity of *tie-1* PTK in gastric cancer tissue revealed fine reddish-brown particles. This immunoreactivity was totally blocked by specific *tie-1*-blocking peptide (Fig. 1B) but not *tie-2*-blocking peptide (Fig. 1C), attesting to the specificity of the antibodies. *tie-1* protein expression was observed in

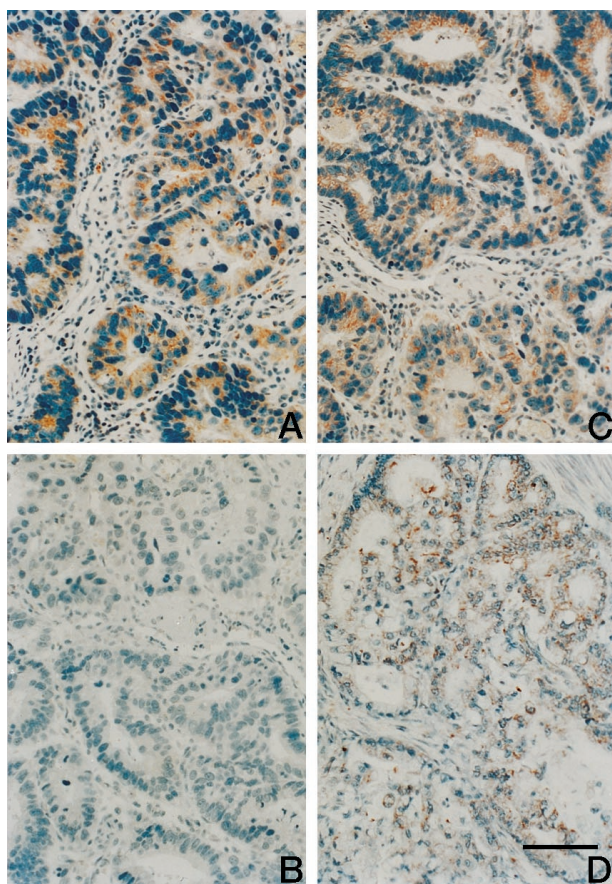


Fig. 1 Immunohistochemical staining of tie-1 and axl/ufo tyrosine kinases in human gastric adenocarcinoma sections. Anti-tie-1 and anti-axl/ufo protein antibodies were used for immunohistochemical staining in human gastric cancer tissues by ABC methods as described in "Materials and Methods." **A**, positive tie-1 PTK immunoreactivity existed in the cytoplasm, not in the nucleus of gastric cancer cells. **B**, absence of tie-1 immunoreactivity was observed in the presence of tie-1-blocking peptide in the staining reaction. **C**, tie-2-blocking peptides fail to block tie-1 immunoreactivity in the anti-tie-1 antibody stain reaction. **D**, positive axl/ufo PTK immunoreactivity in the cancer cells (original magnification, $\times 400$). Scale bar, 100 μm .

69 of 97 (71.1%) gastric adenocarcinoma. It is noteworthy that, in the original gastric cancer specimen where we identified tie-1 protein by RT-PCR, the immunostaining also revealed a positive staining result. The tie-1 immunoreactivity was found in the cytoplasm region of gastric cancer cells, especially on the luminal side of adenocarcinoma. tie-1 protein expression was also observed in microvessels in the tissue sections examined, consistent with the reported role of tie-1 in angiogenesis. Previously, Salven *et al.* (26) used tie-1 as antigenic marker for assessment of breast cancer angiogenesis. Unlike breast cancer, most gastric adenocarcinomas are of the ulcerated type, undergoing inflammatory reaction and neovascularization. Therefore, microvessel counts assessed for gastric cancer were not considered in this study. It is, however, clear that, in gastric adenocarcinoma, tie-1 is expressed in tumor cells themselves in addition to the vascular endothelial cells.

Positive axl/ufo PTK immunoreactivity was demonstrated

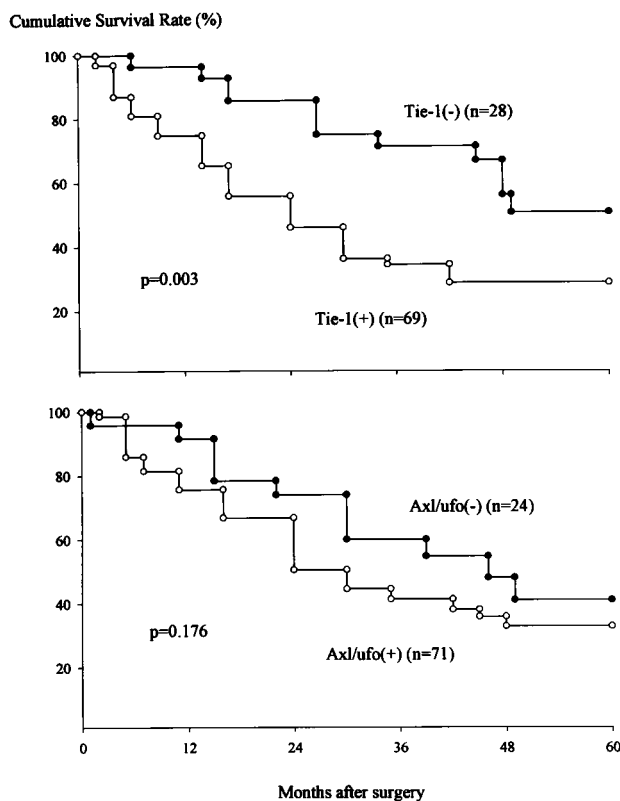


Fig. 2 Cumulative survival curve in gastric adenocarcinoma patients according to tie-1 (*top*) or axl/ufo (*bottom*) PTK immunoreactivity. The survival rate was calculated by the Kaplan-Meier method. Statistical comparisons were made with the general Wilcoxon test. $P < 0.05$ was considered significant.

in Fig. 1D. Among 95 gastric cancer tissue blocks examined, axl/ufo proteins were observed in 71 of 95 (74.7%) gastric adenocarcinoma. Although the number of cancer tissues expressing axl/ufo is comparable to those expressing tie-1, as shown below, associations with clinicopathological features showed distinct results upon statistical analysis.

Patients who expressed tie-1 kinase in their gastric cancer tissues had a worse survival outcome than did those who did not ($P = 0.003$; Fig. 2, *top*). On the contrary, patients with or without axl/ufo kinase expression in gastric cancer tissue had no difference in survival outcome ($P = 0.176$; Fig. 2, *bottom*). Multivariate survival analysis was performed, which includes several clinicopathological factors such as: age; sex; site, size, and gross appearance of tumor; stromal reaction pattern; depth of cancer invasion; cancer cell grading; lymph node or liver metastasis; peritoneal seeding; lymphatic duct or vessel of the stomach wall invasion; and tumor-node-metastasis stage. The results showed that tie-1 protein expression in gastric adenocarcinoma tissues is an independent factor affecting gastric cancer patients' survival (hazard ratio = 2.55; Table 2). To our knowledge, this is the first report of tie-1 tyrosine kinase used as an independent prognostic maker for human gastric adenocarcinoma. Its clinical implications and significance will be further explored.

Table 2 tie-1 immunoreactivity associated with survival after uni- and multivariate analyses using the Cox stepwise proportional hazards model^a

Variable	β	SE	P	Hazard ratio	95% confidence interval
TNM stage	1.10	0.17	<0.001	3.01	2.15–4.23
tie-1 immunoreactivity	0.93	0.34	0.006	2.55	1.30–4.97

^a β , coefficient; TNM, tumor-node-metastasis.

DISCUSSION

Here, we report the tyrosine kinase expression profile of a stage IIIB gastric cancer tissue. Takeshima *et al.* (32) observed that three of six human gastric cancer cell lines showed enhanced tyrosine phosphorylation patterns by Western blot analysis with antiphosphotyrosine antibody, but no specific PTKs were identified. Iwase *et al.* (33), using cDNA library screening method with antiphosphotyrosine antibody as probes, identified only four PTK genes from a human gastric cancer cDNA library, presumably due to the inherent inefficiency of the approach. On the other hand, RT-PCR using degenerate primers has been used effectively to identify novel kinases; but in most instances, a small number of tyrosine kinases have been uncovered (34, 35). By using RT-PCR with a degenerate primer set that encodes invariable sequences of the DFG and DVW regions, coupled with an improved vector system (19), we have identified 17 PTKs in the gastric adenocarcinoma tissue studied here.

We previously identified 13 known PTK genes and 2 novel PTKs in a human gastric cancer cell line with the same set of degenerate primers (20). Interestingly, different PTK expression patterns were observed from these two studies. Although the gastric cancer cell lines provide a homogeneous cancer cell population, it is conceivable that *in vitro* culture conditions would alter the growth conditions as well as PTK gene expression profiles of gastric cancer cells. On the contrary, clinical tissue samples would provide a better vehicle for assessment of PTK gene expression *in vivo*; however, the mixture of cancer cells and other normal host gastric tissue cells would hamper the interpretation of profile results. This is clearly confirmed here. Furthermore, the gastric cancer cell line used in our previous study was expanded from liver metastases of a gastric cancer patient (36). However, we did observe the expression of *axl/ufo* PTK family in several gastric cancer cell lines examined by gene-specific RT-PCR primers (not shown), including *axl/ufo*; *mer/nyk*, and *rse/sky*. It should be noted that *mer/nyk* and *rse/sky* were also identified in previous study (20). This indicates the ubiquitous expression nature of the *axl/ufo* PTK family in human gastric cancer cells and tissues. tie-1 expression was not found in human gastric cancer cell lines cultured in our laboratory under normal conditions (10% fetal bovine serum). However, upon sodium butyrate or tetradecanoyl phorbol acetate treatment, tie-1-specific messages were detected in three human gastric cancer cell lines (AGS, AZ521, and HR) by RT-PCR methods (data not shown). tie-1 was first isolated from a human erythroleukemia cell line K562, and its expression was only detected under tetradecanoyl phorbol acetate induction (25).

This result implies that human gastric cancer cells are able to express tie-1, which could have been lost in the establishment of gastric cancer cell lines *in vitro*, because they were grown in the presence of bovine serum and tissue culture medium. As discussed below, tie-1 may function in the differentiation of endothelial cells during angiogenesis. It is possible that tie-1 may be involved in the *in vivo* neoangiogenesis activity during gastric cancer progression and not in the promotion of growth of gastric cancer cell lines. Therefore, we could not observe the expression of tie-1 in cultured gastric cancer cell lines under standard conditions. tie-1 PTK could have important biological functions *in vivo* during gastric cancer progression.

Interestingly, the *axl/ufo* and *tie-1* genes examined here were both originally uncovered from leukemia cells (22, 25). *axl/ufo* was isolated as a transforming gene from the DNA of two patients with chronic myelogenous leukemia using a transfection assay. tie-1 was recovered from K562 human leukemia cells, and its expression is up-regulated when megakaryocytic differentiation is induced. Further studies of *axl/ufo* showed that this gene may be operative in normal and malignant myeloid biology (37). Challier *et al.* (38) reported that *axl/ufo* is expressed solely in myeloid and erythromegakaryocytic leukemias but not in lymphoid malignancies. These data suggest *axl/ufo* protein kinase is involved in leukemogenesis, although the *axl/ufo* family of PTKs are ubiquitously expressed in nearly all tissues. We found that *axl/ufo* PTK is expressed in most of human gastric cancer tissues examined, but its expression does not appear to be significant with respect to gastric tumor progression or patients' survival outcome.

tie-1 expression is more restricted in endothelial cells and hematopoietic cells (39–41). In this study, we found tie-1 protein expression in individual normal gastric mucosal gland (<25%) and microvessels (data not shown). tie-1 and the other member of this PTK family, tie-2/*tek*, are well studied in the context of blood vessel formation and maturation during embryonic development (40, 42, 43). Recently, the ligand of tie-2, angiopoietin-1 (44), was isolated. It binds specifically to the tie-2 receptor and induces tyrosine phosphorylation of the receptor. The binding of angiopoietin-1 to tie-2 does not directly promote the growth of cultured endothelial cells. However, it is required for embryonic angiogenesis (45). It has been suggested that angiopoietin-1/tie-2 pathway may function in the differentiation rather than growth of endothelial cells during angiogenesis. Because the ligand for tie-1 has not been identified, it is too early to tell whether tie-1 is functionally similar to tie-2. The molecular mechanisms of tie-1 signaling pathway need to be further elucidated to provide a better interpretation.

Recent studies of human brain tumors showed higher amounts of tie-1 mRNA or protein in tumors than in respective normal brain tissues. Using *in situ* hybridization technique, we found tie expression in tumor endothelial cells and not in tumor cells (46, 47). These data support the hypothesis that tie-1 protein plays an important role in the development of embryonic vasculature and possible also in angiogenesis associated with tumorigenesis (40, 43). In this study, we have detected tie-1 protein in not only microvessels but also gastric cancer cells. Easty *et al.* (27) found that tie-1 message was absent in normal melanocytes and primary melanomas but was present in metastatic melanomas. This implies that tie-1 PTK could be involved

in the melanoma progression and metastasis. It is conceivable that tie-1 is also involved in the progression of human gastric adenocarcinoma because we found tie-1 protein is an independent factor negatively affecting survival as well as a prognostic marker in gastric adenocarcinoma. The involvement of tie-1 in human gastric cancer progression and metastasis must be studied further.

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