Intestinal Trefoil Factor: A Marker of Poor Prognosis in Gastric Carcinoma[1]

Takasuke Yamachika, J. Lawrence Werther, Carol Bodian, Mark Babyatsky, Masae Tatematsu, Yoshitaka Yamamura, Anli Chen, and Steven Itzkowitz[2]

Gastrointestinal Research Laboratory, Departments of Medicine [T. Y., J. L. W., M. B., A. C., S. I.] and Biomathematical Sciences [C. B.], Mount Sinai School of Medicine, New York, New York 10029, and Laboratory of Pathology [M. T.] and Department of Surgery [Y. Y.], Aichi Cancer Center, Nagoya 464, Japan

ABSTRACT

Purpose: **Intestinal trefoil factor (ITF) is a marker of intestinal differentiation that may also play a role in cancer cell biology by inhibiting cell adhesion, promoting cell invasion, and blocking apoptosis. Gastric adenocarcinomas can arise through a process of intestinalization, but no study has yet comprehensively examined the expression of ITF in gastric cancer or correlated ITF expression with clinical outcome in any cancer type.**

Experimental Design: **Patients (209) with primary gastric adenocarcinoma were evaluated for ITF expression by immunohistochemistry. Results of immunostaining were correlated with clinicopathological variables and overall survival.**

Results: **In normal gastric mucosa, ITF expression was absent, whereas areas of intestinal metaplasia revealed strong ITF expression by goblet cells. A portion of gastric cancers (55%) demonstrated ITF expression. Women were more likely than men to express ITF in gastric cancers. However, in men, the expression of ITF correlated with aggressive phenotype of tumors (advanced stage, infiltrative growth pattern, and positive lymph nodes). Multivariate analysis revealed that expression of ITF was associated with a poor prognosis, independent of tumor stage.**

Conclusions: **This is the first study to correlate ITF expression with clinicopathological features or outcome in any cancer type. ITF expression in gastric cancer exhibited a curious gender-associated relationship, being more frequently expressed in tumors of women, but associated with more aggressive pathological features in men. The poor prognosis of patients with ITF-positive gastric cancers further implicates ITF in cancer cell biology.**

INTRODUCTION

The trefoil factors comprise a unique family of abundant GI[3] peptides with a distinct three-loop structure formed by a highly conserved motif of cystine disulfide bonds, which confer them with remarkable luminal stability (1). The trefoil factor family consists of three members: TFF1 (pS2), TFF2 (spasmolytic polypeptide), and TFF3 (ITF). The genes for these peptides are located within a 55 kb region on human chromosome 21q22.3 (2).

In the GI tract, TFF1 and TFF2 are normally expressed in gastroduodenal epithelium (3–6) but can be expressed throughout the entire GI tract in inflammatory lesions (3, 7). In contrast to TFF1 and TFF2, ITF is expressed predominantly in the small intestine and colon (3, 6, 8, 9). Outside of the GI tract, ITF expression in normal human tissues appears to be limited to the uterus (9), breast (10), hypothalamus/pituitary (11), salivary glands (12), and respiratory tract (13). The functions of the trefoil peptides in the GI tract are being elucidated gradually. Most of the emphasis to date has revealed important roles for the trefoil peptides in protection and repair against injury to the GI mucosa (14–19).

Less is known about the role of trefoil peptides in cancer biology. In cancer tissues, trefoil peptide expression has been demonstrated in several types of epithelial carcinomas. TFF1 was cloned originally from a breast cancer cell line as a factor induced by estrogen (20), but it is also expressed in gastric, pancreatic, biliary tract, colon, and esophageal cancers (21, 22). Mice that are rendered null for the *pS2* (TFF1) gene develop adenomas and intramucosal carcinomas of the stomach (23), and mapping studies indicate that TFF1 resides in a region of human chromosome 21q22 that frequently demonstrates allelic loss in primary human gastric carcinoma (24). TFF2 expression has been recognized in gastric, pancreatic, and biliary tract cancers (22, 25, 26). Although ITF has not been studied in cancer as extensively as the other trefoil peptides, it is expressed at high levels in breast carcinoma, mucinous carcinomas of the skin, and in the colonic adenoma to carcinoma sequence (10, 27–30). In addition, we have noted that ITF is preserved in all cases of human colon cancer liver metastases.[4]

The precise roles that ITF and the other trefoil peptides play in neoplasia remain unclear. ITF participates in epithelial

Received 10/16/01; revised 2/22/02; accepted 2/28/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by Grants RO1 CA81363 (to S. H. I.) from the National Cancer Institute and The Peptic Ulcer and Gastric Cancer Research Fund (to J. L. W. and S. H. I.).
[2] To whom requests for reprints should be addressed, at GI Division, Box 1069, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY 10029. Phone: (212) 241-6749; Fax: (212) 348-7428; E-mail: steven.itzkowitz@mssm.edu.

[3] The abbreviations used are: GI, gastrointestinal; ITF, intestinal trefoil factor.
[4] M. Babyatsky, J. Lin, A. Chen, M. Schwartz, S. Thung, and S. Itzkowitz. Intestinal trefoil factor: potential for tissue-specific gene therapy of human colon cancer liver metastasis, submitted for publication.

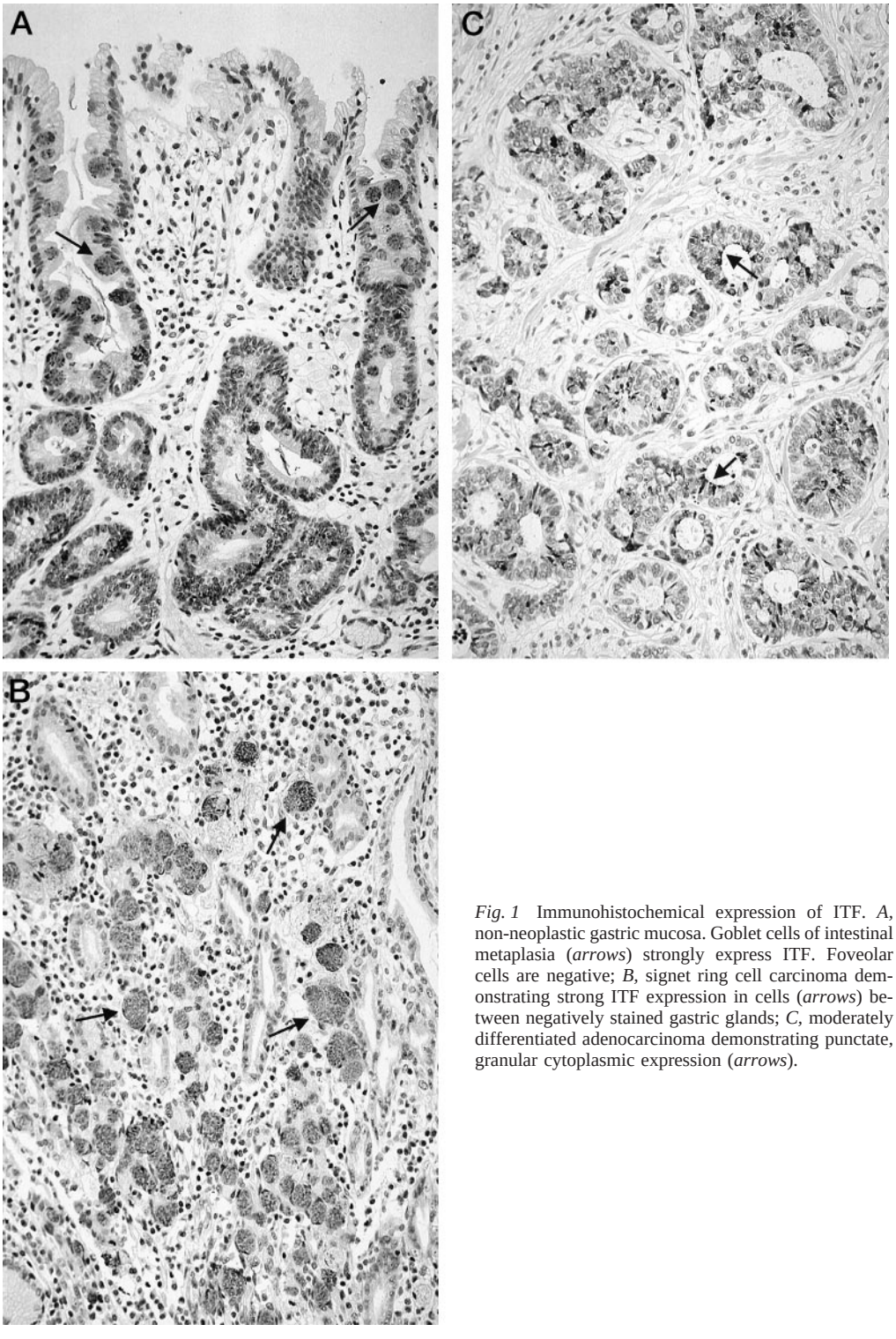

Fig. 1 Immunohistochemical expression of ITF. *A*, non-neoplastic gastric mucosa. Goblet cells of intestinal metaplasia (*arrows*) strongly express ITF. Foveolar cells are negative; *B*, signet ring cell carcinoma demonstrating strong ITF expression in cells (*arrows*) between negatively stained gastric glands; *C*, moderately differentiated adenocarcinoma demonstrating punctate, granular cytoplasmic expression (*arrows*).

Table 1 Summary of clinicopathologic features

Variable	No. of cases (%)
Median age; yr	61 (range:29–85)
Gender	
Male	134 (64)
Female	75 (36)
Tumor location	
Upper third	34 (17)
Middle third	112 (54)
Lower third	63 (30)
Clinical stage	
Ia	116 (56)
Ib	20 (10)
II	25 (12)
IIIa	18 (9)
IIIb	7 (3)
IVa	12 (6)
IVb	11 (5)
Histologic type	
Well differentiated	34 (16)
Moderately differentiated	60 (29)
Papillary	5 (2)
Poorly differentiated	84 (40)
Signet ring cell	23 (11)
Mucinous	3 (1)
Depth of invasion	
pT1	121 (58)
pT2	67 (32)
pT3	16 (8)
pT4	5 (2)
Growth pattern	
Expansive	22 (11)
Intermediate	119 (57)
Infiltrative	68 (33)
Liver metastasis	
Negative	207 (99)
Positive	2 (1)
Peritoneal metastasis	
Negative	198 (95)
Positive	11 (5)
Lymph node metastasis	
Negative	134 (64)
Positive	75 (36)

Table 2 ITF expression in gastric cancer

Variable	No. of ITF-positive cases (%)	*P*
Total positive	114/209 (55)	
1+	61/209 (29)	
2+	37/209 (18)	
3+	16/209 (8)	
Age		NS
≤60	58/102 (57)	
>60	56/107 (52)	
Gender		<0.05
Male	65/134 (49)	
Female	49/75 (65)	
Tumor location		NS
Upper third	18/34 (53)	
Middle third	62/112 (55)	
Lower third	34/63 (54)	
Clinical stage		NS
I, II	84/161 (52)	
III, IV	30/48 (63)	
Histologic type		NS
Differentiated type	50/99 (51)	
Well	19/34 (56)	
Moderate	27/60 (45)	
Papillary	4/5 (80)	
Undifferentiated type	64/110 (58)	
Poor	45/84 (54)	
Signet ring cell	16/23 (70)	
Mucinous	3/3 (100)	
Depth of invasion		NS
pT1	62/121 (51)	
pT2, pT3, pT4	52/88 (59)	
Growth pattern		<0.05
Noninfiltrative	70/141 (50)	
Infiltrative	52/68 (59)	
Peritoneal metastasis		NS
Negative	107/198 (54)	
Positive	7/11 (64)	
Lymph node metastasis		<0.05
Negative	66/134 (49)	
Positive	48/75 (64)	

cell migration and restitution (17, 31, 32). In colon cancer cell lines, ITF mediates tyrosine phosphorylation of β-catenin and the epidermal growth factor receptor, resulting in reduced membranous E-cadherin expression, altered intercellular adhesion, and enhanced cell motility (33, 34). Furthermore, ITF promotes invasion of transformed colonic epithelial cells (35) and can prevent colonic epithelial cells from undergoing apoptosis (36, 37). These observations suggest that ITF may offer the cancer cell a survival advantage while also allowing it to invade and metastasize.

Very little is known about ITF expression in human gastric carcinoma. In a small number of human biopsies and surgical specimens, ITF expression was notable in gastric mucosa that demonstrated intestinal metaplasia and was conserved in gastric carcinoma (38). To our knowledge, no study has systematically compared ITF expression with clinicopathological features or patient outcome in any tumor type, including gastric cancer. Because gastric carcinomas may develop from intestinal metaplasia, and ITF is a good marker of intestinal phenotype, we postulated that ITF might be a useful tumor marker in gastric cancer. In the present study, we analyzed ITF expression immunohistochemically in human gastric carcinomas and correlated its expression with clinicopathological features of these tumors and with patient survival.

MATERIALS AND METHODS

Patients. Approval to conduct this study was obtained from the Mount Sinai Institutional Review Board. We evaluated specimens of surgically resected primary gastric carcinoma from 209 patients at Aichi Cancer Center Hospital between January 1993 and January 1994. This period was selected to provide a sufficient period of follow-up to determine patient prognosis. The median period of follow-up was 4.9 years (range: 0.2–6.4 years). All deaths were attributable to gastric cancer. Tumor-Node-Metastasis classification was applied with regard to depth of tumor invasion (39), and other clinicopathologic data were judged according to the criteria of the Japanese Classification of Gastric Carcinoma (40). For purposes of statistical analysis, stage I and II tumors were combined, as were stage III and IV tumors. Tumors that were histologically well differentiated, moderately differentiated, and papillary were designated "differentiated type," whereas those that were poorly

Table 3 ITF expression by age and gender

Age	No. of ITF-positive cases/total (%)		
	Male	Female	P^a
≤45	6/11 (55)	8/11 (73)	
45–50	1/9 (11)	6/9 (67)	
50–55	7/14 (50)	10/14 (71)	
55–60	12/23 (52)	8/11 (73)	
60–65	6/17 (35)	7/10 (70)	
>65	33/60 (55)	10/20 (50)	
Overall			<0.05

[a]Mantel-Haenszel test.

Table 4 Factors associated with ITF expression

Variable	No. of ITF-positive cases (%)	
	Male	Female
Clinical stage		
I, II	46/107 (43)	38/54 (70)
III, IV	19/27 (70)	11/21 (52)
P	<0.01	NS
Growth pattern		
Noninfiltrative	44/101 (44)	26/40 (65)
Infiltrative	21/33 (64)	23/35 (66)
P	<0.05	NS
Lymph node metastasis		
Negative	39/92 (42)	27/42 (64)
Positive	26/42 (62)	22/33 (67)
P	<0.05	NS

differentiated, signet ring cell, and mucinous were considered "undifferentiated." Growth pattern was considered as two categories: (*a*) infiltrative; and (*b*) noninfiltrative (the latter comprising expansive and intermediate patterns).

Immunohistochemistry. All specimens were routinely fixed in 10% formalin and embedded in paraffin. Specimens were examined after staining with H&E and those through the longest tumor diameters were selected for immunohistochemistry of ITF. Rabbit polyclonal anti-ITF antibody (HM89) was kindly provided by Dr. D. K. Podolsky (Massachusetts General Hospital, Boston, MA). After deparaffinization and dehydration, sections were heated in a microwave oven for 8 min at 100°C in 10 mM citrate buffer (pH 6.0) and incubated sequentially with fresh 3% hydrogen peroxide in PBS, 5% normal goat serum, HM89 antibody (1:200), biotinylated goat antirabbit IgG (1:200), and streptavidin-peroxidase (1:200), with three PBS washes between each step. The sites of peroxidase binding were visualized using diaminobenzidine. Sections were counterstained lightly with hematoxylin.

Assessment of ITF Immunostaining in Carcinomas. The degree of HM89 reactivity in each tissue section was assessed without knowledge of clinicopathological features of the tumor or patient survival. The degree of positivity was initially classified according to the percentage of positive tumor cells as follows: (−) no tumor cells positive, (1+) 1–10% cells positive, (2+) 10–50% cells positive, and (3+) >50% cells positive. For statistical analyses, a tumor was considered positive if it demonstrated any degree of ITF staining.

Statistical Analysis. The χ^2 test was applied for comparison between individual clinicopathologic features and ITF expression. The Mantel-Haenszel test was used for the association between ITF expression and gender, stratified on age. Because this was an exploratory analysis, no correction was made for multiple testing, and all *P*s < 0.20 are shown. Survival was estimated by lifetable analysis and compared by Log-rank tests. The proportional hazards model was used to test for the independent influence of several factors on survival.

RESULTS

ITF Expression in Non-neoplastic Gastric Mucosa. Normal gastric glands without intestinal metaplasia showed no HM89 reactivity except for weak expression in parietal cells. Of 209 specimens, 149 (71%) contained areas of intestinal metaplasia. Goblet cells in all glands exhibiting intestinal metaplasia were strongly ITF positive, and almost all goblet cells in such glands expressed ITF (Fig. 1*A*). ITF was distributed diffusely as coarse granules in the cytoplasm of goblet cells in intestinal metaplasia but not in absorptive cells or Paneth cells. Curiously, acini of esophageal glands and duodenal Brunner's glands also demonstrated HM89 reactivity. ITF expression in gastric mucosa adjacent to malignant ulcers was similar to that seen in normal gastric mucosa.

ITF Expression in Gastric Carcinoma Cells. Two patterns of ITF expression were noted in carcinoma cells. In mucinous cancer cells, ITF expression was noted in intracellular mucus or cytoplasm of signet ring cells (Fig. 1*B*) and in mucous-secreting cells resembling goblet cells. In these mucinous cells, ITF was distributed diffusely as coarse granules similar to normal goblet cells. In nonmucinous cancer cells, ITF was expressed in the cytoplasm sometimes diffusely as fine granules or occasionally in the apical portion of the cytoplasm (Fig. 1*C*).

Clinicopathological Features of Tumors. The study cohort consisted of 134 male and 75 female patients with a median age of 61 years (Table 1). Most tumors were in the mid-region of the stomach, with approximately one-third of cancers located in the antrum. Almost two-thirds of cancers were stage I, and 90% of cancers did not invade beneath the muscularis propria (pT1–2). One-third of cases had lymph node involvement, whereas liver and peritoneal metastases were uncommon.

ITF Expression in Relation to Clinicopathological Features. The relation between ITF expression and clinicopathologic features is summarized in Table 2. ITF was expressed in 114 of 209 (55%) of gastric carcinoma cases. Of the ITF-positive cases, 53 (46%) expressed ITF in ≥10% of cancer cells. No appreciable relationship was demonstrated between ITF expression and either patient age, tumor location, clinical stage, histological type, depth of invasion, or peritoneal metastasis. Of note, however, ITF expression was gender related, with 65% of women compared with 48% of men expressing ITF in their tumors ($P < 0.05$). When data were controlled for age, women demonstrated a higher frequency of ITF-positive tumors than men in all age groups (Table 3; $P < 0.05$). Other variables that were significantly associated with greater ITF expression were infiltrative growth pattern and positive lymph node involvement (Table 2).

Because of the observation that ITF expression was more frequent among women, we decided to reexamine the associa-

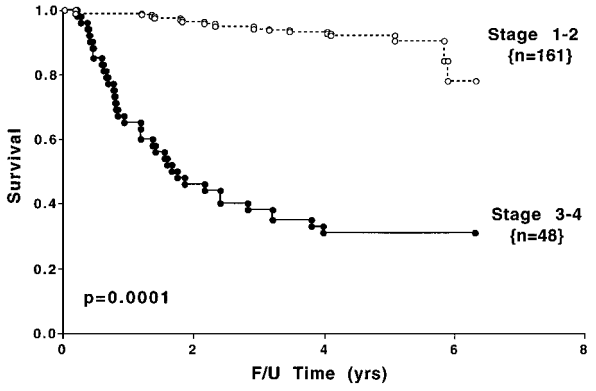

Fig. 2 Effect of tumor stage on survival.

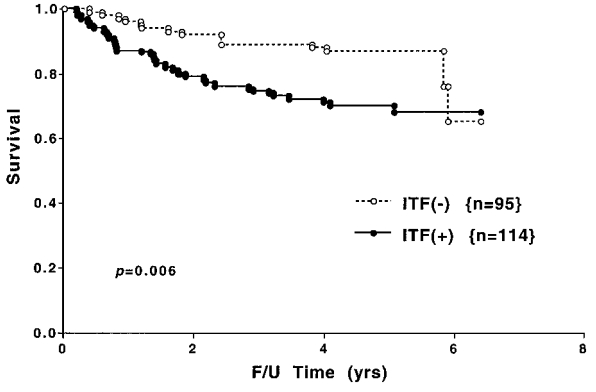

Fig. 3 Effect of ITF status on overall survival.

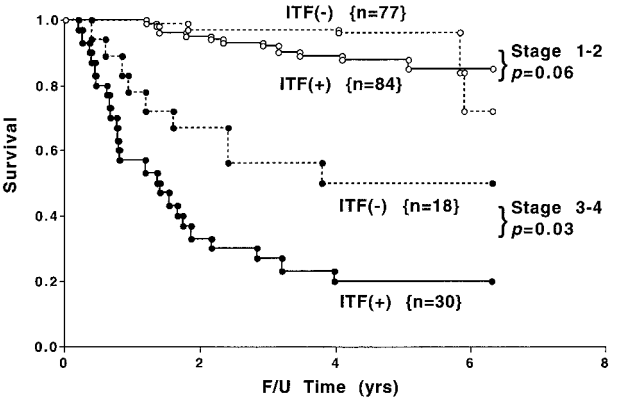

Fig. 4 Effect of ITF status on survival according to early stage (*1–2*) and late stage (*3–4*) disease.

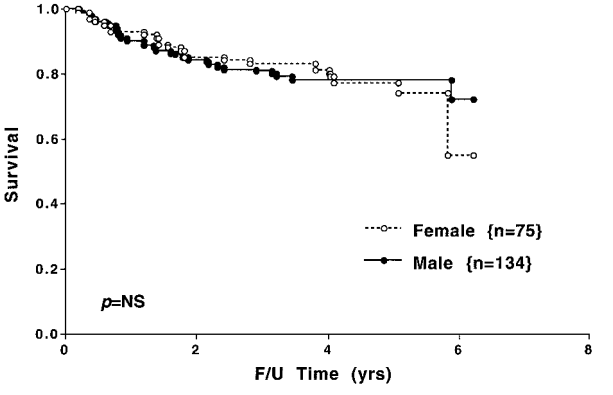

Fig. 5 Effect of gender on survival.

tion of other factors with ITF, stratifying the data by gender (Table 4). This analysis revealed that ITF expression was significantly associated with more advanced tumor stage ($P < 0.01$), infiltrative growth pattern ($P < 0.05$), and positive lymph node metastasis ($P < 0.05$) in men. Among women, however, these same factors were not associated with increased ITF expression.

ITF Expression in Relation to Patient Survival. As expected, patients with later stage tumors had a significantly worse survival than those with early stage disease ($P = 0.0001$; Fig. 2). The overall survival of all patients with ITF-positive tumors regardless of stage was significantly less than for patients with ITF-negative tumors ($P = 0.006$; Fig. 3). This effect of ITF expression on survival was seen both in early stage (stages 1 and 2; $P = 0.06$) and late stage (stages 3 and 4; $P = 0.03$) disease (Fig. 4).

Having observed gender-related differences in ITF expression, survival was also analyzed according to gender. The overall survival curves of men and women were nearly identical, ruling out any influence of gender alone on survival (Fig. 5). Men with ITF-positive tumors had a significantly worse prognosis than those with ITF-negative tumors ($P = 0.002$; Fig. 6). In contrast, the survival of women with ITF-positive tumors was not significantly different from those with ITF-negative tumors (Fig. 7).

Multivariate analysis, using the proportional hazards method and controlling for gender, revealed that tumor stage had the strongest influence on prognosis (risk ratio 12.3; $P < 0.0001$). ITF positivity was also found to be an independent predictor of poor prognosis (risk ratio 2.15; $P < 0.02$).

DISCUSSION

Although most studies of ITF have focused on its role in mucosal restitution and repair, a role for ITF in tumor biology is receiving increasing attention, *e.g.*, expression of ITF is not only observed in normal colonic mucosa, but its expression is preserved in all premalignant adenomatous polyps, primary colonic adenocarcinomas, and metastatic colon carcinomas (29, 30). Other studies have reported ITF expression in breast cancers and mucinous skin cancers (27, 28). It is not known whether other types of epithelial cancers can also express ITF. Furthermore, no study has yet examined large enough numbers of specimens to permit meaningful correlations between ITF expression and clinicopathological features of tumors.

The present study was conducted to investigate the frequency and clinicopathological relationships of ITF in a large collection of well-characterized primary gastric adenocarcinomas. We found that ~55% of gastric carcinomas expressed ITF.

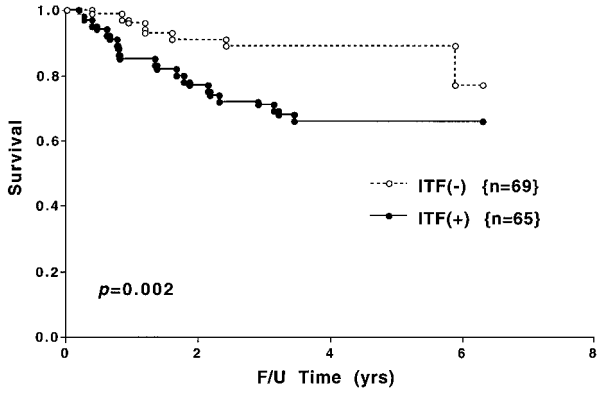

Fig. 6 Effect of ITF status on survival in males.

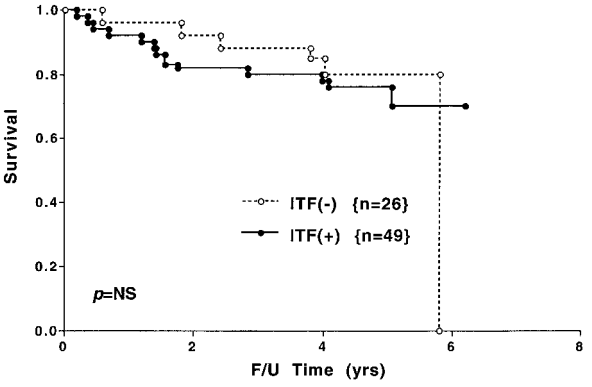

Fig. 7 Effect of ITF status on survival in females.

Importantly, ITF expression in gastric cancer was associated with a poor survival regardless of tumor stage. This is the first study to observe a relationship between ITF expression and survival in any cancer type. One of the curious features of our study was the gender-related difference in survival based on ITF expression. Although women were more likely than men to express ITF in their gastric cancers than men at any given age, it was mainly in men that ITF expression was correlated with a more aggressive tumor phenotype (tumor stage, infiltrative growth pattern, and positive lymph nodes) and a significantly worse survival. Because gastric cancer incidence and mortality is higher in men than women in the United States (41), this could have important clinical implications.

The more frequent expression of ITF among women was a somewhat unexpected observation. It is supported, however, by previous data that indicate that like TFF1, ITF expression is induced by estrogen in breast carcinoma cells (27). Moreover, ITF expression has been associated with expression of estrogen receptor in mucinous skin cancers, a nontarget organ of estrogen (28). Curiously, in gastric cancer, estrogen receptor expression has been associated with a scirrhous, interstitial connective tissue type and infiltrative growth pattern (42). Moreover, global analysis of gene expression using a high-density oligonucleotide array to compare gene expression between a scirrhous gastric cancer cell line and its derivative with high metastatic potential to the peritoneum revealed a 7–8-fold higher expression of ITF (and TFF1) in the metastatic cell line (43). Given the association between ITF expression and infiltrative growth pattern in the present study, it is intriguing to speculate that estrogen receptor and ITF might interact to produce this cancer phenotype. However, the fact that most of the women in our study were in the postmenopausal age group and still expressed ITF in their gastric cancers suggests that other mechanisms besides estrogen are likely to be operative. Additional research will be required to determine whether regulation of ITF occurs through the estrogen receptor and whether this pathway is similar in cancer cells derived from target *versus* nontarget organs. It also remains to be clarified whether ITF expression might be regulated by estrogen receptor expression in men with gastric cancer. The present data suggest an alternative mechanism for ITF regulation in gastric cancer cells.

We also focused on cellular differentiation of gastric carcinoma. The phenotypic expression of tumor cells usually resembles that of the tissue of origin. Accordingly, examination of the phenotypic expression of gastric carcinoma cells should reveal the histogenesis of gastric carcinoma. Human gastric carcinoma may be classified into four cell types: gastric cell type (surface mucous cell type and pyloric gland cell type) or intestinal epithelial cell type (goblet cell type and intestinal absorptive cell types; Refs. 44–47). In addition, human gastric carcinomas have been classified into two histological types. The intestinal and diffuse types of the Lauren classification are considered differentiated and undifferentiated types by Sugano *et al.* (48, 49). As for the histogenesis of gastric cancer, it has generally been concluded that differentiated type (intestinal type) carcinomas arise from areas of intestinal metaplasia, whereas undifferentiated (diffuse type) lesions originate from normal gastric mucosa. However, contrary to this hypothesis, we reported previously a high incidence of differentiated type gastric cancers showing gastric phenotypic markers, with a phenotypic shift from gastric to intestinal type expression in tumors manifesting greater depth of invasion (50, 51). Furthermore, Machado *et al.* (52) used pS2 immunohistochemistry to show that differentiated type carcinomas exhibited focal (43% of cases) or extensive (11%) gastric type differentiation. Moreover, Fiocca *et al.* (53), using pepsinogen II as a marker, reported gastric type differentiation in 55% of differentiated type gastric carcinomas.

Because ITF is strongly expressed by goblet cells in normal small and large intestine, it seemed like a useful marker to shed more light on the issue of gastric cancer histogenesis. Our observation of strong ITF protein expression in goblet cells of intestinal metaplasia of the stomach confirms previous reports of ITF mRNA expression in intestinal metaplasia (9) and supports the notion that this trefoil peptide is correlated with intestinal type differentiation. However, ITF expression in our study was not restricted to the mucinous or even to the undifferentiated phenotype. The present study would therefore support the conclusion that both differentiated (intestinal type) and undifferentiated type (diffuse type) carcinomas arise mainly from the normal gastric mucosa and that the change from gastric to intestinal phenotype with time occurs independently of and separately from the corresponding metaplastic shift in the background mucosa (50, 51).

In conclusion, ITF expression occurs in over half of human gastric cancers and is associated with a poor outcome. Although ITF expression was found more commonly in gastric cancers from women than from men, in men, ITF expression was significantly correlated with more aggressive tumors and poor survival. Although it remains to be determined whether ITF will have an effect on survival in other cancer types, future studies of this marker should consider possible gender-related differences.

REFERENCES

1. Thim, L. A new family of growth factor-like peptides: "trefoil" disulphide loop structures as a common feature in breast cancer associated peptide (pS2), pancreatic spasmolytic polypeptide (PSP), and frog skin peptides (spasmolysins). FEBS Lett., *250:* 85–90, 1989.

2. Seib, T., Blin, N., Hilgert, K., Seifert, M., Theisinger, B., Engel, M., Dooley, S., Zang, K. D., and Welter, C. The three human trefoil genes *TFF1, TFF2,* and *TFF3* are located within a region of 55 kb on chromosome 21q22.3. Genomics, *40:* 200–202, 1997.

3. Williams, G. R., and Wright, N. A. Trefoil family domain peptides. Virchows Arch., *431:* 299–304, 1997.

4. Rio, M-C., Bellocq, J. P., Daniel, Y. C., Tomasetto, C., Lathe, R., Chenard, M. P., Batzenschlager, A., and Chambon, P. Breast cancer-associated pS2 protein: synthesis and secretion by normal stomach mucosa. Science (Wash. DC), *241:* 705–708, 1988.

5. Jeffrey, G. P., Oates, P. S., Wang, T. C., Babyatsky, M. W., and Brand, S. J. Spasmolytic polypeptide: a trefoil peptide secreted by gastric mucous cells. Gastroenterology, *106:* 336–345, 1994.

6. Modlin, I. M., and Poulsom, R. Trefoil peptides: mitogens, motogens, or mirages? J. Clin. Gastroenterol., *25* (Suppl.)*:* S94–S100, 1997.

7. Wright, N. A., Poulsom, R., Stamp, G., Van Noorden, S., Sarraf, C., Elia, G., Ahnen, D., Jeffry, R., Longcroft, J., Pike, C., Rio, M-C., and Chambon, P. Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease. Gastroenterology, *104:* 12–20, 1993.

8. Podolsky, D. K., Lynch-Devaney, K., Stow, J. L., Oates, P., Murgue, B., DeBeaumont, M., Sands, B. E., and Mahida, Y. R. Identification of human intestinal trefoil factor: goblet cell-specific expression of a peptide targeted for apical secretion. J. Biol. Chem., *268:* 6694–6702, 1993.

9. Hauser, F., Poulsom, R., Chinery, R., Rogers, L. A., Hanby, A. M., Wright, N. A., and Hoffmann, W. hP1. B, a human P-domain peptide homologous with rat intestinal trefoil factor, is expressed also in the ulcer-associated cell lineage and the uterus. Proc. Natl. Acad. Sci. USA, *90:* 6961–6965, 1993.

10. Poulsom, R., Hanby, A. M., Lalani, E-N., Hauser, F., Hoffmann, W., and Stamp, G. W. H. Intestinal trefoil factor (TFF3) and pS2 but not spasmolytic polypeptide are co-expressed in normal, hyperplastic, and neoplastic human breast epithelium. J. Pathol., *183:* 30–38, 1997.

11. Jagla, W., Wiede, A., Dietzmann, K., Rutkowski, K., and Hoffmann, W. Co-localization of TFF3 peptide and oxytocin in the human hypothalamus. FASEB J., *14:* 1126–1131, 2000.

12. Devine, D. A., High, A. S., Owen, P. J., Poulsom, R., and Bonass, W. A. Trefoil factor expression in normal and diseased human salivary glands. Hum. Pathol., *31:* 509–515, 2000.

13. Dos Santos Silva, E., Ulrich, M., Doring, G., Botzenhart, K., and Gott, P. Trefoil factor family domain peptides in the human respiratory tract. J. Pathol., *190:* 133–142, 2000.

14. Wright, N. A., Poulsom, R., Stamp, G. W., Hall, P. A., Jeffery, R. E., Longcroft, J. M., Rio, M. C., Tomasetto, C., and Chambon, P. Epidermal growth factor (EGF/URO) induces expression of regulatory peptides in damaged human gastrointestinal tissues. J. Pathol., *162:* 279–284, 1990.

15. Kindon, H., Pothoulakis, C., Thim, L., Lynch-Devaney, K., and Podolsky, D. K. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. Gastroenterology, *109:* 516–523, 1995.

16. Babyatsky, M. W., Debeaumont, M., Thim, L., and Podolsky, D. K. Oral trefoil peptides protect against ethanol- and indomethacin-induced gastric injury in rats. Gastroenterology, *110:* 489–497, 1996.

17. Dignass, A., Lynch-Devaney, K., Kindon, H., Thim, L., and Podolsky, D. Trefoil peptides promote epithelial migration through a transforming growth factor β-independent pathway. J. Clin. Investig., *94:* 376–383, 1994.

18. Alison, M. R., Chinery, R., Poulsom, R., Ashwood, P., Longcroft, J. M., and Wright, N. A. Experimental ulceration leads to sequential expression of spasmolytic polypeptide, intestinal trefoil factor, epidermal growth factor, and transforming growth factor α mRNAs in rat stomach. J. Pathol., *175:* 405–414, 1995.

19. Mashimo, H., Wu, D. C., Podolsky, D. K., and Fishman, M. C. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science (Wash. DC), *274:* 262–265, 1996.

20. Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A., and Chambon, P. Cloning of cDNA sequences of hormone-regulated genes from the MCF-7 human breast cancer cell lines. Nucleic Acids Res., *10:* 7895–7903, 1982.

21. Henry, J. A., Bennett, M. K., Piggott, N. H., Levett, D. L., May, F. E., and Westley, B. R. Expression of the pNR-2/pS2 protein in diverse human epithelial tumours. Br. J. Cancer, *64:* 677–682, 1991.

22. Theisinger, B., Welter, C., Seitz, G., Rio, MC., Lathe, R., Chambon, P., and Blin, N. Expression of the breast cancer associated gene *pS2* and the pancreatic spasmolytic polypeptide gene (*hSP*) in diffuse type of stomach carcinoma. Eur. J. Cancer, *27:* 770–773, 1991.

23. Lefebvre, O., Chenard, M. P., Masson, R., Linares, J., Dierich, A., LeMeur. M., Wendling, C., Tomasetto, C., Chambon, P., and Rio, M. C. Gastric mucosa abnormalities and tumorigenesis in mice lacking the pS2 trefoil protein. Science, *274:* 259–262, 1996.

24. Park, W. S., Oh, R. R., Park, J. Y., Yoo, N. J., Lee, S. H., Shin, M. S., Kim, S. Y., Kim, Y. S., Lee, J. H., Kim, H. S., An, W. G., and Lee, J. Y. Mapping of a new target region of allelic loss at 21q22 in primary gastric cancers. Cancer Lett., *159:* 15–21, 2000.

25. Seitz, G., Theisinger, B., Tomasetto, G., Rio, M. C., Chambon. P., Blin, N., and Welter, C. Breast cancer-associated protein pS2 expression in tumor of biliary tract. Am. J. Gastroenterol., *86:* 1491–1494, 1991.

26. Welter, C., Theisinger, B., Seitz, G., Tomasetto, G., Rio, M. C., Chambon, P., and Blin, N. Association of the human spasmolytic polypeptide and an estrogen-induced breast cancer protein (pS2) with human pancreatic carcinoma. Lab. Investig., *66:* 187–192, 1992.

27. May, F. E. B., and Westley, B. R. Expression of human intestinal trefoil factor in malignant cells and its regulation by oestrogen in breast cancer cells. J. Pathol., *182:* 404–413, 1997.

28. Hanby, A. M., Mckee, P., Jeffery, M., Grayson, W., Dublin, E., Poulsom, R., and Maguire, B. Primary mucinous carcinomas of skin express TFF1, TFF3, estrogen receptor, and progesterone receptors. Am. J. Surg. Pathol., *22:* 1125–1131, 1998.

29. Taupin, D., Ooi, D., Yeomans, N., and Giraud, A. Conserved expression of intestinal trefoil factor in the human colonic adenoma-carcinoma sequence. Lab. Investig., *75:* 25–32, 1996.

30. Uchino, H., Kataoka, H., Itoh, H., and Koono, M. Expression of intestinal trefoil factor mRNA is downregulated during progression of colorectal carcinomas. J. Clin. Pathol., *50:* 932–934, 1997.

31. Poulsom, R., Begos, D. E., and Modlin, I. M. Molecular aspects of restitution: functions of trefoil peptides. Yale J. Biol. Med., *69:* 137–146, 1996.

32. Wright, N. A., Hoffmann, W., Ottto, W. R., Rio, M. C., and Thim, L. Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. FEBS Lett., *408:* 121–123, 1997.

33. Liu, D., Chinery, R., Wilding, J., Gullick, W. J., Kmiot, W., and Pignatelli, M. Phosphorylation of β-catenin and epidermal growth-factor receptor by intestinal trefoil factor. Lab. Investig., *77:* 557–563, 1997.

34. Efstathiou, J. A., Noda, M., Rowan, A., Dixon, C., Chinery, R., Jawhari, A., Hattori, T., Wright, N. A., Bodmer, W. F., and Pignatelli, M. Intestinal trefoil factor controls the expression of the adenomatous

polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. Proc. Natl. Acad. Sci. USA, *95:* 3122–3127, 1998.

35. Emami, S., LeFloch, N., Bruynell, E., Thim, L., May, F., Westley, B., Rio, M. C., Mareel, M., and Gespach, C. Induction of scattering and cellular invasion by trefoil peptides in src- and RhoA-transformed kidney and colonic epithelial cells. FASEB J., *15:* 351–361, 2001.

36. Taupin, D. R., Kinoshita, K., and Podolsky, D. K. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. Proc. Natl. Acad. Sci., USA, *97:* 799–804, 2000.

37. Chen, Y. H., Lu, Y., DePlaen, I. G., Wang, L. Y., and Tan, X. D. Transcription factor NF-kB signals antianoikic function of trefoil factor 3 on intestinal epithelial cells. Biochem. Biophys. Res. Commun., *274:* 576–582, 2000.

38. Taupin, D., Pedersen, J., Familari, M., Cook, G., Yeomans, N., and Giraud, A. S. Augmented intestinal trefoil factor (TFF3) and loss of pS2 (TFF1) expression precedes metaplastic differentiation of gastric epithelium. Lab. Investig., *81:* 397–408, 2001.

39. Hermanek, P., and Sobin, L. H. (eds.). UICC TNM Classification of Malignant Tumours, Ed. 4. 2nd revision. Berlin: Springer-Verlag, 1992.

40. Japanese Research Society for Gastric Cancer. Japanese Classification of Gastric Carcinoma, 1st English ed. Tokyo: Kanehara, 1995.

41. Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer Statistics, 2001. CA Cancer J. Clin., *51:* 15–36, 2001.

42. Kojima, O., Takahashi, T., Kawakami, S., Uehara, Y., and Matsui, M. Localization of estrogen receptors in gastric cancer using immunohistochemical staining of monoclonal antibody. Cancer (Phila.), *67:* 2401–2406, 1991.

43. Hippo, Y., Yashiro, M., Ishii, M., Taniguchi, H., Tsutsumi, S., Hirakawa, K., Kodama, T., and Aburatami, H. Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res., *61:* 889–895, 2001.

44. Fiocca, R., Villani, L., Tenti, P., Solcia, E., Cornaggia, M., Frigerio, B., and Capella, C. Characterization of four main cell types in gastric cancers: foveolar, mucopeptic, intestinal columnar and goblet cells. An histopathologic, histochemical and ultrastructural study of "early" and "advanced" tumors. Pathol. Res. Pract., *182:* 308–326, 1987.

45. Fujimori, Y., Akamatsu, T., Ota, H., and Katsuyama, T. Proliferative markers in gastric carcinoma and organoid differentiation. Hum. Pathol., *26:* 725–733, 1995.

46. Tatematsu, M., Ichinose, M., Miki, K., Hasegawa, R., Kato, R., and Ito, N. Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pepsinogen immunohistochemistry and mucin histochemistry. Acta Pathol. Jpn., *40:* 494–504, 1990.

47. Tatematsu, M., Hasegawa, R., Ogawa, K., Kato, T., Ichinose, M., Miki, K., and Ito, N. Histogenesis of human stomach cancers based on assessment of differentiation. J. Clin. Gastroenterol., *14* (Suppl.)*:* 1–7, 1992.

48. Lauren, P. The two histological main types of gastric carcinomas: diffuse and so called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand., *64:* 31–49, 1965.

49. Sugano, H., Nakamura, K., and Kato, Y. Pathological studies of human gastric cancer. Acta Pathol. Jpn., *32* (Suppl.)*:* 329–347, 1982.

50. Yoshikawa, A., Inada, K., Yamachika, T., Shimizu, N., Kaminishi, M., and Tatematsu, M. Phenotypic shift in human differentiated gastric cancers from gastric to intestinal epithelial cell type during disease progression. Gastric Cancer, *1:* 134–141, 1998.

51. Yamachika, T., Inada, K., Fujimitsu, Y., Nakamura, S., Yamamura, Y., Kitou, T., Itzkowitz, S. H., Werther, J. L., Miki, K., and Tatematsu, M. Intestinalization of gastric signet ring cell carcinomas with progression. Virchows Arch., *431:* 103–110, 1997.

52. Machado, J. C., Carneiro, F., Ribeiro, P., Blin, N., and Sobrinho-Simões, M. pS2 protein expression in gastric carcinoma. An immunohistochemical and immunoradiometric study. Eur. J. Cancer, *32A:* 1585–1590, 1996.

53. Fiocca, R., Carnaggia, M., Villani, L., Capella, C., Solcia, E., and Samloff, I. M. Expression of pepsinogen II in gastric cancer. Its relationship to local invasion and lymph node metastases. Cancer, *61:* 956–962, 1988.