# RUNX3 expression correlates
# with chief cell differentiation in human gastric cancers

**Naotaka Ogasawara[1,2], Tetsuya Tsukamoto[1], Tsutomu Mizoshita[1,2],**
**Ken-ichi Inada[1,3], Hisayo Ban[1], Shinya Kondo[1], Shinji Takasu[1], Toshikazu Ushijima[4],**
**Kosei Ito[5,6], Yoshiaki Ito[5,6], Masao Ichinose[7], Takafumi Ogawa[8], Takashi Joh[2] and Masae Tatematsu[1,9]**

[1]Division of Oncological Pathology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya, Japan [2]Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Science, Mizuho-cho, Mizuho-ku, Nagoya, Japan, [3]Department of Pathology, Fujita Health University School of Medicine, Toyoake, Aichi, Japan, [4]Carcinogenesis Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan [5]Institute of Molecular and Cell Biology, Proteos, [6]Oncology Research Institute, National University of Singapore, Singapore, [7]Second Department of Internal Medicine, Wakayama Medical University, Kimiidera Wakayama-city, Japan, [8]Management Division, Institute of Pathology, Kyodo Byori, Otsuwa, Nishi-ku, Japan and [9]Division of Cancer Genetics, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Japan.

**Summary.** RUNX3 is a novel tumor suppressor in gastric carcinogenesis and an important factor for differentiation of chief cells in the normal gastric fundic mucosa. In this study, we confirmed RUNX3 immunolocalization in the fundic gland (bottom part) but minimum in surface mucous cell epithelium (top part) in the isolated gland from fundic mucosa. We also analyzed RUNX3 expression by immunohistochemistry in 102 gastric cancers and made a histological assessment of the expression of differentiation markers to evaluate interrelations. Among them, 45 and 57 cases were judged to be RUNX3 positive and negative, respectively, and 33 and 69 cases were pepsinogen I positive and negative, with no link to histological types. RUNX3 expression was significantly associated with that of pepsinogen I ($P<0.001$), but not mucins, including MUC5AC and MUC6, or the parietal or intestinal phenotypes. In conclusion, the present study showed, for the first time to our knowledge, a relation between RUNX3 and pepsinogen I expression in human gastric cancers. RUNX3 is strongly associated with chief cell phenotypic expression in human gastric cancers, as well as in normal gastric mucosa, and could be considered to play an important role in maintaining the chief cell phenotype.

**Key words:** Gastric cancer, RUNX3, Pepsinogen I, Chief cell differentiation

## Introduction

A lack of RUNX3 (runt-related family of transcription factor 3) functions is causally related to the genesis and progression of human gastric cancer, indicating roles as a novel tumor suppressor (Li et al., 2002). RUNX3 shows remarkable down regulation in gastric cancers compared to surrounding mucosa, correlating with cancer stage progression. Furthermore, RUNX3 expression is reduced in intestinal metaplasia, commonly considered as a precancerous state, as compared with normal mucosa (Li et al., 2002). In addition, the gastric mucosa of Runx3-null mice is hyperplastic with suppression of transforming growth factor ß (TGF-ß)-mediated apoptosis. Analyses with a primary culture system for gastric epithelial cells also demonstrated Runx3(-/-) gastric epithelial cells to have low sensitivity to the growth-inhibiting and apoptosis-inducing activities of TGF-ß, so that RUNX3 is generally considered a major growth regulator of gastric epithelial cells (Li et al., 2002; Fukamachi et al., 2004).

Histochemical and immunohistochemical analyses have been developed for identification of cellular mucin in both gastric and intestinal epithelium. Recently, mucin-type molecules have been revealed to consist of a core protein moiety (apomucin), where a number of carbohydrate chains are attached to serines and threonines by glycosidic bonds. In the gastrointestinal tract, the MUC5AC and MUC6 genes are mainly expressed in gastric foveolar mucosa and pyloric glands, respectively. Pepsinogen I and the proton pump are expressed in the chief cells (Huang et al., 1988; Takahashi, 1992) and the parietal cells (Takubo et al.,

2002) of gastric fundic glands, respectively. The MUC2 gene encodes a typical secretory gel-forming mucin, which represents the predominant form in human intestinal and colon tissues (Seregni et al., 1997). Cells of intestinal absorptive cell type demonstrate sucrase and intestinal-type alkaline phosphatase activity (Tatematsu et al., 1990). Several reports have indicated that it is possible to analyze the phenotypic expression of each gastric cancer cell using gastric and intestinal epithelial cell markers (Tatematsu et al., 1986, 1990, 1992; Koseki et al., 2000; Tajima et al., 2001; Kawachi et al., 2003) and various authors have demonstrated correlations between prognosis and such phenotypic markers in gastric cancers (Tsuchiya et al., 1995; Utsunomiya et al., 1998; Lee et al., 2001; Tajima et al., 2001; Baldus et al., 2002). However, there has been no report regarding the relationship between RUNX3 and mucin expression in gastric cancers. Also, information on the characteristics of gastric fundic gland cell types in gastric cancers is limited (Muller-Hocker and Rellecke, 2003). In the present study, we therefore analyzed RUNX3 expression in 102 gastric cancers, together with histological evaluation and assessment of mucin expression by immunohistochemistry. The main purpose was to evaluate the relation between RUNX3 expression and gastric differentiation, especially of chief cells.

**Materials and methods**

*Samples and tissue collection*

We examined 102 primary gastric cancers surgically resected at Aichi Cancer Center Hospital between 1992 and 2003. The patients were 60 men with an average age of 62.8±9.80 (SD) years (range, 49-79 years) and 42 women aged 61.3±11.1 years (range, 23-84 years). All specimens were fixed in 10% buffered formalin and embedded in paraffin, and then stained with hematoxylin-eosin (H&E). Histopathological classification was made into differentiated (papillary and well and moderately differentiated tubular adenocarcinomas) and undifferentiated (poorly differentiated adenocarcinomas and signet-ring cell carcinomas) types according to the Japanese Classification of Gastric Carcinomas (Japanese Gastric Cancer Association, 1998).

*Gland isolation*

Normal portions of resected epithelium were injected with 30 mM ethylene diamine tetraacetic acid in Hanks' balanced salt solution (EDTA-HBSS) submucosally and incubated at 37°C for 15 min in EDTA-HBSS solution in a water bath. Then the mucosa was scraped off with a scalpel. Isolated glands were washed in PBS, fixed in 70% ethanol for a few hours, dehydrated with 95% ethanol, and stored at -20°C until use (Tsukamoto et al., 2001, 2004). Groups of isolated classified fundic glands were transferred to 0.5-ml microfuge tubes under an inverted microscope (Axiovert 200, Carl Zeiss, Jena, Germany), ethanol fixed, and subjected to immunohistochemstry for pepsinogen I and RUNX3, as well as HE staining, as documented previously (Tsukamoto et al., 2001, 2004).

*Immunohistochemistry*

Immunohistochemical staining was carried out with monoclonal antibodies against the following antigens: Cdx2 (CDX2-88; 1:50, BioGenex, CA, USA); MUC5AC (CLH2; 1:500, Novocastra Laboratories, Newcastle upon Tyne, UK); MUC6 (CLH5; 1:500, Novocastra Laboratories); pepsinogen I (mouse monoclonal; 1:30,000) (Huang et al., 1988); the proton pump (H+, K+ ATPase) α subunit (2B6; 1:500, Medical & Biological Laboratories, Japan); RUNX3 (6E9; 1:3,000) (Ito et al., 2005); MUC2 (Ccp58; 1:500, Novocastra Laboratories); and villin (12; 1:20,000, Transduction Laboratories, Lexington, KY, USA). With regard to gastric and intestinal phenotypic markers, we used normal gastric mucosa and normal ileum as positive and negative controls. The precise procedures for immunohistochemical techniques were as previously described (Tsukamoto et al., 2005, 2006). Briefly, 5 μm-thick consecutive sections were deparaffinized and hydrated through a graded series of ethanol. After inhibition of endogenous peroxidase activity by immersion in 3% $H_2O_2$/methanol solution, antigen retrieval was conducted for detection of binding of the above-mentioned antibodies with 10 mM citrate buffer (pH 6.0) in a microwave oven for 10 minutes at 98°C, or a pressure cooker for 10 minutes at 121°C. Sections were incubated with primary antibodies, thoroughly washed in phosphate-buffered saline (PBS), then incubated with biotinylated secondary antibody, followed by the avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, CA, USA). Finally, immune complexes were visualized by incubation with 0.01% $H_2O_2$ and 0.05% 3,3'-diaminobenzidine tetrachloride (DAB). Nuclear counterstaining was accomplished with Mayer's hematoxylin. The results for each antibody staining were evaluated in terms of the percentage of positively stained cancer cells, with 10% and above considered positive, as previously described (Kawachi et al., 2003; Mizoshita et al., 2003).

*Immunohistological markers for gastric cancer cells*

MUC5AC and MUC6 are markers of gastric epithelial and pyloric gland cells respectively, whereas MUC2 and villin are typical of the intestinal epithelial cell phenotype (Tatematsu et al., 2003). Cdx2 is a homeobox gene related to intestinalization of gastric mucosa. Pepsinogen I and the proton pump α subunit are markers of chief cells and parietal cells, respectively, in

the gastric fundic glands.

## Dual immunofluorescent staining

The procedure for double immunofluorescent staining was as previously documented (Suzuki et al., 2005). Briefly, sections were antigen-retrieved, incubated with anti-Pepsinogen I primary antibody (dilution 1:5000) overnight at 4°C, incubated with biotinylated secondary antibody, and then fluorescein isothiocyanate (FITC)-labled streptavidin (DAKO). To inactivate the antigenicity of the primary antibody, sections were heated in 10 mM citrate buffer (pH 6.0) in a microwave oven for 15 minutes at 90°C. After that sections were incubated with anti-RUNX3 primary antibody (dilution 1:500) for 1 hour at 37°C and with secondary antibody labeled with rhodamine (CHEMICON). Nuclei were counter-stained with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes).

## Statistical analysis

The data were analyzed by the Fischer's exact test. P-values <0.05 were considered as statistically significant.

## RUNX3 and pepsinogen I mRNA expressions in gastric cancer cell lines

Three human gastric carcinoma cell lines (MKN45, MKN74, and THK1) were cultured in RPMI1640 supplemented with 10% fetal bovine serum (FBS). Total RNA was isolated and cDNA was synthesized using the Thermoscript RT-PCR System (Invitrogen, Carlsbad, CA, USA), which were subjected for 35 cycles of PCR analysis of RUNX3 and pepsinogen I mRNA expression using primers listed in Table 1 using acid ribosomal phosphoprotein PO (ARP) as an internal control.

## Results

### RUNX3 and pepsinogen I expression in nomal fundic glands

RUNX3 staining was detected mainly in the chief cells of fundic glands (Fig. 1). Staining was also detected in monocytes and lymphocytes in the stromal tissue. Pepsinogen I cytoplasmic staining was detected only in the chief cells of fundic glands (Fig. 1E), similar to RUNX3 cytoplasmic expression (Fig. 1D). Immunofluorescent dual staining revealed that pepsinogen I and RUNX3 merged mostly in the same cells in fundic gland (Fig. 2A-C). To further confirm the immunolocalization of pepsinogen I and RUNX3 proteins, isolated glands from fundic mucosa were used for their evaluation; these differentiation markers are limited to the basal region of the fundic gland but demonstrate mimimal expression in the upper part (Fig. 3).

### Immunohistochemical analysis of RUNX3 in gastric cancers

Of the 102 gastric cancers, 45 and 57 cases were judged as RUNX3 positive and negative (Fig. 4), this feature appearing independently of the histological type (Table 2).

### Correlation between RUNX3 and pepsinogen I expression in gastric cancers

Of the 102 gastric cancers, 33 and 69 cases were judged to be pepsinogen I positive and negative, respectively (Table 3). In the pepsinogen I-positive gastric cancers, the areas of pepsinogen I and RUNX3 positive staining were strongly correlated (Fig. 4), being consistent with immunofluorescent staining at least in part (Fig. 2D-F). The rates for RUNX3 positive cases in the pepsinogen I-positive and negative cancers were 79% and 26%, respectively, with a significant positive correlation (Table 3, P<0.001).

### No relationship between RUNX3 expression and MUC5AC or MUC6

51 and 51 cases were judged to be MUC5AC positive and negative (Table 3). The rates for RUNX3 positive cases in the MUC5AC-positive and negative cancers were 41% and 47%, respectively, the difference not being significant. Also, 19 and 83 cases were judged to be MUC6 positive and negative (Table 3). The rates for RUNX3 positive cases in the MUC6-positive and

**Table 1.** Primer sequences for analysis of mRNA levels of Pepsinogen I and RUNX3 in human gastric cancer cell lines.

| Genes | Directions | Sequences | Gene Bank accession numbers |
|---|---|---|---|
| Pepsinogen I | Upper | CAACCACAACCGCTTCAACCCTGAGGA | BC29055 |
| | Lower | GCCGGTGCCGTAGGTGATGGAGACTGT | |
| RUNX3 | Upper | GCCAACCCGTCCCCCTACCACCTCTACT | NM_004350 |
| | Lower | GCTGCCGGCCACCATGGAGAACT | |
| ARP | Upper | CGAAGCCACGCTGCTGAACATGCTCAAC | M7885 |
| | Lower | GCTGCCATTGTCGAACACCTGCTGGATG | |
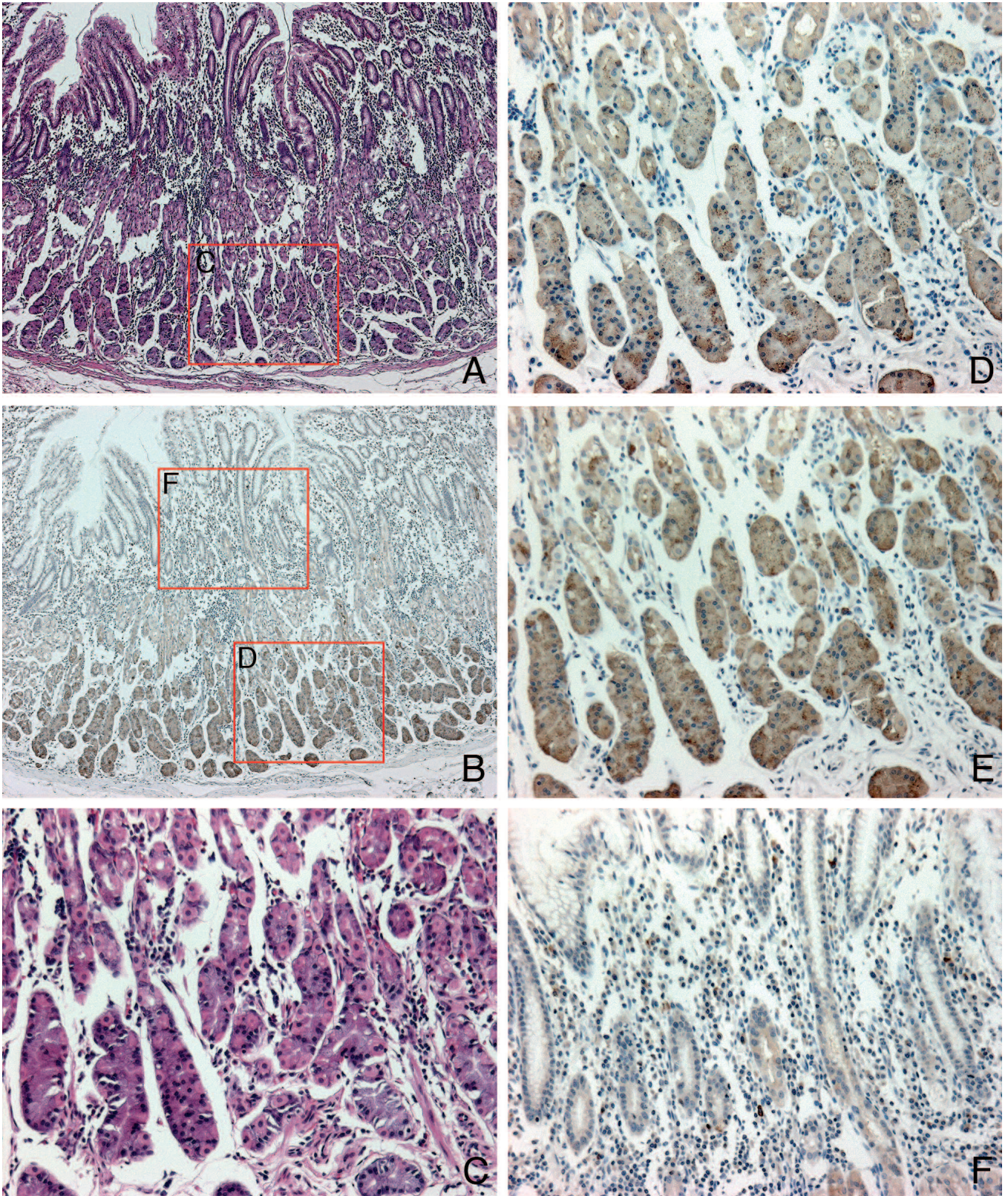
**Fig. 1.** Immunohistochemistry for RUNX3 and pepsinogen I in the fundic gland area of normal gastric mucosa. RUNX3 expression is apparent in the pepsinogen I-expressing chief cells in fundic glands. **A,** H&E staining; **B,** RUNX3 staining, lower magnification; **C,** higher magnification of A, showing basophilic chief cells and acidophilic parietal cells in fundic glands; **D,** RUNX3 staining, higher magnification of B, RUNX3 is positive in the chief cells, but not in the parietal cells of normal gastric mucosa; **E**, Pepsinogen I staining, higher magnification of the area in D, Pepsinogen I is expressed in the fundic chief cells expressing RUNX3; **F**, RUNX3 is weakly stained in the cytoplasm of normal gastric foveolar epithelium, as well as monocytes and lymphocytes in the stromal tissue of the normal gastric mucosa. Original magnification; A and B, x 50; C-E, x 200; F, x 100

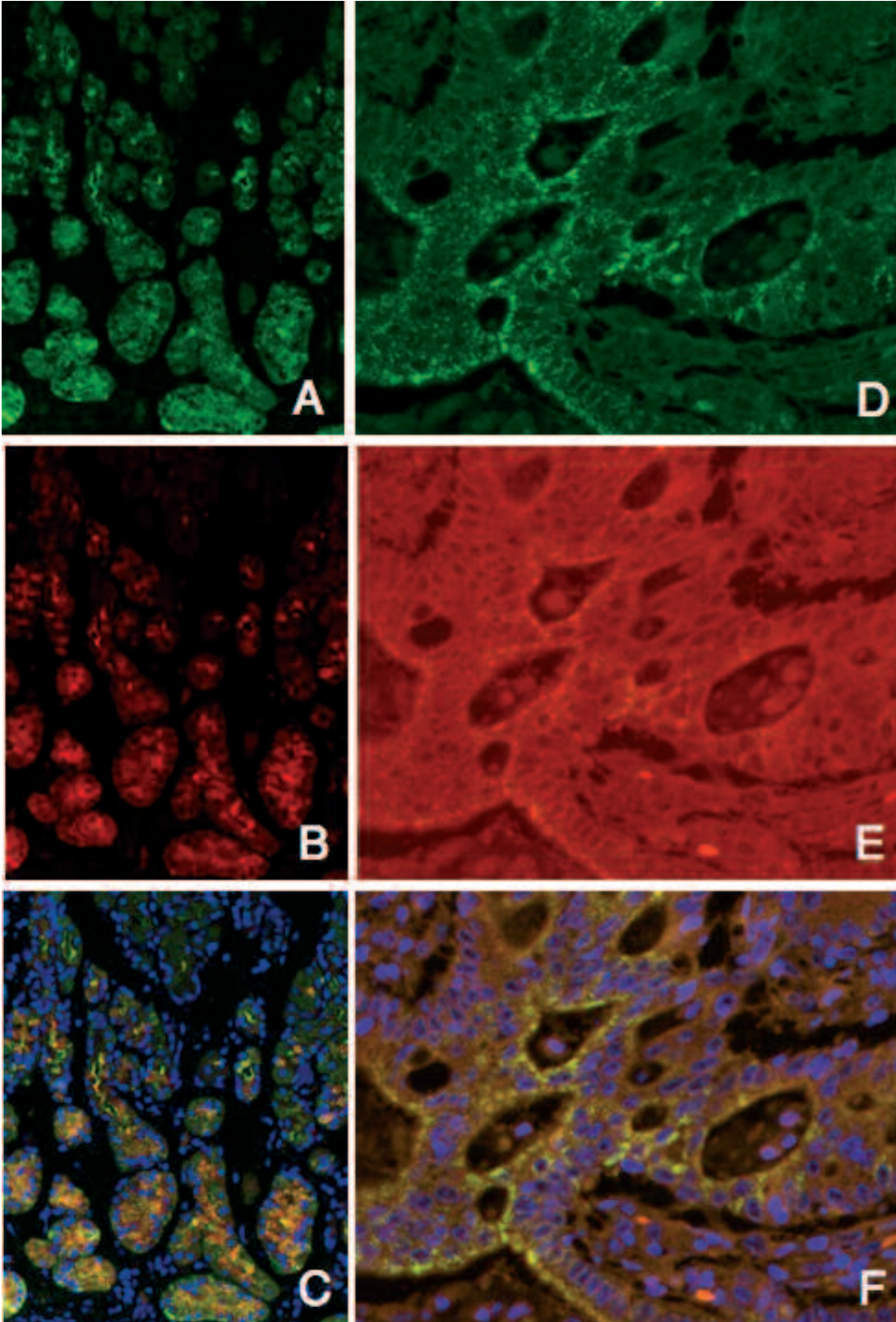**Fig. 2.** Coexpression of pepsinogen I and RUNX3 in normal fundic mucosa and gastric cancers. **A-C**. Bottom part of normal fundic mucosa. **D-F**. Gastric adenocarcinoma. Expression of pepsinogen I (**A and D**, FITC) and RUNX3 (**B and E**, rhodamine). Merged figures are shown with nuclear counter staining (**C and F**, DAPI). Original magnification: A-C, x 200; D-F, x 400

negative cancers were 37% and 46%, respectively, the difference again not being significant.

### No relationship between RUNX3 expression and intestinal phenotypic expression

62 and 40 cases were judged to be MUC2, villin, and/or Cdx2 positive and negative (Table 3). The rates for RUNX3 positive cases were 42% and 48%,

**Table 2.** The relation between RUNX3 expression and the morphological classification.

| | RUNX3* | | Total |
|---|---|---|---|
| | + | − | |
| Differentiated adenocarcinomas | 24 (45%) | 29 (55%) | 53 |
| Undifferentiated adenocarcinomas | 21 (43%) | 28 (57%) | 49 |
| Total | 45 | 57 | 102 |

*P=0.844

**Table 3.** The relation between RUNX3 expression and the phenotypic markers.

| Antigens | Target cells | | RUNX3 | | Total | P values |
|---|---|---|---|---|---|---|
| | | | + | − | | |
| MUC5AC | Gastric foveolar (Surface mucous) cell | + | 21 (41%) | 30 (59%) | 51 (100%) | P=0.69 |
| | | − | 24 (47%) | 27 (53%) | 51 (100%) | |
| MUC6 | Pyloric gland and mucous neck cell | + | 7 (37%) | 12 (67%) | 19 (100%) | P=0.61 |
| | | − | 38 (46%) | 45 (54%) | 83 (100%) | |
| Pepsinogen I | Chief cell | + | 26 (79%) | 7 (21%) | 33 (100%) | P<0.001 |
| | | − | 20 (26%) | 49 (74%) | 69 (100%) | |
| Proton pump | Parietal cell | + | 0 (0%) | 0 (0%) | 0 (100%) | P>0.99 |
| | | − | 45 (44%) | 57 (56%) | 102 (100%) | |
| MUC2, villin, and/or Cdx2 | Intestinal metaplastic cell | + | 26 (42%) | 36 (58%) | 62 (100%) | P=0.68 |
| | | − | 19 (48%) | 21 (52%) | 40 (100%) | |
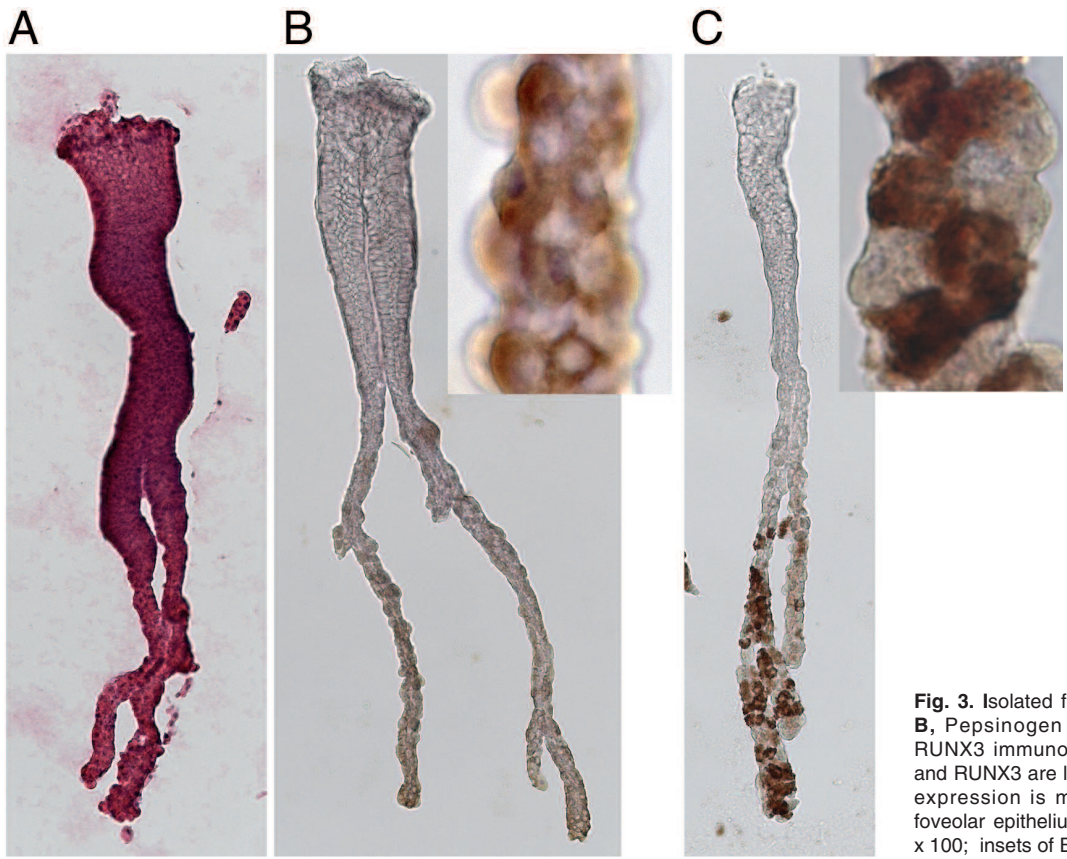


**Fig. 3.** Isolated fundic glands. **A,** H&E staining; **B,** Pepsinogen I immunohistochemistry; **C,** RUNX3 immunohistochemistry. Pepsinogen I and RUNX3 are localized in the basal region but expression is minimal in the upper parts of foveolar epithelium. Original magnification: A-C, x 100; insets of B and C, x 640
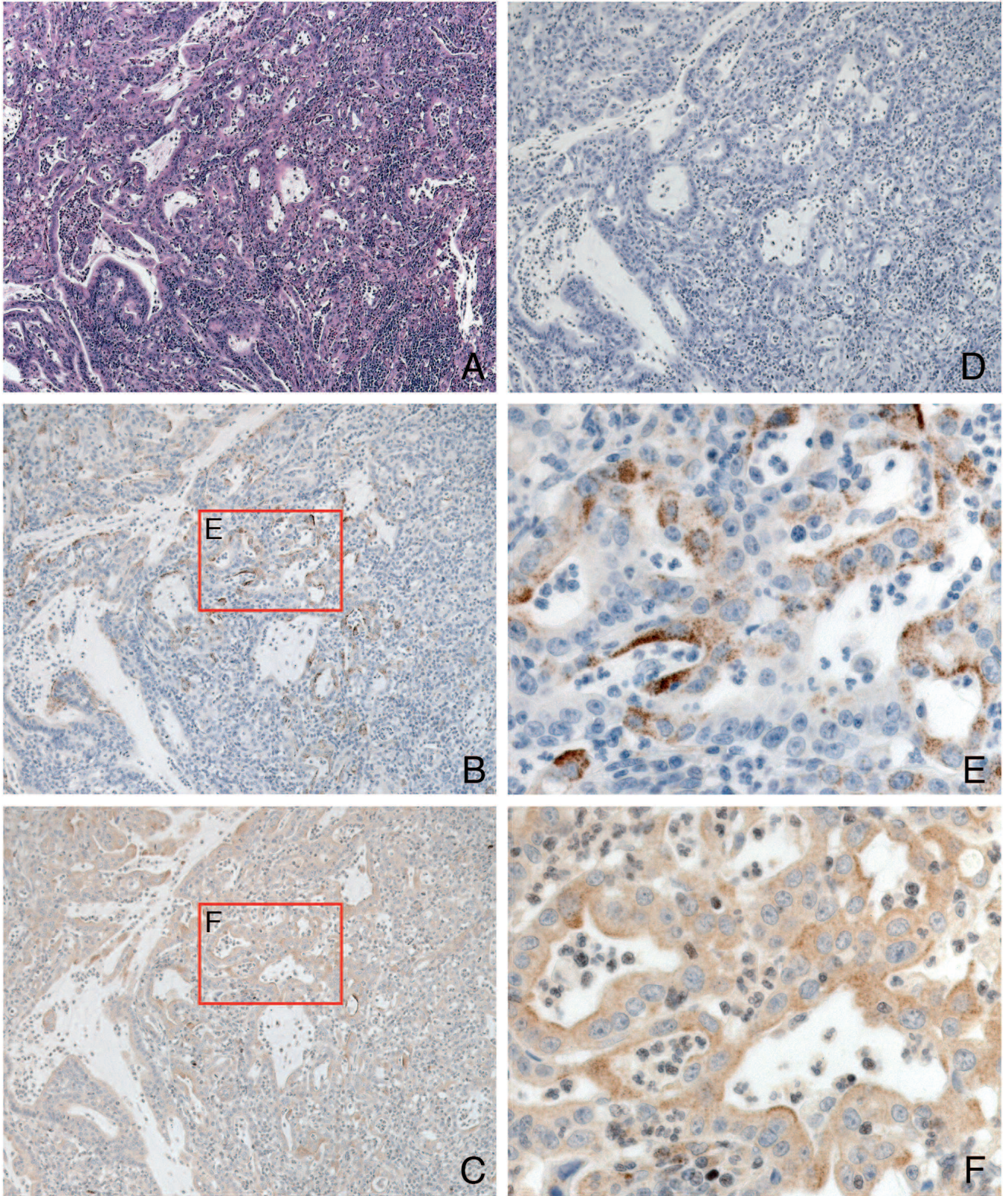
**Fig. 4.** Immunohistochemistry for RUNX3, pepsinogen I, and the proton pump in gastric cancer. Gastric cancer cells with pepsinogen I cytoplasmic staining also express RUNX3, with an almost perfect overlap. **A,** H&E staining; **B and E**, pepsinogen I staining; **C and F**, RUNX3 staining; **D,** Proton pump staining; **E,** higher magnification of B; **F,** higher magnification of C. Original magnification: A-C, x 100; D and E, x 400

respectively, the difference not being significant.

*No relationship between RUNX3 expression and Proton pump expression*

Of the 102 gastric cancers, all cases were judged as proton pump expression negative, indicating no relevance to parietal cells (Table 3).

*RUNX3 mRNA and pepsinogen I mRNA expressions in the gastric cancer cell line*

RUNX3 and pepsinogen I expression were revealed in MKN45 human gastric carcinoma cell line after 35 cycles of PCR reaction. In other cell lines, however, only pepsinogen I was detectable in MKN74 and no visible bands were obtained in TMK1 (Fig. 5). Thus, clear correlation was not assumed between transcriptional expression of RUNX3 and pepsinogen I.

**Discussion**

In the present study, RUNX3 expression was determined immunohistochemically in 102 gastric carcinomas for the purpose of determining interrelations among their RUNX3, pepsinogen I, proton pump, MU5AC, MUC6, and intestinal phenotypic expression.

It was reported recently that loss of expression of RUNX3 is causally related to the genesis and progression of gastric cancer (Li et al., 2002; Ito et al., 2005). About 45% to 60% of surgically resected gastric cancer specimens and associated cell lines do not express RUNX3 due to hemizygous deletion of the gene or hypermethylation of its promoter region (Li et al.,



**Fig. 5.** Transcriptional expression of RUNX3 and pepsinogen I (PgI) in gastric cancer cell lines. Acidic ribosomal phosphoprotein PO (ARP) as an internal control

2002). Inactivation of RUNX3 appears to occur at an early stage, as well as during progression, because silencing of RUNX3 has been observed in 40% of stage I and 90% of stage IV gastric cancers (Li et al., 2002). A mutation found in a gastric cancer patient, RUNX3 (R122C), which causes a single amino acid substitution within the conserved DNA-binding domain, completely abolished the tumor suppressor activity of RUNX3, as assessed in a nude mouse assay. Hyperplasia of the gastric epithelium, observed in the Runx3(-/-) experimental mouse system, seems to be caused by decreased sensitivity to TGF-ß, which inhibits cell cycle progression and induces apoptosis. Furthermore, experiments with stomach epithelial cell lines isolated from Runx3(+/+) and Runx3(-/-) mice with the p53(-/-) background revealed that only those lines derived from Runx3(-/-) p53(-/-) mice were tumorigenic in nude mice (Li et al., 2002). Although these results strongly suggest that RUNX3 is a gastric cancer tumor suppressor and that its loss is involved in roughly half of the cases of gastric cancer, we assumed that it functions normally in the remaining cases (Ito et al., 2005). RUNX3 was here strongly expressed in chief cells in fundic glands, but only to a limited extent in parietal cells in the deeper zone, and was not detectable in cells in the generative zone, suggesting that RUNX3 may play a specific functional role in chief cells (Ito et al., 2005; Osaki et al., 2004). Li et al. reported gastric mucosa to be hyperplastic in RUNX3-deficient mice, leading us to speculate that RUNX3 may be implicated in growth control of chief cells (Li et al., 2002). Further analysis is required to address this possibility.

It is widely thought that the phenotypic expression of tumor cells resembles that of the tissue of origin. Further, it has been shown that gastric cancers at early stages, independent of the histological type, mainly consist of gastric phenotypic cancer cells. A shift from gastric to intestinal phenotypic expression then clearly occurs with progression (Tatematsu et al., 1990, 2003; Yamachika et al., 1997; Yoshikawa et al., 1998; Bamba et al., 2001). The present study showed, for the first time to our knowledge, a relation between RUNX3 and pepsinogen I expression in human gastric cancers, although there have been reports regarding pepsinogen I in gastric cancers (Stemmermann et al., 1985; Huang et al., 1988). Thus the present data provide clear evidence that the RUNX3 is associated with the differentiation of chief cells in cancers, as well as normal gastric mucosa. We also recently experienced a rare case of gastric adenocarcinoma with chief cell differentiation harboring strong pepsinogens I and II, MUC6, and RUNX3 expression characteristic of primitive chief cells (Tsukamoto et al., 2007). Links to gastric foveolar epithelial cells have also been hypothesized (Li et al., 2002; Osaki et al., 2004), but there was no relationship between RUNX3 and MUC5AC or other differentiation markers of gastric epithelial cells in the present study. However, expression analysis of RUNX3 and pepsinogen I using stomach cancer cell lines revealed
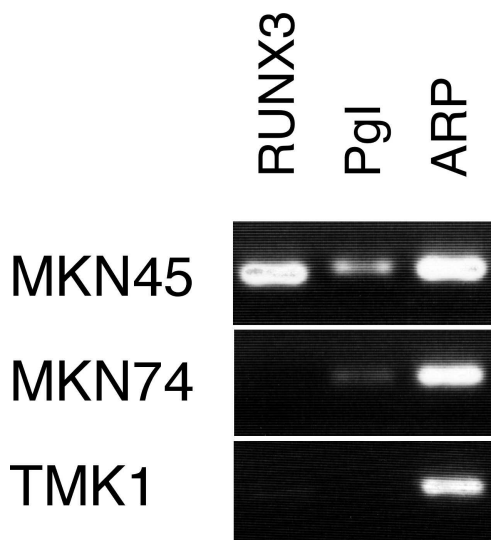
ambiguous correlation between these factors. MKN45 was shown to have significant amount of RUNX3 and pepsinogen I, compatible with the results obtained that in the immunohistochemistry. Nonetheless, MKN74 possessed only pepsinogen I transcript and TMK1 did not harbor both of them. These results suggested that pepsinogen I could not be regulated under RUNX3 but might be coexpressed in the chief cells in the fundic gland.

In conclusion, our data suggest that RUNX3 is strongly associated with chief cell phenotypic expression in human gastric cancers, as well as the human normal gastric mucosa. RUNX3 may have essential roles in cell differentiation in normal fundic glands and gastric cancers.

# References

Baldus S.E., Monig S.P., Arkenau V., Hanisch F.G., Schneider P.M., Thiele J., Holscher A.H. and Dienes H.P. (2002). Correlation of MUC5AC immunoreactivity with histopathological subtypes and prognosis of gastric carcinoma. Ann. Surg. Oncol. 9, 887-893.

Bamba M., Sugihara H., Kushima R., Okada K., Tsukashita S., Horinouchi M. and Hattori T. (2001). Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the human stomach. Virchows Arch. 438, 49-56.

Fukamachi H., Ito K. and Ito Y. (2004). Runx3-/- gastric epithelial cells differentiate into intestinal type cells. Biochem. Biophys Res. Commun. 321, 58-64.

Huang S.C., Miki K., Sano J., Ichinose M., Kawamura N., Oka H., Hirano K., Furihata C., Masugi Y. and Takahashi K. (1988). Pepsinogens I and II in gastric cancer: an immunohistochemical study using monoclonal antibodies. Jpn. J. Cancer Res. 79, 1139-1146.

Ito K., Liu Q., Salto-Tellez M., Yano T., Tada K., Ida H., Huang C., Shah N., Inoue M., Rajnakova A., Hiong K.C., Peh B.K., Han H.C., Ito T., Teh M., Yeoh K.G. and Ito Y. (2005). RUNX3, a novel tumor suppressor, is frequently inactivated in gastric cancer by protein mislocalization. Cancer Res. 65, 7743-7750.

Japanese Gastric Cancer Association (1998). Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1, 10-24.

Kawachi H., Takizawa T., Eishi Y., Shimizu S., Kumagai J., Funata N. and Koike M. (2003). Absence of either gastric or intestinal phenotype in microscopic differentiated gastric carcinomas. J. Pathol. 199, 436-446.

Koseki K., Takizawa T., Koike M., Ito M., Nihei Z. and Sugihara K. (2000). Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. Cancer 89, 724-732.

Lee H.S., Lee H.K., Kim H.S., Yang H.K., Kim Y.I. and Kim W.H. (2001). MUC1, MUC2, MUC5AC, and MUC6 expressions in gastric carcinomas: their roles as prognostic indicators. Cancer 92, 1427-1434.

Li Q.L., Ito K., Sakakura C., Fukamachi H., Inoue K., Chi X.Z., Lee K.Y., Nomura S., Lee C.W., Han S.B., Kim H.M., Kim W.J., Yamamoto H., Yamashita N., Yano T., Ikeda T., Itohara S., Inazawa J., Abe T., Hagiwara A., Yamagishi H., Ooe A., Kaneda A., Sugimura T., Ushijima T., Bae S.C. and Ito Y. (2002). Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell 109, 113-124.

Mizoshita T., Tsukamoto T., Nakanishi H., Inada K., Ogasawara N., Joh T., Itoh M., Yamamura Y. and Tatematsu M. (2003). Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. J. Cancer Res. Clin. Oncol. 129, 727-734.

Muller-Hocker J. and Rellecke P. (2003). Chief cell proliferation of the gastric mucosa mimicking early gastric cancer: an unusual variant of fundic gland polyp. Virchows Arch. 442, 496-500.

Osaki M., Moriyama M., Adachi K., Nakada C., Takeda A., Inoue Y., Adachi H., Sato K., Oshimura M. and Ito H. (2004). Expression of RUNX3 protein in human gastric mucosa, intestinal metaplasia and carcinoma. Eur J. Clin. Invest. 34, 605-612.

Seregni E., Botti C., Massaron S., Lombardo C., Capobianco A., Bogni A. and Bombardieri E. (1997). Structure, function and gene expression of epithelial mucins. Tumori 83, 625-632.

Stemmermann G.N., Samloff I.M. and Hayashi T. (1985). Pepsinogens I and II in carcinoma of the stomach: an immunohistochemical study. Appl Pathol 3, 159-163.

Suzuki T., Tate G., Ikeda K. and Mitsuya T. (2005). A novel multicolor immunofluorescence method using heat treatment. Acta Med. Okayama 59, 145-151.

Tajima Y., Shimoda T., Nakanishi Y., Yokoyama N., Tanaka T., Shimizu K., Saito T., Kawamura M., Kusano M. and Kumagai K. (2001). Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. Oncology 61, 212-220.

Takahashi K. (1992). Gene structures of pepsinogens A and C. Scand J. Clin. Lab. Invest. Suppl. 210, 97-110.

Takubo K., Honma N., Sawabe M., Arai T., Izumiyama-Shimomura N., Kammori M., Sasajima K. and Esaki Y. (2002). Oncocytic adenocarcinoma of the stomach: parietal cell carcinoma. Am. J. Surg. Pathol. 26, 458-465.

Tatematsu M., Furihata C., Katsuyama T., Miki K., Honda H., Konishi Y. and Ito N. (1986). Gastric and intestinal phenotypic expressions of human signet ring cell carcinomas revealed by their biochemistry, mucin histochemistry, and ultrastructure. Cancer Res. 46, 4866-4872.

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

Tatematsu M., Hasegawa R., Ogawa K., Kato T., Ichinose M., Miki K. and Ito N. (1992). Histogenesis of human stomach cancers based on assessment of differentiation. J. Clin. Gastroenterol. 14 (Suppl 1), S1-7.

Tatematsu M., Tsukamoto T. and Inada K. (2003). Stem cells and gastric cancer - Role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci. 94, 135-141.

Tsuchiya A., Kikuchi Y., Ando Y. and Abe R. (1995). Correlation between expression of sialosyl-T antigen and survival in patients with gastric cancer. Br. J. Surg. 82, 960-962.

Tsukamoto T., Fukami H., Yamanaka S., Yamaguchi A., Nakanishi H.,

Sakai H., Aoki I. and Tatematsu M. (2001). Hexosaminidase-altered aberrant crypts, carrying decreased hexosaminidase alpha and beta subunit mRNAs, in colon of 1,2-dimethylhydrazine-treated rats. Jpn. J. Cancer Res. 92, 109-118.

Tsukamoto T., Inada K., Tanaka H., Mizoshita T., Mihara M., Ushijima T., Yamamura Y., Nakamura S. and Tatematsu M. (2004). Down-regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. J. Cancer Res. Clin. Oncol. 130, 135-145.

Tsukamoto T., Mizoshita T., Mihara M., Tanaka H., Takenaka Y., Yamamura Y., Nakamura S., Ushijima T. and Tatematsu M. (2005). Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 46, 649-658.

Tsukamoto T., Mizoshita T. and Tatematsu M. (2006). Gastric-and-intestinal mixed-type intestinal metaplasia: aberrant expression of transcription factors and stem cell intestinalization. Gastric Cancer 9, 156-166.

Tsukamoto T., Yokoi T., Maruta S., Kitamura M., Yamamoto T., Ban H. and Tatematsu M. (2007). Gastric adenocarcinoma with chief cell differentiation. Pathol. Int. 57, 517-522.

Utsunomiya T., Yonezawa S., Sakamoto H., Kitamura H., Hokita S., Aiko T., Tanaka S., Irimura T., Kim Y.S. and Sato E. (1998). Expression of MUC1 and MUC2 mucins in gastric carcinomas: its relationship with the prognosis of the patients. Clin. Cancer Res. 4, 2605-2614.

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

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