# Prognostic Significance of ZNF217 Expression in Gastric Carcinoma

ATSUO SHIDA[1], SHUICHI FUJIOKA[1], HIDEAKI KURIHARA[2], YOSHIO ISHIBASHI[1], NORIO MITSUMORI[1], NOBUO OMURA[1] and KATSUHIKO YANAGA[1]

[1]*Department of Surgery, Jikei University School of Medicine, Tokyo, Japan;*
[2]*Department of Surgery, Kanagawa Prefectural Shiomidai Hospital, Kanagawa, Japan*

**Abstract.** *Background: The zinc finger protein ZNF217 is a candidate oncogene in breast cancer and ovarian clear cell cancer. The purpose of the present study was to clarify the significance of this protein's expression in gastric carcinoma and to evaluate the outcome of these patients. Materials and Methods: Using paraffin-embedded specimens from 84 patients with gastric cancer, ZNF217 protein was detected using an anti-ZNF217 goat polyclonal antibody. We evaluated the ZNF217 protein expression in relation to patient outcome and clinicopathological parameters. Results: The ZNF217 protein was expressed in 34 (40.5%) tumor sections. Patients with ZNF217-negative tumors had better relapse-free survival (RFS) and overall survival (OS) than those with ZNF217-positive tumors by the log-rank test. Notably, multivariate analysis indicated that ZNF217 was an independent prognostic factor for RFS. Conclusion: ZNF217 expression seems to be a novel prognostic biomarker in gastric cancer.*

Gastric cancer is a malignant neoplasm being fourth in incidence worldwide and the second cause of cancer-related deaths, which frequently affects the populations of Latin America, Eastern Europe, China, Korea and Japan (1).

*ZNF217* is a candidate oncogene located on chromosome 20q13.2, a region that is frequently amplified in many tumors (2). ZNF217 is a zinc finger protein that is localized in the nucleus (3) and interacts with co-repressors and histone-modifying proteins, suggesting that it may be part of a transcriptional repressor complex (4-6). The role of ZNF217 in transcriptional regulation is likely to be complex, as ZNF217 has also been shown to induce positive transcriptional regulation of target genes (7, 8). Previous studies have indicated that high ZNF217 expression has been associated with resistance to chemotherapy and with de-regulated apoptotic signals in breast cancer cells (9, 10). High-level amplification of 20q13 is found in 6.8% to 18% (11-13) of breast cancers and this amplification has been associated with poor prognosis in breast cancer (11). Moreover, *ZNF217* gene amplification in ovarian clear cell carcinoma is an independent prognostic factor for progression-free and overall survival (OS) after chemotherapy (14).

To date, there have been no reports on the impact of ZNF217 expression on the outcome of patients with gastric carcinoma. Herein we investigated the expression of ZNF217 in human gastric cancer specimens and evaluated the significance of ZNF217 expression on the outcome and clinicopathological parameters of the patients.

*Correspondence to:* Atsuo Shida, Department of Surgery, Jikei University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo, 105-8461, Japan. Tel: +81 0334331111, Fax: +81 0354724140, e-mail: atsuoshida@ybb.ne.jp

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## Materials and Methods

*Immunohistochemistry*. Using paraffin-embedded specimens from 84 consecutive patients diagnosed and treated by gastrectomy with standard lymph node dissection at the Department of Surgery, Kanagawa prefectual Shiomidai Hospital (Kanagawa, Japan) between January 2006 and February 2011 were studied using immunohistochemistry. Tumor stages and the definition of criteria for histological classification followed those proposed by the Japanese Society for the Research of Gastric Cancer (JRSGC) (15). Histological grade was classified into two groups based on the predominant features: The differentiated group consisted of patients with well differentiated or moderately differentiated adenocarcinoma as well as papillary adenocarcinoma, while the undifferentiated group consisted of patients with poorly differentiated carcinoma, signet-ring cell carcinoma, and mucinous carcinoma.

The ZNF217 protein was detected using the anti ZNF217 goat polyclonal antibody (ab136678, Abcam, Cambridge,UK). Briefly, after microwaving in citrate buffer solution (pH 6.0), de-paraffinized sections were incubated with 1% methanol–hydrogen peroxide for 30 min. The slides were then incubated with goat polyclonal antibody against ZNF217 (×500 dilution) for 60 min. This was followed by incubation with anti-goat secondary antibody (Biotinelated anti-Goat IgG, Vector, address) for 30 min. Staining was visualized by using the DAB method (Dako, address) for 5 min.

Counter-staining was performed lightly with hematoxylin. All incubations were performed at room temperature in a humidified chamber. The examiners were kept unaware of the patients' clinical and histologic (hematoxylin-eosin staining) information. Two investigators (A.S. and Y.I.) evaluated the staining levels independently, after which discordant evaluations were adjusted through the connected microscope. The intensity of ZNF217 staining was graded on a scale of 0 to 3+ under a ×100 field as follows: 0, no detectable nuclear staining of cancer cells; 1+, weak staining; 2+, moderate staining; 3+, strong staining. A specimen was regarded as positive when the intensity of staining was 2+ or 3+ and negative when the intensity of staining was 0 or 1+ (Figure 1).

*Statistics*. The significance of the data was determined using the Chi-square test. The multivariate analysis for patient outcome was determined by the Cox proportional hazards model. Survival curves of the patients were compared using the Kaplan-Meier method and analyzed by the log-rank test. A $p$-value <0.05 indicates significance. All analyses was performed by Excel Statistics 2012 (Social Survey Research Information Co. Ltd., Tokyo, Japan).

## Results

*Patients' characteristics*. Patients' age ranged from 48 to 94 years (mean; SD, 73.3; 10.2 years), consisting of 58 men and 26 women. After surgery, the 84 patients were followed from 25 to 96 months (median 60.1 months). Altogether, 47 patients survived, and 37 died, with cancer death in 31 (37%) and other causes in 6 (7%).

*Immunohistochemical expression patterns and clinico-pathological variables*. Each clinicopathological variable was compared based on ZNF217 protein expression (Table I). Positive ZNF217 expression was observed in the nucleus in 34 (40.5%) tumor tissues, in which negative or weak ZNF217 was also observed in normal gastric tissues. There was no relationship between ZNF217 expression and clinicopathological parameters (Table I). However, RFS and OS in patients with ZNF217-negative tumors were better than in those patients with ZNF217-positive tumors, as assessed by the log-rank test (Figures 2 and 3). Bi-variate analysis focusing on RFS indicated seven significant variables, consisting of tumor size, differentiation, lymphatic system invasion, venous system invasion, tumor depth, lymph node metastasis and ZNF217 (Table II). On the other hand, significant indicators by bivariate analysis for OS consisted of seven variables: tumor size, differentiation, lymphatic system invasion, venous system invasion, tumor depth, lymph node metastasis and ZNF217 (Table III). The multivariate Cox proportional hazard model identified two independent predictive factors, ZNF217 expression and lymph node metastasis for RFS, as well as three independent prognostic factors including differentiation, venous system invasion and lymph node metastasis for OS (Tables II and III). Candidate patients for analysis of RFS were 75 patients with gastric cancer who were stage I, II and III. The stage IV patients (n=9) were excluded from this analysis, because stage IV patients are potentially cancer-positive.

Table I. *Correlation between ZNF217 expression and clinical features in 84 patients*.

| | | ZNF217 expression | | |
|---|---|---|---|---|
| Category | Cases | Yes | No | *P* |
| Gender | | | | |
| Male | 58 | 23 | 35 | |
| Female | 26 | 11 | 15 | 0.8189 |
| Age | | | | |
| <74 years | 44 | 17 | 27 | |
| ≥75 years | 40 | 17 | 23 | 0.7186 |
| Tumor location | | | | |
| Upper | 12 | 4 | 8 | |
| Middle/Lower | 72 | 30 | 42 | 0.8205 |
| Tumor size | | | | |
| <45mm | 38 | 13 | 25 | |
| ≥45mm | 46 | 18 | 28 | 0.7144 |
| Differentiation | | | | |
| Differentiated | 42 | 17 | 25 | |
| Undifferentiated | 42 | 17 | 25 | 1.0000 |
| Lymphatic system invasion | | | | |
| Yes | 61 | 25 | 36 | |
| No | 23 | 9 | 14 | 0.8774 |
| Venous system invasion | | | | |
| Yes | 44 | 20 | 24 | |
| No | 40 | 14 | 26 | 0.3296 |
| Tumor depth | | | | |
| pT1/pT2 | 37 | 11 | 26 | |
| pT3/pT4 | 47 | 23 | 24 | 0.0750 |
| Lymphnode metastasis | | | | |
| Yes | 43 | 21 | 22 | |
| No | 41 | 13 | 28 | 0.1099 |
| Distant metastsis | | | | |
| M1 | 9 | 4 | 5 | |
| M0 | 75 | 30 | 45 | 0.9182 |
| pTNM stage | | | | |
| StageI | 37 | 11 | 26 | |
| StageII/III/IV | 47 | 23 | 24 | 0.0750 |

## Discussion

*ZNF217* mRNA expression is a prognostic marker that gives an added value to current biomarkers. Vendrell *et al*. (16) demonstrated that overexpression of ZNF217 protein strongly stimulates migration and invasion in several breast cancer cell lines in mice, which was associated with the development of spontaneous lung or lymph node metastases. They also indicated that the ErbB2/ErbB3/FAK signaling pathway is de-regulated in ZNF217-overexpressing breast cancer cells *in vitro* and finally showed that high levels of expression of *ZNF217* mRNA are associated with poor

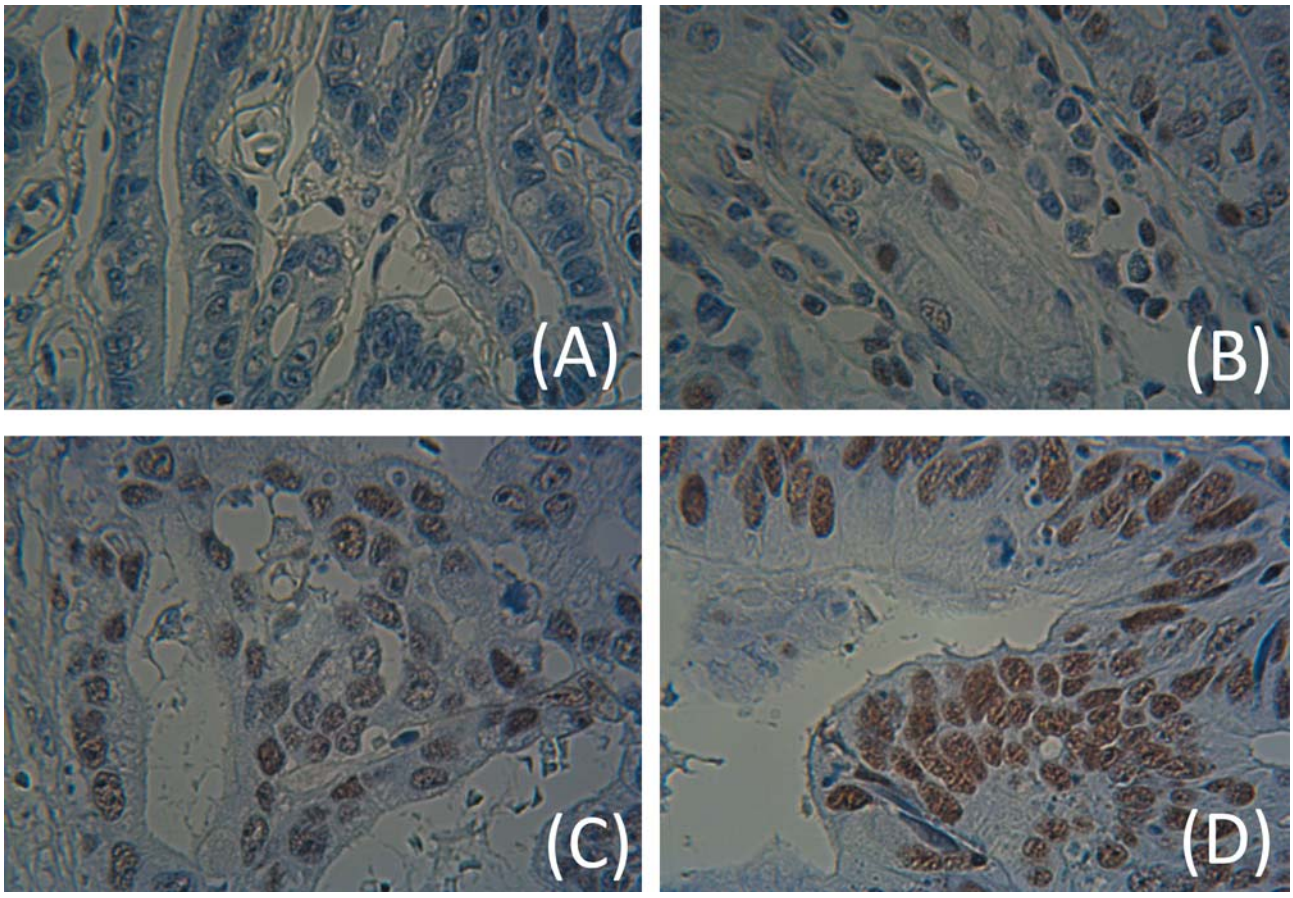


Figure 1. *Representative immunohistochemical staining with ZNF217 polyclonal antibody in gastric cancer, which shows strong staining for ZNF217 in the nuclear region of the tumor tissue (×400 magnification). (A) Negative for ZNF217, (B) 1+ for ZNF217, (C) 2+ for ZNF217, (D) 3+ for ZNF217.*

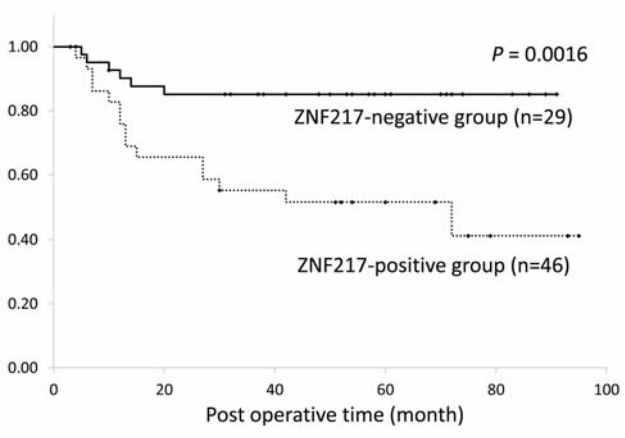


Figure 2. *Kaplan-Meier curves for postoperative relapse-free survival of the patients with or without expression of ZNF217 in gastric carcinoma.*

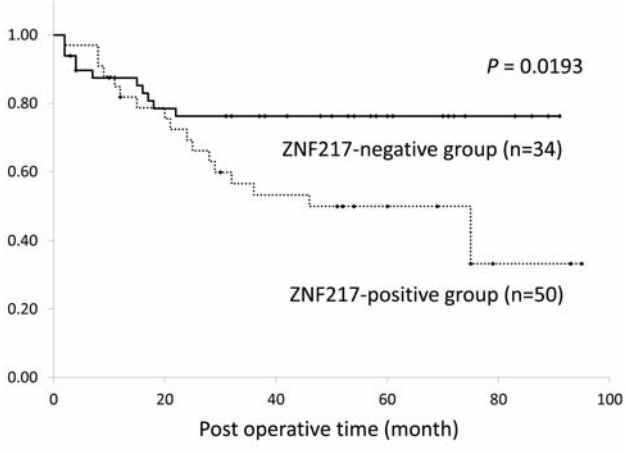


Figure 3. *Kaplan-Meier curves for postoperative overall survival of the patients with or without expression of ZNF217 in gastric carcinoma.*

Table II. *Risk factors affecting relapse-free survival determined by the Cox proportional hazards model in 75 patients with stage I, II and III gastric cancer.*

| | Bivariate analysis for relapse-free survival | | | Multivariate analysis for relapse-free survival | | |
|---|---|---|---|---|---|---|
| Variable | Hazard ratio | 95% CI | *p*-Value | Hazard ratio | 95% CI | *p*-Value |
| Gender (female *vs*. male) | 0.8983 | 0.3539-2.2802 | 0.8214 | | | |
| Age (≥75 *vs*. <74 years) | 2.3494 | 0.9982-5.5294 | 0.0505 | | | |
| Tumor location (ML *vs*. U) | 1.1281 | 0.3817-3.3343 | 0.8274 | | | |
| Tumor size (≥45 mm *vs*. <45 mm) | 7.9891 | 1.7789-35.8799 | 0.0067 | 2.2020 | 0.9056-19.4963 | 0.0667 |
| Differentiation (Undifferentiated *vs*. Differentiated) | 3.6617 | 1.5007-8.9341 | 0.0043 | 1.9090 | 0.7726-4.7168 | 0.1612 |
| Lymphatic system invasion (positive *vs*. negative) | 12.9583 | 1.7446-96.2523 | 0.0123 | 1.3941 | 0.1850-10.5062 | 0.7472 |
| Venous system invasion (positive *vs*. negative) | 4.3325 | 1.6960-11.0680 | 0.0022 | 1.9996 | 0.7185-5.5652 | 0.1845 |
| pT (T3/T4 *vs*. T1/T2) | 14.1349 | 3.3060-60.4349 | 0.0004 | 1.5829 | 0.1951-12.8408 | 0.4237 |
| pN (N+ *vs*. N0) | 11.7048 | 3.4553-39.6502 | 0.0001 | 5.6849 | 1.5197-21.2666 | 0.0098 |
| ZNF217 (positive *vs*. negative) | 3.6926 | 1.5174-8.9860 | 0.0040 | 2.8138 | 1.1414-6.9364 | 0.0246 |

Table III. *Risk factors affecting overall survival determined by the Cox proportional hazards model in 84 patients with stage I, II, III and IV gastric cancer.*

| | Bivariate analysis for overall survival | | | Multivariate analysis for overall survival | | |
|---|---|---|---|---|---|---|
| Variable | Hazard ratio | 95% CI | *p*-Value | Hazard ratio | 95% CI | *p*-Value |
| Gender (female *vs*. male) | 1.1127 | 0.5082-2.4360 | 0.7894 | | | |
| Age (≥75 *vs*. <74 years) | 2.0597 | 0.9335-4.5444 | 0.0735 | | | |
| Tumor location (ML *vs*. U) | 0.9537 | 0.3644-2.4959 | 0.9231 | | | |
| Tumor size (≥45 mm *vs*. <45 mm) | 9.3954 | 2.1541-40.9793 | 0.0029 | 2.7372 | 0.8100-17.2429 | 0.0911 |
| Differentiation (Undifferentiated *vs*. Differentiated) | 4.8593 | 2.0775-11.3661 | 0.0003 | 2.8016 | 1.1815-6.6434 | 0.0194 |
| Lymphatic system invasion (positive *vs*. negative) | 16.4455 | 2.2393-120.7739 | 0.0059 | 2.3430 | 0.2216-24.7756 | 0.4067 |
| Venous system invasion (positive *vs*. negative) | 5.9894 | 2.4332-14.7430 | 0.0001 | 2.9735 | 1.0899-8.1126 | 0.0333 |
| pT (T3/T4 *vs*. T1/T2) | 17.3967 | 4.1270-73.3333 | 0.0001 | 1.2261 | 0.1511-9.9488 | 0.4432 |
| pN (N+ *vs*. N0) | 15.0356 | 4.5219-49.9943 | 0.0000 | 8.0803 | 2.2983-28.4090 | 0.0011 |
| ZNF217 (positive *vs*. negative) | 2.6520 | 1.2693-5.5408 | 0.0095 | 1.5812 | 0.7469-3.3473 | 0.2125 |

prognosis and the development of metastases in patients with breast cancer. Rahman *et al*. (14) reported that *ZNF217* copy number correlated significantly with ZNF217 protein expression, and amplification of this gene correlated significantly with shorter progression-free and overall survival. Kring *et al*. (17) reported that *ZNF217* and *ERBB3* were highly co-expressed in human breast cancer cell lines, as well as in murine and human breast tumor samples. They also showed that ZNF217 binds to the proximal part of *ERBB3* promoter and activates this gene's expression in a reporter gene assay. Conversely, depletion of endogenous ZNF217 resulted in significant decreases in ERBB3 expression, indicating that ZNF217 expression is required for the maintenance of robust ERBB3 in human breast cancer cells. These studies indicated that ZNF217 act as transcriptional factor upstream of *ERBB3*. Gastric cancer patients with nuclear ERBB3 expression showed poor overall survival when compared to those with negative expression by immnohistochemical and fluorescence *in situ* hybridization examination (18).

The current study demonstrated that ZNF217 expression is an independent prognostic factor for RFS, but not for OS. The most reasonable explanation for this may be the small number of patients included in this study.

In summary, we demonstrated that expression of ZNF217 protein is an independent indicator of RFS in patients with gastric carcinoma.

## Conflicts of Interest

None of the Authors has any conflict of interest regarding the manuscript: 'Prognostic significance of ZNF217 expression in Gastric Carcinoma'.

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