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*Original Article*

# Clinical significance of expression level of ZNF471 in gastric cancer

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**Abstract:** Background: As a tumor suppressor gene, zinc finger protein 471 (ZNF471) has an essential role in tumor occurrence and development. Due to promoter hypermethylation, it can be underexpressed or silenced in gastric cancer (GC) cell lines. In this study, we investigated relationships between clinical characteristics and ZNF471 expression levels in tissues of patients with GC. Methods: We used immunohistochemistry (IHC) to detect ZNF471 expression in paraffin tissue specimens, and quantitative real-time PCR (qRT-PCR) and western blot (WB) analysis to measure expression levels of ZNF471 in fresh tissue specimens. We analyzed relationships between ZNF471 expression levels and characteristics, such as tumor size, gender, age, TNM stage, and lymph node metastasis. Results: Immunohistochemistry revealed the expression of ZNF471 protein from paraffin blocks of GC tissues was significantly lower than that of adjacent tissues. Expression levels of ZNF471 mRNA and protein in fresh GC tissues were markedly lower than those in adjacent tissues and in normal gastric mucosal tissues from healthy subjects. ZNF471 expression was significantly correlated with tumor size, lymph node metastasis, and TNM stage (all $P<0.05$). There were no significant associations with gender, age, distant metastasis, or pathologic type. Expression of ZNF471 mRNA and protein was not significantly different between adjacent tissues of patients with GC and normal gastric mucosal tissue from healthy subjects. Conclusion: ZNF471 functions as a tumor suppressor during the pathogenesis of GC. Thus, it is a promising biomarker for diagnosis and therapy of GC.

**Keywords:** Zinc finger protein 471, gastric cancer, clinical significance, biomarker

## Introduction

Gastric cancer (GC) is a common digestive tract malignancy that originates from gastric mucosal epithelial cells [1, 2]. In 2022, global cancer statistics indicated that the incidence and mortality of GC ranked fourth and third, respectively. Patients with advanced GC have a low 5-year survival rate (about 50%) and poor prognosis. Compared to advanced GC, the 5-year survival rate of early GC exceeds 90% [3, 4]. Early diagnosis and treatment of GC are crucial to prolong survival time and improve quality of life.

The onset of GC is usually asymptomatic, and most patients do not have discomfort during early disease stages [5, 6]. Thus, most patients with GC have advanced-stage disease at diagnosis, which is past the optimal time for treatment using endoscopic resection [7]. Serology, imaging, and endoscopy are used for diagnosis of GC. Some early cases of GC can be misdiagnosed due to the lack of use of endoscopy and the skill level of the endoscopist [8]. Abnormalities found using imaging of patients at an advanced stage usually include indications of invasion and metastasis. The sensitivities and specificities of common tumor markers used in the clinic are not high for the diagnosis of GC [9, 10]. Therefore, novel and effective non-invasive prognostic indicators for GC diagnosis, treatment, and prognosis evaluation are needed.

The zinc finger protein (ZFP) family is widely distributed and is the largest transcription factor family in the human genome. It has a variety of biologic functions in the human body, including mediation of protein-protein interactions, chromatin remodeling, and protein chaperoning [11, 12]. Zinc finger protein also participates during transcription and translation due to ZFP binding to DNA. It can inhibit or promote cancer progression by regulation of the cell cycle, inhibiting promoter transcriptional activity, and inducing epithelial-mesenchymal transition [13-15].

As an essential member of the ZFP group, the candidate tumor suppressor gene, ZNF471, belongs to KRAB C2H2 type ZFP, located on the human 19q13.43 chromosome. The N-terminal of ZNF471 contains a KRAB domain, and the C-terminal contains 15 zinc finger structures. ZNF471 can inhibit the growth, proliferation, invasion, and metastasis of cervical cancer cells by up-regulating CDH1 and down-regulating CDH2, VIM, and TW1 to arrest the cell cycle and induce epithelial-mesenchymal transition. In cervical cancer cells, detection of co-methylation of specific 5'-C-phosphate-G-3' (CpG) sites of the ZNF471 promoter can be used to diagnose cancer and identify different stages. It is a possible biomarker for early detection, prognosis, and chemotherapy for cervical cancer [17]. In digestive tract tumors, ZNF471 is associated with esophageal cancer and GC. In esophageal cancer, the CpG site of the ZNF471 promoter is also in a hypermethylation state, and expression is significantly reduced. ZNF471 can have a tumor suppressive role via activation of MAPK10/JNK3 signals [18]. Cao et al. found that ZNF471 is silenced or significantly downregulated in 15 cell lines of GC due to promoter hypermethylation. This change inhibits GC by transcriptional inhibition of the downstream oncogenes PLS3 and TFAP2A. Promoter hypermethylation is an independent risk factor for a poor prognosis for patients with GC [16].

Studies of the relationships between cancer and ZNF471 using basic experiments rather than clinical samples are underway, especially for GC. There are few clinical research studies of the relationships between ZNF471 and GC progression. Based on our previous studies, we aimed to elucidate the relationships between expression levels of ZNF471 and clinical significance in clinical GC tissue samples. First, expression levels of ZNF471 in 40 cases of GC and their adjacent paraffin tissue samples were detected using immunohistochemistry. The samples were collected at The First People's Hospital of Wuhu between January 1, 2021, to December 31, 2021. Real-time fluorescence quantitative PCR (qRT-PCR) and western blot analysis were used to determine the difference between ZNF471mRNA in 21 GC samples, corresponding fresh tissue samples adjacent to cancer, and 21 fresh normal gastric mucosa tissue samples from healthy people. These samples were collected at The First People's Hospital of Wuhu between April 1, 2021, to December 31, 2021. Relationships between the expression of ZNF471 in GC and clinical stage, pathologic findings were examined in detail.

## Materials and methods

### *Sample collection and preservation*

Forty patients who underwent GC surgery at the First People's Hospital of Wuhu between January 1, 2021, and December 31, 2021, were randomly selected for the study. Corresponding GC and adjacent tissue wax blocks were analyzed by the Department of Pathology. All samples were pathologically confirmed as either GC tissues or adjacent tissues.

From April 1, 2021, to December 31, 2021, patients with GC who underwent surgery at our hospital as well as healthy subjects who underwent gastroscopy were selected for the study. Samples of GC and corresponding adjacent tissues from 21 of these patients with GC were collected in the operating room and normal gastric mucinous tissue samples from 21 healthy physical examiners were collected at the Endoscopic Center. Immediately after collection, each specimen was placed into a sterile EP tube and about 1.0 mL RNAlater™ (Invitrogen Company, USA) was added. The sample was stored in a refrigerator at 4°C overnight and then stored in a freezer at -80°C until analysis. The study protocol was approved by the ethics committee of our hospital. All patients agreed to participate and signed an informed consent form before samples were taken.

**Table 1.** qPCR reaction system

| Reagent | Dose |
|---|---|
| 2× AceQ qPCR SYBR Green Master Mix | 10.0 μL |
| Primer F | 0.4 μL |
| Primer R | 0.4 μL |
| 50× ROX Reference Dye 2 | 0.4 μL |
| Template cDNA | 2.0 μL |
| RNase-free $ddH_2O$ | 6.8 μL |

**Table 2.** qPCR reaction program setting conditions

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Pre denaturation | 1 | 95°C | 5 min |
| Cyclic reaction | 45 | 95°C | 10 s |
| | | 60°C | 30 s |
| Melting curve | 1 | 95°C | 15 s |
| | | 95°C | 60 s |
| | | 60°C | 5 s |

*Immunohistochemical stains*

Immunohistochemical stains were performed to detect ZNF471 expression in the GC and corresponding adjacent tissue samples. The paraffin sections were prepared and deparaffinized in xylene and dehydrated in a graded series of ethanols, followed by antigen retrieval in citrate buffer (pH=6.0) for 15 min. Sections were then treated using a 3% hydrogen peroxide peroxidase inhibitor to block endogenous peroxidase. The sections were then incubated overnight at 4°C with ZNF471 antibody (Abcam, 1:50 dilution, USA). Subsequently, horseradish peroxidase-labeled goat anti-mouse or rabbit polyclonal antibody (ZSGB-BIO, China) was dripped onto the slides and incubated at 37°C for 20 min. They were then stained using 3,3'-diaminobenzidine, re-stained with hematoxylin, and scored under a microscope. The results were evaluated by two senior pathologists through an independent double-blind method.

*Quantitative real-time PCR analysis*

Total RNA was extracted from tissues using RNA-easy™ Isolation Reagent (Vazyme, China). Reverse transcription was performed using TP700 (Takara, Japan) according to Hiscript® II Q RT SuperMix for qPCR reagent instructions (Vazyme, China). Real-time qRT-PCR was performed using AceQ® qPCR SYBR Green Master Mix Kit (Vazyme, China). The ZNF471 and GAPDH primers were designed by Sangon Biotech (Shanghai, China), based on ZNF471 mRNA sequences. The synthesized primer sequences were: ZNF471 forward 5'-TGT GCA AAT GTC CTC AGA CAA GA-3' and ZNF471 reverse 5'-TCC ACA GGA CTT GGT GAT GTA GT-3'; GAPDH forward 5'-TCC ACC ACC CTG TTG CTG TA-3' and GAPDH reverse 5'-ACC ACA GTC CAT GCC ATC AC-3'. The qRT-PCR reaction system and reaction procedure settings conditions are presented in **Tables 1** and **2**. Using the original data measured using qPCR, ZNF471 expression in GC, adjacent tissues, and normal gastric mucosa of healthy people was calculated using expression of ZNF471 relative to the internal reference, GAPDH. The calculation method used was: $2^{-\Delta\Delta Ct}$ ($\Delta Ct = Ct_{ZNF471} - Ct_{GAPDH}$) [19].

*Western blot analysis*

The tissue samples from each patient were washed with precooled PBS and then lysed in lysis buffer (RIPA, Beyotime, China). After 30 min at 4°C, the lysate was centrifuged (4°C, 12,000×g for 40 min), and the supernatant was collected. Proteins were separated via 10-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Next, samples were transferred to 0.22 μm-thick polyvinylidene difluoride membranes (Merck Millipore, USA). The membranes were blocked with 5.0% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 at room temperature for 2 h and then incubated with anti-ZNF471 (1:1000, Abcam) overnight at 4°C. After washing with 0.1% Tween-20, the membrane was incubated with horseradish peroxidase-linked GAPDH (1:2000, Abcam). After washing, the bound proteins were detected through an enhanced chemiluminescence reagent.

*Statistical analysis*

The data analysis was performed using SPSS 22.0 software. Paired *t*-tests were used to detect significant differences in levels of ZNF-471 expression in GC tissues, corresponding adjacent tissues, and normal gastric mucosal tissue. Independent sample t-tests were used to detect differences in ZNF471 expression

Table 3. General information and pathologic characteristics of patients with gastric cancer for qPCR and WB analysis

| Information and pathologic characteristic | | Cases (n=21) | Ratio |
|---|---|---|---|
| Sex | Male | 14 | 66.60% |
| | Female | 7 | 33.33% |
| Age | ≥60 | 14 | 66.67% |
| | <60 | 7 | 33.33% |
| Maximum diameter of tumor | ≥5 | 11 | 52.38% |
| | <5 | 10 | 47.62% |
| Lymph node metastasis | Positive | 16 | 76.19% |
| | Negative | 5 | 23.81% |
| Distant metastasis | Positive | 2 | 9.52% |
| | Negative | 19 | 90.48% |
| Pathological type | Adenocarcinoma | 14 | 80.95% |
| | Else | 7 | 19.05% |
| TNM staging | Tis or I-II | 7 | 33.33% |
| | III-IV | 14 | 66.67% |

between GC tissues and normal gastric mucosal tissue of healthy people and between adjacent tissues from patients with GC and normal gastric mucosal tissue of healthy people. Measurement-based results were expressed as mean ± standard deviation (mean ± SD) values; counted data were used to calculate percentages in the statistical analysis. Graphing was performed using GraphPad Prism 9 (v9.0) software. A $p$-value less than 0.05 indicated a difference, and a $p$-value less than 0.001 was considered a significant difference.

## Results

*General and clinicopathologic results of enrolled patients*

Samples from 21 patients with GC and 21 healthy people were used in qPCR analysis. The age range of the GC group was 42-80 years, with an average age of 65.33±9.51 years. There were 14 (66.67%) males and 7 (33.33%) females. The age range of the physical examination group was 26-80 years, with an average age of 50.48±13.70 years; this group included 15 males and 6 females. Among the 21 patients in the physical examination group, there were 7 (33.33%) <60 years of age and 14 (66.67%) ≥60 years of age. The maximum tumor diameter, >5.0 cm, was observed in 11 (52.38%) patients; the largest tumor diameter was ≤5.0 cm in 47.62% (10/21) of patients. Sixteen (76.19%) patients had lymph node metastasis that was detected at diagnosis, and 5 (23.81%) had no lymph node metastasis. Two (9.52%) patients had distant metastasis. Based on histologic type, we found 17 (80.95%) patients with adenocarcinoma and 4 (19.05%) patients with other types of cancer. Based on TNM staging, Tis and stages I-II were found in 7 (33.33%) patients, and stages III-IV were found in 14 (66.67%) patients. The clinicopathologic findings are presented in **Table 3**.

Tissue specimens of GC and paracancerous wax blocks from 40 patients were used for immunostains. These patients' ages ranged from 33 to 86 years, with a mean age of 66.43±12.76 years; there were 30 (75.00%) males and 10 (25.00%) females. There were 29 (72.50%) patients ≥60 years of age and 11 (27.50%) <60 years of age. In 13 (32.5%) patients, the maximum tumor diameter was ≥5 cm; the maximum tumor diameter was <5 cm in 27 (67.5%) patients. Twenty-six (65.0%) patients had lymph node metastasis; 14 (35.0%) had no lymph node metastasis. There were 2 (5.00%) patients with distant metastasis, 38 (95.0%) without distant metastasis, 37 (92.5%) patients with adenocarcinoma, and 3 (7.5%) with another cancer type. Regarding TNM staging, there were 14 (35.0%) patients with Tis and stage I-II, and 26 (65.0%) patients with stage III-IV. These results are presented in **Table 4**.

*Expression levels of ZNF471 in GC and adjacent tissues, by immunohistochemistry*

Immunostaining was performed on paraffin sections of GC and adjacent tissues of 40 patients with GC. ZNF471 was mainly expressed in the nucleus and cytoplasm of cells. Eleven patients with GC had high expression of ZNF471 (27.50%) (**Table 5**; **Figure 1C**). ZNF471 expression was low in 29 (72.50%) patients with GC (**Figure 1A**). In adjacent tissues, high-expression ZNF471 was present in 85.00% (n=34) of the patients (**Figure 1B**); low-expression ZNF471 was present in 15.00% (n=6)

Table 4. General information and pathologic characteristics of patients with gastric cancer for IHC analysis

| Information and pathological characteristic | | Cases (n=40) | Ratio |
|---|---|---|---|
| Sex | Male | 30 | 75.00% |
| | Female | 10 | 25.00% |
| Age | ≥60 | 29 | 72.50% |
| | <60 | 11 | 27.50% |
| Maximum diameter of tumor | ≥5 | 13 | 32.50% |
| | <5 | 27 | 67.50% |
| Lymph node metastasis | Positive | 26 | 65.00% |
| | Negative | 14 | 35.00% |
| Distant metastasis | Positive | 2 | 5.00% |
| | Negative | 38 | 95.00% |
| Pathological type | Adenocarcinoma | 37 | 92.50% |
| | Else | 3 | 7.50% |
| TNM staging | Tis or I-II | 14 | 35.00% |
| | III-IV | 26 | 65.00% |

Table 5. Expression of ZNF471 protein in GC and corresponding adjacent tissues

| | Expression of ZNF471 | Cases | Percentage |
|---|---|---|---|
| GC tissues | High expression | 11 | 27.50% |
| | Low expression | 29 | 72.50% |
| Adjacent tissue | High expression | 34 | 85% |
| | Low expression | 6 | 15% |

(**Figure 1D**). A within-patient paired $X^2$-test was performed on GC and adjacent tissues, and the result was $P<0.001$. This result suggested that expression of ZNF471 protein in GC tissues was significantly lower than in corresponding adjacent tissues (**Table 6**).

*Expression of ZNF471 in fresh GC, adjacent, and normal tissues by qRT-PCR*

Relative expression of ZNF471 mRNA levels in cancer tissues, adjacent tissues from patients with GC, and normal gastric mucosa from healthy people were detected using qRT-PCR; GAPDH was used as an internal reference. The calculation method used was $2^{-\Delta\Delta Ct}$ ($\Delta Ct=Ct_{ZNF471}-Ct_{GAPDH}$), and the results met the assumptions of a normal distribution. The t-test results (**Figure 2**) were: 1) The level of expression of ZNF471 mRNA in GC tissues was significantly lower than in corresponding adjacent tissues ($P<0.001$). 2) The level of ZNF471 mRNA expression in GC was significantly lower than that in normal gastric mucosa of healthy people ($P<0.001$). 3) There was no significant difference in the level of ZNF471 expression between adjacent tissues of patients with GC and normal gastric mucosa of healthy people ($P=0.267$).

*Relationships between clinicopathologic features and ZNF471 mRNA expression*

The results of the analysis of the relationships between expression of ZNF471 mRNA in GC and clinicopathologic features indicated that ZNF471 mRNA expression in GC was associated with smaller tumors, pre-existing lymph node metastasis, and high TNM stage (all $P<0.05$) (**Table 7**). There were no significant correlations with gender, age, distant metastasis, or pathologic type ($P>0.05$).

*Expression of ZNF471 in fresh GC, adjacent, and normal tissues by western blot*

The six cases of GC, six cases of paracancerous tissues, and six cases of normal tissues were subjected to western blot analysis. Expression of ZNF471 in GC was significantly lower than that in paracancerous and normal tissues (**Figure 3**). This western blot result suggested that ZNF471 had a tumor suppression function in GC. The western blot findings were consistent with the immunofluorescence results.

## Discussion

ZNF471 is a tumor suppressor discovered in recent years. ZNF471 is associated with malignant tumors, such as GC, colorectal cancer, esophageal cancer, tongue squamous cell carcinoma, breast cancer, cervical cancer, and non-small cell lung cancer. Tao suggests that ZNF471 inhibited MDA-MB-231, MDA-MB-468, and MCF-7cell growth, induced cell apoptosis, and arrested the cell cycle. Furthermore, Sun's results demonstrate that ZNF471 is an important tumor suppressor and loss of ZNF471 functions hampers MAPK10/JNK3 signaling

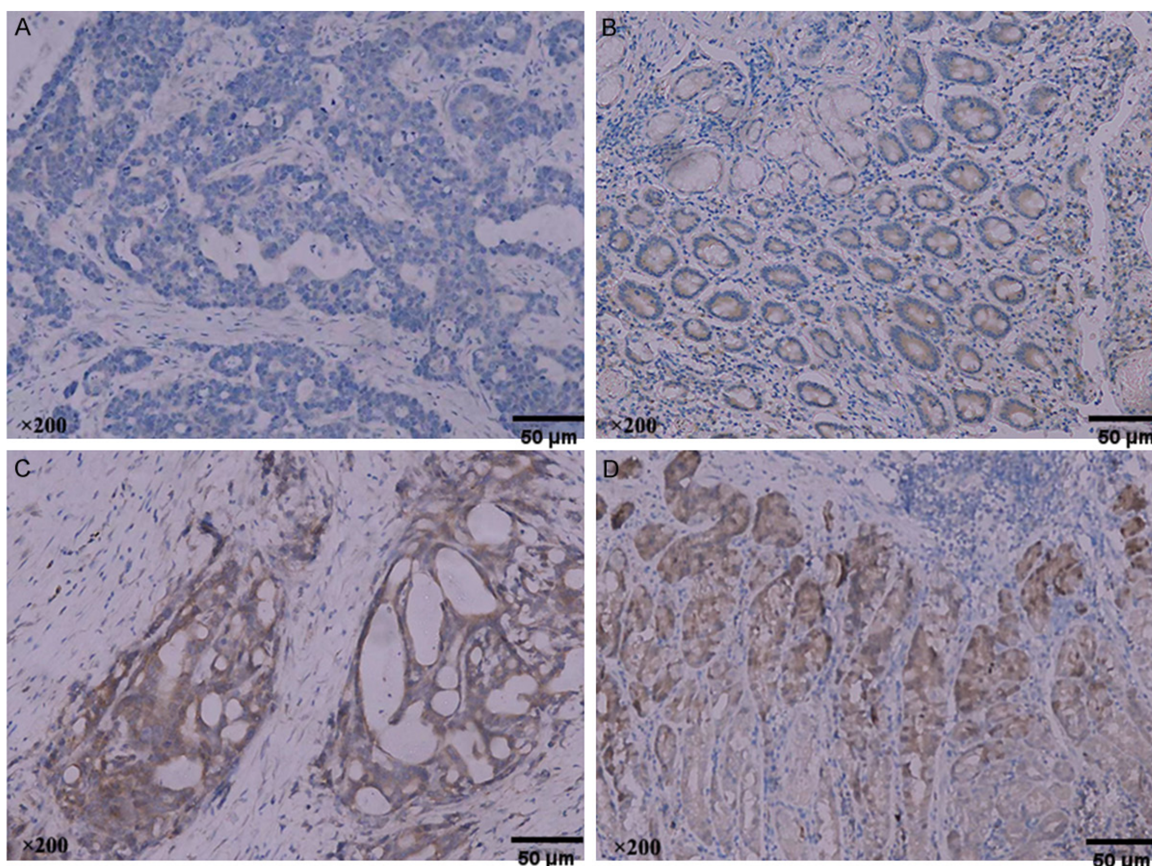


**Figure 1.** Immunohistochemistry showed that the expression of ZNF471 in gastric cancer was significantly lower than that in adjacent tissues. A. ZNF471 showed low expression in gastric cancer tissues (200×). B. ZNF471 showed low expression in the adjacent tissues (200×). C. ZNF471 showed high expression in gastric cancer tissues (200×), mainly localized to the nucleus and cytoplasm. D. ZNF471 showed high expression in the adjacent tissues (200×), mainly localized to the nucleus and cytoplasm.

**Table 6.** Paired $\chi^2$ test results in gastric cancer and corresponding adjacent tissues

| Cancer | Adjacent | | Total | *P* |
|---|---|---|---|---|
| | High expression | Low expression | | |
| High expression | 10 | 24 | 34 | <0.001 |
| Low expression | 2 | 4 | 6 | |
| Total | 12 | 28 | 40 | |

during esophageal carcinogenesis [16-18, 20-23]. Until now, there has been almost no relevant clinical research on the relationships between expression levels of ZNF471 and GC treatment and diagnosis. Its functions and mechanisms in gastric cancer had been unclear. Therefore, we sought clinical results that may support a relationship between ZNF471 and gastric carcinoma.

Immunohistochemical analysis found expression of ZNF471 in GC tissue was significantly lower than that of adjacent tissue ($P<0.001$). Analyses using qRT-PCR and western blot found that expression of ZNF471 was decreased in fresh GC tissues ($P<0.001$). Since downregulation of ZNF471 was observed in gastric cancer and not in normal gastric tissues, ZNF471 may function as a tumor suppressor. These results were consistent with Cao's *in vitro* GC cell line results. Furthermore, to investigate the clinical relevance of ZNF471 expression in GC, we measured the expression levels and clinicopathologic characteristics, of ZNF471 in 40 pairs of GC and corresponding non-cancerous tissues. We also analyzed ZNF471 expression and clinicopathologic characteristics and found expression of ZNF471 mRNA was related to tumor size, lymph node metastasis, and TNM stage ($P<0.05$). However, there were no significant correlations with gen-

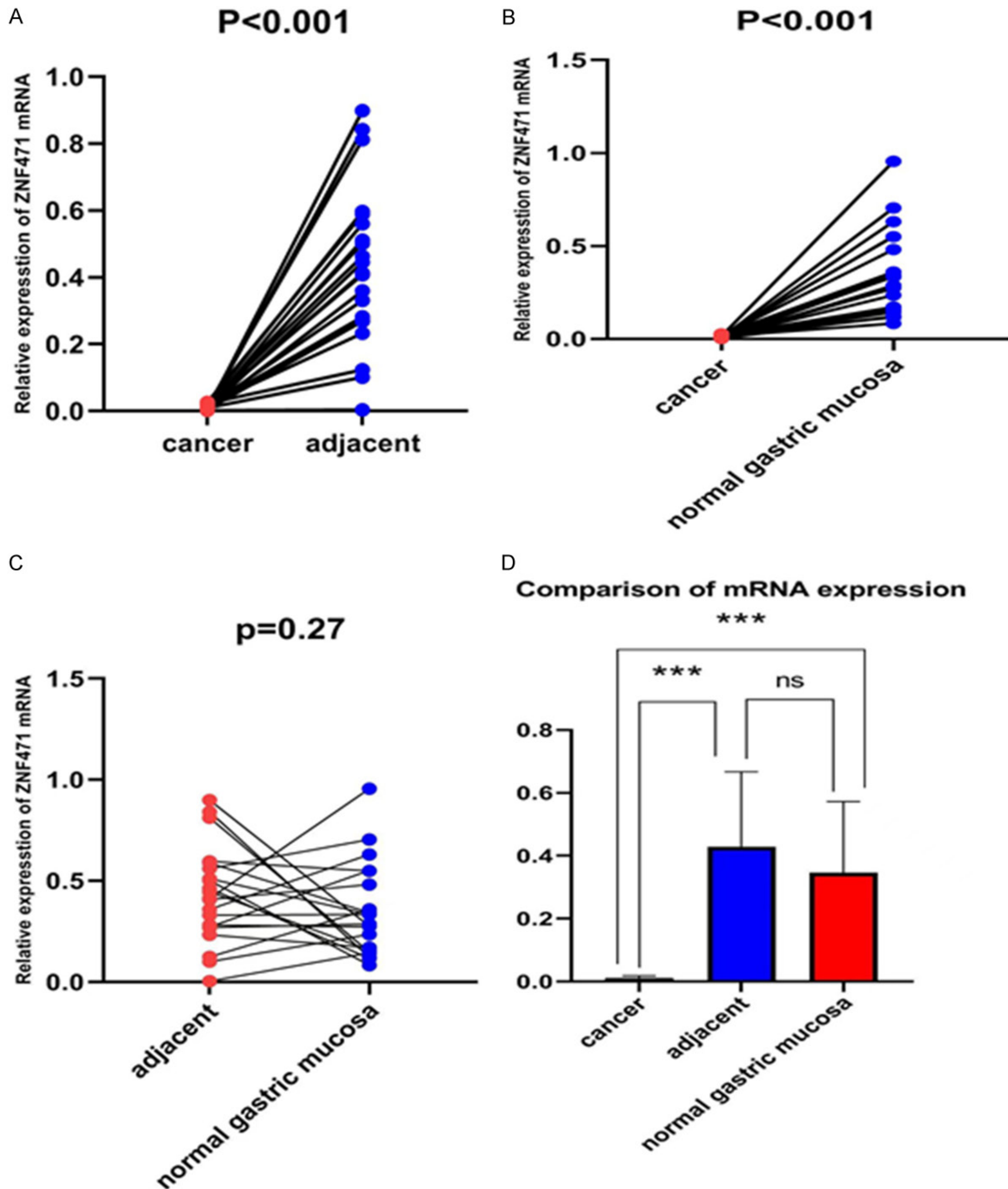


Figure 2. Relative expression of ZNF471 mRNA in tissues for gastric cancer, adjacent tissues, and normal gastric mocusa. A. Relative expression of ZNF471 mRNA between gastric cancer and adjacent tissues. B. Relative expression of ZNF471 mRNA between gastric cancer and normal gastric mocusa. C. Relative expression of ZNF471 mRNA between adjacent tissues and normal gastric mocusa. D. Comparison of ZNF471 mRNA expression in gastric cancer, adjacent tissues, and normal gastric mocusa.

der, age, distant metastasis, or pathologic type ($P>0.05$).

This study was novel because we examined a patient population with clinical GC, and found that the level of ZNF471 expression was significantly decreased in the GC tissues from these patients; expression levels were correlated with tumor size, lymph node metastasis, and TNM stage. These results suggested that ZNF471 may be a biomarker for GC diagnosis, treatment, and prognosis.

**Table 7.** Relationship between ZNF471 mRNA expression and pathologic characteristics in patients with gastric cancer

| Factor | Cases | Mean ± SD | t | *P* |
|---|---|---|---|---|
| Gender | | | 1.07 | 0.30 |
| Male | 14 | 12.33±7.79 | | |
| Female | 7 | 8.68±6.34 | | |
| Age (years) | | | 1.93 | 0.07 |
| <60 | 14 | 13.18±7.55 | | |
| ≥60 | 7 | 6.99±5.38 | | |
| Tumor size (cm) | | | 3.29 | <0.01 |
| ≥5 cm | 11 | 15.25±6.85 | | |
| <5 cm | 10 | 6.56±5.02 | | |
| Lymph node metastasis | | | 2.19 | 0.04 |
| Yes | 16 | 5.33±5.06 | | |
| No | 5 | 12.92±7.17 | | |
| Distant metastasis | | | 0.03 | 0.98 |
| Yes | 2 | 11.10±7.52 | | |
| No | 19 | 11.26±8.61 | | |
| Pathologic type | | | 0.61 | 0.55 |
| Adenocarcinoma | 17 | 11.60±7.42 | | |
| Others | 4 | 9.06±7.98 | | |
| TNM | | | 2.57 | 0.02 |
| Tis or I-II | 7 | 13.70±7.14 | | |
| III-IV | 14 | 5.94±4.99 | | |

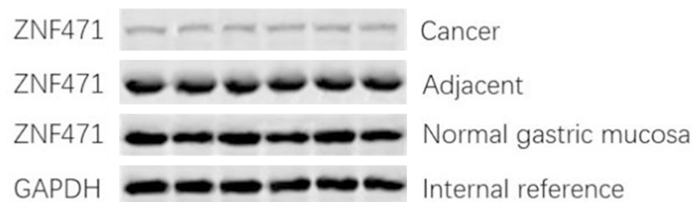


**Figure 3.** Western blot analysis of ZNF471 protein level in tissues of gastric cancer, adjacent tissues, and normal gastric mucosa.

This study had some limitations. First, the small number of tissue samples might have affected the experimental results. Second, due to the small size needed for some tissue samples to ensure accurate qPCR results, only the larger tissues could be extracted for western blot analysis to detect the expression of ZNF-471 protein in fresh tissues. Because the study was time-limited, the relationship between expression and the 5-year survival rate remains unclear.

Similar to our results, other studies found a correlation between the expression of ZNF139 in GC and pathologic characteristics, followed by a decrease in differentiation; after that, expression of ZNF139 then increases. Expression of ZNF139 in GC stages I and II were significantly lower than in stage III. Expression of ZNF139 in GC limited to the T1/T2 phase was significantly lower than that in the T3/T4 phase. Expression of GC without lymph node metastasis was significantly lower than that with lymph node metastasis [24]. These findings suggest that the levels of ZFP expression during development of GC are also different. We speculate that ZNF471 has similar characteristics in GC. That is, ZNF471 has specific differences in expression in normal gastric mucosal tissue, chronic atrophic gastritis, gastric intraepithelial neoplasia, and GC, or the co-methylation of the promoter CpG site is different. Therefore, we plan to continue to expand the sample size and collect chronic atrophic gastritis and intraepithelial neoplasia tissue samples for further detection to examine whether ZNF471 is differentially expressed during the progression of GC.

We will increase the sample size to continue the research and follow up with the enrolled patients to further study the clinical significance of ZNF471 in GC and its effects on prognosis. Samples of chronic atrophic gastritis and gastric intraepithelial neoplasia were collected to examine the role of ZNF471 in the clinical carcinogenesis of GC. In addition, we will knock down, knock out and overexpress ZNF471 in gastric cancer cells to demonstrate the role and significance of ZNF471 in different stages of gastric cancer development by in vitro experiments.

## Conclusion

Our analyses using clinical samples supported a relationship between ZNF471 and GC. Immunohistochemistry found that expression of ZNF471 in the wax block tissue of GC was markedly lower than that of adjacent tissue. The real-time PCR and western blot assay findings revealed that expression of ZNF471 in GC tissues was significantly lower than that in cor-

responding adjacent tissues and normal gastric mucosa tissues. Expression of ZNF471 was associated with tumor size, lymph node metastasis, and TNM stage, but not with gender, age, distant metastasis, or pathologic type. Expression of ZNF471 in paraneoplastic tissues from patients with GC was not obviously changed, compared to normal gastric mucosal tissues. In conclusion, these findings suggest ZNF471 has potential as a novel diagnostic and therapeutic target in patients with GC.

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**Disclosure of conflict of interest**

None.

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