Research Article

ZNF521 Is Correlated with Tumor Immune Cell Infiltration and Act as a Valuable Prognostic Biomarker in Gastric Cancer

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Aim. To explore the correlations between the expression of zinc finger protein 521 (ZNF521) with immune invasion and prognosis of gastric cancer. Methods. Expression of ZNF521 was examined by immunohistochemistry in gastric cancer cases. Kaplan–Meier plotter was used to determine the relationships between ZNF521 and prognosis. TIMER and GEPIA were used to analyze the correlation between ZNF521 expression and gene markers of immune cell infiltration. Results. The expression of ZNF521 was up-regulated in gastric cancer samples. Kaplan–Meier analysis indicated that higher expression of ZNF521 was associated with poor prognosis. The expression of ZNF521 was correlated with infiltrating levels of CD4+ T and CD8+ T cells, macrophages, neutrophils, and dendritic cells in gastric cancer, which also correlated with diverse immune marker sets. Conclusions. ZNF521 is correlated significantly with immune cell infiltration and is a valuable biomarker for prognosis in gastric cancer.

1. Introduction

The incidence and mortality rate of gastric cancer ranked 5th and 3rd worldwide, respectively, in 2018, and their respective rates in China were 44.1% and 49.9% [1]. In recent years, studies have shown that cancer is closely related to autoimmunity, and immunotherapy is a new treatment method that has attracted extensive attention in the field of cancer treatment [2–5]. While immune infiltration in the tumor microenvironment is the basis of immunotherapy and plays a key role in tumorigenesis and development, it also affects patient prognosis [6]. Immune checkpoint inhibitors that target Programmed Cell Death Protein 1 (PD-1) or Programmed Cell Death Ligand 1 have greatly improved outcomes for patients with many types of cancer; however, only 20–40% of patients benefit from these therapies [7]. Therefore, it is necessary to improve the efficacy of immunotherapy and to find indicators of immune infiltration and explore their underlying mechanisms of activity.

Zinc finger protein 521 (ZNF521) encodes a transcription factor with a zinc finger domain that is widely expressed in many tissues and plays an important role in the development and function of immune cells, macrophages, and dendritic cells in gastric cancer, which also correlated with diverse immune marker sets. Conclusions. ZNF521 is correlated significantly with immune cell infiltration and is a valuable biomarker for prognosis in gastric cancer.
invasion of gastric cancer cells via regulating microRNA-204-5p [12].

In this study, we detected ZNF521 expression in gastric cancer by immunohistochemistry (IHC) and analyzed the correlation between its expression with pathological parameters and prognosis. Kaplan–Meier plotter databases were used to analyze the expression of ZNF521 and its correlation with the prognosis of gastric cancer. Moreover, we analyzed the correlation between ZNF521 expression and infiltrating immune cells in different tumor microenvironments through the tumor immune database TIMER. ZNF521 promotes the malignant characteristics of gastric cancer cells in part by interacting with immune infiltrating cells. The findings of this report elucidate the important role of ZNF521 in stomach adenocarcinoma (STAD) and suggest a potential relationship and mechanistic link between ZNF521 and tumor immune interaction. We focused on ZNF521 expression and its relationship with clinicopathological features and prognosis to reveal the relationship between ZNF521 and tumor immune infiltration.

2. Materials and Methods

2.1. Immunohistochemistry. Tissue microarrays were performed as described in our previous study [13]. This study was approved and monitored by the ethics committee of Zhejiang Provincial People’s Hospital. Streptavidin-peroxidase and high pressure immunohistochemical methods were adopted to examine antibody expression. All formalin-fixed, paraffin-embedded tissue sections were deparaffinized in an oven at 60°C overnight, and then further dewaxed in xylene. The pressure cooker antigen repairing method in citrate buffer solution was performed, and then 3% hydrogen peroxide was used to inhibit endogenous peroxidases. Sections were incubated with mouse anti-ZNF521 (1 : 1,000; Novus Biologicals, Littleton, CO, USA) overnight at 4°C.

2.2. Kaplan–Meier Plotter Database. The Kaplan–Meier Plotter can assess the effect of 54,000 genes (mRNAs, miRNAs, and protein-coding) on survival in 21 cancer types including breast (n = 7,830), ovarian (n = 2,190), lung (n = 3,452), and gastric (n = 1,440) cancers. Sources for the databases included the Gene Expression Omnibus, European Genome-Phenome Archive, and The Cancer Genome Atlas (TCGA). The primary purpose of the tool is meta-analysis-based discovery and validation of survival biomarkers. We used this database to assess the relationship between TNF521 expression and patient outcomes (http://kmplot.com/analysis/) [14, 15]. The log-rank test was used for statistical analyses, with P < 0.05 was considered significant differences.

2.3. TIMER Database. TIMER (https://cistrome.shinyapps.io/timer/) is a database designed for analyzing immune cell infiltrates in multiple cancer types [16]. It includes 10,897 samples across 32 different cancer types from TCGA. The correlation between ZNF521 expression levels and immune infiltrates including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (DCs) were explored using the gene module in various cancer types [17, 18]. The correlation between genetic markers of tumor-infiltrating immune cells and ZNF521 expression was analyzed using the correlation module [19–22]. ZNF521 expression was plotted on the x-axis, and the expression of related marker genes were represented as gene symbols on the y-axis.
| Clinical parameters                  | ZNF521 Low | ZNF521 High | \( t/\chi^2 \) | P-value |
|-------------------------------------|------------|-------------|----------------|---------|
| Age (years)                         | 58.21 ± 11.21 | 59.64 ± 12.71 | -1.205       | 0.229   |
| **Gender**                          |            |             |                |         |
| Male                                | 124 (39.9%) | 187 (60.1%) | 0.894         | 0.201   |
| Female                              | 56 (44.8%)  | 69 (55.2%)  |                |         |
| **Location**                        |            |             | 2.819         | <0.01   |
| Proximal                            | 17 (30.9%)  | 38 (69.1%)  |                |         |
| Middle                              | 69 (42.3%)  | 94 (57.7%)  |                |         |
| Distal                              | 94 (43.1%)  | 124 (56.9%) |                |         |
| **Size**                            |            |             | 6.917         | 0.006   |
| <5 cm                               | 119 (46.5%) | 137 (53.5%) |                |         |
| \( \geq 5 \) cm                     | 61 (33.9%)  | 119 (66.1%) |                |         |
| **Lauren classification**           |            |             | 9.616         | 0.001   |
| Intestinal                          | 108 (48.4%) | 115 (51.6%) |                |         |
| Diffuse                             | 72 (33.8%)  | 141 (66.2%) |                |         |
| **Histology classification**        |            |             | 4.326         | <0.01   |
| Papillary adenocarcinoma            | 4 (25.0%)   | 12 (75.0%)  |                |         |
| Tubular adenocarcinoma              | 131 (40.2%) | 195 (59.8%) |                |         |
| Mucinous adenocarcinoma             | 12 (41.4%)  | 17 (58.6%)  |                |         |
| Signet-ring cell carcinoma          | 33 (50.8%)  | 32 (49.2%)  |                |         |
| **Histologic differentiation**      |            |             | 1.572         | <0.01   |
| Well                                | 6 (46.2%)   | 7 (53.8%)   |                |         |
| Moderately                          | 52 (40.6%)  | 76 (59.4%)  |                |         |
| Poorly                              | 122 (41.6%) | 171 (58.4%) |                |         |
| Others                              | 0 (0.0%)    | 2 (100.0%)  |                |         |
| **Invasion depth**                  |            |             | 1.486         | <0.01   |
| T1                                  | 27 (47.4%)  | 30 (52.6%)  |                |         |
| T2                                  | 47 (43.1%)  | 62 (56.9%)  |                |         |
| T3                                  | 96 (39.3%)  | 148 (60.7%) |                |         |
| T4                                  | 10 (38.5%)  | 16 (61.5%)  |                |         |
| **Lymphatic metastasis**            |            |             | 1.200         | 0.160   |
| No                                  | 74 (44.6%)  | 92 (55.4%)  |                |         |
| Yes                                 | 106 (39.3%) | 164 (60.7%) |                |         |
| **Regional lymph nodes**            |            |             | 4.297         | <0.01   |
| PN0                                 | 74 (44.6%)  | 92 (55.4%)  |                |         |
| PN1                                 | 57 (41.9%)  | 79 (58.1%)  |                |         |
| PN2                                 | 40 (40.4%)  | 59 (59.6%)  |                |         |
| PN3                                 | 9 (25.7%)   | 26 (74.3%)  |                |         |
| **Distant metastasis**              |            |             | 6.631         | 0.007   |
| No                                  | 164 (43.7%) | 211 (56.3%) |                |         |
| Yes                                 | 16 (26.2%)  | 45 (73.8%)  |                |         |
| **TNM stages**                      |            |             | 6.783         | <0.01   |
| I                                   | 42 (46.7%)  | 48 (53.3%)  |                |         |
| II                                  | 45 (43.3%)  | 59 (56.7%)  |                |         |
| III                                 | 74 (42.8%)  | 99 (57.2%)  |                |         |
| IV                                  | 19 (27.5%)  | 50 (72.5%)  |                |         |

Bold values indicate that statistical significance was set at \( P < 0.05 \).
Figure 2: Continued.
Correlation coefficients were estimated using Spearman’s correlation test. Gene expression levels were shown as log2 RNA-seq by Expectation-Maximization.

2.4. GEPIA Database. GEPIA is an online database that facilitates the standardized analysis of RNA-seq data from 9,736 tumor samples and 8,587 normal control samples from TCGA and Genotype-Tissue Expression data sets (http://gepia.cancer-pku.cn/index.html) [23]. The RNA-seq data of all 9,736 tumor samples and 8,587 normal control samples were analyzed. Therefore, we used the database to assess the association between ZNF521 expression and prognosis of gastric cancer in the Kaplan–Meier plotter databases (K–M) survival curves of OS, FP, and PPS in Gastric cancer.

3. Results

3.1. ZNF521 Expression in Gastric Cancer and Normal Tissues. We analyzed ZNF521 expression in normal and tumor tissues by IHC. ZNF521 expression levels in gastric cancer tissues were 58.7% (256/436), which was higher than in normal tissues (20.6% [19/92]). ZNF521 was primarily localized within the cytoplasm of cancer cells, and ZNF521 expression in non-tumor mucosa was also recorded (Figure 1). According to the IHC scores, ZNF521 expression was higher in gastric cancer tissues than in non-tumor gastric tissues. There was also a significant difference between the gastric cancer group and non-tumor mucosa (P < 0.05).

3.2. Relationship between ZNF521 Expression and Clinicopathological Parameters. To explore the clinical significance of ZNF521 in gastric cancer and to analyze correlations between ZNF521 expression and the clinical characteristics of gastric cancer patients, patients were divided into two groups according to their ZNF521 expression levels: the high and low ZNF521 expression groups. ZNF521 was highly expressed in 58.7% of patients, and its expression was significantly correlated with tumor
Table 2: The correlation of ZNF521 mRNA expression and clinical prognosis in gastric cancer with different clinicopathological factors by Kaplan–Meier plotter.

| Clinicopathological characteristics | N  | OS n = 881 | HR  | P-value | N  | FP n = 645 | HR  | P-value | N  | PPS n = 503 | HR  | P-value |
|------------------------------------|----|------------|-----|---------|----|------------|-----|---------|----|------------|-----|---------|
| Gender                             |    |            |     |         |    |            |     |         |    |            |     |         |
| Female                             | 236| 2.41 (1.47–3.96) | 0.00031 | 201 | 2.24 (1.38–3.63) | 0.0008 | 149 | 2.61 (1.49–4.59) | 0.00053 |
| Male                               | 544| 1.71 (1.27–2.31) | 0.00036 | 437 | 1.67 (1.24–2.23) | 0.00056 | 348 | 1.86 (1.32–2.63) | 3.00 × 10⁻⁴ |
| Stage                              |    |            |     |         |    |            |     |         |    |            |     |         |
| 1                                  | 67 | 1.76 (0.57–5.42) | 0.32 | 60 | 1.68 (0.54–5.2) | 0.36 | 31 |            |    |            |     |         |
| 2                                  | 140| 2.18 (1.13–4.19) | 0.017 | 131 | 1.99 (1.06–3.73) | 0.029 | 105 | 3.33 (1.65–6.71) | 0.0037 |
| 3                                  | 205| 1.44 (0.98–2.13) | 0.064 | 186 | 1.63 (1.12–2.39) | 0.011 | 142 | 1.6 (1.02–2.5) | 0.037 |
| 4                                  | 148| 1.85 (1.24–2.75) | 0.0022 | 141 | 1.44 (0.98–2.11) | 0.064 | 104 | 1.84 (1.17–2.91) | 0.0076 |
| Stage T                            |    |            |     |         |    |            |     |         |    |            |     |         |
| 1                                  | 14 |            |     |         | 14 |            |     |         |    |            |     |         |
| 2                                  | 241| 1.9 (1.24–2.9) | 0.0025 | 239 | 1.71 (1.13–2.58) | 0.0099 | 196 | 2.29 (1.46–3.59) | 0.0019 |
| 3                                  | 204| 1.22 (0.83–1.78) | 0.37 | 204 | 0.81 (0.56–1.18) | 0.27 | 150 | 1.27 (0.85–1.89) | 0.25 |
| 4                                  | 38 | 3.38 (1.37–8.32) | 0.0051 | 39 | 2.7 (1.19–6.14) | 0.014 | 29 |            |    |            |     |         |
| Stage N                            |    |            |     |         |    |            |     |         |    |            |     |         |
| 0                                  | 74 | 2.15 (0.93–4.98) | 0.068 | 72 | 2.05 (0.89–4.74) | 0.086 | 41 | 4.09 (1.25–13.37) | 0.012 |
| 1                                  | 225| 2.28 (1.46–3.53) | 2.00 × 10⁻⁴ | 222 | 2 (1.32–3.03) | 0.00082 | 169 | 1.98 (1.47–2.66) | 4.40 × 10⁻⁶ |
| 2                                  | 121| 1.53 (0.95–2.47) | 0.08 | 125 | 1.6 (1.03–2.48) | 0.036 | 105 | 2.68 (1.64–4.37) | 4.10 × 10⁻⁵ |
| 3                                  | 76 | 2.05 (1.16–3.63) | 0.012 | 76 | 1.8 (1.01–3.19) | 0.046 | 63 | 1.55 (0.94–2.53) | 0.081 |
| 1 + 2 + 3                          | 422| 1.89 (1.43–2.49) | 4.30 × 10⁻⁶ | 423 | 1.77 (1.36–2.3) | 1.40 × 10⁻⁵ | 437 | 1.91 (1.07–3.42) | 0.026 |
| Stage M                            |    |            |     |         |    |            |     |         |    |            |     |         |
| 0                                  | 444| 1.84 (1.38–2.46) | 2.40 × 10⁻⁵ | 443 | 1.77 (1.34–2.32) | 3.80 × 10⁻⁵ | 342 | 2.03 (1.5–2.74) | 2.50 × 10⁻⁶ |
| 1                                  | 56 | 1.9 (1.05–3.44) | 0.031 | 56 | 1.54 (0.85–2.79) | 0.15 | 36 | 2.65 (1–7.06) | 0.043 |
| Lauren classification              |    |            |     |         |    |            |     |         |    |            |     |         |
| Intestinal                         | 320| 1.74 (1.21–2.5) | 0.0024 | 263 | 1.7 (1.2–2.42) | 0.0026 | 192 | 1.75 (1.16–2.63) | 0.007 |
| Diffuse                            | 241| 1.71 (1.15–2.54) | 0.0079 | 231 | 1.86 (1.24–2.8) | 0.0024 | 176 | 1.89 (1.29–2.77) | 0.00095 |
| Mixed                              | 32 | 3.32 (1.09–10.12) | 0.026 | 28 | 1.9(0.54–6.73) | 0.31 | 16 |            |    |            |     |         |
| Differentiation                    |    |            |     |         |    |            |     |         |    |            |     |         |
| Poor                               | 165| 1.22 (0.75–1.97) | 0.42 | 121 | 1.23 (0.73–2.05) | 0.44 | 49 | 1.31 (0.69–2.48) | 0.41 |
| Moderate                           | 67 | 1.55 (0.79–3.03) | 0.19 | 67 | 1.9 (0.99–3.65) | 0.05 | 24 | 0.42 (0.16–1.16) | 0.086 |
| Well                               | 32 |            |     |         | 5  |            |     |         |    |            |     |         |

Bold values indicate that statistical significance was set at P < 0.05.

location, tumor size, Lauren classification, histological classification, histological differentiation, depth of invasion, regional lymph nodes, distant metastasis, and tumor node metastasis (TNM) stage (P < 0.01; Table 1). ZNF521 expression was not significantly correlated with age or sex (P > 0.05; Table 1).

3.3. Correlation between ZNF521 Expression and Prognosis. The Kaplan–Meier method was used to analyze the effects of ZNF521 on the prognosis of gastric cancer patients. The results indicated that patients with high ZNF521 expression were associated with poor prognosis, and the 5-year survival rate of patients with low ZNF521 expression was significantly higher than that of patients with high ZNF521 expression (Figure 2(a)). Kaplan–Meier curves and univariate analysis (log-rank) were further used to analyze the correlation between ZNF521 expression and patient prognosis according to TNM stage. In stage III gastric cancer, 5-year survival was significantly lower in patients with high ZNF521 expression compared with patients with low expression (P < 0.05; Figure 2(c)). For stage I, II, and IV disease, ZNF521 expression was not correlated with 5-year survival (P > 0.05; Figures 2(b) and 2(d)).

The prognostic value of ZNF521 on the basis of Affymetrix microarray data in gastric cancer was evaluated using the Kaplan–Meier Plotter database. High ZNF521 expression was associated with poor prognosis in gastric cancer (overall survival [OS]: HR = 1.79, 95% CI = 1.43–2.24, P = 3.1 × 10⁻⁷; progression-free survival [PPS]: HR = 1.72, 95% CI = 1.35–2.2, P = 1.2 × 10⁻⁵; post-progression survival [PPS]: HR = 1.94, 95% CI = 1.48–2.56, P = 1.4 × 10⁻⁶; Figures 2(e)–2(g)). The results of analysis of these databases showed that increased ZNF521 expression had a poor prognostic value in gastric cancer.
3.4. Effect of ZNF521 Overexpression on the Prognosis of Gastric Cancer Patients with Lymphatic Metastasis. We used the Kaplan–Meier Plotter database to investigate the relationship between ZNF521 expression and the clinical characteristics of gastric cancer patients. The results showed that higher expression of ZNF521 was associated with sex, stage, and Lauren classification, but not with differentiation (\( p < 0.05 \)). Specifically, higher ZNF521 expression was correlated with worse OS in stage II (HR = 2.18, 95% CI = 1.13–4.19, \( p = 0.017 \)) and stage IV (HR = 1.85, 95% CI = 1.24–2.75, \( p = 0.0022 \)) patients, with PFS in stage II (HR = 1.99, 95% CI = 1.06–3.73, \( p = 0.029 \)) and stage III (HR = 1.63, 95% CI = 1.12–2.39, \( p = 0.011 \)) patients, with PPS in stage II, III, and IV patients (HR = 3.33, 95% CI = 1.65–6.71, \( p = 0.00037 \); HR = 1.6, 95% CI = 1.02–2.5, \( p = 0.037 \); and HR = 1.84, 95% CI = 1.17–2.91, \( p = 0.0076 \), respectively), but was not associated with OS, PFS, or PPS in stage I patients or OS and PPS in stage N0 patients. In particular, we analyzed the prognostic value of ZNF521 expression in gastric cancer patients with lymphatic metastasis. Higher ZNF521 expression was correlated with worse OS in stage N1 (HR = 2.28, 95% CI = 1.46–3.55, \( p = 2.00 \times 10^{-5} \)) and stage N3 (HR = 2.05, 95% CI = 1.16–3.63, \( p = 0.012 \)) patients, worse PFS in stage N1, N2, and N3 patients (HR = 2, 95% CI = 1.32–3.03, \( p = 0.00082 \); HR = 1.6, 95% CI = 1.03–2.48, \( p = 0.036 \); and HR = 1.8, 95% CI = 1.01–3.19, \( p = 0.046 \), respectively), worse PPS in stage N0, N1, and N2 patients (HR = 4.09, 95% CI = 1.25–13.37, \( p = 0.012 \); HR = 1.98, 95% CI = 1.47–2.66, \( p = 4.40 \times 10^{-6} \); and HR = 2.68, 95% CI = 1.64–4.37, \( p = 4.10 \times 10^{-5} \), respectively; Table 2). Thus, ZNF521 expression may affect the prognosis of gastric cancer patients by promoting lymph node metastasis.

3.5. Relationship between ZNF521 Expression and Tumor Immune Infiltration. We next analyzed the relationship between ZNF521 expression and immune infiltration levels in 39 tumor types using the TIMER database (Figure S1). The results showed that ZNF521 expression was significantly correlated with tumor purity in 25 cancer types, B-cell infiltration levels in 23 cancer types, CD4+ T cells in 23 cancer types, CD4+ T cells in 21 cancer types, DCs in 23 cancer types, macrophages in 31 cancer types, and neutrophils in 25 cancer types (Table S1).

Interestingly, we found that ZNF521 expression was associated with higher immune infiltration in gastric cancer patients, worse PFS in stage N1, N2, and N3 patients (HR = 2, 95% CI = 1.32–3.03, \( p = 0.00082 \); HR = 1.6, 95% CI = 1.03–2.48, \( p = 0.036 \); and HR = 1.8, 95% CI = 1.01–3.19, \( p = 0.046 \), respectively), worse PPS in stage N0, N1, and N2 patients (HR = 4.09, 95% CI = 1.25–13.37, \( p = 0.012 \); HR = 1.98, 95% CI = 1.47–2.66, \( p = 4.40 \times 10^{-6} \); and HR = 2.68, 95% CI = 1.64–4.37, \( p = 4.10 \times 10^{-5} \), respectively; Table 2). Thus, ZNF521 expression may affect the prognosis of gastric cancer patients by promoting lymph node metastasis.
3.6. Correlation Analysis between ZNF521 Expression and Immune Marker Sets. To further investigate the relationship between ZNF521 expression and the infiltration of different immune cell types, we analyzed the correlation between ZNF521 expression and markers of different immune cells, including CD8+ T cells, total T cells, B cells, monocytes, tumor-associated macrophages (TAMs), M1 and M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, regulatory T cells (Tregs), and exhausted T cells (Tex) in gastric cancer (Table 3). The results showed that ZNF521 expression was significantly related to most of the markers for various immune cells and T cells in gastric cancer.

ZNF521 expression also had a strong correlation with markers of DCs, such as HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1 (CD1c), BDCA-4 (NRPI), and CD11c (ITGAX; Table 3). ZNF521 expression was also significantly correlated with marker genes of Tex, such as PD-1 (PDCD1), CTLA4, LAG3, TIM-3 (HAVC2), and GZMB (Table 3). We also found that most markers of monocytes, TAMs, and M2 macrophages were significantly correlated with ZNF521 expression, but not with the expression of M1 marker genes (Table 3 and Figure 4). We further analyzed the correlation between ZNF521 expression and markers of monocytes, TAMs, and M1 and M2 macrophages in the GEPIA database of gastric cancer (Table S2). These results suggested that ZNF521 may regulate the polarization of macrophages in gastric cancer.

| Description | Gene markers | STAD purity cor | STAD purity P-value | Description | Gene markers | STAD purity cor | STAD purity P-value |
|-------------|--------------|-----------------|---------------------|-------------|--------------|-----------------|---------------------|
| CD8+ T cells | CD8A -0.219772 *** | | | | | | |
| | CD8B -0.121084 * | | | | | | |
| T cells (general) | CD3D -0.315001 *** | | | | | | |
| | CD3E -0.334622 *** | | | | | | |
| | CD2 -0.302668 *** | | | | | | |
| B cells | CD19 -0.218492 *** | | | | | | |
| | CD79A -0.268382 *** | | | | | | |
| Monocytes | CD86 -0.285611 *** | | | | | | |
| | CD115 (CSF1R) -0.208257 *** | | | | | | |
| | CCL2 -0.204723 *** | | | | | | |
| | CD68 -0.159045 * | | | | | | |
| | IL10 -0.23574 *** | | | | | | |
| M1 macrophage | INOS (NOS2) -0.094312 0.066282 | | | | | | |
| | IRF5 -0.111113 0.0303446 | | | | | | |
| | COX2 (PTGS2) -0.125946 0.0140167 | | | | | | |
| M2 macrophage | CD163 -0.190151 *** | | | | | | |
| | VSG4 -0.16597 * | | | | | | |
| | MS4A4A -0.190671 *** | | | | | | |
| Neutrophils | CD66b (CEACAM8) 0.0206156 0.688722 | | | | | | |
| | CD11b (ITGAM) -0.16399 * | | | | | | |
| | CCR7 -0.291648 *** | | | | | | |
| Natural killer cells | KIR2DL1 -0.075615 0.1365359 | | | | | | |
| | KIR2DL3 -0.131514 0.0102763 | | | | | | |
| | KIR2DL4 -0.164984 * | | | | | | |
| | KIR3DL1 -0.124038 0.0155496 | | | | | | |
| | KIR3DL2 -0.161244 * | | | | | | |
| | KIR3DL3 -0.019629 0.7028965 | | | | | | |
| | KIR2DS4 -0.121695 0.0176306 | | | | | | |
| | KIR2DS5 -0.291648 *** | | | | | | |

**Table 3:** The correlation analysis between ZNF521 and relate genes and markers of immune cells in TIMER.

and poor prognosis. ZNF521 expression was positively correlated with B-cell infiltration \(r = 0.093, P = 0.074\), CD8+ T cells \(r = 0.232, P = 6.80 \times 10^{-6}\), CD4+ T cells \(r = 0.525, P = 2.49 \times 10^{-27}\), macrophages \(r = 0.651, P = 4.93 \times 10^{-46}\), neutrophils \(r = 0.317, P = 3.93 \times 10^{-10}\), and DCs \(r = 0.456, P = 2.09 \times 10^{-20}\) in gastric cancer (Figure 3). These findings strongly suggest that ZNF521 plays a specific role in immune infiltration in gastric cancer.

*; **, and *** indicate that statistical significance was set at \(P < 0.05\).
Figure 4: Continued.
4. Discussion

The tumor microenvironment plays an important role in the dynamic regulation of cancer progression. Therapeutic strategies targeting the tumor microenvironment have emerged as promising cancer treatments. Immunotherapy has been approved in clinical trials and has broad application prospects [24]. Broadly, recent findings, including those presented herein, support the conclusion that immune cells including T and B lymphocytes, TAMs, DCs, natural killer cells, neutrophils, and myeloid suppressor cells playing an important role in the outcomes of gastric cancer patients.

A comprehensive analysis of tumor-infiltrating immune cells will help elucidate the mechanisms of tumor immune escape and provide opportunities for the development of new therapeutic strategies. ZNF521 is a multifunctional transcription cofactor that regulates many biological processes, such as hematopoietic differentiation, cell proliferation, autophagy, inhibiting EBF1, and promoting the development of B-cell leukemia [11, 25–28]. In this study, we found that ZNF521 is a valuable prognostic biomarker that is significantly correlated with cancer immune infiltration. Through database analysis, we showed that high ZNF521 expression was significantly associated with poor survival outcomes in ovarian cancer, gastric cancer, colon adenocarcinoma, bladder cancer, lung squamous cell carcinoma, and thyroid cancer. Specifically, in gastric cancer, higher ZNF521 expression was correlated with worse OS in stage II and stage IV patients, PFS in stage II and stage III patients, PPS in stage II, III, and IV patients, but was not associated with OS, PFS, or PPS in stage I patients or OS and PFS in stage N0 patients.

ZNF521 expression was correlated with levels of infiltrating CD4+ and CD8+ T cells, macrophages, neutrophils, and DCs in bladder cancer, lung squamous cell carcinoma, and gastric cancer, and was also correlated with diverse immune markers. Additionally, the correlation between ZNF521 expression and immune cell markers indicates that ZNF521 regulates tumor immunity in bladder cancer, lung squamous cell carcinoma, and gastric cancer. First, markers of M1 macrophages, such as inducible nitric oxide synthase, interferon regulatory factor 5 (IRF5), and prostaglandin-endoperoxide synthase 2 (PTGS2) were not correlated or were only weakly correlated with ZNF521 expression, while markers of M2 macrophages, such as CD163, V-set immunoglobulin-domain-containing 4 (VSIG4), and MS4A4A were strongly correlated with ZNF521 expression (Table 2). These results reveal the potential regulatory role of ZNF521 in the polarization of TAMs.

DCs are the most powerful full-time antigen-presenting cells of the immune system and play a key role in the initiation and regulation of immune responses [29]. In most cases, vaccines against cancer antigens rely on DCs, which can be used to promote individualized treatment via anti-tumor immunity [30, 31]. ZNF521 is strongly related to markers of DCs including HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA-1 (CD1C), BDCA-4 (NRP1), and CD11c (ITGAX).

Together, these findings suggest that ZNF521 is a valuable prognostic biomarker for gastric and other cancers that is significantly correlated with immune infiltration.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Materials. Figure S1. Correlation analysis between ZNF521 expression and the level of immune infiltration in 39 tumor types using the TIMER database. Supplementary Materials. Table S1. The correlations of ZNF521 expression with immune infiltration levels in 39 cancer types from TIMER. Table S2. Correlation analysis between ZNF521 and relate genes and markers of monocyte and macrophages in GEPIA. *P < 0.01; **P < 0.001; ***P < 0.0001.

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