CD3D Is an Independent Prognostic Factor and Correlates With Immune Infiltration in Gastric Cancer

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The protein encoded by CD3D is part of the T-cell receptor/CD3 complex (TCR/CD3 complex) and is involved in T-cell development and signal transduction. Previous studies have shown that CD3D is associated with prognosis and treatment response in breast, colorectal, and liver cancer. However, the expression and clinical significance of CD3D in gastric cancer are not clear. In this study, we collected 488 gastric cancer tissues and 430 paired adjacent tissues to perform tissue microarrays (TMAs). Then, immunohistochemical staining of CD3D, CD3, CD4, CD8 and PD-L1 was conducted to investigate the expression of CD3D in gastric cancer and the correlation between the expression of CD3D and tumor infiltrating lymphocytes (TILs) and PD-L1. The results showed that CD3D was highly expressed in gastric cancer tissues compared with paracancerous tissues (P<0.000). Univariate and multivariate analyses showed that CD3D was an independent good prognostic factor for gastric cancer (P=0.004, HR=0.677, 95%CI: 0.510-0.898 for univariate analyses; P=0.046, HR=0.687, 95%CI: 0.474-0.994 for multivariate analyses). In addition, CD3D was negatively correlated with the tumor location, Borrmann type and distant metastasis (P=0.012 for tumor location; P=0.007 for Borrmann type; P=0.027 for distant metastasis). In addition, the expression of CD3D was highly positively correlated with the expression of CD3, CD4, CD8, and PD-L1, and the combination of CD3D with CD3, CD4, CD8 and PD-L1 predicted the best prognosis (P=0.043). In summary, CD3D may play an important regulatory role in the tumor immune microenvironment of gastric cancer and may serve as a potential indicator of prognosis and immunotherapy response.

Keywords: gastric cancer, cd3d, tumor infiltrating lymphocytes (TILs), prognosis, PD-L1
INTRODUCTION

Gastric cancer is one of the most common malignant tumors in the world. According to the latest data, there are more than 1 million new cases of gastric cancer in the world, and approximately 770,000 people have died of gastric cancer. Mortality and morbidity rank 4th and 5th, respectively, and seriously threaten human life and health (1). At present, the diagnosis of gastric cancer mainly relies on endoscopy and tissue biopsy. However, due to its invasiveness and high cost, more than 60% of patients with gastric cancer are in the middle or advanced stage at the time of diagnosis, and the prognosis is poor (2). At present, surgery is the main treatment for early gastric cancer, and comprehensive treatments such as surgery, radiotherapy and chemotherapy, targeted therapy, and immunotherapy are the main strategies to combat advanced gastric cancer. However, due to the lack of therapeutic targets and the high tumor heterogeneity of gastric cancer, the population of patients who benefit from targeted therapy and immunotherapy is limited. Therefore, it is urgent to improve the level of early diagnosis and identify new therapeutic targets.

The protein product of the CD3D gene is part of the T-cell receptor/CD3 complex (TCR/CD3 complex) and is involved in T-cell development and signal transduction (3). The integrity of the TCR/CD3 complex is crucial for the effector and regulatory functions of peripheral T lymphocytes (4). An increasing number of studies have shown that CD3D is closely related to the occurrence, development, prognosis, immune microenvironment and treatment response of tumors. Chen et al. have found that CD3D was significantly negatively correlated with PD1 and could predict immunochemotherapy response in diffuse large B-cell lymphoma (5). Kang et al. have demonstrated that CD3D expression reduced the prognostic risk of bladder cancer (6). Saiz-Ladera et al. have confirmed that CD2, CD3D, CD3E and CXCR6 combined gene expression was associated with an improvement in the outcomes of head and neck squamous cell carcinoma patients and an increase in infiltrating immune effector cells (7). However, the expression and clinical significance of CD3D in gastric cancer are still unclear and are worthy of further study.

In this study, we collected 488 gastric cancer tissues and 430 paired adjacent tissues to explore the difference in CD3D expression between tumor tissues and paracancerous tissues, as well as the correlation between the expression of CD3D and clinical information, prognosis, tumor-infiltrating lymph nodes (CD3+ T cells, CD4+ T cells and CD8+ T cells) and PD-L1. The aim of this study was to clarify the value of CD3D in the diagnosis and prognosis of gastric cancer and its role in the tumor immune microenvironment.

MATERIALS AND METHODS

Clinical Specimens

We retrospectively collected 488 patients with gastric cancer treated at The Affiliated Cancer Hospital of the University of Chinese Academy of Sciences (Zhejiang Cancer Hospital) from January 2013 to December 2017. The inclusion criteria were as follows: patients with gastric cancer confirmed by pathological diagnosis; and no prior antitumor treatment, such as chemoradiotherapy, targeted therapy and immunotherapy. The exclusion criteria were as follows: patients with gastric cancer and other types of malignant tumors at the same time; patients with metastasis from other tumor species to the stomach; and patients who received prior antitumor treatment. Overall survival (OS) was defined as the duration from the initial surgery to the date of death or the last follow-up.

All clinical information of the participants was collected, including age, gender, height, weight, family history, smoking status, alcohol consumption, TNM staging, blood tumor markers, etc. Pathological staging was based on the American Joint Committee on Cancer 8th edition staging (8). The study was approved by the Research Ethics Committee of the Zhejiang Cancer Hospital (IRB-2021-431).

Tissue Microarray (TMA) Construction and Immunohistochemistry Analysis

We collected 488 formalin-fixed, paraffin-embedded gastric cancer tissue specimens and 430 corresponding adjacent noncancerous tissue specimens. Two pathologists independently selected the most representative tumors and paired adjacent tissues, and TMAs were performed as described previously (9). For immunohistochemistry assays, after treatment with 3% H2O2/methyl alcohol solution for 10 min at room temperature, 5% normal goat serum buffer was used to block the tissue at 37 °C for 30 min. Slides were then incubated with primary antibodies at 4°C overnight. After washing, the slides were incubated with biotin-labeled goat anti-rabbit IgG and HRP-conjugated streptavidin at 37°C for 1 h. Immunoreactivity was visualized by diaminobenzidine (DAB) (Cat# ZLI-9065, ZSGB-BIO Corp., Shanghai, China). After DAB staining, all tissues were counterstained with hematoxylin (Cat# ZLI-9609 ZSGB-BIO Corp., Shanghai, China), dehydrated and then blocked. The antibodies against CD3D (Cat# 109531), CD3 (Cat# 16669), CD4 (Cat# 133616), and CD8 (Cat# 17147) were purchased from Abcam (Cambridge, UK). PD-L1 (Cat# SK006) was purchased from Dako Denmark A/S (Copenhagen, Denmark).

The immunohistochemical staining results were interpreted by two experienced pathologists. The expression of PD-L1 was evaluated by the combined positive score (CPS) score (CPS=PD-L1-positive cells (tumor cells, lymphocytes, macrophages)/total tumor cells). The CPS evaluation criteria were as follows: tumor cells with membrane staining at any intensity directly related to tumor cells, and membrane/cytoplasmic staining of lymphocytes/macrophages. These stained cells accounted for a percentage of the total tumor cells. The stained cells should exclude all necrotic cells, mesenchymal cells, carcinoma in situ and other immune cells (including but not limited to neutrophils, eosinophils and plasma cells). The expression of CD3D, CD3, CD4, and CD8 was divided into a high-expression group and a low-expression group according to the median number of positive cells (10, 11). The median numbers of
CD3D+, CD3−, CD4+, and CD8-positive cells were 120, 190, 30 and 110, respectively.

**Statistical Analysis**

SPSS 23.0 software was used for statistical analysis of all data, and the measurement data are expressed as the median ± standard error. GraphPad Prism 8.3.0 was used for mapping. The relationship between CD3D expression and clinicopathological features, tumor-infiltrating lymphocytes and PD-L1 expression was analyzed by the chi-square test or Fisher’s exact test. Kaplan–Meier analysis was used to draw survival curves, and univariate and multivariate Cox regression analyses were used to determine the prognostic factors of gastric cancer. P<0.05 indicates a significant difference.

**RESULTS**

**Characteristics of the Participants**

A total of 488 gastric cancer patients were recruited, ranging in age from 28 to 91, with an average age of 64.05 ± 0.48, including 360 males (73.77%) and 128 females (26.23%), which is consistent with the epidemiological situation of gastric cancer, indicating that the included cases were generally representative. In terms of TNM staging, 22 patients had stage I, 70 patients had stage II, 357 patients had stage III, and 32 patients had stage IV disease. In terms of the pathological types, gastric adenocarcinoma was the main type, accounting for 454 cases (93.03%), and only 34 (6.97%) were of other types. More detailed information is shown in Table 1 and Table 1S in Supplementary Material.

**CD3D Is Highly Expressed in Gastric Cancer Tissues and Predicts a Good Prognosis**

As shown in Figure 1A, CD3D was mainly expressed on lymphocytes. The average number of CD3D-positive cells in the tumor tissues was 195.45 ± 10.96, while that in the paracancerous tissues was 107.23 ± 6.05, indicating a significant difference (Figure 1B and Table 2, P<0.000). Kaplan–Meier survival analysis indicated that the 5-year overall survival rate of patients with high CD3D expression in tumor tissues was 57.4%, while that of patients with low CD3D expression was 46.1%, showing a significant difference (Figure 1C, P=0.006). We also analyzed the effect of CD3D expression in paracancerous tissues on prognosis, and the results showed that the expression of CD3D in paracancerous tissues was not associated with prognosis (Figure 1D, P=0.154).

**Expression of CD3D Is Correlated With the Tumor Location, Borrmann Type and Distant Metastasis**

To further observe the significance of CD3D expression in gastric cancer, we analyzed the correlation between its expression and all clinical information, such as age, gender, smoking status, and alcohol consumption. We found that The expression of CD3D was correlated with tumor location, and is significantly higher in proximal gastric cancer than in distal gastric cancer (Table 3, P=0.012). Besides, the CD3D positivity rate was 65.63% among patients with Borrmann type I+II but only 49.55% among those with type III+IV, showing a significant difference (Table 3, P=0.004). In addition, the CD3D positivity rate was 31.25% in gastric cancer patients with distant metastases, while it reached as high as 51.45% in patients without distant metastases, indicating a significant difference (Table 3, P=0.027). To summarize, the expression of CD3D was found to be significantly reduced in patients with distant metastasis or in patients with Borrmann type III+IV. In addition, the expression of CD3D was not associated with clinical characteristics such as age, gender, height, weight, family history, smoking status, alcohol consumption, tumor size, or serum tumor markers (Table 3).
CD3D Regulates the Immune Microenvironment of Gastric Cancer

Some studies have shown that CD3D is involved in regulating the tumor immune microenvironment in cervical cancer, liver cancer, breast cancer and other tumors (12–14). Therefore, we further detected the expression of CD3, CD4, CD8 and PD-L1 in the tumor tissues of the included gastric cancer patients and evaluated their relationship with CD3D to explore the regulatory role of CD3D in the immune microenvironment of gastric cancer. We divided the patients into a high-expression group and a low-expression group (Figures 2A, C, E). For PD-L1 interpretation, a CPS score greater than or equal to 10 was considered positive, and a CPS score less than 10 was considered negative (15–17) (Figure 2G). Kaplan–Meier survival analysis showed that the expression of CD4 and CD8 was correlated with the survival of gastric cancer patients, while the expression of CD3 and PD-L1 was not (Figures 2B, D, F, H). The 5-year overall survival rates of the CD4 and CD8 high-expression groups were 60.2% and 60.3%, respectively, while those of the CD4 and CD8 low-expression groups were 48.2% and 48.0%, respectively (Figure 2D, P=0.024 and 2F, P=0.034).

As shown in Figure 3, the expression of CD3D was closely related to that of CD3, CD4, CD8 and PD-L1. We found that CD3D was positively correlated with the expression of CD3, CD4, and CD8, and the expression of CD3, CD4, and CD8 in the CD3D high-expression group was higher than that in the CD3D low-expression group, with significant differences observed (Figures 3A–C, all P<0.000). Again, Pearson correlation coefficients showed the same result (Table 4). In addition, we found that CD3D was positively correlated with the expression of PD-L1. The PD-L1 positivity rate was 34.34% in the group with high CD3D expression but only 17.5% in the group with low CD3D expression (Figure 3D, P<0.000). The Pearson correlation coefficient also found a positive correlation between CD3D and PD-L1 expression (Table 4). In conclusion, CD3D may play an important regulatory role in the tumor immune microenvironment by regulating tumor infiltrating lymphocytes (TILs) and immune checkpoints and may be a potential immunotherapy target.

CD3Dhigh Combined With CD4high, CD8high and PD-L1- Predicts the Best Prognosis of Gastric Cancer

Risk factors were determined by univariate and multivariate logistic regression analyses. Univariate logistic regression analysis found that CD3D, CD8, CD4, age, family history, tumor location, tumor size, grade of differentiation and TNM stage were all prognostic factors for gastric cancer (Table 5). In addition, we further carried out multivariate analysis with these important factors, and the results

![CD3D is overexpressed in gastric cancer tissues compared with adjacent tissues, and a high expression of CD3D is associated with a good prognosis in gastric cancer patients. (A) Representative images of CD3D staining in TMAs as determined by immunohistochemical analysis. (B) Differential expression of CD3D in gastric cancer tissue and paracancerous tissue. (C) The overall survival (OS) curves of gastric cancer patients with different CD3D expression levels in tumor tissues as determined by Kaplan–Meier analysis (log-rank test). (D) The OS curves of gastric cancer patients with different CD3D expression levels in paracancerous tissues as determined by Kaplan–Meier analysis (log-rank test). ***p < 0.001.](image)

| Parameters          | N   | CD3D expression | Number of positive cells (Mean ± standard error) | T      | P value |
|---------------------|-----|-----------------|-----------------------------------------------|--------|---------|
|                     |     | High            | Low                                           |        |         |
| Tumor tissue        | 488 | 244             | 244                                           | 195.45 ± 10.96 | -7.046  | <0.000  |
| Paracancerous tissue| 430 | 146             | 284                                           | 107.23 ± 6.05  |        |         |

TABLE 2 | The differential expression of CD3D in tumor tissues and paracancerous tissues.
| Parameters | CD3D expression | Total | Positive rate | $\chi^2$ | $P$ value |
|------------|-----------------|-------|---------------|---------|-----------|
|            | High | Low |            |         |           |
| Age(years) |      |     |            |         |           |
| ≥65        | 113  | 130 | 243         | 46.50%  | 2.369     | 0.124     |
| <65        | 131  | 114 | 245         | 53.47%  |           |           |
| Gender     |      |     |            |         |           |
| Female     | 71   | 57  | 128         | 55.47%  | 2.076     | 0.150     |
| Male       | 173  | 187 | 360         | 48.06%  |           |           |
| Family history |    |     |            |         |           |
| Yes        | 24   | 15  | 39          | 61.54%  | 0.715     | 0.398     |
| No         | 173  | 145 | 318         | 54.40%  |           |           |
| Unknown    |      |     | 131         |         |           |           |
| Smoking history |    |     |            |         |           |
| Yes        | 54   | 51  | 105         | 51.43%  | 0.847     | 0.357     |
| No         | 143  | 109 | 252         | 56.75%  |           |           |
| Unknown    |      |     | 131         |         |           |           |
| Drinking history |    |     |            |         |           |
| Yes        | 41   | 36  | 77          | 53.25%  | 0.149     | 0.700     |
| No         | 156  | 124 | 280         | 55.71%  |           |           |
| Unknown    |      |     | 131         |         |           |           |
| Weight loss |      |     |            |         |           |
| Yes        | 60   | 45  | 105         | 57.14%  | 0.196     | 0.658     |
| No         | 137  | 114 | 251         | 54.58%  |           |           |
| Unknown    |      |     | 132         |         |           |           |
| Tumor location |    |     |            |         |           |
| Proximal   | 104  | 135 | 239         | 43.51%  | 6.360     | 0.012     |
| Distal     | 130  | 106 | 236         | 55.08%  |           |           |
| Unknown    |      |     | 13          |         |           |           |
| Borrmann type |     |     |            |         |           |
| I/II       | 84   | 44  | 128         | 65.63%  | 8.515     | 0.004     |
| III/IV     | 111  | 113 | 224         | 49.55%  |           |           |
| Unknown    |      |     | 136         |         |           |           |
| Lauren type |      |     |            |         |           |
| Intestinal | 110  | 87  | 197         | 55.84%  | 3.146     | 0.207     |
| Diffuse    | 66   | 42  | 108         | 61.11%  |           |           |
| Mixed      | 21   | 25  | 46          | 45.65%  |           |           |
| Unknown    |      |     | 137         |         |           |           |
| Tumor size (cm) |    |     |            |         |           |
| >5cm       | 110  | 123 | 233         | 47.21%  | 1.299     | 0.254     |
| ≤5cm       | 131  | 119 | 250         | 52.4%   |           |           |
| Unknown    |      |     | 5           |         |           |           |
| Grade of differentiation |    |     |            |         |           |
| Poor       | 96   | 76  | 172         | 55.81%  | 2.475     | 0.290     |
| Moderate-poor | 61 | 39  | 100         | 61.00%  |           |           |
| Moderate+Well | 30 | 32  | 62          | 48.39%  |           |           |
| Unknown    |      |     | 154         |         |           |           |
| T stage    |      |     |            |         |           |
| T1/2       | 21   | 20  | 41          | 51.22%  | 0.009     | 0.926     |
| T3/4       | 220  | 216 | 436         | 50.46%  |           |           |
| Unknown    |      |     | 11          |         |           |           |
| N stage    |      |     |            |         |           |
| N0/1       | 89   | 79  | 168         | 52.98%  | 0.854     | 0.356     |
| N2/3       | 151  | 160 | 311         | 48.55%  |           |           |
| Unknown    |      |     | 9           |         |           |           |
| M stage    |      |     |            |         |           |
| M0         | 231  | 218 | 449         | 51.45%  | 4.874     | 0.027     |
| M1         | 10   | 22  | 32          | 31.25%  |           |           |

(Continued)
showed that CD3D, CD4, and CD8 were independent prognostic factors for gastric cancer in both the univariate and multivariate analyses (Table 6).

Since CD3D was positively correlated with the expression of CD3, CD4, CD8 and PD-L1, the effect of CD3D in combination with these factors on the prognosis of gastric cancer was analyzed. As shown in Figures 4A–D, the 5-year overall survival rate of CD3D<sup>high</sup> + CD3<sup>high</sup> patients was 59.5%, that of CD3D<sup>high</sup> + CD4<sup>high</sup> patients was 65.4%, that of CD3D<sup>high</sup> + CD8<sup>high</sup> patients was 63.1%, and that of CD3D<sup>high</sup> + PD-L1<sup>-</sup> was 60.7%, which was higher than all other groups. As shown in Figures 4E–H, the 5-year overall survival rate of CD3D<sup>high</sup> + CD4<sup>high</sup> + CD8<sup>high</sup> patients was 68.0%, that of CD3D<sup>high</sup> + CD4<sup>high</sup> + CD8<sup>high</sup> + CD3<sup>high</sup> was 66.1%, that of CD3D<sup>high</sup> + CD4<sup>high</sup> + CD8<sup>high</sup> + PD-L1<sup>-</sup> was 69.8%, and that of CD3D<sup>high</sup> + CD4<sup>high</sup> + CD8<sup>high</sup> + CD3<sup>high</sup> + PD-L1<sup>-</sup> was 67.7%. Thus, the combination of CD3D<sup>high</sup> with CD4<sup>high</sup>, CD8<sup>high</sup> and PD-L1<sup>-</sup> predicts the best prognosis of gastric cancer.

**DISCUSSION**

The exploration of gene expression profiles, the tumor mutational burden, PD-L1 and other biomarkers have yielded great progress in predicting patient prognosis and immunotherapy efficacy, but there are still some problems that make it difficult to meet clinical needs (18, 19). In addition to clarifying the role of TILs, a comprehensive understanding of the tumor immune microenvironment can help to guide individualized immunotherapy, making it a hot spot in cancer immunotherapy research (20). Multiple studies have shown that TILs are often associated with better treatment response and prognosis (21–23). However, due to the heterogeneity of gastric cancer and the complexity of the immune microenvironment, PD-L1-based immune checkpoint inhibitors have limited benefits in the treatment of gastric cancer (24). Moreover, TILs have also been found to play a limited role in the prognosis and efficacy prediction of gastric cancer. Therefore, it is urgent to find new targets to aid in diagnosis and in predicting prognosis and immunotherapy response to improve the diagnosis and treatment of gastric cancer patients.

CD3D is part of the T-cell receptor/CD3 complex (TCR/CD3 complex), and defects in this gene cause severe combined immunodeficiency (25). CD3D has been found to be associated with prognosis in various tumors. Shi et al. have demonstrated that the CD3D/CD4 ratio was a stable independent prognostic factor in muscle-invasive bladder cancer (26). In addition, other studies have shown that high CD3D expression is strongly
associated with poor survival in breast carcinoma (27). In contrast, in patients with colorectal cancer, high CD3D expression was found to predict better clinical outcomes (28). In other words, CD3D may play completely opposite roles in different tumors. In our study, we found that CD3D was highly expressed in gastric cancer tissues compared with paracancerous tissues. Univariate and multivariate analyses showed that CD3D was an independent prognostic factor for gastric cancer, and patients with high expression of CD3D had a better prognosis. In addition, CD3D was negatively correlated with the Borrmann type and distant metastasis.

TILs refer to lymphocytes that leave the blood stream to enter the tumor, constituting an important part of the tumor microenvironment (29). They have been found to have an important role in predicting tumor prognosis and have even been employed as a means of cell therapy (30). The PD-1/PD-L1 signaling pathway has been shown to be important for tumor immunosuppression, inhibiting the activation of T lymphocytes and enhancing the immune tolerance of tumor cells, exhibiting an important regulatory role in the tumor immune microenvironment. Many studies have shown that the expression of TILs (CD3, CD4, CD8) is correlated with a favorable prognosis in gastric cancer (31–33). However, the relationship between PD-L1 and gastric cancer prognosis is controversial. Some studies have shown that positive PD-L1 expression is significantly related to poor overall survival (34–36), but some studies have failed to confirm this finding (37, 38).

In our study, we found that CD4 and CD8 were good prognostic factors for gastric cancer in both univariate and multivariate analyses. CD3 was not found to be associated with prognosis in the univariate analysis but was identified as a good prognostic factor for gastric cancer in the multivariate analysis. In contrast,
The expression of CD3D is highly positively correlated with the expression of CD3, CD4, CD8, and PD-L1. (A) The relationship between the expression of CD3D and CD3. (B) The relationship between the expression of CD3D and CD4. (C) The relationship between the expression of CD3D and CD8. (D) Relationship between the expression of CD3D and PD-L1. ***p < 0.001.

### TABLE 4 | The pearson correlation coefficients between CD3D expression and CD3, CD4, CD8 and PD-L1 expression in gastric cancer.

| Parameters          | CD3D vs CD3 | CD3D vs CD4 | CD3D vs CD8 | CD3D vs PD-L1 |
|---------------------|-------------|-------------|-------------|---------------|
| Pearson Correlation | 0.668       | 0.466       | 0.623       | 0.166         |
| P                   | <0.0001     | <0.0001     | <0.0001     | 0.002         |
| 95% CI              | 0.625-0.723 | 0.411-0.535 | 0.584-0.672 | 0.064-0.277   |

The results with statistical differences are bold values.

### TABLE 5 | Univariate Cox regression analysis of overall survival in gastric cancer patients.

| Parameters          | Univariate COX regression analysis |
|---------------------|-----------------------------------|
|                     | P value   | HR       | (95% CI)           |
| CD3D expression     | 0.007     | 0.677    | 0.510-0.898        |
| High vs Low         |           |          |                    |
| Gender              | 0.963     | 1.008    | 0.734-1.384        |
| Female vs. Male     |           |          |                    |
| Age(year) ≥85 vs <65| 0.035     | 1.357    | 1.022-1.800        |
| Gastric history     | <0.0001   | 1.444    | 1.195-1.744        |
| Yes vs No Smoking   | 0.006     | 1.322    | 1.082-1.615        |
| Drinking history    | 0.006     | 1.319    | 1.082-1.607        |
| Yes vs No           |           |          |                    |

(Continued)
### TABLE 5 | Continued

| Parameters | Univariate COX regression analysis |
|------------|-----------------------------------|
|            | $P$ value | HR (95%CI) |            |
| Weight loss | 0.001     | 1.391      | 1.142-1.694|
| Yes vs No   | 0.018     | 0.707      | 0.591-0.943|
| Tumor location |          |            |            |
| Proximal vs distal | 0.634 | 1.137      | 0.671-1.925|
| Pathological type | 0.038 | 1.410      | 1.019-1.950|
| Adenocarcinoma/Others | 0.051 | 1.349      | 0.999-1.823|
| Borrmann type | 0.027   | 1.378      | 1.037-1.831|
| I+II vs III+IV | 0.048   | 1.387      | 1.003-1.918|
| Lauren type |          |            |            |
| Intestinal vs diffuse | 0.027 | 1.378      | 1.037-1.831|
| Tumor size (cm) | 0.001   | 3.208      | 2.248-4.576|
| ≤8 vs >8 | 0.001     | 3.208      | 2.248-4.576|
| Grade of differentiation | 0.001 | 1.754      | 1.278-2.408|
| Poor vs moderate-poor+ | 0.001  | 1.818      | 1.370-2.412|
| Moderate+well |         |            |            |
| T stage | 0.006     | 2.711      | 1.335-5.502|
| T1+T2 vs T3+T4 | <0.0001 | 3.208      | 2.248-4.576|
| N stage | 0.009     | 1.483      | 1.102-1.996|
| ND+N1 vs N2+N2 | <0.0001 | 1.694      | 1.279-2.243|
| M stage | 0.001     | 1.779      | 1.284-2.466|
| M0 vs M1 | 0.001     | 1.779      | 1.284-2.466|
| TNM stage | 0.001   | 1.779      | 1.284-2.466|
| I+II vs III+IV | 0.001 | 1.779      | 1.284-2.466|
| AFP(ng/ml) | 0.001    | 1.733      | 1.283-2.342|
| ≤5 vs >5 | 0.001     | 1.733      | 1.283-2.342|
| CA199 (U/ml) | 0.001  | 1.733      | 1.283-2.342|
| ≤37 vs >37 | <0.0001  | 1.733      | 1.283-2.342|
| CAY24 (U/ml) | 0.001  | 1.733      | 1.283-2.342|
| ≤6.9 vs >6.9 | 0.001  | 1.733      | 1.283-2.342|
| CA125(U/ml) | 0.001   | 1.733      | 1.283-2.342|
| ≤35 vs >35 | 0.001    | 1.733      | 1.283-2.342|
| CA100 | 0.001      | 1.733      | 1.283-2.342|
| ≤25 vs >25 | <0.0001  | 1.733      | 1.283-2.342|
| PD-L1 | 0.001      | 1.733      | 1.283-2.342|
| Negative vs Positive | 0.938 | 0.986      | 0.693-1.403|
| CD3+T cells | 0.946     | 0.989      | 0.725-1.351|
| Low vs High | 0.026  | 0.700      | 0.512-0.957|
| CD8+T cells | 0.036     | 0.715      | 0.523-0.978|
| Low vs High | 0.048    | 1.387      | 1.003-1.918|
| HDH-A |          |            |            |
| Positive vs Negative | 0.602 | 1.104      | 0.760-1.604|

*The results with statistical differences are bold values.*

### TABLE 6 | Multivariate Cox regression analysis of overall survival in gastric cancer patients.

| Parameters | Multivariate COX regression analysis |
|------------|--------------------------------------|
|            | $P$ value | HR (95%CI) |            |
| CD3D expression | 0.046 | 0.687      | 0.474-0.994|
| High vs Low | 0.013     | 0.617      | 0.421-0.903|
| CD4+T cells | 0.001     | 0.332      | 0.193-0.570|
| Low vs High | 0.001     | 3.485      | 1.911-6.355|
| CD8+T cells | 0.001     | 1.104      | 0.760-1.604|
| Low vs High |            |            |            |
| CD3+T cells | 0.001     | 1.104      | 0.760-1.604|
| Low vs High |            |            |            |
| PD-L1 | 0.938      | 1.104      | 0.760-1.604|

*(Continued)*
PD-L1 was not associated with gastric cancer prognosis in either the univariate or multivariate analysis.

Previous studies have shown that CD3D can modulate the tumor immune microenvironment and affect the prognosis and immune response of breast cancer and cervical squamous cell carcinoma (39–41). In gastric cancer patients, we found that the expression of CD3D was highly positively correlated with the expression of CD3, CD4, CD8, and PD-L1. In addition, the combination of CD3D with CD3, CD4, CD8 and PD-L1 predicted the best prognosis, with the 5-year survival rate of the CD3Dhigh + CD4high + CD8high + PD-L1- group being the highest, reaching 69.8%. Thus, CD3D may play an important role in the regulation of the immune microenvironment in gastric cancer.

TABLE 6 | Continued

| Parameters | Multivariate COX regression analysis |
|------------|-------------------------------------|
|            | P value | HR (95%CI) |
| Gender     |          |            |
| Female vs. Male | 0.726   | 1.075      | 0.717-1.612 |
| Age (year) |          |            |
| ≥65 vs. <65 | 0.142   | 1.278      | 0.922-1.772 |
| Gastric history |        |            |
| Yes vs No | 0.005    | 1.883      | 1.205-2.941 |
| Smoking history |        |            |
| Yes vs No | 0.771    | 0.942      | 0.631-1.407 |
| Drinking history |        |            |
| Yes vs No | 0.722    | 1.081      | 0.704-1.660 |
| Weight loss |        |            |
| Yes vs No | 0.188    | 1.256      | 0.895-1.763 |
| TNM stage |        |            |
| I+II vs III+IV | <0.0001 | 2.787      | 1.640-4.736 |

The results with statistical differences are bold values.

FIGURE 4 | CD3Dhigh combined with CD4high, CD8high and PD-L1- predicts the best prognosis of gastric cancer. (A) The overall survival (OS) curves of gastric cancer patients with different CD3D combined with CD3 expression levels in tumor tissues. (B) The OS curves of gastric cancer patients with different CD3D and CD4 expression levels in tumor tissues. (C) The OS curves of gastric cancer patients with different CD3D and CD8 expression levels in tumor tissues. (D) The OS curves of gastric cancer patients with different CD3D combined with PD-L1 expression levels in tumor tissues. (E) The OS curves of gastric cancer patients with different CD3D, CD4 and CD8 expression levels in tumor tissues. (F) The OS curves of gastric cancer patients with different CD3D, CD4, CD8 and CD3 expression levels in tumor tissues. (G) The OS curves of gastric cancer patients with different CD3D, CD4, CD8 and PD-L1 expression levels in tumor tissues. (H) The OS curves of gastric cancer patients with different CD3D, CD4, CD8, CD3 and PD-L1 expression levels in tumor tissues. E-H: Because of the large number of groups, we only show some groups with large number of cases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article SUPPLEMENTARY material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The study was approved by the research ethics committee of Cancer Hospital of Zhejiang Cancer Hospital (IRB-2021-431). The patients/participants provided their written informed consent to participate in this study.
AUTHOR CONTRIBUTIONS

QY, BZ, and JQ conceived the study and acquired the funding. LY and JX carried out clinical research, collected clinical samples, analyzed clinical data, and wrote articles. YS, ZJ, ZB, YW, PY, and YX participated in clinical sample collection. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.913670/full#supplementary-material
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