Research Article

DOK5 as a Prognostic Biomarker of Gastric Cancer Immunoinvasion: A Bioinformatics Analysis

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Background. Docking protein 5 (DOK5) is a member of the docking protein group of membrane proteins and is an adapter protein involved in signal transduction. Nevertheless, the role of DOK5 expression in the prognosis of gastric cancer (GC) remains unclear. Methods. In this study, clinical prognostic parameters and survival data related to DOK5, in patients with GC, were analyzed using bioinformatics analysis comprising Oncomine and TIMER, UALCAN database, Kaplan-Meier plotter, GEPIA, GSEA, DAVID, and cBioPortal websites. Results. In our study, GC contained various DOK5 expressions, which forecasted poor survival outcomes. Moreover, our research showed that high DOK5 could predict high-level infiltration of several GC immune cells, as evidenced by M1, TAM, M2, B cell, and T cell failure. Hence, DOK5 might become a new gastric cancer biomarker and therapeutic target. In the following analysis, in order to explore the prognostic value of DOK5 in GC, more clinical trials are needed to validate our results. Conclusions. Through multiple database verifications, DOK5 was found to be part of the pathogenic genes for GC. Thus, it can change the formation and progression of tumors by acting on human immunity.

1. Introduction

Gastric cancer (GC) is globally significant. It is the 5th most diagnosed cancer and 3rd major cause of cancer-related deaths. It is twice as common in men than in women [1]. GC is deemed a high-mortality cancer due to delayed diagnosis, as no certain clinical symptom appears during the early stage [2]. Hence, exploring certain sensitive biomarkers is highly important for early diagnosis as well as the prognostic evaluation of patients with GC.

Docking protein 5 (DOK5), which was first reported in 2001, is a member of a subgroup of the DOK family that has been expressed using c-Ret in several neuronal tissues. The receptor, tyrosine kinase c-Ret, had been explored as an oncogene, which also has been mutated in patients with multiple endocrine neoplasia and familial medullary thyroid cancer syndromes. DOK5 enhances c-Ret-dependent activation of mitogen-activated protein kinase [3]. Favre et al. had shown that DOK5 are expressed in T cells and their expression is regulated upon T cell activation [4]. Pothlitchet et al. suggest that DOK5 upregulation might also be associated with metastasis, in human melanoma [5].

The above findings indicate that DOK5 plays a key role in the invasion, progression, and the metastasis of cancer. In this study, we systematically assessed DOK5 expression in a variety of tumor forms involving GC, as well as its association with prognosis. We also assessed its status regarding distinct tumor-infiltrating immune cells, using...
the Oncomine database, TIMER databases, GEPIA, Kaplan-Meier plotter, and UALCANCAN database. Our results clarified the significant role of DOK5 in the prognosis of GC and offer a potential mechanism by which DOK5 expression might monitor tumor immunity—regulation of the infiltration of immune cells in GC.

2. Methods and Materials

2.1. Oncomine. The DOK5 GC and normal tissues’ mRNA expression levels were checked using the Oncomine database [website address (https://www.oncomine.org/resource/login.html)]. We selected $P$ value = $1E - 4$; two-fold change in the study and top 10% gene rank had been utilized for the threshold. Wang’s studies were used to assess the differential expression levels of GC genes.

2.2. GEPIA. Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) is a modern web-based tool containing data on gene expression in normal tissues and tumors, shared from TCGA (The Cancer Genome Atlas), as well as the Genotype-Tissue Expression project; thus, it implements a standard processing pipeline [6]. It gives optional functions like differential expression analysis in tumors as well as normal tissues. We could also illustrate DOK5 expression in GC, as well as normal tissues.

2.3. TIMER. The Tumor Immune Estimation Resource (TIMER) platform has been used to assess the tumor-infiltrating immune cells of 32 cancer types in a comprehensive way. It used 10,000+ samples from TCGA platform (https://cistrome.shinyapps.io/timer/). TIMER assesses a mass of tumor-infiltrating immune cells using the statistical analysis of the gene expression profiles [7]. We examined the link shared by the DOK5 gene expression level along with the abundance of infiltrating immune cells, comprising CD8+ T cells, CD4+ T cells, neutrophils, B cells, and macrophages and dendritic cells based on the expression regarding marker genes in various cancers involving GC. Those marker genes utilized the analysis of the tumor-infiltrating immune cells involving B cells, T cells, monocytes, TAMs, M2 and M1 macrophages, natural killer (NK) cells, neutrophils, dendritic cells (DCs), T-helper 17 (Th17) cells, T-helper (Th) cells, exhausted T cells, and follicular helper T (Tfh) cells, along with Tregs that had based on data taken from past researches. DOK5 gene was on the x-axis, and related marker genes were on the y-axis.

2.4. Kaplan-Meier Plotter. The Kaplan-Meier plotter platform (https://kmplot.com/analysis/) integrates information from TCGA, EGA, and GEO databases and translates the impact of target genes on patients with cancer. To evaluate the impact of DOK5 on the prognosis of patients with GC, Kaplan-Meier plotter was used, using various pathological parameters.

2.5. UALCANCAN. The UALCANCAN platform (http://ualcanc.path.uab.edu) makes use of RNA-seq as well as the clinical data of 31 different cancer categories through TCGA [8]. It is capable of analyzing the tumor and normal sample’s relative gene expression, at varying tumor stages, tumor grades, and other clinicopathological features.

2.6. Functional Enrichment Analyses of Gastric Cancer. We ran Kyoto Encyclopedia of Genes and Genomes (KEGG) as well as Gene Ontology (GO) functional enrichment assessment on DOK5. The Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) was utilized for the identification of enriched pathways as well as terms of GO and KEGG.

2.7. Gene Set Enrichment Analysis. We used the Perl software to compile the expression dataset file along with the phenotype data file of the target gene for the single gene enrichment analysis. We downloaded and installed the GSEA software (http://software.broadinstitute.org/ gsea) and ran it in a Java8 environment. The target gene was enriched by KEGG pathway analysis, and the path for analysis was obtained through the c2.cp.kegg.v7.2.symboIs.gmt dataset in the MsigDB database. Using weighted enrichment analysis technology and random combination enrichment detection a thousand times, we calculated the value of FDR and $P$ through GSEA. We then visualized the outcomes using R (plyr, ggplot2, grid, grid Extra package) software. Cut-off criteria include gene set size < 15 and >500, nominal $P$ value < 0.05, and FDR < 0.25.

2.8. Genetic Alteration Analysis. As we logged onto the cBioPortal website (https://www.cbioportal.org/), we opted for the TCGA Pan Cancer Atlas Studies from the quick selection section and went into DOK5 in order to determine the DOK5 genetic change characteristics. We observed the change frequency, mutation type, and CNA (copy number change) results of all TCGA tumors in the Cancer Type Summary module.

2.9. Clinical Specimens. This study used 24 postoperative tissue samples of patients with GC treated in Changzhou No. 2 People’s Hospital from 2019 to 2021. Further, during the operation, the adjacent tissues were collected and stored at 80°C immediately.

2.10. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) Analysis. The tissues had been preprocessed to extract total RNA. Through the utilization of a PrimeScript RT reagent kit (TaKaRa, Dalian, China), the cDNA was synthesized. Quantitative PCR was carried out with a 7500 real-time PCR system (ABI, Waltham, MA, USA). The PCR primers were synthesized and purchased by Sangon Biotechnology Company (Shanghai, China). DOK5: forward: GGTGAAGGGCTGTTTAATCTTTTC, reverse: TTTCCTACA CTCCTGAGCAAGC; GAPDH: forward: CATGGTCCAT ATGATTCCAC, reverse: CCTGGAAGATGGTGATG. GAPDH served as an internal control, and fold change was calculated using the $2^{-\Delta\DeltaCT}$ technique.

3. Results

3.1. DOK5 mRNA Expression Levels in Different Types of Human Cancers. In order to determine the difference in
Figure 1: DOK5 expression levels in different types of human cancers. (a) Increased or decreased DOK5 in datasets of different cancers compared with normal tissues in the Oncomine database. (b) Human DOK5 expression levels in different tumor types from TCGA database were determined by TIMER (*P < 0.05, **P < 0.01, and ***P < 0.001).
the expression of DOK5 in tumors and normal tissues, we used the Oncomine database to analyze the DOK5 mRNA levels in normal tissues of different tumors and multiple cancer types. Analysis shows that in contrast to the normal tissues, DOK5 was better expressed in GC, leukemia, lymphoma, and pancreatic cancer tissues (Figure 1(a)). The in-depth outcomes of DOK5 expression in varying cancer types have been illustrated in Supplementary Table 1.

For more assessment of the DOK5 expression in human cancers, we made use of RNA-seq data using several malignant tumors found in TCGA for identifying the DOK5 expression. In all TCGA tumors, the differential tumors found in TCGA for identifying the expression of DOK5 was significantly reduced in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), head and neck cancer (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), osteosarcoma (OS), and uterine corpus endometrial carcinoma (UCEC), compared to adjacent normal tissues. However, DOK5 expression was significantly increased in cholangiocarcinoma (CHOL), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), and stomach adenocarcinoma (STAD), compared to adjacent normal tissues.

3.2. Genetic Change Analysis Data of DOK5. In different tumor samples in the TCGA cohort, we observed the genetic changes of DOK5. The highest alteration frequency of DOK5 (>6%) appeared for patients with colorectal tumors, with “amplification” as the primary type. We also observed that the genetic alteration status of DOK5 was mainly amplified in GC (>4%), which probably explains the changes of DOK5 in GC tissues at the gene level and gives the foundation for further study (Figure 2).

3.3. Effects of DOK5 on the Prognosis of Different Types of Human Cancers. In order to study whether the expression of DOK5 is related to the prognosis of cancer patients, we used GEPIA and Kaplan-Meier plotter to evaluate the effect of DOK5 expression on survival. Using the data for STAD, LIHC, and LUAD from TCGA in the GEPIA database, we assessed the correlation between differential expressions of DOK5 and clinical outcomes. Based on results from 381 patients with GC, poorer prognoses in terms of OS and DFS (P < 0.05) were associated with higher mRNA expression levels for DOK5 (Figures 3(a) and 3(b)). However, in liver cancer, different results have emerged. Based on results from 364 patients with liver cancer, poorer prognoses in terms of OS and DFS (P < 0.05) were associated with lower mRNA expression levels for DOK5 (Figures 3(c) and 3(d)).

In order to further study the prognostic potential of DOK5 in different cancers, the Kaplan-Meier plotter database was used to evaluate the prognostic value of DOK5 based on Affymetrix microarray. It is worth noting that the poor prognosis in GC (OS: HR = 1.32, 95%CI = 1.12 to 1.57, P = 0.0012; DFS: HR = 1.02, 95%CI = 1.02 to 1.52, P = 0.033; PFS: HR = 1.35, 95%CI = 1.08 to 1.69, P = 0.0075) was shown to correlate with higher DOK5 expression (Figures 3(e)–3(g)). Poor prognosis is associated with low DOK5 expression in liver cancer (OS: HR = 0.61, 95%CI = 0.43 to 0.87, P = 0.0057; PFS: HR = 0.7, 95%CI = 0.52 to 0.94, P = 0.016; RFS: HR = 0.58, 95%CI = 0.50 to 0.81, P = 0.0011; Figure 3(h)–3(j)). Sawant et al.’s studies have also
Overall survival

Logrank p = 0.035
HR (high) = 1.4
p (HR) = 0.035
n (high) = 190
n (low) = 191

Disease free survival

Logrank p = 0.01
HR (high) = 1.7
p (HR) = 0.011
n (high) = 190
n (low) = 191

Logrank p = 0.0035
HR (high) = 0.6
p (HR) = 0.004
n (high) = 182
n (low) = 182

Logrank p = 0.01
HR (high) = 0.68
p (HR) = 0.011
n (high) = 182
n (low) = 182

Figure 3: Continued.
**Figure 3: Continued.**

(g) DOK5 (214844_s_at)

- **HR**: 1.35 (1.08-1.69)
- **logrank P**: 0.0075

(h) DOK5 (55816)

- **HR**: 0.61 (0.43-0.87)
- **logrank P**: 0.0057

(i) DOK5 (55816)

- **HR**: 0.7 (0.52-0.94)
- **logrank P**: 0.016

(j) DOK5 (214844_s_at)

- **HR**: 0.58 (0.42-0.81)
- **logrank P**: 0.0011

(k) DOK5 (214844_s_at)

- **HR**: 0.85 (0.75-0.96)
- **logrank P**: 0.012

(l) DOK5 (214844_s_at)

- **HR**: 1.02 (0.84-1.24)
- **logrank P**: 0.83

Table for (g):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 249       | 21 14 2       |
| High 249      | 34 13 10 6    |

Table for (h):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 184 80    | 34 10 8 3 1    |
| High 180 102  | 50 22 11 3 0   |

Table for (i):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 188 45    | 22 9 3 2 1     |
| High 182 65   | 25 11 3 1 0    |

Table for (j):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 159 40    | 19 7 2 2 1     |
| High 157 65   | 28 13 5 1 0    |

Table for (k):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 963 422   | 135 44 5       |
| High 962 405  | 68 13 2        |

Table for (l):

| Time (months) | Number at risk |
|---------------|----------------|
| Low 492 184   | 25 8 1         |
| High 490 186  | 19 1 0         |
shown that high DOK5 expression is associated with poor prognosis in liver cancer [9]. The expression of DOK5 was also correlated with the patients’ survival in the lung cancer (OS: HR = 0.85, 95% CI = 0.75 to 0.96, P = 0.0126; PFS: HR = 1.02, 95% CI = 0.84 to 1.24, P = 0.83; PPS: HR = 0.86, 95% CI = 0.67 to 1.11, P = 0.25; Figures 3(k)–3(m)). Conversely, DOK5 expression was not related with PFS and PPS in lung cancer (Figures 3(l) and 3(m)). These results suggest that DOK5 expression is of prognostic significance in GC, liver cancer, and lung cancer.

3.4. Expression and Clinical Features of DOK5 in Patients with Gastric Cancer. We analyzed the influence of DOK5 expression on different types of clinical patients using the Kaplan-Meier plotter database (Table 1). High DOK5 expression correlated with both poorer OS and PPS in stage 3 patients (OS: HR = 1.54, P < 0.05; PFS: HR = 1.77, P < 0.01), stage T2 patients (OS: HR = 1.87, P < 0.01; PFS: HR = 1.66, P < 0.05), stage M0 patients (OS: HR = 1.8, P < 0.001; PFS: HR = 1.71, P < 0.001), intestinal patients (OS: HR = 1.95, P < 0.001; PFS: HR = 1.73, P < 0.01), diffuse patients (OS: HR = 1.63, P < 0.01; PFS: HR = 1.67, P < 0.01), and HER2-negative patients (OS: HR = 1.47, P < 0.001; PFS: HR = 1.35, P < 0.05). It is worth noting that among patients with lymph node metastasis, patients with high DOK5 expression have a poorer prognosis (OS: stage N1, HR = 2.43, P < 0.001; stage N2, HR = 1.78, P < 0.05; stage N3, HR = 1.88, P < 0.05; stage N1+2+3, HR = 1.97, P < 0.001. PFS: stage N1, HR = 2.49, P < 0.001; stage N2, HR = 1.56, P < 0.05; stage N1+2+3, HR = 1.9, P < 0.001). However, in stages 1, 2, T3, T4, N0, M1, and HER2-positive patients, DOK5 expression was not related to OS and PFS. The above data shows that according to the clinical characteristics of patients with GC, DOK5 expression is related to patients with GC lymph node metastasis.

3.5. DOK5 Expression in Patients with STAD. In the UAL- CAN database, we further analyzed various clinicopathological characteristics of TCGA-STAD specimens and it was found that compared with normal tissues, the expression level of DOK5 mRNA was higher in STAD tissues (Figure 4(a)). The expression of DOK5 in patients with STAD of different stages (2–4) was significantly higher than that of normal controls (Figure 4(b)). In addition, in the assessment made on the basis of race, the expression of DOK5 in patients with STAD was essentially high in contrast to that in the control group (specifically in Caucasians and Asians (Figure 4(c)) and sex (Figure 4(d))). The expression of DOK5 in patients having different grades (1, 3) of STAD was significantly more than that of the normal controls (Figure 4(e)). In the end, in patients with lymph node metastasis, DOK5 expression level is also higher (Figure 4(f)). Hence, the expression level of DOK5 is expected to be a potential diagnostic indicator for tumor staging in patients with GC.

3.6. DOK5 Expression Is Associated with Immune Cell Infiltration in GC. The number and active state of tumor-infiltrating lymphocytes can determine the survival time of some patients with cancer [10]. Therefore, we made use of the TIMER database for the identification of the relationship shared by the DOK5 expression and infiltrating immune
cells in 32 cancers, including GC. The results showed that in 32 tumor types, DOK5 expression was crucially related to CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells (Figure 5(a)). Furthermore, we also explored the link shared by SCNA (somatic copy number alteration) of DOK5 gene and the level of GC tumor invasion. It is worth noting that the results show that the CNA of DOK5 is significantly related to the infiltration level of CD8+ T cells, B cells, neutrophils, macrophages, and dendritic cells (Figure 5(b)).

### Table 1: Kaplan-Meier plotter was used to analyze the correlation between DOK5 mRNA expression and different clinicopathological factors in gastric cancer.

| Clinicopathological factors | N   | Overall survival Hazard ratio | P value | N   | Progression-free survival Hazard ratio | P value |
|-----------------------------|-----|------------------------------|---------|-----|----------------------------------------|---------|
| Sex                         |     |                              |         |     |                                        |         |
| Female                      | 244 | 1.47 (1.03-2.08)              | *       | 201 | 1.46 (1.2-1.13)                        | 0.051   |
| Male                        | 566 | 1.28 (1.03-1.59)              | *       | 437 | 1.08 (0.85-1.37)                       | 0.55    |
| Stage                       |     |                              |         |     |                                        |         |
| 1                           | 69  | 0.63 (0.23-1.71)              | 0.36    | 60  | 0.66 (0.22-2)                          | 0.46    |
| 2                           | 145 | 1.74 (0.93-3.25)              | 0.077   | 131 | 1.5 (0.8-2.79)                         | 0.2     |
| 3                           | 319 | 1.54 (1.15-2.05)              | *       | 186 | 1.77 (1.21-2.58)                       | **      |
| 4                           | 152 | 1.5 (1.02-2.21)               | *       | 141 | 1.41 (0.96-2.07)                       | 0.083   |
| Stage T                     |     |                              |         |     |                                        |         |
| 1                           | 14  | —                            |         | 14  | —                                      |         |
| 2                           | 253 | 1.87 (1.21-2.9)               | **      | 239 | 1.66 (1.09-2.54)                       | *       |
| 3                           | 208 | 1.27 (0.9-1.8)                | 0.17    | 204 | 1.22 (0.87-1.7)                        | 0.24    |
| 4                           | 39  | 1.59 (0.69-3.62)              | 0.27    | 39  | 2.49 (1.14-5.47)                       | *       |
| Stage N                     |     |                              |         |     |                                        |         |
| 0                           | 76  | 1.04 (0.44-2.47)              | 0.92    | 72  | 1.21 (0.52-2.8)                        | 0.66    |
| 1                           | 232 | 2.43 (1.57-3.75)              | ***     | 222 | 2.49 (1.64-3.79)                       | ***     |
| 2                           | 129 | 1.78 (1.13-2.81)              | *       | 125 | 1.56 (1.01-2.41)                       | *       |
| 3                           | 76  | 1.88 (1.1-3.22)               | *       | 76  | 1.59 (0.93-2.72)                       | 0.085   |
| 1+2+3                       | 437 | 1.97 (1.51-2.58)              | ***     | 423 | 1.9 (1.47-2.47)                        | ***     |
| Stage M                     |     |                              |         |     |                                        |         |
| 0                           | 459 | 1.8 (1.36-2.39)               | ***     | 443 | 1.71 (1.3-2.24)                        | ***     |
| 1                           | 58  | 1.76 (0.98-3.17)              | 0.0573  | 56  | 1.4 (0.77-2.53)                        | 0.27    |
| Lauren classification        |     |                              |         |     |                                        |         |
| Intestinal                  | 336 | 1.95 (1.41-2.7)               | ***     | 263 | 1.73 (1.2-2.47)                        | **      |
| Diffuse                     | 248 | 1.63 (1.16-2.3)               | **      | 231 | 1.67 (1.18-2.37)                       | **      |
| Mixed                       | 33  | 2.02 (0.72-5.71)              | 0.1743  | 28  | 1.67 (0.6-4.68)                        | 0.32    |
| Differentiation             |     |                              |         |     |                                        |         |
| Poorly differentiated        | 165 | 1.19 (0.8-1.78)               | 0.39    | 121 | 1.35 (0.85-2.15)                       | 0.2     |
| Moderately differentiated    | 67  | 1.41 (0.73-2.69)              | 0.3     | 67  | 1.58 (0.85-2.96)                       | 0.15    |
| Well differentiated          | 32  | 2.45 (1.01-5.95)              | *       | 5   | —                                      | —       |
| HER2 status                 |     |                              |         |     |                                        |         |
| HER2 negative               | 532 | 1.47 (1.17-1.85)              | ***     | 408 | 1.35 (1.04-1.75)                       | *       |
| HER2 positive               | 343 | 1.16 (0.9-1.51)               | 0.26    | 232 | 1.24 (0.9-1.71)                        | 0.19    |

*P < 0.05, **P < 0.01, and ***P < 0.001.

### 3.7. Correlation between Immune Marker Sets and DOK5 Expression.

In exploring the relationship shared by DOK5 as well as various immune infiltrating cells, using GEPIA and TIMER databases, we paid attention to the relationship shared by various immune cell marker sets and DOK5 in GC tissues. In STAD, we examined the link shared by DOK5 expression and varying immune cells, like CD8+ T cells, B cells, T cells (general), TAMs, M1 macrophages, M2 macrophages, monocytes, neutrophils, NK cells, and DCs (Table 2 and Figures 6(a)–6(h)). We examined T cells with different
Expression of DOK5 in STAD based on sample types

(a) TCGA samples

Expression of DOK5 in STAD based on individual cancer stages

(b) TCGA samples

Expression of DOK5 in STAD based on patient’s race

(c) TCGA samples

Expression of DOK5 in STAD based on patient’s gender

(d) TCGA samples

Expression of DOK5 in STAD based on tumor grade

(e) TCGA samples

Expression of DOK5 in STAD based on nodal metastasis status

(f) TCGA samples

Figure 4: DOK5 expression in subgroups of patients with STAD (UALCAN database). Relative expression of DOK5 in (a) STAD and normal samples; (b) normal individuals and patients with STAD at different stages; (c) normal individuals and Caucasian, African American, and Asian patients with STAD; (d) male and female normal individuals and patients with STAD; (e) normal individuals and STAD patients of different tumor grades; and (f) nodal metastasis status of patients with STAD (STAD: stomach adenocarcinoma; *P < 0.05, **P < 0.01, and ***P < 0.001).
functions, involving Th1 cells, Th2 cells, Tfh cells, Th17 cells, Treg cells, and exhausted T cells. The results showed that in STAD, the expression level of DOK5 is related to most immune marker sets of different T cells and various immune cells (Table 2).

It is worth noting that in GC, we explored the levels of expressions of most marker groups of monocytes; TAMs and M2 macrophage held a significant correlation with DOK5 expression (Table 2). We showed that CD86 and CSF1R of monocytes; IRF5 and PTGS2 of M1 macrophages; CD19 and CD79A of M2 macrophages; CD8A and CD8B of CD8+ T cell; CCL2, CD68, and IL10 of TAM; PDCD1 and HAVCR2 of T cell exhaustion; and CD3D, CD3E, and CD2 of T cell (general) are crucially linked to DOK5 expression in GC (P < 0.001; Figures 6(a)–6(h)). We further examined the link shared by DOK5 expression with monocytes markers and TAM markers in the GEPIA database. Correlation outcomes of DOK5 with monocyte markers and TAM markers matched the TIMER (Table 3). The above outcomes suggest that in GC, DOK5 may be related to the regulation of macrophage polarization.

DOK5 expression was positively correlated with dendritic cell infiltration in GC; for example, HLA-DPB1, HLA-DRA, HLA-DQB1, NRP1, CD1C, and ITGAX are also related to the expression of DOK5. The above outcomes also disclosed the proximate link shared by DOK5 and dendritic cell infiltration. In terms of Treg cells, DOK5 is positively correlated with FOXP3, CCR8, and TGFB1 in GC. In tumors, dendritic cells can promote metastasis through the reduction of cytotoxicity of CD8+ T cells and increasing Treg cells [11]. Moreover, we explored a strong link shared by DOK5 and T cell exhaustion and Treg, for instance, CCR8, FOXP3, STAT5B, and ITGAX are also related to the expression of DOK5. The above outcomes further indicate that DOK5 has a crucial immune role in the microenvironment of GC.
Table 2: Correlation analysis between DOK5 and related genes and markers of immune cells in TIMER.

| Description       | Gene markers | STAD | None | P   | STAD | P   |
|-------------------|--------------|------|------|-----|------|-----|
|                   |              | Core | P    | Core | P    |     |
| CD8+ T cell       | CD8A         | 0.319| ***  | 0.277| ***  |     |
|                   | CD8B         | 0.216| ***  | 0.185| ***  |     |
|                   | CD3D         | 0.286| ***  | 0.233| ***  |     |
| T cell (general)  | CD3E         | 0.318| ***  | 0.271| ***  |     |
|                   | CD2          | 0.348| ***  | 0.307| ***  |     |
|                   | CD19         | 0.288| ***  | 0.262| ***  |     |
| B cell            | CD79A        | 0.333| ***  | 0.292| ***  |     |
|                   | CD86         | 0.464| ***  | 0.426| ***  |     |
| Monocyte          | CD115 (CSF1R)| 0.55 | ***  | 0.523| ***  |     |
|                   | CCL2         | 0.594| ***  | 0.56 | ***  |     |
| TAM               | CD68         | 0.291| ***  | 0.257| ***  |     |
|                   | IL10         | 0.457| ***  | 0.438| ***  |     |
|                   | INOS (NOS2)  | -0.065| 0.183| -0.09| 0.08 |     |
| M1 macrophage     | IRF5         | 0.301| ***  | 0.291| ***  |     |
|                   | COX2 (PTGS2) | 0.216| ***  | 0.217| ***  |     |
|                   | CD163        | 0.448| ***  | 0.416| ***  |     |
| M2 macrophage     | VSIG4        | 0.492| ***  | 0.47 | ***  |     |
|                   | MS4A4A       | 0.526| ***  | 0.501| ***  |     |
| Neutrophils       | CD11b (ITGAM)| 0.496| ***  | 0.482| ***  |     |
|                   | CCR7         | 0.398| ***  | 0.357| ***  |     |
|                   | KIR2DL1      | 0.142| **   | 0.124| *    |     |
|                   | KIR2DL3      | 0.079| 0.11 | 0.043| 0.405|     |
|                   | KIR2DL4      | -0.017| 0.73 | -0.052| 0.316|     |
| Natural killer cell| KIR3DL1     | 0.159| **   | 0.134| **   |     |
|                   | KIR3DL2      | 0.175| ***  | 0.14 | **   |     |
|                   | KIR3DL3      | -0.107| *   | -0.102| *   |     |
|                   | KIR2DS4      | 0.059| 0.229| 0.04 | 0.437|     |
|                   | HLA-DPB1     | 0.394| ***  | 0.35 | ***  |     |
|                   | HLA-DQB1     | 0.205| ***  | 0.154| **   |     |
|                   | HLA-DRA      | 0.285| ***  | 0.241| ***  |     |
| Dendritic cell    | HLA-DPA1     | 0.324| ***  | 0.28 | ***  |     |
|                   | BDCA-1 (CD1C)| 0.455| ***  | 0.431| ***  |     |
|                   | BDCA-4 (NRP1)| 0.627| ***  | 0.615| ***  |     |
|                   | CD11c (ITGAX)| 0.459| ***  | 0.432| ***  |     |
|                   | T-bet (TBX21)| 0.301| ***  | 0.264| ***  |     |
|                   | STAT4        | 0.358| ***  | 0.327| ***  |     |
| Th1               | STAT1        | 0.033| 0.507| 0.012| 0.815|     |
|                   | IFN-γ (IFNG)| 0.064| 0.196| 0.0036| 0.482|     |
|                   | TNF-α (TNF)| 0.158| **   | 0.109| *    |     |
| Th2               | GATA3        | 0.363| ***  | 0.337| ***  |     |
|                   | STAT6        | 0.154| **   | 0.148| **   |     |
3.8. Gene Set Enrichment Analysis of DOK5. According to TCGA information, the ability to search for DOK5 and its related symbol transmission is realized using GSEA. As per NES, nominal $P$ value, and FDR $q$ value, fundamentally advanced flagging pathways had been elected. In this study, 19 signaling measures were differentially enhanced in the profoundly communicated phenotypes of DOK5. We discovered that most of these pathways are immune-related and involve cell adhesion molecules (CAMS), gap junction, complement and coagulation cascades, ECM receptor interaction, cytokine-cytokine receptor interaction, hedgehog signaling pathway, and leukocyte transendothelial migration (Figure 7(a)).

3.9. Functional Enrichment Analysis of DOK5 Gene. To understand the biological properties of DOK5 completely, we carried out GO and KEGG analyses. On the basis of the outcomes of DAVID’s research, we explored biologically enriched genes that are positively linked to the DOK5 expression levels. In GO analysis, the two biological processes contained by genes that are positively associated with DOK5 expression are as follows: immune response and the inflammatory response. Nine cellular components have been included in these coexpressed genes: cytoplasm, cytosol, nucleoplasm, extracellular exosome, membrane, extracellular space, protein complex, cell-cell adherents’ junction, and melanosome. Moreover, these coexpressed genes have three main molecular functions: sequence-specific DNA binding, identical protein binding and protein kinase activity, and transcription factor activity. Genes positively correlated with DOK5 expression in KEGG pathway analysis were as follows: cytokine-cytokine receptor interaction, transcriptional misregulation in cancer, TNF signaling pathway, malaria, and Chagas disease (American trypanosomiasis) (Figures 7(b)–7(e)).

3.10. qRT-PCR Experiments Show That DOK5 Expression Is Upregulated in Gastric Cancer. In order to further confirm that the expression level of DOK5 in GC tissues is higher than that in adjacent tissues, we used qRT-PCR technology to reveal the expression of DOK5 at the transcription level. The results showed that compared with adjacent nontumor tissues, the expression level of DOK5 mRNA in GC tissues was significantly increased (Figure 7(f)).

4. Discussion

GC is a tumor of the digestive system with high morbidity and mortality worldwide [13]. At present, surgery can be said to be the most efficient treatment for early GC, and 90% of patients will have a good prognosis [14]. However, resulting from the lack of early diagnosis, many patients are diagnosed at advanced stages, for example, about 65% of patients with stage 3 and stage 4 tumors, and nearly 85% of patients have lymph node metastasis [15]. In addition, treatment resistance often appears during the treatment of advanced GC. Immunomodulation has been applied to various types of cancer in preclinical models and has achieved good results [16]. It is of great significance for exploring an efficient method for the early diagnosis, as well as treatment of GC.

| Description        | Gene markers       | STAD         |
|--------------------|--------------------|--------------|
|                    |                    | Core | None | $P$ | Core | Purity | $P$ |
|                   | STAT5A             | 0.384 | *** |     | 0.383 | ***    |     |
|                   | IL13               | 0.179 | *** |     | 0.207 | ***    |     |
|                   | BCL6               | 0.416 | *** |     | 0.396 | ***    |     |
|                   | IL21               | 0.116 | *   |     | 0.0096 | 0.063 |
|                   | STAT3              | 0.336 | *** |     | 0.337 | ***    |     |
|                   | IL17A              | -0.148 | ** |     | -0.158 | **     |     |
|                   | FOXP3              | 0.33   | *** |     | 0.287 | ***    |     |
|                   | CCR8               | 0.397 | *** |     | 0.384 | ***    |     |
|                   | STAT5B             | 0.462 | *** |     | 0.457 | ***    |     |
|                   | TGFβ (TGFβ1)      | 0.576 | *** |     | 0.552 | ***    |     |
|                   | PD-1 (PDCD1)      | 0.221 | *** |     | 0.182 | ***    |     |
|                   | CTLa4              | 0.17   | *** |     | 0.125 | *      |     |
|                   | LAG3               | 0.179 | *** |     | 0.136 | **      |     |
|                   | TIM-3 (HAVCR2)    | 0.456 | *** |     | 0.433 | ***    |     |
|                   | GZMB               | 0.119 | *   |     | 0.066 | 0.203  |     |

STAD: stomach adenocarcinoma; TAM: tumor-associated macrophage; Th: T helper cell; Tfh: follicular helper T cell; Treg: regulatory T cell; Cor: $R$ value of Spearman’s correlation; None: correlation without adjustment; Purity: correlation adjusted by purity ($^*P<0.05$, $^{**}P<0.01$, and $^{***}P<0.001$).
Figure 6: Continued.
In this study, the Oncomine database and the TCGA database analyses showed that DOK5 expression was higher in GC than in normal tissues; these complied with the results of related research reports [17]. It has also been studied in breast cancer, liver cancer, and colorectal cancer, and DOK5 gene expression in cancer tissues is higher than that in normal tissues [18]. The receptor, tyrosine kinase c-Ret, has been found to be an oncogenic mutation in patients with multiple endocrine tumors and cancer syndromes with familial medullary thyroid carcinoma, and DOK5 can be directly associated with Y1062 of c-Ret, thereby enhancing the effect of c-Ret [3]. Our research shows that DOK5 is...
likely to be an oncogene that holds a crucial part in the development and occurrence of cancer. Moreover, in patients with stomach cancer, the DOK5 expression level is also significantly different in different pathological stages, tumor differentiation, and T stages. Therefore, we further studied the link shared by the expression of DOK5 and icopathological boundaries and found that the DOK5 expression level had been associated with lymph node metastasis and T stage. Moreover, for patients with lymph node metastasis, patients with high DOK5 expression have a poor prognosis, while patients with low DOK5 expression have a better prognosis. The pathological, T, N, and M stages are linked to the prognosis of GC patients. Our research shows that DOK5 is related to the survival and prognosis of GC patients; therefore, this suggests that DOK5 may be a specific marker of gastric cancer. Nineteen signal pathways were enriched by GSEA to analyze the signal pathway of different pathological stages, tumor differentiation, and T stages. Therefore, we further studied the link shared by the expression of DOK5 and clin-icopathological boundaries and found that the DOK5 expression level had been associated with lymph node metastasis and T stage. Moreover, for patients with lymph node metastasis, patients with high DOK5 expression have a poor prognosis, while patients with low DOK5 expression have a better prognosis. The pathological, T, N, and M stages are linked to the prognosis of GC patients. Our research shows that DOK5 is related to the survival and prognosis of GC patients; therefore, this suggests that DOK5 may be a specific marker of gastric cancer. Nineteen signal pathways were enriched by GSEA to analyze the signal pathway of DOK5 in GC. Melanoma, basal cell carcinoma, and hematopoietic cell lineage all proved that DOK5 influences the progression as well as the occurrence of tumors. In addition, we use the research method of Dr. Sun and his colleagues as a reference to conduct correlation analysis on DOK5 [19–21].

Recently, the role of the immune system in the development, as well as occurrence of cancer, was given more attention [22, 23]. Exploring the tumor microenvironment is a new hot research field for tumor diagnosis and treatment [24]. Focal adhesion was found to affect cell migration [25, 26]. Studies have shown that focal adhesion is linked to several biological pathways like cell differentiation, cell proliferation, and cell survival [27]. Simultaneously, focal adhesions are also related to the invasion of cancer cells [28]. Studies have shown that in the tumor microenvironment, ECM receptor interaction plays a significant part in tumor metastasis and recurrence [29]. Tumor angiogenesis and tumor local invasion along with distant metastasis are closely associated with the CAM pathway [30]. Studies have shown that the expression of the CAM pathway can help identify the prognosis of tumors and is expected to turn into a new target for GC treatment. Moreover, the cytokine-cytokine receptor interaction signaling pathway plays a crucial role in cancer pathogenesis [31]. There are also research results showing that the restoration of gap junction will affect the growth of tumor cells and the differentiation of tumor tissues [32]. Therefore, the role of gap junctions can affect the efficiency of antitumor drugs, thereby providing new targets for tumor treatment [33]. There are related research reports on transcription regulation errors playing very important roles in the development of tumors [34]. Tumor necrosis factor (TNF) has a main part in the development of inflammation, cell proliferation, and cell death [35]. For a long time in the past, the TNF family signaling pathway has been a double-edged sword in the process of tumor occurrence and clearance [36]. In the pathogenesis of oral squamous cell carcinoma, TNF-α can regulate EMT through the MAPK signaling pathway to promote cancer cell invasion and metastasis, and DOK5 also involved in MAPK (mitogen-activated protein kinase) signal pathway activation [37]. Therefore, we have reason to speculate that DOK5 may also participate in tumor progression through the MAPK pathway. Therefore, the above results explain the high expression of DOK5 and the high level of immune cell infiltration can reduce the survival rate of patients with GC. These results

| Description        | Gene markers | Tumor | STAD | Normal | P       |
|--------------------|--------------|-------|------|--------|---------|
| Monocyte           | CD86         | 0.26  | ***  | −0.11  | 0.53    |
|                    | CD11b        | 0.26  | ***  | 0.57   | ***     |
| Neutrophils        | CCR7         | 0.19  | ***  | −0.14  | 0.42    |
| TAM                | CD68         | 0.18  | ***  | −0.41  | *       |
|                    | IL-10        | −0.024| 0.63 | 0.099  | 0.57    |
|                    | IFN-γ (IFNG) | −0.042| 0.4  | −0.058 | 0.74    |
| Th1                | STAT1        | −0.083| 0.095| 0.18   | 0.3     |
|                    | T-bet (TBX21)| 0.15  | *    | −0.052 | 0.76    |
|                    | TNF-α (TNF) | 0.11  | *    | −0.15  | 0.37    |
| Th2                | STAT6        | 0.058 | 0.24 | 0.48   | **      |
|                    | CCR8         | 0.18  | ***  | −0.27  | 0.11    |
| Treg               | STAT5B       | 0.3   | ***  | 0.77   | ***     |
|                    | TGF-β (TGFB1)| 0.45  | 0    | 0.066  | 0.7     |
|                    | CTLA4        | −0.049| 0.33 | −0.15  | 0.39    |
| T cell exhaustion  | PD-1 (PDCD1)| 0.03  | 0.55 | −0.16  | 0.34    |
|                    | TIM-3 (HAVCR2)| 0.22 | ***  | 0.02   | 0.91    |

* P < 0.05, ** P < 0.01, and *** P < 0.001.
Enrichment Score

KEGG_CELL_ADHESION_MOLECULES_CAMS
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
KEGG_ECM_RECEPTOR_INTERACTION
KEGG_GAP_JUNCTION
KEGG_HEDGEHOG_SIGNALING_PATHWAY
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION

Figure 7: Continued.
Figure 7: Continued.
Figure 7: Continued.
transcription factor activity, sequence–specific
Identical protein binding
Sequence–specific DNA binding
Protein kinase activity
Protein domain specific binding
Cadherin binding involved in cell–cell adhesion
Enzyme binding
Ubiquitin protein ligase binding
Protein complex binding
Kinase activity
Growth factor activity
Cytokine activity
Protein N–terminus binding
Chemokine activity
Damaged DNA binding
Spectrin binding

gene ratio

Gene count
5 25
10 30
15 35
20

Figure 7: Continued.
may bring new methods for the immunotherapy of patients with GC.

Without doubt, after the above results and demonstrations, we have reason to believe that DOK5 is linked to the GC development as well as occurrence. The high expression of DOK5 further impairs the prognosis of patients with GC of by participating in immune-related mechanisms. However, our research also has certain limitations. Our data comes from different databases, and the information in each database may have some differences. Fortunately, through mutual verification of the different databases, we finally got our results.

5. Conclusions

Using multiple database verifications, we found that DOK5 is an oncogene of GC. Hence, DOK5 can reduce the prognostic effect of GC in patients through immune response. DOK5 is expected to become a new target for GC treatment and provide a new direction for GC treatment.

Data Availability

All data were acquired from public databases, including TCGA, Oncomine, GEPIA, Kaplan-Meier plotter, cBioPortal, TIMER, and UALCAn database.

Ethical Approval

The Ethics Committee of the Affiliated Changzhou No. 2 People’s Hospital of Nanjing Medical University approved all collections (No. [2020] KY125-01).

Consent

All patients provided written informed consent.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.
Authors’ Contributions

Fengyong Luo drafted the manuscript. Zhihuai Wang and Shuai Chen provided the design idea of this study. Zhenbo Luo, Gaochao Wang, Haojun Yang, and Liming Tang processed the data and supplemented the ideas. All authors read and approved the final manuscript. Fengyong Luo and Zhihuai Wang contributed equally to this work.

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Supplementary Materials

Supplementary Table 1: DOK5 expression in cancers versus normal tissue in Oncomine database. (Supplementary Materials)

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