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Analysis of the expression, function, prognosis and co-expression genes of DDX20 in gastric cancer

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ABSTRACT

DDX20 (DEAD-box polypeptide 20) is implicated in many cellular processes involving alteration of RNA secondary structure. The role of DDX20 in gastric cancer is still unknown. In the research, the expression of DDX20 and the functional roles of DDX20 in gastric cancer were detected. The increased DDX20 expression in gastric cancer tissue compared with normal gastric tissue was observed. Functional experiments indicated that DDX20 promoted gastric cancer MGC-803 and AGS cells growth, migration, and invasion in *vitro*. Surprisingly, survival analysis showed that high expression of DDX20 is a favorable prognostic factor for patients with gastric cancer. In addition, enrichment analysis revealed that there is a positive correlation between DDX20 expression and T cell activation in gastric cancer. but not in normal gastric tissues. Furthermore, we found that DDX20 expression level has significant positive correlations with activated CD8 + T cells and activated CD4 + T cells in gastric cancer. Therefore, we hypothesize that the prognostic role of DDX20 in gastric cancer patients may be due to patients with high DDX20 expression contained better immune activation. Taken together, these findings suggest that DDX20 can promote the progression of gastric cancer in *vitro* and its prognostic value in gastric cancer may be related to many factors, including immune activation.

1. Introduction

Gastric cancer is the fourth most common cancer worldwide. More than 70% of gastric cancers happen at developing countries and half the total located in Eastern Asia, especially in China, South Korea and Japan [1,2]. The histological classification of gastric carcinoma has been classified into two major histological subtypes, namely diffuse and intestinal type [3]. Although lifestyle and smoking are important causes of gastric cancer, the major risk factor for gastric cancer is *Helicobacter pylori* infection [4]. *Helicobacter pylori* infection is common in human but only 1% of infected people develop gastric cancer due to the persistent infection [5]. In spite of the great advances in the treatment of patients with gastric cancer, the clinical outcome of gastric cancer remains poor. Therefore, there is still an urgent need to explore new diagnostic and prognostic biomarkers and effective therapeutic targets for gastric cancer.

DEAD-box polypeptide 20 (DDX20), also known as DP103 or Gemin3, is a DEAD-box RNA helicase that is involved in multiple cellular processes. Like other members of the DEAD-box RNA helicase family, DDX20 has nine conserved motifs including Q, I, Ia, Ib,

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II, III, IV, V and VI, which are located on its N-terminus [6]. The DEAD-box RNA helicase family has attracted great interest because they play an important role in all aspects of RNA synthesis and function, including pre-mRNA processing, RNA turnover, ribosome biogenesis, RNA export and translation, and dissociation of large ribonucleoprotein (RNP) complexes. It is known that many of the disorders of DEAD-box helicase have deleterious effects on normal cell homeostasis, leading to uncontrolled proliferation, which is one of the factors leading to the development and progression of cancer. Previous studies found that oncogenic viruses can drive cell proliferation and anti-apoptotic activity by targeting DDX20 [7]. In addition, DDX20 protein levels are up-regulated in distant metastatic tissues in patients with breast cancer, prostate cancer and colorectal cancer [8,9]. The discovery of DDX20-NF-κB-MMP9 axis enhances the role of DDX20 in tumor metastasis. In contrast, DDX20 acts as a tumor suppressor role in liver cancer. The decreased level of DDX20 was observed in liver cancer, and it was found to inhibit NF-κB activity by regulating miRNA-140 in liver cancer cell [10,11]. However, the association between DDX20 and gastric cancer remains to be investigated.

This research aims to investigate the role of DDX20 in gastric cancer. The expression and prognostic significance of DDX20 in gastric cancer tissues and matched adjacent normal tissues were studied. In addition, enrichment analysis of genes co-expressed with DDX20 was performed to identify potential downstream signaling pathways.

2. Materials and methods

2.1. Extraction of clinical and microarray gene expression data from gastric cancer patient datasets

The clinical and microarray datasets of gastric cancer patients were extracted from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). Eight microarray gene expression datasets of gastric cancer patient, GSE54129, GSE13199, GSE112369, GSE118916, GSE19826, GSE79973, GSE56807 and GSE62254, were obtained from GEO database. The method for extracting microarray gene expression values is based on our previous research [12].

The prognostic values of the DDX20 mRNA level for gastric cancer patients were obtained from GSE62254 dataset. The survival analyses were performed using the expression value of DDX20 in gastric cancer patients. According to the expression value of DDX20, samples were divided into high expression group, middle expression group and low expression group.

2.2. DNA constructs

The pCMV-DDX20-FLAG (Sino Biological, Beijing, China) was constructed for expressing full-length DDX20 with a Flag tag at the carboxy-terminal end. The sequence of shRNA targeting DDX20 (5′-GCTGAATTGGTAGAGGATTATG-3′) was cloned into

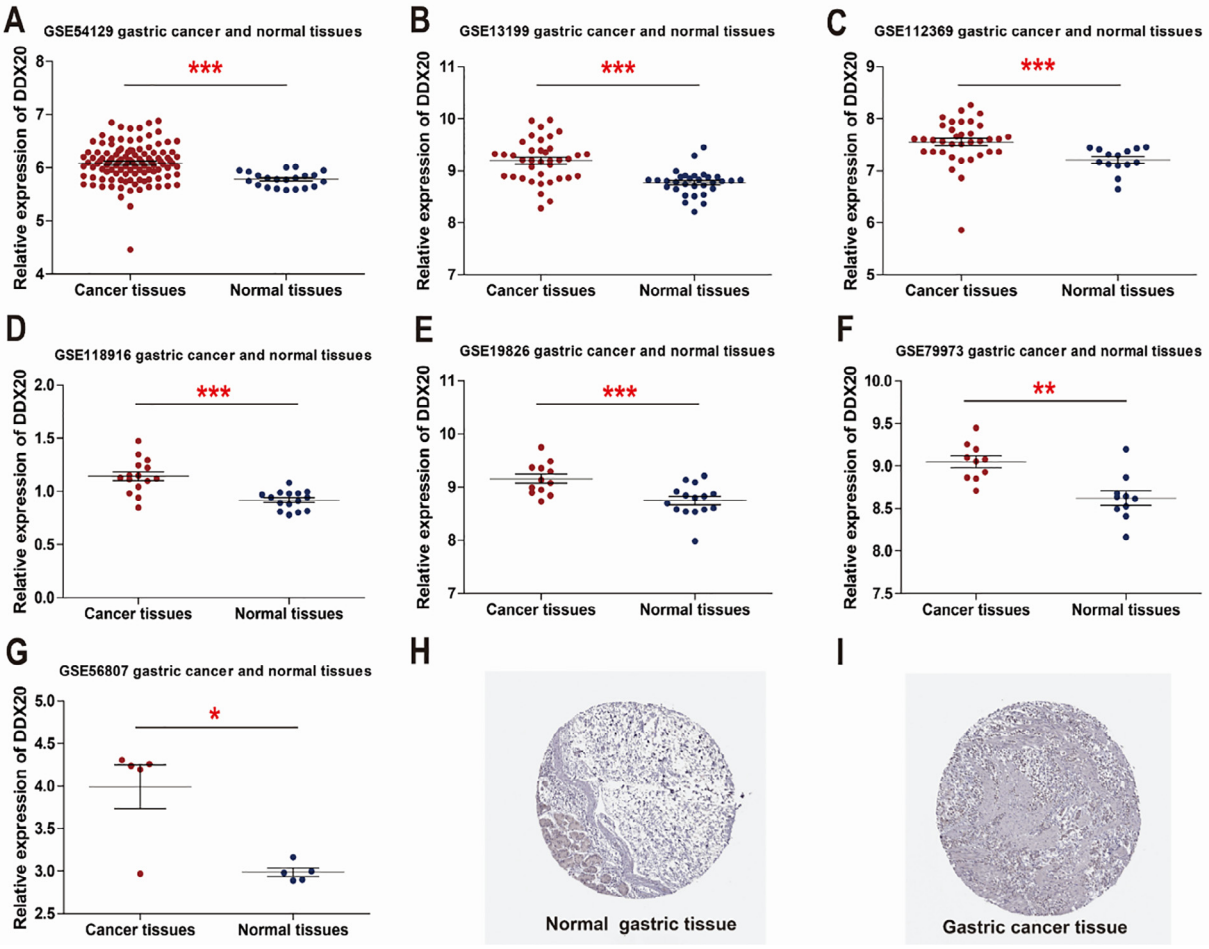

Fig. 1. The mRNA level of DDX20 in gastric cancer based on GEO database. The mRNA level of DDX20 in gastric cancer tissues and adjacent normal gastric tissues were compared. Seven mRNA datasets were employed including (A) GSE54129, (B) GSE13199, (C) GSE112369, (D) GSE118916, (E) GSE19826, (F) GSE79973 and (G) GSE56807. (H&I) The Human Protein Atlas database indicated that DDX20 protein was strongly higher in gastric cancer tissues compared with that in normal tissues.

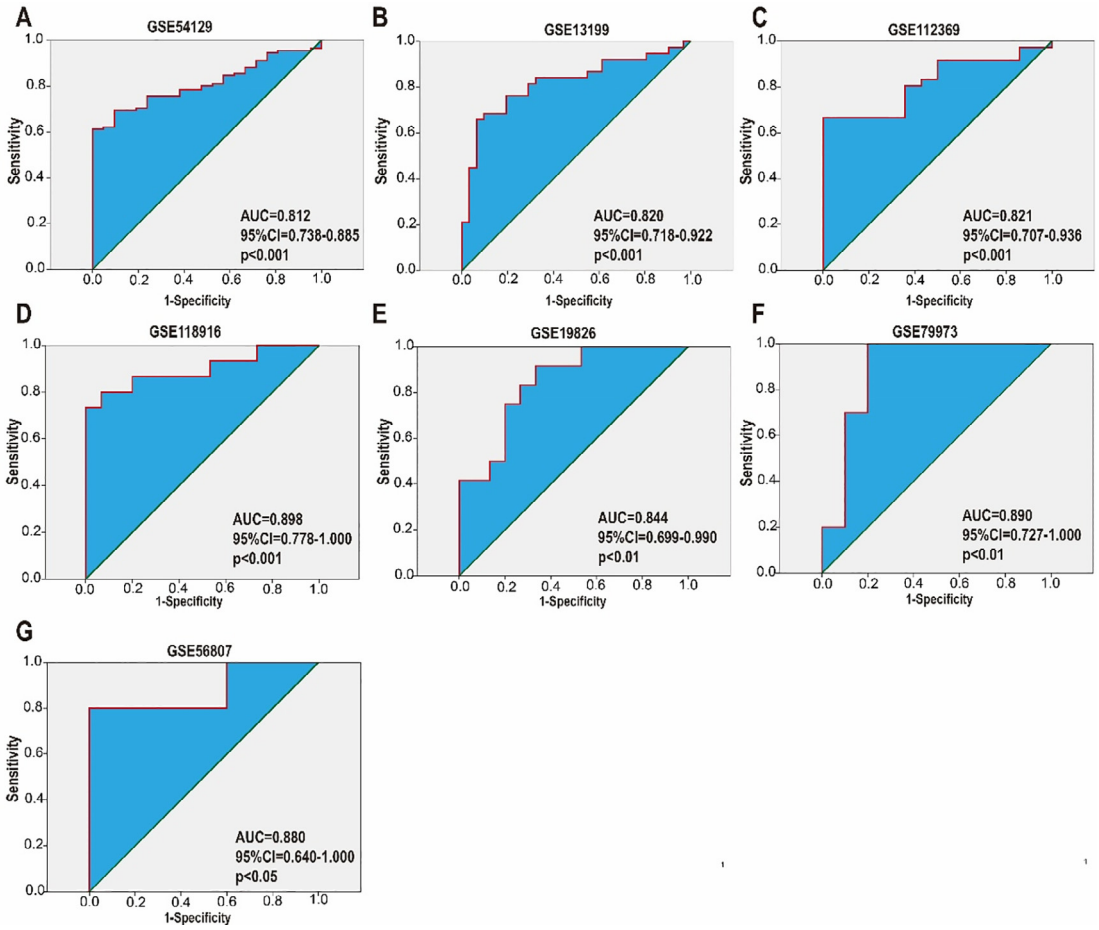

Fig. 2. High levels of DDX20 mRNA as a diagnostic marker in patients with gastric cancer. (A) GSE54129, (B) GSE13199, (C) GSE112369, (D) GSE118916, (E) GSE19826, (F) GSE79973 and (G) GSE56807.

Table 1
Association between DDX20 expression and clinical parameters.

	Number	Mean expression of DDX20	sem	p value
Age				
<60	106	2.071	0.01149	0.0996
≥60	194	2.094	0.00809	
Tumor type				
Diffuse	134	2.077	0.01016	0.3713
Intestinal	146	2.089	0.009116	
Stage				
I&II	126	2.109	0.01063	0.0036
III&IV	172	2.07	0.008367	
Recurrence				
No	157	2.1	0.009118	0.0148
Yes	125	2.067	0.009899	
EBV ISH				
Negative	257	2.084	0.006951	0.1414
Positive	18	2.124	0.02872	

pLVX vector through restriction enzyme sites BamH I and EcoR I site.

2.3. Cell culture and transfection

MGC-803 and AGS cells were obtained from ATCC. MGC-803 cells were cultured in RPMl 1640 Medium (Biological Industries (BI), Israel) and AGS cells were cultured in DME/F12 medium (BI) supplemented with 10% fetal bovine serum (FBS, BI), 100U/mL penicillin and 100 μg/mL streptomycin (1% Pen/Strep) (BBI life sciences, shanghai, China) at 37 °C in a humidified atmosphere containing 5% CO2. Cells were transfected by using VitalGENE-II In Vitro DNA Transfection Kit (biocanaan). The kit was used according to the manufacturer's instruction.

2.4. Western blot

Cells were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer containing protease inhibitors (Roche Ltd, Basel, Switzerland). Protein concentrations in lysate were measured by Micro

BCA protein assay kit (Pierce Biotechnology). Samples were separated on a 10% SDS-PAGE, and subsequently transferred to Amersham Protran nitrocellulose membrane (GE Healthcare Life Sciences, Fairfield, USA). The nitrocellulose membrane was then incubated with the primary antibodies (Proteintech, Wuhan, China) against target protein DDX20 and GAPDH (Abmart) at 1:1000 dilutions. IRDye® 800CW Goat Anti-Rabbit lgG or IRDye® 680RD Goat anti-Mouse lgG (LI-COR Biosciences) was used as the secondary antibody at 1:5000 dilutions. Detection and quantification of proteins were performed using Odyssey® CLx Infrared Imaging System (LI-COR Biosciences).

2.5. CCK8 assay

Taking out a 96-well plate once every 24 h, added 10 μL CCK8 (Transgen) solution to each of the test sample, the 96-well plate at 37 °C and 5% CO2 in the condition of incubation 1.5 h. OD-value was measured with the microplate reader, set the wavelength to 450 nm, OD-value of each well was measured, and the date was collected and analyzed. The experiment was conducted for at least three times.

2.6. Cell migration assays

Cells were first seeded at a 1.5×10^5 cells/well in six-well plates. After 24 h of culture, when the cells fit perfectly with the cells and covered the culture well, a 10 μL pipette tip was used to form cell free area. After the scratch, the cells were rinsed with PBS several times until the dead cells or fragments floating in the culture holes were washed away. Replace the cultured cells with serum-free medium. The six-well plate was photographed with inversion phase contrast microscope and this time point we called it "0h". And the cells were placed in the condition of 5% CO2 and 37 °C. After culture for 48 h, rinse with PBS and take photos again. Multiple views of each well were documented and three independent experiments were performed. The experiment was repeated at least three times.

2.7. Invasion assays

Adding 300 μL complete medium to the 24-well plate, the Transwell chamber with matrigel (Corning, New York, USA) was placed into the 24-well plate. Dilute the cell density to 1×10^4 cells/mL. And 0.5 mL of cell diluent was added to each well in the Transwell chamber, with three repeated controls in each group. The culture medium was discarded, and the cells in the upper layer of the basement membrane were erased 24 h later. After the cells were fixed, about 300 μL gentian violet dye solution was added to the new 24-well plate to dye the treated Transwell chamber for 8 min. Discard the stain solution after staining and use a cotton swab to remove excess dye on the upper layer of the substrate film and the surroundings. The Transwell chamber was photographed with inverted phase contrast microscope (Carl Zeiss).

2.8. Functional enrichment analysis

To further analyze the potential functions of genes positively associated with DDX20 expression, the DAVID (http://david.abcc.ncifcrf.gov/) database was used with gene KEGG pathway analysis and GO functional enrichment analysis. $p < 0.01$ was set as the cut-off criteria.

2.9. Statistical analysis

The comparison between gastric cancer tissues and normal gastric tissues was conducted using the Student's *t*-test to generate a p value. Receiver operating characteristics (ROC) curve analysis was performed to analyze the ability of DDX20 as a biomarker for gastric cancer. All p values < 0.05 were considered statistically significant.

3. Results

3.1. The expression and prognosis of DDX20 in gastric cancer

To explore the expression pattern of DDX20 in gastric cancer, a total of 7 related GEO datasets (GSE54129, GSE13199 [13], GSE112369 [14], GSE118916, GSE19826 [15], GSE79973 [16] and GSE56807 [17]) were employed. The GSE54129 dataset contained 111 cases of gastric cancer and 21 matched adjacent normal gastric tissues. For the remaining six datasets. 38 cases of gastric cancer and 31 matched adjacent normal gastric tissues in GSE13199 dataset, 36 cases of gastric cancer and 14 matched adjacent normal gastric tissues in GSE112369 dataset, 15 cases of gastric cancer and 15 matched adjacent normal gastric tissues in GSE118916 dataset, 12 cases of gastric cancer and 15 matched adjacent normal gastric tissues in GSE19826 dataset, 10 cases of gastric cancer and 10 matched adjacent normal gastric tissues in GSE79973 dataset,

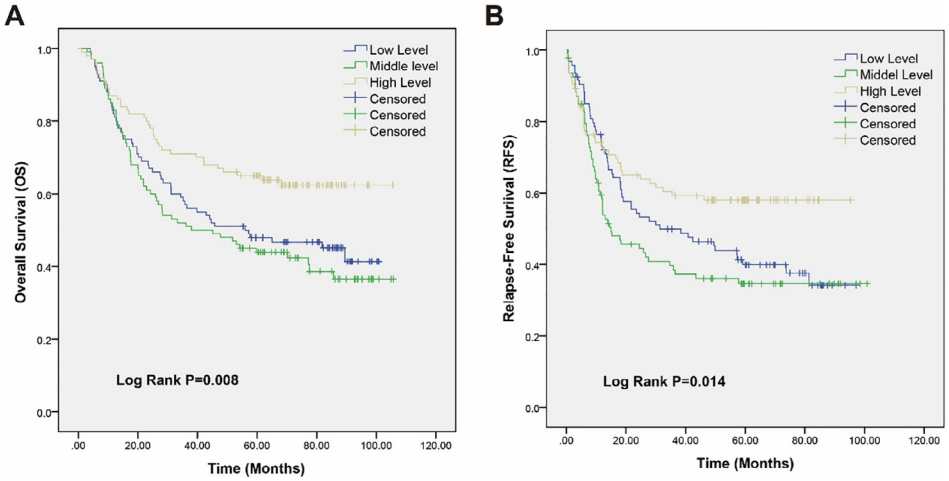

Fig. 3. Increased level of DDX20 is a favorable prognostic factor for gastric cancer. (A) Survival analysis of the association of DDX20 expression with overall survival time in gastric cancer. (B) Survival analysis of the association of DDX20 expression with relapse-free survival time in gastric cancer.

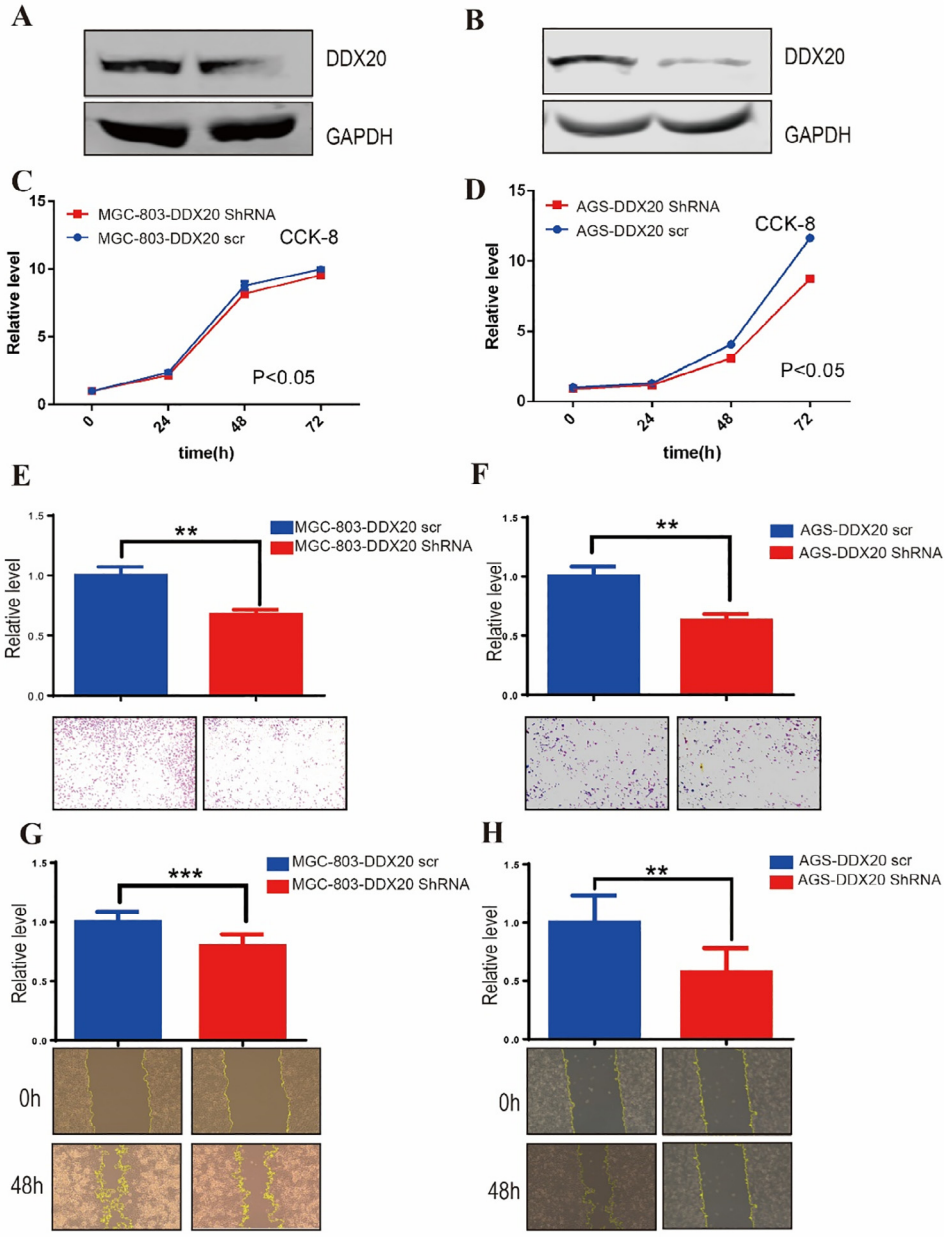

Fig. 4. Downregulation of DDX20 inhibits gastric cancer cell growth, invasion and migration. (A) Decreased protein level of DDX20 in MGC-803 cells transduced with ShDDX20 using western blot. (B) Decreased protein level of DDX20 in AGS cells transduced with ShDDX20 using western blot. (C) Decreased expression of DDX20 inhibited MGC-803 cell growth. Cell growth was assessed using the CCK-8 assay. (D) Decreased expression of DDX20 inhibited AGS cell growth. Cell growth was assessed using the CCK-8 assay. (E) Decreased expression of DDX20 inhibited MGC-803 cell invasion. (F) Decreased expression of DDX20 inhibited AGS cell invasion. (G) Decreased expression of DDX20 inhibited MGC-803 cell migration. (H) Decreased expression of DDX20 inhibited AGS cell migration. **: $p < 0.01$, ***, $p < 0.001$.

and 5 cases of gastric cancer and 5 matched adjacent normal gastric tissues in GSE56807 dataset. Analysis of these datasets showed that the level of DDX20 mRNA was significantly higher in gastric cancer tissues compared with matched adjacent normal gastric tissue (GSE54129, GSE13199, GSE112369, GSE118916, GSE19826, $p < 0.001$; GSE79973, $p < 0.01$; GSE56807, $p < 0.05$) (Fig. 1A-G), which indicated that DDX20 gene was upregulated during tumorigenesis in gastric cancer. Consistent with the mRNA data, immunohistochemistry staining from the Human Protein Atlas database revealed that DDX20 protein was significantly higher in gastric cancer tissues compared with normal gastric tissues (Fig. 1H&I).

ROC curve analysis suggested that the DDX20 mRNA expression possess potential diagnostic value for gastric cancer. Data from GSE54129 dataset showed that DDX20 mRNA expression was a significant diagnostic factor for gastric cancer (Area Under Curve (AUC) = 0.812; 95% CI, 0.738–0.885; $p < 0.001$, Fig. 2A). For the remaining six datasets studied, the AUC was 0.820 in GSE13199 dataset ($p < 0.001$, Fig. 2B), 0.821 in GSE112369 dataset ($p < 0.001$, Fig. 2C), 0.898 in GSE118916 dataset ($p < 0.001$, Fig. 2D), 0.844 in GSE19826 dataset ($p < 0.01$, Fig. 2E), 0.890 in GSE79973 dataset ($p < 0.01$, Fig. 2F) and 0.880 in GSE56807 dataset ($p < 0.05$, Fig. 2G). Based on the above data, DDX20 mRNA expression might be used as a new biomarker to distinguish between gastric cancer tissue and matched adjacent normal gastric tissue. Analysis of the clinical data based on GSE62254 revealed that DDX20 expression was associated with tumor grade and tumor recurrence but not age, tumor type and EBV ISH. Results showed that DDX20 expression decreases with increasing tumor grades and tumor recurrence in gastric cancer (Table 1). The association between DDX20 mRNA expression and patient survival

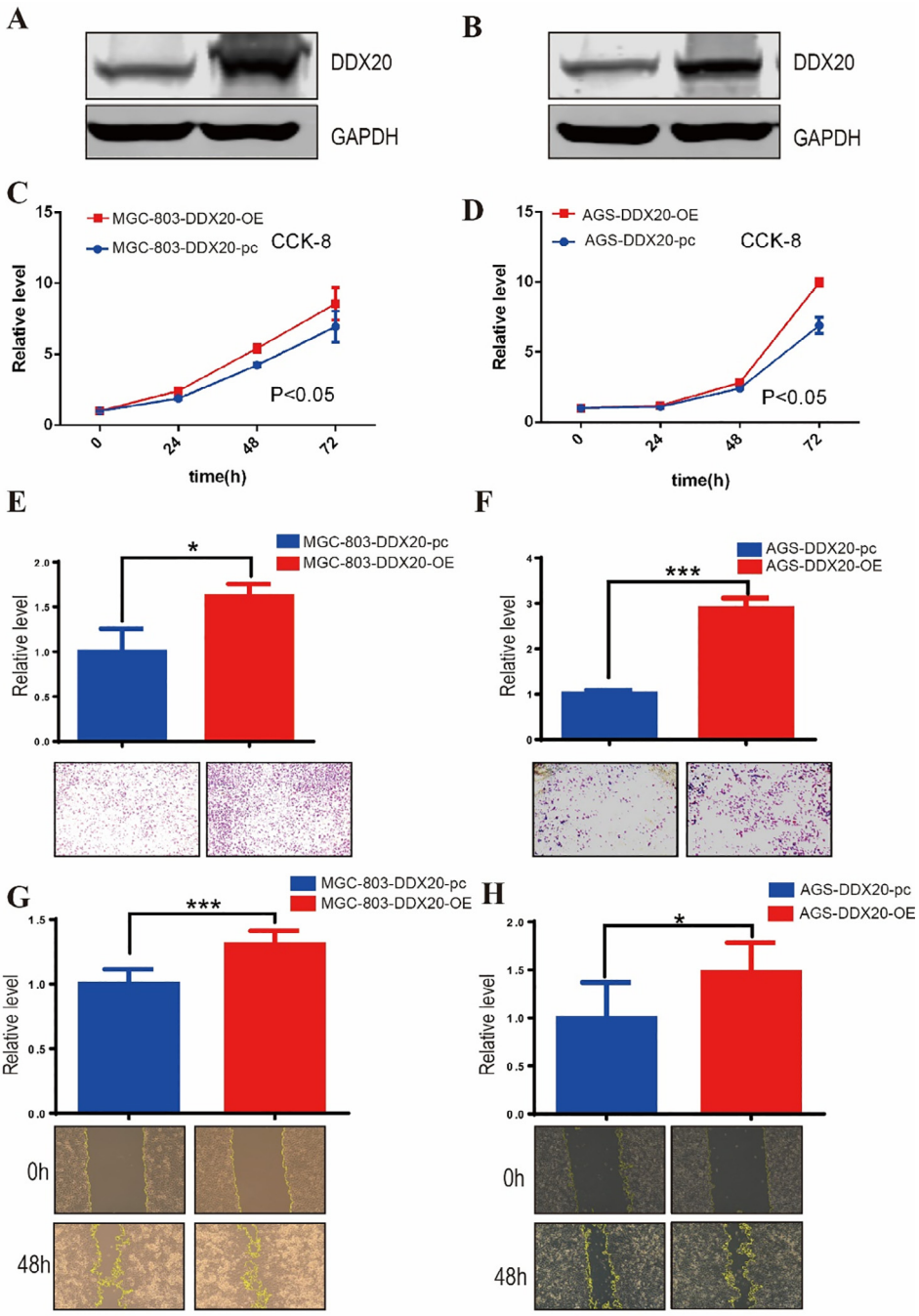

Fig. 5. Overexpression of DDX20 inhibits gastric cancer cell growth, invasion and migration. (A) Increased protein level of DDX20 in MGC-803 cells transduced with ShDDX20 using western blot. (B) Increased protein level of DDX20 in AGS cells transduced with ShDDX20 using western blot. (C) Increased expression of DDX20 inhibited MGC-803 cell growth. Cell growth was assessed using the CCK-8 assay. (D) Increased expression of DDX20 inhibited AGS cell growth. Cell growth was assessed using the CCK-8 assay. (E) Increased expression of DDX20 inhibited MGC-803 cell invasion. (F) Increased expression of DDX20 inhibited AGS cell invasion. (G) Increased expression of DDX20 inhibited MGC-803 cell migration. (H) Increased expression of DDX20 inhibited AGS cell migration. *: $p < 0.05$, ***, $p < 0.001$.

were also analyzed using GSE62254 database. The gastric cancer patients with higher DDX20 expression levels had longer overall survival and disease-free survival time than patients with lower or middle DDX20 expression levels (Fig. 3A&B).

3.2. DDX20 promotes the proliferation, migration, and invasion of gastric cancer cells

To study the biological function of DDX20 in gastric cancer, we knocked down DDX20 expression by shDDX20 plasmid in MGC-803 and AGS cells. Western blot analysis showed that shDDX20 had a strong inhibitory effect on DDX20 protein expression (Fig. 4A&B). As shown in Fig. 4C and D, knockdown of DDX20 significantly suppressed MGC-803 and AGS cell growth. In addition, suppression of DDX20 with shDDX20 downregulated MGC-803 and AGS cells migration and invasion (Fig. 4E-H).

In order to further confirm the effects of DDX20 on gastric cancer cells, MGC-803 and AGS cells were transfected with DDX20 overexpression plasmid. Western blot analysis confirmed there was an increase of DDX20 protein levels in the transfected MGC-803 and AGS cells (Fig. 5A&B). The CCK-8 assays showed that overexpression of DDX20 promoted MGC-803 and AGS cells growth

(Fig. 5C&D). We also evaluated the effects of DDX20 on cell migration and invasion. As shown in Fig. 4E-H, overexpression of DDX20 promoted migration and invasion of MGC-803 and AGS cells.

3.3. Enrichment analysis of co-expressed genes of DDX20 (CEGD) in gastric cancer and adjacent normal gastric samples

Although DDX20 promotes gastric cancer cell proliferation, migration and invasion, the higher expression of DDX20 is actually a favorable prognostic factor for gastric cancer patients. The mechanisms underlying this discrepancy is not clear. Thus, 15 pairs of gastric cancer and matched normal gastric samples obtained from GSE118916 were used to explore genes co-expressed with DDX20. We analyzed the CEGD in gastric cancer and adjacent normal gastric samples respectively. In total, 1157 mRNAs were found to be positively related to DDX20 expression in gastric cancer, whereas 538 mRNAs positively related to DDX20 expression in normal gastric samples (Fig. 6A). Each of the top 10 positive related genes were shown in Fig. 6B&C.

KEGG analysis results showed that the positive CEGDs in gastric cancer were mostly enriched in Type I diabetes mellitus, T cell receptor signaling pathway, ribosome, olfactory transduction, primary immunodeficiency, natural killer cell mediated cytotoxicity, inflammatory bowel disease (IBD), hematopoietic cell lineage, B cell receptor signaling pathway and antigen processing and presentation (Fig. 6D). GO analysis results showed that the positive CEGDs in gastric cancer were mostly enriched in the T cell receptor signaling pathway, T cell differentiation, T cell activation, regulation of T cell activation, positive regulation of T cell activation, positive regulation of leukocyte cell–cell adhesion, positive regulation

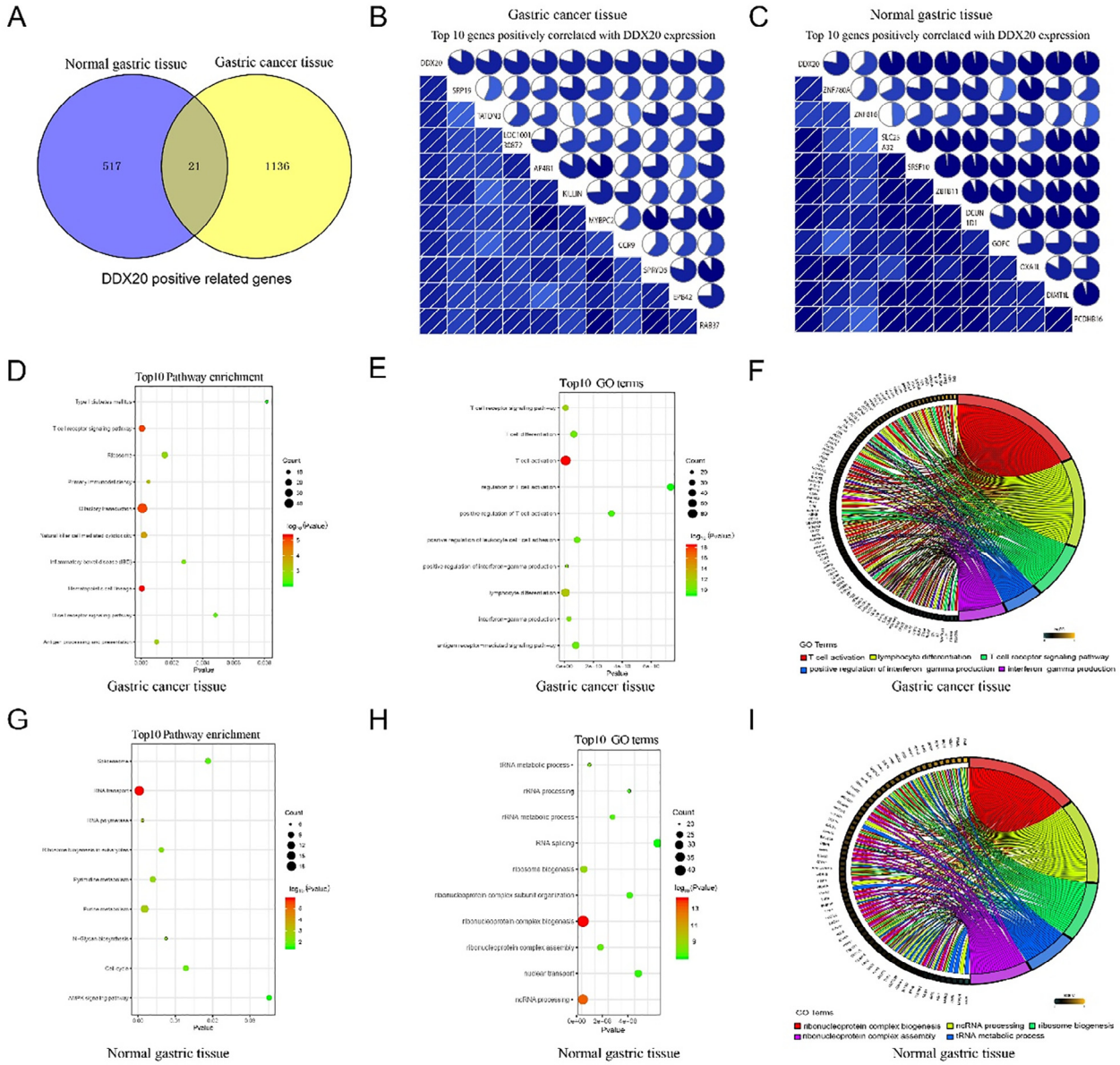

Fig. 6. Enrichment analysis of genes that were co-expressed with DDX20 in gastric cancer tissue and gastric normal tissue. (A) Venn diagram shows genes positively associated with DDX20 expression in gastric cancer and normal gastric tissues. (B) Top 10 genes positively correlated with DDX20 expression in gastric cancer tissues. (C) Top 10 genes positively correlated with DDX20 expression in gastric normal tissues. (D) Scatter plot of the top 10 enriched KEGG pathways for genes positively associated with DDX20 expression in gastric cancer tissue. (E) Scatter plot of the top 10 enriched GO terms for genes positively associated with DDX20 expression in gastric cancer tissue. (F) The network of top 5 mostly enriched GO terms for genes positively associated with DDX20 expression in gastric cancer tissue. (G) Scatter plot of the top 10 enriched KEGG pathways for genes positively associated with DDX20 expression in gastric normal tissue. (H) Scatter plot of the top 10 enriched GO terms for genes positively associated with DDX20 expression in gastric normal tissue. (I) The network of top 5 mostly enriched GO terms for genes positively associated with DDX20 expression in gastric normal tissue.

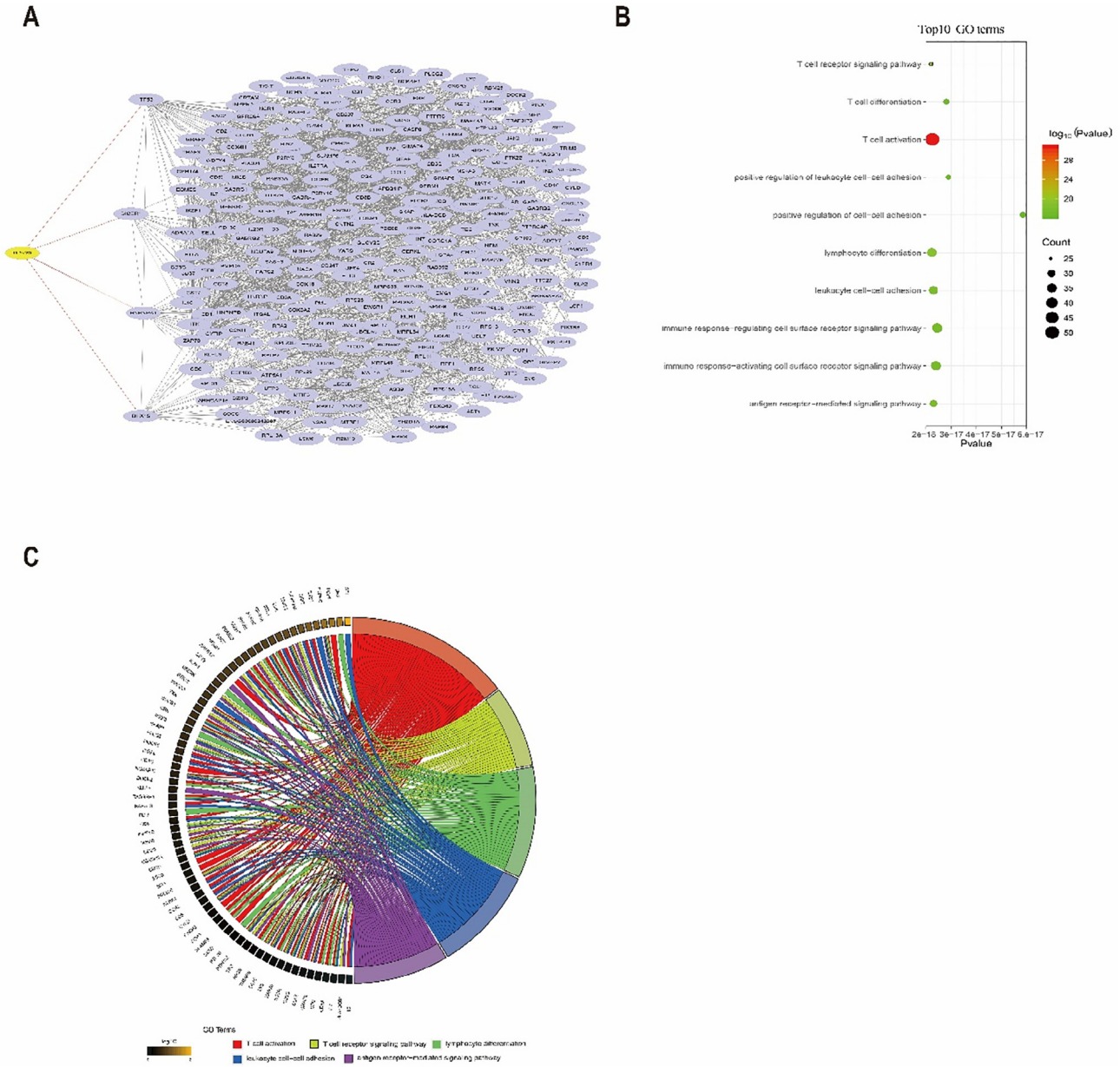

Fig. 7. PPI network analysis of DDX20 positive related genes in gastric cancer. (A) Protein interaction network map positively correlated with DDX20 expression. (B) Scatter plot of the top 10 enriched GO terms for DDX20 PPI protein in gastric cancer tissue. (C) The network of top 5 mostly enriched GO terms for DDX20 PPI protein in gastric cancer tissue.

of interferon–gamma (IFN-γ) production, lymphocyte differentiation, IFN-γ production and antigen receptor–mediated signaling pathway (Fig. 6E). The top five mostly enriched GO terms T cell activation, lymphocyte differentiation, T cell receptor signaling pathway, positive regulation of IFN-γ production and IFN-γ production were employed to construct a network (Fig. 6F).

KEGG analysis results showed that the positive CEGDs in normal gastric tissues were mostly enriched in spliceosome, RNA transport, RNA polymerase, ribosome biogenesis in eukaryotes, pyrimidine metabolism, purine metabolism, N–Glycan biosynthesis, cell cycle and AMPK signaling pathway (Fig. 6G). GO analysis results showed that the positive CEGDs in normal gastric were mostly enriched in tRNA metabolic process, rRNA processing, rRNA metabolic process, RNA splicing, ribosome biogenesis, ribonucleoprotein complex subunit organization, ribonucleoprotein complex biogenesis, ribonucleoprotein complex assembly, nuclear transport and ncRNA processing (Fig. 6H). The top five mostly enriched GO terms ribonucleoprotein complex biogenesis, ncRNA processing, ribosome biogenesis, ribonucleoprotein complex assembly and tRNA metabolic process were used to construct a network (Fig. 6I).

3.4. Protein-protein interaction (PPI) network analysis of positive CEGDs in gastric cancer

Based on the STRING database, a co-occurrence network of the positive CEGDs in gastric cancer was constructed. From the PPI network, the 4 genes interacting with DDX20 were filtered, including tumor protein p53 (TP53), dicer 1, ribonuclease type III (DICER1), Heterogeneous Nuclear Ribonucleoprotein A1 (HNRNPA1), and DEAH-box helicase 15 (DHX15) (Fig. 7A). GO analysis results showed that these PPI genes with DDX20 were mostly enriched in the T cell receptor signaling pathway, T cell differentiation, T cell

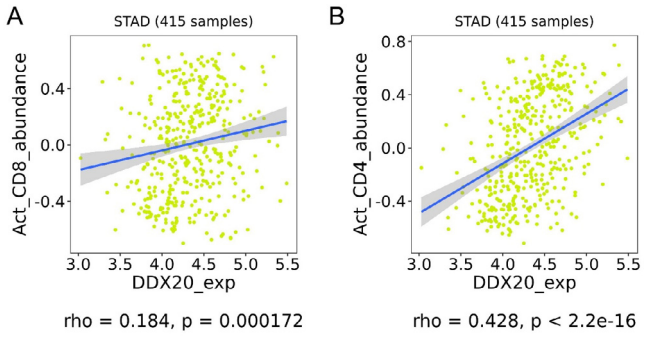

Fig. 8. Correlation of DDX20 expression with activated T cells in gastric cancer. DDX20 expression is significantly positive related to activated CD8 + T cells in gastric cancer. (B) DDX20 expression is significantly positive related to activated CD4 + T cells in gastric cancer.

activation, positive regulation of leukocyte cell–cell adhesion, positive regulation of cell–cell adhesion, lymphocyte differentiation, leukocyte cell–cell adhesion, immune response–regulating cell surface receptor signaling pathway, immune response–activating cell surface receptor signaling pathway and antigen receptor–mediated signaling pathway (Fig. 7B). The top five mostly enriched GO terms T cell activation, T cell receptor signaling pathway, lymphocyte differentiation, leukocyte cell–cell adhesion and antigen receptor–mediated signaling pathway were used to construct a network (Fig. 7C). Moreover, we found that DDX20 expression level has significant positive correlations with activated CD8 + T cells ($r = 0.184$, $p < 0.001$; Fig. 8A)) and activated CD4 + T cells using TISIDB analysis ($r = 0.428$, $p < 0.001$; Fig. 8B). These findings strongly suggest that DDX20 plays an important role in T cell activation in gastric cancer.

4. Discussion

DDX20 is a DEAD-box RNA helicase protein which is involved in multiple cellular processes. Previous studies have shown that DEAD-box RNA helicase proteins have an important role in cellular RNA metabolism. Thus, they are critical in several physiological RNA-centric functions, including transcription, pre-mRNA splicing, ribosome biogenesis, spliceosome assembly, mRNA export, protein translation and RNA turnover. In addition to participating in the regulation of cellular RNA metabolism, DEAD-box RNA helicase proteins also exhibit other functions. For example, they can participate in the regulation of tumor cell proliferation and metastasis by being a part of protein complex. It is known that the dysregulation of DDX20 has a detrimental effect on many normal cellular homeostasis, leading to uncontrolled proliferation, inappropriate invasion and survival of damaged cells, which are events leading to cancer development and progression.

Gastric cancer is the second leading cause of cancer-related deaths in the world, and there are many factors affecting the progression and prognosis of gastric cancer patients, such as depth of invasion, TNM staging and lymph node metastasis rate [18]. In this research, bioinformatics analysis of gastric cancer related GEO dataset (GSE54129, GSE13199, GSE112369, GSE118916, GSE19826, GSE79973 and GSE56807) showed that DDX20 mRNA expression was significantly up-regulated in gastric cancer tissues when compared with matched adjacent normal gastric tissues. We also used the Human Protein Atlas database to find that the DDX20 protein expression was significantly higher in gastric cancer tissues. Usually a gene aberrantly highly expressed in cancer tissues acts as a negative prognostic factor. However, Kaplan-Meier analysis showed that gastric cancer patients with high levels of DDX20 had longer overall survival than those patients with low and middle levels in the testing set GSE62254. Similar results have been encountered in our previous studies. Cyclin-D1 (CCND1) was highly expressed in renal clear cell carcinoma, whereas high level of CCND1 is a favorable prognostic factor for renal clear cell carcinoma patients [19]. Further research indicated that DDX20 expression was associated with tumor grade and tumor recurrence but not age and tumor type. We found that the level of DDX20 decreased with the increase of gastric cancer grades, and patients with recurrence had a low DDX20 expression compared to those with non-recurrence. Therefore, the poor prognosis of patients with low level of DDX20 may be due to the higher recurrence rate.

The role of DDX20 in carcinogenesis differs between different cancer types. In breast cancer and prostate cancer, DDX20 promotes the metastasis of breast and prostate cancer cells by affecting the NF-κB signaling pathway. In contrast with its role as a tumor promoter in breast and prostate cancer, DDX20 acts as a tumor suppressor in liver cancer. DDX20 was found to reduce NF-κB activity by regulating miRNA-140 in order to inhibit liver cancer progression [20]. However, the effect of DDX20 on gastric cancer is less known. In current study, we showed that the increased expression of DDX20 stimulated tumor cell proliferation of gastric cancer cells. Moreover, transwell assay revealed that the increased expression of DDX20 fostered the migration and invasion of gastric cancer cells. Our experimental results suggested DDX20 acts as an oncogene in gastric cancer cells. Therefore, the favorable impact of DDX20 on gastric cancer patient prognosis may be due to the impact of DDX20 on tumor microenvironment.

T cell immunity is an important part of the human immune system, and imbalance of T cell immunity leads to serious physical consequences [21,22]. T cell immunity includes many different types of cells, including CD4 + T cells, CD8 + T cells, and so on [23]. T cell play important roles in several types of cancers, including breast cancer [24], colorectal cancer [25], ovarian cancer [26] and lung cancer [27]. According to recent researches, T cell may have an important role in the progress and prognosis of gastric cancer. Previous study reported that high densities of cytotoxic T cells and memory T cells are associated with favorable survival for gastric cancer patients [28]. IFN-γ is an important cytokine produced by activated CD4 + or CD8 + T cells and natural killer cells and is recognized as an important mediator of immunity. Tu et al reported that IFN-γ overexpression inhibited gastric carcinogenesis induced by IL-1beta (IL-1β) and/or *Helicobacter pylori* infection [29]. Enrichment analysis of genes that were co-expressed with DDX20 in gastric cancer tissues showed that the significant GO functional annotation included T cell activation, lymphocyte differentiation, T cell receptor signaling pathway, positive regulation of IFN-γ production and IFN-γ production. Moreover, based on the TISIDB database, we found that there is a significant positive correlation between DDX20 expression and activated T cells in gastric cancer. These results suggested that the DDX20 levels in gastric cancer cells may affect the T cell activation in tumor tissues. Taken together, these results suggested that DDX20 may regulate the tumor microenvironment by affecting IFN-γ production and T cell activation in gastric cancer.

In contrast, the enrichment analysis of genes that were co-expressed with DDX20 in normal gastric tissues showed that the significant GO functional annotation was ribonucleoprotein complex biogenesis, ncRNA processing, ribosome biogenesis, ribonucleoprotein complex assembly and tRNA metabolic process. These results indicated that the expression of DDX20 was consistent with its known function in normal gastric tissues, whereas high expression of DDX20 was positively correlated with T cell activation in gastric cancer tissues. Therefore, the influence of DDX20 on the prognosis of gastric cancer patients may be

dependent on tumor microenvironment, and gastric cancer patients with high expression of DDX20 may have better tumor immunity activation.

The dysregulation of DDX20 expression was found in a variety of cancers, and it displays different roles in different types of cancer. In the research, the increased level of DDX20 was observed in gastric cancer patients' samples and increased level of DDX20 promoted proliferation, invasion and migration of gastric cancer cells. However, survival analysis showed that high level of DDX20 is a favorable prognostic factor in patients with gastric cancer. Enrichment analysis revealed that DDX20 expression were associated with T cell activation and IFN-γ production in gastric cancer samples, but not in normal gastric tissues. Therefore, we hypothesize that the high expression of DDX20 may be associated with better immune activation in gastric cancer tissues.

CRediT authorship contribution statement

Qingshui Wang: Conceptualization, Writing - original draft. **Yan Ye:** Data curation, Writing - original draft. **Rongbo Lin:** Conceptualization, Writing - review & editing. **Shuyun Weng:** Methodology. **Fan Cai:** Data curation. **Mei Zou:** Methodology. **Haitao Niu:** Resources. **Lilin Ge:** Conceptualization, Writing - review & editing. **Yao Lin:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csbj.2020.09.002.

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