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Comprehensive bioinformatic analysis of mind bomb 1 gene in stomach adenocarcinoma

Wang D *et al*. Analysis of *MIB1* gene in STAD

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Abstract

BACKGROUND

The carcinogenesis of stomach adenocarcinoma (STAD) involves many different molecules and multiple pathways, including the NOTCH signaling pathway. As a key factor that functions as a critical link in the NOTCH pathway, mind bomb 1 (*MIB1*) is upregulated in various tumors and has been reported to promote cell metastasis and invasion. However, studies on the role of *MIB1* in STAD are limited. Here, we evaluated the prognostic value of *MIB1* in STAD and its association with immune infiltration and copy number variation.

AIM

To elucidate the relationship between *MIB1* gene and gastric cancer (GC) and provide a new idea for the treatment of GC.

METHODS

We identified mutations in the *MIB1* gene by searching the cBioPortal database and then analyzed their relationship with the overall survival rate and disease-free survival rate using the Kaplan-Meier method. The Cancer Genome Atlas (TCGA) database provided transcript levels for *MIB1* in STADs and normal tissues. As a method of distinguishing the STAD tissues from adjacent normal tissues, a receiver operating characteristic (ROC) curve was generated. Kaplan-Meier plotter was used to determine

the effect of *MIB1* expression on survival. Based on the LinkedOmics database, we were able to identify the coexpressed genes of the *MIB1* gene, the top 50 positively correlated genes, and the top 50 negatively correlated genes. STRING was used to construct protein-protein interaction networks related to the *MIB1* gene. An analysis of functional enrichment was carried out using the R package "Cluster Profiler". The relationships between mRNA expression of *MIB1* and immune infiltrates were assessed by Tumor IMmune Estimation Resource (TIMER) and the "GSVA package" in R.

RESULTS

According to the cBioPortal database, the *MIB1* mutation rate in 287 patients in the TCGA dataset was approximately 6%. Kaplan-Meier survival analysis showed that patients with STAD in the mutated group had a worse prognosis than those in the unmutated group (P = 0.0156). There was a significant upregulation of *MIB1* expression in STAD tissues compared to adjacent normal tissues. A high T stage was associated with increased *MIB1* mRNA expression. The ROC curve analysis revealed 59.4% sensitivity and 85.6% specificity of *MIB1* for differentiating STAD tissues from adjacent normal tissues at a truncation level of 2.248. Kaplan-Meier plotter indicated that patients with higher *MIB1* levels had a worse prognosis than those with lower levels (26.4 mo *vs* 56.2 mo, P = 0.0330). A correlation analysis demonstrated an association between immune infiltrates and *MIB1* mRNA expression.

CONCLUSION

Upregulation of *MIB1* expression is significantly associated with poor survival rate and immune infiltration in gastric adenocarcinoma. *MIB1* may be a biomarker for the poor prognosis of STAD patients and a potential immunotherapeutic target.

Key Words: Stomach adenocarcinoma; Mind bomb 1; Mutation; Prognosis; Biomarker; Immune infiltration

INTRODUCTION

Gastric cancer (GC) was the world's leading cause of cancer deaths until the 1980s, when it was surpassed by lung cancer. Currently, the incidence of GC ranks fifth in the world and the fatality rate ranks fourth[1]. Despite its worldwide decline in morbidity and mortality rates in the past five years, GC has maintained a high mortality rate of 75% in most parts of the world, which is also the main cause of the global DALY-adjusted life year burden[2], and it is the most burdensome gastrointestinal disease in China[3]. Despite worldwide advances in clinical diagnosis and treatment, GC is still characterized by a low early diagnosis rate, low radical resection rate, and low 5-year survival rate, and most patients are first diagnosed when the disease is in an advanced stage[4]. Although many therapeutic advances, including surgical treatment, targeted therapy and immunological therapy, have been made in GC[5,6], the 5-year survival rate of patients primarily diagnosed with advanced stage is still as low as 18%[7], and the peritoneal recurrence rate after surgery is as high as 60%[8]. These findings indicate that there is a huge demand for more precise diagnosis and treatment of GC. Therefore, there is an urgent need to find new molecular markers to judge the prognosis of patients with GC.

GC is a multifactorial disease, and the recognized risk factors include age, male sex, genetic predisposition, *Helicobacter pylori* (*H. pylori*) infection, gastroesophageal reflux disease, and lifestyle factors such as smoking, alcohol consumption, and dietary composition[9,10]. Among the different types, 95% of GC cases are stomach adenocarcinoma (STAD)[11]. The combination of several variables, including genetics, epigenetics and the external environment, that may collectively result in the unregulated signaling pathway of cancer pathogenesis can be characterized as the pathogenesis of GC[12,13]. In addition, it is widely believed that dysfunctional oncogenic pathways contribute to the pathogenesis of GC, which might include the epidermal growth factor receptor, Notch, Hedgehog, nuclear factor-κB, and Wnt/β-catenin

pathways[14]. Among these pathways, the Notch signaling pathway is involved in direct cell-to-cell communication, cell differentiation, proliferation and apoptosis[15].

Noch signaling is a highly conserved pathway in multicellular animals that regulates the cell fates and upholds homeostasis in adult tissues. Numerous reports have confirmed the role of Notch signaling in both carcinogenesis and antitumor effects in different backgrounds[16,17]. Notch secretion signaling can modulate heterotypic interactions between the stroma and tumor and vice versa. These interactions have been shown to regulate many aspects of oncobiology, such as angiogenesis, cancer stem cell maintenance, immune infiltration, and resistance to therapy. These functions provide evidence for the environmental dependence of Notch-induced cellular responses[18].

Mind bomb 1 (*MIB1*), a large multidomain RING-type E3 ubiquitin-protein ligase[19], which activates Notch signaling by promoting ubiquitination, endocytosis and subsequent activation of Notch ligands, plays a central role in the conduction of Notch signaling pathway. Inhibition of *MIB1* Leaded to the decrease of Notch signal activation in mammalian cells, which was fatal to mouse embryos with Notch activation deficiency[20,21]. Vitro experiments confirmed that *MIB1* can induce degradation of suppressor of tumorigenicity 7 protein (ST7) to upregulate the IQ motif containing GTPase activating protein 1 (IQGAP1) in pancreatic cancer cells to promote tumor growth and progression, and also regulate the resistance of pancreatic cancer cells to gemcitabine[22,23]. It has been reported that *MIB1* was ubiquitous in breast cancer to mediate JAG1 ubiquitination and activate Notch signal[24]. Aside from ubiquitinating the NOTCH ligand, *MIB1* also ubiquitinated Ctnnd1 to regulate the migration of cells[25]. However, it remains unclear that the influence of *MIB1* gene on GC because of the limited research on *MIB1*. Our study aimed to determine whether *MIB1* was associated with prognosis in STAD and whether *MIB1* could be regarded as a potential therapeutic target.

MATERIALS AND METHODS
Study design

Briefly, the design of this study was as follows: First, the mutation of *MIB1* in The Cancer Genome Atlas (TCGA)-STAD data was investigated, and the differences in overall survival (OS) and disease-free survival (DFS) between the patient group with mutations and the group without mutations in *MIB1* were obtained. Second, data on the expression of *MIB1* in pancancer and STAD were acquired. Survival analysis was performed to study the prognostic value of *MIB1* in STAD from the aspect of receiver operating characteristic (ROC) curve and OS. Then, we obtained coexpressed genes from LinkedOmics, conducted Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on the top 200 related genes, and subsequently displayed the top 50 positively and negatively correlated genes. Finally, enrichment analysis of differentially expressed genes was conducted to determine the biological function of *MIB1* significantly differentially enriched genes (DEGs). In addition, the role of *MIB1* in STAD was explored by studying the correlation between *MIB1* and immune infiltrating cells.

cBioPortal analysis

The cBioPortal for Cancer Genomics (https://www.cbioportal.org/) was used to study the relationship between the mutation of the *MIB1* gene in STAD and the OS or DFS of patients, and visual analysis was performed. In this database, STAD (TCGA, Nature 2014) was selected for analysis this study.

Expression of MIB1 gene

The official website of the TCGA (https://portal.gdc.cancer.gov/) was used to download the RNA-seq expression data of *MIB1* for STAD. Thirty-two examples of neighboring normal tissues and a total of 375 cases of gastric adenocarcinoma were preserved. The chosen samples included data on *MIB1* gene expression as well as pertinent clinical data, such as age, sex, HP, T stage, N stage, and M stage. The mean and standard deviation were used to describe the mRNA expression data. No

permission from the ethical committee was needed for this investigation because all of the data were downloaded from the public database.

Survival analysis

Kaplan-Meier curves were drawn using the Kaplan-Meier Plotter Web tool (https://kmplot.com/). Based on median gene expression, patients were split into two groups, and the log-rank test was used to compare the survival rates between the "high" expression group (red line) and the "low" expression group (blue line). We evaluated predictive factors, specifically OS.

LinkedOmics database and protein-protein interaction networks

With the use of LinkedOmics database (http://www.linkedomics.org/), a volcano plot showing the relationship between *MIB1* members and 200 co-expressed genes in GC was created and the top 50 positively and negatively correlated genes were analyzed. The Metascape database (https://metascape.org/) was used to provide GO enrichment analysis and KEGG pathway keywords for these top 200 genes. STRING (https://string-db.org/) was used to find the genes having the strongest interactions with *MIB1*, and generated the associated protein-protein interaction network with an interaction score > 0.4.

Functional enrichment analysis

The median *MIB1* expression level was used to categorize expression data (HTseq-Counts) into high and low expression groups, which were then further examined using the DESeq2 R package (3.6.3). Adjusted $P < 0.05$ and $|\log2(FC)| > 1.5$ were considered the thresholds to obtain DEGs, and GO enrichment analysis and KEGG pathway analysis of DEGs were performed by the "Cluster Profiler" package and visualized by the "ggplot2" package.

Tumor Immune Evaluation Resource database

Tumor Immune Evaluation Resource (TIMER, https://cistrome.shinyapps.io/timer/) is a comprehensive online resource for systematically analyzing immune infiltration in various cancer types. The connection between *MIB1* expression and six different immune infiltrating cells in gastric adenocarcinomas, including B cells, CD4$^+$ T cells, CD8$^+$ T cells, neutrophils, macrophages, and dendritic cells, was examined in our study by using TIMER. We also obtained the correlation between *MIB1* and 24 tumor-infiltrating lymphocytes (TILs) by single-sample gene set enrichment analysis (ssGSEA), which was realized by the GSVA package. The Spearman test was used to measure the correlation between *MIB1* and TILs.

Statistical analysis

All statistical analyses were performed using R (V 3.6.3). The differences between the gastric adenocarcinoma tissues and surrounding normal tissues were assessed using paired *t* tests and Mann-Whitney tests. The pROC software program was used to create a ROC curve in order to determine the *MIB1* cutoff value. The impact of *MIB1* on survival was assessed using Kaplan-Meier and log-rank testing.

RESULTS

Mutation and mRNA expression of the MIB1 gene in STAD

Mutation of the *MIB1* gene was analyzed in STAD patients using the online cBioPortal database, and the genetic alterations of *MIB1* in STAD were 6% (Figure 1A). Mutation data and copy number alteration (CNA) data are shown in Figure 1B. Patients with and without mutations did not have a significantly different OS rate ($P = 0.8900$). Nevertheless, the DFS rate of the group with mutations was much lower than that of the group without mutations ($P = 0.0156$) (Figure 1C and D). As shown in Figure 2A, *MIB1* was considerably upregulated in a range of tumor tissues when compared to adjacent normal tissues, demonstrating that mRNA expression of *MIB1* was abnormally expressed in several cancer types. Analysis of unpaired data showed that the *MIB1* mRNA in STAD tissues ($n = 375$) was significantly higher than that in adjacent normal

tissues (n = 32) (Figure 2B; 2.807 ± 0.584 *vs* 2.218 ± 0.495, Mann-Whitney *U* test, $P <$ 0.001). Paired data analysis also showed that the mRNA expression level of *MIB1* in gastric adenocarcinoma tissues was significantly higher than that in adjacent normal tissues (n = 27) (Figure 2C; 2.562 ± 0.696 *vs* 2.239 ± 0.506, $P < 0.01$).

Relationship between the mRNA level of MIB1 and clinicopathological features in patients with gastric adenocarcinoma

The Mann-Whitney *U* test and logistic regression analysis were performed to evaluate the relationship between the mRNA expression of *MIB1* and the clinicopathological characteristics of gastric adenocarcinoma samples. As shown in Table 1, the expression level of *MIB1* was higher in patients with a high T stage (P = 0.017) and pathological stage (P = 0.032). However, the expression level of *MIB1* was associated with other clinicopathological features, such as age (P = 0.423), sex (P = 0.884), N stage (P = 0.433), M stage (P = 1.000), tissue type (P = 0.448), and *H. pylori* infection (P = 0.470). In conclusion, *MIB1* was associated with high T stage and pathological stage, which further suggested that *MIB1* might be used as a biomarker for the poor prognosis of gastric adenocarcinoma.

Diagnostic value of MIB1 gene expression in STAD

The usefulness of *MIB1* in separating GC samples from normal samples was investigated using ROC curve analysis. As shown in Figure 2D, the ROC curve showed that the area under the curve (AUC) value of *MIB1* was 0.783 (95%CI: 0.704-0.861). The sensitivity and specificity of *MIB1* were respectively 59.4% and 85.6% at the cutoff value of 2.248. Positive and negative predictive values were 26.0% and 96.1%, respectively. These findings suggested that *MIB1* might be a potential biomarker to differentiate between normal tissues and stomach cancer tissues.

Relationship between gene expression level of MIB1 and OS

As shown in Figure 2E, patients with gastric adenocarcinoma who had high *MIB1* Levels compared to those who had low *MIB1* Levels had significantly worse OS (26.4 mo *vs* 56.2 mo, *P* = 0.033). This suggested that high mRNA expression of *MIB1* was a biomarker for poor prognosis in gastric adenocarcinoma.

Correlation and interaction analyses

A volcano plot of *MIB1* and coexpressed genes in GC was generated in the LinkedOmics database (Figure 3A). The Metascape database was then used to examine the GO and KEGG pathway terms of these top 200 genes. GO analysis was used to investigate the functional mechanism of *MIB1* in GC. The BP terms "proteolysis involved in cellular protein catabolic process", "mitochondrion organization", "phosphatidylinositol-3-phosphate biosynthetic process", and "protein phosphorylation" were significantly enriched (Figure 3B). Among the enriched CC terms was "mitochondrial protein-containing complex" (Figure 3C). "Protein serine/threonine/tyrosine kinase activity", "ubiquitin-like protein transferase activity", and "enzyme activator activity" were the most commonly enriched MF phrases (Figure 3D). The target genes were primarily linked to the phrases "chemical carcinogenesis-reactive oxygen species", "platinum drug resistance", and "ubiquitin mediated proteolysis", according to KEGG pathway analysis (Figure 3E). The top 50 genes with positive relationship (Figure 3F) and the top 50 genes with negative relationship (Figure 3G) with *MIB1* were displayed in a heatmap to further investigate the processes of *MIB1* and its coexpressed genes. The ten coexpressed genes of *MIB1* in the STRING database were NOTCH1, NOTCH2, NOTCH3, DLL1, DLL4, UBB, MARK2, JAG1, JAG2, and RPS27A (Figure 3H). These genes were analyzed by GO and KEGG, most of which were related to NOTCH pathway (Figure 3I and J).

Volcano map and enrichment analysis of the differentially expressed genes

With a threshold of |logFC|< 1.5 and adjusted *P* < 0.05, 506 DEGs in total were discovered, of which 454 showed upregulation and 52 showed downregulation. The

DEG expression was visualized in a volcano diagram (Figure 4A). The DEG-related *MIB1* had strongly regulatory effects on the endoplasmic reticulum lumen, cornified envelope, keratin filament, endosome lumen, epidermal cell differentiation, and keratinocyte differentiation, keratinization, cornification and peptide cross-linking processes, according to GO enrichment analysis (Figure 4B). KEGG analysis showed that DEG-related *MIB1* was associated with protein digestion and absorption, pancreatic secretion, and cholesterol metabolism pathways (Figure 4C).

Correlation between MIB1 expression and immune cell infiltration in gastric adenocarcinoma

We analyzed the correlation between the expression of *MIB1* and six types of tumor invasive immune cells in the TIMER database. As demonstrated in Figure 5A, *MIB1* expression was favorably linked with B cells ($r = 0.222$, $P = 1.61E-05$), CD4$^+$ T cells ($r = 0.201$, $P = 1.08E-04$), and macrophages ($r = 0.139$, $P = 7.22E-03$) and negatively connected with CD8$^+$ T cells ($r = -0.143$, $P = 5.77E-03$). Figure 5B shows the relationship between *MIB1* and 24 kinds of tumor immune infiltrating cells. Tcm, helper T cells, Tem, Tgd and NK CD56 bright cells were positively correlated with *MIB1*. *MIB1* was inversely linked with cytotoxic cells, NK CD56dim cells, pDCs, aDCs, CD8 T cells, and Th17 cells. The relationship between the level of *MIB1* expression and the degree of immune cell infiltration as measured by the ssGSEA score was investigated using Spearman correlation. The extent of NK CD56dim cell infiltration was negatively correlated with *MIB1* expression ($r = -0.292$, $P < 0.001$) (Figure 5C) and considerably decreased in the *MIB1* high expression group ($P < 0.001$) (Figured 5D). These results demonstrated that *MIB1* played an important role in the immune infiltration of GC. The proportions of 24 different subsets of tumor-infiltrating immune cells were compared using a heatmap to ascertain the degrees of association (Figure 5E).

DISCUSSION

Notch signaling is an evolutionarily conserved pathway that controls cell fate, determines cell differentiation, proliferation, tumor angiogenesis, stem cell maintenance, apoptosis and other cellular processes, and promotes the occurrence of GC through crosstalk with different signaling pathways, such as the Wnt, Ras, and NF-KB pathways[26,27]. Studies have indicated that endocytosis of Notch ligands is required to activate the receptor in the Notch pathway[28]. However, ubiquitination of the intracellular tail of Notch ligands is a critical event in the subsequent endocytosis and signal transduction of these molecules[29]. Initial genetic studies in flies and zebrafish identified two E3 ubiquitin ligase families capable of ligand ubiquitination: Mind Bomb (Mib) and Neuralized (Neur) proteins[30]. Then a series of studies concluded that *MIB1*, a member of the E3 ubiquitin-protein ligase family, played a major, possibly exclusive role in Notch ligand ubiquitination and transport in mammals[31].

The roles of some Notch family members in GC have not yet been fully understood, despite the fact that the impact of Notch signaling on GC has been extensively established[32,33]. In this work, we performed a thorough examination of *MIB1*, a NOTCH family member, in patients with GC to determine its mutation, expression, prognostic value, and immune infiltration.

The mRNA expression of *MIB1* was observed to be increased in STAD tissues in our research. And positive correlation was found between the increase of mRNA expression and a high T stage. *MIB1* might be a viable diagnostic biomarker for separating stomach cancer tissues from normal tissues, according to a ROC curve analysis. Kaplan-Meier curves and univariate analysis allowed us to demonstrate that increased mRNA expression of *MIB1* was associated with short OS and might be used as a feasible biomarker for poor prognosis of GC. Additionally, *MIB1* might have a unique function in the immunological infiltration of GC.

MIB1 is a ubiquitin-protein ligase. It was reported that overexpression of *MIB1* significantly promoted cell proliferation, migration and invasion[25]. Recent studies have found that *MIB1* plays a carcinogenic function in a variety of human malignancies, including pancreatic, prostate, and lung cancer[23,34,35]. Studies have shown that *MIB1*

was an important biomarker leading to poor prognosis, and upregulation of *MIB1* expression is associated with poor OS[22]. Furthermore, mutation of the *MIB1* gene could lead to congenital heart disease by reducing Notch signaling activation[36]. Overexpression of E3 ubiquitin ligase *MIB1* could reduce the apoptosis and inflammation of cardiac microvascular endothelial cells in coronary microvascular dysfunction[37].

In this study, analysis of the cBioportal database showed that the *MIB1* mutation rate in 287 patients in the TCGA dataset was approximately 6%, and most of the changes were copy number amplification (CNA). In cancer, CNAs and deletions result in altered expression of tumor suppressor genes and oncogenes, respectively. It was reported that copy number variation of E3 ubiquitin ligase was associated with the occurrence and development of colorectal cancer[38]. We further analyzed the correlation between *MIB1* gene changes and prognosis. We found no significant difference between *MIB1* gene changes and OS, but the DFS of the patient group with mutations was much shorter than that of the group without mutations. Moreover, what we discovered were consistent with previous studies that *MIB1* mRNA was abnormally expressed in many cancers, and we found that *MIB1* was greatly increased in gastric adenocarcinoma *via* the TCGA database. According to this, gastric adenocarcinoma with a poor clinical prognosis might be identified using *MIB1* as a possible biomarker for poor prognosis.

At present, the role of *MIB1* in tumors and whether it acts through the NOTCH pathway have not been fully reported. There have been relatively many studies on *MIB1* in pancreatic cancer. Some studies have shown that *MIB1* can be used as a direct target of miRNA-198 and miRNA-195-5p. *MIB1* has been considered a new target of miRNA-198, which reduced the proliferation, migration and invasion of prostate cancer. However, this tumor inhibition role appeared to be independent of the Notch pathway[39]. MicroRNA-195-5p might regulate the proliferation and invasion of tumor cells by regulating *MIB1*, suggesting that miRNA-195-5p might be used to treat prostate cancer in the future[34]. Our results indicated that *MIB1* may be an intriguing biomarker or an emerging target for cancer therapy. In addition, ectopic expression of *MIB1* could

induce epithelial-to-mesenchymal transition and stimulate cell migration through the Notch-dependent pathway, which might provide new insights into the treatment of MIB1-overexpressing cancer[35]. Other studies have shown that MIB1 promotes the progression of pancreatic cancer by inducing ST7 degradation and downregulating IQGAP1, suggesting that the MIB1/ST7/IQGAP1 axis is crucial in the advancement of pancreatic cancer and that inhibiting MIB1 might become a new therapeutic strategy for pancreatic cancer, and inhibiting MIB1 might become a new therapeutic strategy for pancreatic cancer patients[22]. A study proved that MIB1 promoted pancreatic cancer proliferation by activating the β-catenin signaling pathway[23]. Therefore, whether MIB1 affects the progression of GC through the NOTCH pathway needs further in vivo and in vitro experiments.

A ROC curve analysis was performed to verify the clinical value of MIB1 in diagnosing gastric adenocarcinoma. With a sensitivity of 59.4 and a specificity of 85.6%, our findings demonstrated that MIB1 had a relatively higher AUC value to discover the patients with GC. Based on our research, we came to the conclusion that MIB1 might function as an applicable diagnostic biomarker to separate gastric adenocarcinoma from normal controls.

In addition, MIB1 was highly expressed in patients with gastric adenocarcinoma. MIB1 was correlated with multiple clinical features, such as pathological stage, T stage and OS, further suggesting that MIB1 is a prospective biomarker that merits additional clinical testing.

Cytotoxic cells, NK CD56dim cells, pDCs, aDCs, CD8 T cells and Th17 cells were negatively correlated with MIB1. Antitumor immunity was influenced by cytotoxic cells, including NK cells. The function of NK cells in innate immune surveillance is crucial in the fight against cancer[24]. In the course of transformation into toxic T cells, CD8$^+$ T cells display cytotoxic abilities against tumor cells[25]. IFN-I produced by pDCs has antitumor activity[26], Th17 cells have a close connection to neutrophils and are essential for the immune response to tumors[27]. The decrease of these immune cells

might contribute to the further development of GC. The results of ssGSEA further demonstrated that *MIB1* was essential in controlling immune infiltration.

Our research revealed the complex role of *MIB1* gene mutation and abnormal expression in the prognosis of GC. In addition, we also preliminarily discussed the relationship between the *MIB1* gene and immune infiltration, as well as its mechanism and biological function in GC. However, our research also had some limitations. This study lacked *in vivo* or *in vitro* experiments to verify the role of the *MIB1* gene, which will allow us to draw more general and accurate conclusions.

CONCLUSION

In brief, we found that *MIB1* mRNA expression increased in STAD was positively attached with high T stage and pathological stage and negatively correlated with OS. According to our research, higher expression of *MIB1* may be a useful predictive biomarker for identifying individuals with gastric adenocarcinomas who have a poor clinical prognosis and may have a special function in immune infiltration.

Figure Legends

Figure 1 Mutation of mind bomb 1 gene in stomach adenocarcinoma is found in cBioPortal database. A: OncoPrint indicate different types and proportions of mind bomb 1 (*MIB1*) mutations; B: Summary of cancer types shows the type of genomic alterations in stomach adenocarcinoma; C: Kaplan-Meier showed the overall survival rate of patients with and without copy number alteration (CNA) of *MIB1*; D: Kaplan-Meier showed the disease-free survival rate of patients with and without CNA of *MIB1*.

Figure 2 Expression,diagnostic value and prognosis of mind bomb 1 gene. A: Mind bomb 1 (*MIB1*) expression from pan-cancer perspective; B: *MIB1* mRNA expression levels in 375 gastric adenocarcinoma samples and 32 normal samples; C: *MIB1* mRNA expression levels in 27 gastric adenocarcinomas and matched adjacent normal samples; D: Receiver operating characteristic curve showed that the area under the curve value

of *MIB1* in distinguishing gastric adenocarcinoma tissues from healthy controls was 0.783. The cutoff was 2.248, and the sensitivity, specificity and accuracy were 59.4% and 85.6%, respectively; E: Kaplan-Meier showed that the overall survival of gastric adenocarcinoma patients with high mRNA expression of *MIB1* was shorter than that of patients with low expression (26.4 mo *vs* 56.2 mo, *P* = 0.033). [a]*P* < 0.05; [b]*P* < 0.01, [c]*P* < 0.001.

Figure 3 Coexpression analysis of mind bomb 1 gene. A: A volcano plot of the mind bomb 1 (*MIB1*) and its co-expressed genes in gastric cancer; B: The Gene Ontology (GO) enrichment of the BP terms of 200 co-expressed genes; C: The GO enrichment of the CC terms of 200 co-expressed genes; D: The GO enrichment of the MF terms of 200 co-expressed genes; E: The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment of the 200 co-expressed genes; F: The top 50 genes with a positive correlation with *MIB1* gene are visualized in a heatmap; G: The top 50 genes with a negative correlation with the *MIB1* gene are visualized in a heatmap; H: The protein-protein interaction network associated with the *MIB1* in gastric cancer; I: The GO enrichment of the 11 genes with the strongest interaction with *MIB1* proteins; J: The KEGG pathway terms of the 11 genes with the strongest interaction with *MIB1* proteins. MIB1: Mind bomb 1.

Figure 4 Enrichment analysis of mind bomb 1 in stomach adenocarcinoma. A: Volcanic map of differential expression in gastric adenocarcinoma with |logFC| < 1.5 and adjusted *P* < 0.05; B: Gene Ontology enrichment analysis of differentially expressed genes; C: Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially expressed genes.

Figure 5 Analysis of the relationship between mind bomb 1 expression and immune infiltration. A: In gastric adenocarcinoma, the expression of mind bomb 1 (*MIB1*) was negatively correlated with CD8$^+$ T cells, and correlated with B cells, CD4$^+$ T cells and macrophages; B: The correlation between the expression level of *MIB1* and the relative abundance of 24 immune cells; C: The expression of *MIB1* was negatively correlated with NK CD56dim cells; D: NK CD56dim cell infiltration level in different expression groups of *MIB1*; E: Heat map of 24 immune infiltrating cells in stomach adenocarcinoma.

Table 1 Demographic and clinicopathological parameters of patients with gastric cancer with high and low expression of mind bomb 1 in The Cancer Genome Atlas-stomach adenocarcinoma, *n* (%)

Characteristics	Total	Low expression of *MIB1*	High expression of *MIB1*	*P* value
T stage				**0.017**
T1	19 (5.2)	11 (3.0)	8 (2.2)	
T2	80 (21.8)	37 (10.1)	43 (11.7)	
T3	168 (45.8)	99 (27.0)	69 (18.8)	
T4	100 (27.2)	40 (10.9)	60 (16.3)	
N stage				0.433
N0	111 (31.1)	61 (17.1)	50 (14.0)	
N1	97 (27.2)	49 (13.7)	48 (13.4)	
N2	75 (21.0)	32 (9.0)	43 (12.0)	
N3	74 (20.7)	38 (10.6)	36 (10.1)	
M stage				1.000
M0	330 (93.0)	164 (46.2)	166 (46.8)	
M1	25 (7.0)	12 (3.4)	13 (3.7)	
Pathologic stage				**0.032**
Stage I	53 (15.6)	24 (6.8)	29 (8.2)	
Stage II	111 (31.5)	69 (19.6)	42 (11.9)	
Stage III	150 (42.6)	72 (20.5)	78 (22.2)	
Stage IV	38 (10.8)	15 (4.3)	23 (6.5)	
Gender				0.884
Female	134 (35.7)	68 (18.1)	66 (17.6)	
Male	241 (64.3)	119 (31.7)	122 (32.5)	
Histological type				0.448
Diffuse type	63 (16.8)	36 (9.6)	27 (7.2)	
Mucinous type	19 (5.1)	11 (2.9)	8 (2.1)	
Not otherwise	207 (55.3)	103 (27.5)	104 (27.8)	

Specified				
Papillary type	5 (1.3)	1 (0.3)	4 (1.1)	
Signet ring type	11 (2.9)	4 (1.1)	7 (1.9)	
Tubular type	69 (18.4)	32 (8.6)	37 (9.9)	
Histologic grade				0.305
G1	10 (2.7)	4 (1.1)	6 (1.6)	
G2	137 (37.4)	63 (17.2)	74 (20.2)	
G3	219 (59.8)	117 (32.0)	102 (27.9)	
H. pylori infection				0.470
No	145 (89.0)	63 (38.7)	82 (50.3)	
Yes	18 (11.0)	10 (6.1)	8 (4.9)	
OS event				**0.022**
Alive	228 (60.8)	125 (33.3)	103 (27.5)	
Dead	147 (39.2)	62 (16.5)	85 (22.7)	
Age, mean ± SD		65.39 ± 10.80	66.28 ± 10.51	0.423

MIB1: Mind bomb 1; OS: Overall survival; *H. pylori*: *Helicobacter pylori*.

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