OncoTargets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

MAM Domain Containing 2 (MAMDC2) Affects Invasion and Metastasis of Human Gastric Cancer

Jiaofeng Shen[1,*], Guangyu Gao[1,*], Songtao Liu[1,2]

[1]Department of Oncology, The Second Affiliated Hospital of Soochow University, Suzhou, 215004, People's Republic of China; [2]Department of Ultrasound, The Second Affiliated Hospital of Soochow University, Suzhou, 215004, People's Republic of China

*These authors contributed equally to this work

Correspondence: Songtao Liu, Department of Ultrasound, The Second Affiliated Hospital of Soochow University, Suzhou, 215004, People's Republic of China, Email 20185233105@stu.suda.edu.cn

Background: Gastric cancer (GC) is the fifth most prevalent cancer worldwide and the fourth leading cause of cancer-related mortality. MAM Domain Containing 2 (MAMDC2) has been involved in many cancers. However, the impact of MAMDC2 on gastric cancer was unclear. This study aimed to investigate the role and mechanism of MAMDC2 in gastric cancer.

Methods: Differential genes in gastric cancer are analyzed by GEO, TCGA, MSigDB database, R-packet limma, and Wilcoxon test. Survival analysis is performed through the R package survival. Construct a PPI network through the STRING database. Enrichment analysis is performed by Metascape. The infiltration level of gastric cancer immune cells is calculated by CIBERSORT. In addition, the expression of MAMDC2 was analyzed by immunohistochemistry and quantitative real-time polymerase chain reaction (RT-qPCR). siMAMDC2 was used to knock down the specific gene. Cell counting kit-8 (CCK-8) assay, colony formation assay, and cell migration were applied to evaluate the function of MAMDC2 in gastric cancer cells.

Results: In the present study, we revealed a significant upregulation of MAMDC2 in gastric cancer tissues and cells. Knocking down MAMDC2 inhibited the proliferation and migration of gastric cancer cells, while overexpression of MAMDC2 produced the opposite results. Furthermore, MAMDC2 may be an independent factor in poor prognosis in gastric cancer patients.

Conclusion: These results illustrated that MAMDC2 promoted the proliferation and migration of gastric cancer cells. The newly identified MAMDC2 provides novel insight into the pathogenesis of gastric cancer.

Keywords: MAMDC2, gastric cancer, GEO, CCK-8, invasion

Background

Gastric cancer (GC) is a global health challenge that has been recognized as the fifth most prevalent malignancy in terms of incidence and the fourth in cancer-related mortality.[1,2] In 2020, there were 479000 new cases and 374000 deaths of GC in China, accounting for approximately 44.0% and 48.6% of the world's new cases and deaths of GC. The situation is very severe.[3] The early screening rate for GC in our country is relatively low, and most GC patients only seek medical treatment in the late stage, resulting in a lower overall survival rate for GC patients. Therefore, the key to diagnosing and treating GC lies in early diagnosis of GC.[4] In the process of gastric cancer occurrence and development, due to genetic instability and differences in the tumor microenvironment, cell clones with completely different biological behaviors can be formed.[5] The proliferation rate, invasion, and metastasis ability of these cell subpopulations have obvious heterogeneity, and their drug sensitivity is also different. Moreover, there is heterogeneity between the primary and metastatic lesions, as well as between the primary and recurrent lesions of gastric cancer. Therefore, gastric cancer is a highly heterogeneous tumor, resulting in unsatisfactory drug development and clinical research progress in gastric cancer.[6,7] In recent years, with the progress of immunotherapy and the exploration of new targets in gastric cancer, significant breakthroughs have been made in the treatment of gastric cancer. At present, the screening of tumor molecular markers

Received: 11 January 2025
Accepted: 22 May 2025
Published: 26 May 2025

and gene therapy through genetic technology is an emerging research direction and hotspot in the diagnosis and treatment of GC.[8]

The exploration of new targets for gastric cancer and the development of new drugs are still ongoing.[9] In the FIGHT trial, IHC detection found that approximately 30% of HER2-negative gastric and gastroesophageal junction adenocarcinoma patients overexpress FGFR2b, and these patients showed clinically and statistically significant improvements in PFS, OS, and ORR after adding Bemarituzumab to mFOLFOX6 chemotherapy. The median PFS was extended by 2.1 months compared to the placebo group (9.5 vs 7.4 months, HR=0.68, P=0.073), and the median OS had not yet been reached, compared to 12.9 months in the placebo group (HR=0.58, P=0.027); ORR increased by 13% (47% vs 33%, P=0.106). Further analysis revealed that patients with IHC 2+/3+≥ 10% had a higher survival rate (70.2% vs 49.5% at 12 months).[10] DKK1 is Wnt/ β- A typical inhibitor of the catenin signaling pathway, research has found that DKK1 is strongly expressed in gastric cancer cells and weakly expressed in normal gastric mucosa. High expression of DKK1 is associated with advanced gastric cancer, distant metastasis, lymphatic infiltration, and vascular invasion.[11] Sirexatamab (DKN-01) is a targeted drug targeting DKK1.[12] DisTinGuish's study found that the first-line treatment of advanced gastric cancer with tirzelumab+DKN-01+XELOX has a high ORR of 62%, which is 90% in the DKK1 high expression population. In addition, the application value of targets such as cMET, TROP-2, and FAK in gastric cancer is actively being explored.[13]

MAMDC2 (containing 2 MAM domains) is a member of the MAM family, which is a secreted protein composed of 686 amino acids, consisting of a short N-terminal signal sequence and four consecutive MAM domains.[14] Previous studies have shown that the expression of the MAMDC2 gene is regulated differently in different types of human carcinomas, including CML, head and neck squamous cell carcinoma, and breast carcinoma.[15–17] However, the expression regulation of MAMDC2 in gastric cancer has not been reported yet.

The study aims to analyze and detect the expression of differentially expressed genes in GC through bioinformatics and immunohistochemistry methods, providing potential biomarkers such as MAMDC2 for early screening, prognosis, and drug development in GC.

Methods

Database Screening

Download the gene expression profile datasets GSE208099 and GSE172196 for gastric cancer from the GEO database (http://www.ncbi.nlm.nih.gov/geo). The GSE208099 dataset contains 16 gastric cancer tissue samples and 16 matched adjacent nontumor tissue samples; The GSE172196 dataset contains 4 gastric cancer tissue samples and 4 matched adjacent nontumor tissue samples.

Difference Analysis

The GEO2R online tool was used to analyze the DEGs of gastric cancer samples and adjacent noncancer tissue samples from two datasets, adj. P<0.05 and | logFC |>2 were used as the criteria for identifying DEGs, and uDEGs were defined as upregulated DEGs, while dDEGs were defined as downregulated DEGs. Volcanic cartography was meticulously constructed leveraging the robust data resources and analytical tools provided by the Xiantao online data platform (https://www.xiantao.love). It can perform common bioinformatics analysis and result visualization, helping researchers quickly complete bioinformatics analysis. Then, overlapping DEGs between the two datasets were identified using the Venn diagram online tool (https://www.bioinformatics.com.cn) and drew the Venn diagram.

GO and KEGG Pathway Analysis

LinkedOmics database (http://www.linkedomics.org) is a new and unique tool in the software ecosystem for analyzing data from large-scale cancer omics projects. This study searched for co-expressed genes of MAMDC2 through the LinkedOmics database, drew heat maps of co-expressed genes positively and negatively correlated with MAMDC2, and performed GO enrichment analysis on them [Gene Ontology, Gene Ontology database, referring to standardized descriptions of gene products in biological processes (BP), cellular components (CC), molecular functions (MF), and

Kyoto Encyclopedia of Genes and Genomes database, respectively]. A systematic analysis of metabolic pathways and the functions of these gene products.

The Relationship Between DEG Expression and Clinical Characteristics in TCGA

GEPIA database (http://gepia.cancer-pku.cn) is a publicly available database for detecting RNA sequences from 9736 tumor samples and 8587 normal samples in TCGA and GTEx. This study used it to perform a single-gene survival analysis on PRDX4. The Kaplan-Meier survival analysis method was used to calculate overall survival (OS) and disease-free survival (DFS), and complete curve drawing. The survival differences at different expression levels were compared, and the correlation between MAMDC2 and OS and DFS in GC patients was obtained.

Immunohistochemical Staining

Paraffin specimens of gastric cancer tissues and adjacent tissues were prepared into sections with a thickness of 4μm. The sections were subjected to conventional baking, xylene dewaxing, gradient alcohol water hydration treatment, incubated with 3%H2O2 for 10 min, blocked endogenous catalase, and, after antigen repair by the microwave oven, the antigens were blocked with 5% BSA for 1h. MAMDC2 antibody (1:1000) was added and incubated at 4°C overnight, the sections were fully washed with PBS the next day, the corresponding species of secondary antibody was added and incubated at room temperature for 30min, and the color was developed under the microscope of diaminophenylenediamine (DAB), the nuclei were restained with hematoxylin, and the tablets were sealed by routine after the tap water returned to blue. The results were scored according to the improved immunohistochemical results scoring method: 0, 1, 2, and 3 points were scored according to the staining intensity, respectively, no staining, light staining, moderate staining, and deep staining. 0~1 was classified as a negative expression, and 2~3 was classified as a positive expression.

Cell Culture and Transfection

The human gastric cell lines (MKN-45, HGC-27, GES-1) were provided by Professor Li Qin Shen (Soochow University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Suzhou, China). Gastric cancer cell lines MKN-45, HGC-27, and normal cell lines GES-1 were removed for routine resuscitation and cultured with 1640 and DMEM medium containing 10% fetal bovine serum, respectively, and then incubated in a constant temperature incubator at 37°C and 5% CO2. After the cell fusion rate reached 95%, the pancreatic enzyme was added for digestion and passage. 400ul of nuclease-free water was added into the tube, shaken for 10 seconds, and the lipid substance was removed. The reagent was stored at −20°C following vigorous agitation. An optimal volume of serum-free culture medium was subsequently introduced into a transfection tube. Concurrently, Enhanced Green Fluorescent Protein (EGFP) DNA and the transfection reagent (Lipofectamine 3000) were incorporated into the tube. The mixture was then incubated at room temperature for 15 minutes. Meanwhile, the culture medium was aspirated from the culture plate, and the plate was gently washed once with phosphate-buffered saline (PBS). Ultimately, the prepared mixture was added to the cells, which were then returned to the incubator for a 1-hour incubation period. RT-PCR amplification was performed using a monoplex RT-PCR with 2X TOPsimple™ DyeMix-multi HOT premix (Enzynomics, Korea).

Plasmids and siRNAs

The full-length cDNA of human MAM Domain Containing 2 (GeneBank accession number NM153267) was obtained from total RNA of MDA-MB-231 cells. Then, the MAMDC2 gene was amplified and inserted into pcDNA3-3FLAG13 or pEGFP-N3 (Clontech). For siRNA treatment, siMAMDC2 #1 (CGAGUGAAAGUAAAACCAA) and siMAMDC2 #2 (CUACAUUGGAAGGCUCUAU) were synthesized by Bioneer (Korea). The small interfering RNA constructs targeting MAMDC2, specifically siMAMDC2#1 and siMAMDC2#2, were employed in the knockdown experiments.

Cell Viability and Invasion Assays

Log-cycle AGS and HGC-27 cells were inoculated in 96-well plates with 2×104 cells/well. The cells were cultured overnight, diluted with EdU solution, added 100μL50μmol/L/LEdU medium per well, incubated in the incubator for 2h,

and washed with PBS 3 times. 4% paraformaldehyde was added and fixed for 30 min. 0.5%TritonX-100100μL was added to each well and incubated in a decolorizing shaker for 10 min. 1×Apollo staining reaction solution was prepared, 100μL was added to each well, and incubated at room temperature for 30 minutes away from light. Discard the reaction solution, add 100μL1×Hoechst33342 reaction solution to each well, and incubate at room temperature and away from light for 30 min, then discard the reaction solution, and add 100μL PBS to each well 3 times. Fluorescence microscopy was taken, and the percentage of EDU-positive cells was calculated. HGC-27 and AGS cells transfected with MAMDC2, and Vector were inoculated with 500 cells/well in a 6-well plate and cultured for 2 weeks. After 2 weeks, the naturally formed cell colonies were washed twice with PBS, fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet for 10 min. The stained cell colonies were washed again with PBS, dried, and counted under a microscope. The effects of overexpression of MAMDC2 on the growth ability of HGC-27 and AGS cells were compared. After the matrix glue was diluted with a serum-free medium at the ratio of 1:10, 100μL was absorbed and added to the Transwell chamber, and the matrix glue was solidified in an incubator at 37°C for 4~6h. The density of cells in the control group and the experimental group was adjusted to 1.5×105 cells /mL, 200μL was inoculated on the upper layer of the cell, and 500μL was added to each well in the lower layer of the cell, cultured in the incubator for 16~20h, fixed with 4% paraformaldehyde for 20min, the fixed solution was discarded, cleaned twice with PBS, and stained with 0.1% crystal violet for 20min. After PBS cleaning 3 times, cotton swabs were used to gently wipe the crystal violet that was not bound to the cells in the chamber. 5 fields were randomly selected under a high-power microscope to observe the cells and take photos, then counted and analyzed by Adobe Photoshop.

Wound Healing Assay

A black marker and a straight ruler were used to mark the bottom of the 6-well plate. The AGS and HGC-27 cells of groups MAMDC2 and Vector were inoculated into the 6-well plate with 6×105 cells/holes. After 24 hours of culture, the cells were overfilled with marks at the vertical bottom of the 10μL gun head. The floating cells were gently washed with PBS for 2 to 3 times, and cultured in an incubator after adding 2 mL of 2% fetal bovine serum fresh medium. The cells were observed and photographed under a 20× microscope at 0h, 24h, and 48h, respectively. The scratch area was calculated using ImageJ software.

Statistical methods

SPSS 25.0 and GraphPad Prism 9.0 statistical software were used to analyze the experimental data. Quantitative data was represented by (x±s), and counting data was represented by percentage (%). Each experiment was repeated at least three times. The expression and clinical correlation of MAMDC2 were analyzed by Fisher's exact probability method. $P<0.05$ was considered to be statistically significant.

Results

Identification of Differential Genes

GEO2R was used to analyze the differentially expressed genes from GSE208099 and GSE172196. After the screening, 426 differentially expressed genes, such as ANXA9, EXO1, MAMDC2, BCHE, LINGO2, MMP7, and LRRC3B, were identified from GSE172196. By the same method, 1498 differentially expressed genes, such as TIGD6, TEX22, PRPF40B, PLA2G7, PROK1, and RNASE6, were found in GSE208099 (Figure 1). The Venn plot showed that 59 genes were screened in both sets of chips, and we selected 9 of them for subsequent survival analysis (Figure 2).

GO and KEGG Enrichment Analysis of Overlapping DEGs

To further explore the biological function of overlapping DEGs in gastric cancer, we conducted GO and KEGG enrichment analysis of overlapping DEGs through the Xiantao online data platform (https://www.xiantao.love). The GO analysis of uDEGs was mainly concentrated on Immune response, Cell communication, Signal transduction, Extracellular, Plasma membrane, Extracellular region, Calcium ion binding, Motor activity, Complement activity, and Chemokine activity (Figure 3A–C). KEGG analysis is mainly concentrated on protein digestion and absorption, and extracellular matrix receptor

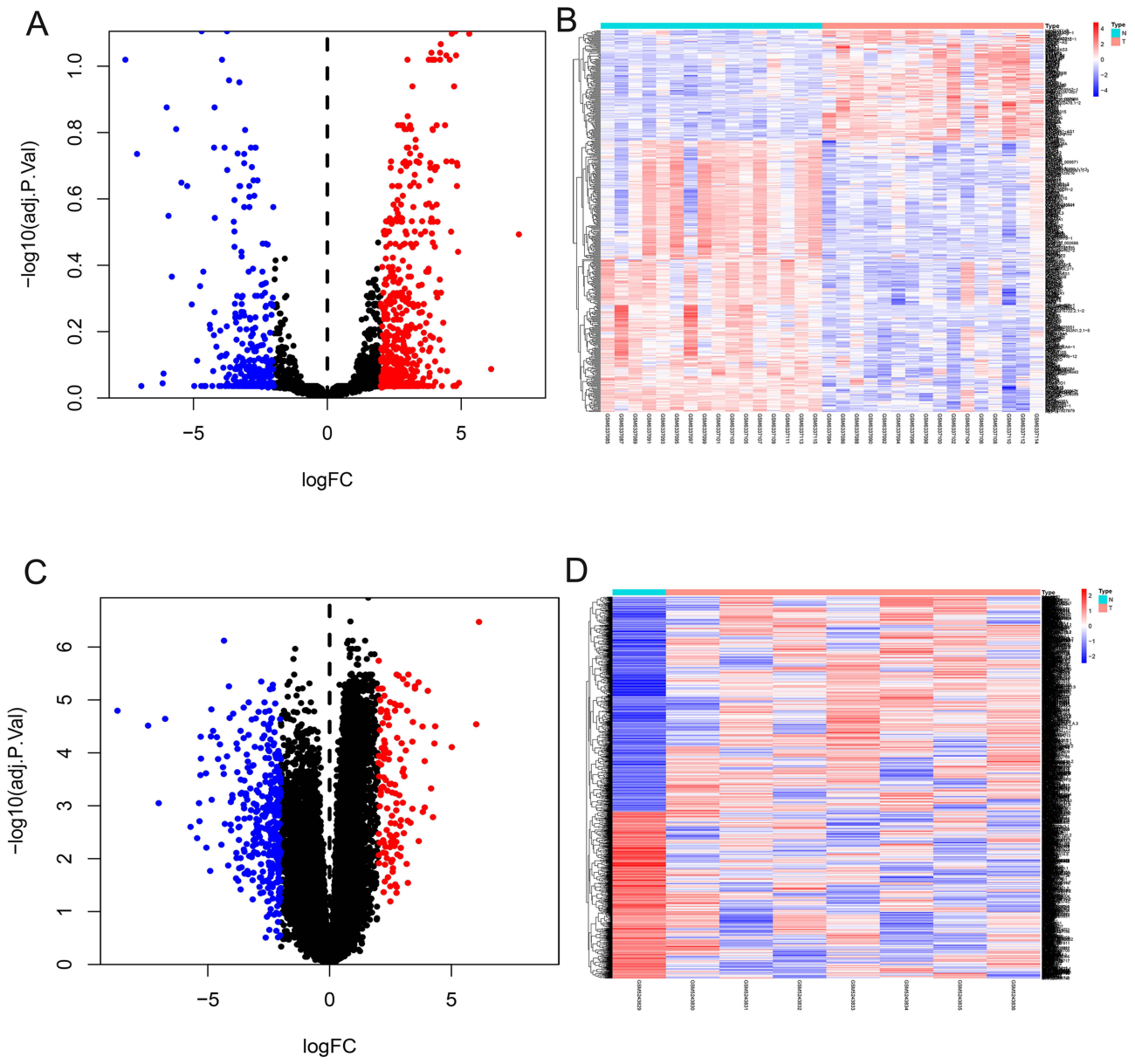

Figure 1 Heat map and Volcano map of differentially expressed genes of GSE208099 and GSE172196. (**A** and **B**). Heat map and volcano map of DEGs in GSE208099. (**C** and **D**). Heat map and volcano map of DEGs in GSE172196.

interactions. KEGG analysis of dDEGs was mainly concentrated in Cytokine-cytokine receptor interaction, Complement and coagulation cascades, Hematopoietic cell lineage, and Staphylococcus aureus infection (Figure 3D).

Survival Analysis

To evaluate the prognostic value of the hub gene in gastric cancer, we further plotted the OS survival curve via the Kaplan-Meier Plotter online data platform (Figure 4). The results showed that 6 hub genes were significantly associated with poor overall survival ($P < 0.05$), suggesting that these genes may be biomarkers of poor prognosis of gastric cancer.

Immunohistochemical Study

The Human Protein Atlas database (https://www.proteinatlas.org/) was founded in 2003 by the Swedish Knut & Alice Wallenberg Foundation. It aims to provide tissue and cellular distribution information for all 24,000 + human proteins, examining the distribution and expression of each protein in a variety of normal human tissues, tumor tissues, cell lines, and blood cells, with

https://doi.org/10.2147/OTT.S516982

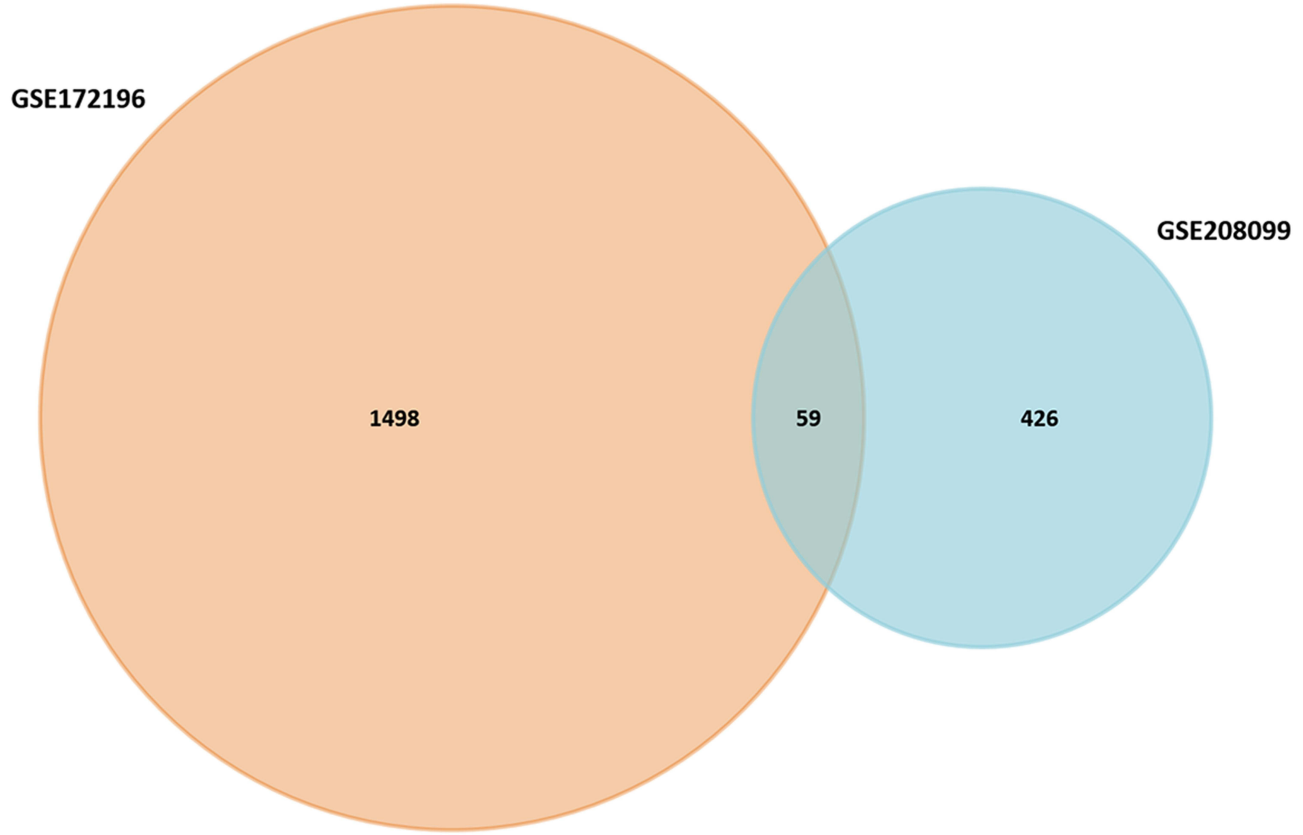

Figure 2 Venn Diagram of GSE208099 and GSE172196.

the results represented by immunohistochemical staining maps. The HPA database website lists detailed information on protein antibodies, including suppliers (Sigma Aldrich), source species (rabbit/mouse), monoclonal/multi antibodies, purification methods, etc. Provided specific experimental results for each antibody, including immunofluorescence, immunohistochemistry, WB, etc. From a visual and statistical perspective, MAMDC2 is significantly differentially expressed in GC tissue (n=346) (Figure 5).

The Relationship Between Clinical Characteristics and MAMDC2 Expression in Gastric Carcinoma

TCGA (Cancer Genome Atlas) is a project jointly initiated by the National Cancer Institute (NCI) and the National Human Genome Institute (NHGRI) in 2006. It collects various sequencing data of human cancers (tumors, including subtypes), including RNA sequencing, microRNA sequencing, DNA sequencing, and SNP-based platforms. Array-based DNA methylation sequencing, reverse array. This includes genomic, transcriptomic, proteomic, and epigenetic data, as well as information on biospecimens and clinical samples. Clinical and gene expression information for patients with gastric cancer was downloaded from the TCGA database (Table 1). To better treat cancer, cancer is usually staged clinically to determine whether it is early cancer or late cancer. At present, the international common cancer staging method is the TNM staging method, where T represents the primary cancer, N represents the lymph node metastasis, and M represents the distant metastasis. The results indicated that the TNM stage ($P<0.05$), Primary therapy outcome ($P<0.05$), and MAMDC2 expression level ($P<0.05$) were related to the OS of patients with GC (Table 2).

Knockdown of MAMDC2 Suppresses GC Cell Growth

In gastric cancer cell lines HGC-27, blank plasmas pcDNA3.1 (Vector) and PCDNA3.1-MAMDC2 expression vectors were transfected, respectively. After 2 weeks of G418 screening, a stable polyclonal (pooled) expression strain was

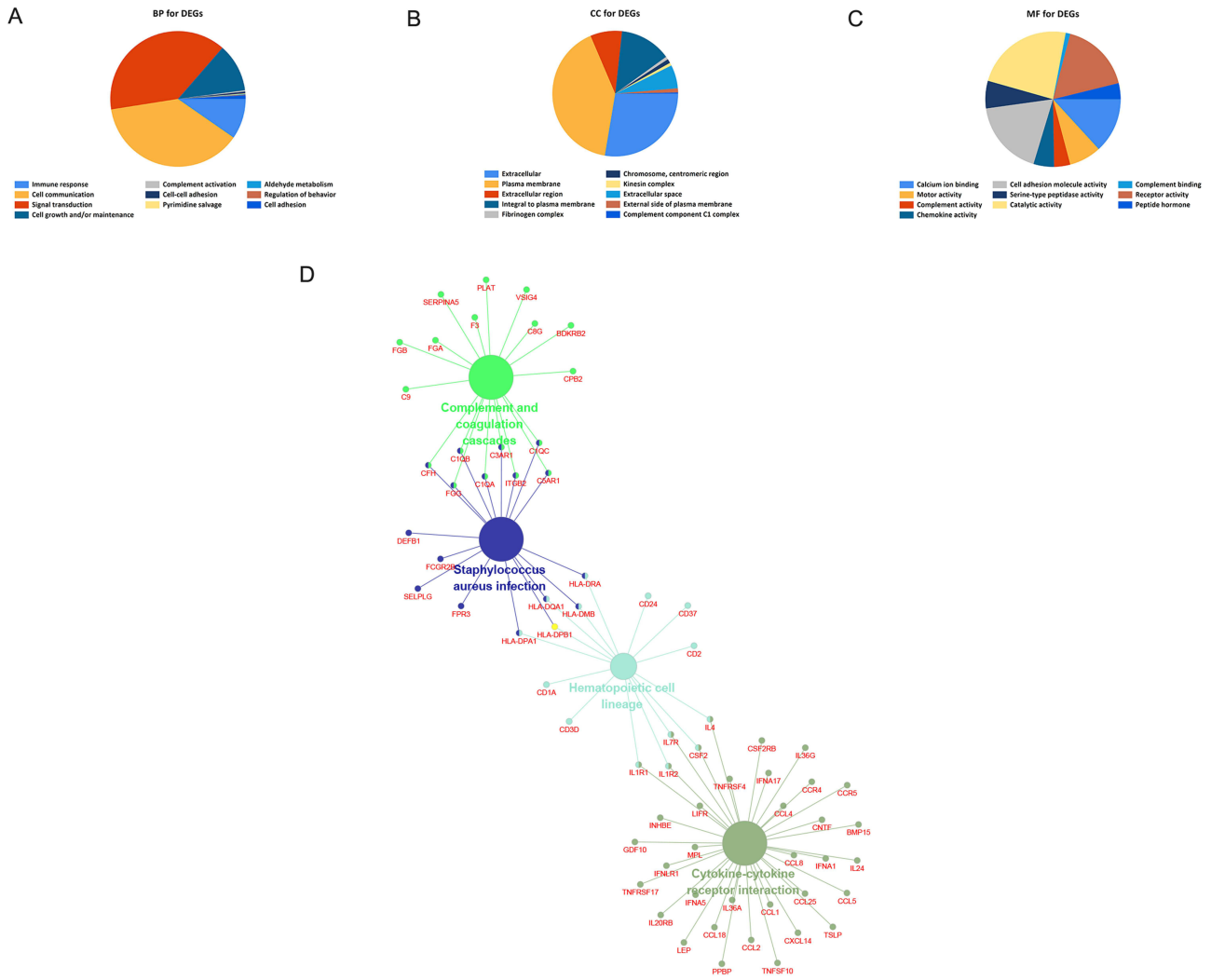

Figure 3 GO and KEGG enrichment analysis. (**A**) Bioprocess enrichment analysis; (**B**) Cell component enrichment analysis; (**C**) Molecular functional enrichment analysis; (**D**) KEGG signaling pathway enrichment analysis.

established, and the expression efficiency was verified by a Quantitative Real-time PCR assay (Figure 6A). Next, CCK-8 showed that the knockdown of the MAMDC2 was sufficient to inhibit GC cell viability (Figure 6B). Compared with the control group, the cell migration ability was significantly decreased in the low-expressed group (HGC-27, P<0.01). The transwell invasion chamber showed that, compared with the Vector group, the migration and invasion values of cells in group MAMDC2 were significantly decreased (P<0.0001), as shown in Figure 6C. This indicated that the high expression of MAMDC2 significantly promoted the migration and invasion ability of gastric cancer cells in vitro. Then, after incubating with EdU for 2h and coloring with Apollo fluorescent solution, the cells whose DNA was replicating were treated with both EdU (red) and Hoechst 33442 (blue). After that, we conducted the EdU cell proliferation experiment, and the results showed that the EdU positive rate of cells in the Knockdown group was significantly lower than that in the control group (HGC-27, P<0.0001), as shown in Figure 6D.

Overexpression of MAMDC2 Promotes Cell Proliferation and Migration

Next, the impact of MAMDC2 on the proliferation and migration of gastric cancer cells was determined. The mRNA level and protein level of MAMDC2 were dramatically increased in oeMAMDC2#1 and oeMAMDC2#2 transfected oeMAMDC2 cells. Cell viability and cell growth were tested through CCK-8 and colony formation assay, respectively. We can see from Figure 7A and B, the cell viability and cell numbers were greatly increased in two lung cancer cells

https://doi.org/10.2147/OTT.S516982

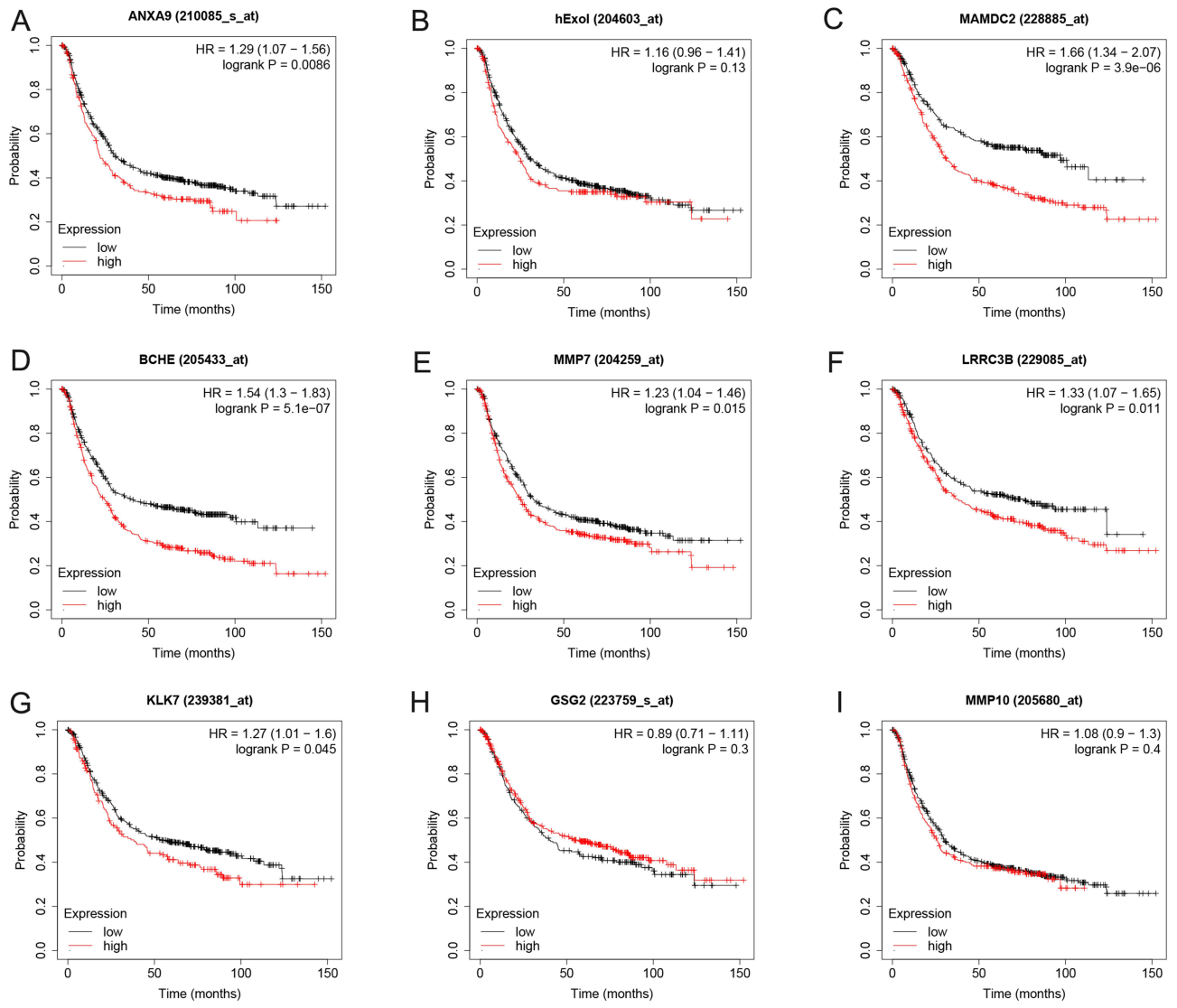

Figure 4 The association between the expression level of selected genes and overall survival of GC patients. (**A**) ANXA9; (**B**) hExol; (**C**) MAMDC2; (**D**) BCHE; (**E**) MMP7; (**F**) LRRC3B; (**G**) KLK7; (**H**) GSG2; (**I**) MMP10.

after MAMDC2 overexpressed. The migration ability of HGC-27 and MKN-45 cells was also greatly enhanced by the Transwell migration assay (Figure 7C). These results illustrated that MAMDC2 participates as a promoter in gastric cancer cells.

Discussion

Gastric cancer is a common malignant tumor of the digestive tract in China. The early symptoms of most patients are insidious or mild, so the diagnosis of gastric cancer is often in the advanced stage of the disease.[18–20] Despite the rapid development of molecular biology, immunology, and surgery in recent years, the survival prognosis and therapeutic effect of gastric cancer patients are still not good.[21–25] Strengthening the research on the occurrence and development of gastric cancer and searching for the biological indicators related to gastric cancer will help the early diagnosis and treatment of gastric cancer and improve the prognosis. In this study, biological markers of gastric cancer were screened by bioinformatics methods. We analyzed the overlapping DEGs of the GSE208099 and GSE172196 datasets, and the GO analysis of DEGs was mainly related to extracellular matrix and collagen. The extracellular matrix is composed of collagen, fibronectin, elastin, laminin, hyaluronic acid, glycosaminoglycan, and other components.[26] In normal tissues

 https://doi.org/10.2147/OTT.S516982

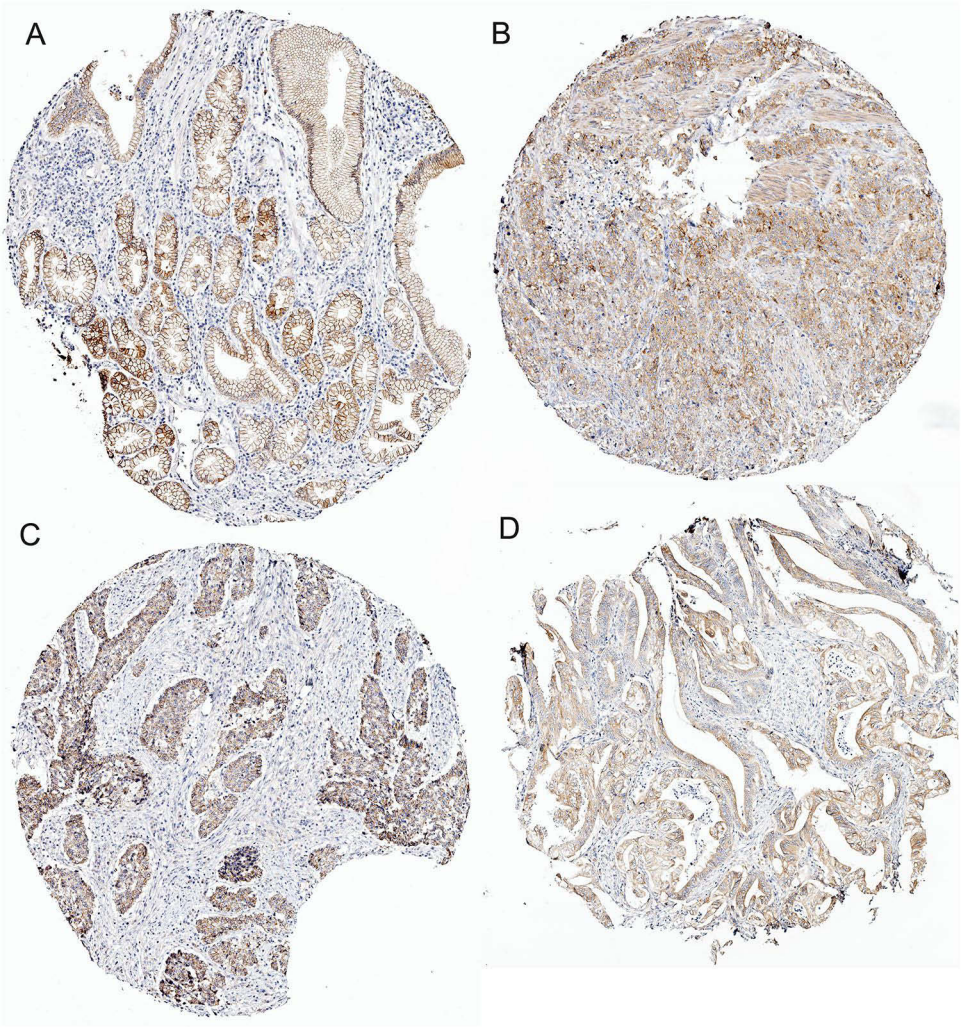

Figure 5 MAMDC2 expression in GC tissues. (**A–D**) MAMDC2 is highly expressed in gastric cancer tissues.

and organs, the extracellular matrix can not only play the role of a physical scaffold and mediate signal transduction but also participate in the regulation of cell shape, migration, survival, proliferation, and other cell biological behaviors.[27] When the hardness and stiffness of the extracellular matrix undergo pathological changes, it can promote the

Table 1 Expression and Clinical Characteristics of MAMDC2 in Gastric Cancer Patients

Characteristics	Low Expression of MAMDC2	High Expression of MAMDC2	p value
n	187	188	
Pathologic T stage, n (%)			0.114893753
T1&T2	46 (12.3%)	53 (14.1%)	
T3	79 (21.1%)	89 (23.7%)	
T4	62 (16.5%)	46 (12.3%)	
Pathologic N stage, n (%)			0.966893633
N0	54 (14.4%)	60 (16%)	
N1	48 (12.8%)	52 (13.9%)	
N2	43 (11.5%)	39 (10.4%)	
N3	42 (11.2%)	37 (9.9%)	

(*Continued*)

https://doi.org/10.2147/OTT.S516982

Table 1 (Continued).

Characteristics	Low Expression of MAMDC2	High Expression of MAMDC2	p value
Pathologic M stage, n (%)			0.543382123
M0	171 (45.6%)	169 (45.1%)	
M1	16 (4.3%)	19 (5.1%)	
Primary therapy outcome, n (%)			0.134754121
SD&PR&CR	157 (41.9%)	135 (36%)	
PD	30 (8%)	53 (14.1%)	
Gender, n (%)			**0.008671561**
Female	79 (21.1%)	55 (14.7%)	
Male	108 (28.8%)	133 (35.5%)	

Note: Bold text represents statistical significance.

Table 2 Univariate and Multivariate Cox Regression Studies on the Correlation Between Clinical Characteristics and Prognosis of Gastric Cancer Patients

Characteristics	Total(N)	HR(95% CI) Univariate Analysis	P value Univariate Analysis	HR(95% CI) Multivariate Analysis	P value Multivariate Analysis
Pathologic T stage	375				
T1&T2	99	Reference		Reference	
T3	168	1.713 (1.103–2.660)	**0.016**	1.825 (1.032–3.226)	**0.039**
T4	108	1.729 (1.061–2.819)	**0.028**	1.776 (0.944–3.342)	0.075
Pathologic N stage	375				
N0	114	Reference		Reference	
N1	100	1.629 (1.001–2.649)	**0.049**	1.134 (0.636–2.023)	0.669
N2	82	1.655 (0.979–2.797)	**0.060**	1.313 (0.722–2.388)	0.372
N3	79	2.709 (1.669–4.396)	**< 0.001**	1.664 (0.927–2.988)	0.088
MAMDC2	375	1.349 (1.079–3.462)	**< 0.001**		
Low	187	Reference			
High	188	0.885 (0.638–1.228)	0.465		
Pathologic M stage	375				
M0	340	Reference		Reference	
M1	35	2.254 (1.295–3.924)	**0.004**	1.615 (0.815–3.200)	0.170
Primary therapy outcome	375				
SD&PR&CR	292	Reference		Reference	
PD	83	4.147 (2.843–6.047)	**< 0.001**	3.590 (2.407–5.353)	**< 0.001**
Gender	375				
Female	138	Reference			
Male	237	1.267 (0.891–1.804)	0.188		

Note: Bold text represents statistical significance.

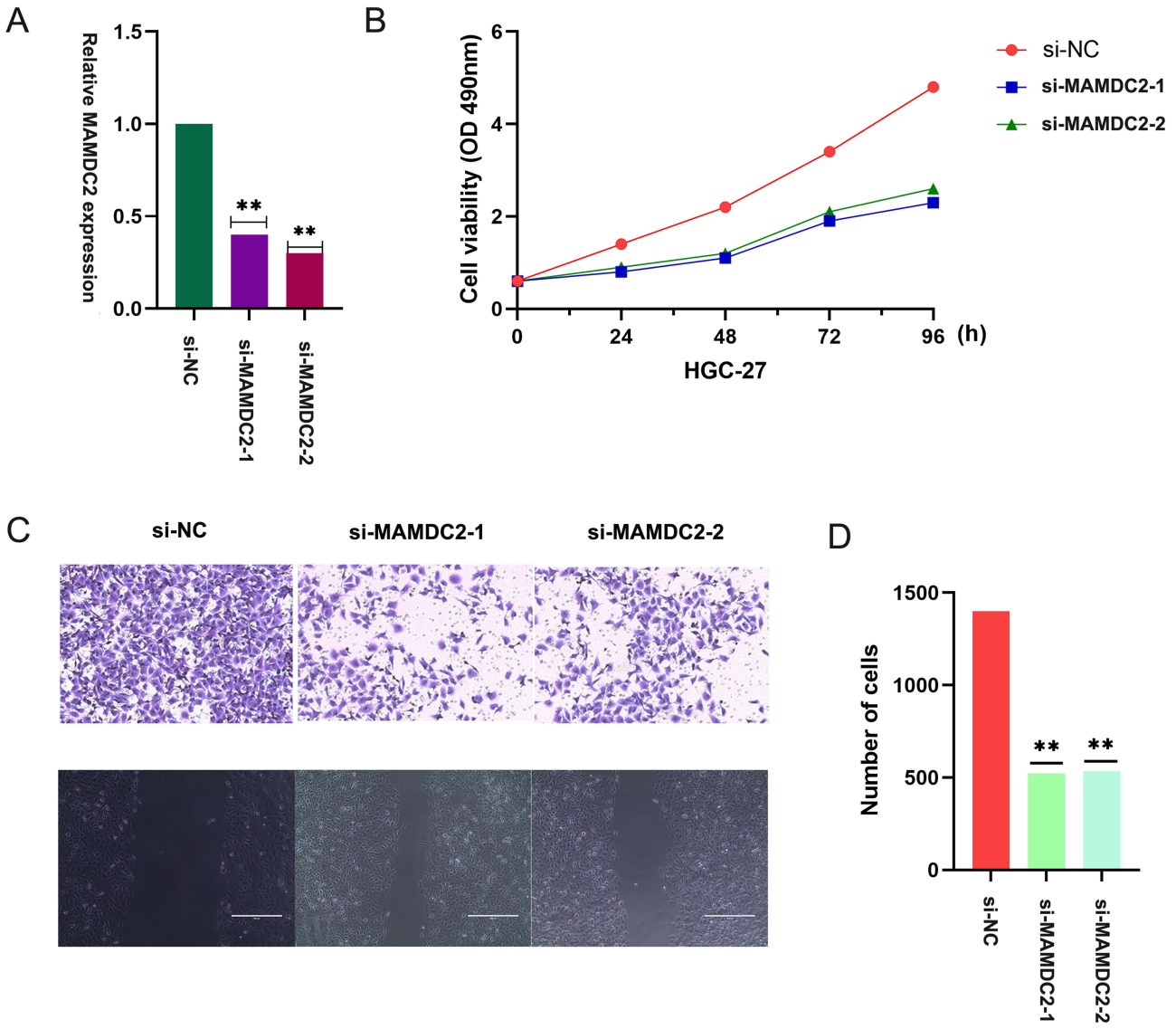

Figure 6 Effects of MAMDC2 knockdown on GC cell viability and migratory capacity in vitro. (**A**) MAMDC2 expression in HGC-27 cells transfected with negative control siRNA (si-NC) or siRNAs targeting MAMDC2 (si-MAMDC2 #1 and #2) for 48h, n=3 for each group. (**B**) Cell viability was assessed using a CCK-8 assay in HGC-27 cells transfected with si-NC or si-MAMDC2#1 and #2 for 48h, n=6 for each group. (**C** and **D**) Transwell invasion assay was performed to determine the invasion ability of si-MAMDC2-transfected HGC-27 cells for 48h. n=3 for each group. **P < 0:01. n=3 for each group.

proliferation, metastasis, and angiogenesis of malignant tumor cells through its physical properties and activation of signaling pathways, and reduce the efficacy of chemoradiotherapy and targeted therapy.[28] As the main component of the extracellular matrix, collagen is closely related to fibrosis of organ tissues, an increase in extracellular matrix hardness, promotion of angiogenesis, and guidance of tumor cell migration and invasion.[29] KEGG enrichment analysis of DEGs showed that it was related to extracellular matrix receptor interaction. The 9 hub genes identified from the overlapping DEGs of the two datasets by the STRING online tool and Cytoscape software are all uDEGs, suggesting that these genes may be closely related to extracellular matrix components and their biological behavior. It has been reported that the extracellular matrix plays a role in promoting the progression of normal gastric epithelial cells, from precancerous lesions to gastric cancer, and is an accomplice in the occurrence and development of gastric cancer.[30] Analysis of online data platforms of GEPIA, Xiantao, and Kaplan-Meier Plotter showed that the hub gene was significantly up-regulated in gastric cancer tissues ($P < 0.01$), which had certain diagnostic value ($AUC > 0.84$) and predicted poor prognosis ($P < 0.01$). Therefore, these 10 hub genes may be potential biomarkers for the diagnosis and prognosis of gastric cancer.

https://doi.org/10.2147/OTT.S516982

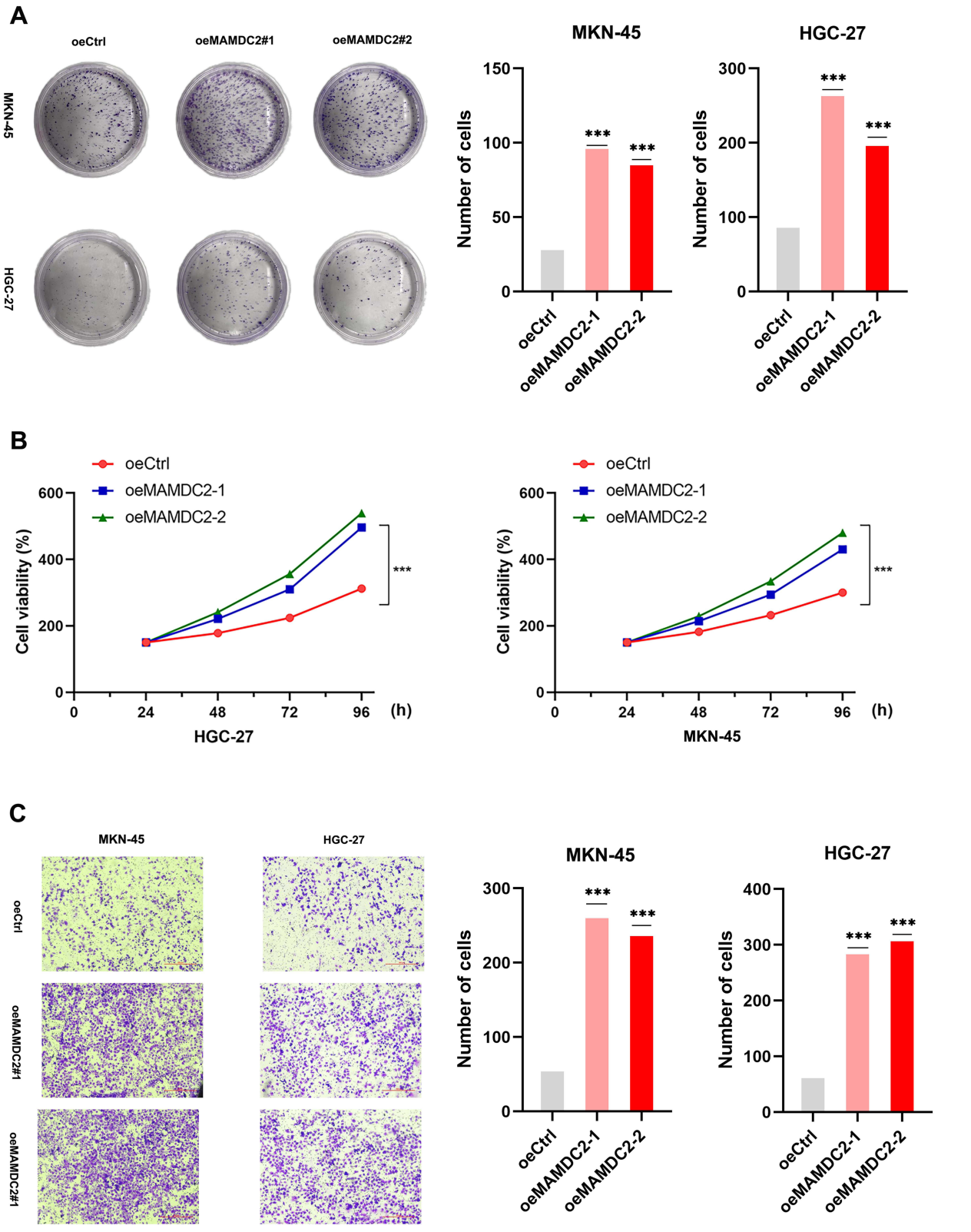

Figure 7 Overexpression of MAMDC2 promoted cell proliferation and migration. (**A**) CCK-8 results of gastric cancer cells transfected with oeMAMDC2. (**B**) Cell colony formation results after overexpressing MAMDC2. (**C**) Cell migration of gastric cancer cells after MAMDC2 overexpressed by Transwell assay. ***P<0.001. n=3 for each group.

https://doi.org/10.2147/OTT.S516982

MAM domain family is A multigene family of calcium-dependent phospholipid binding proteins, which can be divided into groups A, B, C, D, and E5 according to different species. Among them, Group C exists in human tissues and organs, and 12 members have been found (MAMDC1-C11, MAMDC13).[31] MAMs are mainly located on the cytoplasmic side of the plasma membrane and bind to anionic phospholipids in a Ca2+ + dependent manner, which can serve as membrane skeleton to maintain cell membrane homeostasis, participate in regulating cell signal transduction, regulate the inflammatory response, and are related to cell migration.[32,33] MAMDC1 has been confirmed to be associated with the occurrence, development, invasion, and metastasis of various malignant tumors, such as gastrointestinal cancer,[34] breast cancer,[35] esophageal cancer,[36] and pancreatic cancer.[37] MAMDC2 is often overexpressed in pancreatic ductal adenocarcinoma,[38] renal cell carcinoma,[39] and breast cancer[40] and is associated with apoptosis, proliferation, and migration of cancer cells. MAMDC3 overexpression has also been shown to promote the proliferation and metastasis of lung cancer,[41] liver cancer,[42] and ovarian cancer,[43] and is associated with drug resistance. Other members of the MAM family have also been implicated in the development and progression of cancer.

MAMDC2 belongs to the MAM family with a size of 8233bp, and its gene is located in 1q21, which plays an important role in the occurrence, development, and metastasis of tumors.[44,45] Its gene locus is located far from the other 11 parallel human annexins located on different chromosomes, but near the epidermal differentiation gene complex, the S100A gene cluster, and a breast cancer translocation region.[46] Under physiological conditions, MAMDC2 has no calcium-binding site and a low expression level. It cannot bind acidic phospholipids at sub-millimolar Ca2+ concentration and is not regulated by intracellular Ca2+, making it an atypical member of the annexin family.[47] MAMDC2 expression is increased in gastric cancer cells, and the prognosis is poor. Up-regulation of MAMDC2 expression in HGC-27 cells can promote cell migration, while down-regulation of MAMDC2 expression can inhibit the growth and migration of MGC-803 cells. MAMDC2 may influence cell growth, migration, and epithelial-mesenchymal transformation through TGF-β signaling pathways.[48] Yu et al[49] found that the positive expression rate of MAMDC2 in colorectal cancer was significantly higher than that in paracancerous tissue, which is related to the depth of tumor invasion and lymph node metastasis, and is an independent risk factor affecting the survival of patients with colorectal cancer. After inhibition of MAMDC2, the activity, invasion, and metastasis ability of HCT116 cells were significantly reduced, the expression levels of ADAM17 and MMP9 were significantly down-regulated, and the expression levels of TMIP-1 and E-cadherin were significantly up-regulated.[50] In intrahepatic cholangiocarcinoma, the MAMDC2 expression level is higher than that in paracancerous tissue, which is significantly correlated with lymph node metastasis and TNM stage. After silencing the expression of MAMDC2, the invasion ability of HuCCT1 cells is inhibited, and the expression level of MMP9 is down-regulated.[51] MAMDC2 expression is significantly up-regulated in cisplatin-resistant ovarian cancer cells, and the down-regulation of HAS-miR-105-1 expression can promote the up-regulation of MAMDC2 expression in cisplatin-sensitive ovarian cancer cells and enhance the resistance to cisplatin.[52] However, compared with normal tissues, MAMDC2 expression levels in head and neck squamous cell carcinoma are decreased and significantly negatively correlated with the degree of tissue differentiation.[53]

In this study, database and immunohistochemical results showed that MAMDC2 expression was significantly elevated in gastric cancer and could be used as a prognostic marker. However, this bioinformatics study may have limitations in the study of survival prognosis, and further clinical data collection and experimental studies are worthwhile. We also expect to find the correlation between MAMDC2 and the poor prognosis of GC through other research methods.

Conclusion

We have concluded through data analysis and organization that MAMDC2 is highly expressed in gastric cancer cells and significantly affects the prognosis of gastric cancer. The overexpression of MAMDC2 is associated with immune infiltration of tumor cells and may participate in the development of gastric cancer by activating mast cells, which explains the correlation between MAMDC2 and poor prognosis in gastric cancer patients from another perspective. The impact of MAMDC2 on GC phenotype may be due to different cell lines, which need to be confirmed in more cell experiments and animal experiments.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The Ethical Committee of the Second Affiliated Hospital of Soochow University (Suzhou, China) approved all procedures performed in the present study involving human paraffin-embedded tissue specimens and cell lines, which were by the Declaration of Helsinki, and all patients provided written informed consent before participation in this study.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet*. 2020;396(10251):635–648. doi:10.1016/S0140-6736(20)31288-5
2. Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. *Cancer Epidemiol Biomarkers Prev*. 2014;23(5):700–713. doi:10.1158/1055-9965.EPI-13-1057
3. Lopez MJ, Carbajal J, Alfaro AL, et al. Characteristics of gastric cancer around the world. *Crit Rev Oncol Hematol*. 2023;181:103841. doi:10.1016/j.critrevonc.2022.103841
4. Chia NY, Tan P. Molecular classification of gastric cancer. *Ann Oncol*. 2016;27(5):763–769. doi:10.1093/annonc/mdw040
5. Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. *Tumour Biol*. 2017;39(7):1010428317714626. doi:10.1177/1010428317714626
6. Guggenheim DE, Shah MA. Gastric cancer epidemiology and risk factors. *J Surg Oncol*. 2013;107(3):230–236. doi:10.1002/jso.23262
7. Ang TL, Fock KM. Clinical epidemiology of gastric cancer. *Singapore Med J*. 2014;55(12):621–628. doi:10.11622/smedj.2014174
8. Rao X, Zhang C, Luo H, et al. Targeting gastric cancer stem cells to enhance treatment response. *Cells*. 2022;11(18):2828. doi:10.3390/cells11182828
9. Patel TH, Cecchini M. Targeted therapies in advanced gastric cancer. *Curr Treat Options Oncol*. 2020;21(9):70. doi:10.1007/s11864-020-00774-4
10. Wu D, Zhang P, Ma J, et al. Serum biomarker panels for the diagnosis of gastric cancer. *Cancer Med*. 2019;8(4):1576–1583. doi:10.1002/cam4.2055
11. Strong VE. Progress in gastric cancer. *Updates Surg*. 2018;70(2):157–159. doi:10.1007/s13304-018-0543-3
12. Zhao Q, Cao L, Guan L, et al. Immunotherapy for gastric cancer: dilemmas and prospect. *Brief Funct Genomics*. 2019;18(2):107–112. doi:10.1093/bfgp/ely019
13. Lawson JD, Sicklick JK, Fanta PT. Gastric cancer. *Curr Probl Cancer*. 2011;35(3):97–127. doi:10.1016/j.currproblcancer.2011.03.001
14. Beckmann G, Bork P. An adhesive domain detected in functionally diverse receptors. *Trends Biochem Sci*. 1993;18(2):40–41. doi:10.1016/0968-0004(93)90049-S
15. Aviles-Vazquez S, Chavez-Gonzalez A, Hidalgo-Miranda A, et al. Global gene expression profiles of hematopoietic stem and progenitor cells from patients with chronic myeloid leukemia: the effect of in vitro culture with or without imatinib. *Cancer Med*. 2017;6(12):2942–2956. doi:10.1002/cam4.1187
16. Darda L, Hakami F, Morgan R, Murdoch C, Lambert DW, Hunter KD. The role of HOXB9 and miR-196a in head and neck squamous cell carcinoma. *PLoS One*. 2015;10(4):e0122285. doi:10.1371/journal.pone.0122285
17. Sultan G, Zubair S, Tayubi IA, Dahms HU, Madar IH. Towards the early detection of ductal carcinoma (a common type of breast cancer) using biomarkers linked to the PPAR (gamma) signaling pathway. *Bioinformation*. 2019;15(11):799–805. doi:10.6026/97320630015799
18. Hartgrink HH, Jansen EP, van Grieken NC, van de Velde CJ. Gastric cancer. *Lancet*. 2009;374(9688):477–490. doi:10.1016/S0140-6736(09)60617-6
19. Sano T. Gastric cancer: Asia and the world. *Gastric Cancer*. 2017;20(Suppl 1):1–2. doi:10.1007/s10120-017-0694-9
20. Baretton GB, Aust DE. Current biomarkers for gastric cancer. *Pathologe*. 2017;38(2):93–97. doi:10.1007/s00292-017-0271-3
21. Powell AG, Hughes DL, Wheat JR, Lewis WG. The 100 most influential manuscripts in gastric cancer: a bibliometric analysis. *Int J Surg*. 2016;28:83–90. doi:10.1016/j.ijsu.2016.02.028
22. Zhou J, Ma X, Bi F, Liu M. Clinical significance of circulating tumor cells in gastric cancer patients. *Oncotarget*. 2017;8(15):25713–25720. doi:10.18632/oncotarget.14879
23. Poh AR, O'Donoghue RJ, Ernst M, Putoczki TL. Mouse models for gastric cancer: matching models to biological questions. *J Gastroenterol Hepatol*. 2016;31(7):1257–1272. doi:10.1111/jgh.13297
24. Fu DG. Epigenetic alterations in gastric cancer (Review). *Mol Med Rep*. 2015;12(3):3223–3230. doi:10.3892/mmr.2015.3816
25. Crew KD, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol*. 2006;12(3):354–362. doi:10.3748/wjg.v12.i3.354
26. Gu Y, Chen T, Li G, et al. LncRNAs: emerging biomarkers in gastric cancer. *Future Oncol*. 2015;11(17):2427–2441. doi:10.2217/fon.15.175
27. Dunbier A, Guilford P. Hereditary diffuse gastric cancer. *Adv Cancer Res*. 2001;83:55–65.
28. Aytac E, Aslan F, Cicek B, et al. Dealing with the gray zones in the management of gastric cancer: the consensus statement of the Istanbul group. *Turk J Gastroenterol*. 2019;30(7):584–598. doi:10.5152/tjg.2018.18737
29. Hudler P. Outlook on epigenetic therapeutic approaches for treatment of gastric cancer. *Curr Cancer Drug Targets*. 2018;18(1):65–88. doi:10.2174/1568009617666170203163745

30. Shimizu S, Tada M, Kawai K. Early gastric cancer: its surveillance and natural course. *Endoscopy*. 1995;27(1):27–31. doi:10.1055/s-2007-1005628
31. Li YZ, Wang YY, Huang L, Zhao YY, Chen LH, Zhang C. MAM A protein family in atherosclerosis. *Clin Chim Acta*. 2022;531:406–417. doi:10.1016/j.cca.2022.05.009
32. Ural O, Kiratli HE, Sumer S, et al. Evaluation of MAMDC-1 (MAMDC-1), MAMDC-2 (MAMDC-2) and Bone Morphogenetic Protein-7 (BMP-7) serum levels in patients followed up with a diagnosis of COVID-19. *Mikrobiyol Bul*. 2022;56(1):25–35. doi:10.5578/mb.20229903
33. Bourdin M, Perrotin D, Mathieu O, et al. Measuring residual anti-Xa activity of direct factor Xa inhibitors after reversal with andexanet alfa. *Int J Lab Hematol*. 2021;43(4):795–801. doi:10.1111/ijlh.13591
34. Rentero C, Blanco-Munoz P, Meneses-Salas E, Grewal T, Enrich C. Annexins-coordinators of cholesterol homeostasis in endocytic pathways. *Int J Mol Sci*. 2018;19(5):1444. doi:10.3390/ijms19051444
35. Schloer S, Pajonczyk D, Rescher U. Annexins in translational research: hidden treasures to be found. *Int J Mol Sci*. 2018;19(6):1781. doi:10.3390/ijms19061781
36. Xiu D, Liu L, Qiao F, Yang H, Cui L, Liu G. MAMDC2 coordinates STAT3 to regulate the invasion and migration of colorectal cancer cells in vitro. *Gastroenterol Res Pract*. 2016;2016:3521453. doi:10.1155/2016/3521453
37. Liu X, Yang M, Guo Y, Lu X. MAMDC10 is a novel prognostic biomarker of papillary thyroid cancer. *Ir J Med Sci*. 2021;190(1):59–65. doi:10.1007/s11845-020-02263-x
38. Tezuka K, Suzuki M, Sato R, Kawarada S, Terasaki T, Uchida Y. Activation of MAMDC2 signaling at the blood-brain barrier in a mouse model of multiple sclerosis. *J Neurochem*. 2022;160(6):662–674. doi:10.1111/jnc.15578
39. Liu MQ, Weng XY, Sun JY. Expression of recombinant aspergillus Niger xylanase A in Pichia pastoris and its action on xylan. *Protein Expr Purif*. 2006;48(2):292–299. doi:10.1016/j.pep.2006.04.007
40. Pirozzi C, Francisco V, Guida FD, et al. Butyrate modulates inflammation in chondrocytes via GPR43 receptor. *Cell Physiol Biochem*. 2018;51(1):228–243. doi:10.1159/000495203
41. Babur E, Tufan E, Barutcu O, et al. Neurodegeneration-related genes are differentially expressed in middle-aged rats compared to young-adult rats having equal performance on long-term memory and synaptic plasticity. *Brain Res Bull*. 2022;182:90–101. doi:10.1016/j.brainresbull.2022.02.007
42. Fernandez MP, Garcia M, Martin-Almedina S, Morgan RO. Novel domain architectures and functional determinants in atypical annexins revealed by phylogenomic analysis. *Biol Chem*. 2017;398(7):751–763. doi:10.1515/hsz-2016-0273
43. Sun JY, Liu MQ, Xu YL, Xu ZR, Pan L, Gao H. Improvement of the thermostability and catalytic activity of a mesophilic family 11 xylanase by N-terminus replacement. *Protein Expr Purif*. 2005;42(1):122–130. doi:10.1016/j.pep.2005.03.009
44. Zhang T, Yu S, Zhao S. MAMDC2 as a novel prognostic biomarker associated with immune infiltrates in gastric cancer. *PeerJ*. 2021;9:e12605.
45. Zhou X, Zhao J, Yan T, et al. MAMDC2 facilitates S100A4 and promotes breast cancer progression through modulating STAT3 pathway. *Cell Death Dis*. 2024;15(4):260. doi:10.1038/s41419-024-06643-4
46. Lu C, Zhan Y, Jiang Y, Liao J, Qiu Z. Exosome-derived MAMDC2 functions as an oncogene in breast cancer. *J Pathol Clin Res*. 2023;9(5):378–390. doi:10.1002/cjp2.334
47. Wang Z, Zhou X, Deng X, et al. miR-186-MAMDC2 signaling inhibits tumorigenesis in breast cancer. *Front Oncol*. 2023;13:1166666. doi:10.3389/fonc.2023.1166666
48. Miyoshi N, Yamamoto H, Mimori K, et al. MAMDC2 gene expression in colorectal cancer: a novel marker for prognosis. *Oncol Lett*. 2014;8(5):2313–2317. doi:10.3892/ol.2014.2477
49. Zhong VW, Kuang A, Danning RD, et al. A genome-wide association study of bitter and sweet beverage consumption. *Hum Mol Genet*. 2019;28(14):2449–2457. doi:10.1093/hmg/ddz061
50. Pecka-Kielb E, Kowalewska-Luczak I, Czerniawska-Piatkowska E, Kroliczewska B. FASN, SCD1 and MAMDC2 gene polymorphism as genetic predictors of the fatty acid profile of sheep milk. *Sci Rep*. 2021;11(1):23761. doi:10.1038/s41598-021-03186-y
51. Pecka-Kielb E, Czerniawska-Piatkowska E, Kowalewska-Luczak I, Vasil M. Polymorphism in ovine MAMDC2 gene and the physio-chemical properties and the fraction of protein in milk. *J Sci Food Agric*. 2018;98(14):5396–5400. doi:10.1002/jsfa.9081
52. Yu S, Bian H, Gao X, Gui L. MAMDC2 promotes invasion and metastasis of colorectal cancer and predicts poor prognosis. *Int J Mol Med*. 2018;41(4):2185–2192. doi:10.3892/ijmm.2018.3432
53. Zhou Y, Qiu C, Wang T, Tao L, Zhang Z, Yao J. High expression of MAMDC2 promotes cell proliferation and migration in gastric cancer via the TGF-beta signaling pathway. *J Environ Pathol Toxicol Oncol*. 2021;40(3):87–94. doi:10.1615/JEnvironPatholToxicolOncol.2021038527