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Research on the relationship between RAGE and its ligand HMGB1, and prognosis and pathogenesis of gastric cancer with diabetes mellitus

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Abstract. – OBJECTIVE: To investigate the relationship between the expression of receptor for advanced glycation end products (RAGE) and high-mobility group box-1 (HMGB1) and the clinical and pathological parameters and prognosis of the patients with gastric cancer (GC) with diabetes mellitus (DM).

PATIENTS AND METHODS: 30 normal gastric mucosa, 30 tissues with GC, 90 tissues with GC and DM and their clinical data were collected. The expression levels of RAGE and HMGB1 were detected by immunohistochemistry. Kaplan-Meier survival curve was used to analyze the relationship between the expression levels of RAGE and HMGB1 and the 5-year survival rate. MTT and cell scratch assays were used to detect the effects of knockdown RAGE and HMGB1 on the proliferation and migration of BGC-823 cells. Real-Time PCR was used to detect the regulation of RAGE and HMGB1 on PTBP-1, and Spearman correlation analysis was performed to analyze the correlation between RAGE and HMGB1 and Polyprimidine tract protein (PTBP-1).

RESULTS: Compared with the normal gastric mucosa group, the expression levels of RAGE and HMGB1 were significantly higher in the GC group, GC with DM group. The expression of RAGE and HMGB1 was related with lymph node metastasis, TNM staging, and tumor invasion ($p<0.05$). Age, TNM stage, tumor infiltration depth, the expression of RAGE and HMGB1 were related with prognosis of patients with GC and DM ($p<0.05$). Tumor infiltration depth, the expression of RAGE and HMGB1 could affect the 5-year survival rate of patients with GC and DM ($p<0.05$).

CONCLUSIONS: Knockdown RAGE and HMGB1 increased the expression of PTBP-1, and RAGE and HMGB1 were negatively regulated with PTBP-1. RAGE and HMGB1 are independent risk factors for the prognosis of patients with GC with DM. RAGE and HMGB1 may regulate the expression of PTBP-1 and inhibit the glycolysis of cells, which may affect the cell proliferation and migration of GC.

Key Words:
Gastric cancer, Diabetes, Advanced glycation end product receptor, High mobility group protein B1.

Introduction

Gastric cancer (GC) is one of the common malignant tumors of the digestive tract, its onset is concealed, and the prognosis is poor, which is the second largest factor for cancer-related death. Due to the large population in China, the overall incidence of GC is still high. GC is still one of the diseases that threaten the health of Chinese people[1]. Diabetes mellitus (DM) is a metabolic disease characterized by a disorder of glucose metabolism affected by a combination of genetic and environmental factors. With the improvement of living standards, the incidence of DM is increasing. DM can increase the incidence of a variety of malignant tumors, such as pancreatic cancer, colorectal cancer, urinary tumors, etc[2,3]. The occurrence of GC and DM also has a certain correlation[4,5]. In a meta-analysis of 8,558,861 patients, Miao et al[6] found that DM can increase the risk of GC, especially male patients. DM can promote the occurrence of GC. Sekikawa et al[7] found that the rate of catching GC in atrophic gastritis with DM was 16%, and the incidence of GC in patients with atrophic gastritis was 5.1%, which was significantly lower than the former. There is a slight association between GC and DM, which may be related to the risk of HP infection in patients with hyperglycemia, the difficulty of bacterial eradication, insulin resistance, the use of hypoglycemic drugs and side effects[4]. The gastrointestinal tract is the main place where microorganisms and the human immune system interact, and gastrointestinal diseases are

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also significantly related with the pathogenesis of many metabolic diseases including DM[8]. However, there are few reports on the mechanism of GC with DM and its prognostic factors, which have not been fully illustrated.

Inflammatory response is an important pathway in mediating DM and tumorigenesis. Receptor for advanced glycation end products (RAGE) is an important member of the immunoglobulin superfamily, which is widely present in the body. It can induce the formation of advanced glycation end products (AGEs) in the body, activate its surface receptor RAGE, induce the body's inflammatory response, interfere with the normal function of the relevant organs, and further affect the development of DM[9]. RAGE can also participate in regulating the progress of GC[10]. High-mobility group box-1 (HMGB1) is a ligand for RAGE, which has a high affinity for RAGE. HMGB1 is an inflammatory cytokine that can be involved in mediating inflammatory responses. It can participate in the "inflammatory chain" reaction, which is related to the occurrence and progression of malignant tumors. HMGB1 is highly expressed in tumor tissues, which also has a certain correlation with tumor invasion and metastasis[11]. Chen et al[12] showed that the levels of HMGB1 were up-regulated in patients with DM and correlated with interleukin-6 (IL-6) positively. Chhipa et al[13] reported that RAGE and its ligand HMGB1 are up-regulated to DM, tumor, and inflammatory response. They believe that high expression of RAGE and HMGB1 may be a potential factor for GC in patients with DM. Although the expression of RAGE and HMGB1 in GC has been reported, the expression of RAGE and its ligand HMGB1 in GC with DM, the relationship between the expression of RAGE and HMGB1 and the clinicopathological features of patients with GC with DM, the relationship between the survival and prognosis of the patient and the relationship with the biological function of the GC cells are still unclear. This article will further investigate the expression of RAGE and its ligand HMGB1 in GC with DM tissues and its relationship with prognosis, as well as the possible mechanism of RAGE and HMGB1 regulating the occurrence and progression of GC.

Patients and Methods

Clinical Data

One hundred twenty cases of GC-removed pathological specimens were taken from The First Hospital of Lanzhou University from December 2013 to December 2018, including 30 cases in the GC group and 90 cases in the DM group. 30 cases were selected for gastroscopy in our hospital during the same period, and the normal gastric tissue specimens were confirmed by the pathological examination. The above specimens were the remaining paraffin sections for pathological examination. The following inclusion and exclusion criteria were met. Inclusion criteria: (1) GC group was confirmed by histopathological examination after surgery or gastroscopy. The GC with DM group met the above criteria and DM diagnostic criteria of WHO (fasting blood glucose is greater than or equal to 7 mmol/l, or the blood glucose is more than 11.1 mmol/l at 2 hours after the meal, or there is a typical DM symptom accompanied by a random fasting blood glucose greater than 7 mmol/l), and the drugs were used to control blood glucose; (2) the patients who did not receive radiotherapy, chemotherapy or targeted therapy. Exclusion criteria: (1) the patients with DM during pregnancy or DM caused by endocrine diseases; (2) those with severe liver and kidney dysfunction; (3) the patients with severe cardiopulmonary function; (4) the patients with autoimmune diseases. The clinical data of the patients were collected, and all patients were followed up and registered. The follow-up period was started from the diagnosis of the disease, and the deadline was October 30, 2019 or death of the patients. The survival time of patients was recorded. This investigation has been approved by our Ethics Committee.

Culture and Transfection of Cells

Cell Culture: GC cell lines of SGC-7901, BGC-823 and MGC-803 and normal gastric mucosal cells GES-1 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium, and 10% extra fetal bovine serum (FBS; Shanghai Bioengineering, China) and 1% streptomycin culture (Corning, Corning, NY, USA) were added. Culture conditions: at a temperature of 37°C, with 5% CO_2.

Cell transfection: the cells growing in logarithm were inoculated in the culture plate, and the convergence degree of the cells before transfection was about 80%. The empty plasmid group, group-control group, RAGE-siRNA (5'-GCCTAGTGGAGTGCC-3'), HMGB1-siR-

NA lentivirus (5'-CTCCTGGAGTAGT-3') were RAGE-siRNA group and HMGB1-siRNA group, which were synthesized by Shanghai Jikai gene. After 36 hours of transfection, the cells of each group were collected for follow-up experiment.

Immunohistochemistry

Immunohistochemistry was performed using the PV6000 method (general kit (mouse/rabbit polymer assay system)) (Beijing Zhongshan Jinqiao, China). The paraffin slices were baked at 60°C for 2 h, dewaxed by conventional xylene (Shanghai Mingtuo Industry and Trade Co., Ltd.), dehydrated with gradient ethanol and washed by phosphate-buffered saline (PBS), repaired for 2 min under high pressure antigen, soaked in 0.01 M citric acid buffer (pH 6.0) for 3 h, and in water bath for 30 min. The slices were immersed in 3% hydrogen peroxide solution for 15 min to block the activity of exogenous peroxidase. Goat serum was blocked for 15 min, at room temperature and incubated for the night at 4°C with mouse anti-human RAGE antibody (MAB179, Santa Cruz Biotechnology, Santa Cruz CA, USA, 1:100) and sheep anti-human HMGB1 monoclonal antibody (ab79823, Abcam, Cambridge, MA, USA, 1:100), and sheep anti-rabbit second antibody (C86 SSA004, Chuangsai Technology Co., Ltd., Shanghai, China) (1:1000) at room temperature for 30 min. The reaction products were developed with DAB (CAS:7411-49-6, Suzhou Yake Co., Ltd.) for 3 min, and hematoxylin (Shanghai Yinggong Reagent Co., Ltd.) for 20 s. Alcohol was dehydrated, the xylene was transparent, and the neutral resin was sealed and placed under a microscope (Olympus, Tokyo, Japan) for observation. Ten sections were taken from each group, and five non-overlapping fields were randomly selected from each slice, and 100 cells were counted from each visual field. According to the total score of the product of staining area and intensity, the results of immunopathology were taken as the results of immunopathology score. The staining area was < 10% (0), 10% ≤ 49% (1), 50% ≤ 75% (2), > 75% (3); the staining intensity was undyed (0), light yellow (1), brown (2), dark brown (3). The total score ≥ 4 was regarded as high expression, and the total score < 4 was defined as low expression.

Real-Time PCR Method

RNA was extracted by TRIzol method (15596026, Invitrogen, Carlsbad, CA, USA), and RNA was reverse-transcribed into cDNA using TaKaRa Mini BEST FFPE DNA Extraction Kit (RR047A, TaKaRa, Japan), and loaded using SYBR Premix EX Taq kit (RR420A, Takara). PCR amplification was performed using ABI Step One Plus™ Reactor (USA) with β-actin as internal parameter. Reaction system: SYBR Mix 9 ml, positive primer 0.5 ml, negative primer 0.5 ml, cDNA 2 ml, RNase Free dH2O 8 ml. Reaction conditions: 95°C for 10 min, 95°C for 15 s, 60°C for 1 min, for 40 consecutive cycles. The Real-Time PCR reaction was carried out using a 20 μl reaction system. The target genes (RAGE and HMGB1) and the internal reference gene (β-actin) were simultaneously amplified under the same reaction conditions. The relative expression level of the gene of interest was analyzed by the 2-ΔΔCt method. RAGE primer sequence: Forward: AGTGGCATCGTGCAAACCTG-3', Reverse: 5'-CTCCGAATCCATTCGACGATA-3'; β-actin primer sequence: Forward: 5'-ACACAACTGTGTTCACTAGC-3', Reverse: 5'-CAACTTCATCCACGTTCACC-3'; HMGB1 primer sequence: Forward: 5'-CTTCTCCTCCCAGATCCACA-3', Reverse: 5'-CTGTGACAGTCGTGCCAGAT-3'.

MTT Test

When the cell growth density reached 80% after transfection, the cells were digested with trypsin to prepare a Uni cell suspension, vaccinated in 5×10^5 cells per well in 96-well plates at a volume of 0.2 mL per well, incubated in an incubator, and the culture plate was taken out and replaced with 10% MTT solution (GD-Y1317, Gudo Biotechnology Company, Shanghai, China). The absorbance values of each hole at 490 nm were measured by enzyme labeling instrument (BS-1101, Detie Experimental equipment Company, Nanjing, China) for 4 hours. The cell viability curve was plotted with the time point as the abscissa and the optical density (OD) value as the ordinate.

Cell Scratch Test

Logarithmic growth phase cells were taken, which is appropriate to make the degree of cell convergence up to 80%. The cells were gently pushed on the surface to produce scratches and washed 3 times with PBS. The complete medium was replaced, and the scratches were recorded, and continue to culture. After 24 hours, the scratches were photographed, the width of the cell scratches was compared, and the cell migration rate of each group was statistically analyzed.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (IBM, Armonk, NY, USA) software. The experiment was repeated 3 times. The measurement data were analyzed by paired sample *t*-test and variance analysis. The count data were compared by using χ^2-test, the survival analysis was performed by COX regression model, and the correlation analysis was performed by Spearman correlation analysis. α=0.05 was taken as the test standard, and $p<0.05$ was considered statistically significant.

Results

Clinical Data (Table I)

There were 51 males and 39 females in the GC with DM group, 40-80 years old, with an average age of 61.43±5.87 years old; 20 males and 10 females in the GC group, 35-85 years old, with an average age of 64.61±4.56 years old; 17 males and 13 females in the control group, 35-85 years old, with an average age of 62.21±3.45 years. There was no statistical difference in clinical data.

Immunohistochemical Detection of the Expression of RAGE and HMGB1 in Tissue Samples

The results of immunohistochemistry show that the high expression rate of RAGE and HMGB1 was 20% (6/30) and 16.67% (5/30) in normal gastric mucosa tissues, respectively, 46.67% (14/30) and 43.33% (13/30) in the tissues with GC, 64.44% (58/90) and 62.22% (56/90) in the tissues with GC with DM, respectively. Compared with the normal gastric mucosa group, RAGE and HMGB1 were significantly higher in GC group (p=0.018; p=0.021), GC with DM group (p=0.025; p=0.032), and RAGE and HMGB1 in GC with DM group were significantly higher than those in GC group (p=0.036; p=0.040) (Figure 1A and 1B).

Relationship Between the Expression of RAGE and HMGB1 and Clinical Pathological Parameters of Patients with GC with DM

Uni-factor analysis shows that the expression of RAGE and HMGB1 was not related with age, gender, tumor diameter, and differentiation of patients with GC with DM ($p>0.05$). The expression of RAGE and HMGB1 was related with lymph node metastasis, TNM stage, and depth of invasion ($p<0.05$), (Table II).

Relationship Between RAGE and HMGB1 and Prognosis of the Patients with GC with DM

Kaplan-Meier survival curve analysis shows that the 5-year survival rate of patients with GC with DM with high expression of RAGE and HMGB1 was significantly lower than that of patients with low expression of RAGE and HMGB1 (p=0.003; p=0.001, Figure 2A and 2B).

Factors Influencing Prognosis of the Patients with GC with DM

Cox uni-factor survival analysis shows that age, TNM stage, infiltration depth, rage, and HMGB1 expression were related with prognosis in patients with GC with DM. The results of fur-

Table I. Clinical data

		Grouping			
		GC	GC+DM	Control	*p*-value
Gender	Male	20	51	17	0.542
	Female	10	39	13	0.433
Age (years old)	< 60	12	57	14	0.181
	≥ 60	18	33	16	0.237
Lymph node metastasis	Yes	13	56	-	0.085
	No	17	34	-	0.076
TNM staging	I-III	19	58	-	0.226
	IV	9	32	-	0.317
Differentiation	Low	17	42	-	0.298
	High	13	48	-	0.435
Infiltration depth	Mucosa and Submucosa	16	50	-	0.016
	Muscle and Serosa	14	40	-	0.041
Tumor diameter (cm)	≥ 5	12	40	-	0.337
	< 5	18	50	-	0.572

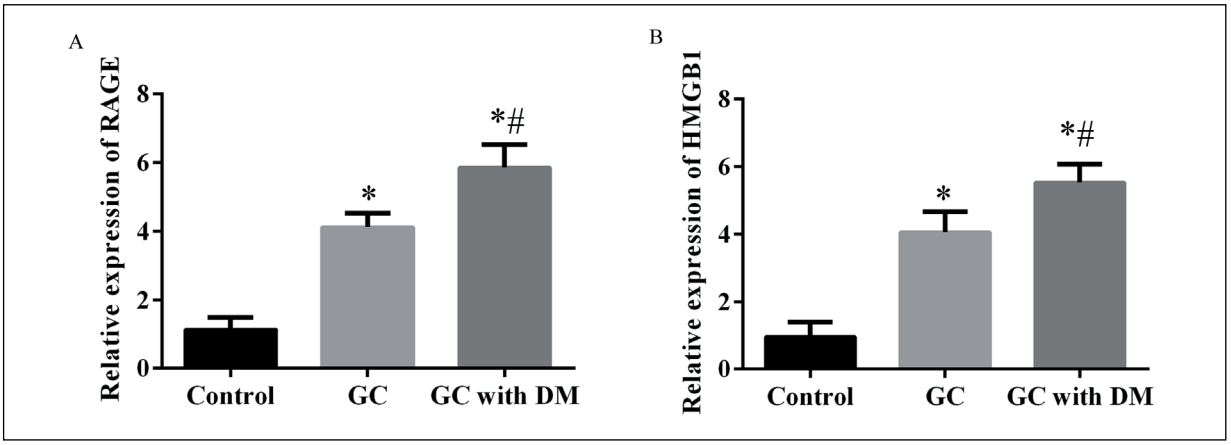

Figure 1. Expression of RAGE and HMGB1 in tissue samples. **A**, The expression of RAGE in tissues was detected by immunohistochemistry. **B**, The expression of HMGB1 in tissues was detected by immunohistochemistry. Control represents the normal gastric mucosa group, GC represents the gastric cancer group, GC with DM represents GC with DM group, * represents $p<0.05$ compared with control group, and # represents $p<0.05$ compared with GC group.

ther multi-factor survival analysis found that the depth of infiltration, the expression of RAGE and HMGB1 could influence the 5-year survival rate of patients with GC with DM (Table III).

Expression of RAGE and HMGB1 in GC Cells

Real-Time PCR results show that RAGE and HMGB1 were significantly increased in GC cells compared to normal cells (Figure 3A and 3B). RAGE-siRNA and HMGB1-siRNA were transfected into BGC-823 cells with the highest expression level, and the expression after transfection was detected by Real-Time PCR. The results show that the expression of RAGE and HMGB1 in the RAGE-siRNA, HMGB1-siRNA group was significantly lower in the group-control group ($p = 0.033$, $p = 0.029$) (Figure 3C and 3D).

Effect of Knockdown RAGE and HMGB1 on Proliferation and Migration of BGC-823 Cells

The results of MTT assay showed that knockdown RAGE and HMGB1 could inhibit the proliferation of tumor cells ($p=0.018$, $p=0.024$) (Figure 4A). The results of scratch test show that the knockdown RAGE and HMGB1 could inhibit the migration of BGC-823 cells ($p=0.026$, $p=0.021$) (Figure 4B and 4C).

Effect of Knockdown RAGE and HMGB1 on PTBP1 Expression

Real-Time PCR results show that the expression of PTBP1 was increased after knockdown RAGE and HMGB1 in BGC-823 cells ($p=0.041$, $p=0.037$) (Figure 5A and 5B). Spearman correlation analysis shows that RAGE and HMGB1 were negatively correlated with PTBP1 expression (r=0.328, $p=0.007$; r=0.349, $p=0.015$) (Figure 5C and 5D). This indicates that HMGB1 and RAGE may have a negative regulatory relationship with PTBP1.

Discussion

GC is a gastric epithelial-derived malignant tumor. In China, the incidence of GC ranks second, only the second to lung cancer[14]. The occurrence of GC is closely related to environmental and dietary, genetic, and HP infection. DM is a metabolic disease characterized by chronic hyperglycemia caused by various etiologies. Epidemiological studies have revealed pancreatic cancer in patients with DM. The incidence of malignant tumors such as breast cancer and colon cancer is significantly increased, and the mortality rate is also increased[15,16]. DNA damage caused by long-term oxidative stress in patients with DM also promotes the occurrence of GC. In addition, persistent hyperglycemia in patients with DM can provide abundant energy substances for the reproduction of tumor cells and promote the metastasis of tumor cells to a distance[17]. The results of a number of independent studies showed that, for patients with DM, the incidence of GC increased and the severity was higher than that of the unincorporated DM[6,18]. The number of patients with

Table II. Relationship between expression of RAGE and HMGB1 and clinicopathological features.

Variable		N	RAGE expression		χ^2-value	*p*-value	HMGB1 expression		χ^2-value	*p*-value
			High	Low			High	Low		
Age	≥ 60	49	26	23	0.467	0.482	27	22	1.024	0.365
	< 60	41	21	20			21	20		
Gender	Male	56	36	20	1.923	0.212	35	21	0.176	0.671
	Female	34	18	16			19	15		
Lymph Node Metastasis	Yes	54	19	35	3.892	0.039	18	36	4.887	0.027
	No	36	19	17			20	16		
TNM Staging	I-III	62	34	28	4.879	0.029	33	29	3.564	0.034
	IV	28	11	17			13	15		
Differentiation	Low	44	24	20	0.176	0.867	25	19	0.475	0.51
	High	46	25	21			24	22		
Degree of Infiltration	Mucosa, Submucosa	51	29	22	5.346	0.0311	33	18	5.177	0.023
	Muscle Layer	39	15	24			17	22		
Tumor Diameter (cm)	≥ 5	35	19	16	0.667	0.41	17	18	0.324	0.376
	< 5	55	27	28			25	30		

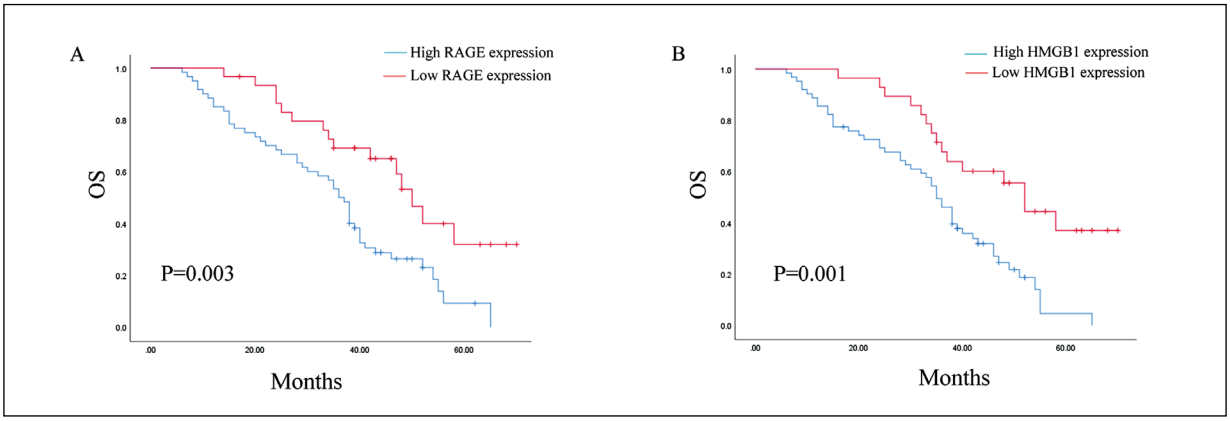

Figure 2. Relationship between RAGE and HMGB1 and the 5-year revival rate of the patients with GC with DM. **A**, Kaplan-Meier survival curve analysis shows that the 5-year survival rate of patients with high RAGE and GC with DM was significantly lower than that of patients with low RAGE expression, $p<0.01$. **B**, Kaplan-Meier survival curve analysis shows that the 5-year survival rate of patients with high HMGB1 expression and DM was significantly lower than that of patients with low HMGB1 expression, $p<0.01$.

GC with DM in China is very large; the incidence of GC combined with DM is high, the prognosis is relatively poor, and the risk factors that influence the occurrence mechanism and prognosis of GC combined DM still need to be further studied.

AGEs are produced by non-enzymatic glycosylation of free energy substances such as galactose and glucose in normal organs. RAGE is a new pattern recognition receptor. RAGE can participate in the pathological development of a variety of diseases, including DM and malignant tumors, by binding specifically to AGEs[19]. The expression level of RAGE in the body is positively correlated with the severity of DM and its complications in their study[20]. RAGE is widely distributed in human tissues, closely related to DM, and is also involved in the occurrence and progression of tumors[21]. Yang et al[22] reported that RAGE acts as a cell signal transduction receptor and interacts with various ligands, such as AGEs and axon growth factors, to promote the pathological processes of DM and various tumors *in vivo*. HMGB1 is the main ligand of RAGE, a highly conserved nuclear protein, belonging to inflammatory factors. HMGB1 and RAGE are combined to participate in the body's signal transmission; on the one hand, they promote the inflammatory response *in vivo*, on the other hand, they regulate the body's energy metabolism process[22,23]. HMGB1 can promote autophagy in the cytoplasm or mitochondria, inhibit apoptosis *in vivo*, regulate the body's inflammatory response, immune response, cell proliferation, apoptosis, autophagy, etc., and may also participate in the malignant evolution of various tumors[24,25]. In this study, immunohistochemistry was used to detect the expression levels of RAGE and HMGB1 in normal gastric mucosa, GC and GC with DM.

Table III. Uni-factor and multi-factor analysis of prognosis of the patients with GC with DM.

Parameter	Uni-factor analysis			Multi-factor analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	1.665	0.847-3.164	0.035	1.137	0.614-2.145	0.13
Gender	0.859	0.358-1.983	0.186	-	-	-
Lymph Node Metastasis	0.547	0.285-1.439	0.323	-	-	-
TNM Staging	1.955	1.148-3.645	0.026	0.875	0.372-2.086	0.176
Differentiation	0.765	0.469-1.887	0.212	-	-	-
Degree of Infiltration	2.128	1.259-3.874	0.021	1.788	0.939-2.843	0.028
Tumor Diameter	0.753	1.251-1.862	0.254	-	-	-
RAGE Expression	2.486	1.236-4.573	0.015	2.137	1.162-3.982	0.023
HMGB1 Expression	2.638	1.450-5.112	0.013	2.346	1.331-4.248	0.018

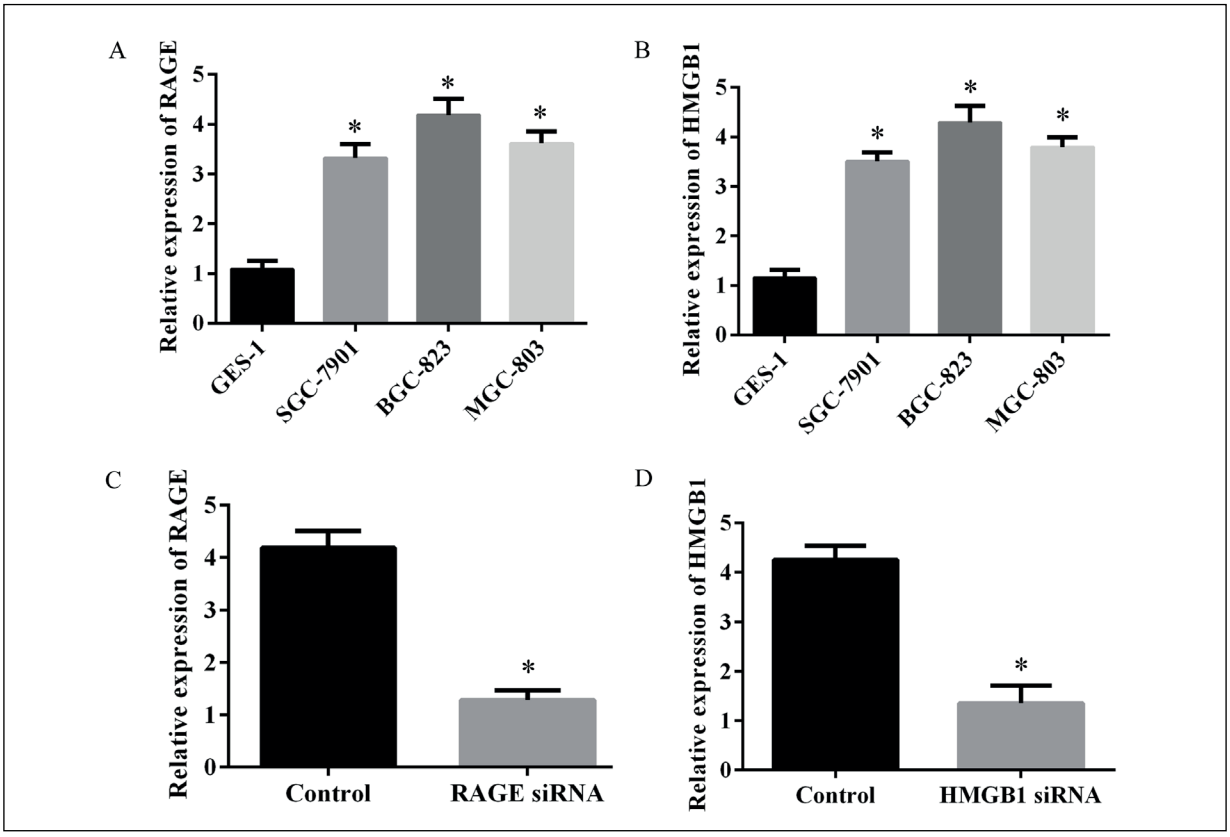

Figure 3. Expression of RAGE and HMGB1 in GC cells. **A**, Real-Time PCR results show that the expression level of RAGE in GC cells was significantly increased, and * represents the comparison with normal gastric epithelial cells, $p< 0.05$. **B**, Real-Time PCR results show that the expression level of HMGB1 in GC cells was significantly increased, and * represents a comparison with normal gastric epithelial cells, $p< 0.05$. **C**, Real-Time PCR results show that the expression of RAGE was significantly decreased after transfection of RAGE-siRNA in BGC-823 cells, and * represents the comparison with control group, $p< 0.05$. **D**, Real-Time PCR results show that the expression of HMGB1 was significantly decreased after transfection of HMGB1-siRNA in BGC-823 cells, and * represents the comparison with control group, $p< 0.05$.

The results show that RAGE and HMGB1 were highly expressed in GC and GC with DM, and the expression level in GC with DM was significantly higher than that in GC alone, which may be related to the low-grade inflammation and the presence of obvious immune dysfunction in DM patients, as well as Tzouvelekis et al[26]. The results of the study are consistent, which means that the expression of RAGE and HMGB1 may be related to the occurrence of GC with DM.

HMGB1-RAGE signaling pathway plays an important role in tumors caused by inflammation[27]. According to Qian et al[28], RAGE and HMGB1 are overexpressed in colorectal cancer and are related with the prognosis of tumor patients. RAGE and HMGB1 may promote tumor progression by targeting YAP1. In addition, Wang et al[29] found that miR-205 can target RAGE and HMGB1 to inhibit proliferation, invasion and epithelial mesenchymal transition (EMT) of breast cancer cells. Li et al[30] reported that circular RNA101368/miR-200a can affect the metastasis of hepatocellular carcinoma by mediating the RAGE-HMGB1 signaling pathway. The relationship between the expression of RAGE and HMGB1 and the combination of GC and DM results in the study of the relationship between the expression of RAGE and HMGB1 and the clinical and pathological parameters and prognosis of the patients with GC. It was found that the expression of RAGE and HMGB1 was closely related to lymph node metastasis, TNM stage, and tumor invasion depth. Thus, it can be seen that the highly expressed malignant potential of RAGE and HMGB1 may be higher. Previous studies have shown that high expression of RAGE and HMGB1 can promote rapid proliferation of colorectal cancer cells[28]. However, the excessive proliferation of tumor

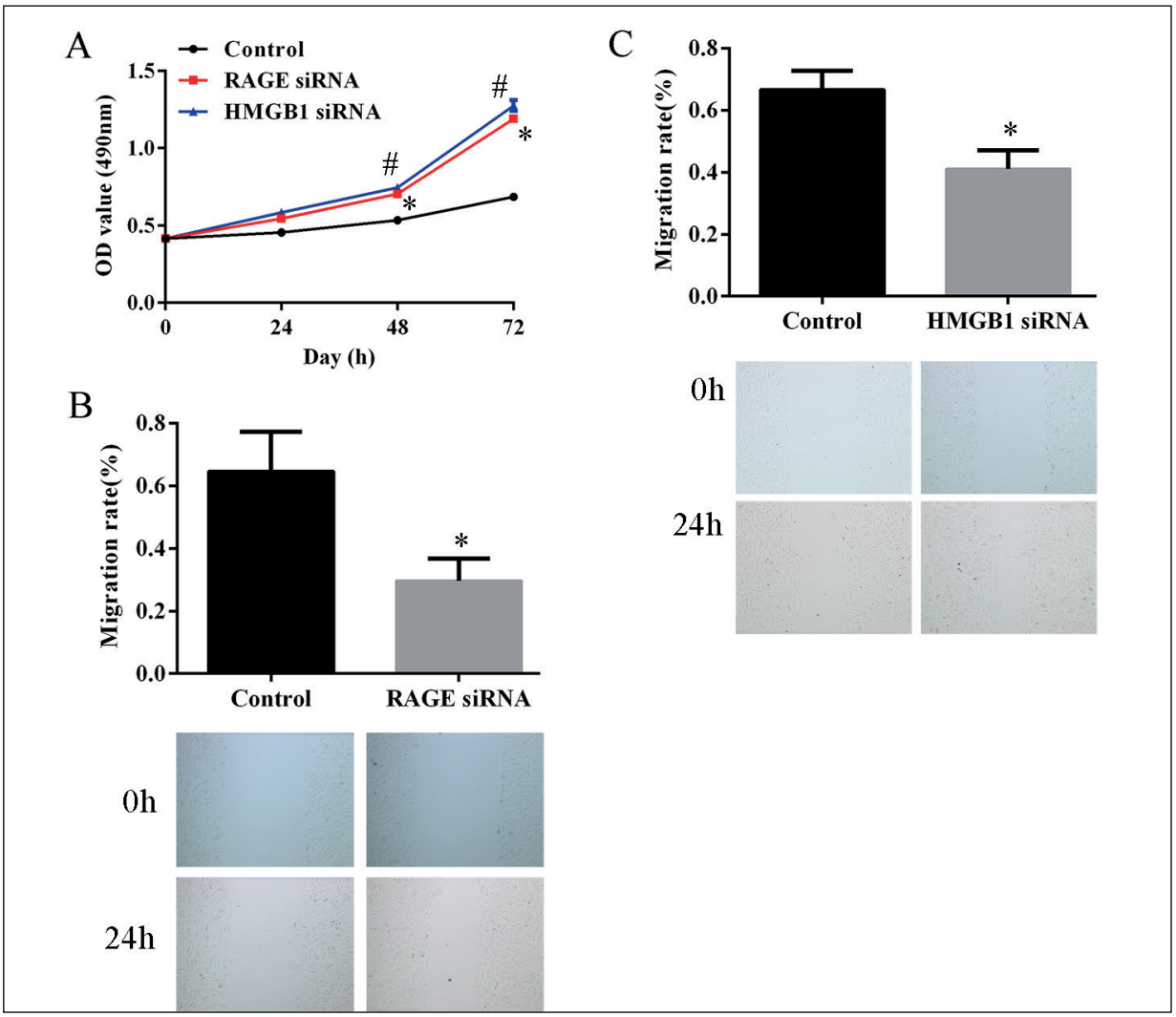

Figure 4. Effect of knockdown RAGE and HMGB1 on proliferation and migration of BGC-823 cells (100X). **A**, MTT assay results show that knockdown RAGE and HMGB1 inhibited the proliferation of BGC-823 cells, and * and # represent comparison with control group, $p< 0.05$. **B**, Knockdown RAGE inhibited the migration ability of BGC-823 cells, and * represents the comparison with the control group, $p< 0.05$. **C**, Knockdown HMGB1 inhibited the migration ability of BGC-823 cells, * represents the comparison with the control group, $p< 0.05$.

cells is prone to lymph node metastasis, which also indicates that the high expression of RAGE and HMGB1 can promote the metastasis of GC to some extent. The early metastasis is one of the most common causes of the failure of the clinical anti-tumor and is one of the important factors that affect the prognosis of the tumor. In addition, the high expression of RAGE and HMGB1 is related to the degree of GC invasion. It is well known that the depth of tumor invasion is related to the stage of GC, and the prognosis of GC patients whose lesions are limited to mucous membrane and submucosa is relatively better. Thus, the up-regulated expression of RAGE and HMGB1 may affect the survival prognosis of patients with GC with DM. In this study, Kaplan-Meier survival analysis found that the 5-year survival rate of patients with GC with DM with high expression of RAGE and HMGB1 was significantly lower than those with low expression of RAGE and HMGB1. Single-factor survival analysis found that age, TNM stage, depth of tumor invasion, the expression of RAGE and HMGB1 were closely related to the prognosis of patients with GC with DM. Further multi-factor survival analysis found that infiltration depth, the expression of RAGE

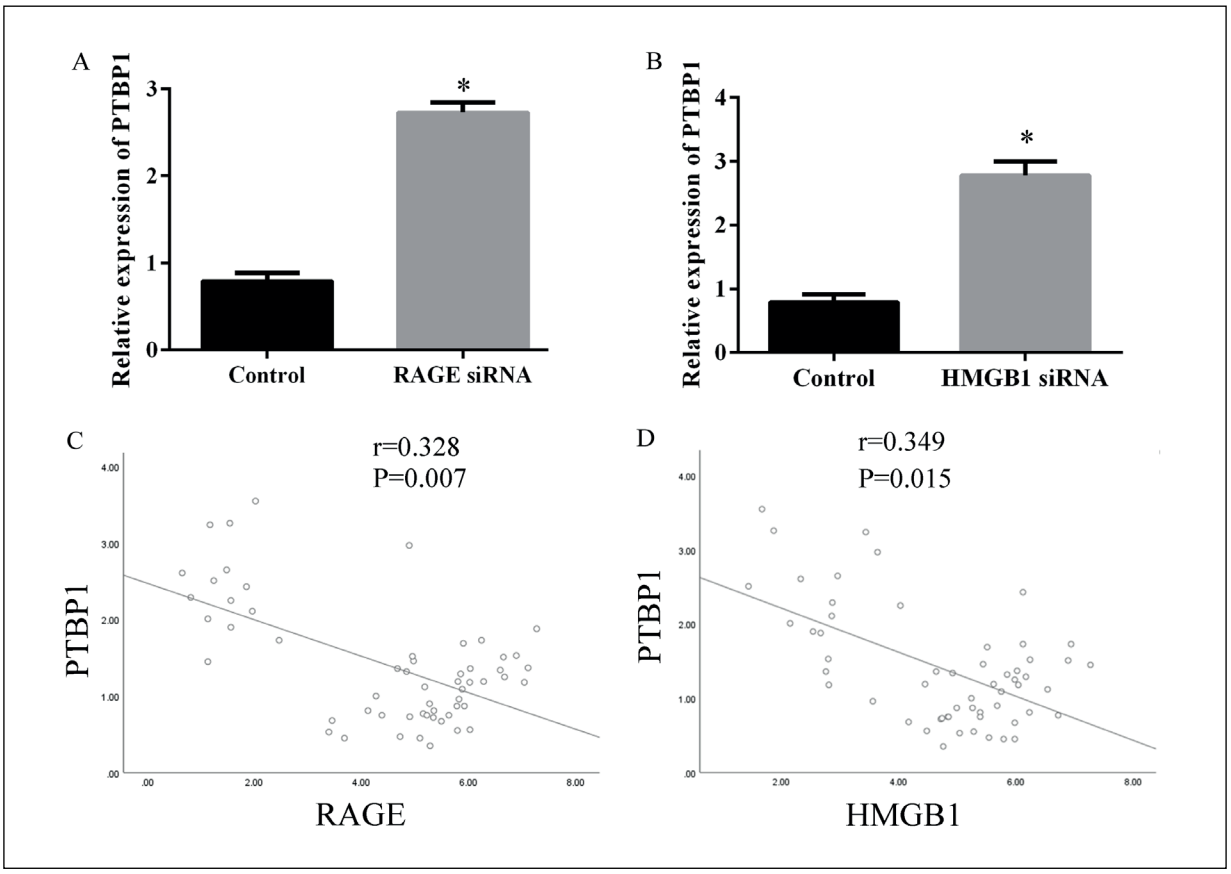

Figure 5. Correlation between RAGE and HMGB1 and PTBP1 expression. **A**, Real-Time PCR results show that in BGC-823 cells, knockdown RAGE increased the expression of PTBP1, and * represents the comparison with control group, $p< 0.05$. **B**, Real-Time PCR results showed that in BGC-823 cells, knockdown HMGB1 increased the expression of PTBP1, and * represents the comparison with control group, $p< 0.05$. **C, D**, Spearman correlation analysis shows that HMGB1 and RAGE were significantly related with PTBP1 expression, $p< 0.05$.

and HMGB1 could affect the 5-year survival rate of patients with GC with DM. The expression of RAGE and HMGB1 was independent risk factor for the prognosis of patients with GC with DM. Spearman correlation analysis also suggested that the expression levels of RAGE and HMGB1 were significantly correlated with GC and DM tissues, and there may be synergistic effects. Therefore, the results of the above studies further confirmed that the relationship between the expression of RAGE and HMGB1 and the prognosis of patients with GC combined with DM.

Pyruvate kinase (PK) is a very important rate-limiting enzyme in the cell glycolytic pathway. There are two major subtypes: PKM-1 and PKM-2. Polyprimidine tract protein 1 (PTBP-1) in cancer cells can promote the expression of PKM-2. Increasing research evidence indicates that regulation of glucose metabolism can affect the occurrence and development of tumors[31-34]. PTBP-1 is also associated to certain biological processes of tumors. He et al reported that knocking out PTBP-1 can inhibit the proliferation and invasive ability of ovarian cancer cells *in vitro*; PTBP-1 overexpression may be one of the important causes of tumor formation[35]. Li et al[36] found that in colon cancer, PTBP-1 can promote tumorigenesis by regulating cell cycle and apoptosis. In this study, it was found that *in vitro* cell experiments, knockdown RAGE and HMGB1 can inhibit the proliferation and migration of GC cells, RAGE and HMGB1 are negatively regulated by PTBP-1, and RAGE and HMGB1 may affect the biological process of tumor cells by regulating PTBP-1. RAGE and HMGB1 regulate the expression of PTBP-1, thus inhibiting the glycolysis process, which may eventually affect the proliferation and migration of GC cells, while

the glycolysis ability of cells in DM patients is inhibited. Therefore, this study found that RAGE and HMGB1 can regulate PTBP-1 to play an important role in the combination of GC and DM.

Conclusions

In summary, RAGE and HMGB1 are significantly higher in GC with DM, which is related with the prognosis of patients with GC with DM and is an independent risk factor affecting their prognosis. RAGE and HMGB1 may regulate the expression of PTBP-1, inhibit cell glycolysis, and eventually affect the proliferation and migration of GC cells. This study is the first report on the expression of RAGE and its ligand HMGB1 in patients with GC and DM and analyzes the relationship between the clinical parameters and survival prognosis of patients. Moreover, this study further explores the effects of RAGE and HMGB on the biological function of GC cells, and conduct preliminary mechanism verification. This study provides a new target for the treatment of GC with DM, and also provides an important basis for the clinical evaluation of the prognosis of patients with DM. However, there are also limitations in this study, such as the lack of large sample research and no further verification of animal experiments, but these problems will provide a certain direction for future research.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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