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HMGA1 accelerates the malignant progression of gastric cancer through stimulating EMT

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Abstract. – OBJECTIVE: To clarify the role of HMGA1 in influencing proliferation and migration abilities, and EMT (epithelial-mesenchymal transition) in gastric cancer (GC) cells.

MATERIALS AND METHODS: Differential expressions of HMGA1 in GC tissues and normal gastric tissues were analyzed in the GEPIA dataset. Its influence on overall survival of GC patients was evaluated as well. Moreover, HMGA1 levels in GC cells and gastric mucosal cells were detected. Regulatory effects of HMGA1 on the proliferation and migration abilities in SGC7901 and MGC803 cells were assessed through a series of functional experiments. At last, influences of HMGA1 on the expression levels of EMT-related genes, E-cadherin, Snail, and Slug were determined in GC cells.

RESULTS: Analysis of data in TCGA GEPIA dataset revealed that HMGA1 was upregulated in GC tissues, and GC patients with a high expression level of HMGA1 suffered poorer prognosis. In addition, HMGA1 was identically upregulated in GC cells, and the overexpression of HMGA1 improved the proliferation and migration abilities of SGC7901 and MGC803 cells, downregulated E-cadherin, and upregulated Snail and Slug in GC cells, while silence of HMAG1 yielded the opposite results

CONCLUSIONS: HMGA1 is upregulated in GC tissues and predicts poor prognosis, and it aggravates the progression of GC *via* stimulating EMT.

Key Words:
Gastric cancer (GC), HMGA1, Proliferation, Migration, EMT.

Introduction

Gastric cancer (GC) is a highly prevalent tumor, and its morbidity and mortality rank the first in all types of malignancies[1]. Symptoms of GC in the early phase are similar to those of digestive system diseases, resulting in a low detection rate of early-stage GC[2]. It is necessary to search for effective targets for screening and diagnosing GC as early as possible, thus improving the prognosis. The carcinogenesis of GC involves both environmental and genetic factors. Obesity, high-salt diet, inadequate intake of vegetables and fruits, and gastroesophageal reflux are risk factors for GC[3]. Helicobacter pylori (Hp) infection is considered to be the leading risk factor for GC. However, individual susceptibility to GC varies greatly even under the same environment, highlighting the significance of genetic variations in the pathogenesis of GC[4].

High mobility group (HMG) proteins are non-histone, which are involved in cell chromatin remodeling, characterized by rapid movement in polyacrylamide gel electrophoresis[5]. HMG proteins include HMGA, HMGB, and HMGN, which are further divided into HMGA1 and HMGA2 according to their encoded genes. HMGA1 is divided into HMGA1a, HMGA1b, and HMGA1c based on different mRNA splicing sequences during protein translation[5]. HMG proteins have homology and are conserved during biological evolution. Each subfamily of HMG has a characteristic functional sequence motif: the AT-hook region of the HMGA1 family, the HMG-box of the HMGB family, and the nucleosome binding region of the HMGN family. Based on this, HMG proteins are able to modify the binding sequences to further affect DNA transcription, replication, recombination, and repair[6,7].

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HMGA1 was first found in the human cervical cancer cell line Hela with highly malignant proliferation[8]. HMGA1 is highly expressed during a variety of biological embryogenesis, but it is barely expressed in most mature tissues. Functionally, HMGA1 participates in a great number of basic cellular processes[9]. Previous studies[8,10-12] have shown that HMGA1 is upregulated in many types of tumors. A recent study[13] has uncovered the role of HMGA1 in regulating cell cycle proteins and proliferation ability of tumor cells by interacting with RB protein. This study mainly investigates the potential function of the underlying mechanism of HMGA1 in GC.

Materials and Methods

GEPIA Data Analysis

Differential expressions and prognostic value of HMGA1 in GC were assessed using GEPIA database (http://GEPIA.cancer-pku.cn/index.html), an online database containing gene sequencing data. Here, TCGA STAD database containing 408 GC tissues and 211 normal tissues were downloaded for analyses.

Cell Culture

Mucosal gastric cells and GC cells were purchased from Cell Bank (Shanghai, China). Cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37°C. Medium was regularly replaced.

Transfection

Overexpression vector of HMGA1 was cloned into pSicoR lentivirus vector (LV-HMGA1), and 293T cells were used for lentivirus production. Then, the cells were transfected with LV-HMGA1 or LV-Vector, respectively. Finally, the stably expressed cells were screened after two-week puromycin selection.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Cells were lysed to harvest RNAs using TRIzol method (Invitrogen, Carlsbad, CA, USA), and the extracted RNAs were subjected to reverse transcription according to the instructions of PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). RNA concentration was detected using a spectrometer. QRT-PCR was then performed based on the instructions of SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan). Finally, the relative level was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers were as follows: HMGA1 F-TTCTCTAAGGAGCAGGTGGAA, R-CGCATTTGCTACCAGCG;

Cell Counting Kit-8 (CCK-8) Assay

Cells were seeded into 96-well plates with 4×10^3 cells per well. At the established time points, 10 μL of CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) was added in each well, and the absorbance at 450 nm of each sample was measured by a microplate reader (Bio-Rad, Hercules, CA, USA).

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

Cells were inoculated into 96-well plates with 1×10^5 cells per well, and labeled with 100 μL of EdU reagent (50 μM) per well for 2 h. After washing with phosphate-buffered saline (PBS), the cells were fixed in 50 μL of fixation buffer, decolored with 2 mg/mL glycine, and permeated with 100 μL of penetrant. After washing with PBS once, the cells were stained with 100 μL of Hoechst33342 in the dark for 30 min. EdU-positive ratio was determined under a fluorescent microscope (magnification 20×).

Wound Healing Assay

Cells were seeded in a 6-well plate with 5.0×10^6 cells/well. An artificial wound was made in the confluent cell monolayer using a 200 μL pipette tip. Wound closure images were taken at 0 and 24 h using an inverted microscope, respectively. Relative distance of wound healing was calculated.

Transwell Assay

Cell density was adjusted to 1×10^5 cells/mL. Subsequently, 100 μL of suspension was applied in the upper side of transwell chamber (Corning, Corning, NY, USA), while 600 μL of medium containing 20% FBS was applied in the bottom side. After 48 h of incubation, the cells penetrating to the bottom side were fixed in 4% paraformaldehyde for 20 min, stained with crystal violet for 20 min, and counted using a microscope. The penetrating cells were counted in 5 randomly selected fields per sample (magnification 20×).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for data analysis. Data were expressed as mean ± standard deviation ($\bar{x} \pm SD$). Survival analysis was performed by introducing the Kaplan-Meier method. Data between the two groups were compared using the *t*-test. $p<0.05$ considered the difference was statistically significant.

Results

HMGA1 Was Upregulated in GC and Related to Prognosis

By analyzing the TCGA STAD database, it was found that HMGA1 was upregulated in GC tissues relative to normal ones (Figure 1A). *In vitro* abundance of HMGA1 was identically upregulated in GC cells compared to that of mucosal gastric cells (Figure 1B). Besides, GC patients with a high expression level of HMGA1 presented shorter overall survival relative to those expressing low level (Figure 1C).

Overexpression of HMGA1 Accelerated Proliferation Ability of GC Cells

To explore potential biological functions of HMGA1 in GC, LV-HMGA1 was constructed. Transfection of LV-HMGA1 markedly upregulated HMGA1 level in SGC7901 and MGC803 cells compared to those transfected with LV-Vector (Figure 2A). CCK-8 assay demonstrated the increased viabilities in GC cells overexpressing HMGA1 (Figure 2B). Moreover, EdU-positive ratio was elevated after transfection with LV-HMGA1 in GC cells (Figure 2C). The above results suggest that HMGA1 accelerates the proliferation of GC cells.

Overexpression of HMGA1 Enhanced the Migration Ability of GC Cells

The regulatory effect of HMGA1 on the migration ability of GC cells was assessed through wound healing assay and transwell assay. After 24-h cell culture, the relative distance of wound healing was much longer in GC cells transfected with LV-HMGA1 (Figure 3A). Additionally, the migratory cell number was elevated by the overexpression of HMGA1 in SGC7901 and MGC803 cells (Figure 3B).

HMGA1 Facilitated EMT in GC

It is well known that EMT is a key link for tumor cells to acquire metastatic ability. Thereafter it was speculated that HMGA1 regulated the metastasis of GC cells *via* influencing EMT. Here, the overexpression of HMAG1 downregulated E-cadherin and upregulated Snail and Slug in GC cells (Figure 4A). Conversely, the transfection with si-HMAG1 achieved the opposite results (Figure 4B). As a result, it is confirmed that HMGA1 is able to accelerate EMT in GC.

Discussion

The etiology and pathology of GC are complex. It is considered that irregular diet, bad eating habit, high-pressure life, Hp infection,

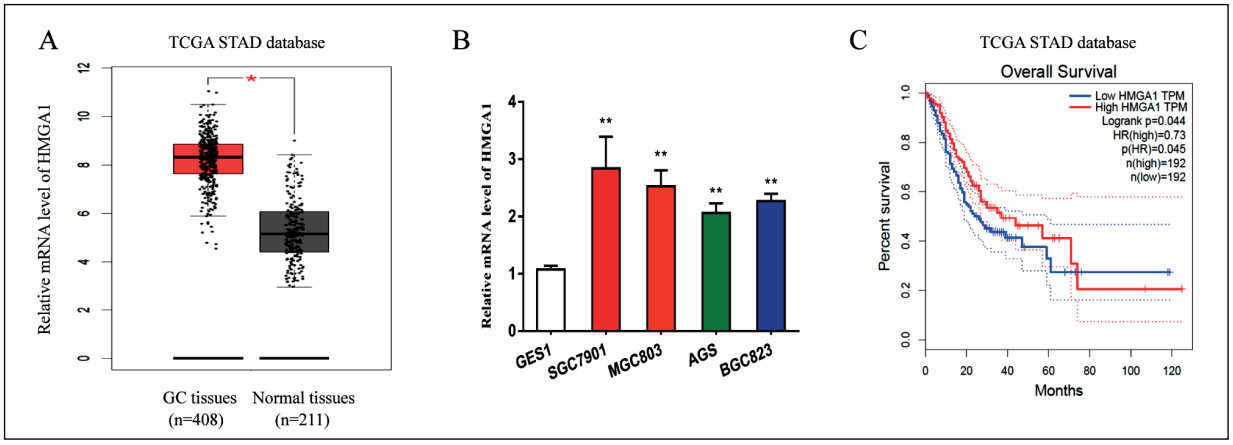

Figure 1. HMGA1 is upregulated in GC and related to prognosis. **A,** HMGA1 levels in GC tissues (n=408) and normal tissues (n=211) analyzed in TCGA STAD database. **B,** HMGA1 level in mucosal gastric cells (GES1) and GC cells (SGC7901, MGC803, AGS and BGC823). **C,** Overall survival in GC patients with a high or low expression level of HMGA1.

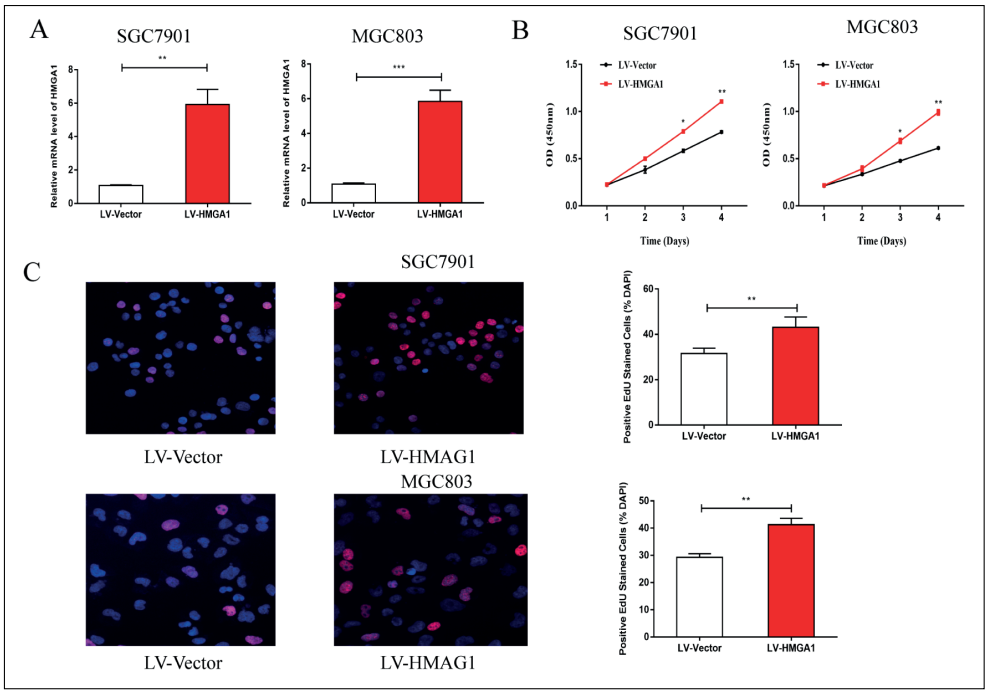

Figure 2. Overexpression of HMGA1 enhances the proliferation ability of GC cells. **A,** Transfection efficacy of LV-HMGA1 in SGC7901 and MGC803 cells. **B,** Viability of SGC7901 and MGC803 cells transfected with LV-Vector or LV-HMGA1. **C,** EdU-positive ratio in SGC7901 and MGC803 cells transfected with LV-Vector or LV-HMGA1 (magnification 20×).

and imbalance between oncogenes and tumor-suppressor genes are all risk factors for GC[14]. Proto-oncogenes, also known as cell oncogenes, are able to promote tumor growth through positively regulating tumor cell growth and differentiation[15]. Abnormal activation of proto-oncogenes leads to dysfunctional cell growth and malignant transformation of cells.

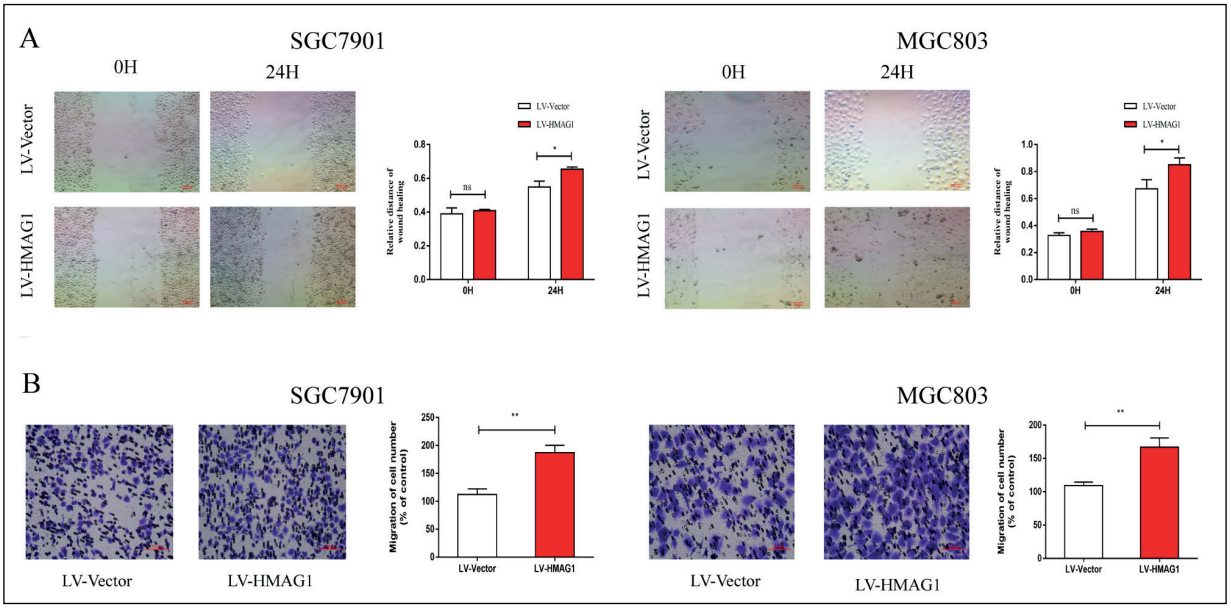

Figure 3. Overexpression of HMGA1 enhances the migration ability of GC cells. **A,** Relative distance of wound healing at 0 and 24 h in SGC7901 and MGC803 cells transfected with LV-Vector or LV-HMGA1. **B,** Migratory cell number in SGC7901 and MGC803 cells transfected with LV-Vector or LV-HMGA1 (magnification 20×).

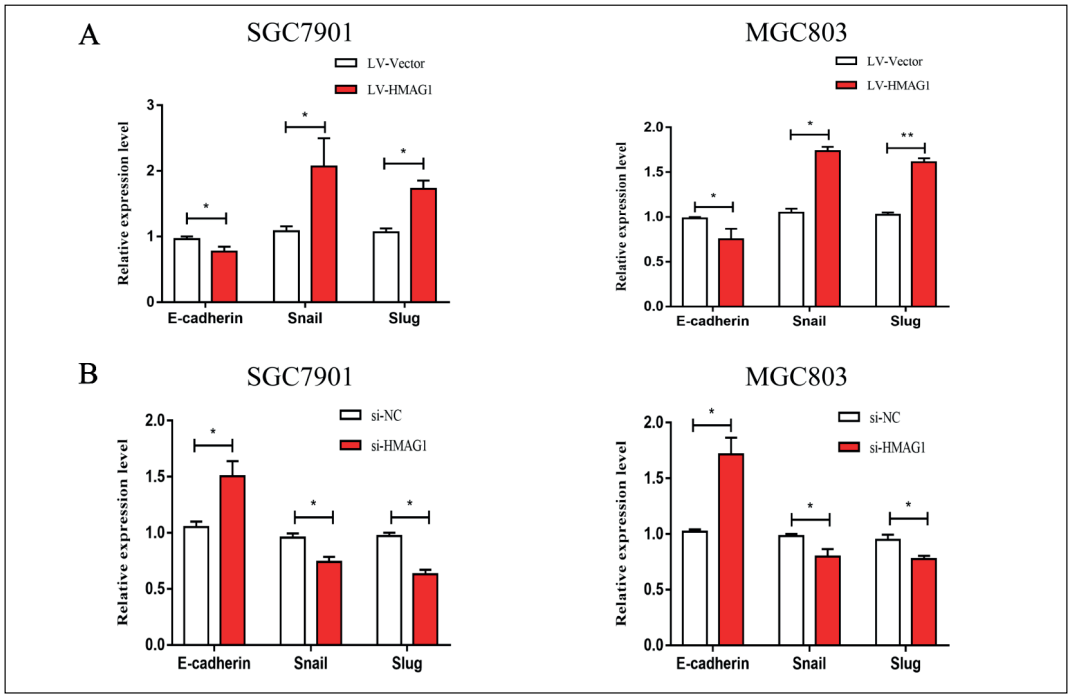

Figure 4. HMGA1 facilitates EMT in GC. **A,** Relative levels of E-cadherin, Snail and Slug in SGC7901 and MGC803 cells transfected with LV-Vector or LV-HMGA1. **B,** Relative levels of E-cadherin, Snail and Slug in SGC7901 and MGC803 cells transfected with si-NC or si-HMGA1.

At present, researches on proto-oncogenes in the pathogenesis of GC have been extensively conducted[16].

HMGA1 is located on human chromatin 6p21 and is of significance in regulating chromatin remodeling, gene transcription, and DNA replication. HMGA1 participates in the malignant phenotypes of tumor cells, which is upregulated in many types of malignant tumors and associated with metastatic rate[9,17]. With the organ development, HMGA1 level is gradually decreased in mature cells. Previous studies[7,18] have uncovered the role of HMGA1 in influencing proliferation and migration abilities of tumor cells, thus aggravating tumor diseases. In this study, HMGA1 was found to be upregulated in GC tissues relative to normal ones by analyzing TCGA STAD database. Consistently, *in vitro* level of HMGA1 was also upregulated in GC cells. Moreover, GC patients expressing high level of HMGA1 had shorter survival as online analysis revealed. Overexpression of HMGA1 remarkably enhanced proliferation and migration abilities of GC cells. The research findings supported that HMGA1 served as an oncogene participating in the progression of GC.

During the development, EMT is classified into three subtypes. Among them, type III EMT is closely linked to tumor recurrence and metastases[19]. Molecular changes of E-cadherin, N-cadherin, and vimentin are key events in EMT, which are regulated by transcription factors Twist, Snail, Slug, etc[20]. EMT results in cell morphology changes, including loss of polarity in epithelial cells, gain of metastatic properties to become mesenchymal stem cells, formation of mesoderm, and other behaviors[21]. Among EMT markers, E-cadherin is a calcium-dependent cell adhesion molecule found in the plasma membrane of most epithelial cells[22]. EMT could be affected by several pathways, including the Wnt, TGF-β and PI3K/AKT pathways[23]. The findings of this study illustrated that the overexpression of HMAG1 downregulated E-cadherin and upregulated Snail and Slug in GC cells. Silencing of HMAG1 yielded the opposite results, demonstrating the promotive effect of HMAG1 on EMT in GC.

Conclusions

Briefly, HMGA1 is upregulated in GC tissues and predicts poor prognosis, and it aggravates the progression of GC *via* stimulating EMT.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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