doi:10.4149/neo_2020_190719N651

MiR-638 regulates gastric cardia adenocarcinoma cell proliferation, apoptosis, migration and invasion by targeting MACC1

C. J. WEI[1,*], Z. W. ZHANG[2], J. H. LU[1], Y. M. MAO[1]

[1]*Department of Cardiothoracic Surgery, Suzhou Kowloon Hospital, Shanghai Jiaotong University Medical School, Suzhou, China;* [2]*Department of Thoracic Surgery, The First Affiliated Hospital of Soochow University, Suzhou, China*

**Correspondence: uzomeq@163.com*

Received July 19, 2019 / Accepted September 11, 2019

Gastric cardia adenocarcinoma (GCA) is one of the most common types of cancer and the incidence is increasing globally. MicroRNAs (miRNAs) have been reported to play critical roles in the progression of GCA. However, the exact role of miR-638 in GCA and its underlying mechanism remain largely unknown. The expression levels of miR-638 and metastasis-associated in colon cancer 1 (MACC1) were measured by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation, apoptosis, migration and invasion were detected by Cell Counting Kit-8 (CCK-8) assay, flow cytometry and transwell assay, respectively. Western blot analysis was performed to determine the protein levels of cleaved-caspase 3 (C-caspase 3) and MACC1. The possible binding sites of miR-638 and MACC1 were predicted by TargetScan online software and confirmed by dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay. A xenograft model was established to investigate the roles of MACC1 in GCA *in vivo*. The expression of miR-638 was evidently reduced and MACC1 expression was obviously enhanced in GCA tissues and cells. Overexpression of miR-638 or knockdown of MACC1 inhibited cell proliferation, migration and invasion but increased apoptosis in GCA cells. Moreover, MACC1 was a direct target of miR-638 and its upregulation attenuated the inhibitory effect of miR-638 overexpression on the progression of GCA. In addition, overexpression of miR-638 significantly decreased tumor growth by downregulating MACCI *in vivo*. In conclusion, miR-638 overexpression suppressed cell proliferation, migration and invasion but induced cell apoptosis by targeting MACC1 in GCA cells, providing a potential therapeutic strategy for the treatment of GCA.

Key words: gastric cardia adenocarcinoma, miR-638, MACC1, apoptosis, migration, invasion

Gastric cancer (GC) is the fourth most common malignancy worldwide and ranks as the second leading cause of cancer-related death [1]. Gastric cardia adenocarcinoma (GCA), a subtype of GC, is an adenocarcinoma that occurs in the transformation zone between the esophagus and the stomach [2]. In developed and developing countries, though the incidence and mortality rates in non-cardia gastric cancer steadily declined worldwide, the incidence rate for GCA continues to present an increase during the few last decades [3, 4]. Despite great advancements in diagnosis and treatment modalities for GCA, including surgery, chemotherapy, and radiotherapy, its survival rate is still very low [5]. Hence, understanding the molecular mechanisms for the development and progression of GCA is critical for developing more effective therapies.

MicroRNAs (miRNAs) are a class of endogenous small non-coding RNAs, which suppress gene expression through binding to the 3' untranslated regions (3'-UTR) of targets, resulting in messenger RNA (mRNA) degradation or translation repression [6]. Recently, miRNAs have been reported to play essential roles in tumorigenesis and progression, including GCA [7]. For example, miR-203a may have therapeutic potential and may be used as a prognostic marker for the patients with GCA [8]. In addition, miR-196a-5p was found to be associated with the age of GCA onset and miR-135b-5p expression was related to GCA survival [9]. As for miR-638, it has been found to be downregulated in several types of cancer, such as lung cancer [10], leukemia [11], and colorectal carcinoma [12]. Besides, it has been reported that the abundance of miR-638 was decreased in gastric cancer tissues and cells [13]. However, the biological function of miR-638 in GCA still needs further research.

MiRNAs combine with specific target genes thus playing essential biological functions in tumor progression [14]. Metastasis-associated in colon cancer 1 (MACC1) is an oncogene that was first revealed in colon cancer. The MACC1

gene is located on chromosome 7 at position 7p21.1 and has been reported to promote tumor proliferation and invasion mediated through HGF/MET signaling pathway signaling [15, 16]. Besides, Ma et al. showed that MACC1 level was notably increased in gastric cancer tissues compared with normal tissues, and its expression may be used as independent prognostic marker in gastric cancer. However, there is no evidence in support of the interaction between MACC1 and miR-638, and the biological functions and underlying mechanism of MACC1 in GCA have not been systematically studied.

In this study, we aimed to determine the expression of miR-638 and MACC1 in GCA tissues or cells, analyze the association between their expression and tumor progression, and to further investigate the regulatory network of miR-638/MACC1. In conclusion, this study may provide a potential therapeutic strategy for GCA treatment.

Materials and methods

Tissue samples. A total of 32 patients with GCA without chemotherapy, radiotherapy or other therapy were enrolled from Suzhou Kowloon Hospital affiliated to Shanghai Jiaotong University School of Medicine. The GCA tissues and adjacent normal samples were collected and immediately frozen in liquid nitrogen and then stored at –80 °C until the RNA and proteins were extracted. The research was approved by the Research Ethics Committee of Suzhou Kowloon Hospital affiliated to Shanghai Jiaotong University School of Medicine. Written informed consent was signed by all patients.

Cell culture and transfection. The human gastric adenocarcinoma cell lines (MKN45 and AGS) and normal gastric mucosal epithelial cell line (GES-1) were purchased from the Shanghai Institute of Cell Biology (Shanghai, China). All cell lines were maintained in Dulbecco's modified Eagle's medium ((DMEM; Hyclone, Logan, Utah, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO, USA) in an incubator with 5% CO_2 at 37 °C.

MiR-638 mimics (miR-638), negative control (NC), miR-638 inhibitor (anti-miR-638), anti-NC, small interfering RNA (siRNA) against MACC1 (si-MACC1), siRNA scrambled control (Scramble), MACC1 overexpression vector (MACC1), and empty vector were obtained from GenePharma (Shanghai, China). MKN45 and AGS cells were transfected with 50 nM oligonucleotides or 2 μg of the vector using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR). Total RNA was extracted from tissues or cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from total RNA using TaqMan microRNA Reverse Transcription Kit or TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Subsequently, qRT-PCR was conducted using SYBR green detection kit (Toyobo, Tokyo, Japan) on ABI 7500 real-time PCR system (Applied Biosystems) following the amplification instructions. The primers sequences were as follows: miR-638 (forward, 5'-ATCCAGTGCGTGTCGTG-3'; reverse, 5'-TGCTAGGGATCGCGGGCGGGTG-3'), U6 (forward, 5'-ATTGGAACGATACAGAGAAGATT-3'; reverse, 5'-GGAACGCTTCACGAATTTG-3'), MACC1 (forward, 5'-TGATTGACATGGAAGCTGGA-3'; reverse, 5'-GGATTTGCAACTTTGGAAGC-3'), GAPDH (forward, 5'-GACTCCACTCACGGCAAATTCA-3'; reverse, 5'-TCGCTCCTGGAAGATGGTGAT-3'). The relative expression levels of miR-638 and MACC1 were evaluated using $2^{-\Delta\Delta Ct}$ method. U6 and GAPDH mRNA levels were used for normalization.

Cell proliferation assay. Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) was used to detect cell proliferation. Briefly, MKN45 and AGS cells (2×10^4 cells/ml) were seeded in 96-well plates (100 μl/well) and transfected with miR-638, si-MACC1, miR-638+MACC1 or their matching controls. After transfection for 24, 48 and 72 h respectively, 10 μl of CCK-8 solution was added to each well. Then, the plates were incubated for another 2 h at 37 °C. The optical density (OD) of each well was measured at 450 nm using a microplate reader (Bio-Teck, Winooski, VT, USA).

Cell apoptosis assay. For the analysis of apoptosis, MKN45 and AGS cells were seeded in 6-well plates and transfected with miR-638, si-MACC1, miR-638+MACC1 or their matched controls. After transfection for 48 h, cells were collected, washed and stained with Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) (Sangon Biotech, Shanghai, China) for 15 min in the dark. The apoptotic cells were analyzed using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Western blot analysis. Total proteins were isolated from tissues and cells by radio-immunoprecipitation assay (RIPA) lysis buffer (Thermo Fisher Scientific, Wilmington, DE, USA) containing protease inhibitor cocktail (Sigma-Aldrich) for 30 min at 4 °C. Subsequently, centrifugation was carried out at 13 000×g for 10 min to collect the supernatant of the protein. Then total protein concentration was quantified by BCA Protein Assay Kit (Pierce, Rockford, IL, USA) and boiled for 10 min with 2× loading buffer (Beyotime, Shanghai, China). Equal amounts of proteins were separated by 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then electrotransferred to PVDF membrane (Millipore Corp, Atlanta, GA, USA). After blocking with 5% (w/v) non-fat milk in Tris-buffered saline and 0.1% Tween 20 (TBST) butter for 2 h, the membranes were incubated overnight at 4 °C with primary antibodies against MACC1 (1:500, ab106579, Abcam, Cambridge, UK), cleaved-caspase 3 (1:1000, ab2302, Abcam), or GAPDH (1:2500, ab9485, Abcam). After being washed the membranes 3 times with TBST, membranes were incubated by horseradish peroxidase (HRP)-conjugated secondary antibodies

(1:4000, Sangon Biotech) for 2 h at room temperature. Finally, the protein bands were examined by an enhanced chemiluminescence system (Thermo Fisher Scientific) and quantitated using ImageJ software.

Transwell assay. The migration and invasion of MKN45 and AGS cells were determined by two chamber transwell assay (Corning Incorporation, New York, NY, USA) with a pore size of 8 mm. For cell migration analysis, the transfected MKN45 and AGS cells were suspended in 100 µl serum-free medium and plated into the upper chamber, and then 600 µl complete DMEM medium was added into the lower chamber. Cells were incubated for 24 h with 5% CO_2 at 37 °C, cells on the top surface were carefully removed using a cotton swab. Adhering to the lower surface were fixed by 4% paraformaldehyde and stained with 0.1% crystal violet (Sigma-Aldrich). For invasion analysis, the upper layer of the polycarbonate membrane was pre-coated with Matrigel (BD Biosciences) and other methods were similar to the cell migration assay.

Dual-luciferase reporter assay. The putative binding sites of miR-638 and MACC1 were predicted by TargetScan software online. The MACC1-wt or MACC1-mut sequences containing putative binding sites of miR-638 were synthesized and inserted into pGL3 vector (Promega, Madison, WI, USA). The constructed pGL3 vectors were then co-transfected into MKN45 and AGS cells with miR-638 or NC using Lipofectamine 3000 (Invitrogen). Firefly luciferase activity was measured at 48 h post-transfection and normalized to the Renilla luciferase activity.

RNA Immunoprecipitation (RIP) assay. The relationship between miR-638 and MACC1 was measured using Magna RIP Kit (Millipore) following the manufacturer's protocol. Briefly, MNK45 and AGS cells transfected with the miR-638 or NC were collected and lysed in RNA immunoprecipitation lysis buffer containing magnetic beads, and then incubated with anti-argonaute 2 (anti-Ago2) or IgG antibodies. Subsequently, the samples were incubated through proteinase K buffer to digest protein, followed by RNA purification. Finally, the purified RNA was further used for the qRT-PCR analysis of the MACC1 level.

Tumor xenograft model. Five weeks old female BALB/c nude mice were maintained under specific pathogen-free conditions. The lentiviral vector pCDH-CMV (pLV, System Biosciences, SBI) was used to construct the pLV-miR-638 plasmid. Empty lentiviral vectors (named LV-NC) were used as controls. AGS cells transfected with LV-miR-638 or LV-NC were harvested, washed with PBS, and re-suspended in normal culture medium at a concentration of 1×10^7 cells/ml. Then, stably transfected cells were transplanted into either side of the posterior flank of the nude mouse (six per group, 1×10^6 cells for each mouse). All animal experiments were performed in accordance to guide for the care and use of laboratory animals as the standard. The xenograft mice experiment was approved by the committee of Animal Research of Suzhou Kowloon Hospital affiliated to Shanghai Jiaotong University School of Medicine. Tumor volume sizes in every group were examined every 5 d for six times with slide calipers and calculated as follows: volume = L (Length) × W (Width)^2 /2. Thirty days after cell implantation, all mice were sacrificed and tumor specimens were weighted and collected for measurement of miR-638 and MACC1 abundances.

Statistical analysis. Data were presented as the mean ± standard deviation (SD) from at least three independent experiments. Statistical analysis was performed using GraphPad Prism software ver. 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). The statistical differences between groups were analyzed using Student's t-test. Spearman rank correlation was employed to detect the correlations between miR-638 and MACC1. A $p<0.05$ was regarded as statistically significant.

Results

The level of miR-638 was downregulated in GCA tissues and cells. qRT-PCR was used to evaluate the expression level of miR-638 in GCA tissues and cells. As displayed in Figure 1A, the level of miR-638 was evidently decreased in GCA tissues (n=32) compared with normal gastric tissues

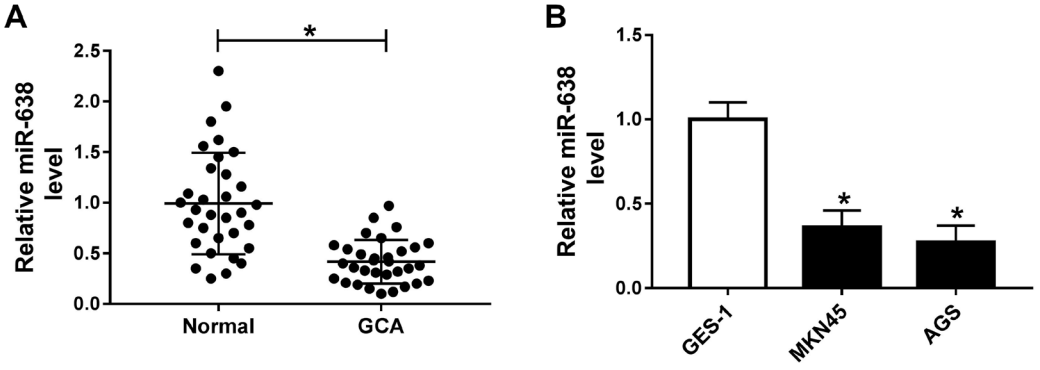

Figure 1. Expression levels of miR-638 in GCA tissues and cells. A) The expression levels of miR-638 in GCA tissues and adjacent normal samples were determined by qRT-PCR. B) The abundance of miR-638 was detected in GCA cells and AGS cells by qRT-PCR, *p<0.05.

(n=32). Similarly, the miR-638 expression was also reduced in MKN45 and AGS cells compared with that in GES-1 cells (Figure 1B). These results indicated that miR-638 might be associated with the progression and development of GCA.

Overexpression of miR-638 inhibited cell proliferation, migration and invasion, but induced apoptosis in GCA cells. To further investigate the function of miR-638 on GCA progression, miR-638 or NC was transfected into MKN45 and AGS cells. As a result, the transfection of miR-638 significantly elevated the level of miR-638 in MKN45 and AGS cells compared with the transfection of NC. CCK-8 analysis displayed that the upregulation of miR-638 prominently restrained the proliferation of MKN45 and AGS cells (Figures 2A and 2B). However, the apoptotic rate was remarkably increased in MKN45 and AGS cells transfected with miR-638 compared with cells transfected with NC group (Figure 2C). Besides, overexpression of miR-638 led to an obvious increase of C-caspase 3 protein level in MKN45 and AGS cells (Figure 2D). In addition, the accumulation of miR-638 resulted in great loss of abilities of migration and invasion in MKN45 and AGS cells (Figures 2E and 2F). Thus, these data demonstrated that the upregulation of miR-638 suppressed GCA cell progression.

The level of MACC1 was increased in GCA tissues and cells, and its inhibition had similar effects with overexpression of miR-638 on GCA cells. To investigate the functional effects of MACC1 in GCA, si-MACC1 or scramble were transfected into MKN45 and AGS cells. Analysis of qRT-PCR showed that the MACC1 expression was markedly higher in ATC tissues or cells than their corresponding control, respectively (Figures 3A and 3B). Similarly, the protein level of MACC1 was also enhanced

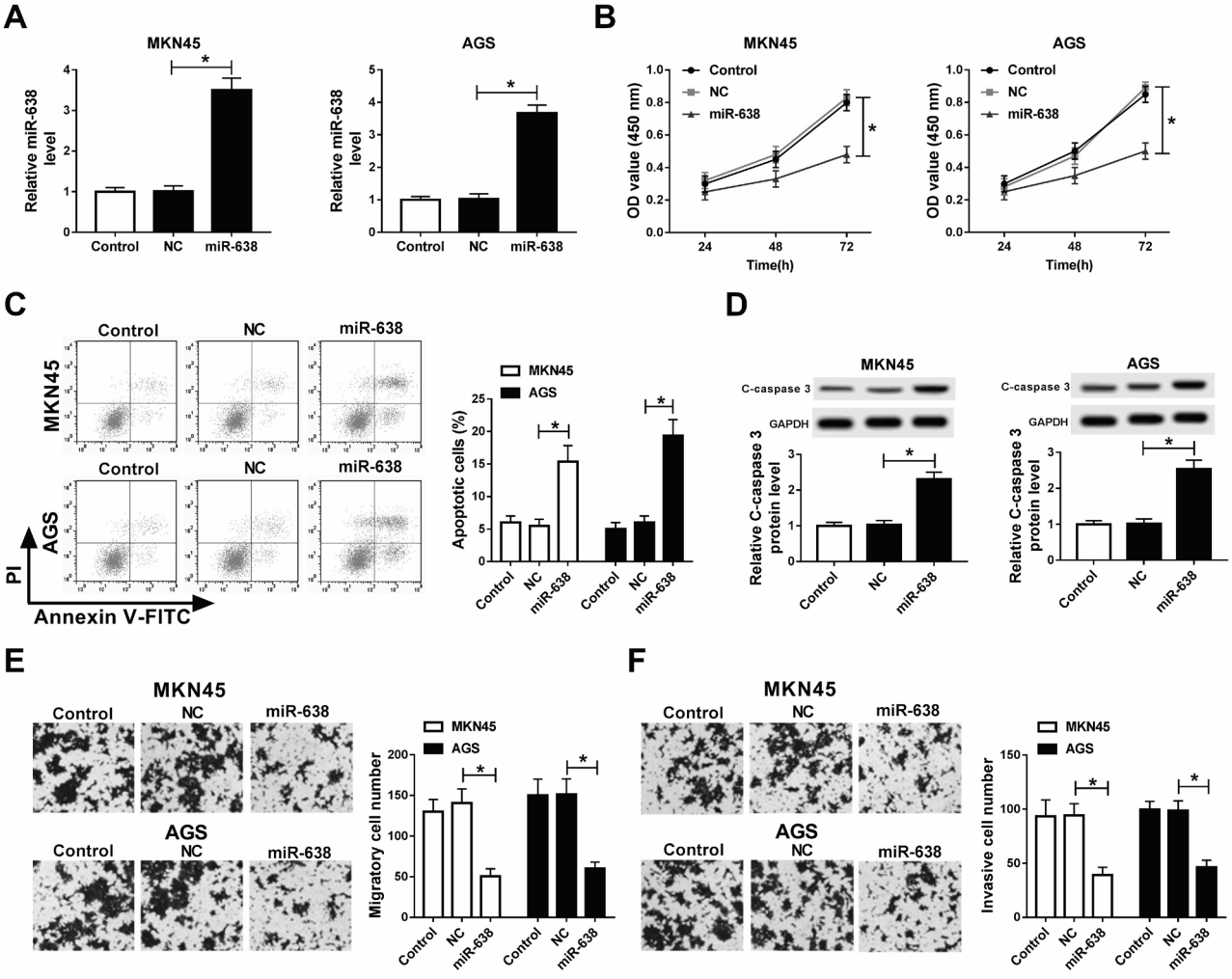

Figure 2. Effects of miR-638 on cell proliferation, migration and invasion, and apoptosis in GCA cells. A-F) MKN45 and AGS cells were transfected with miR-638 or NC. A) The miR-638 level was analyzed by qRT-PCR. B) Cell proliferation was determined by the CCK-8 assay. C) Cell apoptosis was detected by flow cytometry. D) Western blot analysis was performed to detect the protein expression of C-caspase 3. E and F) The number of migrated and invaded cells were determined using transwell assay. *p<0.05.

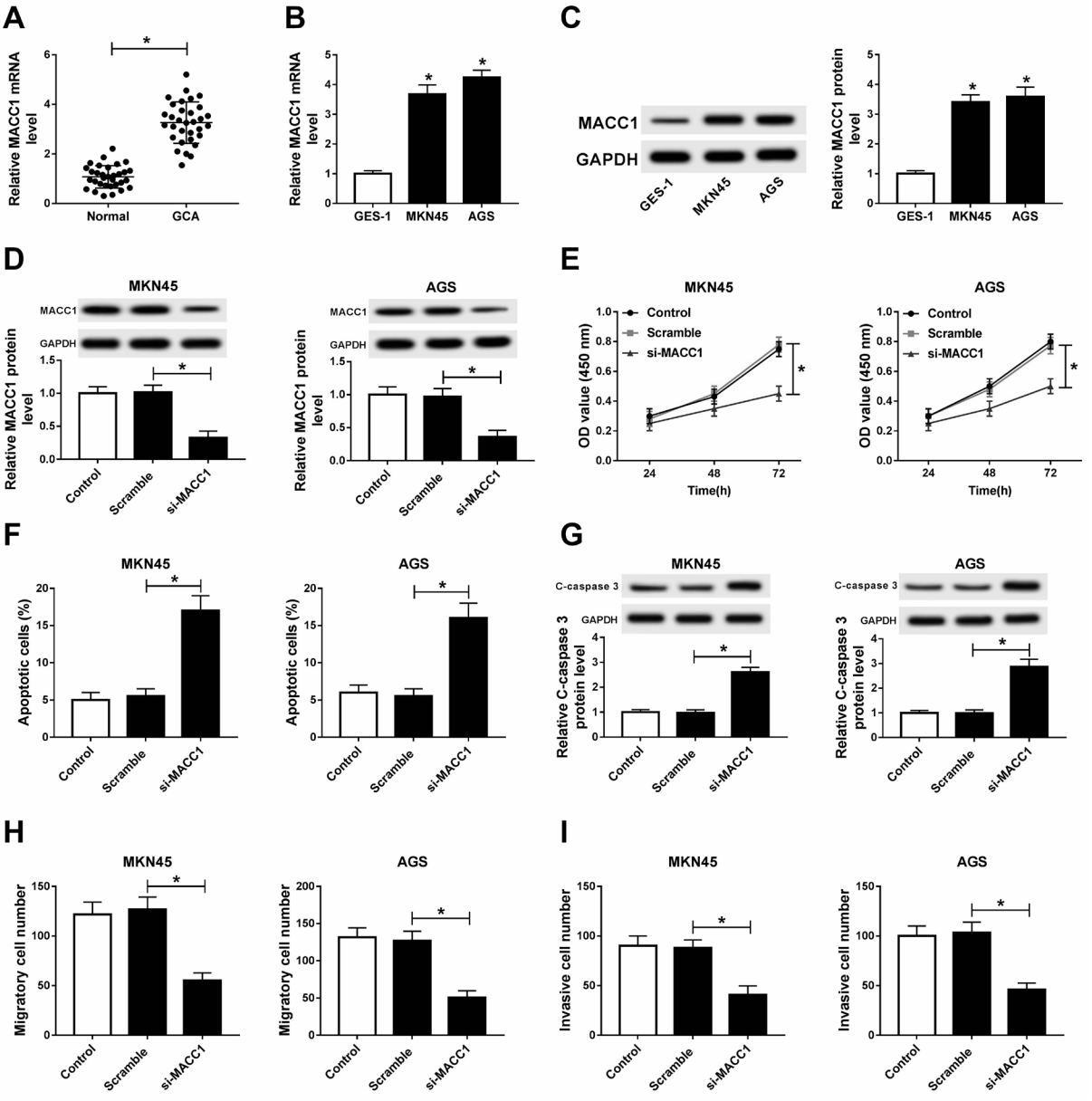

Figure 3. Effects of MACC1 on cell proliferation, migration, and invasion, and apoptosis in GCA cells. A) The MACC1 mRNA levels in GCA tissues and adjacent normal samples were determined by qRT-PCR. B and C) The mRNA and protein level of MACC1 were detected in GCA cells and AGS cells by qRT-PCR and western blot, respectively. D–I) MKN45 and AGS cells were transfected with si-MACC1 or scramble. D) The protein level of MACC1 was analyzed by western blot. E) Cell proliferation was determined by CCK-8 assay. F) Cell apoptosis was detected by flow cytometry. G) Western blot analysis was performed to detect the protein expression of C-caspase 3. H and I) The number of migrated and invaded cells were determined by transwell assay. *p<0.05.

in MKN45 and AGS cells (Figure 3C). The abundance of MACC1 was effectively decreased in MKN45 and AGS cells transfected with si-MACC1 compared with transfected with scramble (Figure 3D). CCK-8 analysis displayed that inhibition of MACC1 apparently limited proliferation of MKN45 and AGS cells (Figure 3E). Nevertheless, the knockdown of MACC1 markedly promoted the apoptosis rate in MKN45 and AGS cells (Figure 3F). Moreover, C-caspase 3 protein level was conspicuously upregulated in MKN45 and AGS cells transfected with si-MACC1 (Figure 3G). Transwell assay indicated that the abrogation of MACC1 strongly reduced the number of migrated and invaded cells in MKN45 and AGS cells (Figures 3H and 3I). Taken together, these above findings suggested that knockdown of MACC1 inhibited GCA cell progression.

MACC1 was a direct target of miR-638 in GCA cells. To explore the interaction between miR-638 and MACC1 in GCA cells, the potential binding sites of miR-638 and MACC1

were predicted by the TargetScan online website, suggesting the potential binding sites between miR-638 and MACC1 (Figure 4A). To validate that MACC1 was a target of miR-638 and regulated by it in GCA cells, the MACC1 3'-UTR was cloned and inserted into a luciferase reporter vector. Results showed that the addition of miR-638 dramatically suppressed the luciferase activity of MACC1-wt, but the luciferase activity of MACC1-mut was unaffected after the transfection with miR-638 in MKN45 and AGS cells (Figure 4B). RIP assay was preformed to further confirm the interaction between miR-638 and MACC1 in GCA cells. Results proved that the enrichment of MACC1 was notably enhanced in the miR-638 group coated with the Ago2 antibody compared to the control group (Figure 4C). In addition, the overexpression of miR-638 apparently downregulated the protein level of MACC1 and its knockdown showed an opposite effect in MKN45 and AGS cells (Figure 4D). Besides, the correlation between miR-638 and MACC1 was analyzed in GCA tissues. Results suggested that the MACC1 expression was negatively correlated with miR-638 abundance in GCA tissues ($p<0.001$, $R^2 = 0.4343$, Figure 4E). Thus, these data demonstrated that miR-638 directly targeted MACC1 in GCA cells.

Overexpression of MACC1 reversed the effects of miR-638 upregulation on GCA cell proliferation, apoptosis, migration, and invasion. To further explore the relationship between miR-638 and MACC1 in GCA cells, miR-638, NC, miR-638+vector, or miR-638+MACC1 were transfected into MKN45 and AGS cells. Western blot analysis displayed that the overexpression of miR-638 suppressed the MACC1 protein level, which was abated by the addition of MACC1 (Figure 5A). CCK8 analysis demonstrated that the upregulation of MACC1 attenuated the anti-proliferative effect of the miR-638 accumulation on MKN45 and AGS cells (Figure 5B). In addition, the overexpression of MACC1 inhibited the apoptotic-promoting effect of miR-638 upregulation on MKN45 and AGS cells (Figure 5C). Likewise, the addition of miR-638 reversed the miR-638 overexpression-mediated promotion of C-caspase 3 expressions in MKN45 and AGS cells (Figure 5D). Besides, the overexpression of MACC1 overturned the inhibitory effect of miR-638 accumulation on migration and invasion of MKN45 and AGS cells (Figures 5E and 5F). These results indicated that MACC1 could reverse the miR-638-mediated progression of GCA.

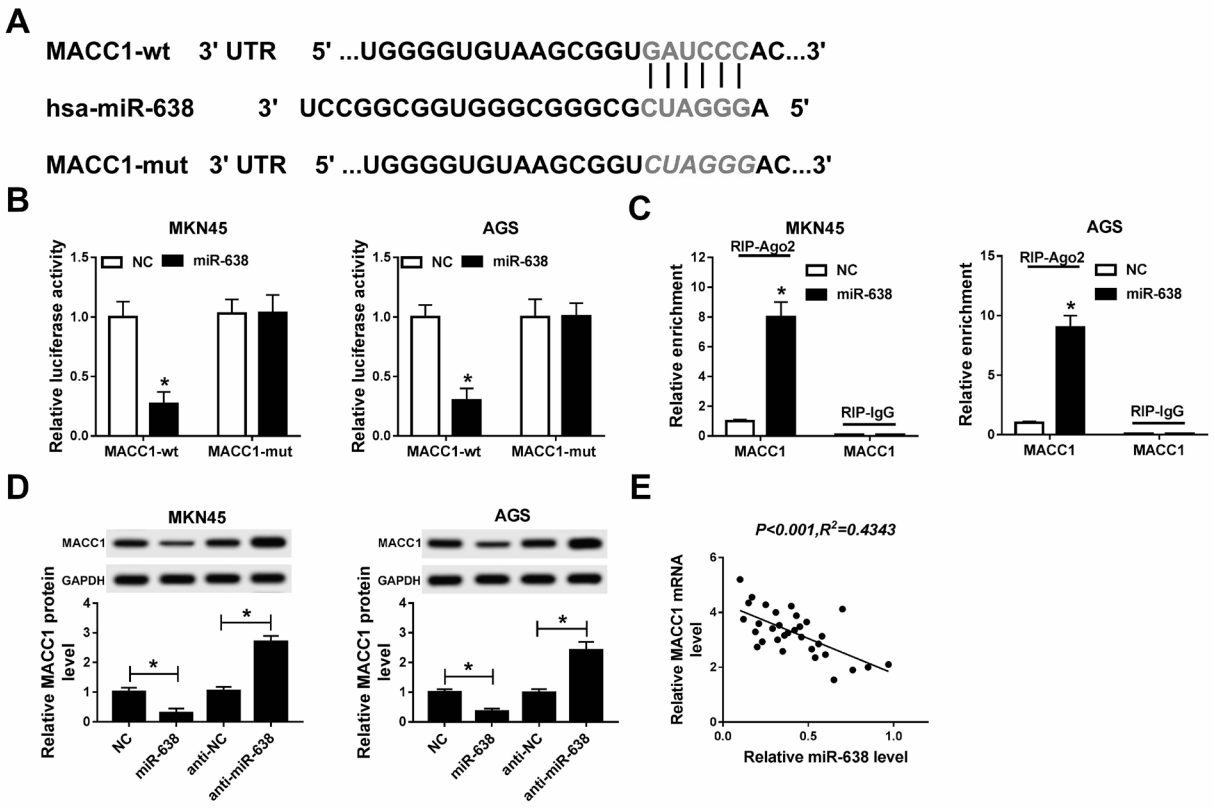

Figure 4. miR-638 directly targeted MACC1 in GCA cells. A) The putative binding sites of miR-638 and MACC1 were provided by TargetScan. B) Luciferase activity was measured in MKN45 and AGS cells co-transfected with MACC1-wt or MACC1-mut and miR-638 or NC. C) RIP assay was performed to detect MACC1 enrichment levels in MKN45 and AGS cells transfected with miR-638 or NC. D) The abundance of MACC1 protein was measured in MKN45 and AGS cells transfected with miR-638, NC, anti-miR-638, or anti-NC by western blot. E) The correlation between the MACC1 mRNA level and the miR-638 level was analyzed in GCA tissues. *p<0.05.

Upregulation of miR-638 decreased the xenograft tumor growth *in vivo*. To explore the effect of miR-638 on ATC *in vivo*, LV-miR-638 or LV-NC-transfected AGS cells were introduced into nude mice. The tumor volume was measured once every 5 days, and tumors weight and volume were gauged 30 days after injection. Overexpression of miR-638 strongly inhibited tumor volume and weight in the xenograft model (Figures 6A and 6B). Moreover, the expression of miR-638 was drastically elevated in tumor tissues (Figure 6C). Besides, the MACC1 protein abundance was greatly decreased in the miR-638 group compared with the NC group (Figure 6D). Collectively, these findings disclosed that the overexpression of miR-638 inhibited the xenograft tumor growth by downregulating MACC1 *in vivo*.

Discussion

GCA is one of the most common malignant types of cancer and the incidence is increasing globally [17, 18]. Many treatments have been used in GCA therapy, while effective and specific strategies are lacked. Aberrant expressions of miRNAs have been identified to be associated with tumor occurrence, progression, and metastasis [19, 20]. Therefore, it is of great significance to understand more about the underlying molecular mechanisms of miRNAs for the treatment of GCA.

Mounting evidence has shown that miRNAs are a class of gene expression-regulating molecules that are closely associated with cancer development and progression [21]. MiR-638 has been suggested to be dysregulated in many cancers and participate in multiple cell behaviors, including proliferation, apoptosis, autophagy, epithelial-mesenchymal transition (EMT), and invasion [10, 22]. For instance, Zhang et al. reported that miR-638 inhibited cell proliferation, invasion and regulated cell cycle via targeting tetraspanin 1 in human colorectal carcinoma [23]. Zhang et al. proved that the downregulation of miR-638 elevated invasion and EMT by targeting SOX2 in hepatocellular carcinoma [24]. Besides, the previous study manifested that miR-638 suppressed cell proliferation by targeting phospholipase D1 in human

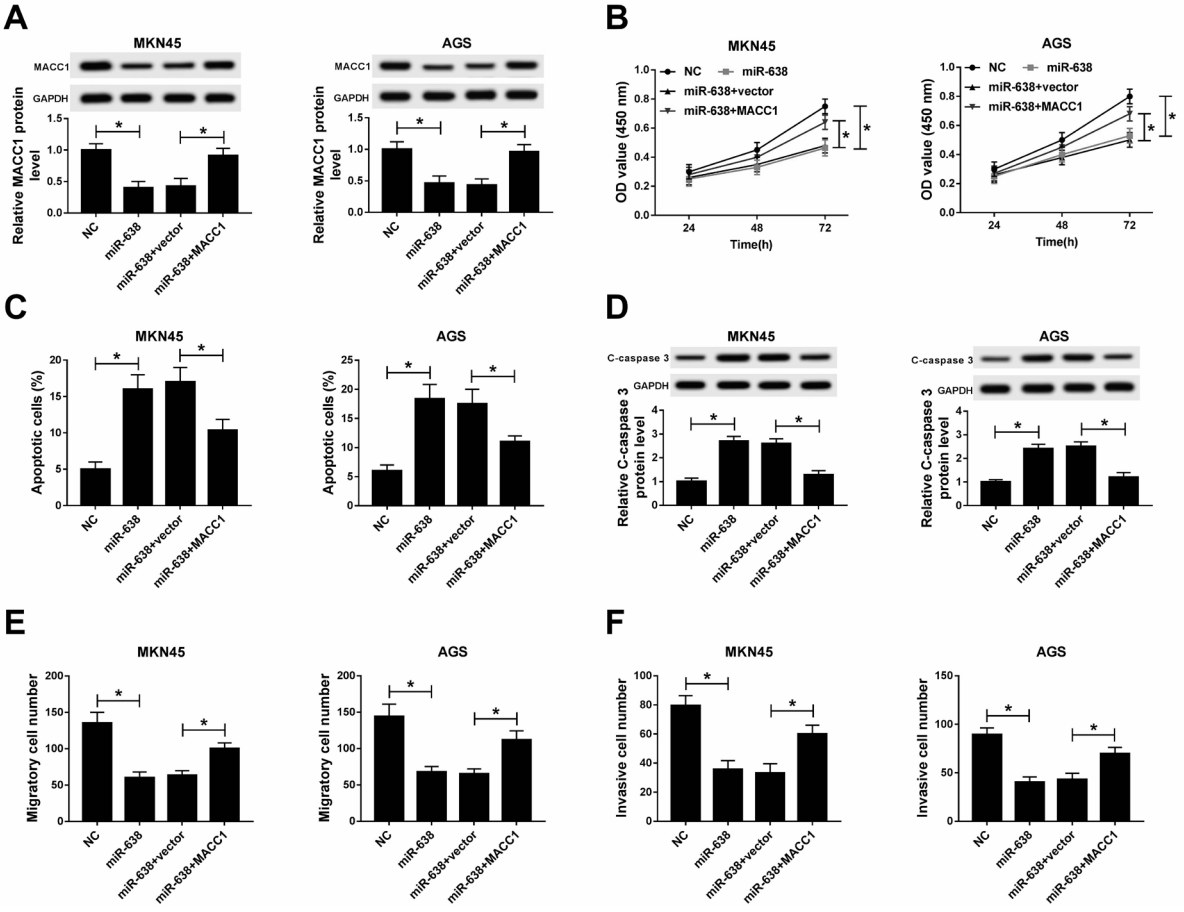

Figure 5. Overexpression of MCM6 reversed the effect of miR-638 upregulation on the progression of GCA. A–F) MKN45 and AGS cells were transfected with miR-638, NC, miR-638+MACC1, or miR-638+vector. A) The protein level of MACC1 was analyzed by western blot. B) Cell proliferation was determined by CCK-8 assay. C) Cell apoptosis was detected by flow cytometry. D) Western blot analysis was performed to detect the protein expression of C-caspase 3. E and F) The number of migrated and invaded cells were determined using transwell assay. *p<0.05.

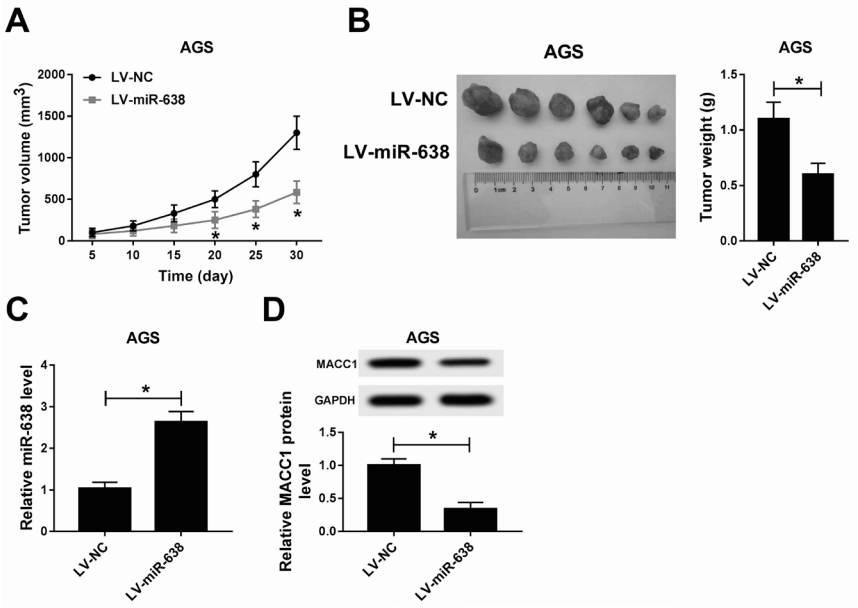

Figure 6. The miR-638 upregulation suppressed tumor growth in the xenograft model. A and B) Tumor volume and weight were measured in the xenograft model. C) The expression of miR-638 was measured in tumor tissues by qRT-PCR. D) The abundance of MACC1 protein was detected in tumor tissues by western blot. *p<0.05.

gastric carcinoma. Additionally, Zhao et al. demonstrated that miR-638 inhibited gastric cancer cell proliferation via targeting Sp2 [25]. However, the roles of miR-638 in GCA remain largely unknown. The aim of this work was to elucidate the more biological functions of miR-638 in GCA. Here, we found that the expression level of miR-638 was prominently downregulated in GCA tissues and cells, which was in line with a previous study. In addition, the overexpression of miR-638 prominently restrained cell proliferation in MKN45 and AGS cells. Moreover, we provided the first evidence that the upregulation of miR-638 remarkably promoted cell apoptosis and inhibited migration and invasion in GCA cells. Besides, we demonstrated that the transfection of miR-638 evidently suppressed tumor growth *in vivo*. Thus, these findings indicated that miR-638 could suppress GCA cell proliferation, migration and invasion, and tumor growth (*in vivo*), but induce apoptosis.

The emerging evidence has already confirmed that most miRNAs can exert their biological functions by binding to their downstream target genes [26]. To investigate, whether MACC1 acts as a sponge of miR-638, the bioinformatics analysis was carried out and provided the putative binding sites of MACC1 and miR-638. Subsequently, it was confirmed that MACC1 was a direct target of miR-638. MACC1 has been found to participate in cell proliferation, invasion, and metastasis in a variety of cancers [27, 28]. Cao et al. showed that the inhibition of MACC1 inhibited hepatocellular carcinoma cell migration, and invasion and limited expression of MMP2 and MMP9 [29]. Sun et al. demonstrated that the silencing of MACC1 expression suppressed proliferation, invasion, and metastasis, and induced apoptosis in U251 human malignant glioma cells [30]. In terms of GCA, the previous research proved that the MACC1 expression was tightly associated with gastric cancer stage and degree of malignancy. Besides, Li et al. further demonstrated that miR-141 inhibited proliferation of GCA by targeting MACC1. Nevertheless, the effects of MACC1 on apoptosis, migration, and invasion of GCA cells have not been reported. Consistent with previous studies, we found that the mRNA and protein levels of MACC1 were notably increased in GCA tissues and cells. Additionally, the inhibition of MACC1 suppressed proliferation, migration, and invasion, but induced apoptosis of GCA cells. Moreover, the overexpression of MACC1 could reverse the miR-638 upregulation-mediated inhibitory effect on the progression of GCA. Taken together, these results suggested that miR-638 exerts its biological functions by directly binding to MACC1 in GCA.

In conclusion, the miR-638 expression was downregulated and the MACC1 level was upregulated in GCA tissues and cells. Overexpression of miR-638 limited cell proliferation, migration, and invasion, but increased apoptosis in GCA cells, which was reversed by the inhibition of MACC1. Moreover, MACC1 knockdown had similar effects with the overexpression of miR-638 in GCA cells. In addition, this study first proved that MACC1 was a direct target of miR-638 in GCA cells. Besides, upregulation of miR-638 suppressed tumor growth *in vivo*. Collectively, miR-638 inhibited proliferation, migration, and invasion, but increased apoptosis via targeting MACC1 in GCA cells, are providing a promising avenue for the treatment of GCA.

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