miR-335-5p Suppresses Gastric Cancer Progression by Targeting MAPK10

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Abstract

Background: In recent years, many microRNAs(miRNAs) involved in cancer progression. The aberrant expression of miR-335-5p in tumorigenesis has been demonstrated. The present study aimed to investigate the molecular mechanisms underlying miR-335-5p- regulated MAPK10 expression in human gastric cancer（GC）.

Methods: The quantitative real-time PCR was used to study the level of miR-335-5p expression in gastric cancer cell lines and tissues. Subsequently, the MTT and cloning formation assays were used to detect cell proliferation, while transwell and wound-healing assays were used to identify invasion and migration of the gastric cancer cells. The correlation between the miR-335-5p and the cell cycle-related target gene mitogen-activated protein kinase 10 (MAPK10) in gastric cancer was analyzed based on the website. In addition, the target gene of miR-335-5p was detected by luciferase reporter assay, qRT-PCR, and western blotting.

Results: The miR-335-5p level was down-regulated in GC tissues and cell lines. Furthermore, miR-335-5p inhibited proliferation, migration of gastric cancer cells, and induced apoptosis. During the G1/S phase, miR-335-5p arrested the cycle of gastric cancer cells in vitro. The correlation between the miR-335-5p and the cell cycle-related target gene MAPK10 in GC was analyzed, MAPK10 was directly targeted by the miR-335-5p.

Conclusion: These data suggested that miR-335-5p acts as a tumor suppressor, and go through the MAPK10 to inhibit the GC progression.

Background

It is widely known that gastric cancer(GC)is still a significant public health problem worldwide and has been endangering human health.[1, 2] GC remains threatening cancer worldwide and is responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths, making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death. [3]Such factors as lifestyle, Helicobacter pylori (HP) infection, polyps, gastric ulcers, genetic diseases and gastric residual tissue may be involved in gastric tumorigenesis. [4] Although there are many methods for the diagnosis and treatment of GC, 30% of patients are still diagnosed with advanced GC .[5]Thus, useful biomarkers for early screening or detection of GC are essential for improving patients' survival rates.[6] Recently, MicroRNAs (miRNAs) have been found to play an important role in tumorigenesis.[7–9]They contribute to gastric carcinogenesis by altering the expression of oncogenes and tumor suppressors.[10]

MicroRNAs (miRNAs) are small, non-protein-coding RNAs that regulate gene expression at the post-transcriptional level. The maturation of miRNA consists of several regulatory steps, and finally a single strand of 22 nucleotides mature miRNA is formed. [11] miRNAs can behave as oncogenes or tumor suppressors in various tumors, such as GC. For example, miR-181d[12], miR-99a[13], miR-105[14], and so forth, had suppressive effects on GC development, whereas other miRNAs, including miR-188-5p [15]and

miR – 221[16], promoted GC growth. MiR-335-5p, as a number of miRNA family, is abnormally expressed in many cancers. For instance, miR-335-5p expression was significantly down-regulated and acted as a vital player in the metastasis of non-small cell lung cancer (NSCLC).[17] miR-335-5p was decreased in breast cancer cells and might be a promising biomarker for breast cancer treatment.[18] Meanwhile, miR-335-5p was down-expressed in renal cell carcinoma and may be used as a novel therapeutic target for renal cell carcinoma.[19] In the GC research, miR-335 downregulated had been found[20], however, the role of miR-335-5p in GC cells has not been fully understood.

MiRNAs can bind to the 3'UTR of the target mRNAs in a complementary base-pairing manner, which has been demonstrated to contribute to cell apoptosis, proliferation, and differentiation.[21–24]Until now, miRNA is thought to regulate more than 50% of protein coding genes. Based on the literature reviews and gene target prediction databases, including TargetScan, miRanda, and miRBASE, we hypothesized that mitogen-activated protein kinase 10 (MAPK10) might be a potential target of miR-335-5p. MAPK10 is a member of the Jun N-terminal kinase subgroup of mitogen-activated protein kinases. MAP kinases acted as integration points for multiple biochemical signals and are involved in a wide variety of cellular processes, such as proliferation, differentiation, transcription regulation and development.[25, 26]The expression of mapk10 was different in different tumors. Lu Zhang et al showed that MAPK10 was poorly expressed in Cervical Cancer tissues and cells.[27] Mapk10 in breast cancer tissues was significantly lower than that in adjacent tissues, which reduced the proliferation, migration, invasion and metastasis of breast cancer cells, and increased apoptosis .[28]However, the percentage of MAPK10 protein-positive cases was significantly higher in ovarian serous, mucinous, and clear cell carcinomas than in normal tissues [29]Hence, this study was designed with the aim of verifying whether miR-335-5p could influence GC progression by targeting MAPK10.

In the present study, we observed that the expression of miR-335 is lower in GC tissues and GC cells than in matched normal tissues and the MKN-28 and SGC-7901 cell line, indicating its role as a tumor suppressor in GC. In addition, using bioinformatics, the luciferase assays qRT-PCR and Western blot, the result showed that the expression of MAPK10 was upregulated in GC and directly targeted by miR-335-5p. Meanwhile, the function of miR-335 and MAPK10 in GC cell progression was investigated. Overexpression of miR-335 and MAPK10 silencing suppressed the proliferation, metastasis and promoted apoptosis of GCs. These results demonstrated that miR-335 suppressed the progression of GCs by targeting MAPK10.

Materials And Methods

Cell Lines and Cell Culture

Human GC cell lines GES-1、AGS、BGC-823、 MKN-45、MKN-28 and SGC-7901, and the HEK-293 model cells, were provided by the Biomedical Experiment Center of Xi’an Jiaotong University (China). The use of these cell lines was approved by Ethics Committee of Yan’an University College of Medicine(China). The human GC cells were cultured in the DMEM medium (PAA Laboratories, Pasching, Australia) containing

10% fetal bovine serum (FBS) and the 1640 medium (PAA Laboratories), in a 37°C, 5%CO2 incubator. The culture medium was changed once every 2–3 d. The MKN-28 and SGC-7901 cells in the logarithmic growth phase were collected and subjected to the following experiments.

Cell Transfection

The GC cells in the logarithmic growth was digested and inoculated onto the 6-well culture plate. When 60–80% confluence was reached, the desired transfected fragments (miR-335-5p -mimics and miR-335-5p -inhibitor were purchased from GenePharma, Shanghai, China) were mixed and added into the corresponding wells, for further culture for 24–48 h.

Quantitative Real-Time PCR

RNA was extracted from GC cells using Trizol. The cDNA was obtained with the reverse-transcription using the commercially available kits, according to the manufacturer's instructions. Quantitative real-time PCR was performed with the PrimeScript^{TM} RT Reagent kit (Takara Bio, Japan) on the iQ5 Optical real-time PCR System machine (Bio-Rad, USA). The following primer sequences were used for amplification: RT miR-335-5p, 5′-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACacatttt-3′; RT U6 5′-CGCTTCACGAATTTGCGTGTCAT-3′; miR-335-5p, forward 5′-ATCCAGTGCGTGTCGTG-3′ and reverse 5′-TGCTTCAAGAGCAATAACGA-3′; U6, forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTCACGAATTTGCGTGTCAT-3′; MAPK10 forward 5′-TTCTCAGGCACGGAATGG -3′ and reverse 5′-TAAGTTGCCATAGTGAAGATCTGAG -3′; and glyceraldehyde-3-phosphate dehydrogenase(GAPDH), forward 5′-TGAAGGTCGGAGTCAACGGATT-3′ and reverse 5′-CCTGGAAGATGGTGATGGGATT-3′. The 20-μL PCR system consisted of 10-μL 2×RealStar Green Power Mixture, 1-μL Forward-primer (10 μM), 1-μL Reverse-primer (10 μM), 2-μL cDNA and 6-μL ddH2O. The amplification conditions were as follows: 95°C for 10 min; 95°C for 15 s, 60°C for 1 min, and 72°C for 30 s, for totally 40 cycles. Relative expression levels of the target genes were calculated with the $2^{-\Delta Ct}$ method. GAPDH was used as internal reference.

Western Blot Analysis

Cells were harvested and lysed with lysis. Total protein concentration was determined with the BCA method. The protein samples were separated with 7.5–12.5% SDS-PAGE, and electronically transferred onto the polyvinylidene fluoride (PVDF) membrane. The membrane was incubated with anti-β-actin, anti-MAPK10 primary antibody (Sanying Biological Co., Ltd., Wuhan, Hubei, China), at 4°C overnight. The membrane was then incubated with tris buffer solution with tween (TBST) -conjugated secondary antibody (Sanying Biological Co., Ltd.) at room temperature for 1 h. Color development was performed with the chemiluminescence detection method, and protein bands were imaged and analyzed with the Q550CW software (Leica, Heidelberg, Germany). β-actin was used as internal reference.

MTT Assay

Cell proliferation was assessed with the MTT kit (Sigma, St Louis, MO, USA). The cells in the logarithmic growth phase were harvested and seeded onto the 96-well plate. At 24 h, 48 h, and 72 h after seeding, respectively, 10 μL MTT was added into each well to incubate the cells for 4 h. The well was added with 150 μL DMSO, and t optical density (OD) was recorded at 490 nm.

Cloning Formation Detection

The transfected cells in the logarithmic growth phase were seeded onto the 6-well plate. After 2 w of culture, the cells were fixed with 4% paraformaldehyde, and stained with crystal violet. The stained cells were then observed, photographed, and counted.

Flow Cytometry

The transfected cells in the logarithmic growth phase were inoculated onto the 6-well plate, and cultured for 1 d. Cells were fixed in 70% ethanol for 24 h, which were then treated with PI and RNase within the kit. Cell cycle distribution was detected by flow cytometry.

Dual Luciferase Reporter Assay

The HEK-293 cells were divided into the miR-30a-3p and pmirGLO empty vector, miR-335-5p and pmirGLO-MAPK10-WT (GenePharma), miR-335-5p and pmirGLO- MAPK10-MuT (GenePharma) co-transfection groups, respectively. Cells without treatment were used as control. The MAPK10 wild-type and mutant fragments were synthesized by Genechem, Shanghai, China, as follows: wild-type MAPK10, up 5′-cATTTAACTTCTAGTTGCTCTTGCc-3′ and down 5′-tcgagGCAAGAGCAACTAGAAGTTAAATgagct-3′; and mutant MAPK10, up 5′-cATTTAACTTCTAGTTGATATCGCc-3′ and down 5′-tcgagGCGATATCAACTAGAAGTTAAATgagct-3′. These cells were inoculated onto the 96-well plates, and cultured for 24 h. The luciferase activity was detected by the microplate reader. *Renilla* was used as internal reference.

Cell invasion assay

The Transwell chambers (8-μm pore size; Millipore, Billerica,MA, USA) were coated with Matrigel (15 μg/filter; BD Biosciences,Franklin Lakes, NJ, USA). Cells (2.0×104) in serum-free medium were plated into the upper chamber, and the bottom wells were filled with complete medium. The cells were allowed to invade across the Matrigel-coated membrane for 48 h.

Wound-healing assay

Awound-healing assay was performed to examine the capacity for cell metastasis. Briefly, once the cells had grown to 90 % confluence in 12-well plates, a single scratch wound was generated with a 200-μl disposable pipette tip. The extent of wound closure was measured 48 h after wounding.

Statistical Analysis

The SPSS22.0 software was used for statistical analysis. Bioinformatics analysis was performed by the R language ggstatsplot package. Experimental data were processed by the GraphPad Prism7.0 software. Comparison was conducted with the Independent *t*-test. $P<0.05$ was considered as statistically significant.

Results

miR-335-5p inhibits cell proliferation of GC in vitro

To investigate the role and function of miR-335-5p in GC cells, we analyzed the expression of miR-335-5p in 22 pairs of GC tissues and matched adjacent non-cancerous tissue samples using qRT-PCR. miR-335-5p was significantly downregulated in GC samples (Figure 1A) ,compared to normal tissues. This result was also validated in four GC cell lines. miR-335-5p expression was decreased in BGC-823,SGC-7901, MKN-45,MKN-28, and AGS cell lines compared to the GES-1 cell line (Figure 1B). To clarify the function of miR-335-5p in GCs, MKN-28 and SGC-7901 cells were selected for further analyses. qRT-PCR results confirmed that the miR-335-5p mimics successfully elevated miR-335-5p expression in two of the cell lines; the inhibitory effect was moderate due to the low expression of endogenous miR-335-5p in MKN-28 and SGC-7901 cells (Figure 2C). Thus , the miR-335-5p may act as a tumor suppressor in the GCs.

miR-335-5p arrests cell cycling and induces apoptosis in GC

To explore the function and role of miR-335-5p, gain and loss of function analyses were conducted. miR-335-5p inhibitor-ctrl, inhibitor, miR-ctrl and mimics were transfected into MKN-28 and SGC-7901 cells to further investigate the effects of miR-335-5p in GCs. MTT and colony formation assays were applied to verify the effect of miR-335-5p on the proliferation of GCs, the results showed that upregulation of miR-335-5p in MKN-28 and SGC-7901 cells inhibited cell growth and colony formation, while the miR-335-5p inhibitor exerted moderate adverse effects on GC cells that may be caused by the low expression level of miR-335-5p in MKN-28 and SGC-7901 cells (Figure 2A and B). Consistent with this, flow cytometry analysis revealed that upregulation of miR-335-5p arrested cells in the G0/G1 phase and inhibited their transition to the G2/M phase, whereas this did not occur in the miR-ctrl transfected cells (Figure 2C). Furthermore, flow cytometry confirmed that upregulation of miR-335-5p induced apoptosis of GC cells. However, the miR-335-5p inhibitor exhibited a slight difference in cell apoptosis and no significant difference compared to cells transfected with the negative control, which may be due to the low expression level and low inhibition efficiency of miR-335-5p in MKN-28 and SGC-7901 cells. (Figure 2D).Inhibition of MiR-335-5p promoted the proliferation and inhibited the apoptosis of breast cancer cells while it was reciprocal in the miR-335-5p mimics group.

Inhibition of miR-335-5p induces migration and invasion of gastric cancer cells

To further confirm that the miR-335-5p played the role of tumor suppressor in MKN-28 and SGC-7901 cells, the effect of miR-335-5p on the invasion of GC cells has been identified. Two major methods for analyzing cell invasion and metastasis were the transwell invasion assay and wound-healing assay. In

the wound-healing assay, MKN-28 and SGC-7901 cells migrated more slowly in the miR-335-5p transfected. Over time, the difference in metastasis rate increased between the two groups (Figure 3A).In the transwell invasion assay, the transfection of cells with miR-335-5p mimics significantly impaired invasion when compared with miR-335-5p-ctrl group in MKN-28 and SGC-7901 cells. In contrast, knockdown of miR-335-5p enhanced GC cells invasion. When transfected with mir-335-5p inhibitor in MKN-28 and SGC-7901 cells, the invasion rate was significantly increased (Figure 3B). These results support the hypothesis that miR-335-5p played a role in the suppression of invasion and metastasis. To investigate the underlying mechanisms of miR-335-5p in apoptosis and cell cycle regulation, we measured the expression levels of apoptosis- and cell cycle-related proteins in GC cells. MiR-335 transfection in MKN-28/SGC-7901 cells downregulated CDK6,CDK4, CyclinD1 and BCL-2, Whereas, upregulated the expression of BAX. Meanwhile, we found that overexpression of miR-335-5p reduced the expression of Vimetin and β-catenin ,while the relative expressions of E-cadherin significantly increased in MKN-28 and SGC-7901 cells. In contrast, these protein expression levels exhibited opposite trends after miR-335-5p inhibitor transfection. MiR-335-5p inhibitor transfection in MKN-28/SGC-7901 cells upregulated CDK6,CDK4, CyclinD1 and BCL-2, while, downregulated the expression of BAX. We showed that silencing miR-355-5p, the relative expressions of Vimetin and β-catenin significantly increased and downregulation of E-cadherin expression, which were comparable with the effects of miR-355-5p overexpression in MKN-28 and SGC-7901. (Figure 3C) These results suggest that mir-335-5p is involved in the progress, migration and invasion of GCs.

MAPK10 is a direct functional target of miR-335-5p in GC cells

Computer-aided miRNA target prediction programs was used to search for potential miR-335-5p target genes. The correlation between mapk10 expression and mir-335-5p expression in gastric cancer based on TCGA data showed a negative correlation, and the p value was less than or equal to 0.001(Figure 4A). MAPK10 was selected as a candidate because of the potential miR-335-5p-binding site in 3'-UTR. To determine whether MAPK10 was directly targeted by miR-335-5p, we subcloned 3'-UTR MAPK10 fragments including wild-type (MAPK10-WT) and mutant (MAPK10-MUT) miR-335-5p-binding sites into the pmiRGLO dual-luciferase reporter vector (Figure 4B). pre-miR-335 and MAPK10-WT- or MUT-3'-UTR vectors were co-transfected into HEK293 cells. The relative luciferase activity of the MAPK10-WT pmirGLO-3'-UTR vector was significantly reduced in miR-335-overexpressing HEK293 cells. As expected, miR-335-5p failed to inhibit the luciferase activity of MAPK10-MUT pmirGLO-3'-UTR vector, indicating that miR-335-5p binds directly to the 3'-UTR of MAPK10 (Figure 4C). In order to verify the relationship between miR-335-5p and MAPK10, qRT-PCR was used. The mRNA levels of MAPK10 were significantly decreased by miR-335 mimics and increased by miR-335 inhibitors in MKN-28 and SGC-7901 cells. (Figure 4D) The Protein levels of MAPK10 were significantly decreased by miR-335 mimics and increased by miR-335 inhibitors in MKN-28 and SGC-7901 cells (Figure 4E). These findings demonstrated that miR-335-5p could directly target MAPK10 and suppressed MAPK10 expression in GC cells.

Bioinformatics analysis of mapk10 in gastric cancer

TCGA database were used to elucidate the effect of MAPK10 in GC tissues. The expression of MAPK10 was higher in GC tissues than counterparts, and the expression associated with the histologic and pathologic stages of GC. (Figure 5A-C) Bioinformatics were used to elucidate the effect of MAPK10 in GC tissues, the expression of MAPK10 was related with the DFI (disease-free interval event, P=0.033), PFI (progression-free interval event, P=0.013), DSS (disease-specific survival event, P=0.0068) and OS(overall survival, P=0.017) of GC. (Figure 5D- G), suggesting that MAPK10 played the key role of an oncogene in GC progression.

Knockdown of MAPK10 reduces the progression of GC cells

We knocked down MAPK10 expression by RNA interference (small interfering RNA (siRNA)) to confirm that MAPK10 was implicated in the antitumor effects of miR-335-5p. We sought to elucidate the mRNA and protein expression levels of MAPK10 in GC cells using qRT-PCR and western blot. There was greater upregulation of MAPK10 expression in GC cells than in GES-1 cells(Figure 6A)，wherein that expression in MKN-28 and SGC-7901 ranked the top two highest level. Our results showed that MAPK10 was knocked down by siRNA both at the mRNA (Figure 6B). Similar to miR-335-5p-overexpressing cells, downregulation of MAPK10 significantly inhibited proliferation and slightly inhibited colony formation in MKN-28 and SGC-7901 cells (Figure 6C and D). Moreover, the influence of MAPK10 siRNA on the cell cycle was similar to miR-335-5p upregulation (Figure 6E). Consistent with the effect of miR-335-5p on apoptosis of GC cells, knockdown of MAPK10 induced apoptosis in MKN45/SGC-7901 cells (Figure 6F), suggesting MAPK10 involved in the progression of GCs

Knockdown of MAPK10 reduces the migration and invasion of GC cells

We silenced MAPK10 expression using RNA interference (RNAi) to identify whether MAPK10 was involved in the effects of miR-335-5p on invasion and metastasis of MKN-28 and SGC-7901 cells. Using the wound-healing assay, we found that the MAPK10 low expression group migrated more slowly (Figure7A). The results of the transwell assays demonstrated that silencing of MAPK10 led to inhibit invasion and migration ability of GC cells (Figure 7B). Using western blot analysis, we showed that silencing MAPK10, the relative expressions of E-cadherin significantly increased and downregulation of Vimetin and β-catenin expression, The result was consisted with the effects of miR-355-5p overexpression in MKN-28 and SGC-7901. (Figure 7C),suggesting that MAPK10 played the role of an oncogene in GCs progression. Based on the above experimental result, we concluded that miR-335-5p suppresses gastric cancer progression by targeting MAPK10. (Figure 7D)

Discussion

The occurrence of GC is a complex process of multiple factors, including genetic and epigenetic events. In recent years, many miRNAs have been demonstrated to contribute to GC tumorigenesis and development, which serve as a valid diagnostic and therapeutic target for GC.[30] Also, scientists have revealed that some specific microRNAs can regulate the expressions of genes in GC cells at the post transcriptional level, and act as diagnostic biomarkers for GC.[31–33] Therefore, it is of great significance

to explore the potential miRNAs associated with the generation and development of GC, which may provide opportunities for better diagnosis and treatment and improve the prognosis of patients. Therefor, mir-335-5p was selected as the research object.

Recently, miR-335-5p played tumor suppressor and inducer roles in several cancer types.[34–37] The research of the function of miR-335-5p on tumor inhibitionshowed that the expression level of miR-335-5p was downregulated and miR-335-5p could inhibit the proliferation of thyroid cancer cells.[38] miR-335-5p went through LDHB to execute the tumor inhibitor function in colorectal cancer. [39]IThe present study, combined with the results of miR-335-5p expression in tissues, found that miR-335-5p played tumor suppressor in GC progression, overexpression of miR-335-5p inhibited proliferation, invasion, metastasis and induced apoptosis in vitro. Meanwhile, miR-335 could induce cell cycle arrest at the G1 phase, and G0 / G1 phase cycle associated-proteins Cyclin D1, CDK 6 and CDK4 were down regulated when miR-335 transfected. A large number of studies have shown that abnormal activation of CDK and its modulators occured in many tumors.[40, 41] Furthermore, miRNAs participated in the regulation of cell cycle process. [42, 43] miR-15a/16 family has been shown to regulate the G0/G1 cell cycle progression by targeting cyclins D1 (CCND1).[44] Also, miR-16 regulated different mRNA targets, including CDK6, CDC27, as well as G1-related cyclins, which jointly control cell cycle progression. [45] These studies strongly support our observation that miR-335 plays a key role in the proliferation of GCs.

Migration and invasion are closely related to the occurrence and development of tumors. Moreover, many migration and invasion related proteins including E-cadherin ,Vimetin andβ-catenin are important events. For instance, p0071 interacted with E-cadherin in the cytoplasm promoted the invasion and metastasis of NSCLC.[46]Furthermore, USP20 regulated the deubiquitination of β-catenin to control invasion and migration of cancer cells.[47] Our results showed that overexpression of mir-335-5p decreased the expression of vimentin and β - Catenin, while E-cadherin was significantly increased in MKN-28 and SGC-7901 cells. Our data suggested that mir-335-5p was involved in the migration and invasion of GCs.

In addition, our results showed that upregulation of miR-335-5p inhibited the expression of MAPK10 in MKN-28/SGC-7901 cancer cells at RNA and protein levels. Using bioinformatic analyses and a dual-luciferase reporter assay, we demonstrated that miR-335-5p directly targeted MAPK10 by binding its 3'-UTR and inhibiting translation.To further clarify the tumor suppressor role of miR-335-5p by targeting MAPK10, siRNA was used to knock down the expression of MAPK10. Our data showed that MAPK10 silencing inhibited cell proliferation, migration and induced cell apoptosis, which was similar to the effect of miR-335-5p overexpression in GC cells in vitro. Accordingly, the expression levels of related proteins, including CDK6,CDK4, CyclinD1 ,BCL-2,BAX, E-cadherin ,Vimetin andβ-catenin were also altered by siMAPK10. MAPK10 was a member of the Jun N-terminal kinase subgroup of the mitogen-activated protein kinase implicated in important physiological process.[48] MAPK10 regulated the occurrence and development of cancer cells in several types of cancers. The downregulation of MAPK10 contributed to the suppression of ovarian cancer cells.[49] miR-27a-3p promoted the growth and invasion of NPC cells by targeting Mapk10.[50] Thus, these results robustly suggested that MAPK10 downregulation induced by miR-335-5p could inhibit the progression of GC cells.

Conclusion

Our data reported the following new findings: (1) miR-335-5p functions as a tumor suppressor in GC cells; (2) miR-335-5p led to the inhibition of proliferation, metastasis, and the promotion of apoptosis in GCs. (3) MAPK10 is a downstream target gene of miR-335-5p;(4) MAPK10 played the vital role of an oncogene in GCs progression.

Abbreviations

miRNAs: microRNAs

GC: gastric cancer

MAPK10: mitogen-activated protein kinase 10

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

OD: optical density

PVDF: polyvinylidene fluoride

TBST: tris buffer solution with tween

siRNA: small interfering RNA

Declarations

Availability of data and materials

The datasets supporting the conclusions of this study are included in this article. Any requests for data or materials can be sent to the corresponding author.

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Ethics declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figures

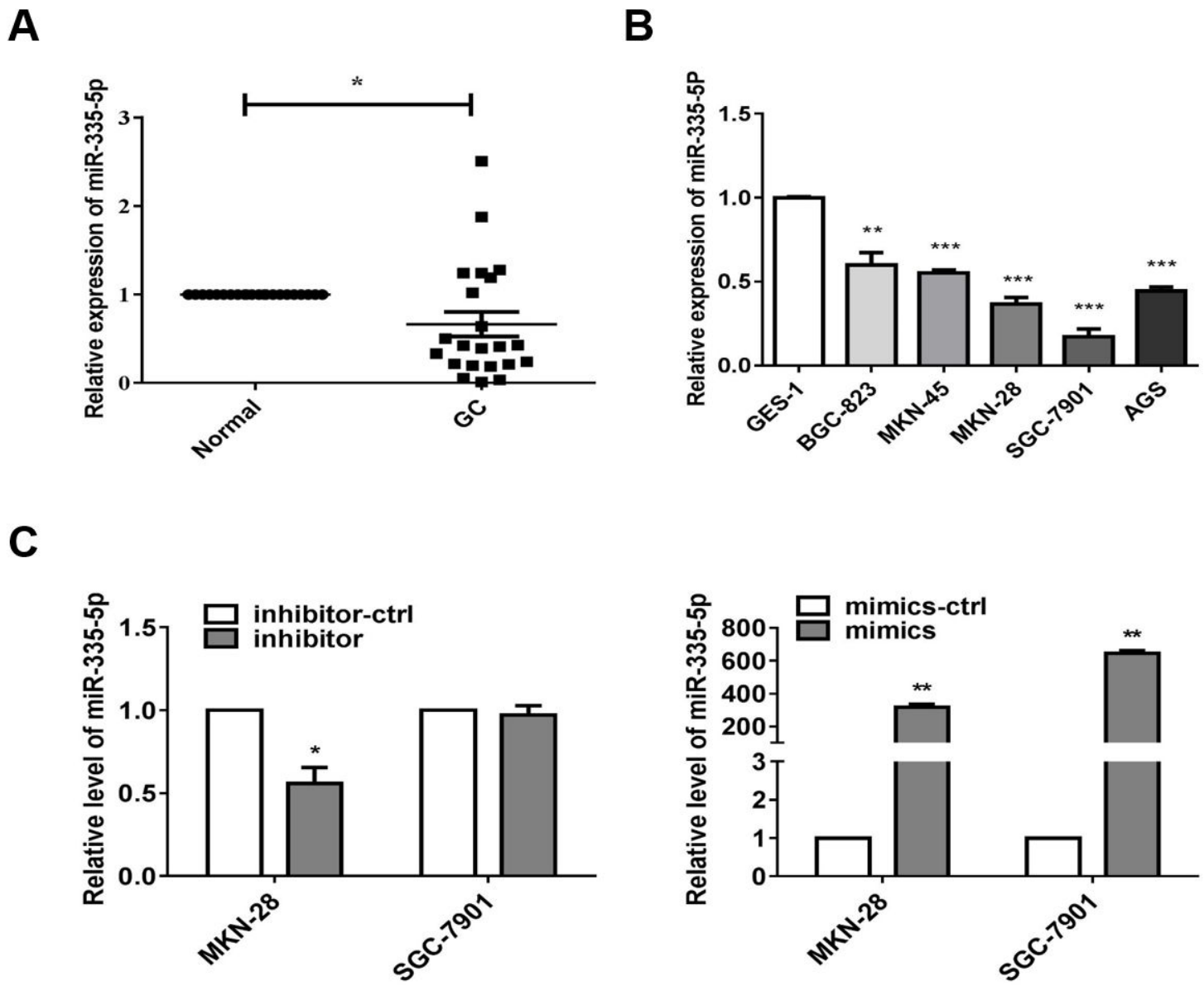

Figure 1

Down-regulation of miR-335-5p in GC tissues and cells. (A) qRT-PCR was performed to examine miR-335-5p expression in 22 paired human gastric cancer and adjacent normal tissues. The expression of miR-335-5p was normalized to U6. (B) qRT-PCR analysis of miR-491-5p expression in normal gastric mucosal and gastric cancer cells and normalized against U6 RNA. (C)The expression levels of miR-335-5p were determined by qRT-PCR in GCs transfected with miR-335-5p mimics, inhibitor or their respective ctrl(*P < 0.05, **P < 0.01 and ***P < 0.005).

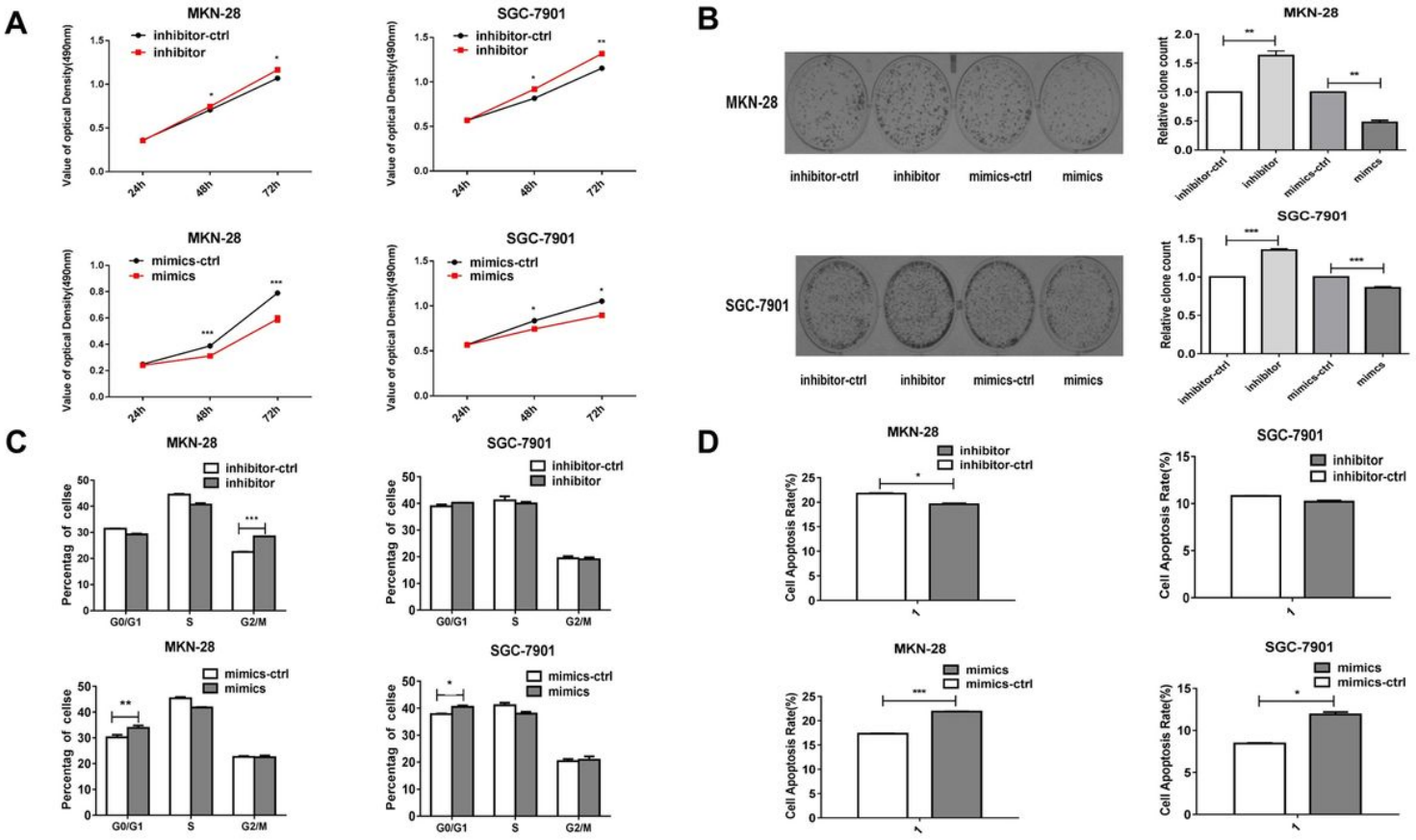

Figure 2

miR-335-5p inhibited cell proliferation and promotes apoptosis in gastric cancer cells. (A) The effects miR-335-5p on gastric cancer cell proliferation were determined by MTT assay after transfection of miR-335-5p mimic and miR-335-5p inhibitor in MKN-28/SGC-7901 cells at 24, 48, and 72 h. (B) The growth of MKN-28/SGC-7901 cells was detected by colony formation after transfection with the miR-335-5p mimic or inhibitor. (C) Cell cycle was determined in MKN-28/SGC-7901 cells transfected with miR-335-5p inhibitor-ctrl, inhibitor miR-335-5p ctrl and mimics. After 48 h, cell cycle distribution was analyzed by flow cytometry. A histogram indicates the percentage of cells in G0/G1, S, and G2/M cell cycle phases. (D) Apoptosis was detected by annexin-V/propidium iodide combined labeling flow cytometry in MKN-28/SGC-7901 cells 48 h after transfection with miR-335-5p inhibitor-ctrl, inhibitor, miR-335-5p ctrl and mimics. Apoptotic evaluation was carried out by calculating the percentage of apoptotic cells (*P<0.05, **P<0.01 and ***P < 0.005).

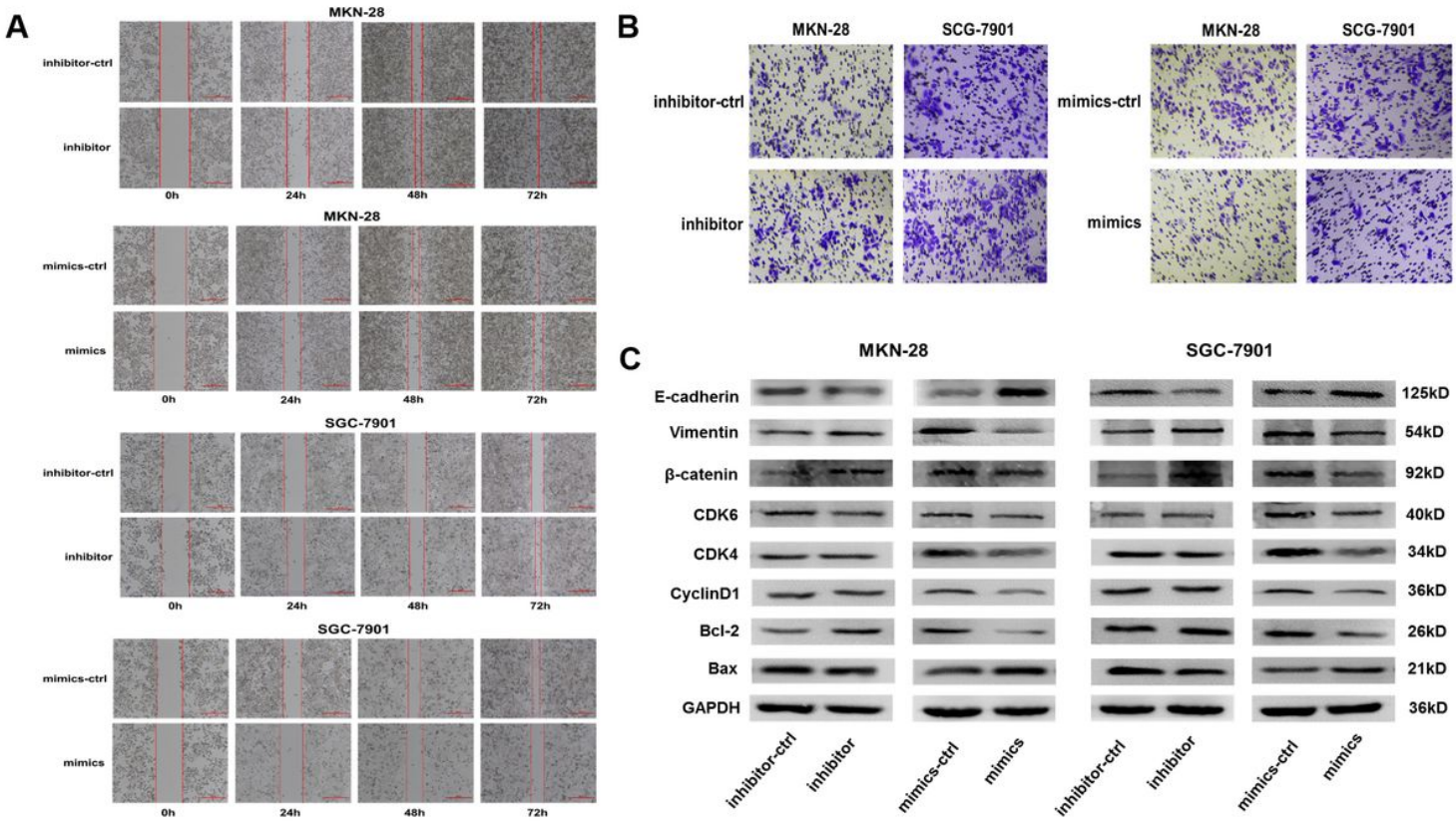

Figure 3

miR-335-5p inhibited the migration and invasion of MKN-28 and SGC-7901 cells.(A)Scratch wound-healing assays of MKN-28 and SGC-7901 cells after treatment with miR-335-5p inhibitor-ctrl, inhibitor, miR-ctrl or miR-335-5p mimics.(B) Transwell analysis of MKN-28 and SGC-7901 cells after transfected with miR-335-5p mimics, inhibitor, or their respective control.(C) Western blot analysis of CDK6,CDK4, CyclinD1 ,BCL-2,BAX , E-cadherin ,Vimetin andβ-catenin expression in MKN-28 and SGC-7901 cells transfected with miR-335-5p, inhibitor, or their respective control.MAPK10 is a direct functional target of miR-335-5p in GC cells.

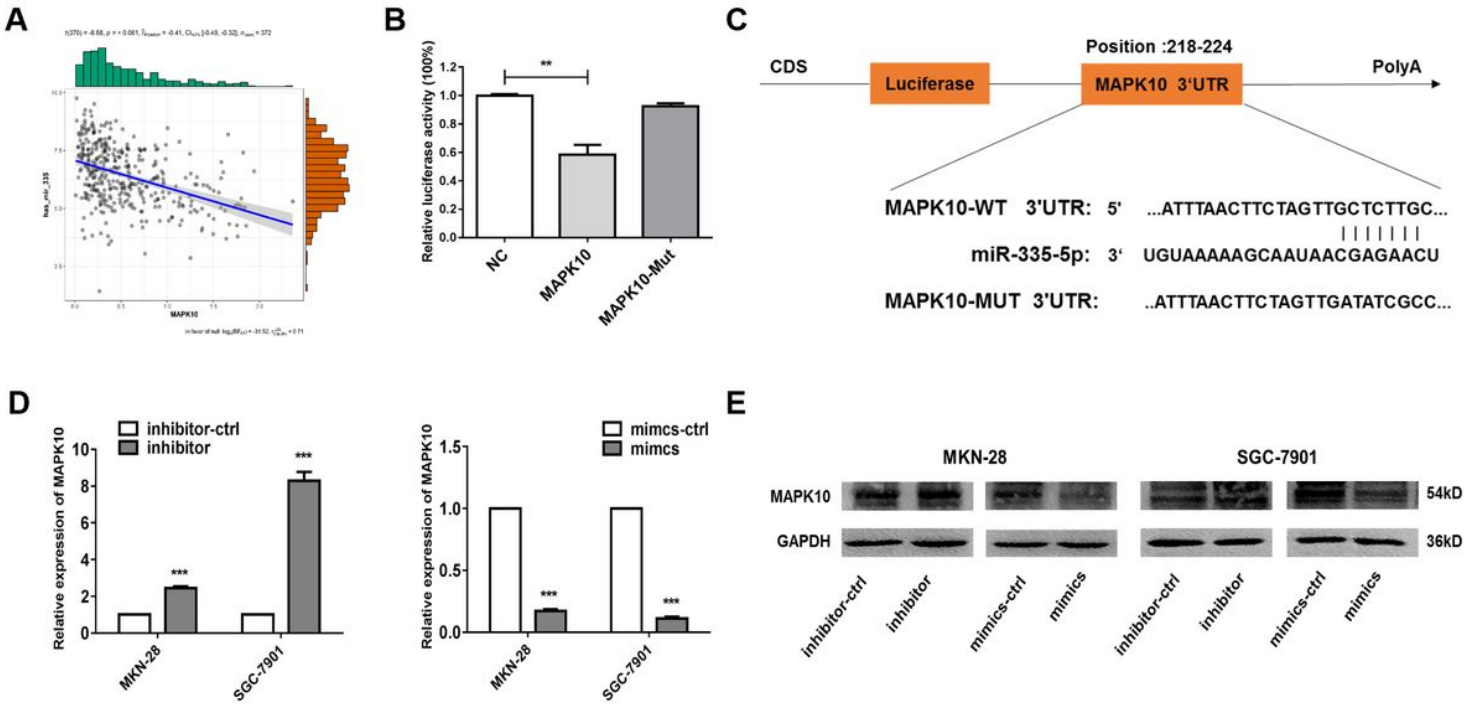

Figure 4

MAPK10 was a direct target of miR-335-5p in gastric cancer cell lines. (A)Correlation between MAPK10 expression and miR-335-5p expression in gastric cancer based on TCGA data. (B) The luciferase assay was performed in HEK293 cells in which miR-335 was co-transfected with pGLO-MAPK10 wild-type or pGLO-MAPK10 mutant vector. (C)miR-335-5p is highly conserved across species and it has binding sites within the 3'-UTR of human MAPK10. (D)mRNA expression levels of MAPK10 were measured by qRT-PCR after transfection with miR-335-5p mimics, miR-335-5pinhibitor, or their respectively negative control in MKN-28 and SGC-7901 cells. (E) Protein expression levels of MAPK10 were measured by western blot after transfection with miR-335-5p mimics, miR-335-5pinhibitor, or their respectively negative control in MKN-28 and SGC-7901 cells(*P<0.05, **P<0.01 and ***P < 0.005).

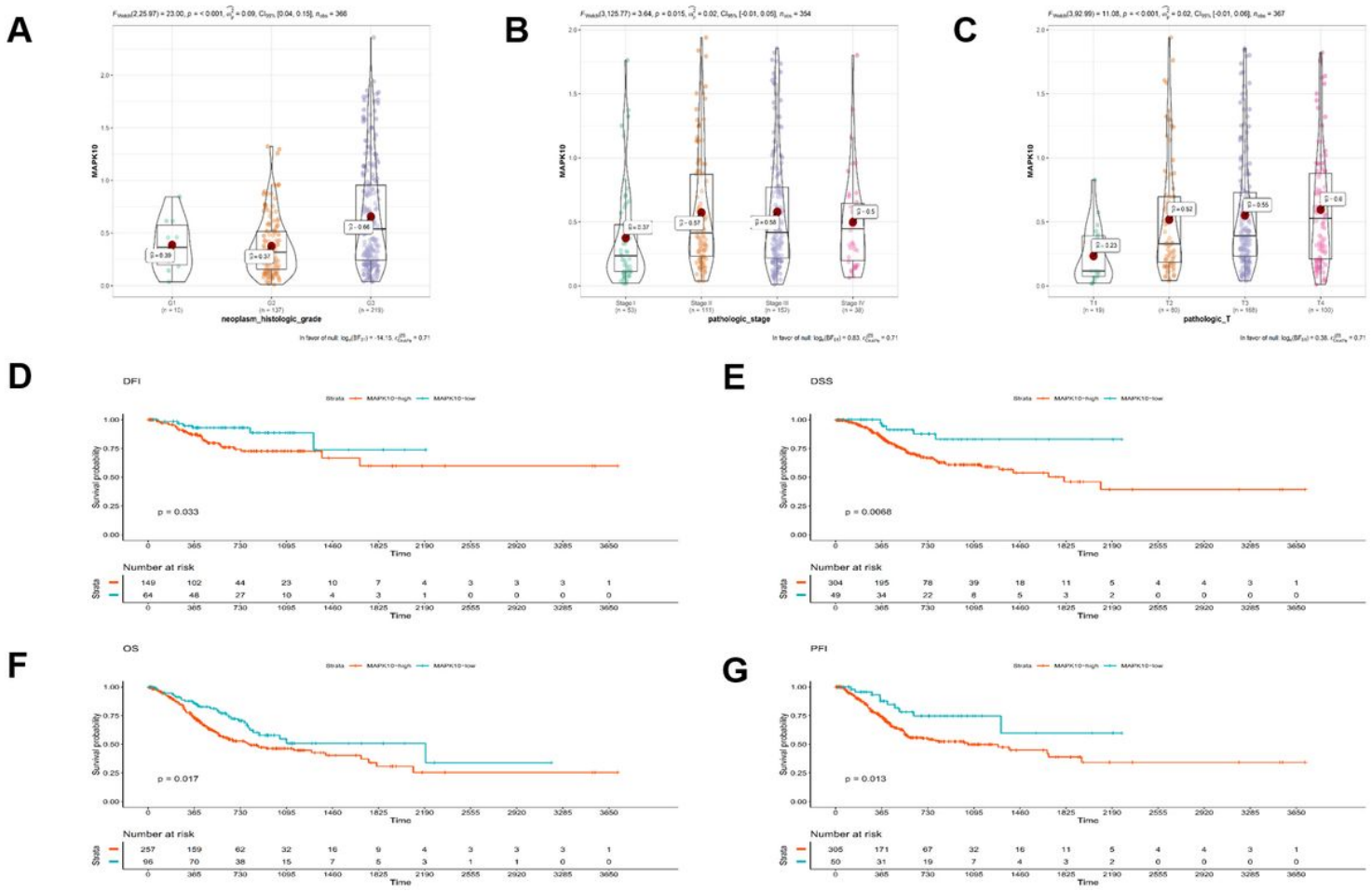

Figure 5

(A-C) The expression of MAPK10 connected with pathologic and histologic grade of GC patients. (D-G) Bioinformatics were used to elucidate the effect of MAPK10 in GC tissues, the expression of MAPK10 was related with the DFI (disease-free interval event), PFI (progression-free interval event), DSS (disease-specific survival event) and OS(overall survival) of GC.

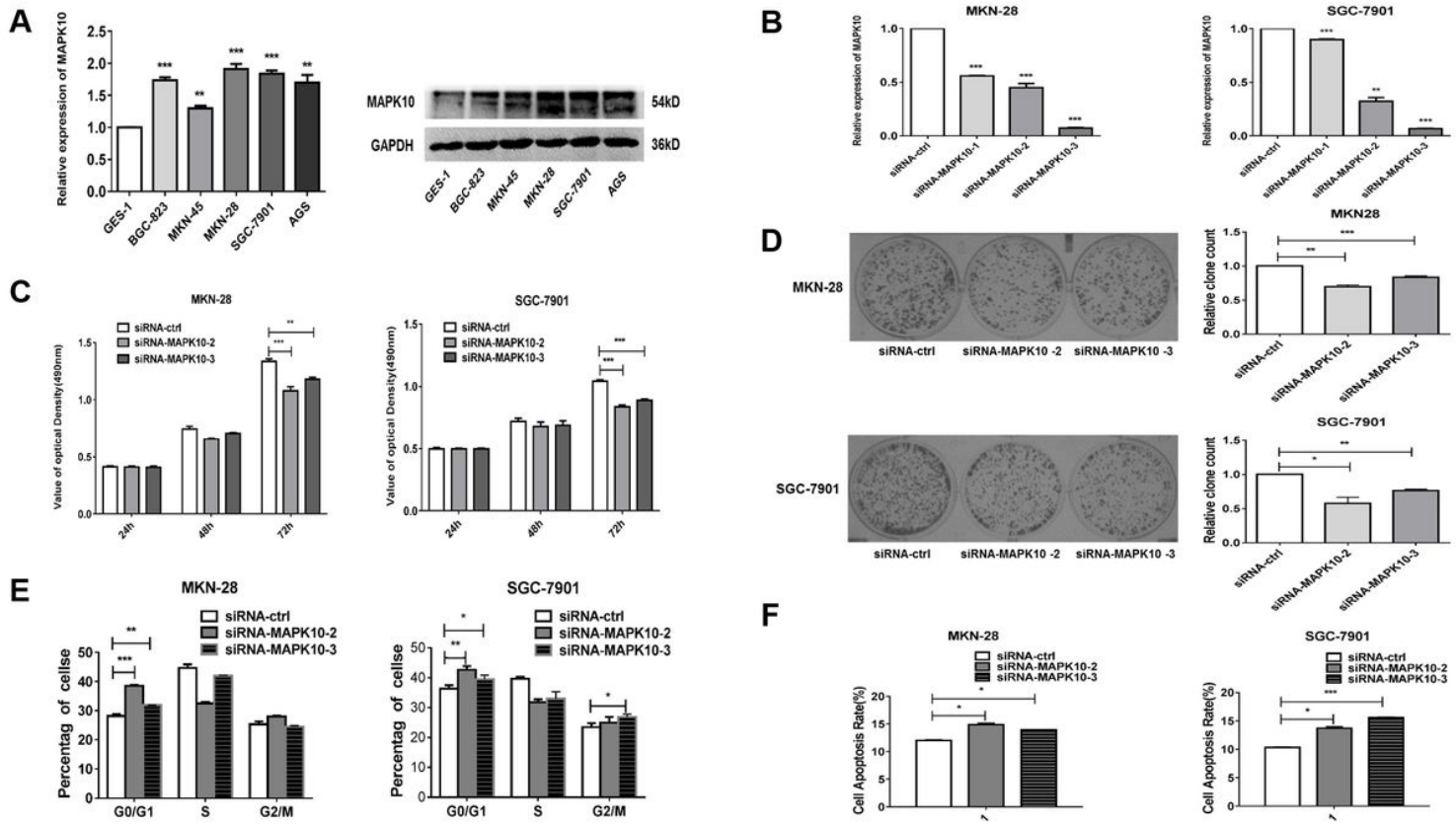

Figure 6

Inhibition of MAPK10 suppressed progression of gastric cancer cells. (A)The mRNA and Protein expression level of MAPK10 in various GC and GES-1 cells. (B) The expression levels of MAPK10 were measured by qRT-PCR in MKN-28 and SGC-7901 cells transfected with siMAPK10. (C) MTT assay was performed to determine the growth of gastric cancer cells treated with siMAPK10 or a negative control (si−ctrl). (D) The colony formation assay was performed several days after transfection of gastric cancer cells with siMAPK10 or a negative control (si−ctrl). (E) Cell cycle distribution was determined in gastric cancer cells 48 h after transfection with siMAPK10 by propidium iodide staining and flow cytometry. The histogram indicates the percentage of cells in G0/G1, S, and G2/M cell cycle phases. (F) Apoptosis was determined in gastric cancer cells at 48 h after transfection with siMAPK10(*P<0.05, **P<0.01 and ***P < 0.005).

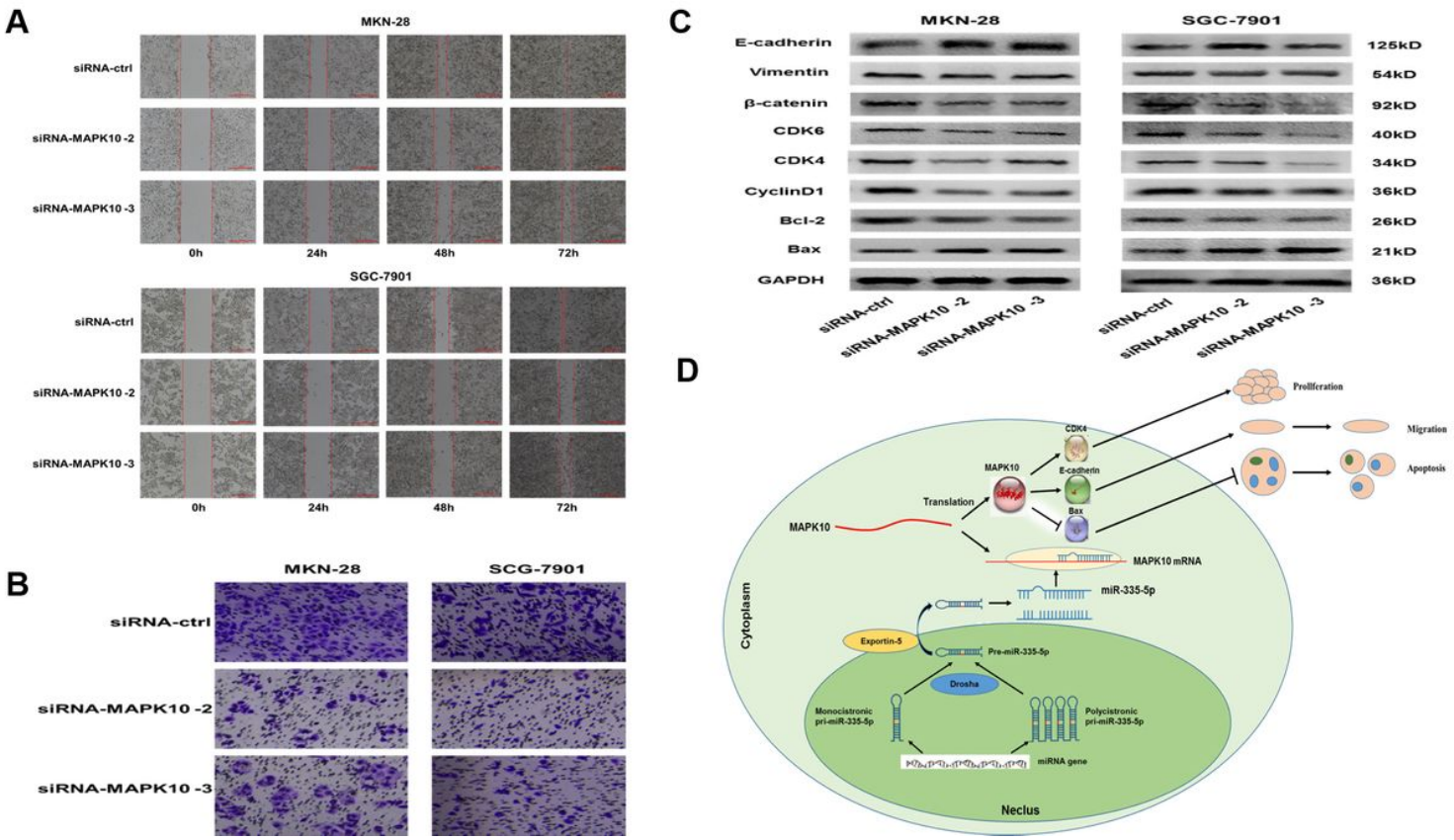

Figure 7

miR-335-5p inhibited cells invasion through MAPK10. (A)The invasion viability of MKN-28 and SGC-7901 cells transfected with siMAPK10 was determined using the Transwell invasion assay.(B)Wound-healing assays of MKN-28 and SGC-7901 cells after treatment with si-ctrl and si-MAPK10.Representative images were captured at 0 h,24 h,48 h and 72 h after transfection of si-ctrl and si-MAPK10.(C) The expression levels of MAPK10 were measured by western blot in MKN-28/SGC-7901 cells transfected with siMAPK10. Protein expression of CDK6,CDK4, CyclinD1 ,BCL-2,BAX , E-cadherin ,Vimetin andβ-catenin in gastric cancer cells transfected with siMAPK10 or si−ctrl was analyzed by western blot. (D) Proposed model for miR-335-5p suppresses gastric cancer progression by targeting MAPK10.