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ORIGINAL RESEARCH

RETRACTED ARTICLE: MiR-138-5p Inhibits the Proliferation of Gastric Cancer Cells by Targeting DEK

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Background: Increasing evidence suggests that microRNAs (miRNAs) play critical roles in cancer progression. Therefore, investigating the function of miRNAs that are aberrantly expressed in gastric cancer (GC) and characterizing the involved underlying mechanism are essential for the treatment of gastric cancer. MiR-138-5p was found to be down-regulated in multiple cancers, which acted as a tumor suppressor in cancer progression; however, whether and how miR-138-5p regulates the malignant behaviors of GC has not been fully understood.

Methods: The level of miR-138-5p in GC tissues and cell lines was detected by RT-qPCR. The effects of miR-138-5p on the growth of GC cells were evaluated by the in vitro Cell Counting Kit-8 (CCK-8) assay, cell apoptosis, cell cycle analysis, wound-healing assay, and in vivo xenograft mice model. The targets of miR-138-5p were predicted using the miRDB online tool, confirmed by luciferase report assay and Western blot.

Results: MiR-138-5p was frequently decreased in GC tissues and cell lines. Decreased expression of miR-138-5p was significantly associated with the lymph node metastasis of GC patients. Overexpression of miR-138-5p suppressed GC cell proliferation, migration, increased cell apoptosis as well as inhibited the tumor growth in vivo. DEK oncogene was predicted as a potential target of miR-138-5p. MiR-138-5p bound the 3′-UTR of DEK and inhibited the level of DEK in GC cells. Restoration of DEK abrogated miR-138-5p over-expression-mediated suppression of GC cell proliferation and cell cycle arrest.

Conclusion: Our results demonstrated the anti-cancer role of miR-138-5p in GC by targeting DEK, which suggested miR-138-5p as a potential therapeutic target for the treatment of patient with GC.

Keywords: gastric cancer, miR-138-5p, DEK

Introduction

Gastric cancer (GC) that originates from the excessive gastric mucosal epithelial cell proliferation is one of the most frequently occurring malignancies and the leading cause of cancer-related fatalities annually.[1–3] Despite great achievements have been made in the treatment of GC, the prognosis and five-year survival rate of GC patients still remain poor mostly due to the metastasis and resistance to the therapy. To improve the intervention of GC, understanding the mechanisms that contribute to the development of GC and identify novel therapeutic targets for GC are urgent.

MicroRNAs (miRNAs) are identified as endogenous small non-coding RNAs that regulate gene expression at the post-transcriptional level.[4–6] Mechanistically, miRNAs bind to the 3′-untranslated region (UTR) of the target mRNAs, resulting

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into the mRNA degradation or translation inhibition.[7,8] Notably, a growing number of studies have identified the tumor-suppressive or oncogenic roles of miRNAs in the development of cancers.[9–13] Many of these miRNAs were associated with the metastasis, invasion, and drug resistance of GC, which made miRNAs as promising targets for the prognosis and treatment of GC.[14–16] Overexpression of miR-96-5p accelerated the growth of GC cells by directly targeting FOXO3.[17] Recent study also demonstrated that miR-183-5p acted as a potential prognostic biomarker for GC and modulated the malignant behaviors of GC cells via regulating EEF2.[18] MiR-138-5p was recently reported to play important roles in human cancers, including lung cancer, cervical cancer, breast cancer, and pancreatic cancer.[19–23] Overexpressed miR-138-5p inhibited the cancer cell proliferation, migration, and invasion. Decreased expression of miR-138-5p was significantly correlated with the advanced progression of cancer patients. These findings suggested the tumor-suppressive roles of miR-138-5p in the progression of cancers. However, the expression, biological function, and underlying molecular mechanisms of miR-138-5p in GC remain largely unknown.

In the present study, we aimed to explore the involvement of miR-138-5p in the malignancy of GC. Our results showed that miR-138-5p was down-regulated in GC tissues and cell lines. Decreased miR-138-5p was significantly correlated with the lymph node metastasis of GC patients. Overexpression of miR-138-5p inhibited the in vitro cell proliferation, migration, induced apoptosis, and suppressed the in vivo tumor growth. Mechanism study identified DEK as a potential target of miR-138-5p and mediated the tumor suppressive function of miR-138-5p in GC. Our findings suggested miR-138-5p as a potential therapeutic target for the treatment of GC.

Materials and Methods

Tissue Samples

A total of 50 paired GC specimens and adjacent non-cancerous tissues were obtained from GC patients at the People's Hospital of Yichun City between January 2011 and December 2013. All tissues were collected via surgical resection prior to the initiation of radiotherapy or chemotherapy and stored at −80ºC until required. The experiment was approved by the Ethics Committee of the People's Hospital of Yichun City. Written informed consents were provided by all enrolled patients.

Cell Lines and Transfection

The GC cell lines (MKN45, MKN28, AGS, and NCI-N87) and normal human gastric epithelial cell line GES-1 were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultured in RPMI-1640 medium (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator with 5% CO_2 at 37ºC.

The miR-138-5p mimics and miR-negative control (NC) were purchased from the GenePharma (Shanghai, China). Cell transfection was performed when the cell confluence reached ~60% using the Lipofectamine 2000 reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol.

Cell Viability Analysis

The viability of GC cells carrying miR-138-5p mimics or miR-NC was assessed via the Cell Counting Kit-8 (CCK-8) assay. Briefly, the transfected GC cells were seeded into a 96-well plate with the density of 2000 cells per well. A total of 10 μL of CCK-8 solution (Beyotime, Shanghai, China) was added into the medium at different time points (1, 2, 3, 4, and 5 days) and incubated for additional 4 h at 37ºC. The absorbance of each well at 450 nm was measured using the microplate reader (Bio-Rad, CA, USA).

Targets Prediction

The potential targets of miR-138-5p were predicted using the miRDB (http://www.mirdb.org/) databases.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from GC tissues or cells using the TRIzol regent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's guidelines. RNA concentration was determined with the NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific, Inc., Waltham, MA, USA). One microgram of total RNA was reversely transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The qPCR analysis of miR-138-5p was performed with the TaqMan miRNA PCR kit (Applied Biosystems,

Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the Option RT-PCR detection system (ABI 7500, Thermo Fisher Scientific, Inc., Waltham, MA, USA). The level of U6 RNA was detected for the normalization. The amplifying reaction was performed with the following conditions: pre-degeneration at 95ºC for 30 sec, followed by denaturation at 95ºC for 5 sec, annealing at 62ºC for 30 sec for 40 cycles. The relative expression of miR-138-5p was determined with the $2^{-\Delta\Delta CT}$ method.

Western Blot

GC cells were lyzed with the Radioimmunoprecipitation Assay (RIPA) lysis buffer (Beyotime, Shanghai, China) containing protease inhibitor (Roche, USA). The protein concentration was determined using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Thermo Fisher Scientific, Inc., Waltham, MA, USA). A total of 20 μg protein was loaded, separated by 15% SDS-PAGE, and transferred onto the polyvinylidene difluoride (PVDF) membrane (EMD Millipore, Billerica, MA, USA). After blocking with 5% non-fat milk at room temperature (RT) for 1 h, the membranes were probed with primary antibody against DEK (1:1000 dilution; ab221545; Abcam, Shanghai, China), cleaved caspase-3 (1:1000 dilution; ab32351; Abcam, Shanghai, China), cleaved caspase-9 (1:1000 dilution; ab2324; Abcam, Shanghai, China), p21 (1:2000 dilution; ab109520; Abcam, Shanghai, China) or GAPDH (1:2000 dilution; ab181602; Abcam, Shanghai, China) overnight at 4ºC. After washing twice with PBST, the membranes were incubated with HRP-conjugated goat anti-Rabbit IgG H&L secondary antibody (1:3000 dilution; ab205718, Abcam, Shanghai, China) for 1 h at RT. The signals were developed using the Enhanced Chemiluminescence kit (Pierce, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and analyzed with the Image J software (Version 5.0; Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cell Cycle Analysis

GC cells were transfected with miR-138-5p mimics or miR-NC using the Lipofectamine 2000. After transfection for 48 h, cells were collected and fixed with 75% ethanol at 4ºC overnight. Cells were then centrifuged at 2000 rpm at 4ºC for 5 min. Followed by washing twice with PBS, cells were treated with RNase A (Solarbio, Beijing, China) for 30 min at 37ºC and stained with propidium iodide (PI) for 15 min in the dark. The cellular DNA content was assessed using the FACScan flow cytometer (BD Bioscience) and the data were analyzed with the ModFit LT software 2.0 (BD Bioscience).

Cell Apoptosis

The apoptosis of GC was determined using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences). Briefly, cells were cultured in 10-cm dish and transfected with miR-138-5p mimics or miR-NC, respectively. After transfection for 48 h, cells were harvested and digested with 0.25% trypsin for 5 min at RT. Cells were then centrifuged and washed with PBS. Ten microliters of fluorescein isothiocyanate (FITC) and 5 μL of PI were added into the cells and incubated at RT for 10 min in the dark. Cell apoptosis was detected using the FACScan flow cytometer (BD Bioscience).

Colony Formation

GC cells transfected with miR-138-5p mimics or miR-NC were seeded into the 6-well plate with the density of 500 cells per well. After cultured for 10 days, colonies were washed twice with PBS and fixed with methanol at RT for 10 min. And then colonies were stained with 0.1% of crystal violet (#C3470, Solarbio, Beijing, China) at RT for 15 min. The colonies were photographed with a light microscope (Olympus Corporation).

Luciferase Reporter Assay

The fragments of wild-type (WT) or mutant (Mut) of 3′-UTR of DEK were inserted into the backbone of pmirGLO dual-luciferase vector (Promega, Madison, Wisconsin, USA). GC cells were co-transfected with miR-138-5p mimics and pmirGLO-WT/Mut-DEK using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After transfection for 48 h, cells were harvested and the luciferase activity was determined using the Dual-Luciferase Reporter Assay Kit (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. *Renilla* luciferase activity was normalized to that of Firefly.

Wound-Healing Assay

Both MKN45 and N87 cells that transfected with miR-138-5p mimics or miR-NC were seeded into the 6-well plate. After the cell confluence reached to monolayer, the wound was generated by starching the cells using a 1000 μL spear head. The debris was removed and cells were cultured overnight. The wound healing was captured with an inverted light microscope (Eclipse TS-100; Nikon Corporation).

In vivo Xenograft Nude Mice Assay

GC cancer cells (1×10^6) with lentivirus stably expressed miR-138-5p mimics or miR-NC were subcutaneously

injected into the flanks of nude mice (female, BALB/c; 5–6 weeks; 17–20 g; 5 mice per group). Mice were fed under sterile-specific pathogen-free condition under a 12-h light/dark cycle with free access to water and food. Tumor growth was measured with a caliper every 5 days. Mice were sacrificed after 30 days via cervical dislocation and tumors were weighted. The tumor volume (V) was calculated with the method: V= Largest diameter×(Smallest diameter)^2/2. The animal experiment was approved by the Ethics Committee of People's Hospital of Yichun City.

Statistical Analysis

All data were presented as the mean ± standard deviation. Difference was determined by Student's *t*-test or one-way analysis of variance followed by Bonferroni test. Spearman correlation test was performed to determine the correlation between the expression of miR-138-5p and DEK in GC tissues. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, Inc.). $P<0.05$ was considered as statistical significance.

Results

MiR-138-5p Was Down-Regulated in GC and Correlated with the Lymph Node Metastasis of GC Patients

To explore the potential role of miR-138-5p in GC, the expression status of miR-138-5p in GC tissues and paired adjacent non-cancerous tissues was detected. The RT-qPCR analysis showed that miR-138-5p expression was frequently down-regulated in GC tissues compared with that of the match normal tissues (Figure 1A). Moreover, the expression of miR-138-5p was significantly decreased in GC patients carrying lymph node metastasis (LNM) than those without LNM (Figure 1B). Meanwhile, the level of miR-138-5p was also detected in GC cell lines and normal cell. The data also showed that miR-138-5p was down-regulated in GC cell lines compared with the normal cells GES-1 (Figure 1C). These results suggested the frequent down-regulation of miR-138-5p in GC, indicating the potential involvement of miR-138-5p in the progression of GC.

MiR-138-5p Inhibited the Malignant Behaviors of GC Both in vitro and in vivo

To investigate how miR-138-5p affects the progression of GC, gain-of-function assays were performed by transfecting miR-138-5p mimics into MKN45 and N87 cells, which harbored relatively lower expression of miR-138-5p among all the GC cell lines we detected. The overexpression of miR-138-5p was validated by RT-qPCR (Figure 2A). The proliferation of GC cells carrying miR-138-5p mimics or miR-NC was measured with the CCK-8 assay. The results showed that MKN45 and N87 cells with overexpressed miR-138-5p exhibited significant growth suppression compared with cells transfected with miR-NC (Figure 2B and C). To determine whether the reduced proliferation of GC cells was associated with cell apoptosis, flow cytometry analysis was performed to assess the apoptotic percentage of GC cells. As indicated in Figure 2D, transfection of miR-138-5p exerted a stimulatory effect on the apoptosis of both MKN45 and N87 cells. Additionally, to detect the effect of miR-138-5p on the migration of GC cells, wound-healing assay was performed with GC cells expressing miR-138-5p mimics or miR-NC. The result indicated that overexpression of miR-138-5p markedly suppressed the migration of both MKN45 and N87 cells (Figure 2E). Meanwhile, the colony formation of GC cells was also significantly inhibited with the transfection of miR-138-5p in comparison with the cells expressing miR-NC (Figure 2F). To provide more evidence regarding the suppressive role of miR-138-5p in GC, in vivo xenograft nude mice model was established by subcutaneously injecting MKN45 and N87 cells that were stably expressed miR-138-5p mimics or miR-NC. The tumor volume was monitored and tumor weight was weighted after injection for 30 days. The overexpression of miR-138-5p in tumors was confirmed by RT-qPCR (Figure 2G). The results showed that overexpression of miR-138-5p significantly inhibited the tumor volume and weight compared with tumors harboring miR-NC (Figure 2H–J). Taken together, these results demonstrated the tumor-suppressive role of miR-138-5p in the progression of GC.

DEK Was a Target of MiR-138-5p in GC

To further understand the underlying mechanism of miR-138-5p in GC, we performed the target prediction using the miRDB databases. A total of 657 targets were predicted containing the potential binding site of miR-138-5p (Supplementary Table 1). The bioinformatics analysis showed that miR-138-5p bound the 3′-UTR of DEK (Figure 3A). Previous studies have demonstrated the important oncogenic function of DEK in tumorigenesis. High level of DEK predicted poor prognosis of GC patients, which may serve as a potential biomarker for GC.[24] However, the involved mechanism that could explain how the expression of DEK is regulated in GC

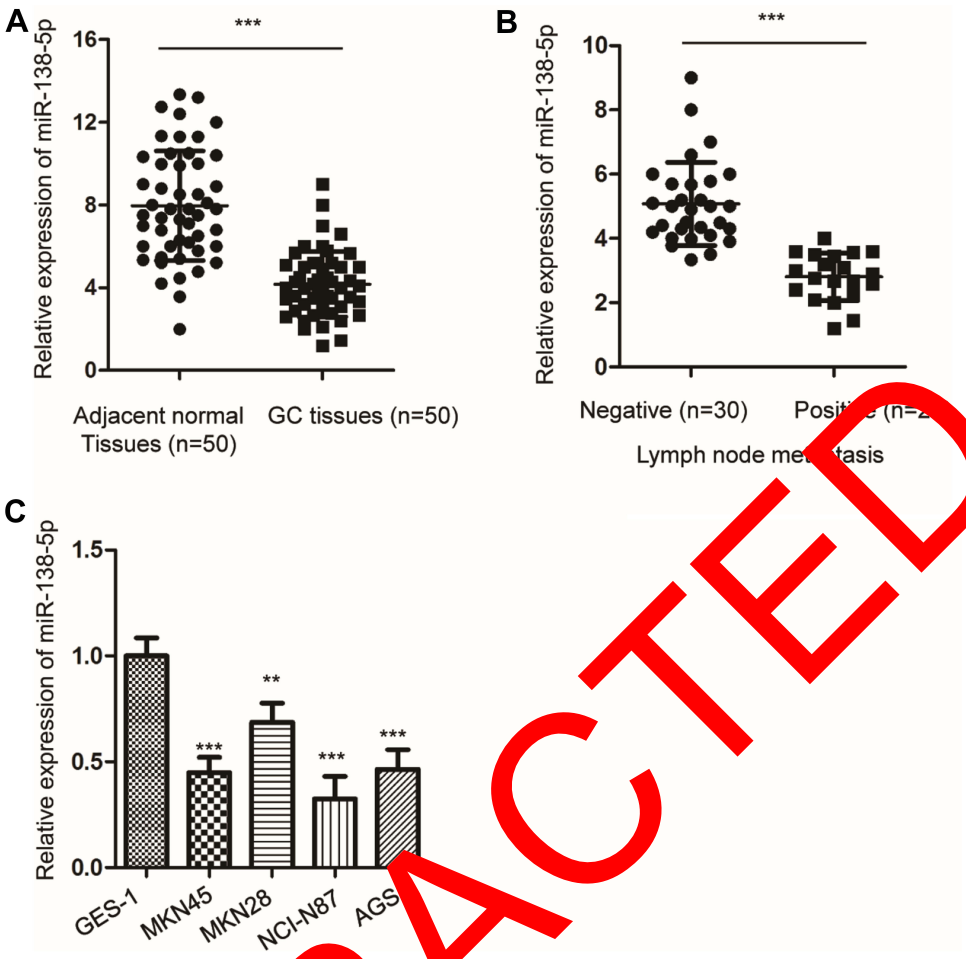

Figure 1 MiR-138-5p was down-regulated in GC. (**A**) RT-qPCR of miR-138-5p expression in GC tissues (n=50) and paired adjacent normal tissues (n=50). ***$P<0.001$ vs normal group. (**B**) The level of miR-138-5p was lower in patients with lymph node metastasis (n=20). (**C**) RT-qPCR analysis of miR-138-5p in normal cell GES-1 and GC cell lines MKN45, MKN28, NCI-N87 and AGS. ***$P<0.001$, $P<0.0$ vs normal. Data were presented as the mean ± standard deviation.

remains unclear. Therefore, DEK was chosen as a potential target of miR-138-5p for further analysis. To validate the prediction, luciferase reporter assay was performed by co-transfecting miR-138-5p mimics and luciferase reporter vector expressing wild type (WT) or mutant (MT) 3′-UTR of DEK. The data indicated that the luciferase activity of cells co-transfected with DEK-WT 3′-UTR and miR-138-5p mimics was decreased significantly (Figure 3B and C). Subsequently, RT-qPCR assay was carried out to examine the mRNA level of DEK with the overexpression of miR-138-5p. The data showed that mRNA level of DEK in MKN45 and N87 cells was reduced with the transfection of miR-138-5p in contrast to the cells expressing miR-NC (Figure 3D). Consistently, the protein expression of DEK was also decreased following the overexpression of miR-138-5p (Figure 3E). These results indicated that miR-138-5p targeted the 3′-UTR of DEK and inhibited the expression of DEK in GC.

To support the conclusion that DEK was a potential target of miR-138-5p, the expression of DEK in GC tissues and matched adjacent non-cancerous tissues was detected by RT-qPCR. The data revealed that the mRNA level of DEK was significantly up-regulated in GC tissues in comparison with that of the normal gastric tissues (Figure 3F). Additionally, the mRNA levels of DEK in GC patients with or without lymph node metastasis (LNM) were compared. As indicated in Figure 3G, the mRNA expression of DEK was significantly higher in GC patient with LNM than patients without LNM. Consistent with the mRNA data, IHC staining also revealed the increased expression of DEK in GC tissues with LNM (Figure 3H). Moreover, the Spearman correlation test showed that DEK expression was inversely proportional to the level of miR-138-5p in GC tissues (Figure 3I). Collectively, these findings demonstrated the overexpression of DEK and its negative correlation with expressions of miR-138-5p in GC.

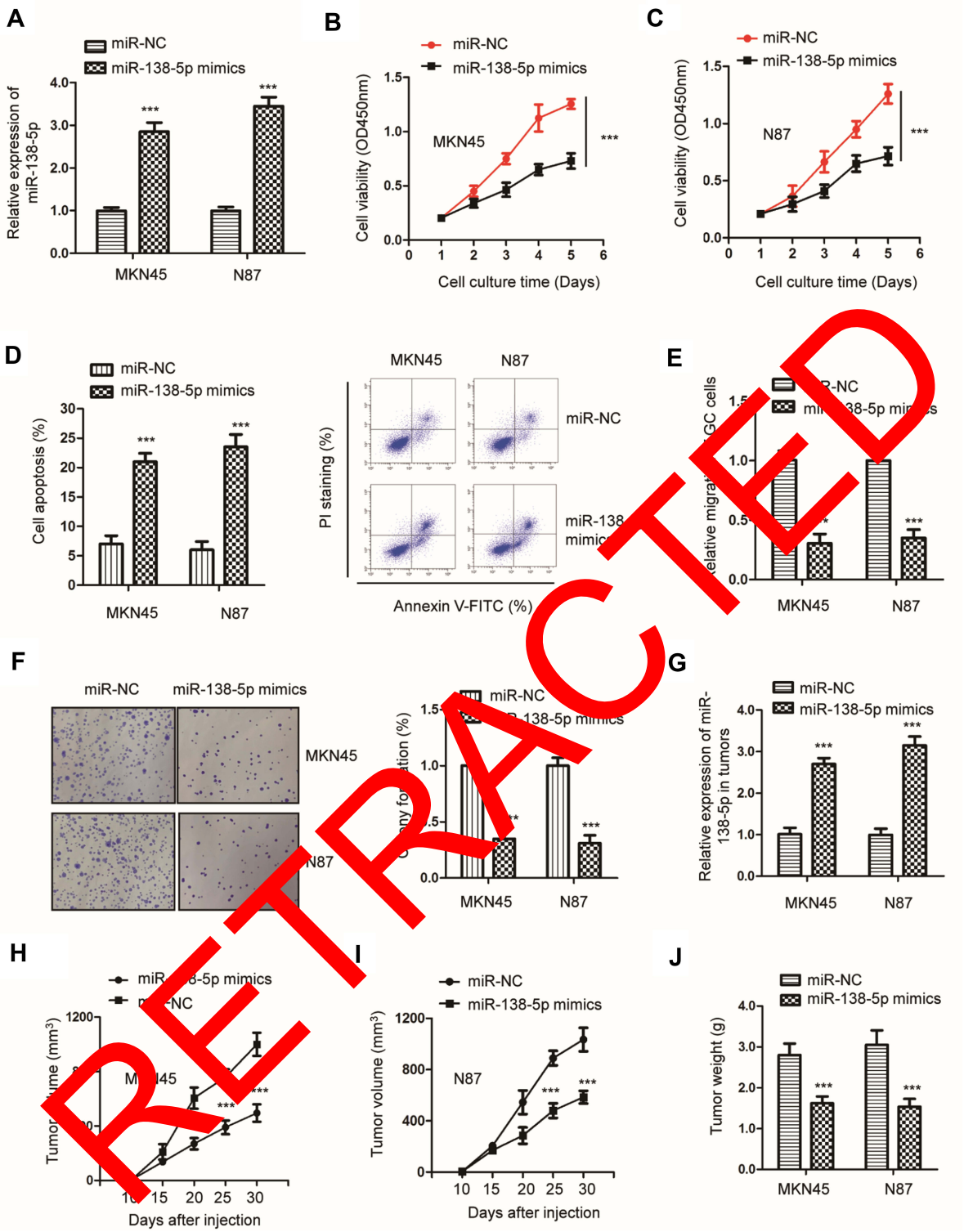

Figure 2 Overexpression of miR-138-5p inhibited the proliferation, colony formation and induced apoptosis of GC cells. (**A**) The overexpression of miR-138-5p with the transfection of miR-138-5p mimics was detected by RT-qPCR. ***P<0.001 vs miR-NC group. (**B** and **C**) CCK-8 assay showed that overexpression of miR-138-5p significantly inhibited the proliferation of MKN45 and N87 cells compared with cells expressing miR-NC. ***P<0.001 vs miR-NC group. (**D**) The apoptosis of cells was detected by the staining of PI and Annexin V-FTIC. Overexpression of miR-138-5p induced a significant increase in the apoptosis (early and late apoptosis) of GC cells. ***P<0.001 vs miR-NC group. (**E**) Transfection of miR-138-5p inhibited the migration of GC cells in comparison with the control cells. ***P<0.001 vs miR-NC group. (**F**) Colony formation assay showed that miR-138-5p overexpression attenuated the proliferative capacity of both MKN45 and N87 cells. ***P<0.001 vs miR-NC group. (**G**) The expression of miR-138-5p in tumors infected with lentivirus expressed miR-138-5p mimics or miR-NC was confirmed by RT-qPCR analysis. ***P<0.001 vs miR-NC group. (**H**–**J**) Overexpressed miR-138-5p significantly inhibited the tumor volume (**H** and **I**) and weight (**J**) compared with that of control group harboring miR-NC, respectively. ***P<0.001 vs miR-NC group. Data were obtained from three independent experiments and presented as the mean ± standard deviation.

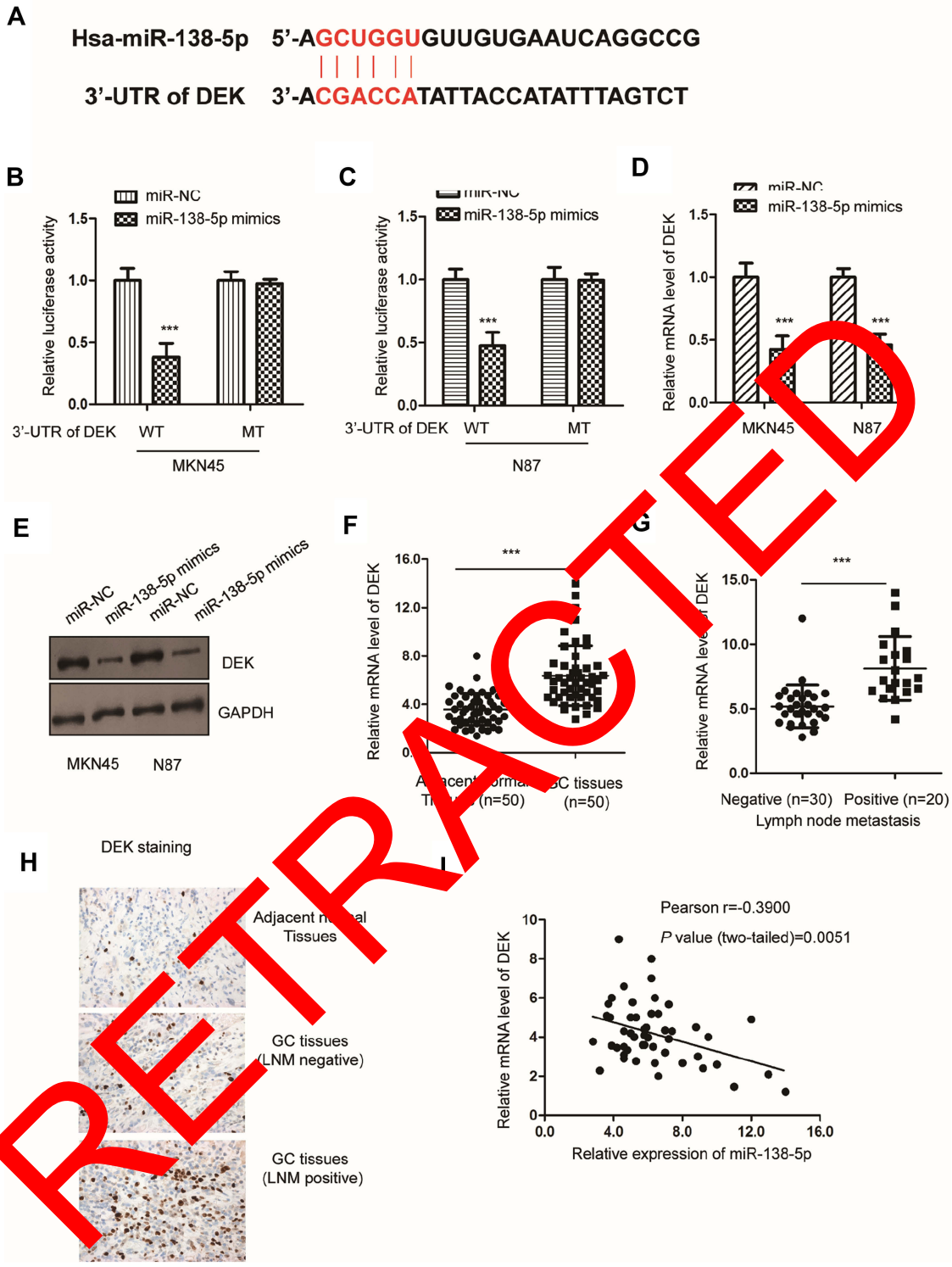

Figure 3 DEK was a target of miR-138-5p in GC. (**A**) Schematic of predicted binding sites of miR-138-5p in the 3′-UTR of DEK predicted by the miRDB database. (**B** and **C**) Luciferase activity in GC cells co-transfected with luciferase vector expressing WT or MT 3′-UTR of DEK and miR-138-5p mimics or miR-NC. ***P<0.001 vs miR-NC group. (**D** and **E**) The mRNA (**D**) and protein levels of DEK (**E**) in GC cells transfected with miR-138-5p mimics were decreased compared with the cells expressing miR-NC. ***P<0.001 vs miR-NC group. (**F**) The mRNA level of DEK in GC tissues (n=50) and paired adjacent normal tissues (n=50) was validated by RT-qPCR. The level of DEK was significantly reduced in GC tissues. ***P<0.001 vs adjacent normal tissues. (**G**) The expression of DEK was increased in GC patients with LNM (n=20) than those patients without LNM (n=30). ***P<0.001 vs LNM negative. (**H**) The IHC staining of DEK was increased in GC tissues especially those with lymph node metastasis. (**I**) The correlation between miR-138-5p and DEK in GC tissues was determined by the Spearman test. Data were obtained from three independent experiments and presented as the mean ± standard deviation.

Restoration of DEK Eliminated the Suppressive Effects of miR-138-5p on the Growth of GC Cells

To investigate whether miR-138-5p inhibited the malignancy of GC by targeting DEK, we overexpressed DEK in both MKN45 and N87 cells by transfecting pcDNA-Flag-DEK. The expression level of ectopic DEK was confirmed by Western blot with anti-Flag antibody (Figure 4A). The CCK-8 assay demonstrated that GC cells transfected with miR-138-5p alone showed reduced cell proliferation, while co-transfection of DEK significantly increased the proliferation of both MKN45 and N87 cells (Figure 4B and C). Because cell proliferation is tightly associated with cell cycle progression, we also evaluated the effects of miR-138-5p/DEK axis on the cell cycle distribution of GC cells. The data showed that overexpressed miR-138-5p significantly accumulated the cells in G1 phase, suggesting G1 cell cycle arrest compared with the cells expressing miR-NC. More importantly, restoration of DEK abrogated the inhibitory effects of miR-138-5p on the cell cycle progression (Figure 4D). Additionally, the effects of DEK on miR-138-5p induced cell apoptosis were determined by flow cytometry. The results showed that co-transfection of DEK significantly inhibited miR-138-5p-mediated apoptosis of both MKN45 and N87 cells (Figure 4E). To explore the possible mechanism underlying these results, the expression of p21 that was involved in G1 cell cycle arrest as well as the cleavage of caspase-3/9 that was associated with cell apoptosis was detected with the co-transfection of miR-138-5p mimics and Flag-DEK. As indicated in Figure 4F, overexpression of miR-138-5p increased the level of p21 in GC cells, while restoration of DEK attenuated the accumulation of p21. Moreover, the up-regulated cleavage of caspase 3/9 due to miR-138-5p was also suppressed with the restoration of DEK (Figure 4F). These data indicated that DEK plays an important role in miR-138-5p mediated growth inhibition of GC cells.

Discussion

Gastric cancer is one of the most frequent malignant cancers with high morbidity and mortality worldwide, especially in developing countries.[25,26] Increasing evidence demonstrates the dysregulation of miRNAs in GC that contributes to the malignancy of GC by targeting tumor-associated proteins.[16] In this study, we investigated the expression, biological function, and possible underlying mechanism of miR-138-5p in the progression of GC. Our findings suggested miR-138-5p might be a potential therapeutic target of GC.

The dysfunction of miR-138-5p has been characterized as an important tumor suppresser in cancer progression. MiR-138-5p was down-regulated in colorectal cancer (CRC) tissues and associated with the advanced clinical stage and poor overall survival of CRC patients.[21] Further study showed that overexpressed miR-138-5p suppressed CRC cell growth via targeting PD-L1, suggesting miR-138-5p as a biomarker and clinically effective anti-CRC therapeutic strategy.[21] It was also found that miR-138-5p reduced the expression of pyruvate dehydrogenase kinase 1 and inhibited the development of retinoblastoma.[27] In this study, miR-138-5p was underexpressed in GC tissues and cell lines. Decreased level of miR-138-5p was associated with the lymph node metastasis of GC patients. Functional analysis revealed that overexpressed miR-138-5p inhibited the proliferation, colony formation, migration, and induced apoptosis of GC cells. In vivo study showed that highly expressed miR-138-5p suppressed the tumor growth. These findings suggested miR-138-5p as a possible therapeutic target for the treatment of GC.

DEK is a ubiquitously expressed protein in multicellular organisms.[28] Increasing evidence has identified DEK as an oncogene and is overexpressed in multiple cancers.[29–34] Overexpression of DEK is implicated in tumor progression, especially the proliferation and migration of cancer cells. Highly expressed DEK was suggested as an unfavorable prognostic biomarker for cancer patients.[35–37] Inhibition of DEK suppressed tumorigenicity and might be a good strategy for cancer intervention. Increasing evidence has illustrated that DEK was targeted by miRNAs and suppressed the cancer development.[38,39] MiR-1204 inhibited the tumorigenesis of non-small cell lung cancer via regulating DEK.[40] Additionally, recent study found that miR-592 suppressed the growth of hepatocellular carcinoma by down-regulating DEK.[38] In the present study, miR-138-5p bound the 3′-UTR of DEK and reduced the expression of DEK in GC cells. DEK was overexpressed in GC tissues and negatively correlated with that of miR-138-5p. Higher level of DEK was also found in GC patients with lymph node metastasis. Restoration of DEK significantly eliminated the inhibitory effects of miR-138-5p on the proliferation and cell cycle progression of GC cells.

In conclusion, the findings of our study provided novel insights into the function of dysregulated miR-138-5p in the progression of GC by targeting DEK. These results

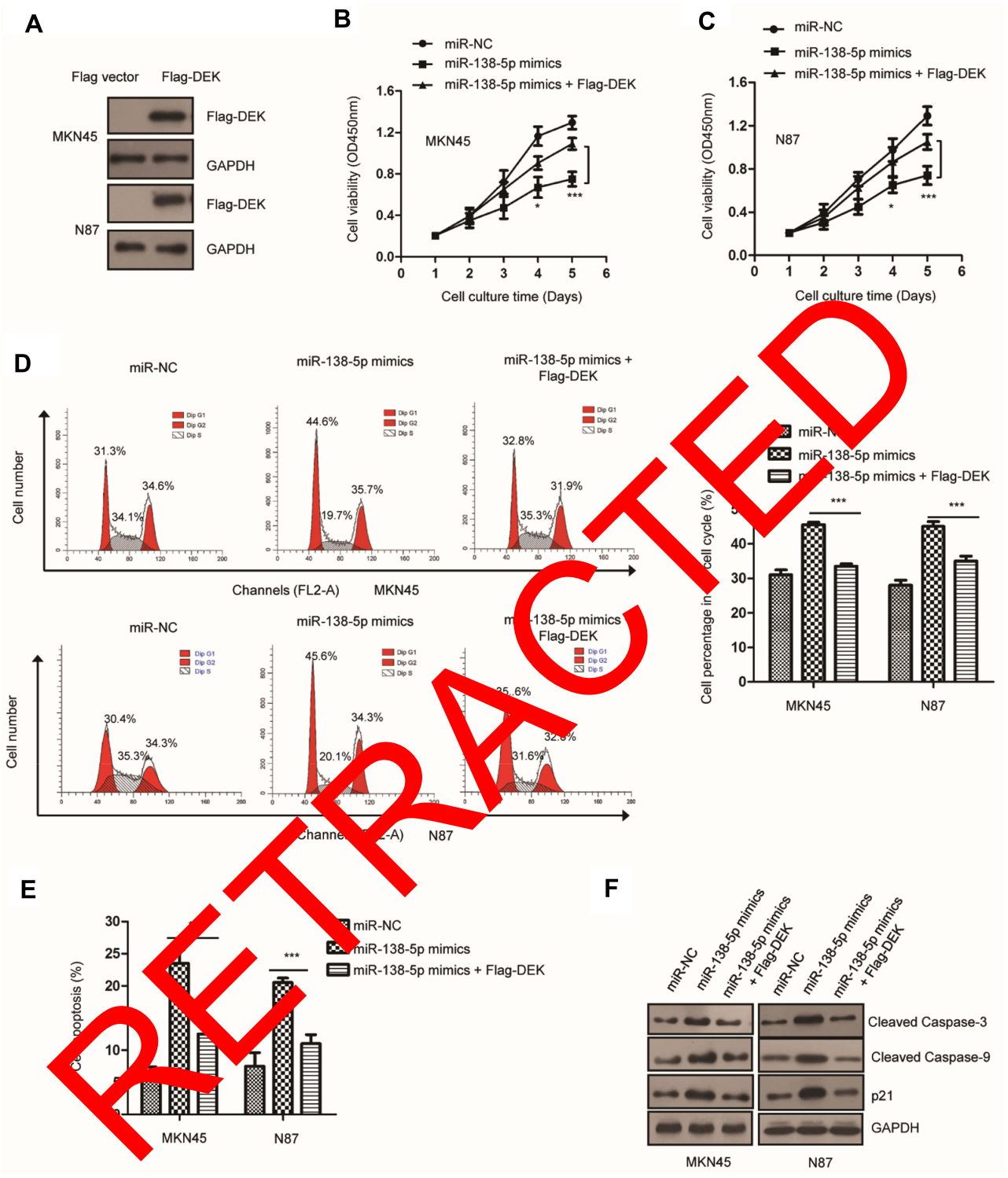

Figure 4 Restoration of DEK reversed the suppressive function of miR-138-5p in GC. (**A**) The transfection efficiency of ectopic expressed Flag-DEK was confirmed by Western blot with anti-Flag antibody. (**B** and **C**) CCK-8 assay was performed to analyze the proliferation of MKN45 and N87 cells that transfected with miR-NC, miR-138-5p mimics or the combination with Flag-DEK. **P*<0.05, ****P*<0.001 vs cells transfected with miR-138-5p mimics alone. (**D**) Overexpression of DEK attenuated the G_1 cell cycle arrest induced by miR-138-5p mimics in GC cells. ****P*<0.001 vs cells transfected with miR-138-5p mimics alone. (**E**) Restoration of DEK significantly reversed miR-138-5p-induced apoptosis of MKN45 and N87 cells. ****P*<0.001 vs cells transfected with miR-138-5p mimics alone. (**F**) Overexpression of miR-138-5p promoted the cleaved caspase-3/9 and the level of p21, while restoration of DEK attenuated the accumulation of cleaved caspase-3/9 and p21 in GC cells. Data were obtained from three independent experiments and presented as the mean ± standard deviation.

suggested miR-138-5p as a possible therapeutic target to improve the outcome of patients with GC.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data, drafting the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that there are no conflicts of interest regarding this paper.

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