Am J Cancer Res 2023;13(6):2670-2680
www.ajcr.us /ISSN:2156-6976/ajcr0147835

Original Article

MiR-410-3p suppresses primary gastric cancer and exosomes regulate endogenous expression of miR-410-3p

Michelle Xin Liu[1], Kent-Man Chu[1,2]

[1]Department of Surgery, The University of Hong Kong, Hong Kong, China; [2]Department of Surgery, Queen Mary Hospital, Hong Kong, China

Received November 17, 2022; Accepted March 23, 2023; Epub June 15, 2023; Published June 30, 2023

Abstract: MicroRNAs play significant roles in cancer initiation and progression. Exosomes are important extracellular vesicles for transporting molecules to distant sites. This study aims to investigate the functional roles of miR-410-3p in primary gastric cancer, as well as the roles of exosomes in regulating expression of miR-410-3p. In this study, forty-seven pairs of human gastric cancer tissue samples were collected. Endogenous expression of miR-410-3p in tissue samples and cell lines, and expression of exosomal miR-410-3p in cell culture medium were evaluated by RT-qPCR. Functional assays including cell proliferation assay by MTT, cell migration and invasion assay by transwell, and cell adhesion assay were performed. Targets of miR-410-3p were screened. Cell culture medium of culturing cell lines established from stomach (AGS and BCG23) was applied for culturing cell lines established from other sites (MKN45 and HEK293T). It was found that miR-410-3p was significantly downregulated in gastric cancer. Overexpression of miR-410-3p inhibited gastric cancer cell proliferation, migration, and invasion. MiR-410-3p mimic enhanced cell adhesion. HMGB1 was a target of miR-410-3p in primary gastric cancer. Expression of exosomal miR-410-3p in cell culture medium was dramatically higher than its endogenous expression. Exosomes from cell culture medium of AGS or BCG23 regulated endogenous expression of miR-410-3p in MKN45. In conclusion, miR-410-3p functioned as a tumor suppressor in primary gastric cancer. MiR-410-3p was higher expressed in exosomes of cell culture medium than its endogenous expression in cells. Endogenous expression of miR-410-3p in a distant site could be regulated by exosomes from the original site.

Keywords: miR-410-3p, metastasis, targets, exosomes, gastric cancer

Introduction

Gastric cancer is the third leading cause of cancer-associated death globally [1]. More than 70% of the worldwide burden of gastric cancer is concentrated in Eastern Asia [2, 3]. Currently, gastrectomy with adjuvant chemotherapy is applied for most of the patients with gastric cancer [4, 5]. However, quite a number of the patients in Stage II or Stage III will develop haematogenous metastasis subsequently even if they undergo a potential curative resection [6, 7]. Most of the metastatic patients are diagnosed until they develop clinical signatures and/or diagnostic images. Therapy for the patients in this situation is limited, making the prognosis of these patients very poor [8]. Metastasis is thus a fatal character of gastric cancer. And promising biomarkers for monitoring metastatic status of gastric cancer is in pressing need. Development of these biomarkers will be beneficial to predict development of metastasis of gastric cancer in order to adjust therapy, reduce mortality rate and improve prognosis.

Previous studies have revealed that dysregulation of miRNAs is actively involved in initiation and progression of gastric cancer [9, 10]. MiRNAs belong to a group of small non-coding RNAs around 18-25 nucleotides in length. They bind to complementary sequences in the 3'-untranslated regions (3'-UTR) of target mRNAs to induce degradation or translational repression [11, 12]. MiRNAs are tissue specific, and even cell specific within those tissues. They

are potentially useful for diagnosis, predicting clinical outcome, or acting as therapeutic targets in patients with cancer [13, 14]. The unique pattern of microRNAs in gastric cancer provides the possibility of applying miRNAs as biomarkers and therapeutic targets for monitoring gastric cancer.

Interestingly, a number of studies indicated that metastasis is an early event in cancer development. Primary cancers would create a favorable microenvironment in secondary organs and/or tissue sites for further metastasis. It is called the seed (pre-metastatic niche) and soil (secondary sites) theory [15]. To transfer the seed to its appropriate soil, primary cancers secret extracellular vesicles [16]. Exosomes are one type of extracellular vesicles. Intriguingly, exosomes secreted from primary cancer cells have a distinct genetic and epigenetic makeup, allowing them to undertake their tumorigenic function [17, 18].

In our previous study, we found that expression of exosomal miR-410-3p was significantly higher in serum of the patients with gastric cancer who developed haematogenous metastasis subsequent of resection. However, expression of endogenous miR-410-3p was significantly downregulated in gastric cancer tissue samples. Expression of endogenous miR-410-3p was much lower in gastric cancer cell lines comparing to its expression in exosomes in cell culture medium. It suggested that miR-410-3p might be translocated from gastric cancer cells to circulation by exosomes [19].

In this study, we investigated the functional roles of miR-410-3p in primary gastric cancer. We also evaluated the expression of miR-410-3p in gastric cancer cell lines and cell culture medium. The culture medium of cell lines established from original site (stomach) was applied for culturing other cell lines, to investigate whether exosomal miRNAs in culture medium could regulate endogenous expression of miRNAs in distant cells. This study will contribute to elucidate the mechanism of down-regulation of miRNAs, cancer metastasis, as well as to provide a potential therapeutic target for gastric cancer.

Materials ad methods

Human tissue samples

Forty-seven pairs of human gastric cancer and non-tumor tissue samples were collected directly after the surgical resection at Queen Mary Hospital, Hong Kong. All of the tumor tissue samples were validated to be malignant by experienced pathologist. All of the samples were obtained with the participants' informed consent and none of the patients received pre-operative treatment. All samples were immediately frozen in liquid nitrogen and stored at -70°C.

Cell lines and cell culture

Human gastric cancer cell lines AGS and SNU1, and human embryonic kidney HEK293T (ATCC, Rockville, MD, USA), MKN45 (RIKEN, Japan) and BCG23 (from Beijing Cancer Institute) were used in this study. Cell lines AGS, SNU1, BCG23 were established from gastric cancer tissue samples of original site (stomach). MKN45 was established from gastric cancer metastasized to liver. Cells were cultivated in RPMI1640 medium (Gibco BRL, Gaithersburg, MD, USA) supplemented with 10% exosomes-depleted fetal bovine serum (FBS) (SBI, System Biosciences, USA). All cells were incubated at 37°C in a humidified incubator which contains 5% CO_2.

miR-410-3p mimic and transfection

The specific miR-410-3p mimic (miRIDIAN™ microRNA Mimics, C-300740-03-0020) was purchased from Dharmacon™ (USA). 1×10^5 cells were seeded into a 6-well plate a day in advance of transfection and transfected with 20 nM mimic or scrambled control using Hiperfect Transfection Reagent (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Transient transfected cells were applied for evaluation of expression levels and functional assays.

Extraction of miRNA and exosomal miRNA

Around 20 mg of each tissue sample or 10^6 cells of each cell line were applied for miRNA extraction via miRNeasy Mini Kit (Qiagen, Hilden, Germany); following the manufacturer's instructions. The concentrations of all miRNA samples were quantified by NanoDrop 1000 (Nanodrop, Wilmington, Delaware, USA). 500 ng of total RNA from each sample were utilized for reversed transcript (RT).

Exosomal miRNAs were extracted using the SeraMir exosome RNA Kit (SBI, Mountain View, CA, USA), following the manufacturer's instructions. The quality and quantity of the miRNAs

were measured by NanoDrop 1000. The same amount of culture medium was applied for extraction of exosomal miRNAs. The same amount of miRNAs according to NanoDrop concentration was applied for Reverse Transcription (RT). This was to make the measurements among different groups were comparable.

RT-qPCR

Total exosomal miRNAs were reverse transcribed to cDNA using miRCURY LNA™ RT Kit (Exiqon), following the manufacturer's instructions. Quantitative PCR was performed using miRCURY LNA™ SYBR Green Mix (Exiqon) in Vii7A real-time PCR system (Applied Biosystems). The miRNA-specific primer sequences were provided by Exiqon based on the miRNA sequences obtained from the miRBase database. Melting curve analyses were performed at the end of the PCR cycles. Fold changes in expression of miR-410-3p were calculated by a comparative threshold cycle (Ct) method using the formula: $2^{-[\Delta Ct\ (Internal\ Control) - \Delta Ct\ (sample)]}$. MiR-16-5p and MiR-93-5p were applied as internal controls for exosomal miR-410-3p. U6 was applied as an internal control for miR-410-3p in tissue samples and cell lines.

Cell proliferation assay

MTT assay was used for cell proliferation assay. 5,000 cells/well were seeded in 96-well plates with transfection of miR-410-3p mimic or scrambled control. After 24 hr and 48 hr, the culture medium was discarded and restained with 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium romide (MTT) (5 mg/ml) for 3 hours. The absorbance was measured at 570 nm on a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific).

Cell migration and invasion assay

Cells with transfection of miR-410-3p mimic or scrambled control for 24 hours were harvested and suspended in RPMI 1640 medium. Transwell culture inserts with 8 μm pore size membrane for 24-well plate (Cat no. 353097, Falcon, NY, USA) were used to analyze the migration activity. Matrigel-coated transwell chambers with 8 μm pore size membrane for 24-well plate (Corning, NY, USA) were applied in invasion assay. 30,000 suspension of cells in 300 μl of serum-free RPMI 1640 medium were seeded into the upper inserted chamber, 700 μl of RPMI containing 10% FBS were added to the well. The plate was then incubated at 37°C for 48 hours. After incubation, the inner wall of the chamber was wiped with swabs to remove un-migrated or on-invaded cells. The outer wall of the chamber was gently rinsed with PBS and stained with Crystal Violet (Sigma-Aldrich, St. Louis, MO) for 10 minutes. Finally, the membrane was rinsed and allowed to air-dry. The stained membrane was photographed and the number of migrated cells was counted.

Cell adhesion assay

Cells with transfection of miR-410-3p mimic or scrambled control for 48 hours were harvested and applied for cell adhesion assay (CBA-070, Cell Biolabs, CA, USA). A 48-well plate with wells coated with Collagen I, Collagen IV, Laminin, Fibronectin, Fibrinogen or BSA was used. 50,000 cells in 150 μl of serum-free RPMI 1640 medium were seeded into the wells coated with ECM. The same number of cells was also seeded into wells coated with BSA as negative controls. The plate was incubated at 37°C for 1 hour, followed by aspiration of the medium from each well and washed by PBS to remove the un-adhesive cells. The wells were stained with the Cell Stain Solution for 10 minutes. The adhesive stained cells were photo-captured. After that, the stained cells were extracted by the Extraction Solution. Lastly, each extracted sample was transferred to a 96-well plate and measured the O.D. 570 nm on a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific).

Targets prediction and analysis

Potential targets of miR-410-3p were predicted by TargetScan (http://www.targetscan.org/), miRDB (http://www.mirdb.org/), and miRANDA (http://www.microrna.org/). There were 601 predicted targets from TargetScan, 1,118 from miRDB, and 8,150 from miRanda. And the overlap numbers of potential targets were 289 in total. Clusters of functions and signal pathways of the potential targets were analyzed by PantherDB (http://www.pantherdb.org/).

Western blot

Western blot was performed for validation of HMGB1 as a target of miR-410-3p. Briefly, protein was extracted and lysed by RIPA Buffer (Sigma Chemical Co., St. Louis, MD, USA).

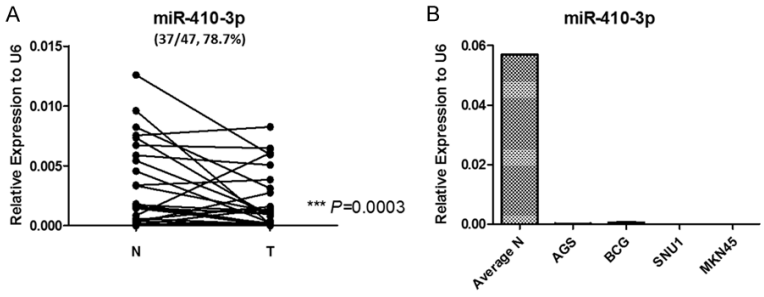


Figure 1. Expression of miR-410-3p in gastric cancer tissue samples and cell lines. A. Expression of miR-410-3p was significantly downregulated in gastric cancer tissue samples (N=47 pairs, N: Non-tumor tissue, T: Tumor Tissue; downregulated in 37 out of 47 pairs, 78.7%, $^{***}P$=0.0003). B. Expression of miR-410-3p was significantly downregulated in gastric cancer cell lines AGS, BCG23, SNU1, and MKN45, comparing with its average expression in non-tumor tissue samples.

Samples containing equal amounts of protein was separated by SDS-PAGE and electro blotted onto Immobilon-P Transfer Membrane (Applied Biosystems). The membrane was blocked with 5% no-fat milk, followed by incubation with antibody specific for anti-HMGB1 (1:1000, Cell-Signaling Technology, Beverly, MA, USA) and anti-β-actin (1:10000, Cell Signaling Technology, Beverly, MA, USA), respectively. Blots were then incubated with anti-rabbit or anti-mouse secondary antibody conjugated to horseradish peroxidase (Amersham Pharmacia, Cleveland, OH) accordingly. The signals were captured by ThermoFisher MyECL digital development system and Fiji film development system.

Statistics

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Student's t test was used to analyze the results expressed as mean ± SD. Wilcoxon sign rank test was used to analyze miR-410-3p in down-regulation of gastric cancer. All *P*-values were two-sided and a value of $P \leq 0.05$ is considered statistically significant.

Results

miR-410-3p was downregulated in gastric cancer tissue samples and cell lines

Expression of miR-410-3p was evaluated in 47 pairs gastric cancer/non-tumor tissue samples by RT-qPCR. The result indicated that miR-410-3p was significantly down-regulated in gastric cancer tissues samples comparing with non-tumor ones (N=47 pairs, downregulated in 37 out of 47 pairs, 78.7%, $^{***}P$=0.0003, **Figure 1A**). In addition, miR-410-3p was significantly downregulated in gastric cancer cell lines AGS, BCG23, SNU1, and MKN45, comparing with its average expression in non-tumor tissue samples (**Figure 1B**). This indicated that expression of miR-410-3p was significantly decreased in gastric cancer.

miR-410-3p mimic inhibited gastric cancer cell proliferation, migration and invasion

MiR-410-3p mimic was transfected into gastric cancer cell lines established from original site (stomach), including AGS and BCG23. Expression of miR-410-3p in transfected cell lines was evaluated by qPCR. The result showed that expression of miR-410-3p in these cell lines was dramatically increased in a time-course manner, and lasted for at least 72 hours (**Figure 2A**). The increase of miR-410-3p was specific as there was almost no increase of other miRNAs in the same transfection ($^{**}P$<0.01, Supplementary Figure 1).

The transfected cell lines were then subjected to functional assays. Cells with miR-410-3p mimic or scrambled control were subjected to MTT assay to evaluate cell proliferation. The result showed overexpression of miR-410-3p significantly inhibited gastric cancer cell proliferation by around 20% for AGS and BCG23 in 48 hours ($^{*}P$<0.05, $^{**}P$<0.01, **Figure 2B**).

Cells were pre-treated with Mitomycin to inhibit cell proliferation before subjecting to migration/invasion assay. The migrated cells were stained and counted under a microscopy. The representative images of stained cells with scrambled control or miR-410-3p mimic were showed in **Figure 2C**. Migrated cell numbers were also indicated in **Figure 2C**. The migration assay revealed that migrated gastric cancer cells were significantly decreased by 40%-60% with overexpression of miR-410-3p ($^{**}P$<0.01 for AGS and $^{*}P$<0.05 for BCG23, **Figure 2C**). For

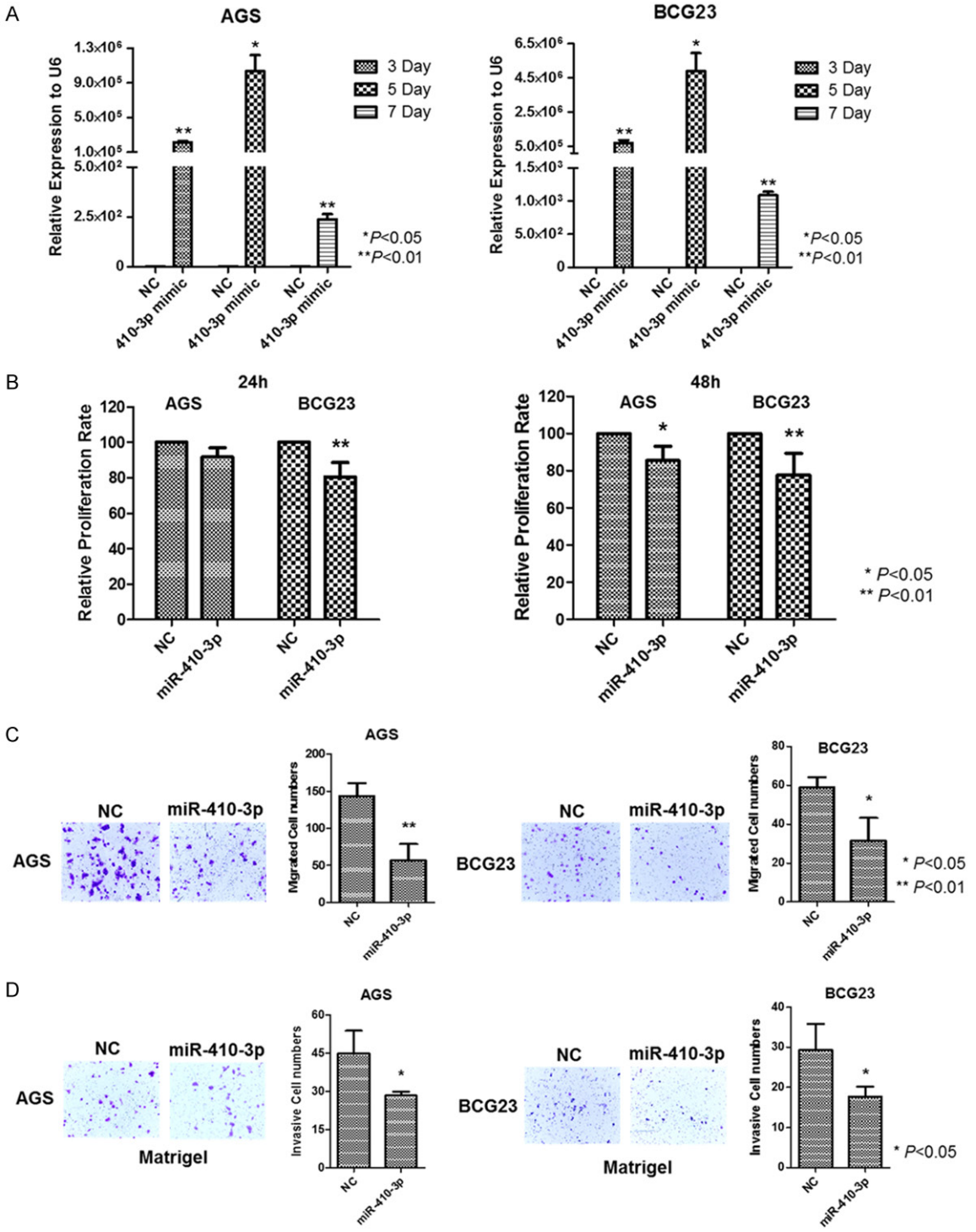


Figure 2. The effect of miR-410-3p mimic in gastric cancer cell proliferation, migration and invasion. (A) A time-course manner of the transfection of control and miR-410-3p for 3 days, 5 days and 7 days. Overexpression of miR-410-3p was significant in AGS and BCG23 cell lines (*P<0.05, **P<0.01). (B) Overexpression of miR-410-3p significantly inhibited cell proliferation of AGS and BCG23. Overexpression of miR-410-3p significantly inhibited cell migration (C) and cell invasion (D) of AGS and BCG23. Migrated and invasive cell numbers were indicated in the figures (*P<0.05, **P<0.01).

invasion assay, the transwell chambers were coated with Matrigel to mimic the situation of basement membrane in tumor microenvironment. The invasion assay showed that the inva-

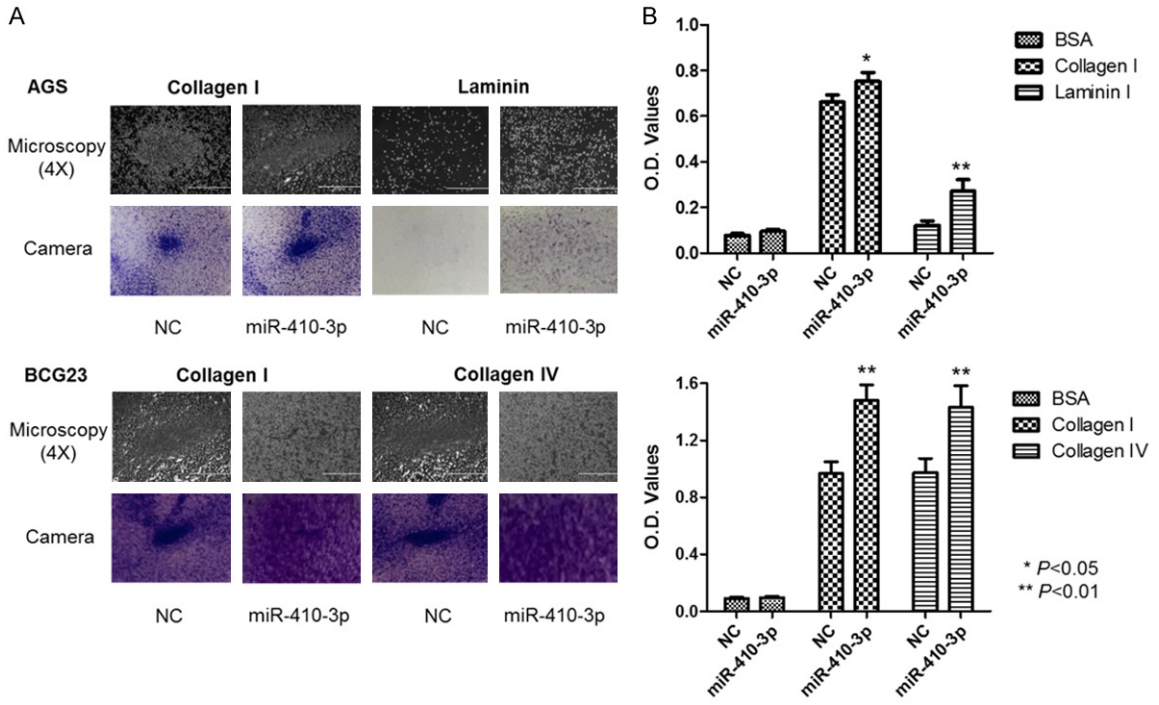


Figure 3. The effect of miR-410-3p mimic in gastric cancer cell adhesion to extracellular matrix. A. Representative images under microscopy (4X) and camera of cell adhesion to certain extracellular matrix (ECM) including Collagen I, Collagen IV and Laminin. B. Colorimetric O.D. values of cell adhesion to ECM of adhesion assay ($^{*}P<0.05$, $^{**}P<0.01$).

sive gastric cancer cells were significantly decreased with overexpression of miR-410-3p in AGS and BCG23 cells by around 30%-40% ($^{*}P<0.05$, **Figure 2D**). The results indicated that overexpression of miR-410-3p inhibited gastric cancer cell proliferation, migration, and invasion.

miR-410-3p mimic enhanced gastric cancer cell adhesion to ECM

In contrast, the transfected cells were subjected to adhesion assay of extracellular matrix (ECM), including Collagen I, Collagen IV, Laminin, Fibronectin and Fibrinogen, with BSA as a blank control. Cells with miR-410-3p mimic or scrambled control were allowed to attach to ECM for an hour, after which cells were stained and washed. The stained cells were photographed under a microscopy and a camera (**Figure 3A**). The stained cells were extracted by solution to evaluate the colorimetric O.D. value. The result indicated that miR-410-3p mimic enhanced AGS cell attachment to Collagen I and Laminin, and enhanced BCG23 cell attachment to Collagen I and Collagen IV ($^{*}P<0.05$, $^{**}P<0.01$, **Figure 3B**).

The above results showed that overexpression of miR-410-3p inhibited gastric cancer cell proliferation, migration and invasion, while enhanced cell attachment to certain ECM. It indicated that miR-410-3p functioned as a tumor suppressor in primary gastric cancer.

Targets of miR-410-3p and analysis of signaling pathways

Targets of miR-410-3p were predicted by TargetScan, miRDB, and miRANDA. There were 601 predicted targets from TargetScan, 1,118 from miRDB, and 8,150 from miRanda. And the overlap numbers of potential targets were 289 in total (Supplementary Table 1). Clusters of functions and signal pathways of the potential targets were analyzed by PantherDB. The analysis revealed that functions of the targets were associated with cancer initiation and progression, such as cell proliferation, biological adhesion and cellular communication. There were also functions associated with cell secretion,

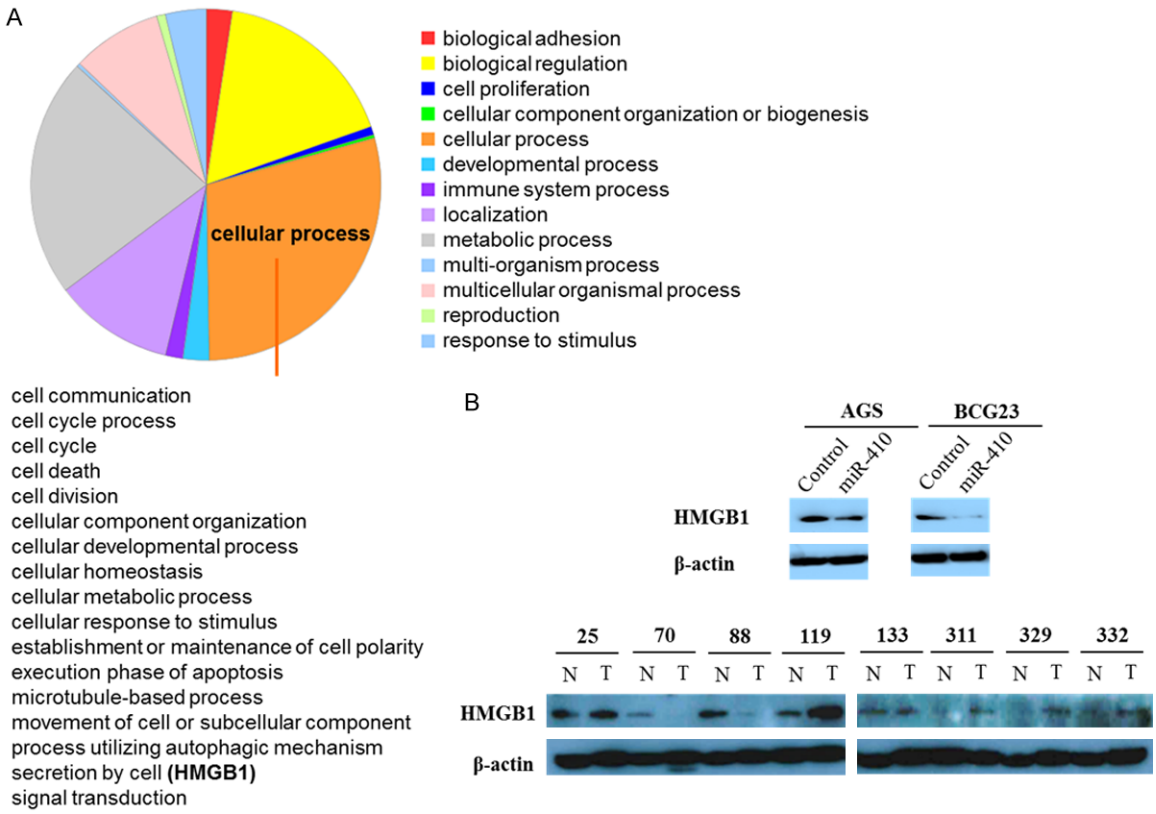


Figure 4. Targets of miR-410-3p and protein expression of HMGB1 in gastric cancer cells. A. Biological classification of targets predicted by TargetScan, miRANDA, and miRDB for miR-410-3p. Potential targets of each classification were indicated. B. Western blot of protein expression of HMGB1. Protein expression of HMGB1 was downregulated with overexpression of miR-410-3p in gastric cancer cell lines AGS and BCG23. Representative figures of protein expression of HMGB1 in paired gastric cancer tissue samples by western blot.

such as cell localization and cell transportation (**Figure 4A**).

miR-410-3p regulated cell mobility and transportation via HMGB1

Among the targets, HMGB1 (high mobility group box-1) was associated with cell mobility/movement. HMGB1 mRNA level in gastric cancer cells with overexpression of miR-410-3p was evaluated by RT-qPCR. The result indicated that HMGB1 mRNA was downregulated by miR-410-3p mimic (Supplementary Figure 2). Protein expression of HMGB1 in these cells was also evaluated by western blot. The result showed that protein expression of HMGB1 could be significantly suppressed by miR-410-3p in AGS and BCG23 cells comparing with scrambled control (**Figure 4B**, upper panel). Protein expression of HMGB1 also indicated that HMGB1 was highly expressed in gastric cancer tissue samples comparing with non-tumor tissue samples by western blot (**Figure 4B**, lower panel). It suggested that HMGB1 was a downstream target of miR-410-3p in primary gastric cancer. Downregulation of miR-410-3p led to increase of HMGB1, further led to enhancement of gastric cancer cell mobility.

miR-410-3p was higher expressed in exosomes of cell culture medium than in cancer cells

The endogenous expression of miR-410-3p in AGS, BCG23 and SNU1, as well as expression of exosomal miR-410-3p in culture medium of these cells, were evaluated by qPCR. Both miR-16-5p and miR-93-5p were used as internal controls. The expressions of these two miRNAs were abundant and relatively consistent in cell lines and exosomes of cell culture medium [19]. This indicated that these two miRNAs highly expressed in cells and were secreted by exosomes from cells to cell culture medium.

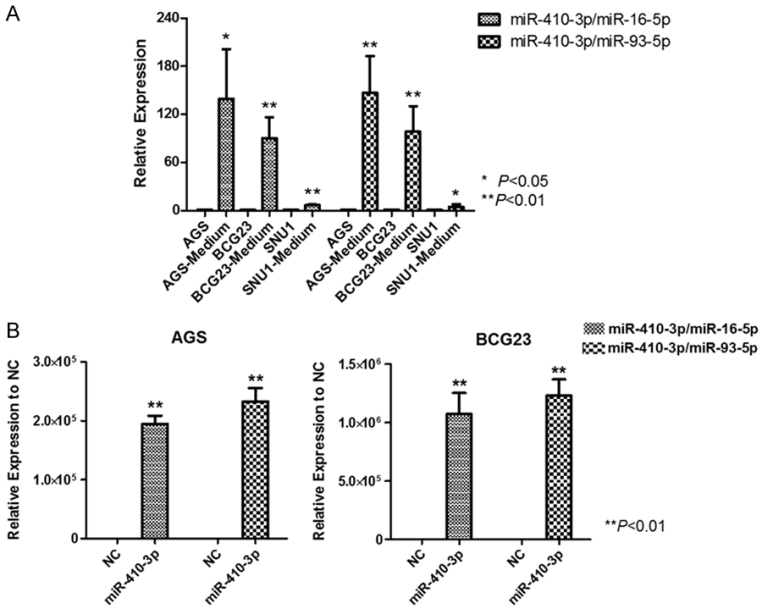


Figure 5. Expression of exosomal miR-410-3p in gastric cancer cell culture medium. A. Expression of exosomal miR-410-3p in cell culture medium was significantly higher than its endogenous expression in gastric cancer cells AGS, BCG23, and SNU1 (*P<0.05, **P<0.01). B. Expression of exosomal miR-410-3p was significantly higher in the cell culture medium with overexpression of miR-410-3p comparing to scrambled control (**P<0.01). MiR-16-5p and miR-93-5p were applied as internal controls for exosomal miRNAs.

The result showed that the expression of exosomal miR-410-3p in cell culture medium was significantly higher than its endogenous expression in gastric cancer cells (*P<0.05, **P<0.01, **Figure 5A**). It suggested that miR-410-3p was secreted by exosomes from gastric cancer cells into cell culture medium. This could be a mechanism of downregulation of miR-410-3p in primary gastric cancer.

MiR-410-3p mimic or scrambled control was transfected into AGS or BCG23 cells for 7 days. The expression of exosomal miR-410-3p in culture medium of AGS or BCG23 was evaluated by qPCR. The result showed that expression of exosomal miR-410-3p was significantly higher in the culture medium of cells with overexpression of miR-410-3p comparing with scrambled control (**P<0.01, **Figure 5B**). It suggested that overexpression of miR-410-3p in gastric cancer cells secreted miR-410-3p via exosomes into cell culture medium. The result was consistent with expression of exosomal miR-410-3p was higher in cell culture medium than endogenous expression of miR-410-3p in gastric cancer cells.

Endogenous expression of miR-410-3p in MKN45 and HEK293T cells

As cell culture medium of AGS or BCG23 contained highly expressing exosomal miR-410-3p, such cell culture medium was used to culture MKN45 and a human embryonic kidney cell line HEK293T. The endogenous expression of miR-410-3p was evaluated in these cell lines cultured with AGS or BCG23 medium. The result showed that miR-410-3p was higher expressed (with lower Ct) in MKN45 with cell culture medium of AGS since week 2 and BCG23 since week 1 (*P<0.05, **P<0.01, **Figure 6A**). The Ct values of miR-16-5p and miR-93-5p were applied as controls to show the alterations of miRNAs other than miR-410-3p (**Figure 6A**). It suggested that miR-410-3p in exosomes from AGS or BCG23 culture medium could enter MKN45 cells to make higher endogenous expression of miR-410-3p. But change of Ct value was not obvious in HEK293T cells (**Figure 6B**). It suggested that attraction of exosomes into cells was different and depending on cell types.

Discussion

In this study, we found that miR-410-3p was significantly downregulated in primary gastric cancer tissue samples and cell lines. Overexpression of miR-410-3p inhibited primary gastric cancer cell proliferation, migration, and invasion. In contrast, miR-410-3p enhanced cell adhesion to certain extracellular matrix including Collagen I, Collagen IV, and Laminin. In our previous study, we found that exosomal miR-410-3p was higher expressed in patients with gastric cancer who developed haematogenous metastasis after surgery, comparing with the patients with no distant metastasis. In this study, we further indicated that miR-410-3p was higher expressed in exosomes of gastric cancer cell culture medium than its endog-

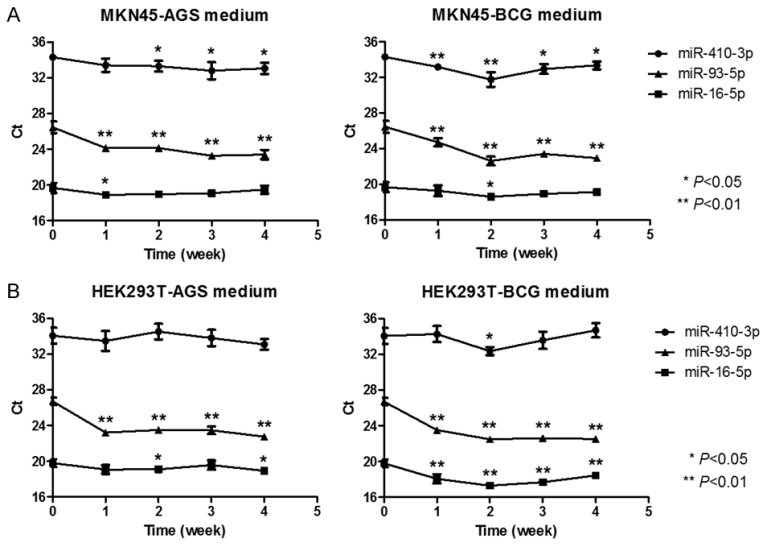


Figure 6. Endogenous expression of miR-410-3p in MKN45 and HEK293T cells. A. Endogenous expression of miR-410-3p was higher expressed (as indicated with lower Ct) in MKN45 cells with cell culture medium of AGS since week 2 and BCG23 since week 1 ($^{*}P<0.01$, $^{**}P<0.05$). B. Endogenous expression of miR-410-3p was not obviously changed in HEK293T cells with cell culture medium of AGS or BCG23. The Ct values of miR-16-5p and miR-93-5p were also showed to indicate the alterations of miRNAs other than miR-410-3p.

enous expression in gastric cancer cells. Endogenous expression of miR-410-3p could be altered when MKN45 cells were cultured with cell culture medium of AGS or BCG23. This suggested that miRNAs could be secreted from gastric cancer cells into cell culture medium via exosomes. Exosomes containing miRNAs could enter recipient cells to execute their roles in regulating expressions and functions of the recipient cells.

In addition, HMGB1 was predicted to be a downstream target of miR-410-3p by algorithm tools including TargetScan, miRDB, and miRANDA. It has been reported that HMGB1 plays significant roles in various cancers, including liver cancer, lung cancer and colorectal cancer [20-23]. Expression of HMGB1 has been reported to be overexpressed in gastric cancer [24, 25]. It has also been shown that HMGB1 functioned as an oncogene in gastric cancer. Upregulation of HMGB1 leads to more cell proliferation, angiogenesis, and metastasis in gastric cancer [26-28]. In this study, we found that overexpression of miR-410-3p mimic inhibited mRNA and protein expression of HMGB1 in gastric cancer cells. As HMGB1 contributed to cell proliferation/invasion/migration, downregulation of miR-410-3p would induce gastric cancer development via HMGB1. It might also enhance gastric cancer cell movement in circulation. This at least partly contributed to gastric cancer progression.

It has been shown that miR-410-3p functions as a tumor suppressor in gastric cancer initiation and progression in its original site (stomach). It has also been reported that miR-410-3p functions as a tumor suppressor in breast cancer, osteosarcoma, and glioma [29-31]. In contrast, miR-410-3p plays an oncogenic role in liver cancer, lung cancer, and colorectal cancer [32-34]. In the current study, we showed that exosomes could translocate miR-410-3p from AGS or BCG23 cells to MKN45 cells through cell culture medium. It suggested that exosomes could transfer molecules from stomach to distant sites via blood circulation. MiR-410-3p translocating by exosomes could regulate its targets in recipient cells to make the tumor microenvironment favorite for later metastasis.

As tumor suppressive miRNAs are downregulated in various cancers, delivery of these miRNAs can be one type of cancer therapies [35-37]. But it should be cautious to deliver tumor suppressor miRNA as therapeutic targets, unless this miRNA can be delivered to a specific site. As in this study, we found that miR-410-3p was a tumor suppressor in primary gastric cancer. However, miR-410-3p was an oncogene in other organs such as in liver or lung. As miR-410-3p was a double sword and it needs more evidence to elucidate its complicated roles in cancer initiation and progression [38].

Further study is needed to elucidate the repression of miR-410-3p in gastric cancer in order to restore the expression of miR-410-3p in stomach. Inhibition of secretion of tumor suppressive miRNAs by exosomes might be a better way for target therapy for gastric cancer.

Conclusion

In conclusion, miR-410-3p was significantly downregulated in gastric cancer. It functioned as a tumor suppressor in primary gastric cancer. MiR-410-3p was higher expressed in exosomes in cell culture medium than in gastric cancer cells. MiR-410-3p could be translocated into distant cells via exosomes. Exosomes played a significant role in translocating miRNAs from primary site to distant sites through circulation. Exosomal miRNAs open a new avenue for elucidating the mechanism of metastasis, also for providing therapeutic targets for gastric cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Kent-Man Chu, Department of Surgery, E501, Queen Mary Hospital, Pokfulam, Hong Kong, China. Tel: +852-22554637; Fax: +852-22554221; E-mail: chukm@hku.hk; Dr. Michelle Xin Liu, Department of Surgery, The University of Hong Kong, L9-57, Laboratory Block, 21 Sassoon Road, Pokfulam, Hong Kong, China. Tel: +852-39179629; Fax: +852-39179629; E-mail: melx1301@hku.hk

References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[2] Ajani JA, Lee J, Sano T, Janjigian YY, Fan D and Song S. Gastric adenocarcinoma. Nat Rev Dis Primers 2017; 3: 17036.

[3] O'Connor A, O'Morain CA and Ford AC. Population screening and treatment of Helicobacter pylori infection. Nat Rev Gastroenterol Hepatol 2017; 14: 230-240.

[4] Fujitani K, Tamura S, Kimura Y, Matsuyama J, Imamura H, Yamamoto K, Fujita J, Iijima S, Ueda S, Kurokawa Y, Shimokawa T and Satoh T; Osaka Gastrointestinal Cancer Chemotherapy Study Group. Five-year outcomes of a phase II study of adjuvant chemotherapy with S-1 plus docetaxel for stage III gastric cancer after curative D2 gastrectomy (OGSG1002). Gastric Cancer 2020; 23: 520-530.

[5] Yang LP, Wang ZX, He MM, Wu HX, Yuan SQ, Wang W, Jin Y, Ren C, Wang ZQ, Wang FH, Li YH, Wang F and Xu RH. A real-world evidence of efficacy of palliative gastrectomy plus chemotherapy in metastatic gastric cancer patients. Cancer Manag Res 2019; 11: 3993-4003.

[6] Spolverato G, Ejaz A, Kim Y, Squires MH, Poultsides GA, Fields RC, Schmidt C, Weber SM, Votanopoulos K, Maithel SK and Pawlik TM. Rates and patterns of recurrence after curative intent resection for gastric cancer: a United States multi-institutional analysis. J Am Coll Surg 2014; 219: 664-675.

[7] Eom BW, Yoon H, Ryu KW, Lee JH, Cho SJ, Lee JY, Kim CG, Choi IJ, Lee JS, Kook MC, Park SR, Nam BH and Kim YW. Predictors of timing and patterns of recurrence after curative resection for gastric cancer. Dig Surg 2010; 27: 481-486.

[8] Li JH, Zhang SW, Liu J, Shao MZ and Chen L. Review of clinical investigation on recurrence of gastric cancer following curative resection. Chin Med J (Engl) 2012; 125: 1479-1495.

[9] Lee YS and Dutta A. MicroRNAs in cancer. Annu Rev Pathol 2009; 4: 199-227.

[10] Peng Y and Croce CM. The role of MicroRNAs in human cancer. Signal Transduct Target Ther 2016; 1: 15004.

[11] Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y and Bentwich Z. Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 2005; 37: 766-770.

[12] Berezikov E, Guryev V, van de Belt J, Wienholds E, Plasterk RH and Cuppen E. Phylogenetic shadowing and computational identification of human microRNA genes. Cell 2005; 120: 21-24.

[13] Wang X, He Y, Mackowiak B and Gao B. MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. Gut 2021; 70: 784-795.

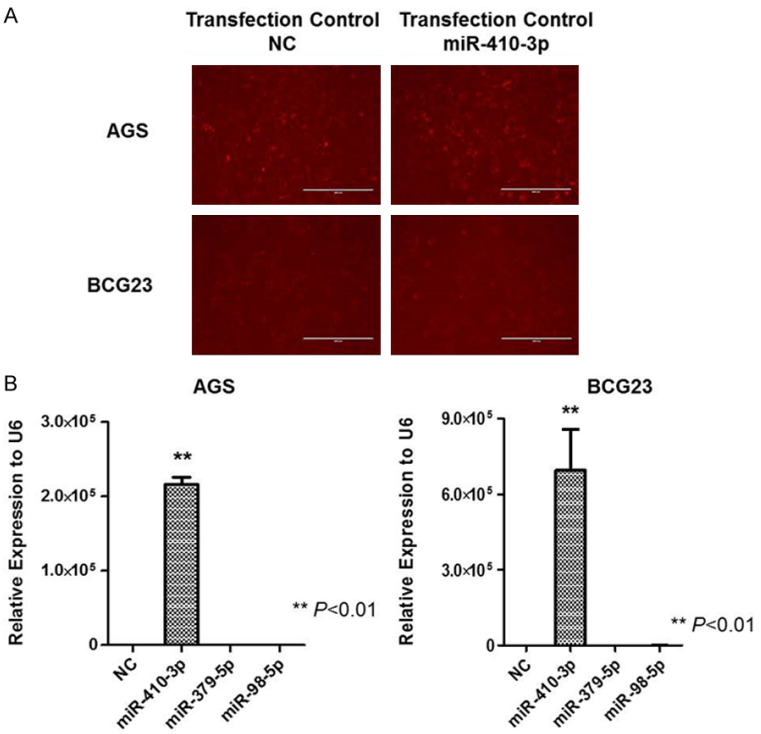
[14] Takahashi RU, Prieto-Vila M, Kohama I and Ochiya T. Development of miRNA-based therapeutic approaches for cancer patients. Cancer Sci 2019; 110: 1140-1147.

[15] Liu Y and Cao X. Characteristics and significance of the pre-metastatic niche. Cancer Cell 2016; 30 : 668-681.

[16] Dos Anjos Pultz B, Andrés Cordero da Luz F, Socorro Faria S, Peixoto Ferreira de Souza L, Cristina Brígido Tavares P, Alonso Goulart V, Fontes W, Ricardo Goulart L and José Barbosa Silva M. The multifaceted role of extracellular vesicles in metastasis: priming the soil for seeding. Int J Cancer 2017; 140: 2397-2407.

[17] Kalluri R. The biology and function of exosomes in cancer. J Clin Invest 2016; 126: 1208-1215.

[18] Ruivo CF, Adem B, Silva M and Melo SA. The biology of cancer exosomes: insights and new perspectives. Cancer Res 2017; 77: 6480-6488.
[19] Liu X and Chu KM. Exosomal miRNAs as circulating biomarkers for prediction of development of haematogenous metastasis after surgery for stage II/III gastric cancer. J Cell Mol Med 2020; 24: 6220-6232.
[20] Chen R, Zhu S, Fan XG, Wang H, Lotze MT, Zeh HJ 3rd, Billiar TR, Kang R and Tang D. High mobility group protein B1 controls liver cancer initiation through yes-associated protein -dependent aerobic glycolysis. Hepatology 2018; 67: 1823-1841.
[21] Liu PL, Liu WL, Chang JM, Chen YH, Liu YP, Kuo HF, Hsieh CC, Ding YS, Chen WW and Chong IW. MicroRNA-200c inhibits epithelial-mesenchymal transition, invasion, and migration of lung cancer by targeting HMGB1. PLoS One 2017; 12: e0180844.
[22] Qian F, Xiao J, Gai L and Zhu J. HMGB1-RAGE signaling facilitates Ras-dependent Yap1 expression to drive colorectal cancer stemness and development. Mol Carcinog 2019; 58: 500-510.
[23] Wu T, Zhang W, Yang G, Li H, Chen Q, Song R and Zhao L. HMGB1 overexpression as a prognostic factor for survival in cancer: a meta-analysis and systematic review. Oncotarget 2016; 7: 50417-50427.
[24] Yue Y, Zhou T, Gao Y, Zhang Z, Li L, Liu L, Shi W, Su L and Cheng B. High mobility group box 1/toll-like receptor 4/myeloid differentiation factor 88 signaling promotes progression of gastric cancer. Tumour Biol 2017; 39: 1010428317694312.
[25] He W, Tang B, Yang D, Li Y, Song W, Cheang T, Chen X, Li Y, Chen L, Zhan W, Li W and He Y. Double-positive expression of high-mobility group box 1 and vascular endothelial growth factor C indicates a poorer prognosis in gastric cancer patients. World J Surg Oncol 2013: 11: 161.
[26] Tang T, Wang S, Cai T, Cheng Z, Meng Y, Qi S, Zhang Y and Qi Z. High mobility group box 1 regulates gastric cancer cell proliferation and migration via RAGE-mTOR/ERK feedback loop. J Cancer 2021; 12: 518-529.
[27] Tian L, Wang ZY, Hao J and Zhang XY. miR-505 acts as a tumor suppressor in gastric cancer progression through targeting HMGB1. J Cell Biochem 2019; 120: 8044-8052.
[28] Chung HW and Lim JB. High-mobility group box-1 contributes tumor angiogenesis under interleukin-8 mediation during gastric cancer progression. Cancer Sci 2017; 108: 1594-1601.
[29] Wu H, Li J, Guo E, Luo S and Wang G. MiR-410 acts as a tumor suppressor in estrogen receptor-positive breast cancer cells by directly targeting ERLIN2 via the ERS pathway. Cell Physiol Biochem 2018; 48: 461-474.
[30] Zhao D, Jia P, Wang W and Zhang G. VEGF-mediated suppression of cell proliferation and invasion by miR-410 in osteosarcoma. Mol Cell Biochem 2015; 400: 87-95.
[31] Chen L, Zhang J, Feng Y, Li R, Sun X, Du W, Piao X, Wang H, Yang D, Sun Y, Li X, Jiang T, Kang C, Li Y and Jiang C. MiR-410 regulates MET to influence the proliferation and invasion of glioma. Int J Biochem Cell Biol 2012; 44: 1711-1717.
[32] Wang Y, Fu J, Jiang M, Zhang X, Cheng L, Xu X, Fan Z, Zhang J, Ye Q and Song H. MiR-410 is overexpressed in liver and colorectal tumors and enhances tumor cell growth by silencing FHL1 via a direct/indirect mechanism. PLoS One 2014; 9: e108708.
[33] Li D, Yang Y, Zhu G, Liu X, Zhao M, Li X and Yang Q. MicroRNA-410 promotes cell proliferation by targeting BRD7 in non-small cell lung cancer. FEBS Lett 2015; 589: 2218-2223.
[34] Wang W, He Y, Rui J and Xu MQ. miR-410 acts as an oncogene in colorectal cancer cells by targeting dickkopf-related protein 1 via the Wnt/beta-catenin signaling pathway. Oncol Lett 2019; 17: 807-814.
[35] O'Neill CP and Dwyer RM. Dwyer, Nanoparticle-based delivery of tumor suppressor microRNA for cancer therapy. Cells 2020; 9: 521.
[36] Boca S, Gulei D, Zimta AA, Onaciu A, Magdo L, Tigu AB, Ionescu C, Irimie A, Buiga R and Berindan-Neagoe I. Berindan-Neagoe, Nanoscale delivery systems for microRNAs in cancer therapy. Cell Mol Life Sci 2020; 77: 1059-1086.
[37] Rupaimoole R and Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203-222.
[38] Wen R, Umeano AC, Essegian DJ, Sabitaliyevich UY, Wang K and Farooqi AA. Role of microRNA-410 in molecular oncology: a double edged sword. J Cell Biochem 2018; 119; 8737-8742.



Supplementary Figure 1. Increased expression of miR-410-3p in transfected cell lines AGS and BCG23 was specific as there was almost no increase in other miRNAs in the same transfection experiment.

Supplementary Table 1. Targets of miR-410-3p overlapping in three databases

Ortholog of target gene	Representative transcript	Gene name
NPPC	ENST00000409852.1	natriuretic peptide C
DCTN6	ENST00000221114.3	dynactin 6
CBFB	ENST00000290858.6	core-binding factor, beta subunit
TRAPPC3	ENST00000373166.3	trafficking protein particle complex 3
ARFIP1	ENST00000451320.2	ADP-ribosylation factor interacting protein 1
TEX14	ENST00000389934.3	testis expressed 14
ZZZ3	ENST00000370801.3	zinc finger, ZZ-type containing 3
OTX2	ENST00000339475.5	orthodenticle homeobox 2
DIMT1	ENST00000199320.4	DIM1 dimethyladenosine transferase 1 homolog (S. cerevisiae)
GRHL3	ENST00000361548.4	grainyhead-like 3 (Drosophila)
TMEM170B	ENST00000379426.1	transmembrane protein 170B
LHX8	ENST00000294638.5	LIM homeobox 8
MCFD2	ENST00000444761.2	multiple coagulation factor deficiency 2
HMGB1	ENST00000399489.1	high mobility group box 1
GLRB	ENST00000541722.1	glycine receptor, beta
TEC	ENST00000381501.3	tec protein tyrosine kinase
C18orf32	ENST00000579820.1	chromosome 18 open reading frame 32
TRPC1	ENST00000273482.6	transient receptor potential cation channel, subfamily C, member 1
DCAF12L1	ENST00000371126.1	DDB1 and CUL4 associated factor 12-like 1
NUP35	ENST00000295119.4	nucleoporin 35kDa
ADM	ENST00000278175.5	adrenomedullin
SMAD7	ENST00000262158.2	SMAD family member 7
PPIL4	ENST00000340881.2	peptidylprolyl isomerase (cyclophilin)-like 4
ATG16L1	ENST00000392017.4	autophagy related 16-like 1 (S. cerevisiae)
NETO1	ENST00000327305.6	neuropilin (NRP) and tolloid (TLL)-like 1

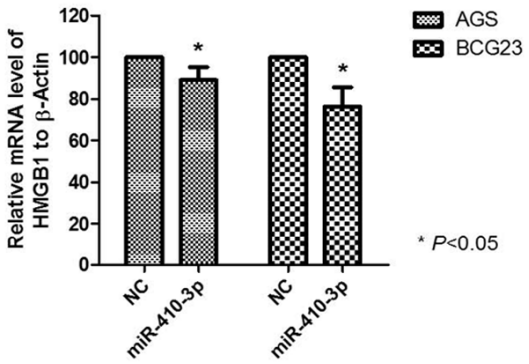
YIPF4	ENST00000238831.4	Yip1 domain family, member 4
UBE2W	ENST00000517608.1	ubiquitin-conjugating enzyme E2W (putative)
EPS8	ENST00000543523.1	epidermal growth factor receptor pathway substrate 8
NDFIP2	ENST00000218652.7	Nedd4 family interacting protein 2
HS3ST1	ENST00000002596.5	heparan sulfate (glucosamine) 3-O-sulfotransferase 1
RPL17-C18orf32	ENST00000584895.1	RPL17-C18orf32 readthrough
KLHL9	ENST00000359039.4	kelch-like family member 9
NUMB	ENST00000554546.1	numb homolog (Drosophila)
PLEKHM2	ENST00000375799.3	pleckstrin homology domain containing, family M (with RUN domain) member 2
TBX5	ENST00000349716.5	T-box 5
PRKD1	ENST00000331968.5	protein kinase D1
RAP1A	ENST00000369709.3	RAP1A, member of RAS oncogene family
CSF2	ENST00000296871.2	colony stimulating factor 2 (granulocyte-macrophage)
MOB1B	ENST00000309395.2	MOB kinase activator 1B
PARG	ENST00000402038.3	poly (ADP-ribose) glycohydrolase
PCDH8	ENST00000377942.3	protocadherin 8
CREB5	ENST00000357727.2	cAMP responsive element binding protein 5
RGMB	ENST00000308234.7	RGM domain family, member B
RAPGEF2	ENST00000264431.4	Rap guanine nucleotide exchange factor (GEF) 2
KLF6	ENST00000542957.1	Kruppel-like factor 6
TMEM106B	ENST00000396667.3	transmembrane protein 106B
YY2	ENST00000429584.2	YY2 transcription factor
TMEFF2	ENST00000392314.1	transmembrane protein with EGF-like and two follistatin-like domains 2
KLHL29	ENST00000486442.1	kelch-like family member 29
SPRED1	ENST00000299084.4	sprouty-related, EVH1 domain containing 1
RGS16	ENST00000367558.5	regulator of G-protein signaling 16
ELAVL4	ENST00000371824.1	ELAV like neuron-specific RNA binding protein 4
ARHGEF40	ENST00000298694.4	Rho guanine nucleotide exchange factor (GEF) 40
SMAD6	ENST00000288840.5	SMAD family member 6
COPS7B	ENST00000373608.3	COP9 signalosome subunit 7B
NFKBIZ	ENST00000394054.2	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
KLHL5	ENST00000261425.3	kelch-like family member 5
CASK	ENST00000421587.2	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
XIAP	ENST00000371199.3	X-linked inhibitor of apoptosis
HDAC1	ENST00000373548.3	histone deacetylase 1
TTYH3	ENST00000258796.7	tweety family member 3
PDE3A	ENST00000359062.3	phosphodiesterase 3A, cGMP-inhibited
ITCH	ENST00000374864.4	itchy E3 ubiquitin protein ligase
BAZ2B	ENST00000392782.1	bromodomain adjacent to zinc finger domain, 2B
DCAF7	ENST00000310827.4	DDB1 and CUL4 associated factor 7
SP3	ENST00000310015.6	Sp3 transcription factor
YY1	ENST00000262238.4	YY1 transcription factor
TBX4	ENST00000393853.4	T-box 4
SMPX	ENST00000379494.3	small muscle protein, X-linked
COL8A1	ENST00000261037.3	collagen, type VIII, alpha 1
C11orf87	ENST00000327419.6	chromosome 11 open reading frame 87
NDNF	ENST00000379692.4	neuron-derived neurotrophic factor
CPEB4	ENST00000265085.5	cytoplasmic polyadenylation element binding protein 4
ACVR1C	ENST00000243349.8	activin A receptor, type IC
LRRC58	ENST00000295628.3	leucine rich repeat containing 58
ITPKB	ENST00000272117.3	inositol-trisphosphate 3-kinase B
RNF214	ENST00000530849.1	ring finger protein 214
GAB1	ENST00000262995.4	GRB2-associated binding protein 1
TM4SF1	ENST00000472441.1	transmembrane 4 L six family member 1
RAB4A	ENST00000366690.4	RAB4A, member RAS oncogene family
NRXN3	ENST00000281127.7	neurexin 3

SCAMP5	ENST00000425597.3	secretory carrier membrane protein 5
FGF7	ENST00000267843.4	fibroblast growth factor 7
TCEAL1	ENST00000372625.3	transcription elongation factor A (SII)-like 1
AFF4	ENST00000265343.5	AF4/FMR2 family, member 4
SERBP1	ENST00000370994.4	SERPINE1 mRNA binding protein 1
GRIA2	ENST00000296526.7	glutamate receptor, ionotropic, AMPA 2
DGKH	ENST00000261491.5	diacylglycerol kinase, eta
ARHGAP24	ENST00000395184.1	Rho GTPase activating protein 24
LPCAT4	ENST00000314891.6	lysophosphatidylcholine acyltransferase 4
TMEM108	ENST00000321871.6	transmembrane protein 108
MED13	ENST00000397786.2	mediator complex subunit 13
GLRA3	ENST00000274093.3	glycine receptor, alpha 3
DIXDC1	ENST00000440460.2	DIX domain containing 1
CPSF6	ENST00000435070.2	cleavage and polyadenylation specific factor 6, 68kDa
PMEPA1	ENST00000341744.3	prostate transmembrane protein, androgen induced 1
SAV1	ENST00000324679.4	salvador homolog 1 (Drosophila)
CNTN4	ENST00000427331.1	contactin 4
DPYSL2	ENST00000311151.5	dihydropyrimidinase-like 2
RORA	ENST00000335670.6	RAR-related orphan receptor A
CBX3	ENST00000337620.4	chromobox homolog 3
RDX	ENST00000343115.4	radixin
RAB8B	ENST00000321437.4	RAB8B, member RAS oncogene family
VWC2	ENST00000340652.4	von Willebrand factor C domain containing 2
MAP3K12	ENST00000267079.2	mitogen-activated protein kinase kinase kinase 12
FAM19A5	ENST00000358295.5	family with sequence similarity 19 (chemokine (C-C motif)-like), member A5
WNT11	ENST00000322563.3	wingless-type MMTV integration site family, member 11
HTR2A	ENST00000378688.4	5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled
SENP3	ENST00000429205.2	SUMO1/sentrin/SMT3 specific peptidase 3
RASSF8	ENST00000541490.1	Ras association (RalGDS/AF-6) domain family (N-terminal) member 8
ITPRIPL2	ENST00000381440.3	inositol 1,4,5-trisphosphate receptor interacting protein-like 2
LCORL	ENST00000326877.4	ligand dependent nuclear receptor corepressor-like
BMPR2	ENST00000374574.2	bone morphogenetic protein receptor, type II (serine/threonine kinase)
PRRX1	ENST00000367760.3	paired related homeobox 1
LOXL3	ENST00000264094.3	lysyl oxidase-like 3
PARD6G	ENST00000353265.3	par-6 family cell polarity regulator gamma
RHEB	ENST00000262187.5	Ras homolog enriched in brain
HBEGF	ENST00000230990.6	heparin-binding EGF-like growth factor
SLC24A2	ENST00000341998.2	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2
CPEB3	ENST00000412050.4	cytoplasmic polyadenylation element binding protein 3
RAPGEF6	ENST00000509018.1	Rap guanine nucleotide exchange factor (GEF) 6
SLC25A42	ENST00000318596.7	solute carrier family 25, member 42
ETS1	ENST00000345075.4	v-ets avian erythroblastosis virus E26 oncogene homolog 1
VAMP2	ENST00000316509.6	vesicle-associated membrane protein 2 (synaptobrevin 2)
CDK14	ENST00000380050.3	cyclin-dependent kinase 14
ZC3H12C	ENST00000278590.3	zinc finger CCCH-type containing 12C
ZNRF1	ENST00000335325.4	zinc and ring finger 1, E3 ubiquitin protein ligase
SLC25A3	ENST00000188376.5	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3
ZFX	ENST00000539115.1	zinc finger protein, X-linked
ROCK2	ENST00000315872.6	Rho-associated, coiled-coil containing protein kinase 2
AGO3	ENST00000373191.4	argonaute RISC catalytic component 3
ST18	ENST00000276480.7	suppression of tumorigenicity 18 (breast carcinoma) (zinc finger protein)
ST6GAL2	ENST00000361686.4	ST6 beta-galactosamide alpha-2,6-sialyltranferase 2
ARHGAP5	ENST00000345122.3	Rho GTPase activating protein 5
RORB	ENST00000376896.3	RAR-related orphan receptor B
VAT1L	ENST00000302536.2	vesicle amine transport 1-like
PBRM1	ENST00000356770.4	polybromo 1

PLXNA2	ENST00000367033.3	plexin A2
ST8SIA4	ENST00000231461.5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4
SNX27	ENST00000368843.3	sorting nexin family member 27
HCN1	ENST00000303230.4	hyperpolarization activated cyclic nucleotide-gated potassium channel 1
TMEM132B	ENST00000299308.3	transmembrane protein 132B
PRKAR2B	ENST00000265717.4	protein kinase, cAMP-dependent, regulatory, type II, beta
SLC34A2	ENST00000382051.3	solute carrier family 34 (type II sodium/phosphate contransporter), member 2
STAT3	ENST00000585517.1	signal transducer and activator of transcription 3 (acute-phase response factor)
IQCK	ENST00000320394.6	IQ motif containing K
PCSK5	ENST00000376752.4	proprotein convertase subtilisin/kexin type 5
TNPO1	ENST00000337273.5	transportin 1
BEND3	ENST00000369042.1	BEN domain containing 3
HTRA2	ENST00000258080.3	HtrA serine peptidase 2
RC3H1	ENST00000367696.2	ring finger and CCCH-type domains 1
GFPT1	ENST00000357308.4	glutamine--fructose-6-phosphate transaminase 1
CHD7	ENST00000423902.2	chromodomain helicase DNA binding protein 7
QKI	ENST00000392127.2	QKI, KH domain containing, RNA binding
CD200	ENST00000315711.8	CD200 molecule
BRWD3	ENST00000373275.4	bromodomain and WD repeat domain containing 3
SETD3	ENST00000331768.5	SET domain containing 3
ITGA9	ENST00000264741.5	integrin, alpha 9
GLIS3	ENST00000324333.10	GLIS family zinc finger 3
SP4	ENST00000222584.3	Sp4 transcription factor
MAFG	ENST00000357736.4	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog G
PDZD2	ENST00000438447.1	PDZ domain containing 2
CEP97	ENST00000341893.3	centrosomal protein 97kDa
GLCE	ENST00000559420.2	glucuronic acid epimerase
NFAT5	ENST00000354436.2	nuclear factor of activated T-cells 5, tonicity-responsive
COBLL1	ENST00000375458.2	cordon-bleu WH2 repeat protein-like 1
SH3GLB1	ENST00000370558.4	SH3-domain GRB2-like endophilin B1
TMTC1	ENST00000256062.5	transmembrane and tetratricopeptide repeat containing 1
KAT6A	ENST00000265713.2	K(lysine) acetyltransferase 6A
CNOT7	ENST00000361272.4	CCR4-NOT transcription complex, subunit 7
ZER1	ENST00000291900.2	zyg-11 related, cell cycle regulator
NR3C1	ENST00000394464.2	nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
GSK3B	ENST00000264235.8	glycogen synthase kinase 3 beta
MNX1	ENST00000252971.6	motor neuron and pancreas homeobox 1
NCOA5	ENST00000290231.6	nuclear receptor coactivator 5
ATN1	ENST00000356654.4	atrophin 1
GUCY1A2	ENST00000526355.2	guanylate cyclase 1, soluble, alpha 2
ARID2	ENST00000457135.1	AT rich interactive domain 2 (ARID, RFX-like)
SFMBT2	ENST00000361972.4	Scm-like with four mbt domains 2
UNC5D	ENST00000287272.2	unc-5 homolog D (C. elegans)
FBXO33	ENST00000298097.7	F-box protein 33
GABPA	ENST00000354828.3	GA binding protein transcription factor, alpha subunit 60kDa
BRD3	ENST00000303407.7	bromodomain containing 3
RERE	ENST00000337907.3	arginine-glutamic acid dipeptide (RE) repeats
AHCTF1	ENST00000366508.1	AT hook containing transcription factor 1
GRIN2B	ENST00000609686.1	glutamate receptor, ionotropic, N-methyl D-aspartate 2B
DLG3	ENST00000194900.4	discs, large homolog 3 (Drosophila)
FOXP2	ENST00000408937.3	forkhead box P2
TSC22D1	ENST00000458659.2	TSC22 domain family, member 1
SLC2A1	ENST00000426263.3	solute carrier family 2 (facilitated glucose transporter), member 1
FBXO3	ENST00000526785.1	F-box protein 3
CCNA2	ENST00000274026.5	cyclin A2
HSPA4L	ENST00000296464.4	heat shock 70kDa protein 4-like

WDR82	ENST00000296490.3	WD repeat domain 82
MATR3	ENST00000510056.1	matrin 3
OSBPL3	ENST00000313367.2	oxysterol binding protein-like 3
LMTK2	ENST00000297293.5	lemur tyrosine kinase 2
ERG	ENST00000398905.1	v-ets avian erythroblastosis virus E26 oncogene homolog
BMPR1A	ENST00000372037.3	bone morphogenetic protein receptor, type IA
STRN	ENST00000263918.4	striatin, calmodulin binding protein
RAI1	ENST00000353383.1	retinoic acid induced 1
LRP6	ENST00000261349.4	low density lipoprotein receptor-related protein 6
PLA2G4A	ENST00000367466.3	phospholipase A2, group IVA (cytosolic, calcium-dependent)
BHLHE40	ENST00000256495.3	basic helix-loop-helix family, member e40
ADCYAP1	ENST00000579794.1	adenylate cyclase activating polypeptide 1 (pituitary)
CTNND1	ENST00000524630.1	catenin (cadherin-associated protein), delta 1
UBR3	ENST00000272793.5	ubiquitin protein ligase E3 component n-recognin 3 (putative)
STARD13	ENST00000336934.5	StAR-related lipid transfer (START) domain containing 13
ATP8B2	ENST00000368489.3	ATPase, aminophospholipid transporter, class I, type 8B, member 2
MPP6	ENST00000222644.5	membrane protein, palmitoylated 6 (MAGUK p55 subfamily member 6)
CDK19	ENST00000368911.3	cyclin-dependent kinase 19
FOXF1	ENST00000262426.4	forkhead box F1
SNIP1	ENST00000296215.6	Smad nuclear interacting protein 1
MIER1	ENST00000357692.2	mesoderm induction early response 1, transcriptional regulator
PDE8B	ENST00000264917.5	phosphodiesterase 8B
ELK3	ENST00000228741.3	ELK3, ETS-domain protein (SRF accessory protein 2)
CNTNAP1	ENST00000264638.4	contactin associated protein 1
SYP	ENST00000263233.4	synaptophysin
HLF	ENST00000226067.5	hepatic leukemia factor
IPO7	ENST00000379719.3	importin 7
ATP2B2	ENST00000352432.4	ATPase, Ca++ transporting, plasma membrane 2
BTG3	ENST00000339775.6	BTG family, member 3
ETV6	ENST00000396373.4	ets variant 6
ABHD13	ENST00000375898.3	abhydrolase domain containing 13
PJA2	ENST00000361189.2	praja ring finger 2, E3 ubiquitin protein ligase
POGZ	ENST00000392723.1	pogo transposable element with ZNF domain
PDPK1	ENST00000441549.3	3-phosphoinositide dependent protein kinase-1
SMAD2	ENST00000262160.6	SMAD family member 2
MAGI3	ENST00000307546.9	membrane associated guanylate kinase, WW and PDZ domain containing 3
PTPRB	ENST00000334414.6	protein tyrosine phosphatase, receptor type, B
RNF44	ENST00000274811.4	ring finger protein 44
SLC8A1	ENST00000406785.2	solute carrier family 8 (sodium/calcium exchanger), member 1
WNT9B	ENST00000290015.2	wingless-type MMTV integration site family, member 9B
LSAMP	ENST00000490035.2	limbic system-associated membrane protein
RCOR1	ENST00000262241.6	REST corepressor 1
PRUNE2	ENST00000376718.3	prune homolog 2 (Drosophila)
MEX3D	ENST00000402693.4	mex-3 RNA binding family member D
FBXO42	ENST00000375592.3	F-box protein 42
WNT3	ENST00000225512.5	wingless-type MMTV integration site family, member 3
TBC1D15	ENST00000550746.1	TBC1 domain family, member 15
RAB21	ENST00000261263.3	RAB21, member RAS oncogene family
SNX2	ENST00000379516.2	sorting nexin 2
DNMT3A	ENST00000380746.4	DNA (cytosine-5-)-methyltransferase 3 alpha
SLC9A7	ENST00000328306.4	solute carrier family 9, subfamily A (NHE7, cation proton antiporter 7), member 7
ARMC8	ENST00000469044.1	armadillo repeat containing 8
KLF3	ENST00000261438.5	Kruppel-like factor 3 (basic)
HERPUD1	ENST00000379792.2	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1
OSBP	ENST00000263847.1	oxysterol binding protein
ZFAND5	ENST00000237937.3	zinc finger, AN1-type domain 5

TMED4	ENST00000457408.2	transmembrane emp24 protein transport domain containing 4
SLIT3	ENST00000519560.1	slit homolog 3 (Drosophila)
DACT1	ENST00000395153.3	dishevelled-binding antagonist of beta-catenin 1
PCNP	ENST00000296024.5	PEST proteolytic signal containing nuclear protein
FRMPD4	ENST00000380682.1	FERM and PDZ domain containing 4
TNRC18	ENST00000399537.4	trinucleotide repeat containing 18
ESM1	ENST00000381405.4	endothelial cell-specific molecule 1
RTN4RL1	ENST00000331238.6	reticulon 4 receptor-like 1
HACE1	ENST00000262903.4	HECT domain and ankyrin repeat containing E3 ubiquitin protein ligase 1
CGNL1	ENST00000281282.5	cingulin-like 1
ZNF384	ENST00000319770.3	zinc finger protein 384
ELMO2	ENST00000290246.6	engulfment and cell motility 2
STRN3	ENST00000355683.5	striatin, calmodulin binding protein 3
FZD8	ENST00000374694.1	frizzled family receptor 8
GRAMD1B	ENST00000529750.1	GRAM domain containing 1B
BEND4	ENST00000504360.1	BEN domain containing 4
USP6NL	ENST00000609104.1	USP6 N-terminal like
UBE2H	ENST00000355621.3	ubiquitin-conjugating enzyme E2H
NMT1	ENST00000592782.1	N-myristoyltransferase 1
RPS6KA3	ENST00000379565.3	ribosomal protein S6 kinase, 90kDa, polypeptide 3
NRDE2	ENST00000354366.3	NRDE-2, necessary for RNA interference, domain containing
MYH9	ENST00000216181.5	myosin, heavy chain 9, non-muscle
IGF2BP1	ENST00000290341.3	insulin-like growth factor 2 mRNA binding protein 1
ZNF592	ENST00000299927.3	zinc finger protein 592
PPFIBP1	ENST00000318304.8	PTPRF interacting protein, binding protein 1 (liprin beta 1)
RHOBTB2	ENST00000251822.6	Rho-related BTB domain containing 2
GABPB2	ENST00000368918.3	GA binding protein transcription factor, beta subunit 2
IGSF3	ENST00000369486.3	immunoglobulin superfamily, member 3
PIK3IP1	ENST00000441972.1	phosphoinositide-3-kinase interacting protein 1
MAPRE2	ENST00000436190.2	microtubule-associated protein, RP/EB family, member 2
CDK13	ENST00000181839.4	cyclin-dependent kinase 13
LGALSL	ENST00000238875.5	lectin, galactoside-binding-like
GTF2B	ENST00000370500.5	general transcription factor IIB
CNOT2	ENST00000229195.3	CCR4-NOT transcription complex, subunit 2
CDH11	ENST00000394156.3	cadherin 11, type 2, OB-cadherin (osteoblast)
COX15	ENST00000016171.5	cytochrome c oxidase assembly homolog 15 (yeast)
FZD5	ENST00000295417.3	frizzled family receptor 5
SNTG1	ENST00000522124.1	syntrophin, gamma 1
ZBTB20	ENST00000462705.1	zinc finger and BTB domain containing 20
RBMS2	ENST00000262031.5	RNA binding motif, single stranded interacting protein 2



Supplementary Figure 2. HMGB1 mRNA was downregulated by miR-410-3p mimic in both AGS and BCG23 cell lines.