Long non-coding RNA MIAT promotes gastric cancer proliferation and metastasis via modulating the miR-331-3p/RAB5B pathway

XIAO-MEI LI1, YAN-YAN JIAO1, BAO-HONG LUAN1, HONG-XIA WU1, RONG-RONG WANG1 and JIE ZHONG2

Departments of 1Oncology and 2Interventional Radiography, Qing Dao Cheng Yang People's Hospital, Qingdao, Shandong 266109, P.R. China

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Abstract. Gastric cancer (GC) remains a threat to the health of the global population. The present study investigated the effects and mechanisms of the long non-coding RNA myocardial infarction associated transcript (MIAT) on the proliferation, apoptosis and metastasis of GC (HGC-27 and AGS) cells. The expression levels of MIAT, microRNA (miR)-331-3p and RAB5B mRNA were analyzed using reverse transcription-quantitative PCR analysis. Cell growth, apoptosis, migration and invasion were measured using 5-ethynyl-2'-deoxyuridine, flow cytometry, wound healing and Transwell assays, respectively. A luciferase assay was used to determine whether miR-331-3p targeted MIAT and RAB5B. The results indicated that MIAT levels were significantly upregulated in GC tissues and cells, correlated with RAB5B levels and inversely associated with miR-331-3p levels. MIAT overexpression promoted proliferation and metastasis, and inhibited the apoptosis of GC cells. MIAT knockdown had the opposite effect on GC cells. The rescue experiments revealed that the effects of MIAT knockdown on the biological behaviour of GC cells were attenuated by RAB5B overexpression. These data suggest that MIAT promotes GC progression via modulating miR-331-3p/RAB5B pathway.

Introduction

Gastric cancer (GC) is a common cancer worldwide and ranks third among the leading causes of cancer-associated mortalities. Incidence rates are markedly elevated in Eastern Asia (incidence rates are 24.7 per 100,000), when compared with Northern America (incidence rates are 8.4 per 100,000) and Northern Europe (incidence rates are 9.3 per 100,000) (1). Targeted therapy with biomarkers for advanced GC has developed rapidly in recent years (2). Due to late diagnosis, patients with extensive invasion and metastasis have poor prognoses (3). Even after a complete resection, recurrence occurs in ~50% of patients (4). As the molecular mechanisms underlying the metastasis and recurrence of GC have not been fully clarified, identifying key GC-promoting molecules may contribute to the understanding of GC pathogenesis and identification of new therapeutic targets.

Long non-coding RNAs (lncRNAs) are a type of RNA transcript that have >200 nucleotides and are not translated into proteins (5). The myocardial infarction associated transcript (MIAT) is first identified to play a role in the pathogenesis of myocardial infarction (6). Recent studies have reported that MIAT is upregulated in several types of cancers including papillary thyroid (7), lung (8) and colorectal cancer (9). However, the underlying molecular mechanism of MIAT in GC remains largely unknown.

MicroRNAs (miRNAs) are 20-23 nucleotides in length and serve a negative regulatory role by binding to the 3' untranslated region (UTR) of target mRNAs, which results in inhibition of mRNA translation or promotion of mRNA degradation (10). lncRNAs can serve as sponges of miRNAs and reduce their regulatory effects on target mRNAs (11). miR-331-3p serves as a potential tumour suppressor in multiple types of human cancers, including pancreatic (12), ovarian (13) and colorectal cancer (14). miR-331-3p has also been demonstrated to inhibit GC cell growth (15). Here, it was speculated that MIAT functions via targeting miR-331-3p.

Ras-related protein Rab-5B (RAB5B), an isoform of RAB5 (16). High expression of RAB5B is associated with cancer progression and poor prognoses in numerous cancer types, including pancreatic (17), breast (18) and ovarian cancer (19). However, the function of RAB5B in GC is yet to be elucidated.

The present study investigated the effects and mechanisms of the lncRNA MIAT on the proliferation, apoptosis and metastasis of GC cells.

Materials and methods

The Cancer Genome Atlas (TCGA) database. Tissues samples in TCGA database (https://cancergenome.nih.gov/) were
Table I. siRNA sequences used in the present study.

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequences</th>
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<tr>
<td>si-MIAT-1</td>
<td>5'-GGUGUUAAGACUGUUGUUCCTT-3'</td>
</tr>
<tr>
<td>si-MIAT-2</td>
<td>5'-ACUUCUUCGUAUUGUGCCCTT-3'</td>
</tr>
<tr>
<td>si-NC</td>
<td>5'-UUCUCGAACGUGACAGGUTT-3'</td>
</tr>
<tr>
<td>miR-331-3p mimics</td>
<td>5'-GCCCCUGGGCCUAAUCCUAGA-3'</td>
</tr>
<tr>
<td>miR-331-3p inhibitors</td>
<td>5'-UUCUAGGAUAGCCAGGCCCCG-3'</td>
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</table>

MIAT, myocardial infarction associated transcript; si, small interfering; NC, negative control.

Table II. Primers used for reverse transcription-quantitative PCR.

<table>
<thead>
<tr>
<th>Primer names</th>
<th>Sequences</th>
</tr>
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<td>lncRNA-MIAT</td>
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<tr>
<td>lncRNA-MIAT</td>
<td>R: 5'-TCCCACTTTTGGCATTCTAGG-3'</td>
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<tr>
<td>miR-331-3p</td>
<td>F: 5'-GGCCCTCTGCGGCTTCTAC-3'</td>
</tr>
<tr>
<td>miR-331-3p</td>
<td>R: 5'-CGATGACCTTATGAATTGACA-3'</td>
</tr>
<tr>
<td>RAB5B</td>
<td>F: 5'-TTCTCACCAGTCGGTTGTG-3'</td>
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<td>RAB5B</td>
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<td>U6</td>
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<tr>
<td>β-actin</td>
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</tr>
<tr>
<td>β-actin</td>
<td>R: 5'-CTGGAAGGTGCGACAGCGGGA-3'</td>
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</table>

lncRNA, long non-coding RNA; miR, microRNA; MIAT, myocardial infarction associated transcript; F, forward; R, reverse.

Patient samples and cell lines. GC tissue samples and paired normal tissue samples were obtained from 47 patients who underwent surgery between March 2017 and March 2018 at Chengyang People's Hospital (Qingdao, Shandong, China) with written informed consent. All samples were frozen in liquid nitrogen and stored at -80°C until use. The present study was approved by the Ethics Committee of Chengyang People's Hospital. GC tissues were fixed in 4% buffered paraformaldehyde for 24 h at 4°C, embedded in paraffin and then sectioned to 5 µm. These sections were stained with hematoxylin for 15 min and eosin for 5 min at 25°C. Samples were examined under a light microscope at a magnification of x400. The human gastric epithelial mucosa cell line GES-1 and GC cell lines HGC-27, AGS, MKN45 and NCI-N87 were purchased from Nanjing Keygen Biotech Co., Ltd. HGC-27, AGS, MKN45 and NCI-N87 cells were maintained in RPMI-1640 medium at 37°C.

Transfection. pcDNA 3.1-MIAT, pcDNA 3.1-RAB5B, pcDNA 3.1-negative control (pcDNA 3.1-NC), small interfering RNA (si)-MIAT-1, si-RAB5B and negative control siRNA (si-NC) were generated by Shanghai Gene Pharma Company. miR-331-3p mimics, inhibitors and their respective negative controls were purchased from Guangzhou RiboBio Co., Ltd. The siRNA, miR-331-3p mimics and inhibitor sequences are listed in Table I. HGC-27, AGS and GES-1 cells were transfected using Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C, and the fresh medium was changed after transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C, and the fresh medium was changed after 6 h. The final concentration for transfection was 50 nM. HGC-27, AGS and GES-1 cells were harvested at 48 h after transfection.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA from all cultured cells lines and human tissue was extracted using TRIzol according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was synthesized from 1 µg total RNA using the PrimeScript® RT reagent kit (Takara Bio, Inc.) according to the manufacturer's instructions. qPCR was performed in an ABI 7500 instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) using SYBR® Green Real-Time PCR Master Mix (Takara Bio, Inc.). Amplification conditions were 94°C for 7 min, followed by 40 cycles of 95°C for 15 sec, and 60°C for 30 sec. Relative expression levels were quantitated using the 2^-ΔΔCq method (20) with β-actin and U6 as reference control. Primer pairs are listed in Table II.

Cell Counting Kit (CCK)-8 assay. The AGS and HGC-27 cells without transfection (5x10^3 cells/well) were cultivated in RPMI-1640 complete medium with 10% FBS or serum-free medium at 37°C for 1, 2 and 3 days. Then, according to the manufacturer's instructions, CCK-8 solution (10 µl; Dojindo Molecular Technologies, Inc.) was supplemented into each well for incubating another 2 h. A microplate reader (BioTek Instruments, Inc.) was used for examining the absorbance at 450 nm. The GES-1 cells (5x10^3 cells/well) were cultivated in DMEM medium with 10% FBS at 37°C for 1, 2 and 3 days. The next steps were similar to the AGS and HGC-27 cells.

Cell proliferation assay. The HGC-27 and AGS cells at 48 h post transfection were seeded into 24-well plates (2x10^5) and cultured with 5-ethyl-2'-deoxyuridine (EdU; 50 µM; Guangzhou RiboBio Co., Ltd.) at 37°C for 2 h. The cells were fixed in 4% paraformaldehyde at 25°C for 30 min and incubated with glycine at 37°C for 4 h. After being submerged
Polymerase chain reaction (PCR). The total RNA was extracted from the GC tissues and cell lines using TRIzol reagent and subjected to RT-qPCR with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal control. Primer sequences are shown in Table II. The RT-qPCR results revealed that MIAT expression was significantly higher in AGS and HGC-27 cells, compared with paratumor tissues (Fig. 1A). Representative staining images are displayed in Fig. 1B. The high expression of MIAT was correlated with late TNM stage and lymph node involvement (Table III). In Fig. 1C, MIAT expression was significantly increased in GC tissue samples (adenocarcinoma) as compared with paratumor tissues (Fig. 1A). Representative staining images are displayed in Fig. 1B. The high expression of MIAT was correlated with late TNM stage and lymph node involvement (Table III). In Fig. 1C, MIAT expression was significantly increased in GC tissue samples (adenocarcinoma) as compared with paratumor tissues (Fig. 1A). Representative staining images are displayed in Fig. 1B. The high expression of MIAT was correlated with late TNM stage and lymph node involvement (Table III).
Table III. The association between lncRNA MIAT expression and clinicopathological variables of patients with gastric cancer.

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^P<0.05. lncRNA, long non-coding RNA; MIAT, myocardial infarction associated transcript.

Figure 1. MIAT is upregulated in gastric cancer (GC) tissues and cell lines. (A) RT-qPCR analysis of MIAT expression in GC tissues and corresponding adjacent tissues (n=47). **P<0.01 vs. adjacent tissues group. n=3. (B) The tissue type of gastric cancer was identified as adenocarcinoma. (C) RT-qPCR analysis of MIAT expression in GC cell lines (HGC-27, AGS, MKN45 and NCI-N87) and the gastric epithelial mucosa cell line GES-1. (D) Kaplan-Meier analysis was used to analyze association between MIAT expression and overall survival of patients with GC. n=3. *P<0.05, **P<0.01 vs. the GES-1 group. GC, gastric cancer; RT-qPCR, reverse transcription-quantitative PCR; MIAT, myocardial infarction associated transcript; NS, not significant.
of MIAT in HGC-27 cells was higher compared with in AGS cells (Fig. S1A). As expected, the proliferation, migration and invasion ability of HGC-27 cells was higher compared with AGS cells (Fig. S1B and C). Kaplan-Meier analysis revealed that high expression of MIAT was associated with poor overall survival time based on data from TCGA database (Fig. 1D). MIAT promotes HGC-27 and AGS cell proliferation and inhibits apoptosis.

Overexpression of MIAT was induced in HGC-27 and AGS cells following transfection with pcDNA3.1-MIAT (Fig. 2A). The results revealed that both si-MIAT-1 and si-MIAT-2 downregulated MIAT expression levels (Fig. 2B). Cell proliferation was significantly increased in cells transfected with pcDNA3.1-MIAT compared with cells transfected with pcDNA3.1-NC. Knockdown of MIAT significantly suppressed cell proliferation in HGC-27 and AGS cells (Fig. 2C). The apoptotic rate of HGC-27 and AGS cells was decreased by overexpression of MIAT and

Figure 2. MIAT promotes GC cell proliferation by inhibiting apoptosis. (A) RT-qPCR analysis of MIAT expression in HGC-27 and AGS cells transfected with pcDNA3.1-NC or pcDNA3.1-MIAT. (B) RT-qPCR analysis of MIAT expression in HGC-27 and AGS cells transfected with si-NC, si-MIAT-1 or si-MIAT-2. (C) EdU analysis of cell proliferation in HGC-27 and AGS cells transfected with pcDNA3.1-NC, pcDNA3.1-MIAT, si-NC or si-MIAT-1. (D) Flow cytometric analyses of HGC-27 and AGS cells transfected with pcDNA3.1-NC, pcDNA3.1-MIAT, si-NC or si-MIAT-1. (E) The protein levels of Bax, Bcl-2 and cleaved caspase-3 in HGC-27 and AGS cells were measured via western blotting. n=3. **P<0.01 vs. the blank or pcDNA3.1-NC group; #P<0.05, ##P<0.01 vs. the blank or si-NC group. GC, gastric cancer; MIAT, myocardial infarction associated transcript; si, small interfering; NC, negative control; RT-qPCR, reverse transcription-quantitative PCR; PI, propridium iodide.
increased by knockdown of MIAT (Fig. 2D). The expression of Bax and cleaved caspase-3 were significantly decreased following pcDNA3.1-MIAT transfection and increased by transfection with si-MIAT-1. By contrast, the expression of Bcl-2 was increased by pcDNA3.1-MIAT transfection and decreased by transfection with si-MIAT-1 (Fig. 2E).

**MIAT promotes HGC-27 and AGS cell migration and invasion.** To determine the effect of MIAT on HGC-27 and AGS cell migration and invasion, wound healing and Transwell assays were performed. HGC-27 and AGS cell proliferation in serum-free medium was assessed via the CCK-8 assay. HGC-27 and AGS cell proliferation in serum-free medium was slightly lower compared with in medium with 10% FBS (Fig. S2). It was demonstrated that HGC-27 and AGS cells overexpressing MIAT exhibited a faster closing of the scratch wound compared with the NC group (Fig. 3A). MIAT overexpression significantly promoted cell invasion relative to the NC group. The opposite results were observed following MIAT knockdown (Fig. 3B).

**MIAT serves as a miR-331-3p sponge in HGC-27 and AGS cells.** Using StarBase 3.0, it was predicted that miR-331-3p had binding sites complementary to MIAT (Fig. 4A). Overexpression of miR-331-3p suppressed the luciferase activity of MIAT WT 3'-UTR in the HGC-27 and AGS cells (Fig. 4B). The expression of miR-331-3p was downregulated in GC tissues compared with adjacent tissues (Fig. 4C), and miR-331-3p expression was negatively correlated with the expression of MIAT in GC tissues (r=-0.5638; P<0.0001; Fig. 4D). miR-331-3p was also significantly decreased in HGC-27 and AGS cells compared with the GES-1 cells (Fig. 4E). Overexpression of MIAT suppressed the expression of miR-331-3p, while MIAT knockdown increased the expression of miR-331-3p in both AGS and HGC-27 cells (Fig. 4F).
**MIAT promotes RAB5B expression via miR-331-3p.** Bioinformatics analysis using the TargetScan 7.2 revealed that miR-331-3p potentially binds to the 3'UTR of RAB5B mRNA, which was significantly reduced by transfection of miR-331-3p mimics (Fig. 5A and B). The expression of RAB5B was markedly higher in GC tissues and HGC-27 and AGS cell lines (Fig. 5C and F, respectively). A correlation analysis revealed that the mRNA expression of RAB5B in GC tissues was inversely correlated with miR-331-3p levels (r=-0.6545; P<0.0001) and positively correlated with MIAT levels (r=0.6543; P<0.0001) (Fig. 5D and E, respectively). The expression of miR-331-3p was upregulated in AGS and HGC-27 transfected with miR-331-3p mimics, whereas it was downregulated in cells transfected with miR-331-3p inhibitors (Fig. 5G). The mRNA and protein expression levels of RAB5B were downregulated in HGC-27 and AGS cells transfected with miR-331-3p mimics, whereas they were upregulated in AGS and HGC-27 transfected with miR-331-3p inhibitors (Fig. 5H and I, respectively). The mRNA and protein expression of RAB5B was upregulated in AGS and HGC-27 transfected with pcDNA-RAB5B (Fig. 5J and K, respectively). Subsequently, the mRNA and protein expression of RAB5B were determined after transfection of si-MIAT and pcDNA-RAB5B into HGC-27 and AGS cells and it was demonstrated that the decreased expression of RAB5B in response to si-MIAT was partially reversed by RAB5B overexpression (Fig. 5L and M).

**MIAT knockdown effects are reversed by miR-331-3p inhibitor or RAB5B overexpression in HGC-27 and AGS cells.** The results revealed that MIAT knockdown inhibited cell proliferation (Fig. 6A) and induced apoptosis (Fig. 6B) in HGC-27 and AGS cells, while these functions were abrogated by miR-331-3p inhibitor. It was also demonstrated that MIAT knockdown inhibited cell migration (Fig. 6C) and invasion (Fig. 6D) in HGC-27 and AGS cells, while these functions were reversed by RAB5B overexpression.
Influence of MIAT overexpression on cell proliferation, apoptosis, migration and invasion are reversed by RAB5B knockdown in GES-1 cells. Overexpression of MIAT was induced in GES-1 cells via transfection with pcDNA3.1-MIAT (Fig. 7A). The results revealed that pcDNA3.1-MIAT downregulated miR-331-3p expression (Fig. 7B). Overexpression of MIAT upregulated RAB5B mRNA and protein expression, and knockdown of RAB5B downregulated RAB5B mRNA and protein expression (Fig. 7C, D, respectively). Subsequently, RAB5B mRNA and protein expression levels were assessed after transfection of the pcDNA3.1-MIAT and si-RAB5B into GES-1 cells and it was revealed that
the increased mRNA and protein expression of RAB5B in response to pcDNA3.1-MIAT could be decreased by RAB5B knockdown (Fig. 7E and F). It was also demonstrated that MIAT overexpression induced cell viability (Fig. 7G), inhibited cell apoptosis (Fig. 7H), and promoted migration (Fig. 7I) and invasion (Fig. 7J) in GES-1 cells, while these functions were significantly reversed following RAB5B knockdown.
Figure 7. Influence of MIAT-overexpression on GES-1 cell proliferation, apoptosis, migration and invasion are reversed by RAB5B-knockdown. (A) RT-qPCR analysis of MIAT expression in the gastric epithelial mucosa cell line GES-1. (B) RT-qPCR analysis of miR-331-3p expression in GES-1 cells. (C) RT-qPCR and (D) western blot analysis of RAB5B expression in GES-1 cells, n=3. *P<0.01 vs. the blank or pcDNA3.1-NC group, **P<0.01 vs. the blank or si-NC group. (E) HGC-27 and AGS cells were transfected with pcDNA3.1-NC, pcDNA3.1-MIAT, si-NC, or si-RAB5B, followed by (F) the measurement of RAB5B level. (G) Cell proliferation ability was assessed by the Cell Counting Kit-8 assay. (H) Cell apoptosis was evaluated by flow cytometry. (I) Cell migration capacity was evaluated using a wound healing assay. (J) Cell invasion capacity was evaluated by the Transwell invasion assay. n=3. *P<0.05, **P<0.01 vs. the blank or pcDNA3.1-NC + si-NC group; #P<0.05, ##P<0.01 vs. the pcDNA3.1-MIAT + si-NC group; &P<0.05, &&P<0.01 vs. the pcDNA3.1-NC + si-RAB5B group. RT-qPCR, reverse transcription-quantitative PCR; GC, gastric cancer; MIAT, myocardial infarction associated transcript; si, small interfering; NC, negative control.
Discussion

Long non-coding RNAs (lncRNAs) regulate gene expression via various mechanisms, including transcriptional and post-transcriptional processing, and have extensive regulatory functions in tumour initiation and progression (23). MIAT, a recently identified oncogenic lncRNA, has been reported to be upregulated in several types of cancers, including papillary thyroid cancer (7), lung cancer (8) and acute myeloid leukemia (24). However, the detailed role and molecular mechanisms of MIAT in GC remain to be elucidated. MIAT was revealed to be upregulated in GC tissues, which is consistent with previous reports (25). In the present study, it was also demonstrated that high expression of MIAT was correlated with late TNM stage, lymphatic metastasis and a poor prognosis. Similarly, upregulation of lncRNA LINC00858 is associated with a poor prognosis in patients with GC (26). High expression of LINC00858 is positively associated with TNM stage and lymphatic metastasis (26). In the present study, functional experiments revealed that MIAT knockdown inhibited HGC-27 and AGS cell proliferation, induced GC cell apoptosis and inhibited HGC-27 and AGS cell migration and invasion. These results suggest that MIAT serves an important role in the GC tumorigenesis and metastasis.

Emerging evidence suggests that lncRNAs can serve as competing endogenous RNAs (ceRNAs) to regulate microRNAs, subsequently regulating expression of target genes (27). For instance, HLA-F-AS1 promotes colorectal cancer progression by sponging miR-330-3p to upregulate PFN1 expression (28). Exosome-transmitted IncARSR functions as a sponge of miR-34/miR-449 to induce c-MET and AXL expression and mediates sunitinib resistance in renal cell carcinoma (29). In the present study, it was revealed that MIAT shares miR-331-3p response elements with RAB5B and facilitates RAB5B expression via sponging miR-331-3p. RAB5B was experimentally validated as a genuine target of miR-331-3p. Functional inhibition of miR-331-3p effectively rescued the decreased expression of RAB5B protein that was induced by MIAT knockdown in HGC-27 and AGS cells, indicating that MIAT serves as a ceRNA. Two other targets have been reported to serve roles in gastric cancer downstream of MIAT. Knockdown of MIAT suppresses cell biological behaviours in gastric cancer via a mechanism involving the miR-29a-3p/HDAC4 axis (30). Moreover, MIAT promotes gastric cancer growth and metastasis via regulation of the miR-141/DDX5 pathway (25). The current results also extend the regulatory mechanism of MIAT function.

Recent studies have reported the involvement of miR-331-3p in cancer progression. miR-331-3p inhibits cell proliferation and induces cell apoptosis in nasopharyngeal carcinoma via targeting eIF4B and blocks the PI3K-AKT signalling pathway (31). Reduced expression of miR-331-3p in ovarian cancer promotes proliferation and invasion, due to upregulation of its target RCC2 (13). Guo et al (15) reported that miR-331-3p suppresses GC cell growth via inhibiting E2F1. Zhao et al (32) revealed that miR-331-3p suppresses cell proliferation in triple-negative breast cancer cells via down-regulating NR2F2 (32). The current results also supported the regulatory role of miR-331-3p.

RAB5 has three isoforms (RAB5A, B and C) (33). Rab5B is a member of the Ras superfamily of small Rab GTPases (34). RAB5B is localized at the plasma membrane and early endosomes, and functions as a key regulator of vesicular trafficking during early endocytosis (35). Inhibition of RAB5/7 efficiently eliminates colorectal cancer stem cells and disrupts cancer foci (36). RAB5B expression is elevated in melanoma cells (37). RAB5B regulates cell adhesion and migration by promoting Rac1 activation and cancer cell migration (38). Kong et al (39) reported that RAB5B is directly downregulated by miR-130a-3p, and knockdown of RAB5B inhibits cell proliferation, migration and invasion of breast cancer cells. Wang et al (40) demonstrated that IncRNA-APC1 expression inhibits colorectal carcinoma cell growth, metastasis and tumour angiogenesis via suppressing exosome production through the direct binding of Rab5b mRNA. The present results further confirmed that RAB5B serves a critical role in the progression of GC.

In conclusion, the present study revealed that MIAT is upregulated in GC, which is associated with poor clinical outcomes. MIAT promotes HGC-27 and AGS cell proliferation via RAB5B. MIAT promotes RAB5B activity via sponging miR-331-3p to upregulate RAB5B expression. The present findings provide insight into the MIAT/RAB5B pathway, and indicate it as a promising potential therapeutic target in GC, suggesting important translational implications.

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Funding

No funding was received.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JZ designed the study. XML, YYJ, BHL, HXW and RRW performed the research and analyzed data. XML wrote the manuscript.

Ethics approval and consent to participate

The protocol of this research has been approved by the Ethics Committee of Qing Dao Cheng Yang People's Hospital (approval no. 20170106). All patients have signed written informed consent.

Patient consent for publication

All patients agreed to the publication of the article.

Competing interests

The authors declare that they have no competing interests.
miR-331-3p functions as an oncogene

References


