miR-133b acts as a tumor suppressor and negatively regulates ATP citrate lyase via PPARγ in gastric cancer

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Abstract. MicroRNAs (miRNAs/miRs) are a class of small noncoding RNAs that negatively regulate protein expression by binding to protein-coding mRNAs and suppressing translation. Accumulating evidence suggests that miRNAs are involved in the development and progression of cancer by regulating cancer metabolism. Meanwhile, the cytosolic enzyme ATP citrate lyase (ACLY) is a promising target in the prevention and treatment of cancer. In the present study we revealed by western blot analysis and reverse transcription-quantitative PCR that miR-133b was downregulated in human gastric cancer (GC) tissues and cell lines, while ACLY was upregulated. The overexpression of miR-133b could decrease the proliferation and invasion of MKN-74 cells by inhibiting the expression and activation of ACLY. Furthermore, the nuclear distribution of peroxisome proliferator-activated receptor-γ (PPARγ) in GC tissues and cell lines was markedly decreased, and overexpression of miR-133b could increase the levels of nuclear PPARγ in MKN-74 cells. Additionally, miR-133b decreased the transcriptional activity of ACLY in a PPARγ-dependent manner, as determined by a dual-luciferase reporter assay. These results indicate that miR-133b targets ACLY and inhibits GC cell proliferation by regulating the expression of PPARγ, suggesting that miR-133b may serve as a tumor-suppressive target in GC therapy.

Introduction

Human gastric cancer (GC) is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide, accounting for approximately 10% of newly diagnosed cancers, although the incidence and mortality rates have generally declined during the past few decades (1). The development of optimal strategies for the treatment of GC is a major focus of current clinical research. Numerous efforts have been made to identify microRNAs (miRNAs/miRs) involved in the occurrence and development of GC (2,3), and evidence to date suggests that miRNAs involved in the multifaceted and complex mechanisms of GC are primarily responsible for its metabolism.

miRNAs are a class of endogenous noncoding RNA molecules of approximately 19-25 nucleotides in length that are cleaved from 70-100-nucleotide hairpin precursors (pre-miRNAs). Although miRNAs only account for approximately 1% of all expressed human genes, accumulating evidence indicates that miRNAs may regulate the expression of nearly one-third of all human genes, and may play important roles in cell growth, proliferation, differentiation and death (4,5). Recently, evidence has indicated that alterations in miRNA levels, resulting from mutation or aberrant expression, are associated with various human types of cancers (6). Moreover, numerous miRNAs are aberrantly expressed in human types of cancer, including GC.

miR-133b was initially considered to be a muscle-specific miRNA and was revealed to be involved in the development of skeletal muscle (7). Many studies have now shown that miR-133b is downregulated and inhibits cell proliferation, migration and invasion while promoting cell apoptosis in human malignancies, such as osteosarcoma (8), colorectal (9), lung (10) and bladder cancer (11); however, its molecular mechanism of action and target genes in tumor development remain to be elucidated. In the present study we aimed to reveal the novel regulatory mechanism of miR-133b in the development of GC, with the hope of providing a new approach for clinical treatment.

In this study, we detected differences in the expression levels of miR-133b in human gastric tissues compared with adjacent non-tumor tissues using reverse transcription-quantitative PCR (RT-qPCR), and confirmed the role of miR-133b as a tumor suppressor. As expected, the same result was found in GC cells (MKN-74 and MGC80-3) compared with gastric epithelial cells (GES-1). miRNA-133b mimics and miRNA-133b-inhibitors were used to stimulate the overexpression and knockdown of miR-133b, respectively, and we subsequently identified that ATP citrate lyase (ACLY) was regulated by miR-133b and expressed at a higher level in GC tissues and cell lines. Notably, when ACLY was inhibited by the overexpression of miRNA-133b, MKN-74 cells exhibited decreased

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proliferative and invasive capacities. Furthermore, this process was found to be regulated by the activation of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear transcription factor strongly associated with cancer occurrence and development. These findings contribute to our understanding of the tumor-suppressive function of miR-133b.

Materials and methods

Cell culture. All cell lines were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Human GC cell lines MGC80-3 and MKN-74, gastric epithelial cell line GES-1 were maintained in RPMI-1640 medium (11875085) and Dulbecco's modified Eagle's medium (DMEM; 11995065) (both from Gibco, Grand Island, NY, USA) respectively, and supplemented with 10% (v/v) fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. Cell culture was conducted at 37˚C in a humidified 5 % CO_2 incubator. Cell transfection was performed with a Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. Briefly, cells were plated at a density of $2x10^5$ cells/well in 6-well plates, cultured overnight, then transfected with 100 pmol (final 50 nM) of either scramble, miR-133b-mimic (MC10029, catalog no. 4464066) or miR-133b-inhibitor (MC10029, cat. no. 4464084; both from Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the Lipo 2000. Forty-eight hours later, total protein and RNA samples were prepared; luciferase assay and cell activity assay were carried out as described below. Rosiglitazone (S2556) 1 μM and T0070907 (S2871) 1 μM were purchased from Selleck Chemicals (Shanghai, China) and added to the medium 24 h before harvesting.

Clinical specimens. Human GC tissue and adjacent non-tumor tissue were obtained from patients diagnosed with colon adenocarcinoma at the Department of General Surgery, Second Affiliated Hospital of Anhui Medical University. Stage of disease was reported according to TNM classification (12). The specimens were obtained after surgical resection and immediately frozen at -80˚C until use. The study methodologies conformed to the standards set by the Declaration of Helsinki. Collection and usage of all specimens were approved by the Ethics Committee of The Second Affiliated Hospital of Anhui Medical University.

RT-qPCR. Total RNA was extracted from tissues and cultured cells using TRIzol® reagent (Invitrogen Life Technologies). DNaseI-treated RNA was used for first strand cDNA synthesis using M-MLV reverse transcriptase (Promega Corp., Madison, WI, USA) and oligo $(dT)_{13}$ according to the manufacturer's protocols and 1 μl cDNA samples were used for conventional PCR amplifications. Real-time quantitative PCR analysis was performed on a real-time PCR system (StepOne; Applied Biosystems Life Technologies, Foster City, CA, USA) and the expression levels of ACLY were normalized to GAPDH determined by a SYBR Green-based comparative cycle threshold (CT) method. Real-time PCR primers were: ACLY forward, 5'-GACTTCGGCAGAGGTAGAGC-3', and reverse, 5'-TCAGGAGTGACCCGAGCATA-3'; GAPDH forward, 5'-TGTGGGCATCAATGGATTTGG-3' and reverse, 5'-ACACCATGTATTCCGGGTCAAT-3'; miR-133b forward, 5'-AAGAAAGATGCCCCCTGCTC-3', and reverse, 5'-GTAG CTGGTTGAAGGGGACC-3'; U6 forward, 5'-CTCGCTTCG GCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTG CGT-3'.

Cell lysis and immunoblotting. To obtain total protein lysates, cells were homogenized and dissolved in RIPA buffer [150 mmol/l NaCl, 1.0% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% SDS, 50 mmol/l Tris (pH 8.0)] containing proteinase inhibitors and phosphatase inhibitors. The protein concentration of each lysate was determined using a BCA protein assay kit (Beyotime, Shanghai, China). The total cell lysate was applied on 10% SDS-PAGE. After electrophoresis, the proteins were transferred electrophoretically from the gel to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were then blocked for 1 h in blocking buffer (5% low-fat dried milk in TBS) and probed with the primary antibodies overnight at 4˚C. After washing, horseradish peroxidase-conjugated secondary antibodies were incubated with the membranes at room temperature for 1 h, followed by enhanced chemiluminescence (Amersham). The primary antibodies used were raised against ACLY (1:1,000 dilution; ab40793), PPARγ (1:1,000 dilution; ab209350) and β-actin (1:3,000 dilution; ab8227), and were purchased from Abcam (Cambridge, MA, USA).

ACLY activity assay. ACLY activity was assessed via the malate dehydrogenase-coupled method (13). RIPA whole-cell lysates were added at a 1:19 ratio to the reaction mixture containing 100 mM Tris-HCl (pH 8.7), 20 mM potassium citrate, 10 mM $MgCl_2$, 10 mM DTT, 0.5 U/ml malate dehydrogenase, 0.33 mM CoASH, 0.14 mM NADH, and 5 mM ATP (all from Sigma-Aldrich, St. Louis, MO, USA). The change in absorbance at 340 nm was read every 15 sec over 35 min on a SpectraMax 190 spectrophotometer. Change in absorbance in the absence of exogenous ATP was subtracted from change in the presence of ATP and was normalized to protein concentration to determine the specific ACLY activity.

Cell proliferation assay. Cell proliferation was assessed using the MTT assay. MTT was diluted in phosphate-buffered saline (PBS) to a final concentration of 5 mg/ml and sterile filtered. Cells were incubated with a final concentration of 5 μg/ml MTT at 37˚C for 4 h. Cell culture supernatants were carefully removed, and DMSO was added. The absorbance values were determined using a microplate reader (MK3, Thermo Fisher Scientific) at a wavelength of 570 nm. The experiments were performed three times.

Dual-luciferase reporter assay. Luciferase reporter assays were performed in MKN-74 cells. *Renilla* luciferase (200 ng), luci-ACLY (300 ng), and equal amounts (200 pmol) of miR-133b-mimic, miR-133b-inhibitor or scramble control were transfected into cells in 24-well plates with Lipo 2000. Forty-eight hours after transfection, a luciferase assay was performed with a Dual-Luciferase Reporter Assay system (E1910; Promega). The luciferase activity was assessed using a Victor Luminometer (Perkin Elmer, Inc., Waltham, MA,

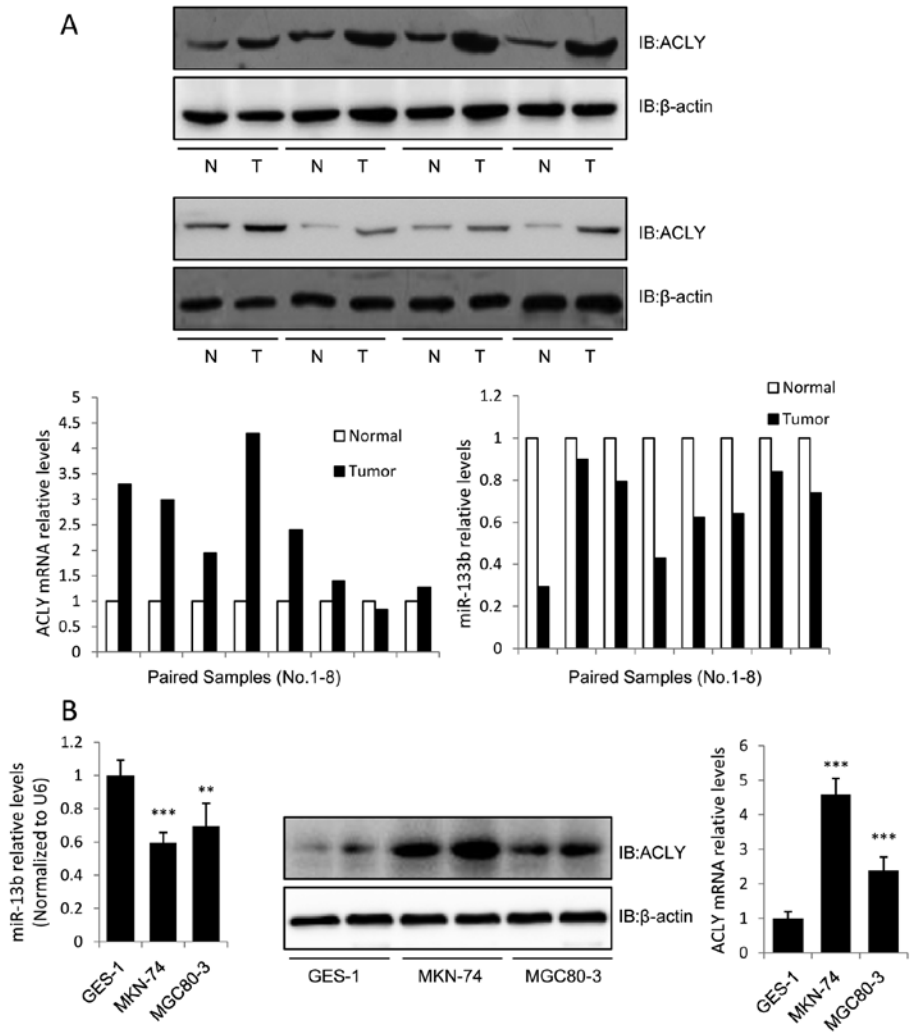

Figure 1. miR-133b is downregulated while ATP citrate lyase (ACLY) is upregulated in human gastric cancer (GC) cell lines and tissues. (A) The expression levels of miR-133b and ACLY in 8 pairs of GC tissues (Tumor) and matched adjacent non-tumor tissues (Normal; control) were detected by RT-qPCR and western blot analysis. (B) The expression levels of miR-133b and ACLY in the human GC cell lines MKN-74 and MGC80-3 and the normal gastric epithelial cell line GES-1 (control) were detected by RT-qPCR and western blot analysis. U6 and GAPDH were used as RT-qPCR endogenous controls, and β-actin was used as the western blotting loading control. Data represent the mean ± standard error of the mean of three biological replicates assayed in technical triplicates. $^{**}p<0.01$ and $^{***}p<0.001$ vs. control determined by a two-tailed Student's t-test.

USA). The firefly luciferase activity was normalized using co-transfected *Renilla* luciferase for transfection efficiency. All experiments were performed in triplicate.

Statistical analysis. Data were presented as the means ± SE. Data between groups were analyzed by Student's t-test or one-way ANOVA followed by Bonferroni-Dunn multiple comparison. P<0.05 was considered as statistically significant.

Results

miR-133b is downregulated while ACLY is upregulated in human GC. To evaluate the association between miR-133b and ACLY in human GC tissues, we assessed the expression levels of miR-133b and ACLY in 8 pairs of GC tissue samples using RT-qPCR and western blot analysis. The 8 GC cases were classified as IV, IIIB, IIA, IIIA, IIB, IIA, IIIA, and IIIC, respectively, according to the seventh edition of the AJCC staging system. The results revealed that miR-133b expression was downregulated while the mRNA and protein expression levels of ACLY were upregulated in all of the screened GC tissues (Fig. 1A). We also investigated the expression levels of miR-133b and ACLY in GC cell lines. Compared with the normal gastric epithelial cell line GES-1, miR-133b was downregulated in the GC cell lines MKN-74 and MGC80-3, while the protein and mRNA levels of ACLY were upregulated (Fig. 1B).

Previous evaluations of ACLY expression in human lung, prostate, bladder, breast, liver, stomach, and colon tumors have identified increased expression levels of this enzyme when compared with normal tissues (14,15). Consistent with these previous results, we observed a high-level of expression of the lipogenic enzyme ACLY in the GC tissues and cell lines. These data demonstrated that ACLY may be involved in the tumor-suppressive effect of miR-133b in GC. As MKN-74 cells expressed higher levels of ACLY than MGC80-3

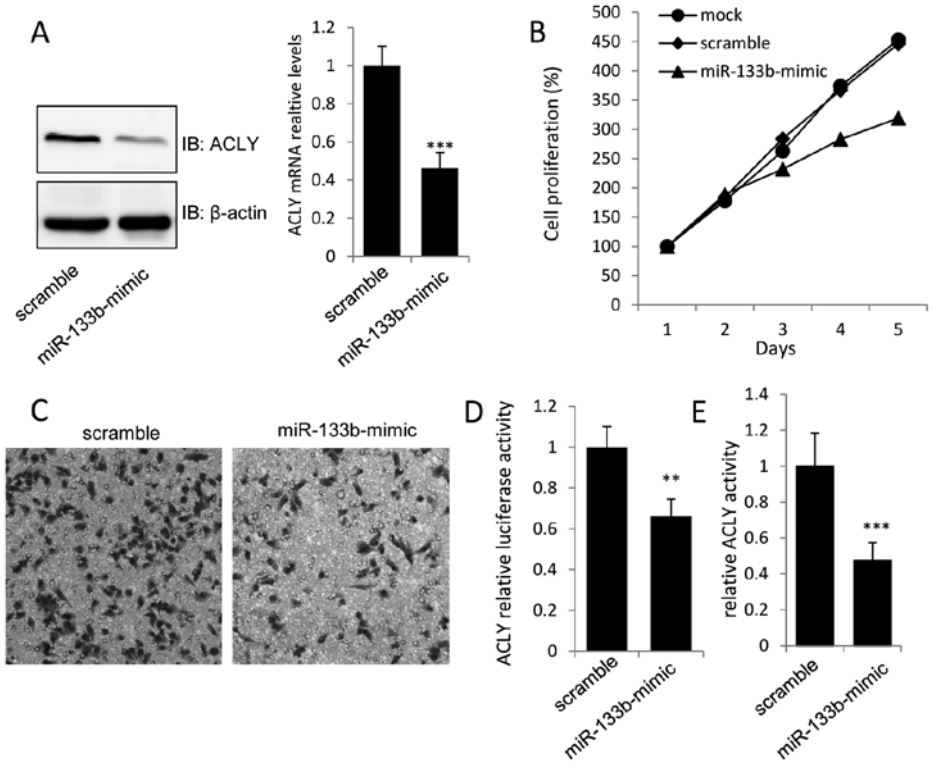

Figure 2. Overexpression of miR-133b inhibits gastric cancer (GC) cell growth by decreasing the expression and activation of ATP citrate lyase (ACLY). (A) Verification of the functions of mature miR-133b after transfection with miR-133b-mimic in MKN-74 cells; both ACLY protein and mRNA levels were analyzed. MKN-74 cell (B) growth activity and (C) invasion ability were determined at the indicated time-points post-transfection by MTT and Transwell assays. (D) miR-133b-mimic (200 pmol), pGL3-ACLY (ACLY-3'UTR-driven luciferase; 300 ng) and *Renilla* luciferase (200 ng) were co-transfected into MKN-74 cells. After 48 h, the luciferase activity was assessed and normalized to the *Renilla* luciferase values. Data are expressed as fold changes relative to the scramble-control group values. (E) ACLY activity was assessed 48 h after transfection with scramble miR and miRNA-133b-mimic. Data represent the mean ± standard error of the mean of three biological replicates assayed in technical triplicates. $^{**}p<0.01$ and $^{***}p<0.001$ vs. scramble determined by a two-tailed Student's t-test.

cells (Fig. 1B), MKN-74 cells were used in subsequent assays to investigate the correlation between ACLY and miR-133b.

Overexpression of miR-133b suppresses GC cell proliferation by inhibiting ACLY. To evaluate the biological significance of miR-133b in GC cell proliferation, miR-133b-mimics were transfected into MKN-74 cells. The overexpression of miR-133b was detected by real-time PCR, and was found to induce ~0.6- and 0.45-fold decreases in the protein and mRNA levels of ACLY, respectively, in miR-133b-mimic transfected MKN-74 cells when compared with the vector control (Fig. 2A). To assess the effects of miR-133b overexpression on the proliferation and invasion of MKN-74 cells, MTT and Transwell assays were performed 48 h after transfection. As shown in Fig. 2B and C, transfection with miR-133b-mimic caused a marked decrease in the growth and invasion of MKN-74 cells. Moreover, the dual-luciferase and ACLY activity assays revealed that transfection of miR-133b-mimic into MKN-74 cells could inhibit the luciferase activity of the luci-ACLY-3'-UTR construct, as well as decrease ACLY activity (Fig. 2D and E). These findings provided evidence that miR-133b could directly regulate the expression and activation of ACLY to suppress gastric tumor growth.

Knockdown of miR-133b promotes GC cell proliferation by increasing ACLY expression and activation. To further validate whether the increased expression of miR-133b in MKN-74 cells mediated the downregulation in ACLY, the functional significance of miR-133b knockdown in MKN-74 cells was investigated. The results revealed that the miR-133b-inhibitor, but not the scramble miR, could markedly increase the expression of ACLY at both the mRNA and protein levels (Fig. 3A).

As predicted, increased levels of cell proliferation and invasion were observed in MKN-74 cells transfected with the miR-133b-inhibitor when compared with the scramble group (Fig. 3B and C). Additionally, the luciferase activity of the ACLY-3'-UTR construct and the relative ACLY activity were significantly higher in the miR-133b-inhibitor group than in the scramble group (Fig. 3D and E), which further demonstrated that ACLY may play an essential role in the tumor-suppressive effect of miR-133b in MKN-74 cells.

miR-133b regulates the expression of PPARγ and its transactivation of ACLY. PPARγ is expressed at high levels in primary colon tumors and colon cancer cell lines, and its agonists have been reported to decrease the growth and induce the differentiation of malignant breast epithelial cells (16,17). Therefore, we aimed to determine whether miR-133b inhibited the activation of ACLY by regulating PPARγ expression. Notably, overexpression of miR-133b was found to stimulate the expression of PPARγ in the nucleus, and knockdown of miR-133b inhibited this phenomenon (Fig. 4A). We further

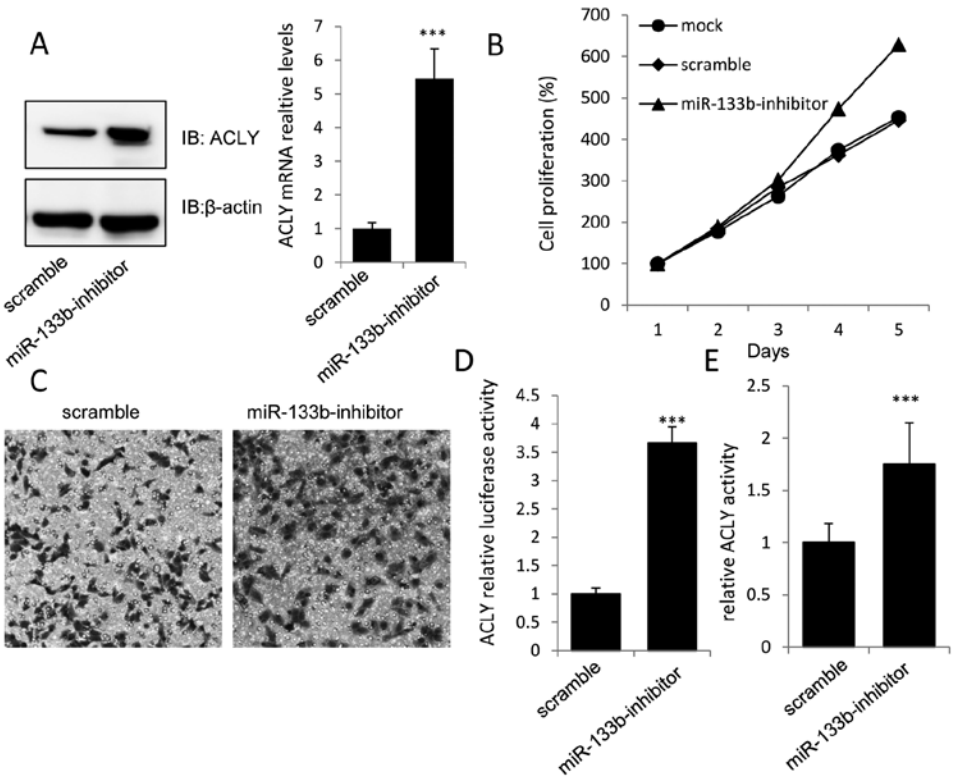

Figure 3. Knockdown of miR-133b increases gastric cancer (GC) cell growth by upregulating the expression and activation of ATP citrate lyase (ACLY). (A) MKN-74 cells were transfected with miR-133b inhibitor and the protein and mRNA levels of ACLY were analyzed. (B) The cell growth activity and (C) invasion ability of MKN-74 cells were determined at the indicated time-points post-transfection by MTT and Transwell assays. (D) miR-133b inhibitor (200 pmol), pGL3-ACLY (ACLY-3'UTR-driven luciferase) (300 ng) and *Renilla* luciferase (200 ng) were co-transfected into MKN-74 cells. After 48 h, the luciferase activity was assessed and normalized to the *Renilla* luciferase values. Data are expressed as fold changes relative to the scramble-control group values.(E) ACLY activity was assessed 48 h after transfection with scramble miR and miR-133b-mimic. Data represent the mean ± standard error of the mean of three biological replicates assayed in technical triplicates. $^{***}p<0.001$ vs. scramble determined by a two-tailed Student's t-test.

evaluated PPARγ expression in the GES-1 and MKN-74 cell lines and GC tissues, and found lower levels of PPARγ in the nuclear lysates of MKN-74 cells and tumor tissues samples, which was consistent with previous research, suggesting that miR-133b may induce PPARγ expression to inhibit tumor growth.

To further assess whether miR-133b inhibited the expression of ACLY in a PPARγ-dependent manner, a dual-luciferase assay was used to detect the transcriptional activity of the ACLY3'UTR. As shown in Fig. 4B, 1 μM of rosiglitazone (PPARγ agonist) could significantly enhance the inhibitory effect of miR-133b-mimic on the luciferase activity of the luci-ACLY 3'UTR construct, while T0070907 (PPARγ inhibitor) could significantly attenuate the inhibitory effect of the miR-133b-mimic. By contrast, rosiglitazone suppressed the stimulatory effect of the miR-133b-inhibitor on the transcriptional activity of the ACLY-3'UTR, while T0070907 enhanced the stimulatory effect of miR-133b-inhibitor. Our data clearly indicated that miR-133b decreased the proliferation of GC cells by targeting the expression of ACLY in a PPARγ-dependent manner.

Discussion

miRNAs are endogenous non-coding RNAs that interact with the 3'UTRs of target mRNAs to induce mRNA cleavage, which enables miRNAs to regulate protein expression at the transcriptional level (18). In recent years, it has been reported that miRNAs can alter the expression of tumor-suppressor genes and oncogenes, thus implicating them in the regulation of tumor development and progression. Therefore, the identification of cancer-related miRNAs and their targets is essential to understand their roles in tumorigenesis, and may be important for diagnosis and targeted therapeutic treatment.

miR-133b is located on chromosome 18 in the same bicistronic unit as miR-133 (19). miR-133b has long been recognized as a muscle-specific miRNA that may regulate myoblast differentiation and participate in many myogenic diseases and in myocardial infarction (20,21). Recently, researchers found that miR-133b was significantly downregulated in a number of types of cancer, such as colorectal cancer, non-small-lung cancer, and GC (5,22-24), but its target genes remain unclear. Therefore, we focused on the downstream target genes of miR-133b and aimed to identify the molecular mechanism underlying its tumor-suppressive effect on GC, since it is the fifth most common malignancy worldwide and the second most common cancer in China (25).

PPARγ is a ligand-activated transcription factor that belongs to the nuclear hormone receptor superfamily (26). Activation of PPARγ results in the inhibition of tumor growth in various types of cancer, and activating ligands of PPARγ have been shown to induce differentiation and inhibit tumor growth in multiple tumor types (27). The miRNA miR-122 can regulate the PPARc/RXRa complex through interactions with

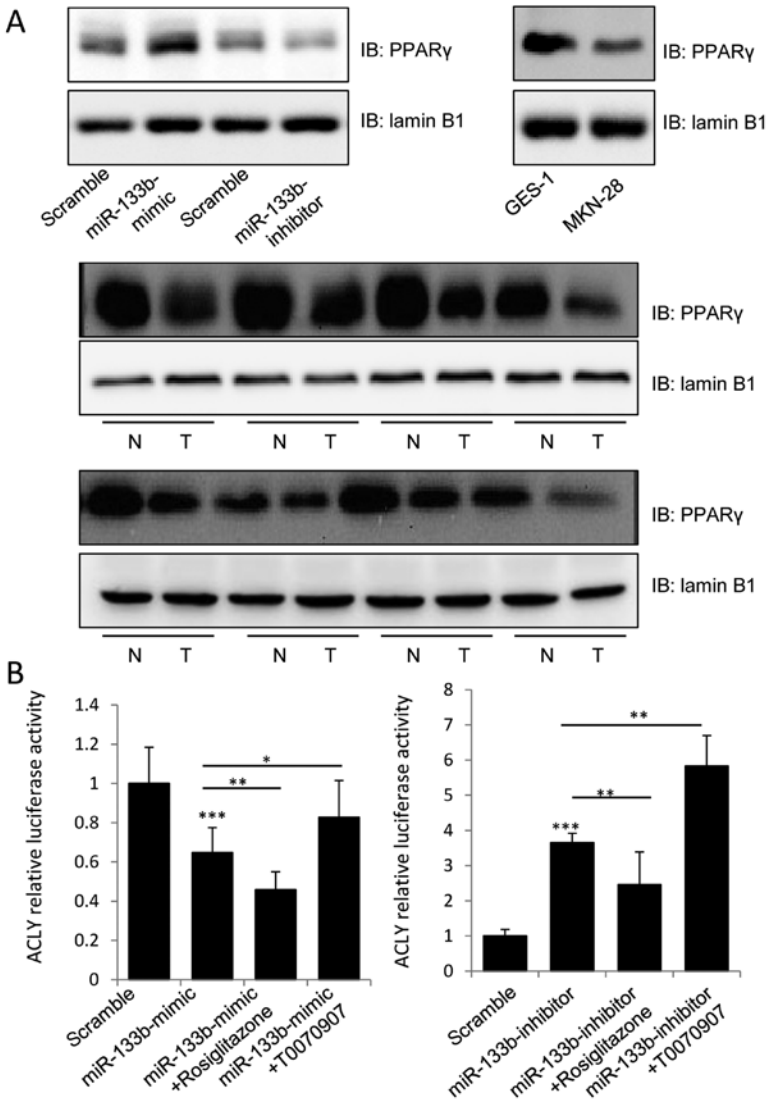

Figure 4. miR-133b induces tumor suppression of gastric cancer (GC) via PPARγ-mediated targeting of ATP citrate lyase (ACLY). (A) The nuclear lysates from MKN-74 cells transfected with miR-133b-mimic or miR-133b inhibitor and from GC tissues were immunoprecipitated with anti-PPARγ and anti-lamin B1 antibodies. (B) miR-133b-mimic or miR-133b inhibitor (50 ng), pGL3-ACLY construct (200 ng) and *Renilla* luciferase (10 ng) were co-transfected into MKN-74 cells. After 36 h, the cells were treated with or without rosiglitazone (1 μM) or T0070907 (1 μM) and cultured for another 24 h. The luciferase activity was normalized to the *Renilla* luciferase values. Data are expressed as fold changes relative to the control values. Data represent the mean ± standard error of the mean of three biological replicates assayed in technical triplicates. *p<0.05, **p<0.01 and ***p<0.001 vs. scramble or as indicated, determined by a two-tailed Student's t-test.

corepressors and H3K9 histone methyltransferase in hepatocellular carcinoma cells (28). In the present study we investigated whether miR-133b could regulate the expression of PPARγ to inhibit tumor growth in GC and, notably, we demonstrated that PPARγ expression was increased by miR-133b overexpression in GC cells.

As a cytosolic enzyme that catalyzes the generation of acetyl CoA from citrate, ACLY has been found to be involved in cancer progression, potentially via its regulatory role in cancer cell metabolism (15). It has been reported that ACLY expression and activity are markedly increased in lung, prostate, bladder, breast, liver, stomach, and colon tumors (14,29). Moreover, ACLY inhibition by siRNAs or the selective inhibitor SB-204990 has been found to suppress the growth and survival of tumor cells *in vitro* and *in vivo* (30).

In the present study we detected increased expression of ACLY in the GC cell lines MKN-74 and MGC80-3 at both the mRNA and protein levels when compared with the gastric epithelial cell line GES-1. Notably, this upregulation was concomitant with decreased expression levels of miR-133b. The *in vivo* findings in GC tissues were consistent with the *in vitro* data. Our subsequent observations indicated that the exogenous expression of miR-133b in MKN-74 cells could inhibit cell proliferation and invasion, which was accompanied by decreased expression of ACLY at both the mRNA and protein levels. Furthermore, the essential role of ACLY in miR-133b-induced tumor suppression was implicated by the findings that knockdown of miR-133b could increase ACLY activity and the growth and invasive capacities of MKN-74 cells *in vitro*. These findings revealed that ACLY could be a critical downstream target of the tumor suppressor activity of miR-133b in GC.

To further elucidate the molecular mechanism of miR-133b with regard to its inhibition of ACLY in GC, the transcription

of PPARγ in the nucleus was analyzed by western blotting, which indicated that miR-133b could induce PPARγ expression to inhibit tumor growth. A luciferase assay also revealed that transfection with miR-133b could decrease the transcriptional activity of the ACLY 3'UTR in a PPARγ-dependent manner within MKN-74 cells, suggesting that the apparent tumor-suppressive effect of miR-133b may be mediated by PPARγ, thus enabling it to regulate the expression of ACLY in GC cells.

Our present study provides evidence that manipulation of the miR-133b/ACLY axis may be a novel strategy for the treatment of GC. However, the extent to which the miR-133b/ACLY axis functionally aids in tumor suppression in GC requires further investigation.

Acknowledgements

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References

1. Murray CJ and Lopez AD: Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 349: 1498-1504, 1997.
2. Guo J, Miao Y, Xiao B, Huan R, Jiang Z, Meng D and Wang Y: Differential expression of microRNA species in human gastric cancer versus non-tumorous tissues. J Gastroenterol Hepatol 24: 652-657, 2009.
3. Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, *et al*: A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103: 2257-2261, 2006.
4. Ambros V: The functions of animal microRNAs. Nature 431: 350-355, 2004.
5. Bandrés E, Cubedo E, Agirre X, Malumbres R, Zárate R, Ramirez N, Abajo A, Navarro A, Moreno I, Monzó M, *et al*: Identification by Real-time PCR of 13 mature microRNAs differentially expressed in colorectal cancer and non-tumoral tissues. Mol Cancer 5: 29, 2006.
6. Zhong X, Coukos G and Zhang L: miRNAs in human cancer. In: Next-Generation MicroRNA Expression Profiling Technology: Methods and Protocols, Methods in Molecular Biology. Fan JB (ed). Vol.822. Humana Press, New York, pp 295-306, 2012.
7. Chiba Y, Tanabe M, Goto K, Sakai H and Misawa M: Down-regulation of miR-133a contributes to up-regulation of Rhoa in bronchial smooth muscle cells. Am J Respir Crit Care Med 180: 713-719, 2009.
8. Zhao H, Li M, Li L, Yang X, Lan G and Zhang Y: MiR-133b is down-regulated in human osteosarcoma and inhibits osteosarcoma cells proliferation, migration and invasion, and promotes apoptosis. PLoS One 8: e83571, 2013.
9. Hu G, Chen D, Li X, Yang K, Wang H and Wu W: miR-133b regulates the MET proto-oncogene and inhibits the growth of colorectal cancer cells in vitro and in vivo. Cancer Biol Ther 10: 190-197, 2010.
10. Crawford M, Batte K, Yu L, Wu X, Nuovo GJ, Marsh CB, Otterson GA and Nana-Sinkam SP: MicroRNA 133B targets pro-survival molecules MCL-1 and BCL2L2 in lung cancer. Biochem Biophys Res Commun 388: 483-489, 2009.
11. Han Y, Chen J, Zhao X, Liang C, Wang Y, Sun L, Jiang Z, Zhang Z, Yang R, Chen J, *et al*: MicroRNA expression signatures of bladder cancer revealed by deep sequencing. PLoS One 6: e18286, 2011.
12. Sobin LH, Gospodarowicz MK and Wittekind C (eds): TNM Classification of Malignant Tumours. 7th edition. Wiley-Blackwell, Hoboken, NJ, 2009.
13. Srere PA: The citrate cleavage enzyme. I. Distribution and purification. J Biol Chem 234: 2544-2547, 1959.
14. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuura M, Ushijima M, Mashima T, Seimiya H, Satoh Y, *et al*: ATP citrate lyase: Activation and therapeutic implications in non-small cell lung cancer. Cancer Res 68: 8547-8554, 2008.
15. Zaidi N, Swinnen JV and Smans K: ATP-citrate lyase: A key player in cancer metabolism. Cancer Res 72: 3709-3714, 2012.
16. Mueller E, Sarraf P, Tontonoz P, Evans RM, Martin KJ, Zhang M, Fletcher C, Singer S and Spiegelman BM: Terminal differentiation of human breast cancer through PPARγ. Mol Cell 1: 465-470, 1998.
17. DuBois RN, Gupta R, Brockman J, Reddy BS, Krakow SL and Lazar MA: The nuclear eicosanoid receptor, PPARγ, is aberrantly expressed in colonic cancers. Carcinogenesis 19: 49-53, 1998.
18. Ha M and Kim VN: Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 15: 509-524, 2014.
19. Kano M, Seki N, Kikkawa N, Fujimura L, Hoshino I, Akutsu Y, Chiyomaru T, Enokida H, Nakagawa M and Matsubara H: *miR-145*, *miR-133a* and *miR-133b*: Tumor-suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma. Int J Cancer 127: 2804-2814, 2010.
20. Williams AH, Liu N, van Rooij E and Olson EN: MicroRNA control of muscle development and disease. Curr Opin Cell Biol 21: 461-469, 2009.
21. Boštjančič E, Zidar N, Štajer D and Glavač D: MicroRNAs *miR-1*, *miR-133a*, *miR-133b* and *miR-208* are dysregulated in human myocardial infarction. Cardiology 115: 163-169, 2010.
22. Xiang KM and Li XR: MiR-133b acts as a tumor suppressor and negatively regulates TBPL1 in colorectal cancer cells. Asian Pac J Cancer Prev 15: 3767-3772, 2014.
23. Liu L, Shao X, Gao W, Zhang Z, Liu P, Wang R, Huang P, Yin Y and Shu Y: MicroRNA-133b inhibits the growth of non-small-cell lung cancer by targeting the epidermal growth factor receptor. FEBS J 279: 3800-3812, 2012.
24. Wen D, Li S, Ji F, Cao H, Jiang W, Zhu J and Fang X: miR-133b acts as a tumor suppressor and negatively regulates FGFR1 in gastric cancer. Tumour Biol 34: 793-803, 2013.
25. Stewart B and Wild CP (eds): World cancer report 2014. International Agency for Research on Cancer (IARC), Lyon, France, 2015.
26. Lemberger T, Desvergne B and Wahli W: Peroxisome proliferator-activated receptors: A nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol 12: 335-363, 1996.
27. Jiang C, Ting AT and Seed B: PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 391: 82-86, 1998.
28. Song K, Han C, Zhang J, Lu D, Dash S, Feitelson M, Lim K and Wu T: Epigenetic regulation of MicroRNA-122 by peroxisome proliferator activated receptor-gamma and hepatitis b virus X protein in hepatocellular carcinoma cells. Hepatology 58: 1681-1692, 2013.
29. Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, Hingorani SR, Tuveson DA and Thompson CB: ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell 8: 311-321, 2005.
30. Bauer DE, Hatzivassiliou G, Zhao F, Andreadis C and Thompson CB: ATP citrate lyase is an important component of cell growth and transformation. Oncogene 24: 6314-6322, 2005.