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Original Article

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miR-373-3p promotes aerobic glycolysis in colon cancer cells by targeting MFN2

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Abstract

MicroRNAs (miRNAs) are implicated in the development of cancers and may serve as potential targets for therapy. However, the functions and underlying mechanisms of miRNAs in cancers are not well understood. This work aims to study the role of miR-373-3p in colon cancer cells. We find that the expression of miR-373-3p mimics promotes and the miR-373-3p inhibitor suppresses aerobic glycolysis and proliferation of colon cancer cells. Mechanistically, miR-373-3p inhibits the expression of *MFN2*, a gene that is known to suppress glycolysis, which leads to the activation of glycolysis and eventually the proliferation of cells. In a nude mouse tumor model, the expression of miR-373-3p in colon cancer cells promotes tumor growth by enhancing lactate formation, which is inhibited by the co-expression of MFN2 in the cells. Administration of the miR-373-3p antagomir blunts *in vivo* tumor growth by decreasing lactate production. In addition, in human colon cancers, the expression levels of miR-373-3p are increased, while those of *MFN2* mRNA are decreased, and the increase of miR-373-3p is associated with the decrease of *MFN2* mRNA. Our results reveal a previously unknown function and underlying mechanism of miR-373-3p in the regulation of glycolysis and proliferation in cancer cells and underscore the potential of targeting miR-373-3p for colon cancer treatment.

Key words miRNA, colon cancer, glycolysis, cell proliferation, miR-373-3p, MFN2

Introduction

MicroRNAs (miRNAs) are a subclass of small noncoding RNAs (ncRNAs). They are single-stranded RNA molecules of approximately 19–24 nucleotides (nt) that are typically derived from 60- to 110-nt RNA hairpin precursors [1]. miRNAs are transcribed as primary miRNAs (pri-miRNAs), which are subsequently cleaved into precursor miRNAs (pre-miRNAs) and further processed into mature single-stranded ~22-nt miRNAs [2]. The biogenesis of miRNAs involves a complex protein system that includes the RNase III enzymes DROSHA and DICER1 [3]. The classic function of miRNAs is post-transcriptionally repressing expressions of specific target proteins by either promoting mRNA decay or dampening translation [1,4]. Recent studies have demonstrated that miRNAs may also be involved in translational upregulation, epigenetic regulation, and transcriptional activation [5].

miRNAs are involved in biological processes, including cell proliferation, differentiation, and apoptosis [1], and are expressed in distinct spatial and temporal patterns, both during embryonic and postnatal development and in adult tissues [6]. miRNAs have regulatory effects on metabolic enzymes, signaling pathways, and transcription factors involved in glucose and lipid metabolism [7,8]. miRNAs are associated with cancers [9,10] and have been proven to drive or repress tumorigenesis [11]. In cancer cells, miRNAs were found to control the Warburg effect, i.e., aerobic glycolysis [12–14]. Dysregulated miRNAs are associated with the clinical pathological features of many tumors, and they may serve as biomarkers for diagnosis and prognosis and as therapeutic targets for tumors [15–17].

Mitofusin 2 (MFN2) is implicated in cancer development [18]. MFN2 is downregulated in some types of cancer and inhibits cancer cells [19]. Bioinformatics analysis indicated that MFN2 is a promising predictive biomarker and therapeutic target for colon cancer [20]. Ovarian cancer patients with higher MFN2 expression have better survival than those with lower MFN2 expression [21]. Both breast and lung cancer patients with low MFN2 expression are associated with poor prognosis as compared to patients with high MFN2 expression [22]. MFN2 functions to mediate mitochondrial fusion [23] and suppress glycolysis [24–26].

Colon cancer is the third leading cause of death among various cancers and one of the leading causes of cancer death. miRNAs have been shown to be involved in colon cancer development, influencing cancer cell proliferation, apoptosis, metastasis, and angiogenesis [27–29]. To date, the role of miRNAs in the regulation of aerobic glycolysis in colon cancer cells remains largely unclear. Herein, we show that miR-373-3p targets MFN2 to promote aerobic glycolysis and proliferation in colon cancer cells. Targeting miR-373-3p inhibited the proliferation of colon cancer cells *in vitro* and tumor growth *in vivo*. Our results revealed a previously unknown function and underlying mechanism of miR-373-3p in colon cancer cells. Our findings also suggest that miR-373-3p may serve as a target for colon cancer treatment.

Materials and Methods

Cell culture and reagents

Human colon cancer SW480 and RKO cells were from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM (Gibco, Grand Island, USA) supplemented with 10% serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin. miR-373-3p mimics, miR-373-3p inhibitor, si-MFN2, and miR-373-3p antagomir were purchased from GenePharma (Shanghai, China). The sequence are as follows: negative control: 5′-UUCUCCGAACGUGUCACGUTT-3′; miR-373-3p mimics: 5′-GAA GUGCUUCGAUUUUGGGGUGU-3′; si-MFN2: 5′-CCCUCAACUAU GACCUAAATT-3′; control microRNA inhibitor: 5′-CAGUACUUUU GUGUAGUACAA-3′; miR-373-3p inhibitor: 5′-ACACCCCAAAAUC GAAGCACUUC-3′; control antagomir: 5′-CAGUACUUUUGUGUA GUACAA-3′; and miR-373-3p antagomir: 5′-GGAAAGCGCCCC CAUUUUGAGU-3′.

Construction of vectors and lentivirus

The vector encoding MFN2 was constructed from the pcDNA3.1-3 × Flag plasmid (Youbio Biological Technology, Changsha, China). The possible miR-373-3p binding sequence identified in the 3′UTR of *MFN2* mRNA was cloned and inserted into a psiCHECK-2 luciferase reporter vector (Youbio Biological Technology) for the construction of the MFN2-3′UTR-WT reporter. The mutated MFN2-3′UTR-Mut luciferase reporter vector was constructed with a site mutagenesis kit (Youbio Biological Technology). The lentiviruses expressing MFN2 and miR-373-3p were constructed using pcSLenti-EF1-EGFP-P2A-Puro-CMV-MCS-3 × FLAG-WPRE and pcSLenti-EF1-EGFP-F2A-Puro-CMV-MCS-WPRE, respectively. The lentiviruses expressing MFN2 and miR-373-3p were prepared by OBiO Technology (Shanghai, China).

Western blot analysis

Western blot analysis was performed in a regular way. Briefly, the cellular proteins were extracted using lysis buffer and the extracted proteins were quantitated using a BCA kit (GlpBio, Montclair, USA). The proteins were separated by 10% SDS-PAGE and transferred to 0.45-μm PVDF membrane. After being blocked with 5% skim milk at room temperature for 1 h, the membrane was incubated with primary antibody at 4°C for 12 h, followed by an incubation with HRP-conjugated secondary antibody (Proteintech, Wuhan, China) at room temperature for 2 h. The anti-MFN2 antibody was obtained from Santa Cruz (Dallas, USA). The anti-beta-actin antibody was obtained from Sigma (St Louis, USA). An enhanced chemiluminescence (ECL) kit (Meilunbio, Dalian, China) was used to visualize the protein bands.

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed as previously described [30]. Total RNA was extracted with TRIzol® (Invitrogen, Carlsbad, USA) and quantified with a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA). Two micrograms of total RNA was reverse-transcribed using EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). qRT-PCR was performed on an Applied Biosystems Step Two Real Time PCR System (Applied Biosystems, Foster City, USA). SYBR® Green Realtime PCR Master Mix (ABclonal, Wuhan, China) was employed to detect the expression levels of the target genes. The relative RNA expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method. *GAPDH* and *U6* were used as the internal control genes for mRNA and miRNA, respectively. The primers used were as follows: *MFN2*: 5′-CTCTCGATGCAACTCTATCGTC-3′ (F), 5′-TCCTGTACGTGTCTT CAAGGAA-3′ (R); *GAPDH*: 5′-CTGACTTCAACAGCGACACC-3′ (F), 5′-TGCTGTAGCCAAATTCGTTGT-3′ (R); miR-373-3p-stem-loop: 5′-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGAT ACGACACACCC-3′; U6-stem-loop: 5′-GTCGTATCCAGTGCGTG TCGTGGAGTCGGCAATTGCACTGGATACGACAAAAATAT-3′; miR-373-3p: 5′-GGGGAAGTGCTTCGATTTTG-3′ (F), 5′-CAGTGC GTGTCGTGGAGT-3′ (R); and *U6*: 5′-GGGGTGCTCGCTTCGGCAG CACA-3′ (F), 5′-CAGTGCGTGTCGTGGAGT-3′ (R).

Glucose and lactate assays

The glucose content was measured as previously described [31]. The lactate production was determined as previously described [32]. Briefly, 1×10^6 cells were seeded in a 60-mm plate and grown for 48 h. The culture medium was collected and subjected for determination of glucose and lactate using a glucose assay kit (Sigma) and a lactate assay kit (BioVision, San Francisco, USA), respectively. The glucose and lactate levels were normalized to the total cellular protein concentration.

Measurement of the pH value of cell culture solution

The pH value of cell culture medium was measured with a pH meter [33]. The pH meter measured results in increments of 0.01 pH units, between 0.00 and 14.00. According to the manufacturer's instructions, before analysis, the pH meter needed to be calibrated with a pH 6.8 buffer solution. Between measurements, the pH sensor and container were rinsed with deionized water.

Cell proliferation assay

Cell proliferation was determined using a CCK8 kit (TargetMol, Shanghai, China) as previously described [34]. In brief, cells were seeded into a 96-well plate at 2500 cells per well. Cell proliferation was detected at different time points. The absorbance of each well

was measured at 450 nm with a VICTOR® Nivo™ microplate reader (PerkinElmer, Waltham, USA).

Nude mouse tumor model

SW480 cells were infected with control, miR-373-3p, MFN2, or miR-373-3p and MFN2 lentivirus, and the cells with stable expression of above-mentioned virus were selected. The selected cells at logarithmic growth phase were harvested and suspended in PBS at 4°C. Two hundred microliters of the cell suspension (containing 6×10^6 cells) were then injected into the left axilla of nude mice with a 1-mL syringe to establish a subcutaneous mouse tumor model. Mice were humanely euthanized 45 days after SW480 cell inoculation, the tumors were harvested and used for subsequent evaluation.

In the miR-373-3p antagomir treatment experiments, 200 μL of untreated SW480 cell suspension (containing 6×10^6 cells) was injected into the left axilla of nude mice. Tumors that grew to approximately 50 mm^3 in volume were injected with miR-373-3p antagomir or control antagomir (10 nmol in 50 μL of diethyl pyrocarbonate-treated water) every 3 days for 4 weeks [35]. After treatment, two groups of nude mice were euthanized. After subcutaneous removal of the tumor, the weight of the tumor was measured and photographed. The tumor was stored in a −80°C freezer. The tumors were measured with a caliper and tumor volume was calculated using the following equation: volume = $a \times b^2/2$, where a is the longer dimension, and b is the shorter one.

The tumor lactate content was determined by using a Lactate Assay Kit (BioVision, San Francisco, USA). Briefly, the tumor tissues suspended in saline (g/mL at 1:9 ratio) were mechanically homogenized in a centrifuge tube in the presence of grinding beads on ice. The homogenate was centrifuged at 13400 *g* 4°C for 10 min. The supernatants were collected for measurement of lactate content. The supernatants were also used for western blot analysis.

The specific-c pathogen-free male BALB/c nude mice (5 weeks old) were purchased from Vital River Laboratory Animal Technology (Beijing, China) and maintained under specific pathogen-free conditions. All procedures were approved by the Ethics Committee of the Medical College of Qingdao University.

Determination of miR-373-3p and MFN2 in human colon cancer tissues

Colon cancer tissues paired with adjacent normal colonic tissues were collected from patients who underwent surgery at the Affiliated Hospital of Qingdao University from 2018 to 2019 after informed consent was obtained, and analyzed by qRT-PCR. The study protocol was approved by the ethical committee of the Medical College of Qingdao University. Patients' information is shown in Table 1.

Bioinformatics analysis

The potential targets of individual miR-373-3p were predicted by starBase (https://rnasysu.com/encori/agoClipRNA.php?source=mRNA) in combination with PITA, RNA22, microT, miRmap, miRanda, PicTar and TargetScan.

Statistical analysis

Data are presented as the mean ± SD. Statistical analyses were mainly conducted with SPSS 21.0 (IBM, Chicago, USA) and GraphPad Prism 8.4.0 (GraphPad Software Inc, La Jolla, USA). The differences between groups were analyzed using Student's *t* test, one-way ANOVA or Chi-square test. Correlations between groups were assessed with Pearson correlation coefficient. $P < 0.05$ was considered statistically significant.

Table 1. Information of patients with colon cancer

Information	Patients (%)
Sex	
Male	8 (44)
Female	10 (56)
Age, year	
≤60	5 (28)
>60	13 (72)
Tumor site	
Colon (R)	5 (28)
Colon (L)	6 (33)
Rectum	6 (33)
Others	1 (6)
Lymph node metastasis	
Yes	6 (33)
No	12 (67)
Invasion subtypes	
Serosal layer	14 (78)
Deep muscular layer	3 (17)
Submucosa	1 (5)

Results

miR-373-3p promotes aerobic glycolysis and proliferation in colon cancer cells

Although miR-373-3p is implicated in cancers, whether it influences aerobic glycolysis in cancer cells remains unknown. To test this, we examined SW480 and RKO colon cancer cells and found that the overexpression of miR-373-3p mimics enhanced lactate production in these cells (Figure 1A). In contrast, the expression of miR-373-3p inhibitor decreased the production of lactate in these cells (Figure 1B). The expression of miR-373-3p mimics enhanced and that of the miR-373-3p inhibitor decreased, the consumption rates of glucose in these cells, respectively (Figure 1C,D). Moreover, the expression of miR-373-3p mimics enhanced the acidification of the cell culture medium (Figure 1E), while the expression of the miR-373-3p inhibitor had the opposite effect (Figure 1F). Together, these data suggest that miR-373-3p promotes aerobic glycolysis in colon cancer cells.

miR-373-3p has been shown to have either tumor-promoting activities [36,37] or anti-tumor effects [34,38,39], depending on the type of cancer. The role of miR-373-3p in colon cancer cells is not clear. As miR-373-3p promoted glycolysis in SW480 and RKO cells, we presumed that it might has tumor-promoting effect on colon cancer cells. As expected, the expression of miR-373-3p mimics promoted the proliferation of SW480 and RKO cells (Figure 1G), while the expression of the miR-373-3p inhibitor had the opposite effect (Figure 1H). These data indicate that miR-373-3p has tumor-promoting effects on colon cancer cells.

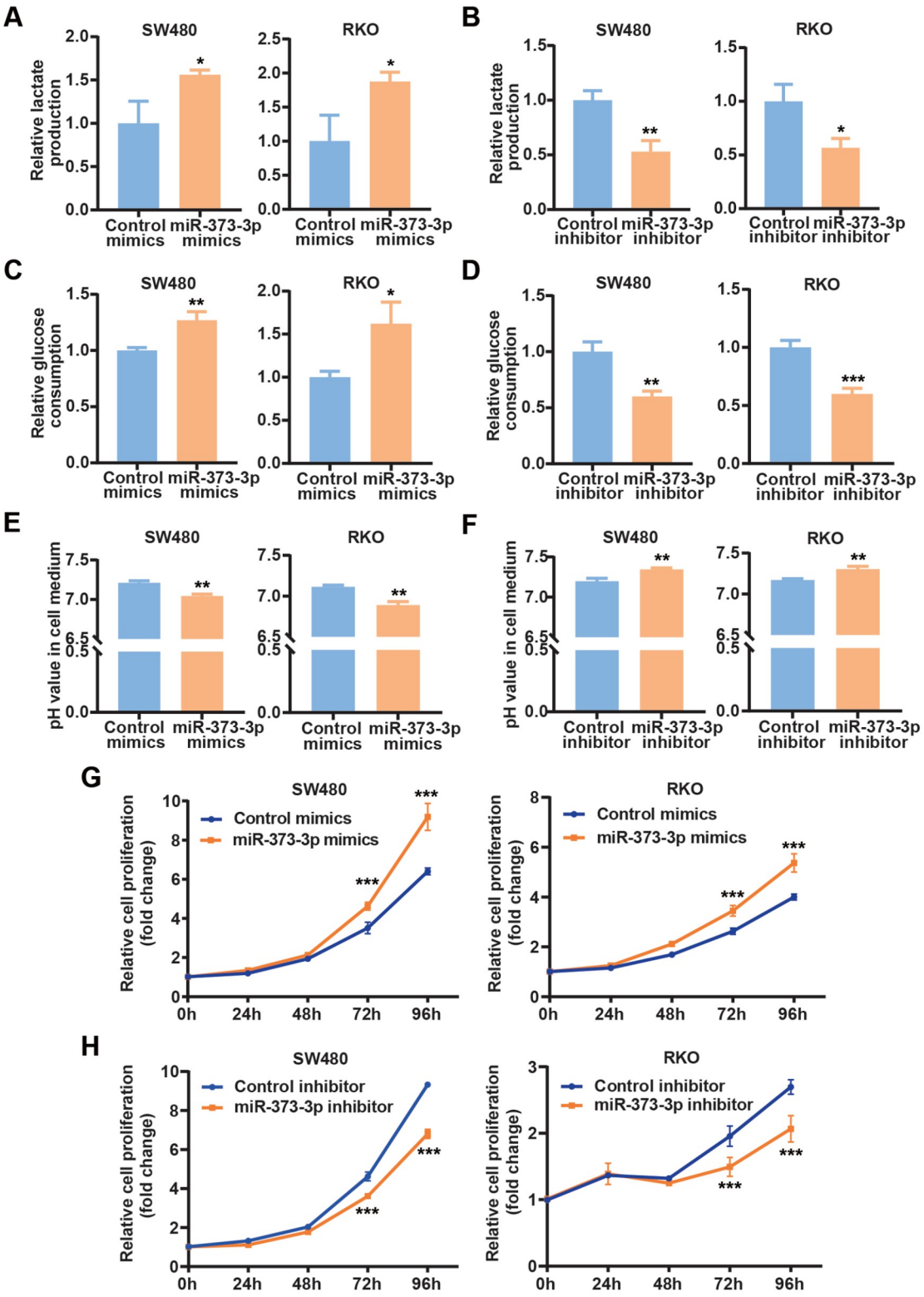

Figure 1. miR-373-3p promotes aerobic glycolysis in colon cancer cells (A) Determination of lactate production by SW480 (left) and RKO (right) cells expressing miR-373-3p mimics. (B) Effect of the miR-373-3p inhibitor on lactate production in SW480 (left) and RKO (right) cells. (C) Effect of the overexpression of miR-373-3p mimics on glucose consumption in SW480 and RKO cells. (D) Determination of glucose consumption by SW480 (left) and RKO (right) cells expressing the miR-373-3p inhibitor. (E) Effect of overexpression of miR-373-3p mimics on the pH of the culture media of SW480 and RKO cells. (F) Effect of the miR-373-3p inhibitor on the pH of the culture media of SW480 and RKO cells. (G) Effects of the expression of miR-373-3p mimics on the proliferation of SW480 cells (left) and RKO cells (right). (H) Effects of the miR-373-3p inhibitor on the proliferation of SW480 cells (left) and RKO cells (right). $*P<0.05$, $**P<0.01$, $***P<0.001$.

MiR-373-3p inhibits the expression of MFN2

Next, we determined the possible mechanism underlying the regulation of aerobic glycolysis by miR-373-3p. We predicted the possible targets of miR-373-3p in PITA, RNA22, microT, miRmap, miRanda, PicTar and TargetScan, and identified the mitofusin 2 (*MFN2*) gene as a possible target of miR-373-3p. As MFN2 inhibits glycolysis [24–26], we therefore presumed that miR-373-3p might modulate aerobic glycolysis by targeting *MFN2*. We found that overexpression of miR-373-3p mimics decreased the protein levels of MFN2 in both SW480 and RKO cells (Figure 2A,B). Treatment with the miR-373-3p inhibitor increased the protein level of MFN2 (Figure 2C,D). The expression of miR-373-3p mimics decreased (Figure 2E), and the expression of the miR-373-3p inhibitor increased the mRNA level of *MFN2* (Figure 2F). These data indicate that miR-373-3p acts as a negative regulator of the expression of MFN2.

A possible miR-373-3p-binding site in the 3′UTR of *MFN2* mRNA

The sequence was cloned and inserted into a luciferase reporter vector for the construction of the MFN2-3′UTR-WT luciferase reporter vector (Figure 3A). A mutated MFN2-3′UTR-Mut luciferase reporter vector was also constructed. The expression of miR-373-3p mimics inhibited the activity of the MFN2-3′UTR-WT reporter but not that of the MFN2-3′UTR-Mut reporter in both SW480 and RKO cells (Figure 3B), providing evidence that miR-373-3p binds to the 3′ UTR of *MFN2* mRNA. The expression levels of miR-373-3p were increased (Figure 3C), and those of *MFN2* mRNA were decreased in colon cancer tissues (Figure 3D). The expression levels of miR-373-3p were negatively associated with those of *MFN2* mRNA (Figure 3E). Together, these data suggest that miR-373-3p targets *MFN2* mRNA to inhibit MFN2 expression.

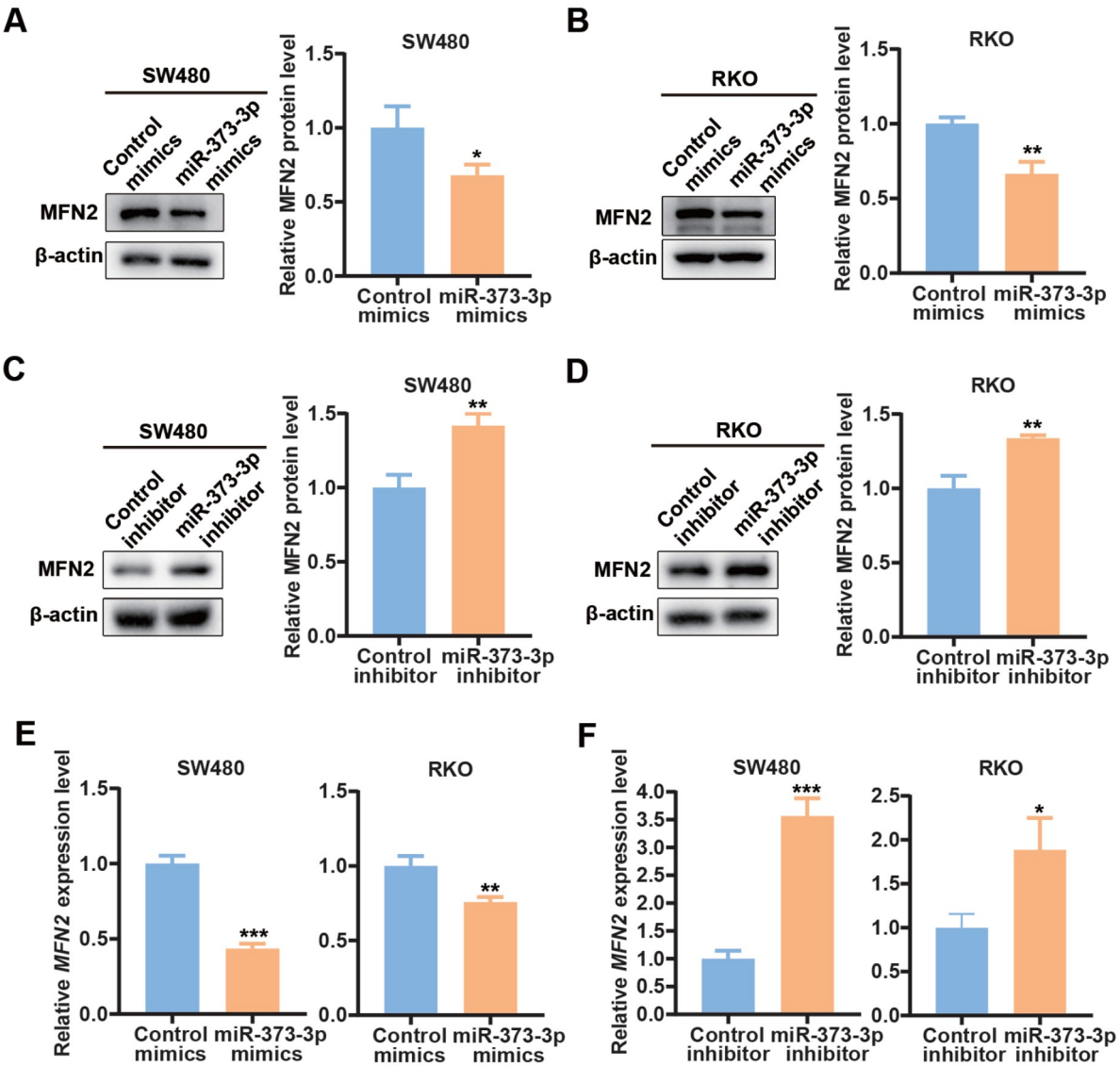

Figure 2. miR-373-3p inhibits the expression of MFN2 (A,B) Expression of miR-373-3p mimics decreased the expression of MFN2 in SW480 (A) and RKO (B) cells. The left panels are representative images of western blots. The right panels show the relative protein levels of MFN2 ($n=3$). (C,D) The miR-373-3p inhibitor increased the expression of MFN2 in SW480 (C) and RKO (D) cells. (E) The mRNA levels of *MFN2* in SW480 and RKO cells expressing miR-373-3p mimics. (F) *MFN2* mRNA levels in SW480 and RKO cells expressing the miR-373-3p inhibitor. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

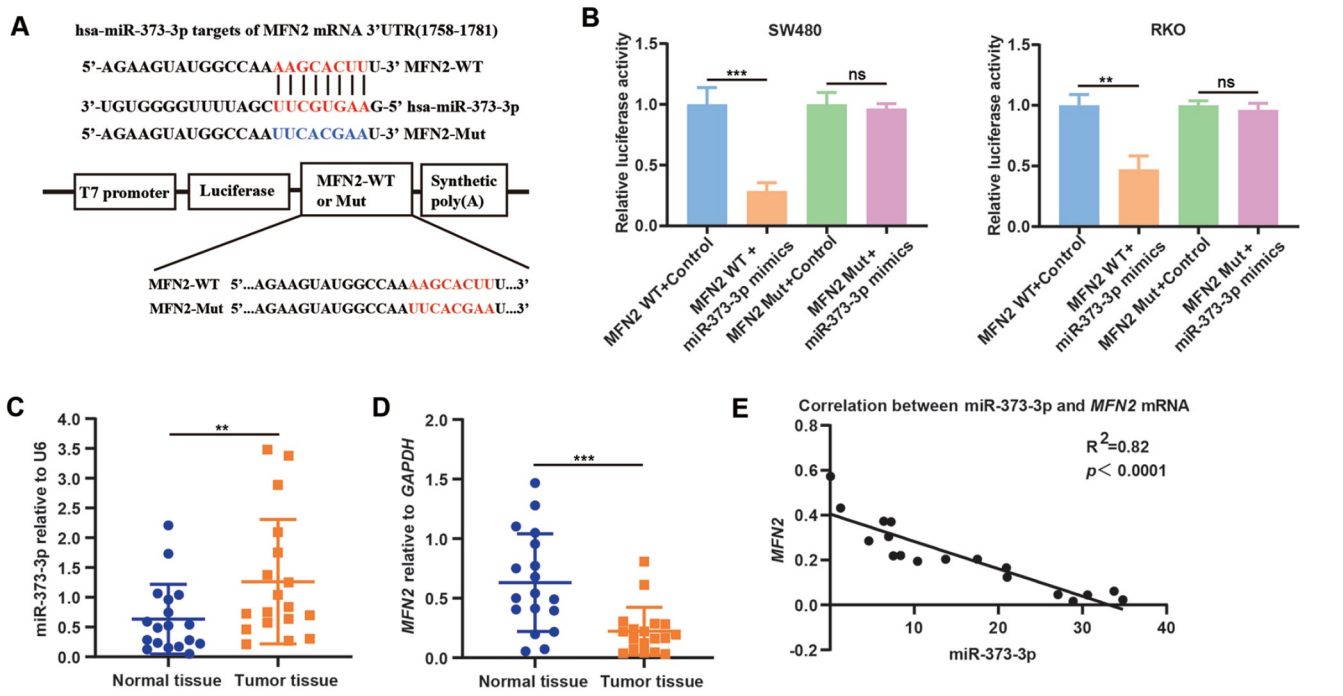

Figure 3. miR-373-3p binds to the 3′UTR of *MFN2* mRNA (A) The schematic diagram shows the predicted miR-373-3p binding sites in the 3′UTR of the human *MFN2* gene and the construction of the MFN2-WT and MFN2-Mut luciferase reporter vectors. (B) Luciferase activity assay in SW480 and RKO cells expressing MFN2-3′UTR-WT with miR-373-3p mimics or MFN2-3′UTR-Mut with miR-373-3p mimics. (C,D) The expression levels of miR-373-3p (C) and *MFN2* mRNA (D) in human colon cancer tissues and adjacent normal colonic tissues ($n=18$). (E) Correlation analysis of miR-373-3p levels and *MFN2* mRNA levels. $^{**}P<0.01$, $^{***}P<0.001$. ns, not significant.

MFN2 inhibits aerobic glycolysis and proliferation in colon cancer cells

Though MFN2 is involved in glycolysis [24–26], it is unknown whether MFN2 modulates glycolysis in colon cancer cells. We found that overexpression of MFN2 decreased the production of lactate in SW480 and RKO cells (Figure 4A). In contrast, knockdown of *MFN2* enhanced the production of lactate in these cells (Figure 4B). Overexpression of MFN2 inhibited and knockdown of *MFN2* promoted glucose consumption in SW480 and RKO cells (Figure 4C,D). Overexpression of MFN2 inhibited and knockdown of *MFN2* promoted acidification of the culture medium of these cells (Figure 4E,F). These data indicate that MFN2 suppresses aerobic glycolysis in colon cancer cells. In line with these results, overexpression of MFN2 inhibited and knockdown of *MFN2* promoted the proliferation of SW480 and RKO cells (Figure 4G,H), respectively.

MiR-373-3p promotes aerobic glycolysis through MFN2

To determine whether miR-373-3p regulates aerobic glycolysis through MFN2, we determined the effects of overexpression of MFN2 on aerobic glycolysis in SW480 and RKO cells expressing miR-373-3p mimics. The results showed that the expression of miR-373-3p mimics promoted lactate production, which was prevented by the overexpression of MFN2 (Figure 5A). Consistent with these results, the expression of the miR-373-3p inhibitor suppressed lactate production in these cells, which was reversed by the knockdown of *MFN2* (Figure 5B).

MiR-373-3p promotes colon cancer cell proliferation through MFN2

We found that the overexpression of miR-373-3p mimics promoted the proliferation of SW480 and RKO cells, which was repressed by the expression of exogenous MFN2 (Figure 5C). Consistent with these results, the expression of the miR-373-3p inhibitor attenuated the proliferation of the cells, which was reversed by the knockdown of MFN2 (Figure 5D).

We next performed an *in vivo* SW480 tumor growth assay in nude mice, and the results showed that the expression of miR-373-3p mimics promoted the growth of SW480 tumors (Figure 5E–G). Overexpression of MFN2 had the opposite effect (Figure 5E–G). The miR-373-3p-induced increase in SW480 tumor growth was blocked by coexpressing exogenous MFN2. Western blot analysis results showed that the expression of miR-373-3p mimics inhibited the expression of MFN2 in tumors (Figure 5H). We determined the lactate levels in tumors and found that the expression of miR-373-3p mimics enhanced the production of lactate (Figure 5I). In contrast, overexpression of MFN2 inhibited the production of lactate in tumors. And the miR-373-3p mimics-enhanced lactate production was inhibited by MFN2 overexpression. Together, these results suggest that miR-373-3p promotes glycolysis and proliferation in colon cancer cells through MFN2.

Administration of miR-373-3p antagomir inhibits *in vivo* tumor growth

To determine whether miR-373-3p could serve as a target for colon cancer therapy, mice harboring SW480 tumors were administered with an miR-373-3p antagomir as described in the Methods section. The results showed that the administration of the miR-373-3p antagomir, but not the control antagomir, to the mice significantly inhibited the growth of the SW480 tumors (Figure 6A–C). Western blot analysis of the tumor extracts indicated that miR-373-3p

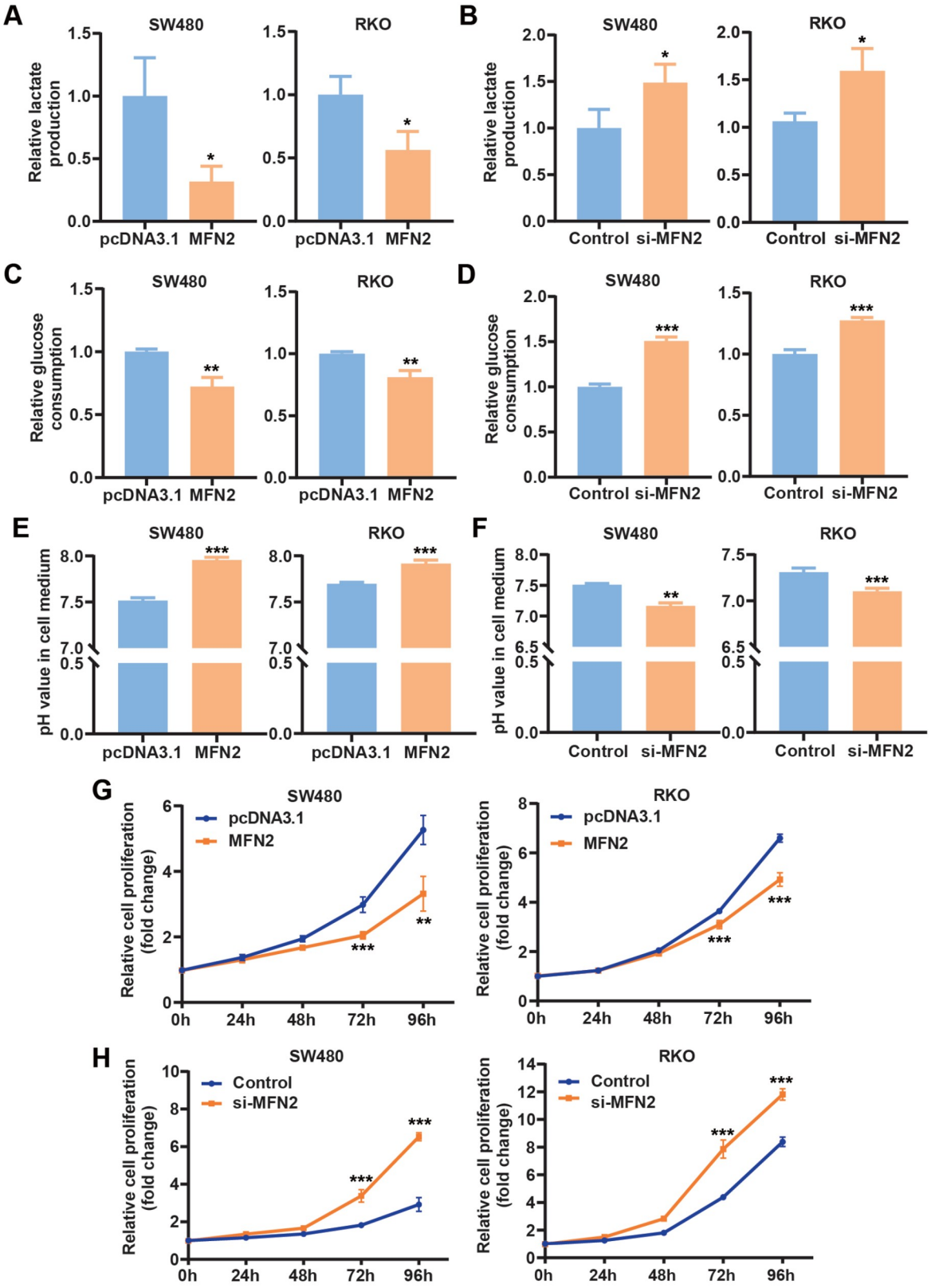

Figure 4. MFN2 inhibits glycolysis in colon cancer cells (A,B) Determination of lactate production by SW480 and RKO cells overexpressing MFN2 (A) or si-MFN2 (B). (C,D) Detection of glucose consumption by SW480 and RKO cells overexpressing MFN2 (C) or si-MFN2 (D). (E,F) Determination of pH values of the cell culture medium of SW480 cells and RKO cells overexpressing MFN2 (E) or si-MFN2 (F). (G,H) Determination of the effects of overexpression of MFN2 (G) or knockdown of MFN2 (H) on the proliferation of SW480 and RKO cells. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

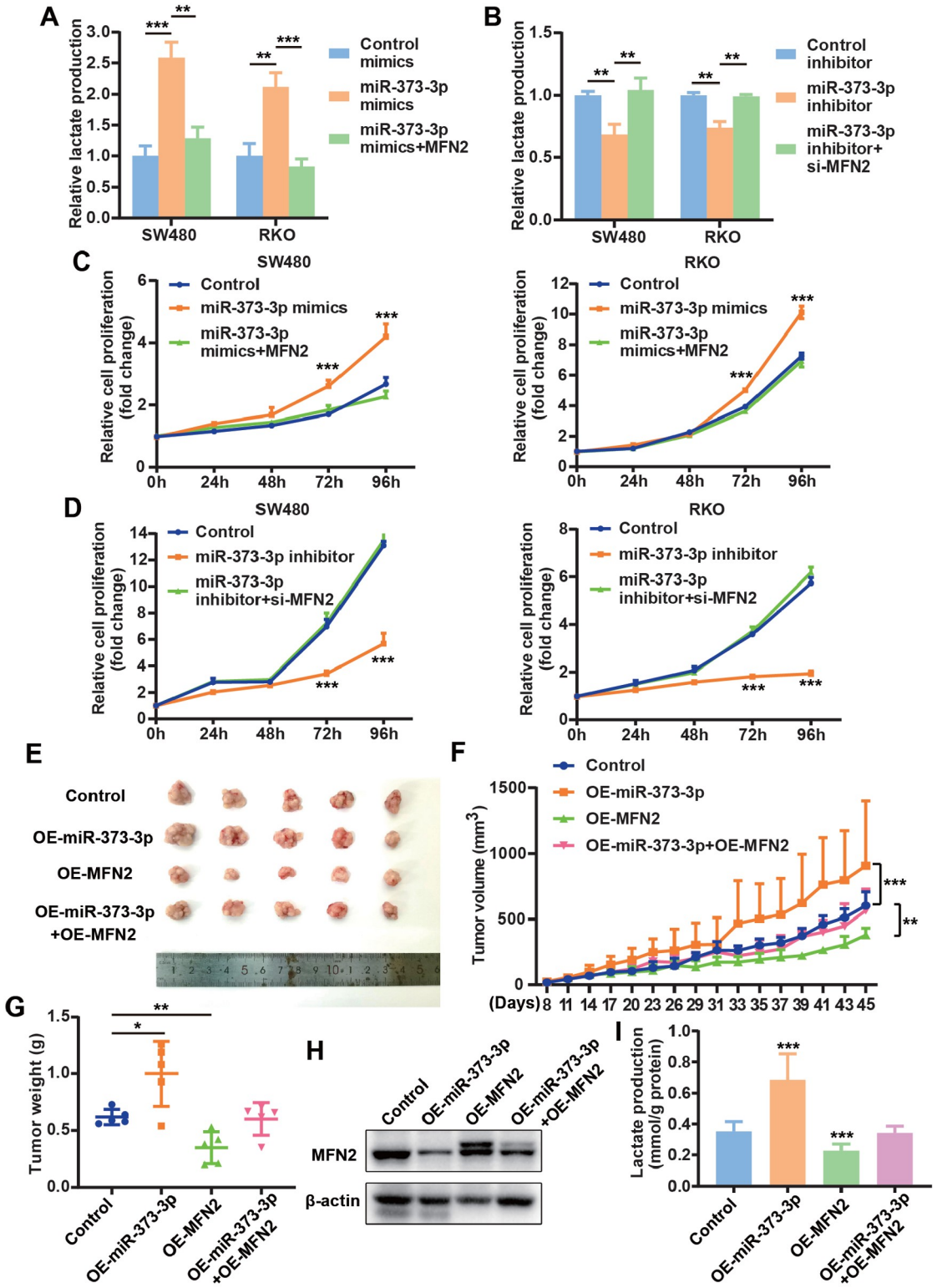

Figure 5. miR-373-3p inhibits colon cancer cell growth through MFN2 (A) Lactate production by SW480 (left) cells and RKO (right) cells expressing miR-373-3p mimics or miR-373-3p mimics and MFN2. (B) Lactate production by SW480 (left) cells and RKO (right) cells expressing the miR-373-3p inhibitor or miR-373-3p inhibitor and si-MFN2. (C) Determination of the proliferation of SW480 (left) and RKO (right) cells expressing miR-373-3p mimics or miR-373-3p mimics and MFN2. (D) Determination of the proliferation of SW480 (left) cells and RKO (right) cells expressing miR-373-3p inhibitor or miR-373-3p inhibitor and si-MFN2. (E) Images of xenografts of SW480 cells overexpressing miR-373-3p (OE-miR-373-3p), MFN2 (OE-MFN2), or miR-373-3p and MFN2 (OE-miR-373-3p + OE-MFN2). (F,G) Volumes (F) and weights (G) of xenografts of SW480 cells expressing miR-373-3p, MFN2, or miR-373-3p and MFN2 ($n = 5$). (H) Protein levels of MFN2 in xenografts. (I) Determination of lactate levels in xenografts. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

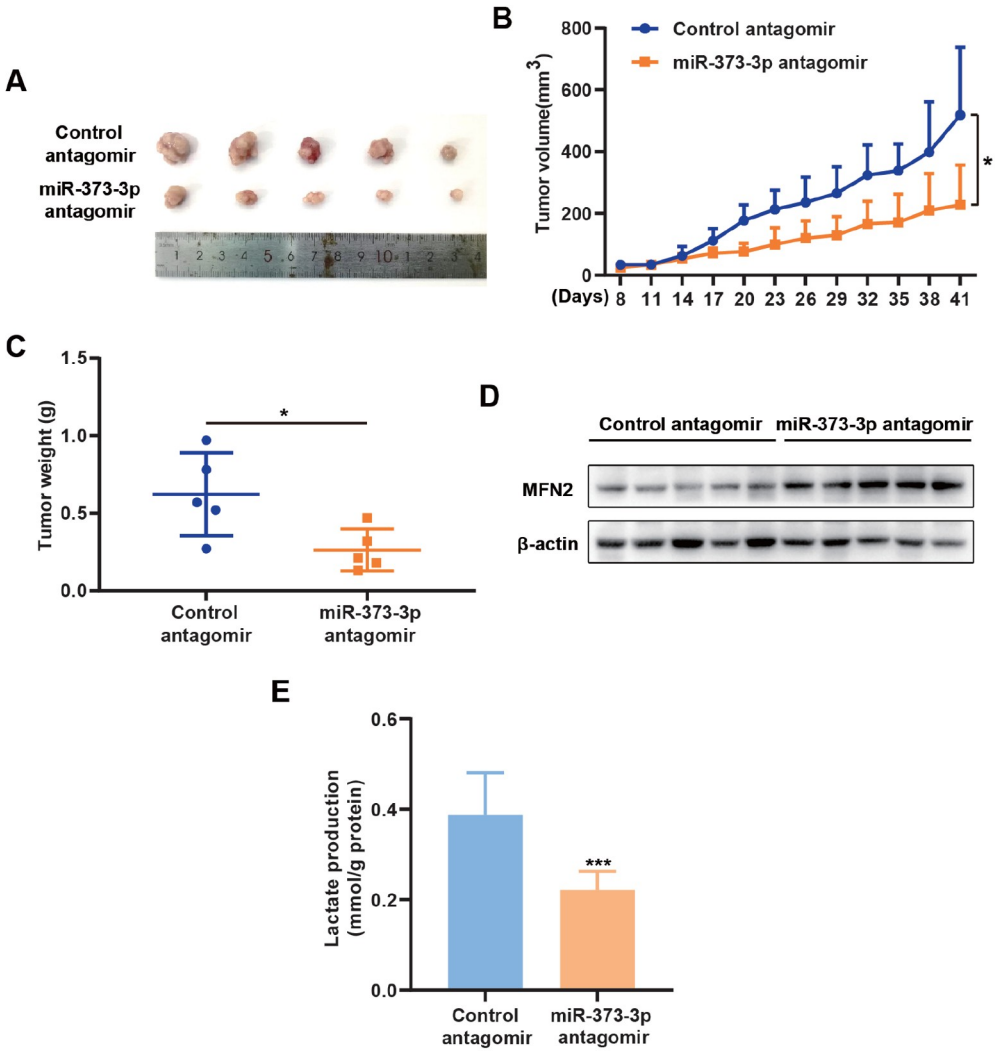

Figure 6. Administration of miR-373-3p antagomir inhibits *in vivo* tumor growth (A) Images of xenografts of SW480 cells treated with or without the miR-373-3p antagomir. (B,C) The volumes (B) and weights (C) of the xenografts ($n = 5$). (D) The protein levels of MFN2 in xenografts. (E) Determination of lactate production in xenografts. $^{*}P < 0.05$, $^{***}P < 0.001$.

antagomir treatment increased the protein level of MFN2 (Figure 6D). miR-373-3p antagomir treatment decreased lactate level in tumors (Figure 6E). These results suggest that miR-373-3p is a potential target for colon cancer treatment.

Discussion

In this study, we demonstrated that miR-373-3p promotes the proliferation of colon cancer cells by promoting aerobic glycolysis. We showed that miR-373-3p modulated aerobic glycolysis in colon cancer cells by inhibiting the expression of MFN2. Our findings reveal a previously unknown function of miR-373-3p in the regulation of aerobic glycolysis and proliferation of cancer cells (Figure 7).

Tumor cells adapt to characteristic metabolic phenotypes during cancer initiation and progression. Aerobic glycolysis is a hallmark of cancer. Unlike normal cells, most cancer cells produce energy by a high rate of glycolic catabolism to lactate in the cytosol rather than by oxidation of pyruvate in mitochondria, even in the presence of oxygen. Many studies have indicated that alterations in oncogenes and tumor suppressors are responsible for such metabolic reprogramming in cancer cells [40–42]. The molecular basis underlying cancer aerobic glycolysis is not well understood. The regulation of aerobic glycolysis is complex, and multiple factors are involved [43].

Increasing evidence has shown that miRNAs are involved in the regulation of aerobic glycolysis in cancers, including colon cancer [44–47]. Wu *et al.* [48] showed that miR-326 inhibits glycolysis by targeting the pyruvate kinase M2 isoform. Qin *et al.* [49] demonstrated that miR-4458 inhibits aerobic glycolysis in colon cancer cells by inhibiting hexokinase 2. Xu *et al.* [50] reported that miR-335-3p regulates lung cancer cells glycolysis through TEAD1. Zhu *et al.* [51] reported that microRNA-98 suppresses the Warburg effect by targeting HK2. Chen *et al.* [52] showed that microRNA-143 inhibits colon cancer cell glycolysis by targeting HK2. All these studies showed that miRNAs modulate aerobic glycolysis in colon cancer cells by controlling the expressions of glycolytic enzymes. In this work, we found that miR-373-3p promotes glycolysis by inhibiting the expression of MFN2, a protein that triggers a shift from aerobic glycolysis to mitochondrial oxidative metabolism [26]. MFN2 is a mitochondrial membrane protein that participates in

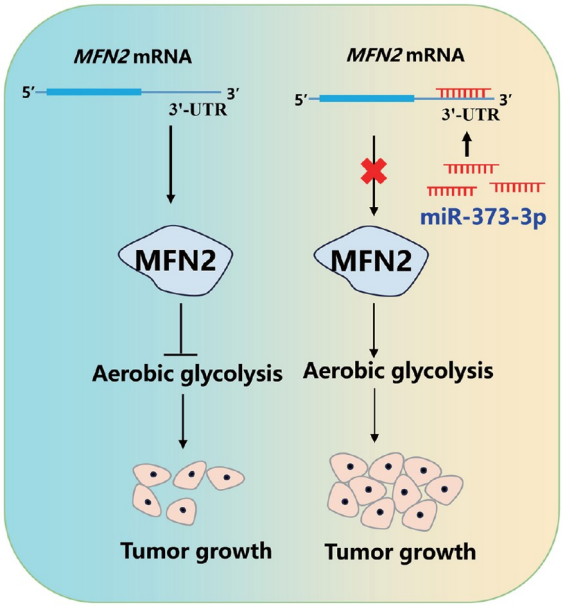

Figure 7. Proposed working model for miR-373-3p In colon cancer cells, miR-373-3p binds to the 3′UTR of *MFN2* mRNA to inhibit the expression of MFN2, which leads to an increase in aerobic glycolysis and ultimately proliferation of the cells.

mitochondrial fusion and contributes to the maintenance and operation of the mitochondrial network [53]. Though MFN2 was shown to have suppressive effects on a few cancers [18–22], there was publication demonstrating that it might have tumor-promoting effects. Ahn *et al.* [54] reported that MFN2 promoted the progression of cervical cancer. These results suggest that the role of MFN2 is tumor context-dependent.

A miRNA may have more than one target. miR-373-3p was demonstrated to target a few genes and to have multiple functions in cancer cells. For example, it was shown that miR-373-3p targeted *DKK1* mRNA for degradation to promote the metastasis of tongue squamous cell carcinoma [37]. In prostate cancer, miR-373-3p was found to target *AKT1* mRNA for degradation to inhibit prostate cancer [55]. These results suggest that miR-373-3p may act as a promoter or suppressor of cancers in a cell context-dependent manner. Here, we show that miR-373-3p inhibits the expression of MFN2 to promote aerobic glycolysis and proliferation in colon cancer cells. Since overexpression of MFN2 prevents miR-373-3p from promoting tumor growth, the results suggest that miR-373-3p promotes colon cancer at least partially through MFN2. MiR-373-3p was shown to affect the phosphorylation of AKT and ERK [56,57]. Therefore, we cannot exclude the possibility that in colon cancer cells, miR-373-3p promotes proliferation through AKT and/or ERK signaling.

Pre-clinical studies have demonstrated that miRNA-based therapeutics, along with various protective coating approaches, can be used for efficient delivery and anti-tumor activity. In fact, some miRNA-based cancer therapeutic strategies have shown promising results even in early-phase human clinical trials [58,59]. In our work, we found that the administration of the miR-373-3p antagomir to mice inhibited *in vivo* tumor growth of colon cancer cells, with increased expression of MFN2 and decreased production of lactate. These results suggest that targeting miR-373-3p might be a potential approach for the treatment of colon cancer.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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