Tropical Journal of Pharmaceutical Research November 2020; 19 (11): 2265-2272
ISSN: 1596-5996 (print); 1596-9827 (electronic)
© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v19i11.3

Original Research Article

MiR-331-5p suppresses gastric cancer cell proliferation, migration, invasion, and glycolysis via targeting PFKFB3

Zhongmin Deng, Guijun Wei*, Lei Qiu, Huifei Lu
Department of Gastroenterology, No. 1 People's Hospital of Huzhou, Huzhou City, Zhejiang Province 313000, China

For correspondence: **Email: guijun_wei@163.com; **Tel:** +86-572-2039321*

Sent for review: 8 July 2020 *Revised accepted: 28 October 2020*

Abstract

Purpose: *To examine the role of microRNAs (miRNAs), miR-331-5p, in gastric cancer (GC).*
Methods: *The mRNA level of miR-331-5p and protein level of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) were determined using quantitative real-time reverse transcription–polymerase chain reaction (qRT–PCR) and western blotting, respectively. The cell viability and proliferation of the two GC cell lines (AGS and MKN45) were evaluated using Cell Counting Kit-8 (CCK-8) and bromodeoxyuridine (BrdU) assays. Cell migration and invasion of AGS and MKN45 were evaluated using wound healing and invasion assays, respectively. Potential interactions between miR-331-5p and PFKFB3 were assessed by luciferase activity assay, while the effects of the interactions on cell physiology and metabolism were investigated in cells overexpressing both miR-331-5p and PFKFB3.*
Results: *MiR-331-5p overexpression inhibited cell proliferation, suppressed migration and invasion, and inhibited glycolysis in AGS and MKN45 cells. The mRNA for the glycolytic regulatory enzyme PFKFB3 was shown to be a direct target of miR-331-5p and modulated by miR-331-5p. In rescue experiments, PFKFB3 reversed the miR-331-5p-induced inhibition of proliferation, migration, invasion, and glycolysis in AGS cells.*
Conclusion: *This work supports a role for miR-331-5p through the modulation of PFKFB3 activity in GC in vivo, thus providing insight into novel potential therapies for the treatment of GC.*

Keywords: *Gastric cancer, MiR-331-5p, Cell proliferation, Glycolysis, PFKFB3*

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Gastric cancer (GC), a common gastrointestinal malignancy, is the second most common cause of cancer-related death [1,2]. Despite improvements in the diagnosis and treatment of cancer [3], gastric cancer-related mortality remains high, particularly in Asian countries [4]. Surgery is the most common and effective strategy for the treatment of GC. However, for patients with advanced gastric cancer, tumor resection may be impossible and treatment options are limited [5]. Thus, it is imperative to explore the molecular mechanism of GC oncogenesis to develop effective targeted therapies. Accumulating evidence has demonstrated that small molecules play important regulatory roles in the occurrence and

© 2020 The authors. This work is licensed under the Creative Commons Attribution 4.0 International License

development of GC [6,7]. Feng et al. have shown that the microRNA (miRNA) miR-126 plays an anticancer role in GC [6]. Moreover, miR-375 has been verified to inhibit the proliferation of GC cells by targeting and regulating the expression of Janus kinase 2, an important oncogenic enzyme [8]. miR-200b and miR-200c can be used as diagnostic markers for gastric cancer development [9]. miR-331-5p is a novel miRNA that is abnormally expressed and participates in several pathologies, including cerebral ischemia, leukemia, and human preterm placenta [10-12]. However, a potential role for miR-331-5p in GC remains to be defined.

The activity of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) controls the level of fructose-2,6-bisphosphate, which, in turn, regulates the activity of phosphofructokinase, a key regulatory enzyme in glycolysis. The process of glycolysis gives tumor cells more energy and opportunities to reproduce and invade. The *PFKFB3* gene is highly expressed in various malignant tumors, such as prostate cancer, colorectal cancer, gastric cancer, and pancreatic cancer [13]. More importantly, its high expression is closely related to a poor prognosis for these diseases [13,14]. PFKFB3 also promotes the proliferation and migration of GC cells, whereas the depletion of PFKFB3 inhibits the malignant phenotype of GC cells [15,16].

In the present study, we investigated the effect of miR-331-5p on proliferation, migration, invasion, and glycolysis in GC cells. Because PFKFB3 was predicted to be the target of miR-331-5p, we also investigated potential interactions between miR-331-5p and PFKFB3 in GC cells.

EXPERIMENTAL

Cell culture and transfection

The GC cell lines (AGS and MKN45) were obtained from the American Type Culture Collection (ATCC, USA) and cultured in RPMI1640 (Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) in 5% CO_2 at 37°C.

The miR-NC and miR-NC inhibitor were used as negative controls for the miR-331-5p mimic and miR-331-5p inhibitor, respectively. The miR-331-5p mimic, miR-331-5p inhibitor, and negative controls (miR-NC and miR-NC inhibitor) were acquired from GenePharma (China) and transfected into AGS and MKN45 cells using Lipofectamine 2000 (Invitrogen, USA).

For co-transfection, the miR-331-3p mimic or miR-NC and pcDNA3.1 or pcDNA3.1-PFKFB3 were co-transfected into AGS cells using Lipofectamine 2000 (Invitrogen, USA).

Cell counting kit-8 (CCK-8) assay

The CCK-8 assay was conducted using the Cell Counting Kit-8 kit [MedChemExpress (MCE), USA] following the manufacturer’s instructions. After transfection, the AGS and MKN45 cells were seeded into a 96-well plate. After 72 h, 10 μl of CCK8 solution was added to each well, and then the plate was incubated at 37°C for 3 h. The absorbance value at 450 nm was evaluated using the Multiscan MS spectrophotometer (Labsystems, Sweden).

Proliferation assay

Bromodeoxyuridine (BrdU) is commonly used to detect cell proliferation because it is readily incorporated into DNA during synthesis. The AGS and MKN45 cells were incubated with BrdU (Sigma-Aldrich, USA) to a final concentration of 50 μM. After incubation for 1 h, the cells were counted under a microscope (Leica, Germany).

Glucose uptake and lactate production detection

After transfection, the glucose uptake and lactate production of AGS and MKN45 cells were detected using the glucose uptake assay kit (Abcam, UK) and lactate assay kit (Abcam, UK), respectively. Finally, the glucose uptake and lactate production values were normalized to the protein concentration.

Wound healing assay

The AGS and MKN45 cells were seeded in 6-well plates and incubated until they reached 90% confluence. At time 0, a wound was scratched across each well using sterile tweezers, after which a photomicrograph was captured. After 24 h, cell migration was observed under a microscope.

Transwell assay

The cell invasion of AGS and MKN45 cells was assayed in 12-well transwell chambers with 8.0-μm pores (Corning, USA). The upper chamber with Matrigel was incubated with 1×10^5 AGS or MKN45 cells and medium, and the lower chamber was filled with medium as the attracting agent. After incubation for 48 h, the cells were fixed in methanol, stained with 0.2% gentian violet (m/v; Sigma-Aldrich; USA) and washed

with phosphate-buffered saline. Finally, the cells were photographed under a microscope, and the number of cells was counted.

Luciferase reporter assay

We verified the Targetscan (www.targetscan.org) prediction that PFKFB3 was a target of miR-331-5p as follows. In total, 2×10^3 AGS cells were seeded in 96-well plates and incubated overnight. The constructed luciferase plasmids (pGL3-PFKFB3-WT or pGL3-PFKFB3-MUT) plus miR-NC or miR-331-5p were co-transfected into AGS cells using Lipofectamine 3000 (Invitrogen, USA) as described earlier. After 48 h, the luciferase activity was detected using the Dual Luciferase Assay Kit (Zeye Biological Technology, China).

Quantitative real-time reverse transcription–polymerase chain reaction (qRT–PCR)

Total RNA was isolated using TRIzol reagent (Invitrogen, USA), and the RNA quality was detected using the NanoDrop 2000 system (Thermo Fisher Scientific, USA). The RNA was then reverse transcribed into cDNA, which was used as a template in combination with gene-specific primers (Table 1) using PowerUp™ SYBR Green (Thermo Fisher Scientific, USA) and amplifying with ABI7500 (Applied Biosystems, Foster City, CA, USA).

Table 1: Primers used in this study

Gene	Primer
miR-331-5p	sense: 5′-GCGCTAGGTATGGTCCCAG-3′ antisense: 5′-GTGCAGGGTCCGAGGT-3′
PFKFB3	sense: 5′-CCTCACTCGCAGCCACTTCT-3′ antisense: 5′-CAGTTCCTACTCAATTCCAA-3′
U6	sense: 5′-TGCTTCGGCAGCACATATAC-3′ antisense: 5′-TTCACGAATTTGCGTGTCAT-3′
β-actin	sense: 5′-GCATCGTCACCAACTGGGAC-3′ antisense: 5′-ACCTGGCCGTCAGGCAGCTC-3′

Western blotting

Cells were lysed with RIPA buffer (Beyotime, China), and the protein extracts were quantified using the BCA kit (Beyotime, China). The proteins (30 μg each lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane. The membrane was washed with Tris-buffered saline buffer (TBST) containing Tween 20 and then incubated in 5% skim milk containing PFKFB3 antibody (1:1000 dilution; Proteintech, USA) or β-actin antibody (1:5000 dilution; Proteintech, USA) at 4°C overnight.

Next, the membranes were probed with HRP-labeled Goat Anti-Mouse IgG (1:10000 dilution; Beyotime, China) or HRP-labeled Goat Anti-Rabbit IgG (1:10000 dilution; Beyotime, China) for 2 h at room temperature. The protein bands were visualized using the ECL chemiluminescent detection system (Beyotime, China).

Statistical analysis

All statistical analyses were performed using SPSS 23.0 software. Comparison between groups was performed using Student's *t*-test, and comparison among three groups was performed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Up-regulation of miR-331-5p inhibits cell proliferation

In the present study, miR-331-5p was overexpressed in AGS and MKN45 cell lines to examine its effect on GC proliferation (Figure 1 A). miR-331-5p overexpression significantly reduced AGS and MKN45 cell activities, as determined by the CCK-8 assay (Figure 1 B). In addition, miR-331-5p overexpression markedly inhibited the proliferation of AGS and MKN45 cells in the BrdU assay (Figure 1 C). These findings indicate that the up-regulation of miR-331-5p inhibits cell proliferation in AGS and MKN45 cells.

Up-regulation of miR-331-5p suppresses cell migration and invasion

The wound width remaining in AGS and MKN45 cells transfected with miR-331-5p mimic was significantly higher than that in cells transfected with miR-NC, indicating that miR-331-5p suppressed the migration of AGS and MKN45 cells (Figure 2 A).

Invasion (as determined by the transwell assay) was also significantly reduced in AGS and MKN45 cells expressing miR-331-5p mimic compared with the negative control (Figure 2 B). These findings show that miR-331-5p up-regulation suppresses cell migration and invasion in AGS and MKN45 cells.

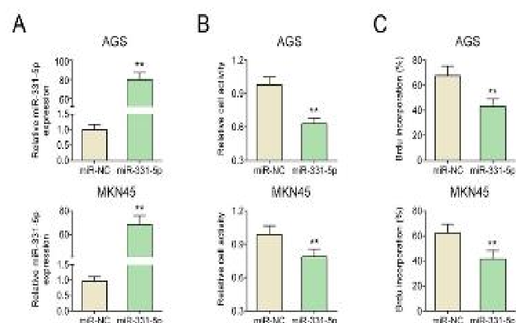

Figure 1: Upregulation of miR-331-5p inhibits cell proliferation. The AGS and MKN45 cell lines were transfected with miR-331-5p mimic or miR-NC. (A) qRT–PCR assay demonstrated that miR-331-5p mimic increased the miR-331-5p levels in AGS and MKN45 cells. (B) The CCK-8 assay revealed that miR-331-5p mimic reduced the cell viability of AGS and MKN45 cells. (C) The Edu staining assay demonstrated that the proliferation of AGS and MKN45 cells was suppressed by miR-331-5p mimic. Each experiment was repeated at least three times. ***p* < 0.01 vs. miR-NC

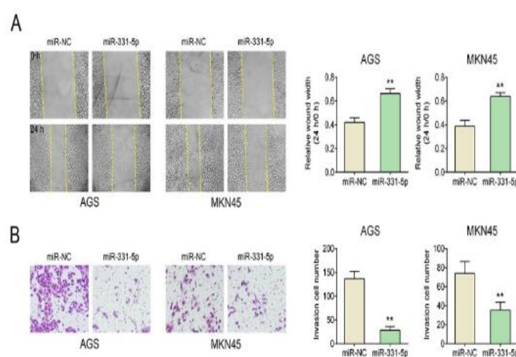

Figure 2: Up-regulation of miR-331-5p suppresses cell migration and invasion. (A) Twenty-four hours after transfection, cell migration was repressed in AGS and MKN45 cells compared that after miR-NC transfection, as indicated by the wound healing assay. (B) After transfection with miR-331-5p mimic, cell invasion was suppressed in AGS and MKN45 cells compared with that after miR-NC transfection, as determined by the transwell assay. Each experiment was repeated at least three times. ***p* < 0.01 vs. miR-NC

miR-331-5p inhibits glycolysis in AGS and MKN45 cells

Overexpression of miR-331-5p caused AGS and MKN45 cells to take up less glucose than the miR-NC group (Figure 3A). Cells overexpressing miR-331-5p mimic also seemed to produce less lactate than the miR-NC group (Figure 3B). These findings suggest that miR-331-5p inhibits glycolysis in AGS and MKN45 cells.

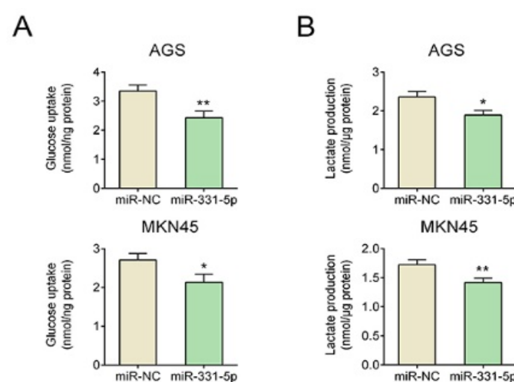

Figure 3: miR-331-5p inhibits glycolysis in AGS and MKN45 cells. AGS and MKN45 cells were transfected with either miR-NC or miR-331-5p. Comparison of the glucose uptake (A) and lactate production (B) between the two samples indicates that miR-331-5p inhibits glucose uptake and lactate production compared with miR-NC. Each experiment was repeated at least three times; **p* < 0.05, ***p* < 0.01 vs. miR-NC.

PFKFB3 is a direct target of miR-331-5p

To determine how miR-331-5p influences the proliferation, migration, invasion, and glycolytic metabolism of AGS cells, its effect on the expression of PFKFB3, a candidate target predicted by Targetscan (Figure 4 A), was investigated. First, the prediction that PFKFB3 was a target of miR-331-5p was confirmed by the overexpression of miR-331-5p in AGS cells significantly reducing the luciferase activity of PFKFB3-WT but not that of PFKFB3-MUT (Figure 4 B). In addition, qRT–PCR showed that the mRNA level of PFKFB3 was induced by miR-331-5p inhibitor but inhibited by miR-331-5p mimic in AGS cells (Figure 4 C). Consistent with the qRT–PCR results, the protein level of PFKFB3 was promoted by miR-331-5p inhibitor but reduced by miR-331-5p mimic in AGS cells (Figure 4 D). These findings suggest that PFKFB3 is a direct target of and is modulated by miR-331-5p.

PFKFB3 reverses the miR-331-5p-induced inhibition of GC cell proliferation, migration, invasion, and glycolysis

Consistent with the results of Figure 4, western blotting and qRT–PCR assay revealed that miR-331-5p mimic decreased PFKFB3 expression. Co-transfection with a PFKFB3 overexpression vector (pcDNA3.1-PFKFB3) increased PFKFB3 expression in AGS cells (Figure 5 A and B) and abrogated the effect of miR-331-5p mimic. Moreover, overexpression of PFKFB3 descended AGS cell and overexpressed PFKFB3 could ascend AGS cell activity (Figure 5 C). The BrdU assay confirmed that the

proliferation of AGS cells was inhibited by miR-331-5p mimic, whereas this effect of the miR-331-5p was reversed by PFKFB3 overexpression (Figure 5 D).

We also examined the interaction of miR-331-5p and PFKFB3 in glycolysis. Cells that overexpressed the miR-331-5p mimic showed reduced glucose uptake and lactate production compared with the negative control. However, if these cells simultaneously overexpressed PFKFB3, glucose uptake and lactate production increased, indicating that the miR-331-5p-induced inhibition of glycolysis was rescued by PFKFB3 overexpression (Figure 5 E and F). Likewise, the inhibition of AGS cell migration and invasion caused by miR-331-5p was reversed by PFKFB3 overexpression (Figure 5 G and H). These findings suggest that PFKFB3 overexpression reverses miR-331-5p-induced inhibition of proliferation, migration, invasion, and glycolysis.

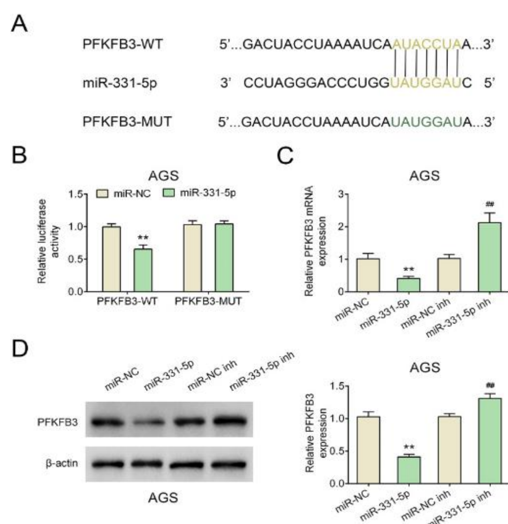

Figure 4: PFKFB3 is a direct target of miR-331-5p. (A) The predicted miR-331-5p-binding sites in the 3′-UTR region of PFKFB3 (PFKFB3-WT) and designed mutant sequence (PFKFB3-MUT), as determined by Targetscan, are indicated. (B) The miR-331-5p mimic significantly reduced the luciferase activity of PFKFB3-WT but not that of PFKFB3-MUT in AGS cells. Luciferase reporter assay in AGS cells co-transfected with miR-331-5p mimic and the indicated luciferase PFKFB3-WT or PFKFB3-MUT. After transfection with miR-331-5p inhibitor or miR-331-5p mimic, the (C) expression of PFKFB3 mRNA (measured by qRT–PCR) was induced by miR-331 inhibitor and inhibited by miR-331 mimic in AGS cells (D) and the expression of PFKFB3 protein (as measured by western blotting) was induced by miR-331 inhibitor and inhibited by miR-331 mimic in AGS cells. Each experiment was repeated at least three times. **$p < 0.01$ vs. miR-NC. ##$p < 0.01$ vs. miR-NC inhibitor

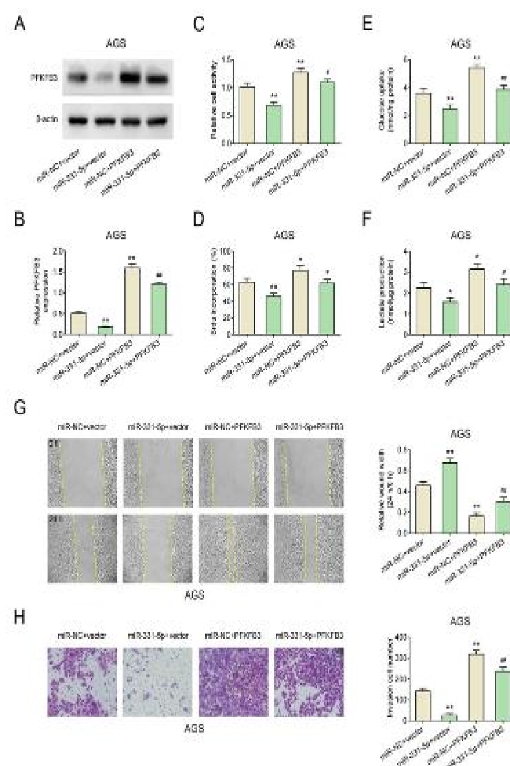

Figure 5: PFKFB3 reverses miR-331-5p-induced inhibition of proliferation, migration, invasion, and glycolysis. Western blot (A) and qRT–PCR (B) analysis of PFKFB3 expression in miR-331-5p-overexpressed AGS cells showed that transfection of cells with pcDNA3.1-PFKFB3 increased the protein and mRNA levels, respectively. (C) Whereas miR-331-5p mimic decreased the number of viable cells (as determined by the CCK-8 assay), PFKFB3 overexpression increased it, restoring the value to that observed in cells not overexpressing miR-331-5p mimic. (D) Whereas miR-331-5p mimic decreased AGS cell proliferation (detected using the BrdU assay), PFKFB3 overexpression increased it to the level of cells not overexpressing miR-331-5p mimic. PFKFB3 overexpression also reversed the miR-331-5p-induced decrease in glucose uptake (E) and lactate production (F) in AGS cells. The inhibition of AGS cell migration (as measured by the wound healing assay) (G) and invasion (as measured by the transwell assay) (H) caused by miR-331-5p overexpression was also reversed by simultaneous PFKFB3 overexpression. Each experiment was repeated at least three times. *$p < 0.05$, **$p < 0.01$ vs. miR-NC + vector. #$p < 0.05$, ##$p < 0.01$ vs. miR-331-5p + vector

DISCUSSION

miRNAs are a class of non-coding RNAs that participates in the regulation of cell proliferation, apoptosis, and differentiation. miR-331-5p is a novel miRNA that is abnormally expressed in multiple diseases. Down-regulation of miR-331-5p is related to middle cerebral artery occlusion (MCAO)-induced tissue infarction, neurological deficits, and inflammasome activation,

suggesting that it has neuroprotective effects in ischemic injury [10]. Guinever has demonstrated that increased miR-331-5p participates in chorioamnionitis by inducing a specific signature of placental ABC transporters [12]. In cancer, down-regulated miR-331-5p is associated with resistance to chemotherapy and relapse in leukemia [11]. To our best knowledge, the function of miR-331-5p in GC has not yet been investigated.

In this study, we showed that miR-331-5p overexpression decreased the viable cell number, inhibited cell proliferation, and suppressed cell migration and invasion in AGS and MKN45 cells. Interestingly, miR-331-5p also inhibited key glycolytic activities in AGS and MKN45 cells. To explore the mechanism by which miR-331-5p inhibits proliferation, migration, invasion, and glycolysis, we predicted that PFKFB3 is a target of miR-331-5p. We confirmed this prediction using the luciferase reporter assay and by determining that PFKFB3 mRNA is, indeed, modulated by miR-331-5p. In rescue experiments, PFKFB3 could reverse miR-331-5p-induced inhibition of proliferation, migration, invasion, and glycolysis. This study has elucidated the mechanism and role of miR-331-5p in GC *in vivo*, thus providing insight into novel potential treatments of GC.

Glycolysis induces increased ATP production and provides a growth advantage to cancer cells [17,18]. In addition, cancer cells need further metabolic intermediates and precursors that are essential for the biosynthesis of macromolecules. Furthermore, the final components are indispensable to increase tumor growth [19]. In GC, glycolysis is related to changes in the activities of key enzymes, mitochondrial protein expression, and levels of non-coding RNAs compared with normal tissues or cells [20]. Not only do these molecules affect the metabolism of GC cells but also they influence the proliferation, migration, and invasion of GC cells [20]. GC is caused by the cumulative dysregulation of multiple signaling pathways, which disrupt cell proliferation, cell cycle, cell death, and metastasis [21]. Our work shows that miR-331-5p a critical factor in causing these effects and changes in glycolysis; thus, it may play an important role in GC progression.

Although investigations about the target of miR-331-5p are limited, PFKFB3 was predicted and proven to be a potential target of miR-331-5p. PFKFB3 is one of four genes encoding 6-phosphofructokinase-2/fructose bisphosphatase-2, an important regulator of glycolysis [22]. It converts glucose-6-phosphoric acid to glucose-3-phosphoric acid and is present at high levels in various tumor cell lines where it causes more glucose to be metabolized through the glycolytic pathway, thus providing energy for tumor proliferation and the synthesis of substrates [23]. Wang et al. have demonstrated that miR-488 suppresses proliferation and glycolysis via targeting PFKFB3 in human prostate cancer cells [24] and miR-206 inhibits proliferation, migration, and glycolysis of breast cancer cells by targeting PFKFB3 [25]. In GC cells, miR-449c inhibits cell migration and invasion by targeting PFKFB3, an effect that can be eliminated by PFKFB3 overexpression [26].

Likewise, this work shows that PFKFB3 overexpression abrogates the inhibitory effects of miR-331-5p on migration and invasion in GC cells as well as the inhibitory effects of miR-331-5p on proliferation and glycolysis. It might be great significance that the role of PFKFB3 in proliferation and glycolysis in GC cells. Notably, given the limitation of miR-331-5p in cancers, miR-331-5p may have different downstream targets in GC, requiring more investigations in the future.

CONCLUSION

The present study has demonstrated that the overexpression of miR-331-5p mimic inhibits proliferation, migration, invasion and glycolysis in two GC cell lines. A target of miR-331-5p (PFKFB3) was identified, and its overexpression was shown to reverse these effects of miR-331-5p in AGS cells. Because the upregulation of miR-331-5p expression inhibits GC progression, possibly via PFKFB3 inhibition, miR-331-5p may be a potential target for GC treatment.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Zhongmin Deng and Guijun Wei designed the study, supervised the data collection, and analyzed the data. Lei Qiu interpreted the data and prepared the manuscript for publication. Huifei Lu supervised the data collection, analyzed the data and reviewed the draft of the manuscript. All

authors have read and approved the manuscript for publication.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. *Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65(2): 87-108.*
2. *Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127(12): 2893-2917.*
3. *Nikkhoi SK, Rahbarizadeh F, Ahmadvand D. Oligo-clonal nanobodies as an innovative targeting agent for cancer therapy: new biology and novel targeting systems. Protein Expr Purif 2017; 129(115-121.*
4. *An JY, Cheong JH, Hyung WJ, Noh SH. Recent evolution of surgical treatment for gastric cancer in Korea. J Gastric Cancer 2011; 11(1): 1-6.*
5. *Xu S, Li D, Li T, Qiao L, Li K, Guo L, Liu Y. miR-494 Sensitizes Gastric Cancer Cells to TRAIL Treatment Through Downregulation of Survivin. Cell Physiol Biochem 2018; 51(5): 2212-2223.*
6. *Feng R, Chen X, Yu Y, Su L, Yu B, Li J, Cai Q, Yan M, Liu B, Zhu Z. miR-126 functions as a tumour suppressor in human gastric cancer. Cancer Lett 2010; 298(1): 50-63.*
7. *Wu JT, Lv SM, Lu CH, Gong J, An JB. Effect of 3,3 '-Biisofraxidin on Apoptosis of Human Gastric Cancer BGC-823 Cells. Tropical Journal of Pharmaceutical Research.*
8. *Ding L, Xu Y, Zhang W, Deng Y, Si M, Du Y, Yao H, Liu X, Ke Y, Si J et al. MiR-375 frequently downregulated in gastric cancer inhibits cell proliferation by targeting JAK2. Cell Res 2010; 20(7): 784-793.*
9. *Tang H, Deng M, Tang Y, Xie X, Guo J, Kong Y, Ye F, Su Q, Xie X. miR-200b and miR-200c as prognostic factors and mediators of gastric cancer cell progression. Clin Cancer Res 2013; 19(20): 5602-5612.*
10. *Zhang HS, Liu MF, Ji XY, Jiang CR, Li ZL, OuYang B. Gastrodin combined with rhynchophylline inhibits cerebral ischaemia-induced inflammasome activation via upregulating miR-21-5p and miR-331-5p. Life Sci 2019; 239(116935.*
11. *Feng DD, Zhang H, Zhang P, Zheng YS, Zhang XJ, Han BW, Luo XQ, Xu L, Zhou H, Qu LH et al. Down-regulated miR-331-5p and miR-27a are associated with chemotherapy resistance and relapse in leukaemia. J Cell Mol Med 2011; 15(10): 2164-2175.*
12. *do Imperio GE, Bloise E, Javam M, Lye P, Constantinof A, Dunk C, Dos Reis FM, Lye SJ, Gibb W, Ortiga-Carvalho TM et al. Chorioamnionitis Induces a Specific Signature of Placental ABC Transporters Associated with an Increase of miR-331-5p in the Human Preterm Placenta. Cell Physiol Biochem 2018; 45(2): 591-604.*
13. *Minchenko OH, Tsuchihara K, Minchenko DO, Bikfalvi A, Esumi H. Mechanisms of regulation of PFKFB expression in pancreatic and gastric cancer cells. World J Gastroenterol 2014; 20(38): 13705-13717.*
14. *Imbert-Fernandez Y, Clem BF, O'Neal J, Kerr DA, Spaulding R, Lanceta L, Clem AL, Telang S, Chesney J. Estradiol stimulates glucose metabolism via 6-phosphofructo-2-kinase (PFKFB3). J Biol Chem 2014; 289(13): 9440-9448.*
15. *Yalcin A, Telang S, Clem B, Chesney J. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. Exp Mol Pathol 2009; 86(3): 174-179.*
16. *Han J, Meng Q, Xi Q, Wang H, Wu G. PFKFB3 was overexpressed in gastric cancer patients and promoted the proliferation and migration of gastric cancer cells. Cancer Biomark 2017; 18(3): 249-256.*
17. *Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4(11): 891-899.*
18. *Lunt SY, Vander Heiden MG. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu Rev Cell Dev Biol 2011; 27(441-464.*
19. *Deberardinis RJ, Sayed N, Ditsworth D, Thompson CB. Brick by brick: metabolism and tumor cell growth. Curr Opin Genet Dev 2008; 18(1): 54-61.*
20. *Liu Y, Zhang Z, Wang J, Chen C, Tang X, Zhu J, Liu J. Metabolic reprogramming results in abnormal glycolysis in gastric cancer: a review. Onco Targets Ther 2019; 12(1195-1204.*
21. *Berger H, Marques MS, Zietlow R, Meyer TF, Machado JC, Figueiredo C. Gastric cancer pathogenesis. Helicobacter 2016; 21 Suppl 1(34-38.*
22. *Boyd S, Brookfield JL, Critchlow SE, Cumming IA, Curtis NJ, Debreczeni J, Degorce SL, Donald C, Evans NJ, Groombridge S et al. Structure-Based Design of Potent and Selective Inhibitors of the Metabolic Kinase PFKFB3. J Med Chem 2015; 58(8): 3611-3625.*
23. *Kim SG, Manes NP, El-Maghrabi MR, Lee YH. Crystal structure of the hypoxia-inducible form of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3): a possible new target for cancer therapy. J Biol Chem 2006; 281(5): 2939-2944.*
24. *Wang J, Li X, Xiao Z, Wang Y, Han Y, Li J, Zhu W, Leng Q, Wen Y, Wen X. MicroRNA-488 inhibits proliferation and glycolysis in human prostate cancer cells by*

regulating PFKFB3. FEBS Open Bio 2019; 9(10): 1798-1807.

25. *Ge X, Lyu P, Cao Z, Li J, Guo G, Xia W, Gu Y. Overexpression of miR-206 suppresses glycolysis, proliferation and migration in breast cancer cells via PFKFB3 targeting. Biochem Biophys Res Commun 2015; 463(4): 1115-1121.*
26. *Chen X, Wang A, Yue X. miR-449c inhibits migration and invasion of gastric cancer cells by targeting PFKFB3. Oncol Lett 2018; 16(1): 417-424.*