MicroRNA-200b reduced ZEB1 to inhibit cell proliferation and migration in human gastric cancer

He Chen

First Affiliated Hospital of Anhui Medical University

Pengcheng Liu

First Affiliated Hospital of Anhui Medical University

Lei Wang

Huaian First People's Hospital

Yanxia Yu

Anhui Medical University

Yan Zhao

First Affiliated Hospital of Anhui Medical University

Yihong Cai

Anhui Medical University

Zhongxin Wang

First Affiliated Hospital of Anhui Medical University

Min Zhang (✉ ahmuzhangmin@163.com)

Research article

Keywords: microRNA-200b, proliferation, migration, gastric cancer

Posted Date: June 8th, 2020

DOI: <https://doi.org/10.21203/rs.2.17514/v4>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

BACKGROUND: Gastric cancer (GC) is one of the most common malignant cancers, with high morbidity and mortality rates worldwide. The present study was to explore whether miR-200b is a tumor suppressor in GC and to unveil the potential mechanisms.

METHODS: Levels of c-Myc, Cyclin D1, MMP-3 and MMP-9 expression were detected respectively by qRT-PCR and Western blot assay. BrdU proliferation assay, Cell cycle analysis, Wound-healing and Transwell assays were used to study the role of miR-200b with inhibitor, mimics or ZEB1-RNAi in TGF- β 1-treated SGC-7901/DDP cells. The xenograft model with nude mice was established to unveil the role of miR-200b *in vivo*.

RESULTS: Compared with the paracancerous tissues, miR-200b was decreased in GC patients and SGC-7901/DDP cells. Lower level of miR-200b induced by its inhibitor promoted TGF- β 1-treated SGC-7901/DDP cells proliferation and migration, and increased the levels of c-Myc, Cyclin D1, MMP-3, MMP-9, β -catenin and APC. Interestingly, miR-200b mimics and ZEB1-RNAi were able to reduce the proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells as well as their levels of c-Myc, Cyclin D1, MMP-3, MMP-9, β -catenin and APC. In addition, ZEB1 was indeed the potential target of miR-200b identified by dual luciferase reporter gene assay. Xenograft model also suggested that over-expression of miR-200b suppressed the growth of tumor *in vivo*.

CONCLUSION: Taken together, our findings suggest that miR-200b be likely to play an important role in activating TGF- β 1-induced SGC-7901/DDP cells and perform as a tumor suppressor by targeting ZEB1 in GC. What's more, miR-200b may modulate Wnt/ β -catenin signaling pathway in TGF- β 1-induced SGC-7901/DDP cells.

Background

Gastric cancer (GC) is a malignant tumor originating from the gastric mucosal epithelium with highest morbidity and mortality [1]. Most GC patients with early have no obvious symptoms and be ignored, however, the 5-year overall survival rate less than 30% with prognosis of advanced GC due to the local and systemic metastasis [2]. Transforming growth factor-beta1 (TGF- β 1), includes polypeptides play important roles in regulating tumor cells, tumor-associated fibroblasts and immunorelated cells, has been shown to play an important role in GC. The TGF- β was increased in the cancer microenvironment after radiotherapy [3] and promoted activation of epithelial-mesenchymal transition in GC [4]. Therefore, it is very important to study the molecular mechanism and effective molecular biomarkers of GC. The microRNAs (miRNAs) are a class of 18-24 nucleotides small noncoding RNAs, which regulate biological processes and pathogenesis of cancer by targeting genes [5, 6], such as miR-124a in non-small cell lung cancer [7] and miR-7 for prostate cancer [8]. On the other hand, accumulating evidence strongly suggests that miR-200c could reverse drug resistance of GC [9]. The miR-200 family members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) [10] could inhibit epithelial-mesenchymal transition (EMT) by

targeting E-cadherin, ZEB1 or ZEB2 [11]. Interestingly, It was found ZEB1 could bind to the promoter regions of miR-200b/200a/429 or miR-200c/141, suggesting that miR-200b could target inhibition of ZEB1 and control cell migration, invasion, and EMT [12].

In this study, we investigated the expressions of miR-200b in GC, paracancerous tissues and SGC-7901/DDP cells, and further explored the functions of miR-200b in GC.

Methods

Human gastric cancer tissue collection.

All GC tissues (n=6) were obtained from GC from First Affiliated Hospital, Anhui Medical University, Hefei, China. After patients who take part in the study were informed and signed written informed consents to their participation, tissues were performed in accordance with the institutional guidelines. The study protocol was approved by the ethics boards of Anhui Medical University (Ethics#LLSC20150030), and specimen acquisition.

Reagents.

Fetal bovine serum (FBS): Millipore (Billerica, MA, USA); High-glucose DMEM medium: Hyclone (Logan, Utah, USA). Rabbit anti-C-Myc, anti-Cyclin D1, anti- β -catenin and APC monoclonal antibodies: Cell Signaling (Danvers, MA, USA); Rabbit anti-MMP-9 antibody: Millipore (Billerica, MA, USA); Rabbit anti-ZEB1 antibody: Abcam (Eugene, USA); , anti-MMP-3 and anti- β -actin monoclonal antibodies: Bioworld (Shanghai, China).

Animal experiments

20g nude mice were subcutaneously injected with 5×10^6 SGC-7901/DDP cells with miR-200b mimics or NC lentivirus. After 2 weeks, the tumor size was observed and measured. At the 3rd week, the mice were sacrificed by neck dragging. tumors were photographed and weighed. All experimental protocols used on the animals were approved by the institutions' subcommittees on animal care of Anhui Medical University

Immunohistochemistry (IHC) staining

Tissues were fixed with 4% paraformaldehyde and embedded with paraffin. IHC was performed according to standard procedures and assessed and photographed under CaseViewer (3DHISTECH Ltd., Hungary).

Cell culture

SGC-7901 cells and SGC-7901/DDP cells were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM supplemented with 10 % (v/v) FBS, 100 U/ml of penicillin, and 100 mg/ml of streptomycin (both from Beyotime, China) at 37 °C and 5 % CO₂.

Cells transient transfection of miR-200b and small interfering RNA silencing

The miR-200b inhibitor and miR-200b mimics (Biomics, Jiangsu, China), small interfering RNA (RNAi) of ZEB1 (GenePharma, Shanghai, China) were transfected by Lipofectamine 2000 (Invitrogen, CA, USA) in SGC-7901/DDP cells according to the manufacturer's instructions. And the sequences of ZEB1-RNAi as follows, sense: 5'-GUCGCUACAAACAGUUGUATT-3', antisense: 5'-UACAACUGUUUGUAGCGACTT-3'.

Cell cycle analysis

After fixed in ethanol overnight, SGC-7901/DDP cells were stained with 0.5 ml of PI (Beyotime, China) and RNase A for 30 min in dark. Flow cytometric analysis was performed on Beckman Coulter. **Quantitative real-time PCR (qRT-PCR)**

After Total RNA was extracted with trizol method in SGC-7901/DDP cells, the miR-200b was measured by EzOmics One-Step qPCR Kit (Biomics, USA) in PikoReal 96 real-time PCR system (Thermo, USA). The fold-change for miR-200b relative to U6 was calculated through the $2^{-\Delta\Delta CT}$ method. The levels of C-Myc, CyclinD1, MMP-3, MMP-9, ZEB1 and β -actin mRNA were determined using the SYBR Green Realtime PCR Master Mix (TOYOBO, Japan). The sequences of primer pairs used were as follows: C-Myc (forward: 5'-GGACTATCCTGCTGCCAAGA-3'; reverse: 5'-CGCCTCTTGACATTCTCCTC-3'), CyclinD1 (forward: 5'-GATCAAGTGTGACCCGGACT-3'; reverse: 5'-TCCTCCTCTTCCTCCTCCTC-3'), MMP-3 (forward: 5'-GGCCAGGGATTAATGGAGAT-3'; reverse: 5'-TGAAAGAGACCCAGGGAGTG-3'), MMP-9 (forward: 5'-GTACCACGGCCAACTACGAC-3'; reverse: 5'-GCCTTGGAAGATGAATGGAA-3') and β -actin (forward: 5'-GCCAACACAGTGCTGTCTGG-3'; reverse: 5'-AGGAGGAGCAATGATCTTG-3').

Western blot

Protein was extracted from SGC-7901/DDP cells with lysis buffer (Beyotime, China). And the nitrocellulose blots were incubated in antibodies dilution at 1:800 (ZEB1), 1:500 (C-Myc, CyclinD1, MMP-3 and MMP-9) and 1:1000 (β -actin) with primary antibodies overnight. After washing and incubated in HRP-conjugating antibody at 1:10000 diluted, the protein blots were detected using the ECL-chemiluminescent kit (ECL-plus, Thermo Scientific). The inclusions of the original and uncropped blots are Additional files.

Luciferase reporter assays

The ZEB1 3'UTR WT plasmid, miR-200b mimics or the negative controls (Gene Pharma, Shanghai) were transfected into SGC-7901/DDP cells using lipofectamine 2000 in 24-well plates. Activation of luciferase was measured consecutively by Dual-Luciferase Reporter 1000 Assay system (Promega, USA). **Wound-**

healing analysis

After cultured 2 days, SGC-7901/DDP cells (5×10^5 /ml cells/well) were scratched with serum deprived In 24-well plate. 24 h later, cells were fixed with methanol, stained with crystal violet, and viewed under an Olympus BX-51 microscope. **Transwell assay**

After coating with matrigel, SGC-7901/DDP cells were placed into the upper chamber (8 μ m pore size; Millipore, USA) in 1% FBS media. 48h later, cells were stained with methanol and 0.1% crystal violet, and

viewed under an Olympus BX-51 microscope.

Statistical analysis.

Data were presented as means $\pm$ SD and analyzed using SPSS16.0 software. Statistical significances were determined by one-way ANOVA with the post-hoc Dunnett's test. In all assays, values of $P < 0.05$ were considered to be statistically significant.

Results

1. The expression of miR-200b was down-regulated in GC and TGF- β 1-induced SGC-7901/DDP cells.

To explore the role of miR-200b, GC and paracancerous tissues were collected. As shown in Fig. 1A, the miR-200b was decreased in GC compared with the paracancerous tissues by qRT-PCR. Likewise, compared with SGC-7901, the miR-200b was also reduced in SGC-7901/DDP cells. Moreover, the miR-200b was down-regulated by various concentrations of TGF- β 1 in SGC-7901/DDP cells (Fig. 1 B). These results demonstrated that the miR-200b may be closely associated with the incidence of GC.

2. The miR-200b inhibitor enhanced proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells

To further identify the functions of miR-200b, the effect of miR-200b on the proliferation and migration were assessed by miR-200b inhibitor in TGF- β 1-induced SGC-7901/DDP cells. Firstly, down-regulation of miR-200b was verified using qRT-PCR in TGF- β 1-induced SGC-7901/DDP cells (Fig. 1C). In addition, c-Myc and Cyclin D1 oncogenes were detected to increase (Figs. 1D and 1E) by miR-200b inhibitor. Notably, the TGF- β 1-induced SGC-7901/DDP cells were promoted to proliferate considerably with miR-200b inhibitor (Fig. 1G) by BrdU cell proliferation ELISA assay. Moreover, as shown in Fig. 1F, the ratios of cells were increased in G2/M phases. More significantly, MMP-3 and MMP-9 expression were up-regulated by miR-200b inhibitor in TGF- β 1-induced SGC-7901/DDP cells (Figs. 1H and 1I). Remarkably, wound-healing and transwell assay proved that the migration was augmented via miR-200b inhibitor in TGF- β 1-induced SGC-7901/DDP cell (Fig. 1J and 1K). Thus, these data indicated that miR-200b inhibitor was potential to dramatically enhance proliferation and migration of TGF- β 1-induced SGC-7901/DDP cell.

3. The miR-200b mimics weakened proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells

The miR-200b mimics were used to over-express miR-200b in TGF- β 1-induced SGC-7901/DDP cells. After TGF- β 1-induced SGC-7901/DDP cells with miR-200b mimics 48 h, the miR-200b was increased remarkably (Fig. 2I). Meanwhile, c-Myc and cyclin D1 were decreased by miR-200b mimics (Figs. 2A and 2B). The proliferation of cells was inhibited observably by miR-200b mimics (Fig. 2D). In addition, miR-200b decreased the proportions of cells in G2/M phase (Fig. 2C) by flow cytometric analysis. On the other hand, MMP-3 and MMP-9 were down-regulated significantly by miR-200b mimics in TGF- β 1-induced SGC-7901/DDP cells as shown in Figs. 2E and 2F. To be noted, wound-healing and transwell assay have proved that the migration was inhibited considerably with miR-200b mimics (Fig. 2G and 2H). These results indicated that miR-200b mimics weaken the proliferation and migration of TGF- β 1-induced SGC-

SGC-7901/DDP cells. The miR-200b might be related to the SGC-7901/DDP cell proliferation and migration, playing a vital role in the pathogenesis of GC.

4. ZEB1 was a target gene of miR-200b.

It has been proved [13] miR-200b was complementary to 3' non-coding regions of ZEB1 mRNA. In addition, it was found that the ZEB1 were high in GC (Figs. 2J, 3A and 3B). Compared with SGC-7901 cells, ZEB1 was also up-regulated in SGC-7901/DDP cells as shown in Fig. 3C and 3E. And, it was also increased significantly by TGF- β 1 in SGC-7901/DDP cells (Figs. 3C - 3E). Moreover, ZEB1 was increased significantly by the miR-200b inhibitor (Figs. 3G and 3H), and decreased substantially by the miR-200b mimics in TGF- β 1-induced SGC-7901/DDP cells (Figs. 3I and 3J). As compared to the control, the co-transfection with miR-200b mimics significantly suppressed the ZEB1 3'UTR activity (Fig. 3F). In conclusion, miR-200b suppressed the expression of ZEB1 by specifically binding to ZEB1 mRNA.

5. Down-regulation of ZEB1 weakened proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells

After ZEB1 siRNA was obtained to knockdown the ZEB1 expression in SGC-7901/DDP cells (Figs. 4A, C and D), c-Myc and CyclinD1 were remarkably reduced in TGF- β 1-induced SGC-7901/DDP cells (Figs. 4C and 4D). Similar to the miR-200b mimics, the ZEB1-RNAi significantly reduced the proportions of G2/M phases as shown in Fig. 4E. In addition, BrdU cell proliferation ELISA showed that the cell proliferation was inhibited substantially by the ZEB1-RNAi (Fig. 4B). Meanwhile, MMP-3 and MMP-9 (Figs. 4F and 4G) were also decreased by the ZEB1-RNAi. Moreover, wound-healing and transwell assay exhibited that the cell migration ability was inhibited significantly with the ZEB1-RNAi (Fig. 4H and 4I). All the findings suggest that ZEB1-RNAi be likely to weaken the ability of TGF- β 1-induced SGC-7901/DDP cells proliferation and migration.

6. MiR-200b suppressed tumor growth *in vivo*.

We established xenograft model with overexpression of mir-200b SGC-7901/DDP cells. The tumor growth was remarkably slower in mir-200b mimics group (Fig 5A). As shown in Fig 5B, the tumor volume was observably decreased at 2 weeks. Interestingly, the average tumor weight was also down-regulated in the mir-200b mimics (Fig 5C). It has proved that mir-200b could suppress tumor growth *in vivo*.

7. miR-200b may modulate proliferation and migration of by SGC-7901/DDP Wnt/ β -catenin signaling pathway

We also found Wnt/ β -catenin signaling was closely associated with the effect of miR-200b in SGC-7901/DDP cells. Western blot have performed that the expression of β -catenin and APC were up-regulated obviously by miR-200b inhibitor, and down-regulated obviously by miR-200b mimics in TGF- β 1-induced SGC-7901/DDP cells, as show as Fig.5D and E. In particular, Fig.5F showed that ZEB1-RNAi also reduced the β -catenin and APC. Taken together, all the above results indicated that miR-200b could modulate

proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells by activation of Wnt/ β -catenin signaling pathway.

Discussion

As all know that miRNAs are involved in biological processes, such as cell proliferation, differentiation and apoptosis, through inhibiting target genes expression [6]. Many miRNAs have been proved to play in cancers, such as breast cancer [14], gastric cancer [15] and hepatocellular carcinoma [16]. It has been proved that miRNAs play important role in GC including miR-15b, miR-16 [17], miR-146a [18] and miR-200 family [19]. And miR-200b is proved widely expressed in many tumors, including breast cancer, pancreatic cancer and GC [20]. Interestingly, miRNA-200b could inhibit the proliferation, invasion, and migration of glioma cells through targeting CD133 in glioma [21]. Moreover, Chen [22] has proved miRNA-200 could inhibit the growth of melanoma cells. The previous study showed that the miRNA-200b were decreased in GC tissues. And Wu et al. [23] also found miRNA-200 family (miRNA-200a, miRNA-200b and miRNA-200c) was down-regulated in GC. Moreover, the miR-200b was also down-regulated in SGC-7901/DDP compared with SGC-7901 cells. Hence, we supposed that miR-200b would play a pivotal role in GC. We also proved that the expression of miR-200b was decreased by various concentrations of TGF- β 1 in SGC-7901/DDP cells. Indeed, it is necessary to consider that miR-200b is likely to be closely related to the tumorigenesis of GC.

To identify the effects of miR-200b in GC, miR-200b inhibitor was used in TGF- β 1-induced SGC-7901/DDP cells. It is generally known that c-Myc and Cyclin D1 are important downstream molecules in proliferation pathway. C-Myc regulates G-phase of cell cycle as proto-oncogene protein, and Cyclin D promotes G1/S phase transition and accelerates the process of cell cycle [24]. The results illustrated that miR-200b inhibitor up-regulated c-Myc and Cyclin D1 in TGF- β 1-induced SGC-7901/DDP cells. Moreover, BrdU cell proliferation assay and cell cycle analysis also showed that miR-200b inhibitor promoted proliferation of TGF- β 1-induced SGC-7901/DDP cells. Migration is another important biological process in malignant tumors, and it was related by matrix metalloproteinases (MMPs) [25, 26]. More significantly, MMP-3 and MMP-9 were up-regulated significantly by miR-200b inhibitor. Remarkably, wound-healing and transwell assay further proved the cell migration was dramatically promoted with miR-200b inhibitor. These data suggest that miR-200b inhibitor might enhance both proliferation and migration abilities of TGF- β 1-induced SGC-7901/DDP cells. The uncontrollable proliferation of cells is always corresponding with maladjusted cell cycle and shorter passage time [27].

Next, miR-200b mimics were transfected into TGF- β 1-induced SGC-7901/DDP cells to simulate endogenous overexpression of miR-200b. The results showed that u miR-200b mimics decreased c-Myc, Cyclin D1, MMP-3 and MMP-9 expressions in TGF- β 1-induced SGC-7901/DDP cells. Similarly, the percentages of cells at G2/M stages were decreased. Moreover, BrdU cell proliferation assay revealed that the cell proliferation was inhibited considerably by miR-200b mimics. Wound-healing and transwell assay proved that the cell migration ability was significantly weakened with miR-200b mimics. Xenograft model was established by SGC-7901/DDP cells with miR-200b mimics. The miR-200b mimics also suppress

tumor growth and weight *in vivo*. These findings indicated that miR-200b mimics could significantly inhibit the proliferation and migration of GC cells.

In this study, the construction and transfection of ZEB1 3'UTR-WT and miR-200b mimics into SGC-7901/DDP cells changed the fluorescence, and ZEB1 was identified as the target of miR-200b. Title et al. [12] reported that regulation of ZEB1 by miR-200 was sufficient to drive EMT in tumor progression and invasion *in vivo*. ZEB1 is a critical member of the ZEB family of transcription factors [28], and involved in regulation of key factors at the invasive front of carcinomas by triggering EMT. EMT could control protein stabilization mechanisms, transcription and translation, alternative splicing and expression of non-coding RNAs [29]. In the present study, after treating the SGC-7901/DDP cells with the miR-200 inhibitor, the ZEB1 were up-regulated in GC. Similarly, miR-200 mimics significantly inhibited the ZEB1 levels. Coincidentally, we also found the ZEB1 mRNA and protein levels were over-expressed in GC. Moreover, it was also increased significantly by TGF- β 1 in SGC-7901/DDP cells. Additionally, inhibition of ZEB1 induced by ZEB1-RNAi significantly suppressed the mRNA and protein expressions of c-Myc, CyclinD1, MMP-3 and MMP-9 in TGF- β 1-induced SGC-7901/DDP cells. Interestingly, ZEB1-RNAi reduced the proportion of G2/M-phase cells remarkably. Notably, BrdU cell proliferation assay proved ZEB1-RNAi significantly the proliferation. Wound-healing and transwell assay further proved that the cell migration was hindered dramatically with the ZEB1-RNAi. These results indicate that the proliferation and migration abilities were compromised by ZEB1-RNAi. Zhou et al. [30] also found that miR-200c/141 decreased ZEB1/2 and increased E-cadherin expressions to repress the migration and invasion of gastric cancer cells. Therefore, we speculate that miR-200b is likely to inhibit cell proliferation and migration of TGF- β 1-induced SGC-7901/DDP cells through targeting ZEB1.

Conclusions

In conclusion, as our study demonstrated, consistent with experiments *in vitro*, miR-200b plays an important role in activating TGF- β 1-induced SGC-7901/DDP cells and may perform as a tumor suppressor by targeting ZEB1 in GC. Therefore, it is likely to provide new insight for the diagnosis and new targeted therapy of GC.

Declarations

Ethics approval and consent to participate

This study was approved by ethical committee of Anhui Medicine University. The informed consent, written or verbal, was obtained from all participants.

Consent for publication.

Not applicable.

Availability of data and material

The data sets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Natural Science Foundation of China (Grants No. 81572801) and Natural Science Foundation of Anhui Province (Grants No. KJ2017A208). These funding bodies had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

HC, ZXW, YHC and MZ conceived and designed the study. HC, PCL, LW, YXY and YZ performed the experiments. HC and PCL analyzed the data. HC, MZ, ZXW and YZ drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Our thanks are given to Dr. Jinxing Xia for his valuable comments to the manuscript.

Authors' Information

Zhongxin Wang: aywzhx87@163.com

Min Zhang: ahmuzhangmin@163.com

Abbreviations

EMT: epithelial-mesenchymal transition; FBS: Fetal bovine serum; GC: Gastric cancer; miRNAs: microRNAs; MMPs: matrix metalloproteinases; PI: propidium iodide; qRT-PCR: quantitative real-time PCR.

References

1. Liu Y, Fan D: **Ginsenoside Rg5 induces G2/M phase arrest, apoptosis and autophagy via regulating ROS-mediated MAPK pathways against human gastric cancer.** *Biochem Pharmacol* 2019, **168**:285-304.
2. Wu WW, Zhang WY, Zhang WH, Yang L, Deng XQ, Ou MC, Yang YX, Liu HB, Zhu T: **Survival analysis of intraoperative blood salvage for patients with malignancy disease: A PRISMA-compliant systematic review and meta-analysis.** *Medicine (Baltimore)* 2019, **98**(27):e16040.

3. Xu L, Liu F, Li C, Li S, Wu H, Guo B, Gu J, Wang L: **Fucoidan suppresses the gastric cancer cell malignant phenotype and production of TGF-beta1 via CLEC-2.** *Glycobiology* 2019.
4. Yang T, Huang T, Zhang D, Wang M, Wu B, Shang Y, Sattar S, Ding L, Liu Y, Jiang H *et al*: **TGF-beta receptor inhibitor LY2109761 enhances the radiosensitivity of gastric cancer by inactivating the TGF-beta/SMAD4 signaling pathway.** *Aging (Albany NY)* 2019, **11**(20):8892-8910.
5. Spizzo R, Nicoloso MS, Croce CM, Calin GA: **SnapShot: MicroRNAs in Cancer.** *Cell* 2009, **137**(3):586-586 e581.
6. Budak H, Akpınar BA: **Plant miRNAs: biogenesis, organization and origins.** *Funct Integr Genomics* 2015, **15**(5):523-531.
7. Yu F, Liu JB, Wu ZJ, Xie WT, Zhong XJ, Hou LK, Wu W, Lu HM, Jiang XH, Jiang JJ *et al*: **Tumor suppressive microRNA-124a inhibits stemness and enhances gefitinib sensitivity of non-small cell lung cancer cells by targeting ubiquitin-specific protease 14.** *Cancer Lett* 2018, **427**:74-84.
8. Pacciez JD, Duncan K, Sekar D, Correa RG, Wang Y, Gu X, Bashin M, Chibale K, Libermann TA, Zerbini LF: **Dihydroartemisinin inhibits prostate cancer via JARID2/miR-7/miR-34a-dependent downregulation of Axl.** *Oncogenesis* 2019, **8**(3):14.
9. Li M, Gao M, Xie X, Zhang Y, Ning J, Liu P, Gu K: **MicroRNA-200c reverses drug resistance of human gastric cancer cells by targeting regulation of the NER-ERCC3/4 pathway.** *Oncol Lett* 2019, **18**(1):145-152.
10. Peng L, Fu J, Ming Y: **The miR-200 family: multiple effects on gliomas.** *Cancer Manag Res* 2018, **10**:1987-1992.
11. Jauhari A, Yadav S: **MiR-34 and MiR-200: Regulator of Cell Fate Plasticity and Neural Development.** *Neuromolecular Med* 2019, **21**(2):97-109.
12. Title AC, Hong SJ, Pires ND, Hasenohrl L, Godbersen S, Stokar-Regenscheit N, Bartel DP, Stoffel M: **Genetic dissection of the miR-200-Zeb1 axis reveals its importance in tumor differentiation and invasion.** *Nat Commun* 2018, **9**(1):4671.
13. Gui Z, Luo F, Yang Y, Shen C, Li S, Xu J: **Oridonin inhibition and miR200b3p/ZEB1 axis in human pancreatic cancer.** *Int J Oncol* 2017, **50**(1):111-120.
14. Rhodes LV, Martin EC, Segar HC, Miller DF, Buechlein A, Rusch DB, Nephew KP, Burow ME, Collins-Burow BM: **Dual regulation by microRNA-200b-3p and microRNA-200b-5p in the inhibition of epithelial-to-mesenchymal transition in triple-negative breast cancer.** *Oncotarget* 2015, **6**(18):16638-16652.
15. Li HL, Xie SP, Yang YL, Cheng YX, Zhang Y, Wang J, Wang Y, Liu DL, Chen ZF, Zhou YN *et al*: **Clinical significance of upregulation of mir-196a-5p in gastric cancer and enriched KEGG pathway analysis of target genes.** *Asian Pac J Cancer Prev* 2015, **16**(5):1781-1787.
16. Yao S, Tian C, Ding Y, Ye Q, Gao Y, Yang N, Li Q: **Down-regulation of Kruppel-like factor-4 by microRNA-135a-5p promotes proliferation and metastasis in hepatocellular carcinoma by transforming growth factor-beta1.** *Oncotarget* 2016, **7**(27):42566-42578.

17. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M *et al*: **miR-15 and miR-16 induce apoptosis by targeting BCL2**. *Proc Natl Acad Sci U S A* 2005, **102**(39):13944-13949.
18. Kogo R, Mimori K, Tanaka F, Komune S, Mori M: **Clinical significance of miR-146a in gastric cancer cases**. *Clin Cancer Res* 2011, **17**(13):4277-4284.
19. Shinozaki A, Sakatani T, Ushiku T, Hino R, Isogai M, Ishikawa S, Uozaki H, Takada K, Fukayama M: **Downregulation of microRNA-200 in EBV-associated gastric carcinoma**. *Cancer Res* 2010, **70**(11):4719-4727.
20. Feng B, Wang R, Chen LB: **Review of miR-200b and cancer chemosensitivity**. *Biomed Pharmacother* 2012, **66**(6):397-402.
21. Zhao C, Ma ZG, Mou SL, Yang YX, Zhang YH, Yao WC: **Targeting effect of microRNA on CD133 and its impact analysis on proliferation and invasion of glioma cells**. *Genet Mol Res* 2017, **16**(1).
22. Chen YN: **Dacarbazine inhibits proliferation of melanoma FEMX-1 cells by up-regulating expression of miRNA-200**. *Eur Rev Med Pharmacol Sci* 2017, **21**(6):1191-1197.
23. Wu XM, Shao XQ, Meng XX, Zhang XN, Zhu L, Liu SX, Lin J, Xiao HS: **Genome-wide analysis of microRNA and mRNA expression signatures in hydroxycamptothecin-resistant gastric cancer cells**. *Acta Pharmacol Sin* 2011, **32**(2):259-269.
24. Zheng L, Liang X, Li S, Li T, Shang W, Ma L, Jia X, Shao W, Sun P, Chen C *et al*: **CHAF1A interacts with TCF4 to promote gastric carcinogenesis via upregulation of c-MYC and CCND1 expression**. *EBioMedicine* 2018, **38**:69-78.
25. Wells JM, Gaggari A, Blalock JE: **MMP generated matrikines**. *Matrix Biol* 2015, **44-46**:122-129.
26. Tan C, Qiao F, Wei P, Chi Y, Wang W, Ni S, Wang Q, Chen T, Sheng W, Du X *et al*: **DIXDC1 activates the Wnt signaling pathway and promotes gastric cancer cell invasion and metastasis**. *Mol Carcinog* 2016, **55**(4):397-408.
27. Scatena R: **Mitochondria and cancer: a growing role in apoptosis, cancer cell metabolism and dedifferentiation**. *Adv Exp Med Biol* 2012, **942**:287-308.
28. Zhang Y, Xu L, Li A, Han X: **The roles of ZEB1 in tumorigenic progression and epigenetic modifications**. *Biomed Pharmacother* 2019, **110**:400-408.
29. Singh M, Yelle N, Venugopal C, Singh SK: **EMT: Mechanisms and therapeutic implications**. *Pharmacol Ther* 2018, **182**:80-94.
30. Zhou X, Wang Y, Shan B, Han J, Zhu H, Lv Y, Fan X, Sang M, Liu XD, Liu W: **The downregulation of miR-200c/141 promotes ZEB1/2 expression and gastric cancer progression**. *Med Oncol* 2015, **32**(1):428.

Figures

Figure 1

miR-200b inhibitor increased proliferation and migration of TGF-β1-induced SGC-7901/DDP cells (A) and (B) The mRNA level of miR-200b was determined. (C) The miR-200b in TGF-β1-induced SGC-7901/DDP cells with miR-200b inhibitor. (D) and (E) The levels of c-Myc and Cyclin D1. (F) Cell cycle of SGC-7901/DDP cells. (G) BrdU proliferation assay. (H) and (I) The expression levels of MMP-3 and MMP-9. (J) Cell migration in SGC-7901/DDP cells (original magnification, 10X). All the data were expressed in the

form of means $\pm$ SD. #P < 0.05, ##P < 0.01 vs. paracancerous, SGC-7901 or normal group; *P < 0.05, **P < 0.01 vs. SGC-7901/DDP or NC-miRNA group. NC-miRNA: Negative control miRNA.

Figure 2

miR-200b mimics inhibited proliferation and migration of TGF-β1-induced SGC-7901/DDP cells. (A) and (B) The levels of c-Myc and Cyclin D1. (C) Cell cycle. (D) BrdU proliferation assay. (E) and (F) The levels of MMP-3 and MMP-9. (G) Cell migration (original magnification, 10X). (H) The expression level of miR-

200b. (I) The expressions of ZEB1 in paracancerous and GC tissues were analyzed by IHC staining analysis (original magnification, 20X). #P < 0.05, ##P < 0.01 vs. normal group; *P < 0.05, **P < 0.01 vs. NC-miRNA.

Figure 3

ZEB1 was a direct target of miR-200b. (A) - (D) The expression of ZEB1. (E) The protein expression of ZEB1 was detected by immunofluorescence analysis in SGC-7901/DDP cells treated with TGF-β1. (F)

ZEB1 3' UTR and miR-200b mimics in SGC-7901/DDP cells were tested by Dual luciferase assay. (G) - (J) The expression of ZEB1. ##P < 0.01 vs. paracancerous, SGC-7901 or NC group; **P < 0.01 vs. SGC-7901/DDP or NC-miRNA group.

Figure 4

ZEB1-RNAi inhibited proliferation and migration of TGF-β1-induced SGC-7901/DDP cells. (A) The expression of ZEB1 was detected by immunofluorescence. (B) BrdU proliferation assay. (C) and (D) The

levels of ZEB1, c-Myc and Cyclin D1. (E) Cells cycle (F) and (G) The levels of MMP-3 and MMP-9. (H) Cells migration (original magnification, 10X). ##P < 0.01 vs. normal group; **P < 0.01 vs. NC-RNAi. NC- RNAi: Negative control RNAi.

Figure 5

miR-200b may modulate Wnt/β-catenin signaling pathway in TGF-β1-induced SGC-7901/DDP cells. (A) The images of tumors after isolated. (B) Tumor volumes in 21d. (C) Tumor weight after isolated. (D) The

expression of β -catenin in TGF- β 1-induced SGC-7901/DDP cells with miR-200b inhibitor. (E) The expression of β -catenin in TGF- β 1-induced SGC-7901/DDP cells with miR-200b mimics. (F) The expression of β -catenin in TGF- β 1-induced SGC-7901/DDP cells with ZEB1-RNAi. ##P < 0.01 vs. normal group; **P < 0.01 vs. NC-miRNA or NC-RNAi.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplement1.docx](#)