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Original Article

MiR-433 functions as a tumor suppressor in glioma cells by regulating YAP1

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Abstract: YAP1 is an oncogene in plenty types of tumors and miR-433 is a tumor suppressor in many types of tumors. Furthermore, YAP1 is overexpressed in glioma and the function mechanism is still not entirely clear. So this study was aimed to investigate whether YAP1 expression in glioma is associated with miRNA. The YAP1 expression in glioma tissues and glioma cells and miR-433 expression in glioma tissues were detected using real-time PCR. Then the relationship between YAP1 and miR-433 was analyzed by dual-luciferase assay. The effects of miR-433 overexpression on the proliferation, apoptosis, migration and invasion of glioma cells (LN382 and AM38) were evaluated using CCK-8 assay, flow cytometry, wound-healing assay and transwell assay, respectively. MiR-433 was lowly expressed in glioma tissues ($P < 0.01$) when YAP1 was highly expressed ($P < 0.01$), and miR-433 could directly target the YAP1 and indirectly downregulate the expression of YAP1. Overexpression of miR-433 would inhibit the proliferation, migration and invasion of glioma cells but promote the apoptosis of glioma cells. MiR-433 targets 3'-UTR of YAP1 and may suppress the proliferation, migration and invasion of glioma cells through downregulating the YAP1 expression. This discovery is helping to understand the regulatory mechanism of YAP1 expression in glioma cells.

Keywords: MiR-433, YAP1, glioma, cell viability

Introduction

Yes-associated protein 1 (YAP1) is a major effector of Hippo pathway and a transcriptional regulator, plays a role in cell proliferation, apoptosis and controlling organ size [1]. Furthermore, YAP1 acts as an oncogene and is highly expressed in various human tumors [2, 3]. Overexpression of YAP promotes the tumorigenesis and is involved in tumor type and grade [4, 5], because YAP can stimulate the cellular transformation and improve cell invasion and metastasize [3].

MiRNA can inhibit the translation of mRNA through recognizing and binding to the target gene and induce the post-transcriptional gene silencing. So miRNAs play an important role in the regulation of the organisms' physiological activities including development and immunoregulation. Most of miRNAs in mammal inhibit gene translation from the transcriptional level through binding to the 3'UTR of mRNA with incomplete base pairing way. About a third gene expressions in the human genome are predicted to be regulated by miRNA. Many miRNAs are expressed with stronger tissue specificity and time phase property [6-8]. Dysregulation of cell differentiation, proliferation, apoptosis and other processes induces the malignant transformation of cells and tumorigenesis. Increasing evidences showed the close relationship between miRNA and tumorigenesis. George showed about 19% miRNAs (35 of 186) were located in the high incidence areas of deletion, amplify and invesion in tumor cells [9]. MiR-143 and miR-145 are down-regulated in rectal carcinoma [10], and let-7 is down-regulated in lung cancer and related to the prognosis [11]. MiR-21 is down-regulated in breast cancer but up-regulated in glioma [12, 13]. Furthermore, the relationship between the abnormal expression of important tumor associated proteins and miRNA has being revealed gradually, miR-127 negatively regulates the oncogene BCL6 [14]

and the inhibitor of apoptosis protein BCL2 is the target gene of miR-15 and miR-16 [15].

YAP1 is up-regulated in human medulloblastomas, human meningioma, ependymoma and infiltrating human astrocytic tumors, and overexpression of YAP1 is involved in the glioma pathogenesis [16]. But the regulatory mechanism of YAP1 expression in gliomas is still not fully understood, and a plenty of oncogenes expression are regulated by miRNAs. So in this study, we screen the miR-433 regulating the YAP1 expression and validate the regulatory function of miR-433 in glioma cells.

Materials and methods

Tissue samples

20 glioma tissues and 20 adjacent normal tissues were collected from 20 patients with glioma, who underwent the tumor resection in Fudan University Shanghai Cancer Center between June 2012 and June 2014. All patients, including 13 males and 7 females, were diagnosed as glioma by nuclear magnetic resonance and histopathological confirmation. All tissues were stored in liquid nitrogen before using in this study. The research was approved by the Institutional Review Board of the Shanghai Cancer Center and all patients have given the written informed consent.

Cell culture and transfection

Normal human astrocytes HEB cells and human astrocytoma cell line U-251MG cells were purchased from ATCC (Rockville, MD, USA), and were cultured in complete medium RPMI-1640 (supplemented with 10% FBS). Other five human astrocytoma cell lines (U-138MG, LN382, A172, AM38, KMG4) were also used in this study, and A172 cells was purchased from ATCC, the others were purchased from Biofavor Company (Wuhan, China). These five cells were all cultured in complete medium DMEM supplemented with 10% FBS. 293T cells were cultured in DMEM medium supplemented with 10% FBS and used in dual-luciferase assay. LN382 cells and AM38 cells were also transfected with miR-433 mimics and mimics NC (GenePharma, Suzhou, China) respectively to detect the effects of miR-433 on YAP1 expression and cell proliferation, apoptosis, migration and invasion *in vitro*. The final concentration of both miR-433 mimics and mimics NC was 50 nM and they were transfected into cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

Real time quantitative PCR

Total RNA was extracted from glioma tissues, adjacent tissues and cells described in above respectively by TRIzol Reagent (Invitrogen) according to the instruction. PrimeScript RT Reagent Kit (Takara, Dalian, China) was used for reverse transcriptions from 1 μg of total RNA, and real time PCR was performed with SYBR Green Premix Ex Taq II (Takara) and Real-Time PCR System (Applied Biosystems, CA, USA). The relative expression of CTGF in cells treated with microRNA mimics was also detected with the same method. The expression of β-actin was also detected as an internal control. The primer sequences were as follows: for YAP1, 5'-TAGCCCTGCGTAGCCAGTTA-3' and 5'-GGTTCGAGGGACACTGTAGC-3'; for CTGF, 5'-CTGCAGGCTAGAGAAGCAGAG-3' and 5'-GATGCACTTTTTGCCCTTCT-3'; forβ-actin, 5'-ACCTTCTACAATGAGCTGCG-3' and 5'-CCTGGATAGCAACGTACATGG-3'. For the analysis of miR-433 expression in tissues, total RNA was reverse-transcribed with gene specific primers (5'-GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACGAATAATG-3') and real time PCR was performed with the primer pair (5'-TGCGGTACGGTGAGCCTGTC-3' and 5'-CCAGTGCAGGGTCCGAGGT-3'). U6 was detected with primer pair (5'-CTCGCTTCGGCAGCACA-3' and 5'-ACGCTTCACGAATTTGCGT-3') to calculate the relative expression of miR-433.

Western blot

YAP1 expression in LN382 cells and AM38 cells transfected with miR-433 mimics and mimics NC respectively was detected using western blot. Total protein was extracted with RIPA buffer (Sigma, MO, USA) and quantified by BCA protein assay kit (Tiangen Biotech, Beijing, China) according to the instructions. Equal amounts of protein samples were loaded on 10% SDS-PAGE and then transferred to a PVDF membrane (Millipore, MA, USA). Subsequently the membranes were blocked in 5% non-fat milk for 1h at room temperature followed by incubating with anti-YAP1 antibody (1:1000 dilution, Abcam, MA, USA) and anti-beta actin antibody (1:500, Abcam) at 4°C for overnight. After

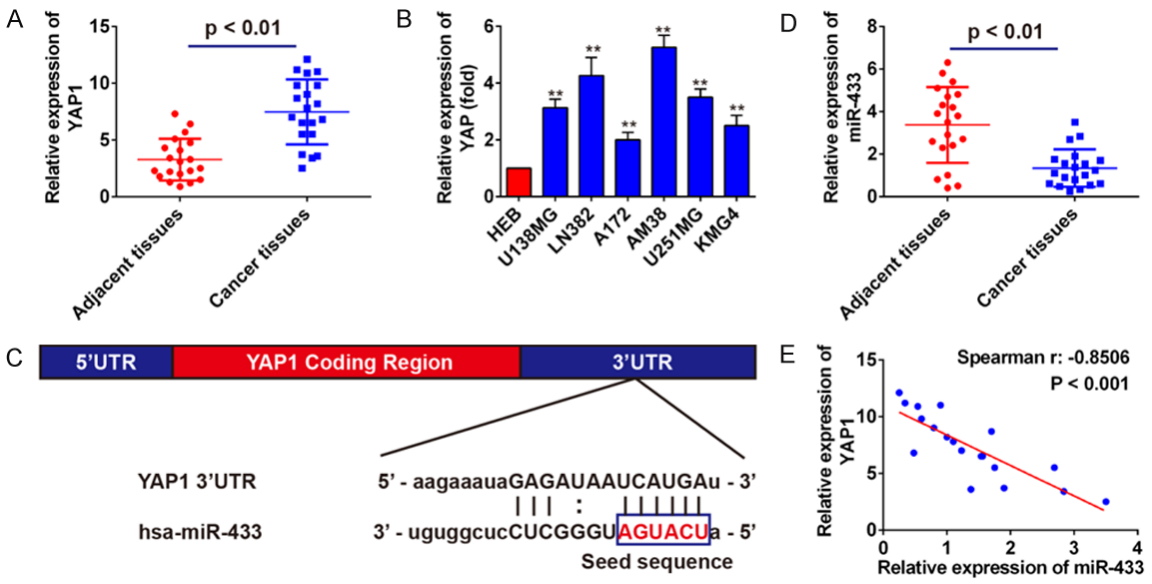

Figure 1. MiR-433 is a potential regulation of YAP1. A: mRNA level of YAP1 detection in glioma tissues and adjacent tissues using RT-qPCR; B: mRNA level of YAP1 detection in normal human astrocytes HEB cells and human glioma cell lines (U138MG, LN382, A172, AM38, U251MG and KMG4) using RT-qPCR; C: Predicted binding sites between YAP1 and miR-433 using TargetScan 4.2; D: MiR-433 detection in glioma tissues and adjacent tissues using RT-qPCR; E: The correlation analysis between YAP1 expression and miR-433 expression in glioma tissues.

washing with TBST buffer for three times, membranes were incubated with HRP-conjugated goat anti-mouse IgG or rabbit anti-goat IgG at 1:5000 dilutions for 1 h at room temperature. Signal was detected with ECL kit (Millipore).

Luciferase reporter assay

YAP1 3'-UTR-wt and YAP1 3'-UTR-mut were cloned into pmirGLO vector (E1330, Promega, WI, USA) respectively. 293T cells (4×10^4 cells) were plated on 96-well plates with serum-free medium and cultivated at 37°C. The next day, 293T cells were cotransfected with 150 ng pmirGLO- YAP1 3'-UTR-wt or pmirGLO- YAP1 3'-UTR-mut and mimics NC or miR-433 mimics or inhibitor NC or miR-433 inhibitor (50 nM) using Lipofectamine 2000 reagent, respectively. After cultivation for 48 h, 293T cells were collected and the luciferase activities in each well were detected with the Dual-Luciferase Reporter Assay system (Promega).

CCK-8 assay

LN382 cells and AM38 cells were seeded in 96-well plates at a density of 4×10^3 cells/well and then transfected with miR-433 mimics or mimics NC (50 nM) respectively. Cells were harvested to estimate the cell viability using a CCK-8 detection kit (Sigma) at the first day, second day, third day, fourth day, fifth day after transfection, respectively. The detection was performed according to the product specification.

Flow cytometry

After LN382 cells and AM38 cells transfected with miR-433 mimics or mimics NC for 48 h, cell apoptosis was analyzed using Annexin V-FITC apoptosis detection kit (BD Biosciences, CA, USA) according to the manufacturer's instruction.

Wound-healing assay

LN382 cells and AM38 cells were seeded in 6-well plates and then transfected with miR-433 mimics or mimics NC when grow to 90% confluence. After 24 h of transfection, a micropipette tip was used to create a wound (0 h) followed by washing three times with PBS and adding appropriate serum-free medium. Capturing the images then cells were cultivated at 37°C and monitored daily. Finally, cells migrated towards the wound was captured at 48 h and the closure of denuded area was calculated.

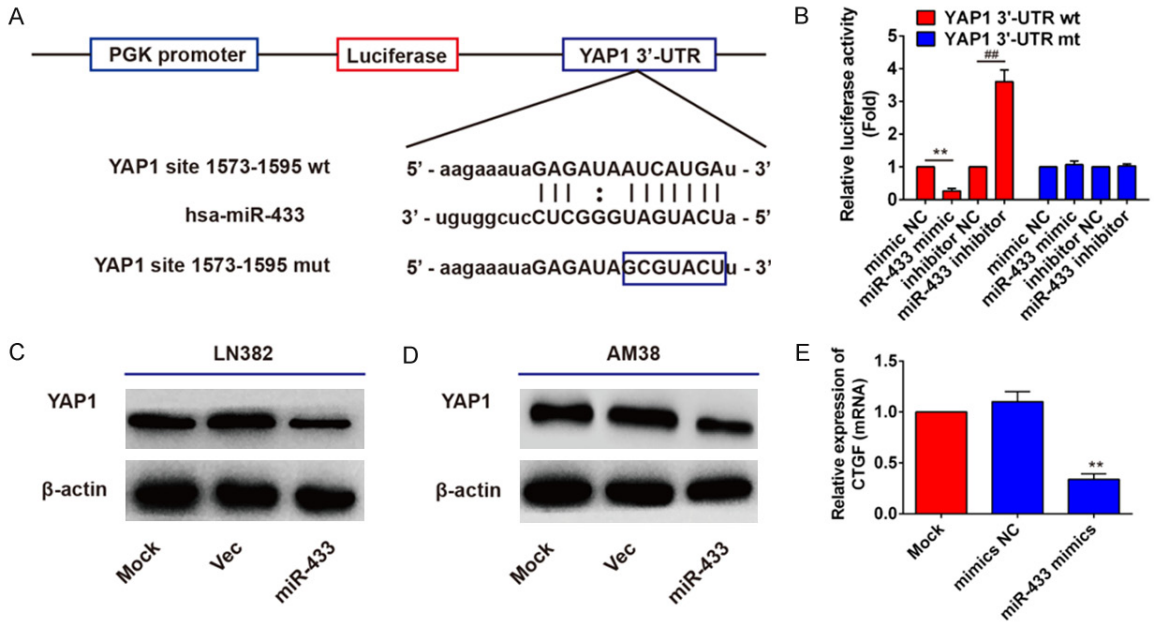

Figure 2. MiR-433 directly target the YAP1. A: The wild type and mutant type of YAP1 3'-UTR cloned into the dual-luciferase reporter vector; B: Relative luciferase activities of reporters with YAP1 3'-UTR wt/ YAP1 3'-UTR mt in 293T cells treated with miR-433 mimic or miR-433 inhibitor; C, D: YAP1 protein expression in LN382 cells and AM38 cells treated with miR-433 mimics or mimics NC; E: mRNA level of CTGF in glioma cells treated with miR-433 mimics.

Transwell invasion assay

Transwell chambers (8 μm, Millipore) were coated with Matrigel and used in this study. LN382 cells and AM38 cells transfected with miR-433 mimics or mimics NC were seeded in the upper chamber with serum-free medium, and complete medium supplemented with 10% FBS was added in the lower chamber. After 24 h cultivation at 37°C, the invaded cells on the bottom of the upper chamber were fixed with methanol and stained with crystal violet. Finally, the invaded cells were observed and counted under an inverted microscope.

Statistical analysis

Data from three independent results was analyzed using Graphpad Prism 6, and values were shown as means ± SD. The correlation analysis between YAP1 expression and miR-433 expression in glioma tissues was performed with Graphpad Prism 6 and the correlation coefficient r was calculated using Spearman method. Statistical significance was evaluated with Student's *t* test and difference was statistically significant when $P < 0.05$.

Results

MiR-433 may regulate the YAP1 expression

The mRNA expression of YAP1 in glioma tissues, adjacent tissues and cells were detected using real time PCR respectively, and the results showed the high level of YAP1 in glioma tissues and glioma cells than that in adjacent normal tissues and human astrocytes ($P < 0.01$) (**Figure 1A** and **1B**). MiR-433 plays a role of tumor suppressor gene with low expression level in gastric cancer, liver cancer and other cancers [17-20], so we predicted the binding site between YAP1 and miR-433 using TargetScan 4.2, the seed sequence of miR-433 could be completely matched to the 3'UTR of YAP1 mRNA as shown in **Figure 1C**. Furthermore, the miR-433 expression in glioma tissues and adjacent tissues was assessed and miR-433 expression in glioma tissues was significantly lower than that in adjacent tissues ($P < 0.01$) (**Figure 1D**). The result of correlation analysis between YAP1 expression and miR-433 expression showed a remarkable negative correlation ($r = -0.8506$, $P < 0.001$) (**Figure 1E**). The matching between YAP1 mRNA and Mir-433 sequence

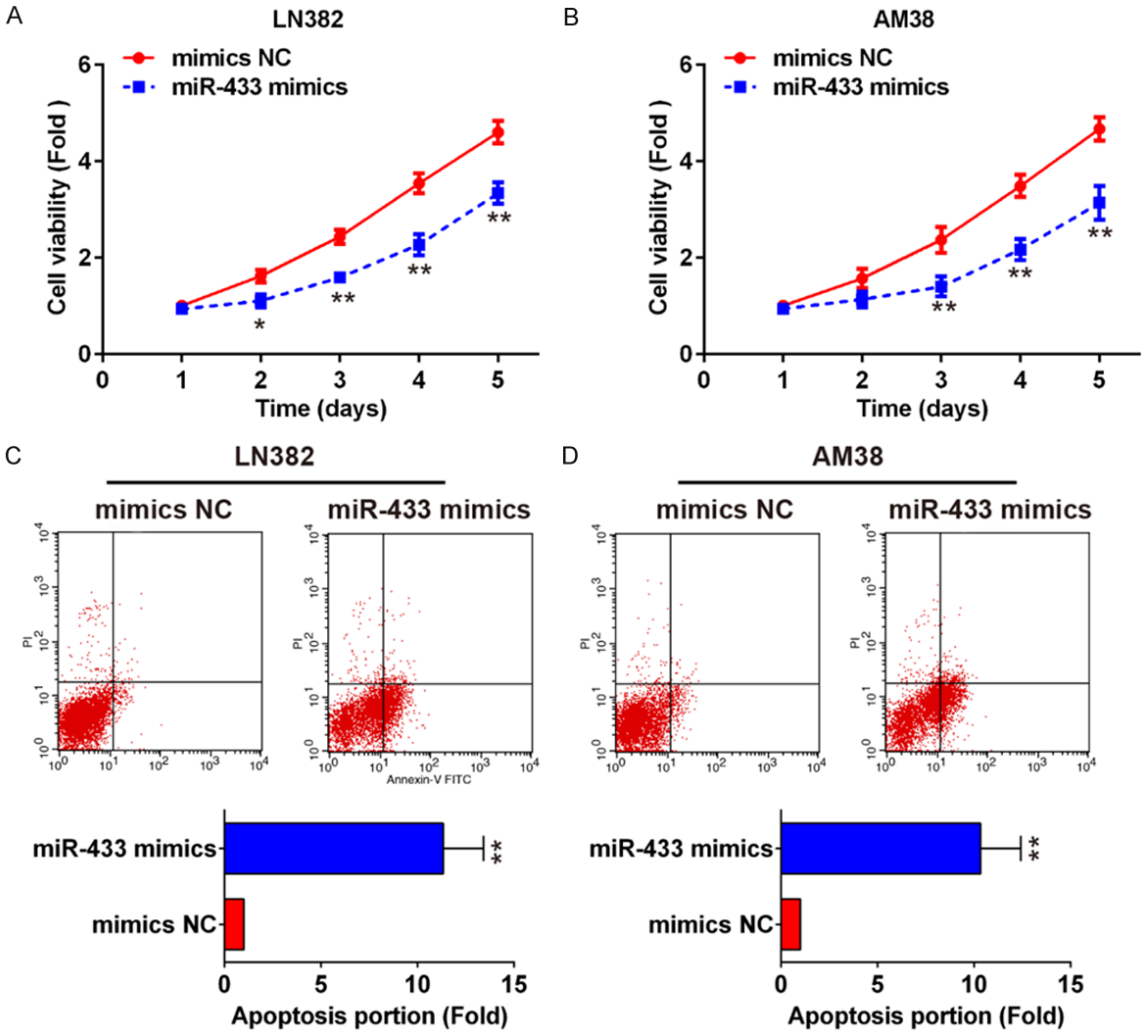

Figure 3. Overexpression of miR-433 inhibits the glioma cells proliferation and promotes cells apoptosis. A, B: Cell viability detection of LN382 and AM38 treated with miR-433 mimics or mimics NC using CCK-8 assay; C, D: Cell apoptosis detection of LN382 and AM38 treated with miR-433 mimics or mimics NC using flow cytometry.

and negative correlation between the expression of YAP1 and miR-433 indicate that miR-433 is a candidate miRNA which can regulate the YAP1 expression.

MiR-433 directly down-regulates the YAP1 expression

To verify the relationship between miR-433 and YAP1, dual-luciferase reporter assay was used. For cells treated with pmirGLO-YAP1 3'-UTR-wt, the relative luciferase activity was obviously decreased when cells cotransfected with miR-433 mimics ($P < 0.01$) and, in contrary, it was significantly increased when cells cotransfected with miR-433 inhibitor ($P < 0.01$). For cells treated with pmirGLO-YAP1 3'-UTR-mut, which didn't match to the miR-433 sequence (**Figure 2A**), the relative luciferase activity showed no difference between different treatments (**Figure 2B**). Furthermore, the YAP1 protein expression and the downstream target CTGF expression in glioma cell lines LN382 and AM38 were both degraded significantly when treated with miR-433 mimics ($P < 0.01$) (**Figure 2C-E**). These suggest YAP1 is a direct target gene of miR-433 and can be down-regulated by miR-433.

MiR-433 inhibits glioma cells proliferation and promotes apoptosis

CCK-8 assay was performed to detect the effect of miR-433 on glioma cells viability. The

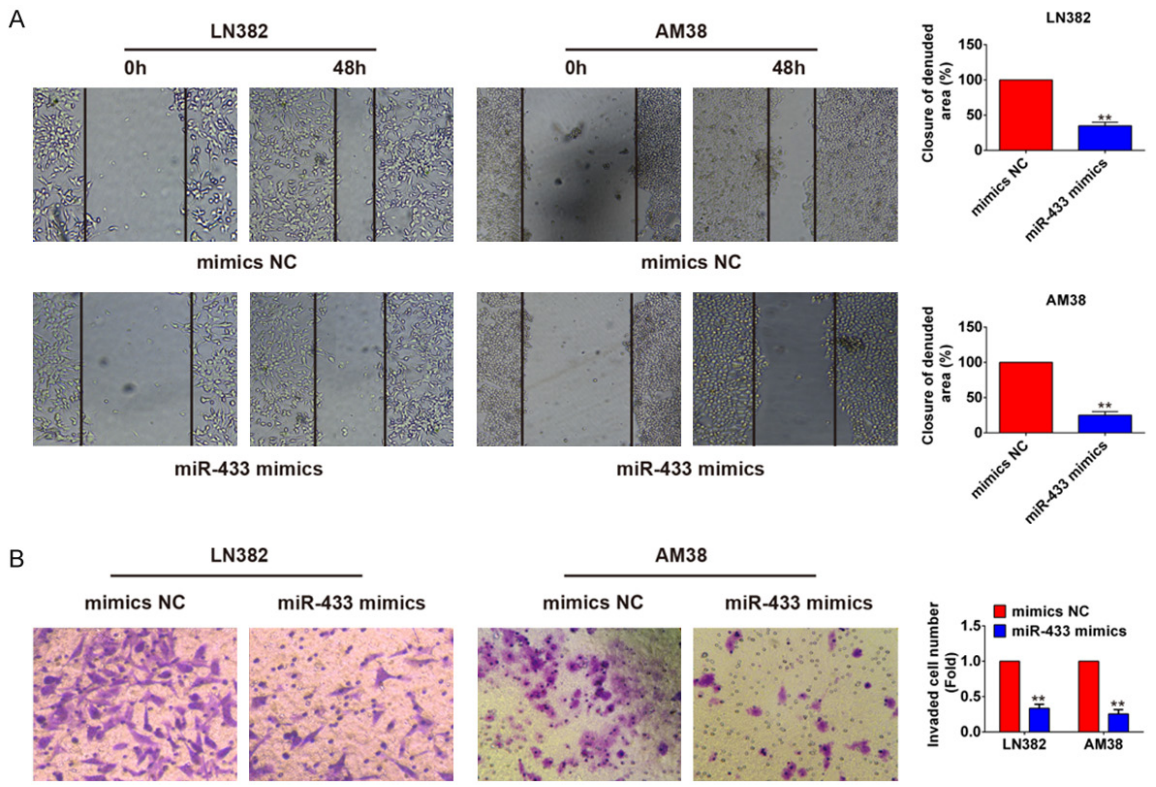

Figure 4. Overexpression of miR-433 inhibits the glioma cells migration and invasion. A: Cell migration ability evaluation of LN382 and AM38 treated with miR-433 mimics or mimics NC using wound-healing assay; B: Cell invasive ability evaluation of LN382 and AM38 treated with miR-433 mimics or mimics NC using transwell assay.

results showed that both LN382 cells and AM38 cells were growth slowly when treated with miR-433 mimics compared with cells treated with mimics NC, the difference was more obvious over the 5 days period (**Figure 3A** and **3B**). The apoptosis of LN382 cells and AM38 cells treated with miR-433 mimics were evaluated using AnnexinV-FITC apoptosis detection kit and flow cytometry. The results showed that the cells apoptosis portions of LN382 cells and AM38 cells were all significantly increased when treated with miR-433 mimics compared to cells treated with mimics NC (**Figure 3C** and **3D**). These results indicate that miR-433 can inhibit glioma cells proliferation and promote cell apoptosis.

MiR-433 inhibits glioma cells migration and invasion

In addition, the effects of miR-433 on glioma cells migration and invasion were evaluated using wound-healing assay and transwell assay, respectively. As shown in **Figure 4A**, when the wounds were created for 48 h, LN382 cells and AM38 cells were both migrated towards to the wound, but the migration rate of cells treated with miR-433 mimics was reduced compared to that of cells treated with mimics NC. Similarly, the invaded cell number of LN382 cells and AM38 cells treated with miR-433 mimics was both decreased significantly compared to that of cells treated with mimics NC ($P < 0.01$) (**Figure 4B**). These results demonstrate that miR-433 can suppress glioma cells migration and invasion.

Discussion

Dysregulation of miRNAs and oncogenes overexpression have been proved in many tumors and many miRNAs play important roles in tumor growth and metastasis. MiR-33a, a tumor suppressor, is downregulated in hepatocellular carcinoma cells and can directly downregulates the β-catenin expression to suppress the cell growth [21]. MiR-448 is also downregulated in several cancers and plays a role as a tumor suppressor; miR-448 can suppress the colorectal cancer cells proliferation, migration and

invasion through inhibiting protooncogene IGF1R [22]. YAP1 is a transcription co-activator which promotes cell proliferation and inhibits apoptosis [1]. YAP1 is also overexpressed in many types of cancers including gliomas and functions as an oncogene involving in oncogenic transformation and epithelial-mesenchymal transition [16]. In this study, we demonstrated the overexpression of YAP1 in glioma tissue and the negative correlation between YAP1 expression and miR-433 expression in glioma tissues, which suggests downregulation of miR-433 may elevate the YAP1 expression and promote the glioma development.

Furthermore, we indicated that YAP1 is a target gene of miR-433 and be downregulated by miR-433 in glioma cells. In addition, the expression of CTGF was also decreased in glioma cells when downregulation of YAP1, and CTGF as a downstream factor of YAP1 involves in cell growth [1]. Our founding indicates that miR-433 not only regulate the YAP1 expression but also influence the YAP1 signaling involving in cell growth.

MiR-433 has been shown as a tumor suppressor in gastric cancer [23], liver cancer [24] and myeloproliferative neoplasms [19]. MiR-433 is also downregulated in bladder cancer tissues and inhibits the bladder cancer cells proliferation, migration and invasion [20]. MiR-433 is also known be associated with the poorer survivals ovarian cancer [25], and the targets of miR-433 are closely related to cancer [26-28]. Overexpression of miR-433 in glioma cells suppressed the cell viability, migration and invasion but promoted cell apoptosis in this study indicating miR-433 may also be a tumor suppressor in glioma. YAP1 is a direct target of miR-433 in this study, and GRB2, TYMS, CREB1 and HDAC6 are also the target of miR-433 in gastric carcinoma, HeLa cells, liver cancer cells and hepatocellular carcinoma cells, respectively. So miR-433 may be closely correlated with tumor development, and more studies should be performed to reveal the action mechanism of miR-433 in cancer.

Conclusion

In summary, we show the decreased expression of miR-433 in glioma tissues with increased expression of YAP1 and miR-433 may inhibit the proliferation, migration and invasion of glioma cells through suppressing the YAP1 expression. Our results indicate the important role of miR-433 in inhibiting glioma cells viability and the potential application of miR-433 in glioma therapy.

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Disclosure of conflict of interest

None.

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References

[1] Zhao B, Ye X, Yu J, Li L, Li W, Li S, Yu J, Lin JD, Wang CY, Chinnaiyan AM, Lai ZC and Guan KL. TEAD mediates YAP-dependent gene induction and growth control. Genes Dev 2008; 22: 1962-1971.

[2] Huang J, Wu S, Barrera J, Matthews K and Pan D. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila Homolog of YAP. Cell 2005; 122: 421-434.

[3] Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, Deng CX, Brugge JS and Haber DA. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A 2006; 103: 12405-12410.

[4] Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA and Anders RA. Expression of Yes-associated protein in common solid tumors. Hum Pathol 2008; 39: 1582-1589.

[5] Cao JJ, Zhao XM, Wang DL, Chen KH, Sheng X, Li WB, Li MC, Liu WJ and He J. YAP is overexpressed in clear cell renal cell carcinoma and its knockdown reduces cell proliferation and induces cell cycle arrest and apoptosis. Oncol Rep 2014; 32: 1594-1600.

[6] Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W and Tuschl T. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002; 12: 735-739.

[7] Lee YS, Kim HK, Chung S, Kim KS and Dutta A. Depletion of human micro-RNA miR-125b re-

veals that it is critical for the proliferation of differentiated cells but not for the down-regulation of putative targets during differentiation. J Biol Chem 2005; 280: 16635-16641.
[8] Houbaviy HB, Murray MF and Sharp PA. Embryonic stem cell-specific MicroRNAs. Dev Cell 2003; 5: 351-358.
[9] Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M and Croce CM. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004; 101: 2999-3004.
[10] Michael MZ, SM OC, van Holst Pellekaan NG, Young GP and James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003; 1: 882-891.
[11] Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T and Takahashi T. Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 2004; 64: 3753-3756.
[12] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M and Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65: 7065-7070.
[13] Chan JA, Krichevsky AM and Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. Cancer Res 2005; 65: 6029-6033.
[14] Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA and Jones PA. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 2006; 9: 435-443.
[15] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005; 102: 13944-13949.
[16] Liu YC and Wang YZ. Role of Yes-associated protein 1 in gliomas: pathologic and therapeutic aspects. Tumour Biol 2015; 36: 2223-2227.
[17] Luo H, Zhang H, Zhang Z, Zhang X, Ning B, Guo J, Nie N, Liu B and Wu X. Down-regulated miR-9 and miR-433 in human gastric carcinoma. J Exp Clin Cancer Res 2009; 28: 82.
[18] Wang W, Zhao LJ, Tan YX, Ren H and Qi ZT. Identification of deregulated miRNAs and their targets in hepatitis B virus-associated hepatocellular carcinoma. World J Gastroenterol 2012; 18: 5442-5453.
[19] Lin X, Rice KL, Buzzai M, Hexner E, Costa FF, Kilpivaara O, Mullally A, Soares MB, Ebert BL, Levine R and Licht JD. MiR-433 is aberrantly expressed in myeloproliferative neoplasms and suppresses hematopoietic cell growth and differentiation. Leukemia 2013; 27: 344-352.
[20] Xu X, Zhu Y, Liang Z, Li S, Xu X, Wang X, Wu J, Hu Z, Meng S, Liu B, Qin J, Xie L and Zheng X. c-Met and CREB1 are involved in miR-433-mediated inhibition of the epithelial-mesenchymal transition in bladder cancer by regulating Akt/GSK-3beta/Snail signaling. Cell Death Dis 2016; 7: e2088.
[21] Fang Y, Feng Y, Wu T, Srinivas S, Yang W, Fan J, Yang C and Wang S. Aflatoxin B1 negatively regulates Wnt/beta-catenin signaling pathway through activating miR-33a. PLoS One 2013; 8: e73004.
[22] Li B, Ge L, Li M, Wang L and Li Z. MiR-448 suppresses proliferation and invasion by regulating IGF1R in colorectal cancer cells. Am J Transl Res 2016; 8: 3013-3022.
[23] Guo LH, Li H, Wang F, Yu J and He JS. The tumor suppressor roles of miR-433 and miR-127 in Gastric Cancer. Int J Mol Sci 2013; 14: 14171-14184.
[24] Yang Z, Tsuchiya H, Zhang Y, Hartnett ME and Wang L. MicroRNA-433 inhibits liver cancer cell migration by repressing the protein expression and function of cAMP response element-binding protein. J Biol Chem 2013; 288: 28893-28899.
[25] Weiner-Gorzel K, Dempsey E, Milewska M, McGoldrick A, Toh V, Walsh A, Lindsay S, Gubbins L, Cannon A, Sharpe D, O'Sullivan J, Murphy M, Madden SF, Kell M, McCann A and Furlong F. Overexpression of the microRNA miR-433 promotes resistance to paclitaxel through the induction of cellular senescence in ovarian cancer cells. Cancer Med 2015; 4: 745-758.
[26] Simon D, Laloo B, Barillot M, Barnetche T, Blanchard C, Rooryck C, Marche M, Burgelin I, Coupry I, Chassaing N, Gilbert-Dussardier B, Lacombe D, Grosset C and Arveiler B. A mutation in the 3'-UTR of the HDAC6 gene abolishing the post-transcriptional regulation mediated by hsa-miR-433 is linked to a new form of dominant X-linked chondrodysplasia. Hum Mol Genet 2010; 19: 2015-2027.
[27] Gotanda K, Hirota T, Matsumoto N and Ieiri I. MicroRNA-433 negatively regulates the expression of thymidylate synthase (TYMS) responsible for 5-fluorouracil sensitivity in HeLa cells. BMC Cancer 2013; 13: 369.
[28] Snyder CM, Rice AL, Estrella NL, Held A, Kandarian SC and Naya FJ. MEF2A regulates the Gtl2-Dio3 microRNA mega-cluster to modulate WNT signaling in skeletal muscle regeneration. Development 2013; 140: 31-42.