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Original Article

MiR-137 regulates the proliferation and invasion of glioma cells through EZH2/Wnt/β-catenin signaling pathway

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Abstract: MicroRNAs (miRNAs) play critical roles in tumor development. In the present study, we explored the role of miR-137 in glioma progression. Our data showed that the miR-137 expression was reduced in glioma tissues and cell lines and reduced expression of miR-137 was corrected with tumor progression. Luciferase assay suggested that EZH2 is a direct target of miR-137, while EZH2 expression was inversely correlated with miR-137 expression in glioma tissues. Overexpression of EZH2 significantly reversed miR-137 mediated inhibition of proliferation and epithelial-mesenchymal transition (EMT), suggesting EZH2 upregulation is involved in the function of miR-137. Furthermore, our data showed that miR-137 inhibited the activity of Wnt/β-catenin signaling pathway in glioma cells. In summary, our study provided that miR-137 could act as a tumor suppressor by targeting EZH2 in glioma cells, and might be useful for developing a novel therapeutic target for glioma.

Keywords: Glioma, EZH2, miR-137, Wnt/β-catenin

Introduction

Glioma is the most common malignant tumors of the brain [1]. According to the World Health Organization (WHO) classification which is based on histogical criteria, human glioma divided into three major histologic groups: includes well differentiated low grade astrocytomas [WHO grade I~II], anaplastic astrocytomas (WHO grade III) and glioblastoma multiforme (GBM, WHO grade IV) [2]. Despite the advances in therapeutic intervention, such as surgery, radiotherapy, chemotherapy, gene therapy, immunotherapy and other novel biological therapies, prognosis and treatment of this tumor type continue to be dismal [3, 4]. Thus, identifying novel molecular biomarker that can inhibit the progression will be urgent for the development of novel therapeutic strategies in glioma.

MicroRNAs (miRNAs), small non-coding RNAs that regulate the translation of many genes by binding to the untranslated region (3'UTR) of target mRNAs, are involved in a variety of physiological and pathological processes, including cancer development [5, 6]. Accumulating evidence shows that miRNAs are aberrantly expressed in many types of cancers, including glioma, and some of these miRNAs function as tumor suppressor genes or oncogenes during tumor development and progression [7, 8]. Distinct miRNAs have been reported to be directly involved in glioma carcinogenesis and progression by regulation of cell proliferation, apoptosis, invasion or drug sensitivity [9]. For example, Gabriely et al showed that miR-21 promoted glioma invasion by targeting matrix metalloproteinase regulators [10]. Zhang et al showed that miR-128 inhibited glioma cells proliferation by targeting transcription factor E2F3a [11]. Bai et al suggested that miR-296-3p regulated cell growth and multi-drug resistance of human glioblastoma by targeting ether-a-go-go (EAG1) [12]. However, the expression and role of miR-137 in glioma remain unclear.

Enhancer of zeste homolog 2 (EZH2) is a catalytic subunit of polycomb repressive complex 2 (PRC2), which represses genes involved in

tumorigenesis via methylation of lysine 27 of histone 3 (H3K27) [13]. Recent studies reported that EZH2 could act as a novel oncogene, playing key roles in the development and progression of human malignant tumors [14]. For example, Choi et al showed that EZH2 was upregulated in gastric cancer and high expression of EZH2 was associated with tumor cell proliferation and metastasis [15]. Huang et al suggested that miR-98 and miR-214 post-transcriptionally regulated EZH2 and inhibited migration and invasion in human esophageal squamous cell carcinoma [16]. Luo et al found that lncRNA H19 increased bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression [17]. However, the underlying molecular mechanism of EZH2 in progression of glioma remains unclear.

In the present study, we aim to identify a novel miRNA involved in cell progression of glioma and investigate their roles in controlling glioma cell proliferation, migration and invasion, indicating miR-137 could act as a promising therapeutic target for the treatment of glioma.

Material and methods

Tissue samples

The tissue collection and analyses were approved by the Ethics Committee of Zhumadian Central Hospital, and written informed consent was obtained from all participants. 33 glioma tissues and 18 normal brain tissues were obtained from patients undergoing surgery at the Department of Neurosurgery, Zhumadian Central Hospital. None of the patients received preoperative treatment, such as radiation or chemotherapy. Pathology specimens from all patients were centrally reviewed and the definitive tumor stage was established on the basis of operative findings according to the 2007 WHO classification. All tissues were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Cell culture and transfection

Human glioma cell lines (U87, U251, A172 and LN18) and normal human glial cell line (HEB) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultured in DMEM medium containing 10% fetal bovine serum (FBS, GBICO), ampicillin and streptomycin at 37°C in a humidified cell incubator with an atmosphere of 5% CO_2 at 37°C.

MiR-137 mimics, miR-137 inhibitor and negative control were obtained from GenePharma (Shanghai) and then transfected into cells to overexpression or knockdown miR-137 expression. Lentiviral vectors mediated EZH2 were obtained from RiboBio Co., Ltd. (Guangzhou, China) and then transfected into cells to overexpression EZH2 expression.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed to detect the relative levels of the transcripts. The transcripts were generated from 2 μg of the large RNA extracted from cells through reverse transcription using M-MLV reverse transcriptase (Promega). The U6 gene was used as an internal control for the PCR reaction. PCR was performed under the following conditions: 94°C for 4 min, followed by 40 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min.

CCK8 assay

Cell growth was measured using the cell proliferation reagent WST-8 (Roche Biochemicals). After plating cells in 96-well microtiter plates (Corning Costar) at 1.0×10^3/well, 10 μl of CCK8 was added to each well at the time of harvest, according to the manufacturer's instructions. 1 h after adding CCK8, cellular viability was determined by measuring the absorbance of the converted dye at 450 nm.

Cell migration and invasion assay

For migration assays, 24 h after transfection, 5×10^4 cells in serum-free medium were added into the upper chamber of an insert (8 um pore size, Millipore). For invasion assays, 1×10^5 cells in serum-free medium were placed into the upper chamber of an insert coated with Matrigel (Sigma-Aldrich). Medium containing 10% FBS were added to the lower chamber as a chemoattractant. After 24 h of incubation, cells remaining on the upper membrane were removed, whereas cells which had migrated or invaded to the lower membrane were stained with 0.1% crystal violet, imaged, and counted using a microscope (Olympus). Experiments were performed independently three times.

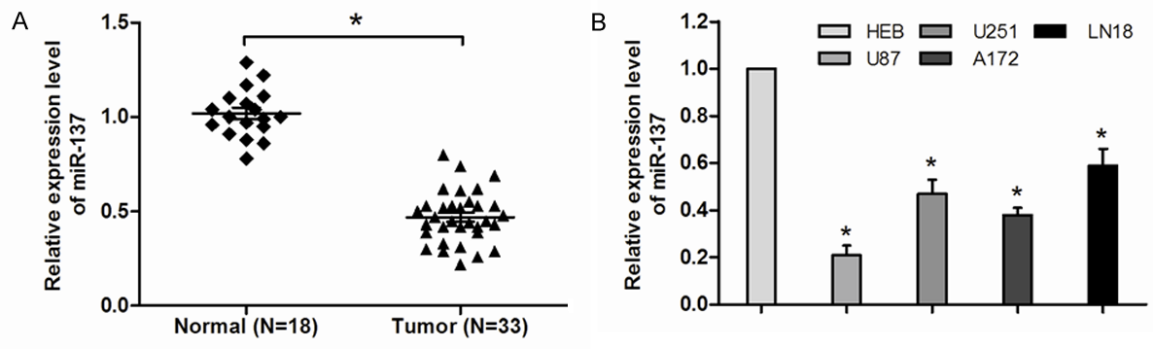

Figure 1. Relative expression of miR-137 in glioma tissues and cell lines. A. qRT-PCR showed downregulated expression of miR-137 in glioma tissues compared with healthy brain tissues. The expression level of miR-137 was measured using qRT-PCR. B. qRT-PCR assay showed downregulation of miR-137 expression in four glioma cell lines (U87, U251, A172 and LN18) compared with normal human glial cell line (HEB). *P<0.05.

Luciferase reporter assay

The 3'-UTR of human EZH2 was amplificated and clone d into the pGL3-luciferase reporter plasmid (Promega). The pGL3-EZH2-mut vector was built by PCR. Cells were co-transfected with wild-type pGL3-EZH2 or mutated pGL3-EZH2-mut vector, and miR-137 mimics, inhibitor or negative control, using Lipofectamine 2000 for 24 h. The luciferase activity was measured using the commercial Dual-Luciferase reporter assay system (Promega) according to the manufacturer's instructions.

Western blot

The cells were lysed in RIPA lysis buffer (Sigma). Protein lysates were prepared, and protein concentration was measured using the BCA Protein Assay kit (Beyotime). Equal quantities of protein samples were loaded on 10% SDS-PAGE and transferred onto PVDF membranes, and Blocking was performed with 5% non-fat milk. The membrane was incubated overnight with anti-EZH2, anti-E-cadherin, anti-vimentin, anti-snail, anti-β-catenin, anti-CyclinD1, anti-c-Myc and anti-GAPDH (Abcam). After washing and incubation with horseradish peroxidase-conjugated antibody (Sigma) for 1.5 h at room temperature, blotted proteins were detected using an enhanced chemiluminescence (ECL) system following the manufacturer's protocol.

Statistical analysis

All the statistics were expressed as mean ± standard deviation (SD) of three independent experiments and analysis by SPSS 18.0 software. One-way ANOVA, Student's t test, and χ^2 test were utilized to analyze the significance of the differences. A *P* value <0.05 was considered statistically significant.

Results

MiR-137 was decreased expression in glioma

To investigate the role of miR-137 in glioma progression, we first determined the expression of miR-137 in 33 glioma tissues and 18 normal brain tissues by using quantitative real-time PCR (qRT-PCR). We concluded that the expression of miR-137 was significantly decreased in glioma tissues compared to normal brain tissues (**Figure 1A**; P<0.05). Furthermore, we tested the expression of miR-137 in glioma cell lines (U87, U251, A172 and LN18) and normal human glial cell line (HEB). Our results showed that miR-137 was downregulated in glioma cell lines (U87, U251, A172 and LN18) compared with normal human glial cell line (HEB) (**Figure 1B**; P<0.05). Those findings indicated that miR-137 play important role in the development and progression of glioma.

MiR-137 inhibited cell proliferation and invasion in glioma

To further explore the function of miR-137 in the progression of glioma, we transfected the U87 cells with miR-137 mimics, miR-137 inhibitors or negative controls. The transfection efficiency of miR-137 mimics and miR-137 inhibitors was validated by qRT-PCR (**Figure 2A**; P<0.05). CCK8 assay showed that the ectopic expression of miR-137 evidently inhibited the

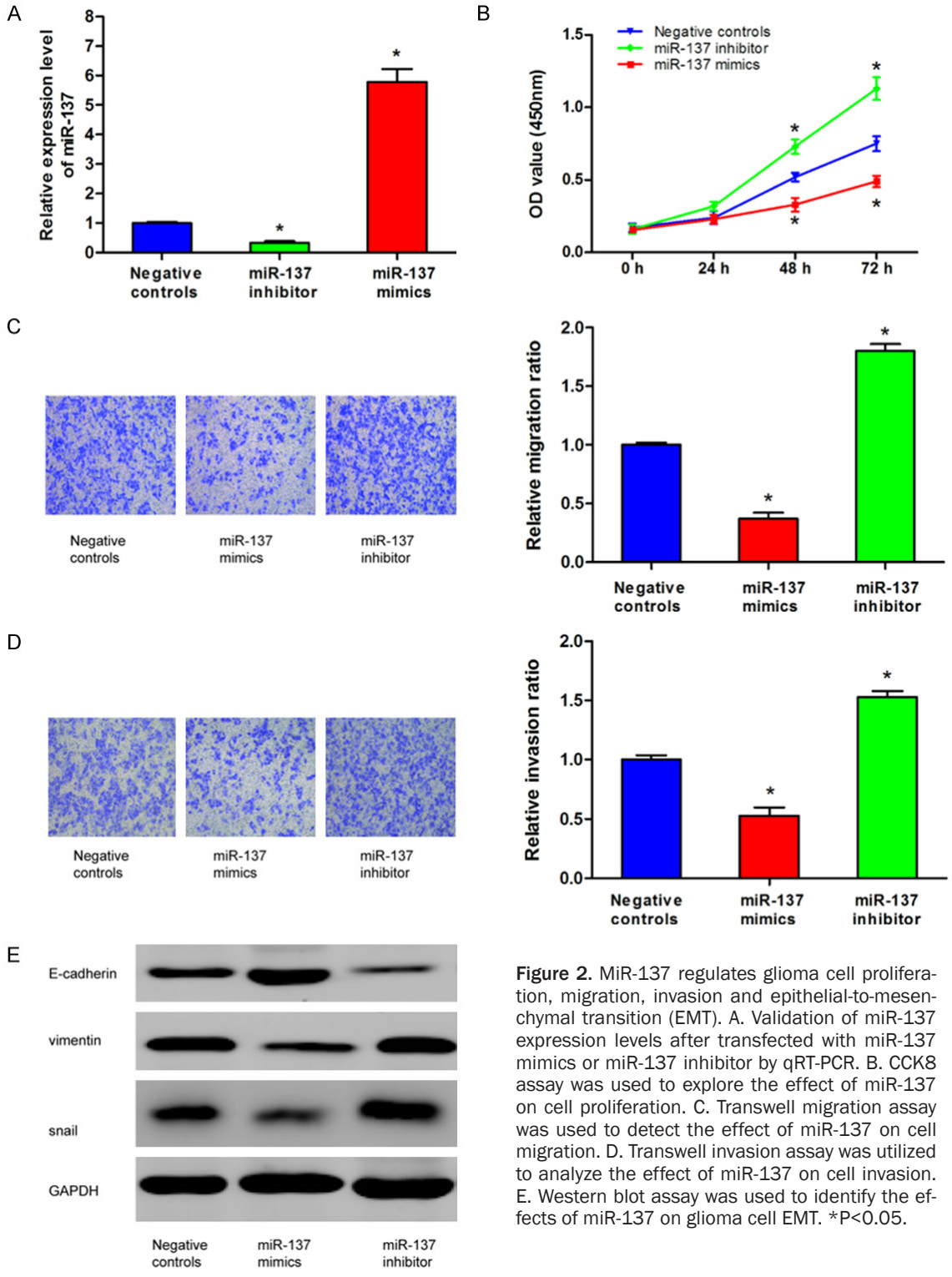

Figure 2. MiR-137 regulates glioma cell proliferation, migration, invasion and epithelial-to-mesenchymal transition (EMT). A. Validation of miR-137 expression levels after transfected with miR-137 mimics or miR-137 inhibitor by qRT-PCR. B. CCK8 assay was used to explore the effect of miR-137 on cell proliferation. C. Transwell migration assay was used to detect the effect of miR-137 on cell migration. D. Transwell invasion assay was utilized to analyze the effect of miR-137 on cell invasion. E. Western blot assay was used to identify the effects of miR-137 on glioma cell EMT. *P<0.05.

proliferation abilities of U87 cells, while miR-137 knockdown promoted the proliferation abilities of in U87 cells (**Figure 2B**; P<0.05). In addition, miR-137 overexpression significantly suppressed the migration and invasion ability of U87 cells compared with control group, while the miR-137 inhibitors showed the opposite effect (**Figure 2C** and **2D**; P<0.05).

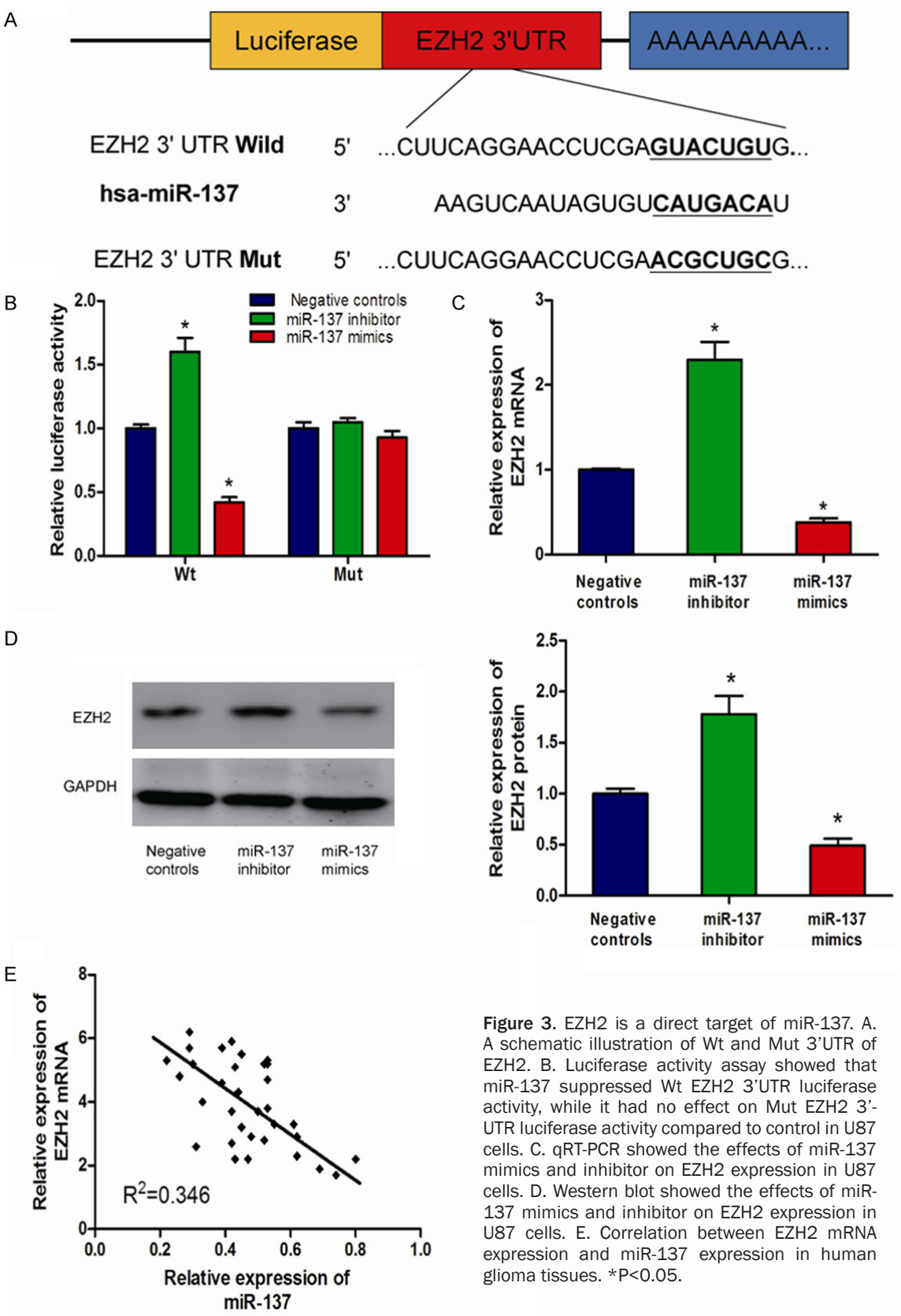

Figure 3. EZH2 is a direct target of miR-137. A. A schematic illustration of Wt and Mut 3'UTR of EZH2. B. Luciferase activity assay showed that miR-137 suppressed Wt EZH2 3'UTR luciferase activity, while it had no effect on Mut EZH2 3'-UTR luciferase activity compared to control in U87 cells. C. qRT-PCR showed the effects of miR-137 mimics and inhibitor on EZH2 expression in U87 cells. D. Western blot showed the effects of miR-137 mimics and inhibitor on EZH2 expression in U87 cells. E. Correlation between EZH2 mRNA expression and miR-137 expression in human glioma tissues. *P<0.05.

The epithelial-mesenchymal transition (EMT) is one of the key initiation steps in the metastasis process, which provides cancer cells with motility, invasion and migration properties.

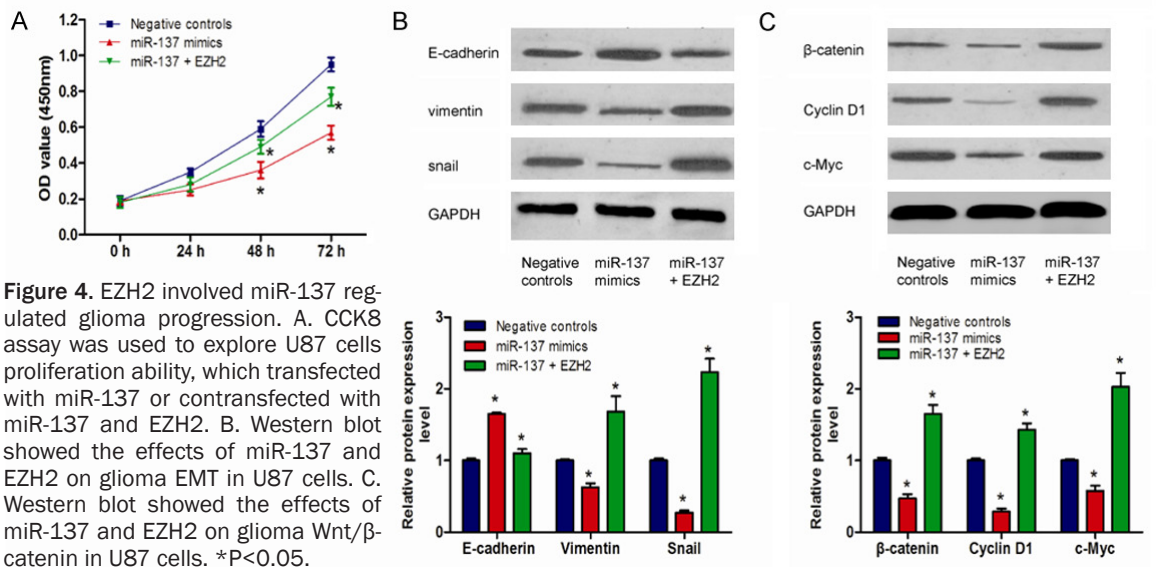

Figure 4. EZH2 involved miR-137 regulated glioma progression. A. CCK8 assay was used to explore U87 cells proliferation ability, which transfected with miR-137 or contransfected with miR-137 and EZH2. B. Western blot showed the effects of miR-137 and EZH2 on glioma EMT in U87 cells. C. Western blot showed the effects of miR-137 and EZH2 on glioma Wnt/β-catenin in U87 cells. *P<0.05.

Therefore, we explored whether miR-137 inhibited gliomas progression is mediated by the EMT process. Western blot showed that the epithelial marker E-cadherin was upregulated after miR-137 overexpression. By contrast, the mesenchymal markers vimentin and snail were inhibited in miR-137 overexpressed U87 cells, while the miR-137 inhibitors showed the opposite effect (**Figure 2E**; P<0.05). Thus, we demonstrated that miR-137 could suppress the glioma progression by repressing cells proliferation, migration and invasion abilities and EMT.

EZH2 is a direct target of miR-137 in glioma

To elucidate the mechanisms through which miR-137 acts on glioma, we predicted the target gene of miR-137 with Targetscan databases. EZH2 3'UTRs was found to have binding sequences for miR-137 (**Figure 3A**). To verify the effects of miR-137 on EZH2, luciferase activity assay was performed. Here, we found that miR-137 significantly inhibited the luciferase activity of the 3'UTR EZH2-Wt group, compared with that in the EZH2-Mut group, indicating that EZH2 is one of the direct targets of miR-137 in glioma (**Figure 3B**; P<0.05). To further confirm EZH2 as a miR-137 target, we determined the expression of EZH2 in miR-137 knockdown or overexpression glioma cells. The EZH2 expression was downregulated at both the mRNA (**Figure 3C**; P<0.05) and protein levels (**Figure 3D**; P<0.05) in U87 cells by miR-137 mimics. While the EZH2 expression was upregulated at both the mRNA and protein levels in U87 cells by miR-137 inhibitors (**Figure 3C**, **3D**; P<0.05). More importantly, there is an inverse correlation between miR-137 expression and EZH2 expression in 33 cases of glioma tissues (**Figure 3E**; P<0.05, R^2=0.346). Taken together, those findings strongly suggested that EZH2 is a direct target of miR-137 in glioma.

EZH2 involved in miR-137 regulated glioma progression

EZH2 has been reported to involve in proliferation and invasion in various cancers. To explore the role of EZH2 in glioma progression, we transfected miR-137 mimics or miR-137 and EZH2 into U87 cells and determined EZH2 expression. Our data showed that EZH2 obviously reversed the inhibition of miR-137 on cell proliferation in vitro (**Figure 4A**; P<0.05). Furthermore, we explore the function of EZH2 in the EMT progression of glioma cells. As showed in **Figure 4B**, our data showed that co-transfection with EZH2 overexpression abolished miR-137 caused EMT-related marker changes at protein levels (**Figure 4B**; P<0.05). Those findings suggested that the miR-137 suppressed glioma cell progression and EMT progression are dependent on EZH2.

Wnt/β-catenin signaling pathway plays important roles in kinds of tumor progression including glioma. Furthermore, recent studies showed that Wnt/β-catenin signaling pathway was a downstream of EZH2 in tumor progression. In the present study, we explored the function of

miR-137 on Wnt/β-catenin signaling pathway in glioma. As shown in **Figure 4C**, western blot revealed that β-catenin, Cyclin D1 and c-Myc were significantly decreased by miR-137 mimics, which was reversed by increased expression of EZH2. Our study indicated that miR-137 inactivated the Wnt/β-catenin signaling pathway via inhibiting EZH2 expression.

Discussion

Increasing studies revealed that dysregulated miRNAs contribute to tumor progression. The present study explored the function of miR-137 in the progression of glioma. We found that the expression of miR-137 was decreased in glioma tissues and cell lines. Increased expression of miR-137 suppressed glioma cell proliferation, migration and invasion in vitro. While, inhibition of miR-137 have the opposite effect. These findings were consistent with previous reports showed that miR-137 was downregulated in several cancers. For example, Balaguer et al found that epigenetic silencing of miR-137 was an early event in colorectal carcinogenesis [18]. Chen et al showed that miR-137 was frequently down-regulated in gastric cancer and was a negative regulator of Cdc42 [19]. Guo et al showed that miR-137 could act as a tumor suppressor to inhibit cell growth in ovarian cancer by targeting AEG-1 [20]. The epithelial-to-mesenchymal transition (EMT) has been confirmed to be important in cell invasion in types of cancer [21]. Recently, miRNAs was reported to involve the metastasis of cancer by regulating EMT [22]. In the present study, our data showed that miR-137 could suppress the EMT progression in glioma for the first time.

It is well known that miRNAs perform their function by regulating the expression of its target gene. Thus, we explore the functional target gene for miR-137 which was involved in glioma progression. Targetscan and luciferase reporter assay suggested that EZH2 might be the functional target gene of miR-137. Furthermore, increased expression or knockdown of miR-137 significantly inhibited or promoted EZH2 expression, respectively. In addition, we found that miR-137 expression was inversely correlated with EZH2 expression in glioma. In vitro assay, our data revealed that EZH2 partially abolished the inhibition of miR-137 on glioma cell progression and reversed miR-137 caused EMT-related marker changes, suggesting that miR-137 could regulate glioma progression via targeting EZH2.

The Wnt/β-catenin signaling pathway is a vital signal transduction pathway, as the activation of this pathway contributes to carcinogenesis and cancer metastasis in certain types of cancer including glioma [23, 24]. Furthermore, recent studies showed that Wnt/β-catenin signaling pathway was a downstream of EZH2 in tumor progression. For example. Cheng et al suggested that EZH2-mediated concordant repression of Wnt antagonists promotes β-catenin-dependent hepatocarcinogenesis [25]. Yuan et al found that down-regulation of EZH2 by RNA interference inhibits proliferation and invasion of ACHN cells via the Wnt/β-catenin pathway [26]. Guo et al indicated that miR-144 down-regulation increased bladder cancer cell proliferation by targeting EZH2 and regulating Wnt signaling [27]. In the present study, our data showed that miR-137 significantly decreased β-catenin, Cyclin D1 and c-Myc expression in glioma, which was reversed by overexpressed EZH2, suggesting that miR-137 inactivated the Wnt/β-catenin signaling pathway by suppressing EZH2.

In summary, the current study provided that miR-137 could serve as a potential tumor suppressor in glioma progression. MiR-137 suppressed glioma cell proliferation, migration, invasion and EMT by targeting EZH2 via Wnt/β-catenin signaling pathway.

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Disclosure of conflict of interest

None.

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References

[1] Wen PY and Kesari S. Malignant gliomas in adults. N Engl J Med 2008; 359: 492-507.
[2] Foote MB, Papadopoulos N and Diaz LA Jr. Genetic classification of gliomas: refining histopathology. Cancer Cell 2015; 28: 9-11.
[3] Zong H, Parada LF and Baker SJ. Cell of origin for malignant gliomas and its implication in therapeutic development. Cold Spring Harb Perspect Biol 2015; 7.

[4] Da Rocha AB, Mans DR, Regner A and Schwartsmann G. Targeting protein kinase C: new therapeutic opportunities against high-grade malignant gliomas? Oncologist 2002; 7: 17-33.
[5] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
[6] Garzon R, Calin GA and Croce CM. MicroRNAs in cancer. Annu Rev Med 2009; 60: 167-179.
[7] Wang X, Tang S, Le SY, Lu R, Rader JS, Meyers C and Zheng ZM. Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS One 2008; 3: e2557.
[8] Zhang B, Pan X, Cobb GP and Anderson TA. MicroRNAs as oncogenes and tumor suppressors. Dev Biol 2007; 302: 1-12.
[9] Ciafre SA, Galardi S, Mangiola A, Ferracin M, Liu CG, Sabatino G, Negrini M, Maira G, Croce CM and Farace MG. Extensive modulation of a set of microRNAs in primary glioblastoma. Biochem Biophys Res Commun 2005; 334: 1351-1358.
[10] Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS and Krichevsky AM. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol 2008; 28: 5369-5380.
[11] Zhang Y, Chao T, Li R, Liu W, Chen Y, Yan X, Gong Y, Yin B, Qiang B, Zhao J, Yuan J and Peng X. MicroRNA-128 inhibits glioma cells proliferation by targeting transcription factor E2F3a. J Mol Med (Berl) 2009; 87: 43-51.
[12] Bai Y, Liao H, Liu T, Zeng X, Xiao F, Luo L, Guo H and Guo L. MiR-296-3p regulates cell growth and multi-drug resistance of human glioblastoma by targeting ether-a-go-go (EAG1). Eur J Cancer 2013; 49: 710-724.
[13] Xiao Y. Enhancer of zeste homolog 2: a potential target for tumor therapy. Int J Biochem Cell Biol 2011; 43: 474-477.
[14] Lu C, Han HD, Mangala LS, Ali-Fehmi R, Newton CS, Ozbun L, Armaiz-Pena GN, Hu W, Stone RL, Munkarah A, Ravoori MK, Shahzad MM, Lee JW, Mora E, Langley RR, Carroll AR, Matsuo K, Spannuth WA, Schmandt R, Jennings NB, Goodman BW, Jaffe RB, Nick AM, Kim HS, Guven EO, Chen YH, Li LY, Hsu MC, Coleman RL, Calin GA, Denkbas EB, Lim JY, Lee JS, Kundra V, Birrer MJ, Hung MC, Lopez-Berestein G and Sood AK. Regulation of tumor angiogenesis by EZH2. Cancer Cell 2010; 18: 185-197.
[15] Choi JH, Song YS, Yoon JS, Song KW and Lee YY. Enhancer of zeste homolog 2 expression is associated with tumor cell proliferation and metastasis in gastric cancer. APMIS 2010; 118: 196-202.
[16] Huang SD, Yuan Y, Zhuang CW, Li BL, Gong DJ, Wang SG, Zeng ZY and Cheng HZ. MicroRNA-98 and microRNA-214 post-transcriptionally regulate enhancer of zeste homolog 2 and inhibit migration and invasion in human esophageal squamous cell carcinoma. Mol Cancer 2012; 11: 51.
[17] Luo M, Li Z, Wang W, Zeng Y, Liu Z and Qiu J. Long non-coding RNA H19 increases bladder cancer metastasis by associating with EZH2 and inhibiting E-cadherin expression. Cancer Lett 2013; 333: 213-221.
[18] Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR and Goel A. Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res 2010; 70: 6609-6618.
[19] Chen Q, Chen X, Zhang M, Fan Q, Luo S and Cao X. MiR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. Dig Dis Sci 2011; 56: 2009-2016.
[20] Guo J, Xia B, Meng F and Lou G. MiR-137 suppresses cell growth in ovarian cancer by targeting AEG-1. Biochem Biophys Res Commun 2013; 441: 357-363.
[21] Christiansen JJ and Rajasekaran AK. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. Cancer Res 2006; 66: 8319-8326.
[22] Katoh Y and Katoh M. Hedgehog signaling, epithelial-to-mesenchymal transition and miRNA (review). Int J Mol Med 2008; 22: 271-275.
[23] Clevers H and Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012; 149: 1192-1205.
[24] Gong A and Huang S. FoxM1 and Wnt/beta-catenin signaling in glioma stem cells. Cancer Res 2012; 72: 5658-5662.
[25] Cheng AS, Lau SS, Chen Y, Kondo Y, Li MS, Feng H, Ching AK, Cheung KF, Wong HK, Tong JH, Jin H, Choy KW, Yu J, To KF, Wong N, Huang TH and Sung JJ. EZH2-mediated concordant repression of Wnt antagonists promotes beta-catenin-dependent hepatocarcinogenesis. Cancer Res 2011; 71: 4028-4039.
[26] Yuan JB, Yang LY, Tang ZY, Zu XB and Qi L. Down-regulation of EZH2 by RNA interference inhibits proliferation and invasion of ACHN cells via the Wnt/beta-catenin pathway. Asian Pac J Cancer Prev 2012; 13: 6197-6201.
[27] Guo Y, Ying L, Tian Y, Yang P, Zhu Y, Wang Z, Qiu F and Lin J. miR-144 downregulation increases bladder cancer cell proliferation by targeting EZH2 and regulating Wnt signaling. FEBS J 2013; 280: 4531-4538.