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EZH2-mediated H3K27me3 enrichment on the lncRNA MEG3 promoter regulates the growth and metastasis of glioma cells by regulating miR-21-3p

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Abstract. – OBJECTIVE: Glioma is one of the most common and invasive brain tumors worldwide. Long non-coding RNAs (LncRNAs) play an important role in the development of glioma. However, the regulatory mechanism of LncRNAs in glioma has not been fully elucidated. This study aimed to explore the interaction of lncRNA maternally expressed gene 3 (MEG3) and aberrant histone modification in glioma.

MATERIALS AND METHODS: The expression levels of MEG3 and miR-21-3p in glioma cells were measured by quantitative polymerase chain reaction (qPCR). EZH2 (enhancer of zeste homolog 2) and H3K27me3 expression in glioma cells were detected by Western Blot (WB). The binding site of the promoter of MEG3 by H3K27me3 was confirmed by ChIP-Real-time PCR. The direct target of MEG3 and miR-21-3p in glioma cells was measured by a luciferase reporter assay. Cell proliferation was detected by Cell Counting Kit-8 (CCK8), and cell invasion and migration were measured by Transwell assays.

RESULTS: EZH2 and miR-21-3p were upregulated and MEG3 was downregulated in glioma cells. Silencing of EZH2 inhibited cell proliferation, migration, and invasion in U87 and U251 cells. Meanwhile, the expression of H3K27me3 could be significantly inhibited by EZH2 interference. H3K27me3 protein can bind to MEG3 promoter directly. EZH2 inhibition and MEG3 down-expression in U87 cells reversed the effects of silencing of EZH2 on glioma cell growth and metastasis. However, EZH2 inhibition and MEG3 overexpression in U251 cells restricted cell proliferation, migration, and invasion. Furthermore, miR-21-3p was verified to interact with MEG3 by direct binding. Inhibition of MEG3 promoted U87 cell growth and metastasis, which was further strengthened following the co-transfection of si-MEG3 and miR-21-3p. Overexpressed MEG3 inhibited U251 cell growth and metastasis and a complete reversal of the results observed in the co-transfection of LV-MEG3 and miR-21-3p.

CONCLUSIONS: EZH2 was highly expressed in glioma cells and EZH2-mediated H3K27me3 enrichment on the MEG3 promoter regulated the growth and metastasis of glioma cells by targeting miR-21-3p. It potentially provided a new therapeutic marker targeting glioma.

Key Words:
Glioma, H3K27me3, EZH2, LncRNA MEG3, MiR-21-3p, Growth, Metastasis.

Introduction

Glioma is a common primary tumor of the central nervous system[1]. Glioma has a fast growth rate and a strong invasive ability, and the patient has a poor prognosis[2]. Surgical treatment and postoperative combined radiotherapy and chemotherapy are currently the standard treatment for glioma, but the average median survival of patients is still less than 15 months[3]. The tolerance of glioma cells to chemotherapeutic drugs is one of the important reasons for patients with chemotherapy failure and poor prognosis[4]. Therefore, it is pivotal to further clarify the pathogenesis of glioma and find a new drug target that more effectively blocks this progressive disease.

Histone methylation/demethylation modification balance dysfunction plays a pivotal role in various tumors[5]. Studies have shown that in human and mouse ovarian cancer models, EZH2 (enhancer of zeste homolog 2) is a human homolog of the Drosophila zeste gene enhancer, belonging to the PcG (Polycomb Group) gene family[6,7]. EZH2

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can catalyze the lysine trimethylation of nucleosome histones and mediate the high expression of H3K27me3 in the promoter region of CXCL9/10 gene[8]. H3K27me3 is a transcriptional repressor signal, regulates CXCL9/10 gene expression to inhibit tumor growth[9]. Liu et al[10] reported that EZH2 phosphorylation promoted self-proliferation and persistence of stemness in glioma stem-like cells through NF-κB methylation.

Long non-coding RNAs (lncRNAs) are a subclass of non-coding RNAs and have stronger tissue specificity and cell specificity than the coding RNA[11]. lncRNAs are expressed in different cells and tissues, which are closely related to the occurrence and development of many diseases[12, 13]. LncRNAs play an important regulatory role in cell differentiation, proliferation, apoptosis, and metabolism. lncRNAs have been identified as a novel epigenetic regulation. Histone methylation can regulate the expression of lncRNAs to participate in various important regulatory processes such as chromatin remodeling, transcriptional activation, and transcriptional interference[14]. lncRNA maternally expressed gene 3 (MEG3) has been shown to have tumor suppressor function[15]. MEG3 significantly lower expression in glioma, but its mechanism is still unclear[16]. The present study aimed to investigate the interaction of MEG3 and H3K27me3 mediating miR-21-3p in glioma cell proliferation, migration, and invasion, thus elucidating the molecular mechanisms of H3K27me3 in the progression of glioma and provide a new insight for glioma treatment.

Materials and Methods

The Online Database Gene Expression Profiling (the GEPIA Web Tool)

Based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects, the RNA sequencing expression data related to our project was analyzed using the GEPIA web tool (http://gepia.cancer-pku.cn).

Cell Culture

Normal human astrocyte cell line (A735) and two human glioma cell lines (U87 and U251) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone, South-Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, GE Healthcare Life Science, South-Logan, UT, USA), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) and incubated in a 5% CO_2 incubator at 37°C.

Cell Transfection

For cell transfection, cells in the logarithmic growth phase were transfected with corresponding constructs when the confluence was up to 80% following the instructions of Lipofectamine 2000 (Life Technology, Carlsbad, CA, USA). At 6-h post-transfection, the culture medium was replenished with fresh DMEM containing 10% FBS. MEG3-small interfering RNA (si-MEG3), EZH2-small interfering RNA (si-EZH2), negative control (si-NC), MEG3 overexpression model (LV-MEG3 or LV-NC) and miR-21-3p were constructed by Gene Pharma (Shanghai, China).

Chromatin Immunoprecipitation (ChIP)

The ChIP assay was performed using the ChIP assay kit (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Cells were crosslinked with 1% formaldehyde for 10 min at room temperature and the reaction was terminated with 125 mM glycine treatment for 10 min. Chromatin extracts were immunoprecipitated with anti-H3K27me3 and anti-IgG antibodies on Protein-A/G-Sepharose beads. After washing, elution, and de-crosslinking, PCR was performed using the primers spanning the putative H3K27me3-binding site on MEG3 promoter.

RT-qPCR Assays

The total RNA was extracted from the glioma cell lines with a RNeasy Mini kit (Invitrogen, Carlsbad, CA, USA) and then transcribed reversely with a SuperScript III kit (Invitrogen, Carlsbad, CA, USA). QRT-PCR was performed with a polymerase chain reaction instrument (Opticon CFD-3200, Waltham, MA, USA). The primer sets included: MEG3: forward, 5'-GCCCTA GGGGAGTGACTACA-3'; reverse, 5'-ACTCGGGACATACCTGCTCT-3'. miR-21-3p: forward, 5'-ACACTCCAGCTGGGCAACACCAGTCGATGGGC-3'; reverse, 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGACAGCCC-3'. EZH2: forward, 5′-GGACCACAGTGTTACCAAGCAT-3′; reverse, 5′-GTGGGGTCTTTATCCGCTCAG-3′. β-actin: - forward, TCAGGTCATCACTATCGGCAAT; reverse, AAAGAAAGGGTGTAAAACCA. The relative expression of amplified RNA samples was calculated using the $2^{-\Delta\Delta CT}$ method and β-actin or U6 was used as the internal control.

CCK-8 Assays

Cell proliferation capacity of treated U87 and U251 cells was evaluated using Cell Counting Kit-8 (CCK-8, CK04, Dojindo Molecular Technologies, Kumamoto, Japan) referring to the manufacturer's instructions at days 1, 2, 3 and 4 post-inoculation. Briefly, cells after transfection were seeded in 96-well plates in growth medium at approximately 6×10^3 cells/well. Then, 10 µl of CCK-8 solution was added into each well at each time-point and incubated at 37°C for 2 hours in dark. The optical density (OD) value (450 nm) was evaluated by a microplate reader (9200, Bio-Rad Laboratories, Hercules, CA, USA).

Transwell Assay

Transwell assay was used to investigate the invasion and migration of glioma cells. Cells were put on the upper the Matrigel-coated invasion chambers or non-coated migration chambers (BD Biosciences, Franklin Lakes, NJ, USA). 500 µl of DMEM medium containing 10% FBS was put in the lower chamber and serum-free medium was put in the upper chamber. The non-invasive cells were wiped off by cotton swabs after 48 hours of incubation. The invading or migrating cells were fixed with 95% ethanol, stained with 0.1% crystal violet. The number of invasive and migratory cells in the lower chamber were counted under an inverted microscope.

Western Blot Assays

Total protein was obtained from U87 and U251 cells using RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, USA) and the protein concentrations were determined by a BCA protein assay kit (Pierce, Rockford, IL, USA). The sample of protein was separated by electrophoresis on 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Followed by the blocks with skimmed milk, the membranes were incubated with primary antibodies (Abcam, Cambridge, UK) overnight at 4°C. After being washed with TBS-T (Tris-buffered Saline with Tween 20) for 3 times, the membranes were incubated with secondary antibody at 24°C for 1 h. The protein blot was visualized by using ECL Reagents (Pierce) followed by densitometry using the ImageJ software (NIH, Bethesda, MD, USA).

Luciferase Reporter Assays

Luciferase assay was used to investigate the interaction among MEG3 and miR-21-3p with a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). The wild-type MEG3 and mutant MEG3 sequence (mutant in miR-21-3p binding site) were cloned into pmirGLO plasmid. The pmirGLO- MEG3 or pmirGLO- MEG3-mut was co-transfected with miR-21-3p mimics or miR-NC by Lipofectamine 2000 (Life Technology, Carlsbad, CA, USA). Subsequently, the recombinant vectors were co-transfected with miR-NC or miR-21-3p mimics into HEK 293 cells. Luciferase activity was detected by the Dual-luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocols after transfection for 48 h.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) and analyzed using SPSS 20.0 software (SPSS IBM, Armonk, NY USA). The significance of differences was determined using Student's *t*-test for two groups and one-way analysis of variance (ANOVA) was used for more than two groups. A Student-Newman-Keuls (SNK) test was performed following ANOVA. $p < 0.05$ was considered to indicate a statistically significant difference.

Results

EZH2 Was Upregulated and MEG3 Was Downregulated in Glioma

We investigated the expression levels of EZH2 and MEG3 in glioma. In the GEPIA database, the mRNA levels of EZH2 were significantly upregulated in brain lower-grade glioma (LGG) tissues (Figure 1A) and glioblastoma multiforme (GMB) tissues (Figure 1B), and the mRNA levels of MEG3 were significantly downregulated in LGG (Figure 1C) and GMB (Figure 1D) tissues. Meanwhile, Kaplan–Meier analysis suggested that high expression of EZH2 was associated with reduced overall survival (Figure 1E). Consistently, the mRNA and protein expression level of EZH2 was also significantly higher in glioma cell lines (U87 and U251) than that in a normal human astrocyte cell line (A735) (Figure 1F and 1G). MEG3 expression was decreased in U87 and U251 compared with A735(Figure 1H). Taken together, these data revealed that EZH2 is a potential oncogene in glioma and correlated with poor prognosis of glioma patients.

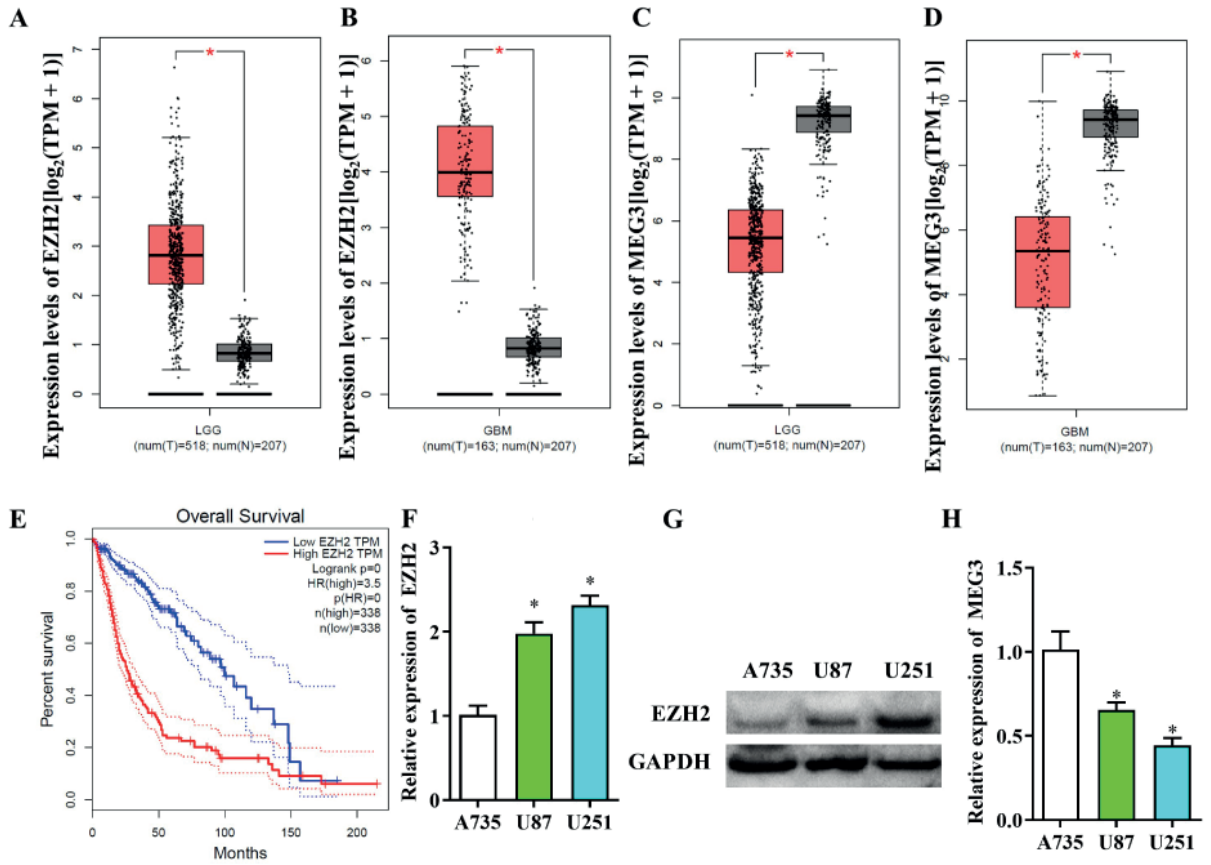

Figure 1. EZH2 was upregulated and MEG3 was downregulated in glioma. **A.** The mRNA levels of EZH2 in LGG tissues from GEPIA database. **B.** The mRNA levels of EZH2 in GMB tissues from GEPIA database. **C.** The mRNA levels of MEG3 in LGG tissues from GEPIA database. **D.** The mRNA levels of MEG3 in GMB tissues from GEPIA database. **E**. Overall survival of glioma patients in different expression of EZH2 from GEPIA database. **F.** The mRNA expression levels of EZH2 were measured by qRT-PCR in glioma cell lines (U87 and U251) and normal human astrocyte cell line (A735). **G.** The protein expression levels of EZH2 were measured by WB in U87, U251, and A735 cells. **H.** The mRNA expression levels of MEG3 were measured by qRT-PCR in U87, U251, and A735 cells. The data are expressed as mean ± SD. *$p<0.05$.

EZH2 Inhibition in Glioma Cells Depressed Cell Proliferation, Invasion and Migration

To further explore the function of EZH2 in glioma cells, si-EZH2 was used to decrease the expression of EZH2 in U87 and U251 cells and the transfection efficiency was tested using qRT-PCR and WB. The mRNA and protein expression levels of EZH2 were suppressed in U87 and U251 cells after transfection with si-EZH2 (Figure 2A and 2B). The CCK8 showed that EZH2 inhibition significantly decreased the proliferative ability of U87 cells (Figure 2C) and U251 cells (Figure 2D). Transwell assays were used to detect the cell invasive and migratory abilities in si-EZH2-transfected glioma cells, and EZH2 inhibition significantly suppressed cell invasion (Figure 2E) and migration (Figure 2F) in U87 and U251 cells. These results indicated that the silencing of EZH2 inhibited glioma cell proliferation, migration, and invasion.

EZH2-Mediated H3K27me3 Enrichment on the MEG3 Promoter

EZH2 is a specific H3K27me3 histone methyltransferase. To investigate whether EZH2-mediated H3K27me3 enrichment on the MEG3 promoter regulated the growth and metastasis of glioma, si-MEG3 or LV- MEG3 was introduced into si- EZH2 transfected U87 and U251 cells. The expression level of H3K27me3 was decreased in U87 and U251 cells after transfection with si-EZH2 (Figure 3A). ChIP assay was performed to evaluate the binding of H3K27me3 to the promoter region of MEG3. We observed an increase in the binding between H3K27me3 and

MEG3 promoter in U87 and U251 cells (Figure 3B). Furthermore, the silencing of EZH2 dramatically suppressed MEG3 expression level in U87 and U251 cells (Figure 3C). MEG3 was low-expressed in U87(Figure 3D) and over-expressed in U251 cells (Figure 3E). The transfection efficiency was determined using RT-qPCR. Besides, EZH2 inhibition and MEG3 down-expression in U87 cells reversed the effects of silencing of EZH2 on glioma cell proliferation (Figure 3E), invasion (Figure 3H), and migration (Figure 3J). However, EZH2 inhibition and MEG3 overexpression in U251 cells further inhibited cell proliferation (Figure 3G), invasion (Figure 3I), and

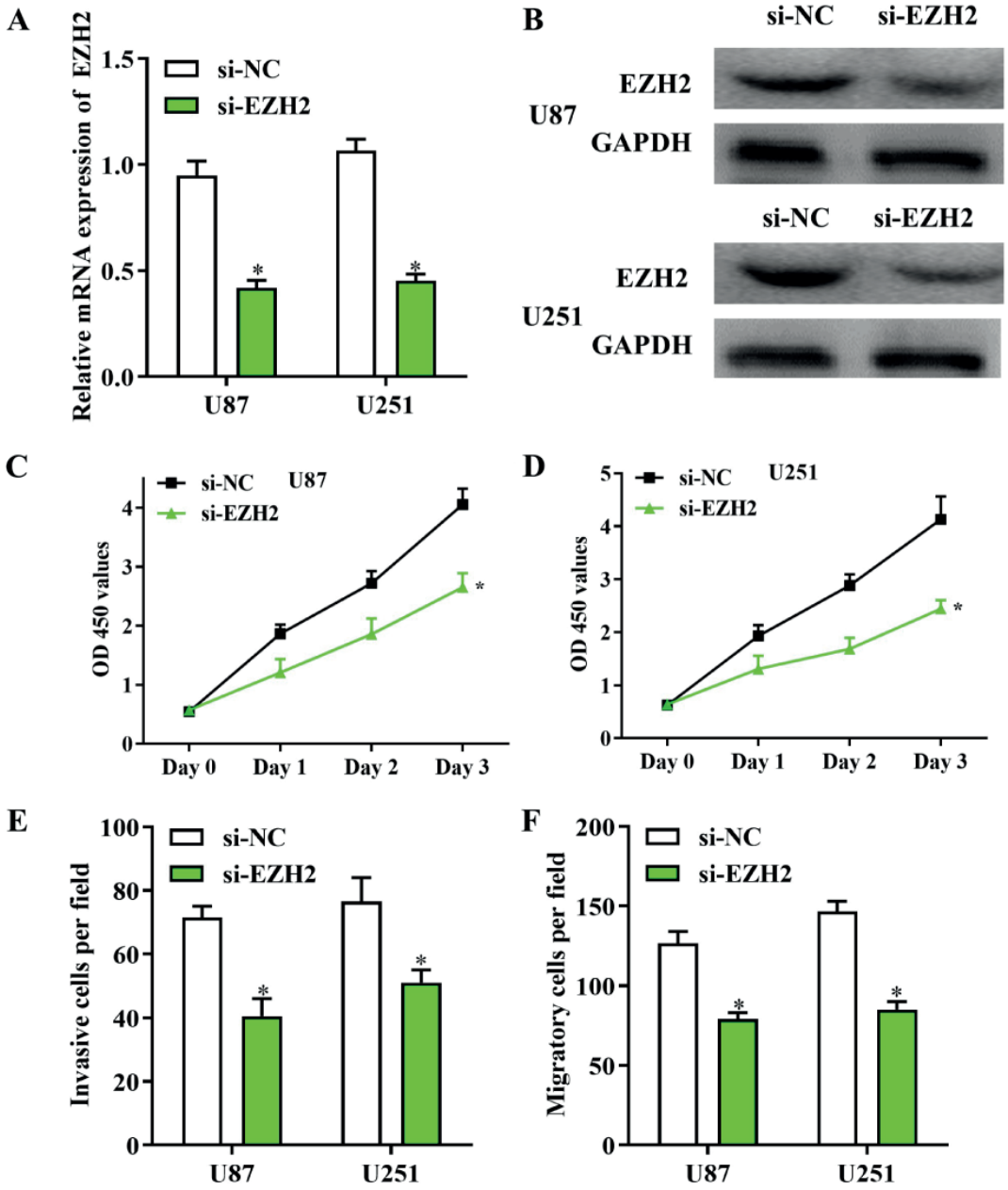

Figure 2. EZH2 inhibition in glioma cells depressed cell proliferation, invasion and migration. **A.** The mRNA expression levels of EZH2 in U87 and U251 cells after transfection with si-EZH2 were measured by qRT-PCR. **B.** The protein expression level of EZH2 in U87 and U251 cells after transfection with si-EZH2 were measured by WB. **C** and **D**. The cell proliferation of U87 and U251 cells after transfection with si-EZH2 was measured using CCK-8 assay. **E**. The cell invasion of U87 and U251 cells after transfection with si-EZH2 was measured using transwell assay. **F**. The cell migration of U87 and U251 cells after transfection with si-EZH2 was measured using transwell assay. The data are expressed as mean ± SD. *p<0.05.

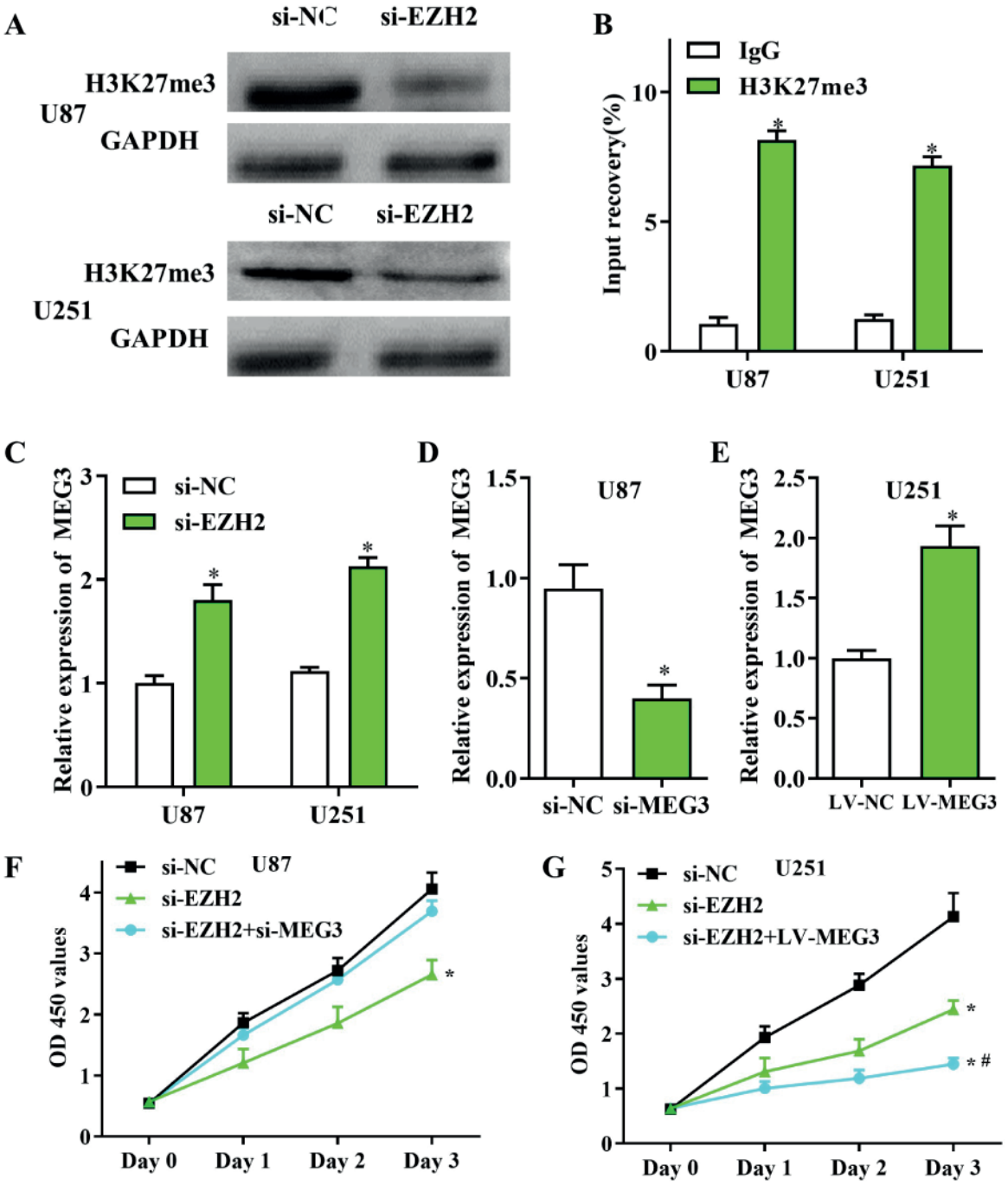

Figure 3. EZH2-mediated H3K27me3 enrichment on the MEG3 promoter. **A.** The protein expression levels of H3K27me3 in U87 and U251 cells, after transfection with si-EZH2, were measured by WB. **B.** The binding of H3K27me3 to the promoter region of MEG3 was performed by ChIP assay. **C.** The mRNA expression levels of MEG3 in U87 and U251 cells after transfection with si-EZH2 were measured by qRT-PCR. **D.** The mRNA expression levels of MEG3 in U87 cells after transfection with si-MEG3 were measured by qRT-PCR. **E.** The mRNA expression levels of MEG3 in U251 cells after transfection with LV-MEG3 were measured by qRT-PCR. **F**. The cell proliferation of U87 cells after transfection with si-EZH2 or si-EZH2 and si-MEG3 was measured using CCK-8 assay. **G.** The cell proliferation of U251 cells after transfection with si-EZH2 or si-EZH2 and LV-MEG3 was measured using CCK-8 assay. **H.** The cell invasion of U87 cells after transfection with si-EZH2 or si-EZH2 and si-MEG3 was measured using transwell assay. **I.** The cell invasion of U251 cells after transfection with si-EZH2 or LV-EZH2 and si-MEG3 was measured using transwell assay. **J.** The cell migration of U87 cells after transfection with si-EZH2 or si-EZH2 and si-MEG3 was measured using transwell assay. **K.** The cell migration of U251 cells after transfection with si-EZH2 or LV-EZH2 and si-MEG3 was measured using transwell assay. The data are expressed as mean ± SD. *p<0.05.

migration (Figure 3K). These findings indicated that EZH2-mediated H3K27me3 enrichment on the MEG3 promoter regulated the growth and metastasis of glioma cells.

MEG3 Suppressed miR-21-3p Expression by Direct Interaction

To further investigate the effects of MEG3 in glioma progression, the putative binding site between MEG3 and miR-21-3p was predicted by bioinformatics (TargetScan, http://www.targetscan.org/) (Figure 4A). Luciferase reporter gene assay demonstrated that miR-21-3p over-expression led to a significant decrease in luciferase activity of MEG3-WT, but not in MEG3-MUT (Figure 4B). Moreover, the mRNA level of miR-21-3p was remarkably elevated in U87 cells after transfection with si-MEG3 (Figure 4C) and the mRNA level of miR-21-3p was reduced in U251 cells after transfection with LV-MEG3 (Figure 4D). Furthermore, the silencing of EZH2 dramatically suppressed miR-21-3p expression level in U87 and U251 cells (Figure 4E). To further verify the expression levels of miR-21-3p in glioma cells, qPCR results showed miR-21-3p expression was increased in U87 and U251 compared with A735 (Figure 4F). To explore the function of miR-21-3p in glioma cells, miR-21-3p mimics were used to elevate the expression of miR-21-3p in U87 and U251 cells and the transfection efficiency was tested using qRT-PCR.

MiR-21-3p Was the Functional Target of MEG3 that Affects the Growth and Metastasis of Glioma Cells

To further explore whether the effects of MEG3 in glioma progression were mediated by miR-21-3p, si-MEG3 mimics and miR-21-3p were co-transfected into U87 cells and LV-MEG3 mimics, and miR-21-3p was co-transfected into U251 cells. MEG3 down-expression in U87 cells promoted cell proliferation (Figure 5A), invasion (Figure 5C), and migration (Figure 5E), which was further strengthened following the co-transfection of si-MEG3 and miR-21-3p. Overexpressed MEG3 inhibited U251 cell proliferation (Figure 5B), invasion (Figure 5D), and migration (Figure 5F) and a complete reversal of the results seen in co-transfection of LV-MEG3 and miR-21-3p. All these data suggested that MEG3 blocked glioma cell growth and metastasis via down-regulating miR-21-3p. Taken together, EZH2-mediated H3K27me3 enrichment on the lncRNA MEG3 promoter, regulating the proliferation, invasion, migration of glioma cells through targeting miR-21-3p (Figure 6).

Discussion

Glioma is a heterogeneous and multifactorial disease which threatens public physical and psychosocial wellbeing and results in a significant burden in public health and economy. Although in recent years, the 5-year survival rate for glioma has ascended a little, it remains not optimistic. Thus, our study aimed at seeking a practicable alternative to glioma treatment. Epigenetics has important effects on biological cytology by regulating the expression of genes that do not alter the sequence of the coding gene. Epigenetic regulation includes DNA methylation, histone modification, regulation of non-coding RNA, and chromatin remodeling. At present, studies[17,18] have shown that histone modification is closely related to the occurrence and development of tumors. Wang et al[19] reported that histone demethylase NO66 was highly expressed in glioma tissues and required for EGFR expression and the proliferation of glioma cells. In our study, we found that EZH2 was significantly overexpressed in glioma cell lines and associated with reduced overall survival of glioma patients. Meanwhile, the silencing of EZH2 dramatically suppressed glioma cell proliferation, invasion, and migration. Collectively, these findings suggested that EZH2 may function as an oncogene in glioma and EZH2 contributes to the progression of glioma.

EZH2 is a specific H3K27me3 histone methyltransferase and EZH2 can promote the expression of H3K27me3. ChIP assay showed the binding of H3K27me3 to MEG3 promoter region, thus indicating EZH2-mediated H3K27me3 enrich on the MEG3 promoter to down-regulate the expression level of MEG3. Studies[20,21] showed that MEG3 expression was downregulated in glioma, which could contribute to the development of glioma patients. In our research, the silencing of EZH2 dramatically suppressed MEG3 expression levels in glioma cells. Meanwhile, the effects of si-EZH2 on glioma cell proliferation, invasion, and migration could be reversed in U87 cells after transfection with si-MEG3 and further promoted in U251 cells after transfection with LV-MEG3. These findings indicated that EZH2-mediated H3K27me3 enrichment on the MEG3 promoter, regulating the growth and metastasis of glioma cells.

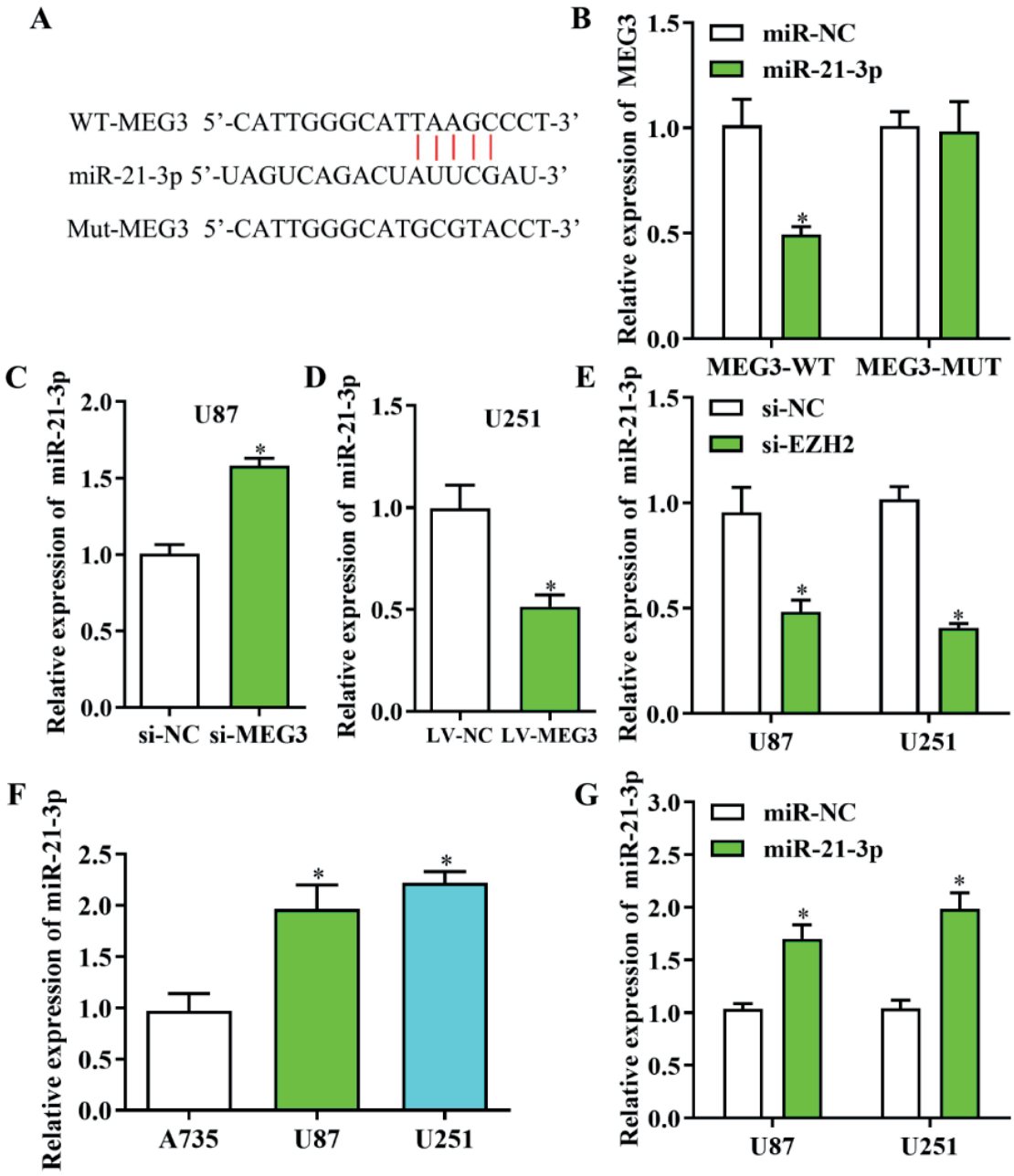

Figure 4. MEG3 suppressed miR-21-3p expression by direct interaction. **A.** The predicted miR-21-3p binging sites in MEG3 3'-UTR. **B.** Effects of miR-21-3p on the luciferase activity of MEG3 -WT and MEG3 -MUT reporter were detected by luciferase assays. **C.** The expression levels of miR-21-3p were determined by qRT-PCR in U87 cells after transfection with si- MEG3. **D.** The expression levels of miR-21-3p were determined by qRT-PCR in U251 cells after transfection with LV- MEG3. **E.** The expression levels of miR-21-3p were determined by qRT-PCR in U87 and U251 cells after transfection with si-EZH2. **F.** The mRNA expression levels of miR-21-3p were measured by qRT-PCR in U87, U251, and A735 cells. **G.** The mRNA expression levels of miR-21-3p were measured by qRT-PCR in U87 and U251 cells after transfection with miR-21-3p mimics. The data are expressed as mean ± SD. *p<0.05.

MicroRNAs (miRNAs) are defined as small non-coding RNAs which regulate gene expression through post-transcriptional modulation, and progresses cell differentiation, growth, and invasion[22]. MiRNAs can act as oncogenes or tumor suppressors in human cancers by modulating the processes associated with tumorigenesis[23]. miR-21-3p is one of the oncogenes miRNAs[24],

which associated with multiple tumors, such as paraganglioma, esophageal squamous cell carcinoma, liver cancer[25-27]. Studies showed that miR-21-3p could regulate glioma cell proliferation and apoptosis by downregulating PTEN protein[28,29]. Our data showed that MEG3 was the molecular sponge of miR-21-3p, and regulated glioma cell growth and metastasis. To further explore wheth-

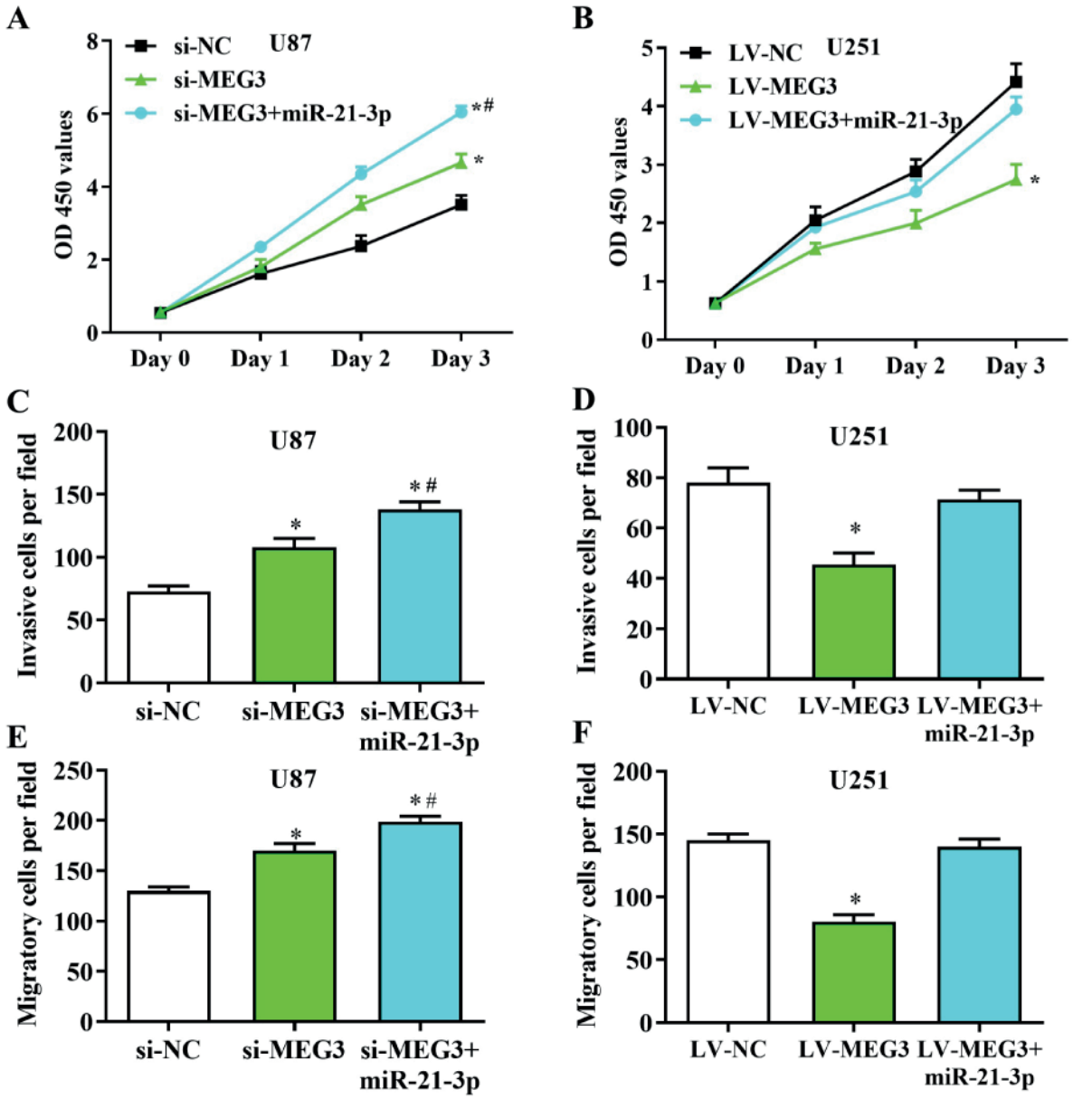

Figure 5. MiR-21-3p was the functional target of MEG3 that affect the growth and metastasis of glioma cells. **A**. The cell proliferation of U87 cells after transfection with si- MEG3 or si- MEG3 and miR-21-3p mimics was measured using CCK-8 assay. **B.** The cell proliferation of U251 cells after transfection with LV- MEG3 or LV- MEG3 and miR-21-3p mimics was measured using CCK-8 assay. **C.** The cell invasion of U87 cells after transfection with si- MEG3 or si- MEG3 and miR-21-3p mimics was measured using transwell assay. **D.** The cell invasion of U251 cells after transfection with LV- MEG3 or LV- MEG3 and miR-21-3p mimics was measured using transwell assay. **E.** The cell migration of U87 cells after transfection with si- MEG3 or si- MEG3 and miR-21-3p mimics was measured using transwell assay. **F.** The cell migration of U251 cells after transfection with LV- MEG3 or LV- MEG3 and miR-21-3p mimics was measured using transwell assay. The data are expressed as mean ± SD.*$p<0.05$.

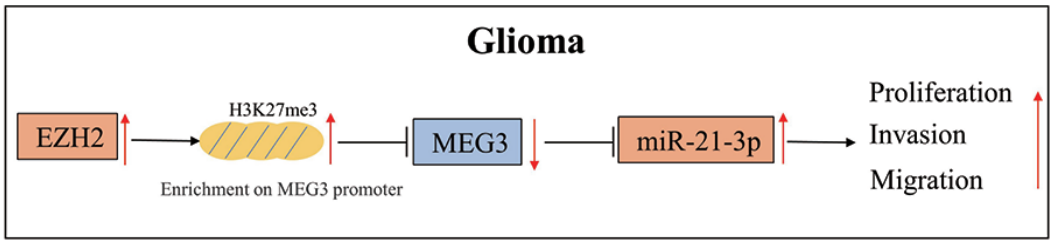

Figure 6. Schematic illustration of EZH2-mediated H3K27me3 enrichment on the MEG3 promoter regulates the growth and metastasis of glioma cells by targeting miR-21-3p.

er the effects of MEG3 in glioma progression was mediated by miR-21-3p, si-MEG3 mimics and miR-21-3p were co-transfected into U87 cells, and LV-MEG3 mimics and miR-21-3p were co-transfected into U251 cells. The results showed MEG3 down-expression in U87 cells promoted cell proliferation, invasion, and migration, which was further strengthened following the co-transfection of si-MEG3 and miR-21-3p. Overexpressed MEG3 inhibited U251 cell growth and metastasis and a complete reversal of the results seen in co-transfection of LV-MEG3 and miR-21-3p. All these data suggested that MEG3 blocked glioma cell growth and metastasis via down-regulating miR-21-3p.

Conclusions

The above data revealed that EZH2 was up-regulated and MEG3 was downregulated in glioma cells, and EZH2-mediated H3K27me3 enrichment on the MEG3 promoter, regulating the growth and metastasis of glioma cells by targeting miR-21-3p. Thus, our study provides valuable clues for understanding the regulatory network of EZH2 in tumor carcinogenesis and identifying a new therapeutic marker targeting glioma.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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