

Original Article

LncRNA MEG3 inhibits glioma progression by inducing pyroptosis through the miR-223-3p/FOXO1/PARP1 axis

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Abstract: Glioma, particularly glioblastoma (GBM), is a highly aggressive malignancy with limited therapeutic options. Pyroptosis, a pro-inflammatory regulated cell death, represents a promising tumor-suppressive mechanism; however, its precise regulatory networks in glioma remain incompletely defined. Although lncRNA MEG3 is a recognized tumor suppressor, whether it dictates glioma pyroptosis through a competing endogenous RNA (ceRNA) mechanism has yet to be characterized. In the study, we measured the expression profiles of MEG3, miR-223-3p, FOXO1 and PARP1 in glioma tissues and cultured cell lines. In vitro, we evaluated the biological effects of MEG3 modulation. Subcutaneous xenograft tumor models were established to investigate tumor progression in vivo. Dual-luciferase reporter and RIP assays validated the molecular interactions, and rescue experiments confirmed the functional importance of this axis. In comparison to normal counterparts, glioma specimens and cell models displayed a prominent reduction in MEG3 expression. Crucially, restoring MEG3 levels thwarted aggressive cellular traits (including proliferation, migration, and invasion) by prompting pyroptosis, a process validated by the elevated abundance of mature IL-1 β , cleaved caspase-1, and GSDMD-N. Mechanistically, as a ceRNA molecule, MEG3 exerts its regulatory role by sponging and functionally neutralizing miR-223-3p, thus derepressing FOXO1 and PARP1 post-transcriptionally. Knockdown of FOXO1 or PARP1 effectively abrogated the pro-pyroptotic and anti-tumor effects induced by MEG3 overexpression. Consistently, MEG3 upregulation hindered tumor growth and triggered pyroptosis in vivo. In conclusion, our findings unveil a novel regulatory MEG3/miR-223-3p/FOXO1-PARP1 axis that drives pyroptosis to restrain glioma progression, which identifies this axis as a potential therapeutic target with translational value for glioma therapy.

Keywords: Glioma, LncRNA MEG3, Pyroptosis, miR-223-3p, FOXO1, PARP1, ceRNA mechanism

Introduction

Glioblastoma (GBM, WHO grade IV) is the most prevalent and aggressive primary CNS malignancy. Despite the integration of maximal safe resection, radiotherapy, and temozolomide, clinical outcomes remain dismal [1, 2]. In addition to its infiltrative growth pattern and rapid development of chemoresistance, glioma exhibits profound intratumoral heterogeneity, which further complicates effective clinical management [3]. Thus, delineating the molecular basis of glioma development and identifying actionable therapeutic targets represent a critical step toward addressing this clinical challenge.

Pyroptosis represents a specific type of regulated cell death that manifests as cytoplasmic swelling and membrane lysis, thereby triggering the liberation of inflammatory mediators like IL-1 β and IL-18. Its status as a pivotal orchestrator of malignant progression has gained widespread acknowledgment in recent years [4, 5]. Unlike apoptosis, which is typically immunologically silent, pyroptosis triggers a robust inflammatory response that can reshape the tumor microenvironment and modulate cancer progression [6]. Within the standard pyroptotic signaling pathway, the proteolytic cleavage of pro-GSDMD, pro-IL-1 β , and pro-IL-18 is orchestrated by activated caspase-1; these functional products then initiate membrane pore assem-

bly and eventual cell rupture [7, 8]. Although pyroptosis exerts anti-neoplastic effects in multiple cancer types [9], its precise mechanisms and regulatory networks in glioma remain largely uncharacterized. Elucidating the molecular signals that govern pyroptosis in glioma cells may provide novel strategies for clinical intervention.

Long non-coding RNAs (lncRNAs) - defined as transcripts spanning more than 200 nucleotides with scarce protein-coding potential - exert profound control over gene expression at the epigenetic, transcriptional, and post-transcriptional layers [10, 11]. Accumulating evidence indicates that dysregulated lncRNAs play critical roles in glioma initiation, progression, and chemoresistance [12, 13]. The lncRNA MEG3, located on chromosome 14q32, exerts a vital tumor-suppressive role and routinely exhibits diminished expression levels in glioma [14, 15]. MEG3 has been demonstrated to inhibit cell proliferation while triggering apoptotic cell death; however, whether it modulates glioma progression through pyroptosis remains unexplored.

lncRNAs exert diverse functions through a “sponge” mechanism within the ceRNA framework by sequestering miRNAs and thereby blocking their interactions with target mRNAs [16]. MiR-223-3p, an identified oncomiR, is frequently upregulated in glioma, where it promotes tumor growth and invasion [17, 18]. In this study, bioinformatic analysis and preliminary results indicate that MEG3 harbors binding motifs for miR-223-3p, suggesting a potential regulatory interaction. FOXO1 (Forkhead box O1) and PARP1 (Poly (ADP-ribose) polymerase 1) regulate cell cycle progression, DNA repair, and apoptosis [19, 20]. FOXO1 and PARP1 are both predicted downstream targets of miR-223-3p, however, their participation in a MEG3-driven ceRNA network controlling glioma pyroptosis remains unverified.

This study tested the hypothesis that lncRNA MEG3 suppresses glioma by sponging miR-223-3p and subsequently upregulating FOXO1 and PARP1 expression, which in turn initiates pyroptosis. We utilized glioma tissue samples and cell lines to examine the expression of MEG3, miR-223-3p, FOXO1, and PARP1 for validation of our hypothesis. Elucidation of the mechanistic crosstalk within this regulatory

network was achieved via gain- and loss-of-function approaches in conjunction with dual-luciferase reporter assays, RIP, and rescue studies. The effects of this axis on glioma cell proliferation, migration, invasion, and pyroptosis were assessed and validated in a xenograft mouse model. This study identifies the MEG3/miR-223-3p/FOXO1-PARP1 axis as a promoter of pyroptotic cell death in glioma, providing insights into glioma pathogenesis and indicating potential therapeutic targets.

Materials and methods

Cell lines and culture

Human glioma cell lines (U87, U251, T98G, and A172) and the normal human astrocyte cell line (HEB) were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cell propagation was carried out in DMEM (Gibco, Grand Island, NY, USA) containing 1% penicillin-streptomycin (Beyotime, Shanghai, China) and 10% fetal bovine serum (FBS; Gibco). All cultures were incubated in a humidified atmosphere at 37°C containing 5% CO₂. Routine mycoplasma testing was performed periodically using a PCR-based detection kit (Beyotime) to ensure contamination-free conditions.

Animal models and xenograft assay

Male 7-week-old BALB/c nude mice (Vital River, Beijing, China) were acclimated under SPF conditions (22-25°C, 40-60% humidity, 12-h light/dark cycle). For the xenograft model, mice (n=6/group) received a subcutaneous injection of 5×10^6 U251 cells (stably expressing oe-Vector or oe-MEG3) or PBS (Control) into the right flank. Tumor volume was measured with calipers every 3 days and calculated as $(\text{length} \times \text{width}^2)/2$. For tissue harvesting, mice were humanely euthanized via gradual CO₂ inhalation (20% chamber volume/min). Death was confirmed by total immobility, respiratory arrest, and bilateral pupil dilation, after which tumors were excised and weighed. Animal protocols were approved by the IACUC of The Affiliated Hospital of Yan'an University and complied with NIH guidelines.

Cell transfection

Upregulation of MEG3 was accomplished by transfecting cells with pcDNA3.1 plasmids con-

taining the full-length construct (pcDNA-MEG3) or empty vector using Lipofectamine 3000 (Invitrogen). For knockdown, shRNAs targeting FOXO1 or PARP1 and sh-NC were synthesized by GenePharma (Shanghai, China); miR-223-3p mimics and NC mimics were from the same vendor. Transfections followed the manufacturer's protocols, and cells were harvested at 48 h for subsequent analyses. The specific shRNA sequences are listed in [Table S1](#).

RT-qPCR

Isolation of total RNA from the collected cells and specimens was achieved utilizing TRIzol reagent (Invitrogen, China). To analyze lncRNA and mRNA expression, reverse transcription was executed with the PrimeScript RT reagent kit (Takara, Dalian, China) in a 20- μ L reaction volume following the manufacturer's protocol. For miRNA profiling, cDNA was generated employing the All-in-One miRNA RT-qPCR Detection kit (GeneCopoeia), followed by real-time PCR amplification using SYBR Green Master Mix (Applied Biosystems) on an ABI 7500 platform. The endogenous references GAPDH and U6 were used to normalize mRNA/lncRNA and miRNA data, respectively. Relative fold changes were determined via the comparative 2- $\Delta\Delta$ Ct method. Primers are listed in [Table S2](#).

Western blotting

Total proteins were extracted with RIPA buffer (Beyotime) containing protease/phosphatase inhibitors and quantified using a BCA kit (Beyotime). Equal protein was resolved by SDS-PAGE and transferred to PVDF membranes (Millipore). Following a 1 h blocking step at room temperature with 5% non-fat milk in TBST, the membranes were exposed to primary antibodies overnight at 4°C. FOXO1 (ab179450, 1:1000, Abcam), PARP1 (6704, 1:1000, CST), pro-caspase-1 (ab179515, 1:1000, Abcam), cleaved-caspase-1 (89332, 1:1000, CST), pro-IL-1 β (83186, 1:1000, CST), IL-1 β (12242, 1:1000, CST), GSDMD (ab219800, 1:1000, Abcam), GSDMD-N (ab215203, 1:1000, Abcam), and GAPDH (ab181602, 1:5000, Abcam). Subsequent to the washing steps, the blots were exposed to HRP-conjugated secondary antibodies (1:5000, Proteintech) for 1 h under room temperature conditions, with protein bands developed by ECL and densitometric analysis performed using ImageJ.

Dual-luciferase reporter assay

The pmirGLO dual-luciferase vector (Promega) was utilized to clone wild-type (WT) or mutant (MUT) segments of MEG3, FOXO1 3'UTR, and PARP1 3'UTR, all of which encompassed the predicted binding region for miR-223-3p. Co-transfection of these reporter vectors was performed with miR-223-3p mimics or negative controls (NC mimics). After 48 hours, evaluation of luciferase activities was executed via the Dual-Luciferase Reporter Assay System (Promega); normalization of firefly luciferase against Renilla luciferase was applied to derive the final values.

RIP assay

RIP assays were performed using the Magna RIP Kit (Millipore). Magnetic beads conjugated with anti-Ago2, anti-FOXO1, anti-PARP1, or isotype IgG antibodies were introduced into tissue lysates, followed by an overnight incubation at 4°C. After washing and RNA purification, RT-qPCR was used to assess the enrichment of lncRNA MEG3 and miR-223-3p.

Colony formation assay

Following transfection, cells were plated into 6-well plates at a density of 500 cells per well. After a 14-day incubation period, the cells underwent fixation with 4% PFA and were subsequently visualized via 0.1% crystal violet staining.

Wound healing assay

The mechanical introduction of a linear wound was performed using a sterile 200 μ L pipette tip on cell monolayers that had attained 90% confluence in 6-well plates. Following a PBS rinse, the medium was replaced with a serum-free alternative for continued cultivation. Wound images were captured at 0 and 24 h using an inverted microscope, and migration distance was measured with ImageJ.

Transwell invasion assay

Cell invasion capacity was assessed using Matrigel-coated Transwell chambers (Corning, NY, USA). A suspension of cells (5×10^4) was added to the upper chamber and the lower well was filled with complete medium containing

FBS. Subsequent to 24 h of culture, non-migrated cells on the upper side of the membrane were cleared via a cotton tip; meanwhile, the invaded cells adhering to the underside were sequentially subjected to fixation, crystal violet staining, and microscopic enumeration.

Flow cytometry on pyroptosis

Pyroptosis was assessed using a Caspase-1/Propidium Iodide (PI) Flow Cytometry Assay Kit (BestBio). Cells were dual-labeled with FLICA (FAM-YVAD-FMK) and PI according to the manufacturer's instructions. Samples were run on a BD FACSCanto II flow cytometer and analyzed with FlowJo v10.8. Cells double-positive for caspase-1 and PI were considered pyroptotic.

Histological analysis

Tumor samples were fixed with 4% PFA, paraffin-embedded, and sectioned at a thickness of 4 μ m, prior to undergoing hematoxylin and eosin (H&E) staining. Microscopic changes, including altered cellularity, nuclear condensation, and chromatin dispersal, were observed under light microscopy (LM).

Statistical analysis

Data collected from three independent replicates are represented as the mean $\pm$ SD. The assessment of variations between two groups was executed via Student's t-test; meanwhile, multigroup datasets were analyzed using one-way ANOVA followed by Tukey's post hoc comparison. Correlations among molecular expressions were assessed using Pearson correlation. $P < 0.05$ was considered statistically significant.

Results

Expression profiles of lncRNA MEG3, miR-223-3p, FOXO1 and PARP1 in mouse glioma xenograft tissues and cell lines

Aiming to decipher the phenotypic impact of lncRNA MEG3 on glioma pathogenesis, we first quantified its expression levels in a murine xenograft model and various human glioma cell lines. As quantified by RT-qPCR, lncRNA MEG3 expression was profoundly diminished across 15 paired mouse glioma xenograft specimens relative to their corresponding non-tumor tissue counterparts (**Figure 1A**). Among the four

types of human glioma cell lines analyzed (U87, U251, T98G, and A172), lncRNA MEG3 was notably decreased compared with the normal human astrocyte reference (HEB), with U251 cells exhibiting the lowest expression level (**Figure 1B**). Conversely, miR-223-3p demonstrated an opposing expression profile, characterized by significant upregulation in murine xenograft tumors relative to matched normal tissues (**Figure 1C**), and the highest expression was observed in U87 cells among the tested cell lines (**Figure 1D**). Given the characterized roles of FOXO1 and PARP1 in malignancies, we assessed their protein expression patterns within the same samples. Murine glioma tissues exhibited a significant deficit in the protein abundance of both FOXO1 and PARP1 when contrasted with normal control specimens (**Figure 1E, 1G**). Similarly, consistently reduced levels of FOXO1 and PARP1 proteins were observed in all glioma cell lines compared with HEB cells (**Figure 1F, 1H**). In the 15 paired mouse xenograft tissues, miR-223-3p and MEG3 expression were negatively correlated (**Figure 1I**). Furthermore, the expression of both FOXO1 (**Figure 1J**) and PARP1 (**Figure 1K**) was found to be negatively associated with miR-223-3p abundance, suggesting a potential regulatory network among these molecules during glioma progression in vivo.

miR-223-3p directly interacts with lncRNA MEG3, FOXO1, and PARP1

Based on the inverse expression patterns, we hypothesized that miR-223-3p could exert direct regulatory control over lncRNA MEG3, FOXO1 and PARP1. As demonstrated by the dual-luciferase reporter assays, the relative luciferase activity of vectors harboring the WT binding sequences for lncRNA MEG3, FOXO1, and PARP1 was markedly suppressed upon co-transfection with miR-223-3p mimics (**Figure 2A-C**); in contrast, mutation of the expected binding sequences (MUT) abolished such suppression. Ago2-RIP experiments were conducted to verify the physical interaction within the RNA-induced silencing complex. Both lncRNA MEG3 and miR-223-3p were significantly enriched in Ago2 immunoprecipitates relative to IgG controls, confirming that they directly interacted (**Figure 2D**). Furthermore, RIP assays using specific antibodies against FOXO1 and PARP1 revealed significant enrichment of miR-223-3p in the anti-FOXO1 (**Figure 2E**) and anti-

MEG3/miR-223-3p axis drives glioma pyroptosis

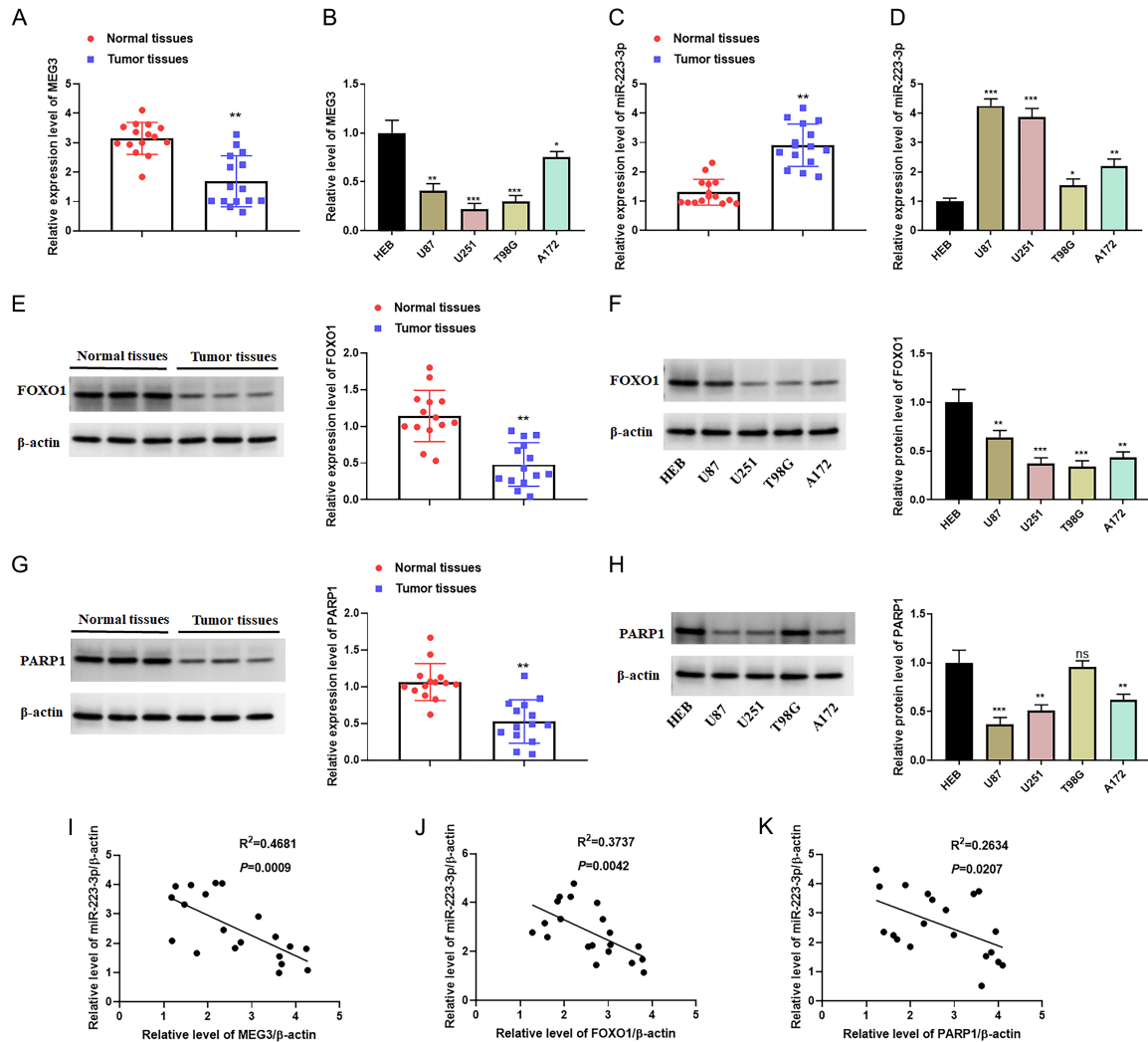


Figure 1. Expression profiles of lncRNA MEG3, miR-223-3p, FOXO1 and PARP1 in glioma xenograft specimens and cell models. (A, B) lncRNA MEG3 was detected by RT-qPCR in glioma samples and corresponding non-tumor tissues from (A) nude mice (n=15), as well as in (B) human glioma cell lines (U87, U251, T98G, A172) relative to the normal human Astrocyte control (HEB). (C, D) Quantified miR-223-3p abundance through RT-qPCR among (C) glioma specimens and corresponding non-malignant tissues derived from nude mice (n=15) plus (D) specified glioma cell lines alongside HEB cells. (E-H) Representative Western blot bands and densitometric analysis of FOXO1 (E, F) as well as PARP1 (G, H) proteins' levels in (E, G) murine glioma tissue versus healthy tissue; also (F, H) glioma cell strains versus HE B cells. GAPDH is chosen as the internal reference. (I-K) Correlation analysis via Pearson methods among miR-223-3p expression and (I) lncRNA MEG3, (J) FOXO1, or (K) PARP1 levels in collected xenografts. Results are shown as means $\pm$ S.D. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

PARP1 (Figure 2F) immunoprecipitates, respectively. Collectively, these results demonstrate that miR-223-3p directly interacts with lncRNA MEG3, FOXO1 and PARP1, confirming the existence of a ceRNA regulatory network.

lncRNA MEG3 suppresses glioma cell proliferation, migration, and invasion by sponging miR-223-3p

After confirming the molecular interactions, we evaluated the biological effects of the lncRNA

MEG3/miR-223-3p axis on glioma cell phenotypes. MEG3 overexpression via pcDNA-MEG3 significantly upregulated MEG3 and downregulated miR-223-3p in U87 and U251 (Figure 3A, 3B). Transfection with miR-223-3p mimics effectively elevated the miR-223-3p levels (Figure 3C) and reduced lncRNA MEG3 expression (Figure 3D), further confirming their inverse relationship. Functional assays demonstrated that overexpression of lncRNA MEG3 significantly inhibited colony formation, migration and invasion (Figure 3E-G). The tumor-suppres-

MEG3/miR-223-3p axis drives glioma pyroptosis

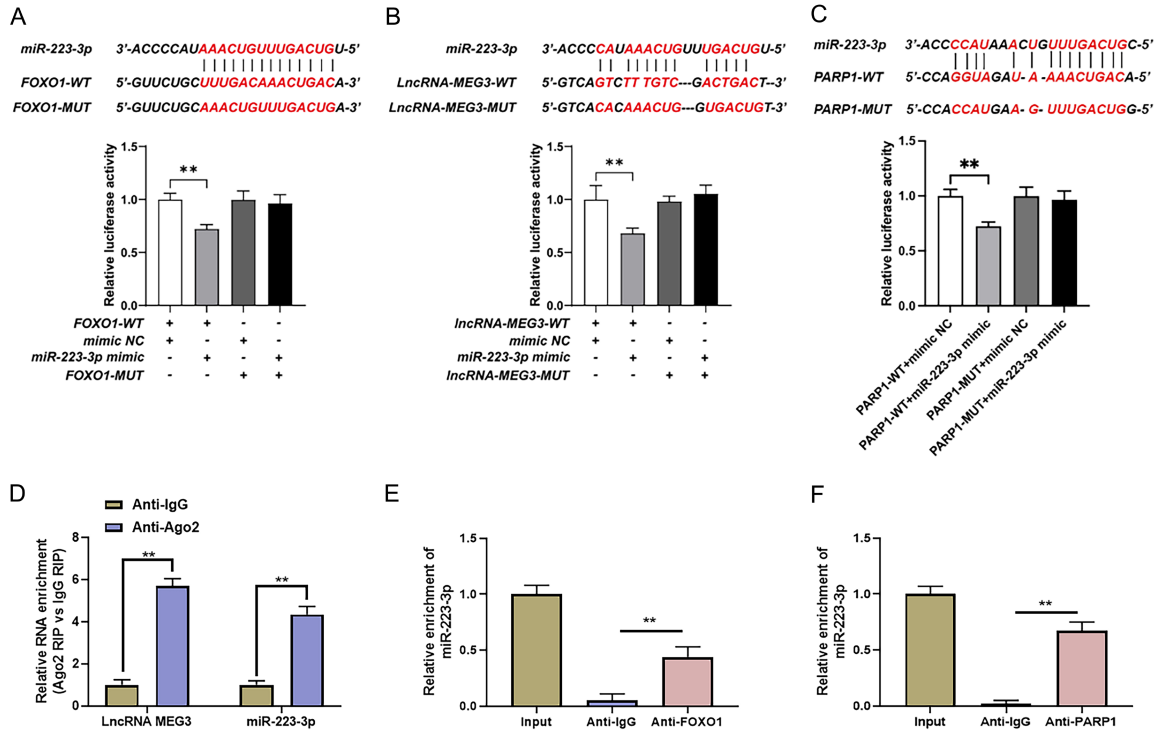


Figure 2. Direct association confirmation of miR-223-3p and lncRNA MEG3, FOXO1 and PARP1. To assess the relative luciferase activity in cells co-transfected with miR-223-3p or NC mimics together with luciferase reporters with either WT or MUT binding sites for (A) FOXO1, (B) lncRNA MEG3, or (C) PARP1. (D) The Ago2-RIP experiment showed that lncRNA MEG3 and miR-223-3p were more enriched in Ago2 immunoprecipitates than in IgG controls. (E, F) RIP experiments showing miR-223-3p accumulated in pulled-down samples by either using anti-(E) FOXO1 or (F) PARP1 antibodies compared to IgG isotypes. Results expressed as mean $\pm$ SD. *** P < 0.001.

sive effects of lncRNA MEG3 overexpression were partially reversed by co-transfection with miR-223-3p mimics (Figure 3E-G). These outcomes collectively underscore the tumor-suppressive role of MEG3 in glioma, which represses malignant advancement via its competitive binding to miR-223-3p.

lncRNA MEG3 promotes pyroptosis in glioma cells via the miR-223-3p pathway

Since tumor suppression is related to cell death, we investigated the modulatory function of the lncRNA MEG3/miR-223-3p axis in pyroptosis in glioma cells. Western blotting was performed to examine pyroptosis-related proteins. Overexpression of lncRNA MEG3 markedly elevated the protein expression of GSDMD-N, mature IL-1 β , and cleaved caspase-1 in both U87 (Figure 4A, 4C) and U251 (Figure 4B, 4D) cells; conversely, the levels of their respective precursors (total GSDMD, pro-IL-1 β , and pro-caspase-1) remained unaltered across the

experimental groups. The activation of pyroptotic factors induced by lncRNA MEG3 was reversed by co-transfection with miR-223-3p mimics. Consistent with the protein expression data, flow cytometry using PI and caspase-1 dual staining revealed a significant increase in the pyroptosis rate in lncRNA MEG3-overexpressing U251 and U87 cells (Figure 4E, 4F), which was reversed by miR-223-3p reintroduction. TUNEL staining further confirmed this trend (Figure 4G, 4H). These results indicate that lncRNA MEG3 promotes pyroptosis in glioma cells by enhancing caspase-1 and IL-1 β cleavage and GSDMD cleavage through the inhibition of miR-223-3p.

FOXO1 and PARP1 are essential downstream effectors of the tumor-suppressive effects of lncRNA MEG3

To determine whether FOXO1 and PARP1 are downstream targets of the lncRNA MEG3/miR-223-3p pathway, shRNAs were selected based

MEG3/miR-223-3p axis drives glioma pyroptosis

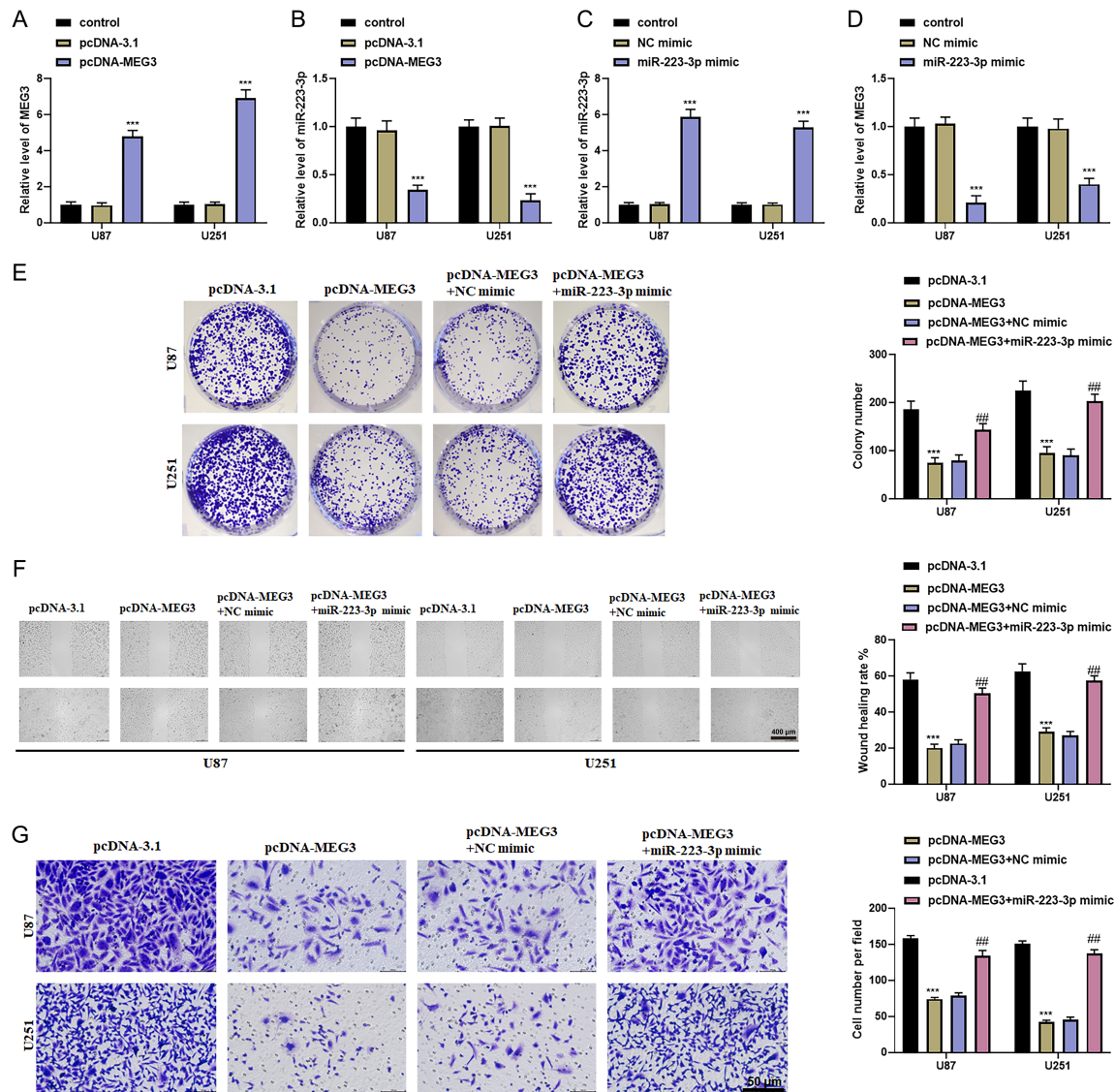


Figure 3. Effects of the lncRNA MEG3/miR-223-3p axis on glioma cell proliferation, migration, and invasion. (A, B) RT-qPCR analysis of (A) MEG3 lncRNA expression and (B) miR-223-3p expression in U87 and U251 cells transfected with pcDNA-3.1 (Control), empty vector alone or pcDNA-MEG3. Real-time quantitative PCR was conducted to detect the expression levels of (C) miR-223-3p and (D) lncRNA MEG3 in cells transfected with non-targeting controls (NC) mimics and miR-223-3p mimics. (E) Typical micrographs and statistical results of the colony formation experiments carried out on U87 and U251 cell lines in each of the following groups: pcDNA-3.1, pcDNA-MEG3, pcDNA-MEG3 + NCmimic, pcDNA-MEG3 + miR-223-3p mimic. (F) Representative images and quantitative analysis of wound healing assays assessing cellular mobility among the designated groups - U87 and U251 lines. Scale: 400 μ m; original magnification $\times 100$. (G) Representative images and quantitative analysis of Transwell invasion assays assessing cell invasiveness in U87 and U251 lines for the specified groups. Scale: 50 μ m; original magnification $\times 400$. Results are expressed as mean $\pm$ SD. * P < 0.05, ** P < 0.01, *** P < 0.001.

on their knockdown efficiency in the U87 and U251 cell lines. FOXO1 shRNA#2 showed potent suppression of FOXO1 mRNA levels (Figure 5A), and effective knockdown of both FOXO1 and PARP1 proteins was confirmed by Western blot in the context of MEG3 overexpression (Figure 5B). Subsequently, rescue

experiments were performed in U251 cells. MEG3 overexpression suppressed cell proliferation, migration, and invasion; however, these tumor-suppressive effects were significantly reversed by knockdown of FOXO1 or PARP1, with the most pronounced reversal observed upon simultaneous depletion of both genes

MEG3/miR-223-3p axis drives glioma pyroptosis

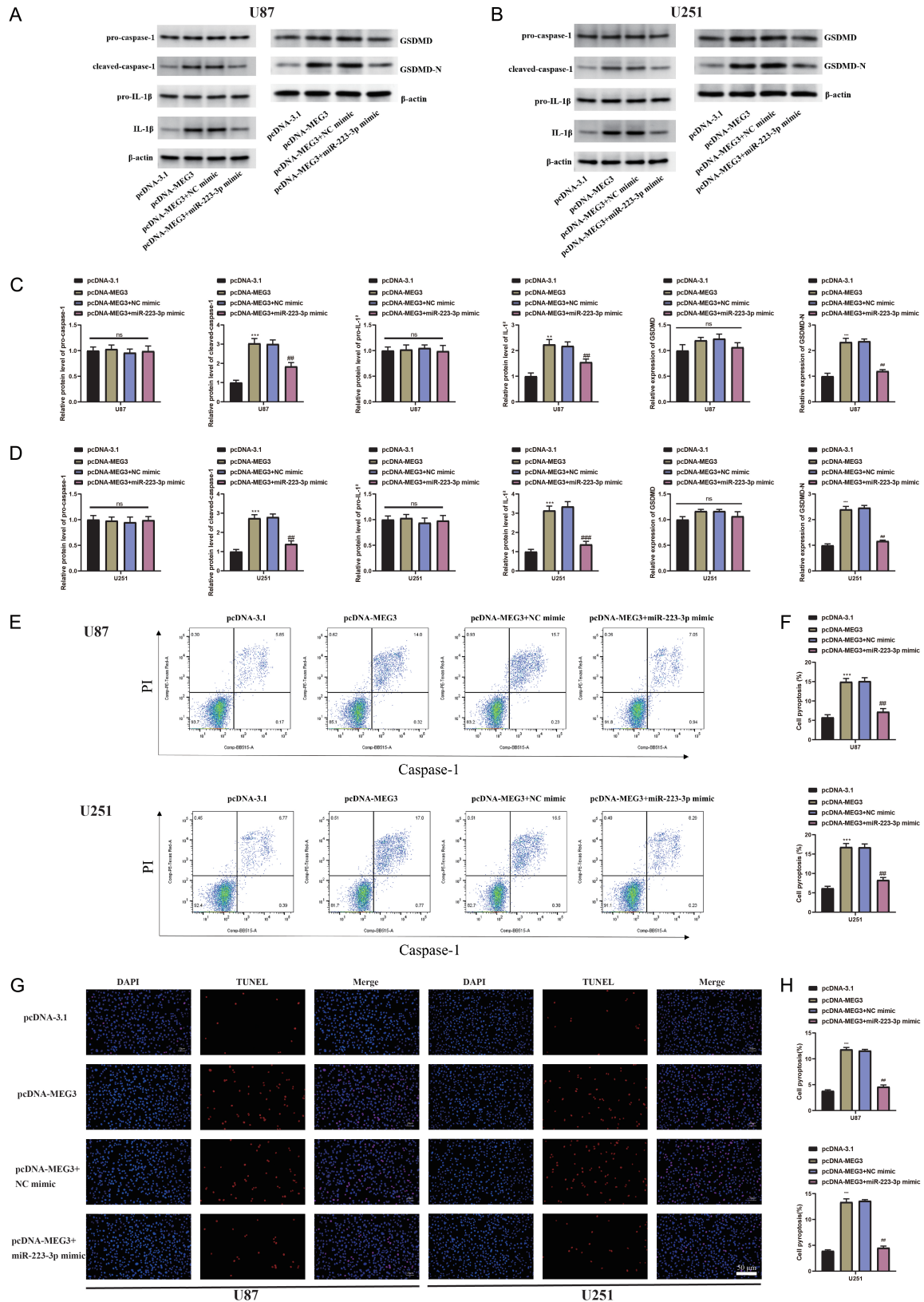


Figure 4. Modulation of pyroptosis by the MEG3/miR-223-3p lncRNA axis in glioma cells. (A-D) Immunoblotting analysis of pyroptosis-related molecules (pro-caspase-1, activated-caspase-1, pro-IL-1 β , IL-1 β , GSDMD, and GSDMD-N) in (A, C) U87 and (B, D) U251 cells transfected with pcDNA-3.1, pcDNA-MEG3, pcDNA-MEG3 + NC mimic, or

pcDNA-MEG3 + miR-223-3p mimic. GAPDH was used as the internal loading control. (E, F) Flow cytometric detection of pyroptosis in U251 and U87 cells stained with PI and anti-caspase-1 reagents. (E) Representative flow cytometry plots. (F) Quantification of the pyroptosis proportion. (G, H) TUNEL staining detection of pyroptosis in U251 and U87 cells. (G) Representative TUNEL staining images. Scale: 50 μ m; original magnification $\times 400$. (H) Quantification of the pyroptosis proportion measured by TUNEL assay. All methods were performed as previously described. Results are expressed as the means $\pm$ SD. $^{**}P < 0.01$, $^{***}P < 0.001$.

(**Figure 5C-E**). Furthermore, flow cytometry and TUNEL staining revealed that lncRNA MEG3-induced pyroptosis was markedly inhibited when FOXO1 and/or PARP1 were silenced (**Figure 5F, 5G**). These findings confirm that FOXO1 and PARP1 are key mediators of the tumor-suppressive and pro-pyrototic functions of lncRNA MEG3 in glioma.

Enhanced expression of lncRNA MEG3 suppresses tumor growth and induces pyroptosis in vivo

Finally, we validated the therapeutic significance of MEG3 in a xenograft mouse model. BALB/c nude mice received subcutaneous injections of U251 cells stably overexpressing MEG3 (oe-MEG3) or empty vector (oe-Vector). Macroscopically, Tumors in the oe-MEG3 group were significantly smaller, with both volume and weight markedly reduced compared to the oe-Vector and Control groups (**Figure 6A**). As demonstrated by H&E staining, tumors in the oe-LncRNA-MEG3 group had reduced cellularity, altered tissue architecture and necrotic features such as nuclear pyknosis and karyorrhexis, and quantitative analysis confirmed a significant decrease in cell density (**Figure 6B**). Western blotting of tumor samples showed elevated protein levels of GSDMD-N, mature IL-1 β , and cleaved caspase-1 in the oe-LncRNA-MEG3 group, whereas the levels of GSDMD, pro-IL-1 β , and pro-caspase-1 remained unchanged, indicating enhanced pyrototic activity (**Figure 6C**). Furthermore, RT-qPCR analysis of the tumor tissues confirmed the effective overexpression of lncRNA MEG3, accompanied by decreased miR-223-3p expression and increased FOXO1 and PARP1 expression (**Figure 6D**). These in vivo findings corroborate our in vitro data, demonstrating that lncRNA MEG3 can suppress glioma growth by modulating the miR-223-3p/FOXO1/PARP1 pathway and inducing pyroptosis.

Discussion

Gliomas remain a challenging clinical target due to their highly invasive nature and intrinsic

resistance to chemotherapeutics [21]. This study reveals a novel regulatory axis composed of lncRNA MEG3, miR-223-3p, FOXO1, and PARP1 that suppresses glioma progression by inducing pyroptosis. A prominent deficit in MEG3 expression was identified across glioma specimens and cell models. Crucially, upregulating this lncRNA was shown to thwart malignant progression both in culture and in animal models through triggering pyroptosis. Our data indicate that MEG3 functions as a ceRNA by sponging miR-223-3p, thereby relieving the suppression of its target genes, FOXO1 and PARP1, which are essential for pyroptosis activation.

Accumulating evidence has demonstrated the tumor-suppressive role of MEG3 across multiple cancer types. Wu *et al.* [22] showed that MEG3 was downregulated in prostate cancer and modulated proliferation, migration, invasion, and apoptosis. Zhang *et al.* [23] demonstrated that MEG3 functions as a ceRNA to sponge miR-23a, thereby regulating APAF-1 expression in laryngeal cancer. Wei *et al.* [24] demonstrated that lncRNA MEG3 restricted the progression of hepatocellular carcinoma by promoting M1 macrophage polarization and modulating immunity via CSF-1 suppression. Moreover, Jia *et al.* [25] demonstrated that MEG3 suppresses glioma cell proliferation and growth, likely through Bcl-xL downregulation in the PI3K/Akt/NF- κ B pathway. Consistent with these findings, MEG3 was markedly downregulated in glioma specimens relative to matched normal tissues. Significantly, this study establishes a precedent, as far as we are aware, by deciphering the regulatory impact of MEG3 on the execution of glioma cell pyroptosis. Pyroptosis exerts dual roles in tumors. While chronic pyroptosis-associated inflammation may promote tumor progression, acute induction of pyroptosis in neoplastic cells typically leads to rapid cell death and suppression of tumor growth [26]. Our data confirmed that overexpression of MEG3 significantly increased the abundance of key pyrototic execution molecules, including cleaved caspase-1, mature

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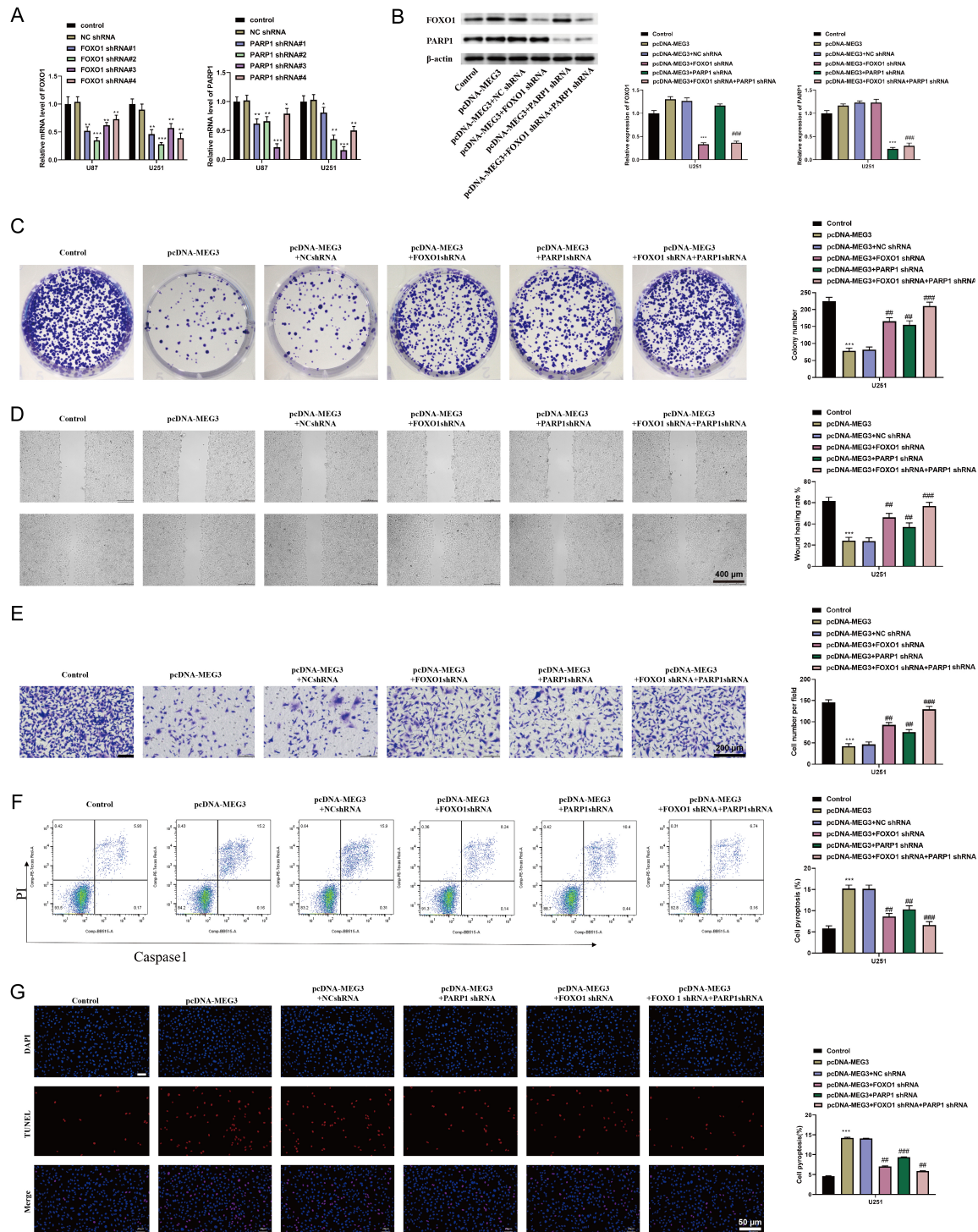


Figure 5. FOXO1 and PARP1 are downstream mediators in the lncRNA MEG3/miR-223-3p regulatory network. **A.** RT-qPCR analysis of FOXO1 mRNA expression levels in U87 and U251 cells transfected with control, NC shRNA, or four different FOXO1-specific shRNAs (#1-#4). **B.** Western blot analysis and quantitative densitometry of FOXO1 and PARP1 protein expression in the indicated groups (Control, pcDNA-MEG3, pcDNA-MEG3 + NC shRNA, pcDNA-MEG3 + FOXO1 shRNA, pcDNA-MEG3 + PARP1 shRNA, and pcDNA-MEG3 + FOXO1 shRNA + PARP1 shRNA). β -actin was used as an internal loading control. **C.** Representative images and quantitative analysis of colony formation assays in U251 cells subjected to the indicated treatments. **D.** Representative bright-field images and quantitative analysis of wound healing assays evaluating cell migration ability in U251 cells from the indicated groups. Scale: 400 μ m; original magnification $\times 100$. **E.** Representative crystal violet-stained images and quantitative analysis of Transwell

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invasion assays assessing cell invasion ability in U251 cells from the indicated groups. Scale: 200 μ m; original magnification $\times 400$. F, G. Flow cytometric analysis of pyroptosis in U251 cells following different treatments using Caspase1/PI double staining. F. Representative flow cytometry dot plots; G. TUNEL staining results and quantitative analysis of the proportion of pyroptotic cells. Scale: 50 μ m; original magnification $\times 400$. Results are shown as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

IL-1 β and the N-terminal cleavage product of GSDMD. Furthermore, both our in vitro and in vivo experimental platforms robustly substantiated the endoproteolytic cleavage of GSDMD into its active N-terminal moiety - a process widely recognized as the quintessential execution event of pyroptosis. In addition, TUNEL staining detected increased DNA fragmentation in MEG3-overexpressing cells, which, together with elevated GSDMD-N cleavage and caspase-1 activation, supports the induction of pyroptosis. These findings indicate that MEG3 upregulation triggers pyroptosis to restrain glioma growth.

The ceRNA hypothesis postulates that lncRNAs competitively bind microRNAs via conserved response elements by sharing miRNA response elements [27]. Accumulating evidence indicates that miR-223-3p expression is subject to regulation by MEG3. MiR-223-3p is commonly upregulated in malignancies and has been associated with enhanced cell survival and chemoresistance. In this study, dual-luciferase reporter assays and Ago2-RIP experiments confirmed that MEG3 directly binds to miR-223-3p. MEG3 prevents miR-223-3p from targeting the 3'UTRs of FOXO1 and PARP1 mRNAs, thereby restoring the expression of FOXO1 and PARP1, which were identified as key functional mediators of the MEG3-driven tumor-suppressive effects. Western blot analysis confirmed that FOXO1 and PARP1 proteins were effectively knocked down under the rescue experimental conditions. Subsequently, rescue experiments demonstrated that knockdown of FOXO1 or PARP1 abrogated both the pro-pyroptotic and anti-proliferative effects of MEG3 overexpression, confirming their essential roles in this process.

FOXO1 is a well-established transcription factor that governs cell cycle arrest, apoptosis, and oxidative stress responses [28]. Accumulating evidence suggests that FOXO1 can transcriptionally transactivate inflammasome components, thereby driving pyroptotic cell death [29]. In our experimental setting, the transcrip-

tional upregulation of the NLRP3 machinery, or related inflammasome elements, is presumably promoted by the MEG3-dependent recovery of FOXO1, a process that subsequently prompts caspase-1 activation. Similarly, PARP1, which is conventionally associated with genome maintenance, has been reported to interact with NLRP3 and facilitate inflammasome assembly under conditions of excessive DNA damage and oxidative stress [30]. The simultaneous upregulation of FOXO1 and PARP1 via the MEG3/miR-223-3p pathway suggests a cooperative mechanism between DNA damage signaling (PARP1) and transcriptional reprogramming (FOXO1) that triggers robust pyroptosis in glioblastoma cells. This dual-target regulatory mode underscores the complexity and robustness of the MEG3-driven tumor-suppressive network.

In vivo data support the clinical potential of targeting this pathway. Xenograft tumors derived from MEG3-overexpressing cells exhibited significantly smaller size and lower weight than those in the control group. Histopathological examination revealed pronounced cellular damage and inflammatory features consistent with pyroptosis in the MEG3-overexpressing group, and quantification of H&E sections revealed a significant drop in tumor cell density. Furthermore, the elevated expression of pyroptosis-related markers in these tumors validated that the mechanism identified in vitro operates effectively in a physiologically relevant context. These observations provide a rationale for developing strategies that induce pyroptosis in treatment-refractory malignant cells.

Despite these encouraging results, several limitations of our investigation should be acknowledged. First, although we focused on the MEG3/miR-223-3p/FOXO1-PARP1 axis, glioma pathogenesis is highly complex, and other miRNAs or proteins may also participate in MEG3-mediated functions. Second, the precise upstream mechanisms responsible for MEG3 silencing in glioma, such as promoter methylation or histone modifications, were not investi-

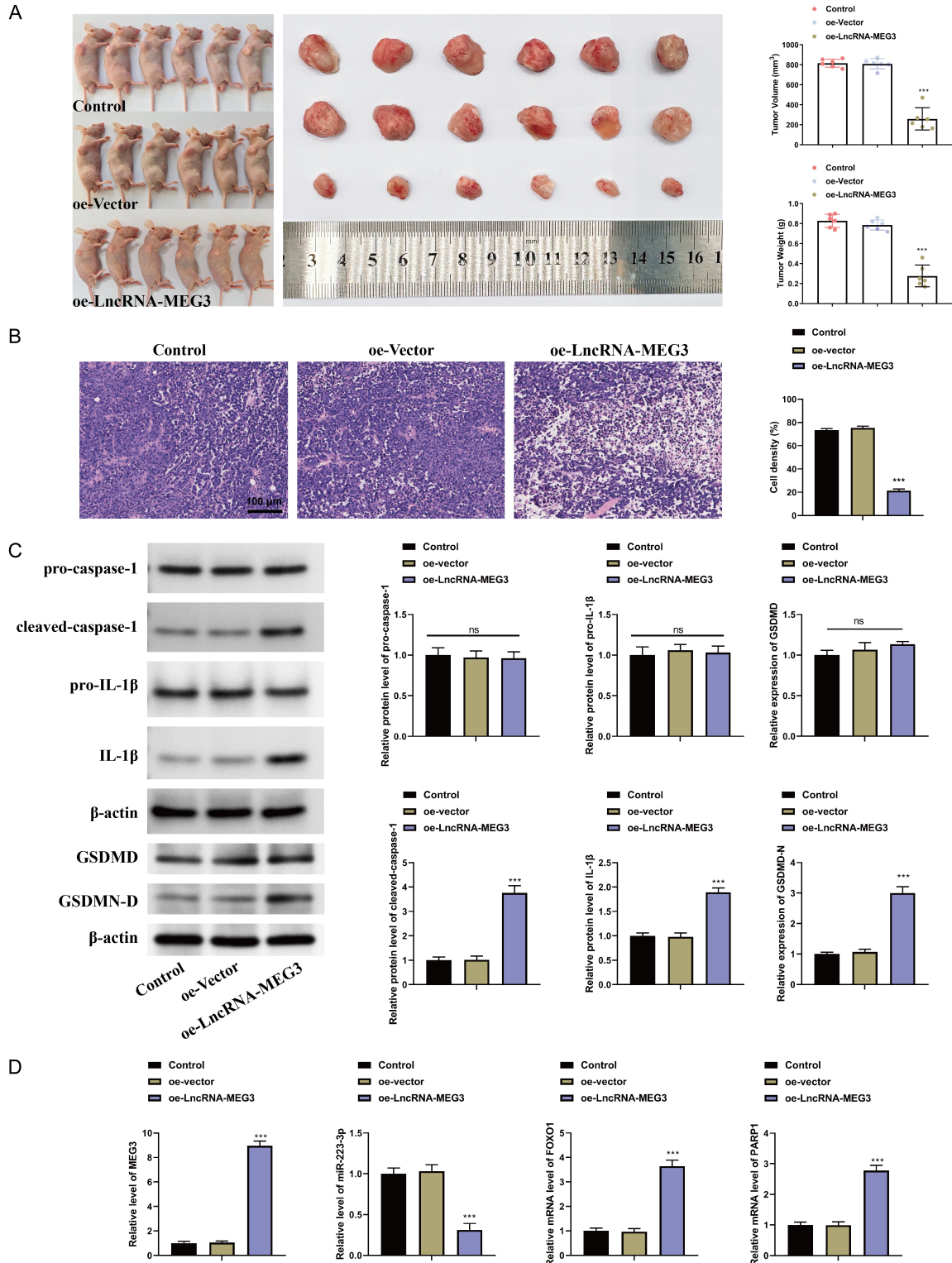


Figure 6. In vivo effects of lncRNA MEG3 overexpression on glioma tumor growth and pyroptosis. A. Representative images of nude mice, excised tumor tissues (n=6 per group), and quantitative analysis of tumor volume and weight in the Control, oe-Vector, and oe-LncRNA-MEG3 groups. B. Representative images of H&E-stained tumor tissues showing histological morphology and cell density, with quantitative analysis of cell density in each group. Scale: 100 µm; original magnification ×200. C. Western blot analysis of pyroptosis-related proteins (pro-caspase-1, cleaved-caspase-1, pro-IL-1β, IL-1β, GSDMD, and GSDMD-N) in tumor tissues from each group, with β-actin as the internal

reference. The corresponding quantitative densitometric analysis results are shown below. D. RT-qPCR detection of the transcript levels of lncRNA MEG3, miR-223-3p, FOXO1, and PARP1 in tumor tissues from the indicated groups. Results are shown as the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

gated in this study and warrant further exploration. Third, although MEG3 restoration showed promise in a xenograft model, translating these findings to clinical applications will require safe and efficient delivery systems for lncRNAs, e.g., nanoparticles or exosomes. Finally, the impact of MEG3-induced pyroptosis on the tumor immune microenvironment, including the recruitment and activation of immune cells, remains to be elucidated. Given the inflammatory nature of pyroptosis, MEG3 upregulation may enhance anti-tumor immunity, a hypothesis that requires further validation in immunocompetent animal models.

In summary, the present work delineates a novel regulatory cascade governing the anti-tumorigenic properties of lncRNA MEG3 in glioma. Through the targeted decoy of miR-223-3p, MEG3 sustains the expression of FOXO1 and PARP1, a network that ultimately prompts pyroptosis induction to curtail tumor growth. These findings deepen our insight into the molecular mechanisms underlying glioma pathogenesis and indicate that the MEG3/miR-223-3p/FOXO1-PARP1 axis holds promise as a candidate therapeutic target. Future translational studies focused on MEG3-targeted therapies, alone or in combination with existing treatments, may provide new hope for patients afflicted with this devastating disease.

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Disclosure of conflict of interest

None.

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MEG3/miR-223-3p axis drives glioma pyroptosis

Table S1. The shRNA sequences and vector sequences

	Forward (5'-3')	Reverse (5'-3')
sh MEG3	GGGUUGUUGUGAGAAUU	UUAAUUCUCACAACAACCC TA
sh POX01	CAGGUGGAGGUUGGUUUUGUATT	UACAAAACCAACCUCCACCUGTT
sh PARP1	ACGGUGAUCGGUAGCAACAAATT	UUUGUUGCUACCGAUCACCGUTT

Table S2. Sequences of the primers for RT-qPCR

	Forward (5'-3')	Reverse (5'-3')
H-miR-223-3p	ACACTCCAGCTGGGTGTCAGTTTGTCAAAT	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTTGAGTGGGGTAT
M-miR-223-3p	GTGCAGGGTCCGAGGT	CGGGCTGTCAGTTTGTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
MEG3	GCATTAAGCCCTGACCTTTG	TCCAGTTTGCTAGCAGGTGA
GAPDH	GAGTCAACGGATTGGTCGT	GACAAGCTTCCCGTTCTCAG
FOXO1	AGATGAGTGCCCTGGGCAGC	GATGGACTCCATGTCACAGT
PARP1	TCGGGTCTCCCTGAGATGTG	GGAAAGGGATCTACTTTGCCG