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Short communication

MEOX2 promotes glioma growth and temozolomide chemoresistance☆

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Glioblastoma (GBM) is a primary and fatal subtype of adult brain tumors. Despite standard treatments, including surgical resection and temozolomide (TMZ) chemotherapy, overall survival is only 16 months [1]. Profound genomic heterogeneity and altered transcriptional profiles drive chemoresistance, leading to tumor recurrence and a poor prognosis. Gain of chromosome 7 is a pivotal event in initiation and recurrence [2]. Our previous studies highlighted that engrailed 2 (*EN2*), a homeobox transcription factor at 7q36.3, is negatively correlated with glioma malignancy [3]. This led us to explore additional genes associated with GBM chemoresistance. At the 7p21.2 locus, mesenchyme homeobox 2 (*MEOX2*), another homeobox factor, has emerged as a candidate gene. Recent studies have yielded conflicting results regarding the influence of *MEOX2* on glioma, underscoring the need to understand its intricate expression patterns and biological functions in GBM [4,5]. In this study, we comprehensively investigated the expression patterns and functions of *MEOX2* in gliomas, particularly in terms of chemoresistance. Our findings reveal a positive correlation between *MEOX2* expression and glioma malignancy. Increased *MEOX2* expression is concomitant with accelerated glioma growth and decreased sensitivity to TMZ chemotherapy. Mechanistically, *MEOX2* exerts oncogenic effects by modulating protein kinase B (AKT) activity, thereby orchestrating glioma chemoresistance.

To evaluate the clinical significance of *MEOX2* in gliomas, we analyzed its expression profile in The Cancer Genome Atlas (TCGA) glioma dataset. All the *in silico* and following experiment methods are shown in the Supplementary data. The results showed that *MEOX2* expression increased in parallel with World Health Organization (WHO) grades and histological features (Figs. 1A, S1A, and S1B). Elevated *MEOX2* expression was relevant to crucial molecular biomarkers, including wild-type isocitrate dehydrogenase 1 (*IDH1*), 1p/19q non-co-deletion, and methylguanine DNA methyltransferase (*MGMT*) promoter unmethylation, which are associated with high malignancy and poor prognosis (Fig. 1A). *MEOX2* was significantly upregulated in GBM (WHO grade IV) in TCGA cohort (Fig. S1C). Moreover, *MEOX2* expression was upregulated in wild-type *IDH* gliomas (Fig. S1D). Glioma patients with high *MEOX2* expression exhibited significantly shorter overall survival (OS) than those with low *MEOX2* expression ($P < 0.0001$) (Figs. 1B, S2A, and S2B). To validate the correlation between *MEOX2* and glioma malignancy, we analyzed *MEOX2* expression in the Chinese Glioma Genome Atlas (CGGA) dataset. Similarly, the expression pattern of *MEOX2* in CGGA was consistent with that in TCGA (Figs. S2C and D). We then examined the *MEOX2* expression profiles in the glioma cohort of our institution and found that the expression levels of *MEOX2* were higher in tumor tissues than in paired adjacent tissues (Figs. 1C–E, S3A, and S3B), suggesting that *MEOX2* expression is a predictive biomarker of glioma (area under the curve (AUC) = 0.840) (Fig. S3C).

We investigated whether changes in *MEOX2* expression affect the chemoresistance of glioma cells. Using a lentivirus-based strategy to manipulate *MEOX2* expression in glioma cell lines (Figs. S4A–C), we found that *MEOX2* overexpression (OE) increased cell growth, whereas *MEOX2* knockdown (KD) decreased the growth of glioma cells (Figs. 1F, 1G, and S4D–G). Wound healing and Transwell assays further showed that *MEOX2* enhanced the migration/invasion of glioma cells (Fig. S5). Next, we examined whether *MEOX2* regulated glioma growth *in vivo*, by transplanting *MEOX2*-OE and KD cells into the cortex of immunocompromised nude mice. As anticipated, *MEOX2* OE significantly increased tumor progression, as indicated by the maximal coronal sectional area of

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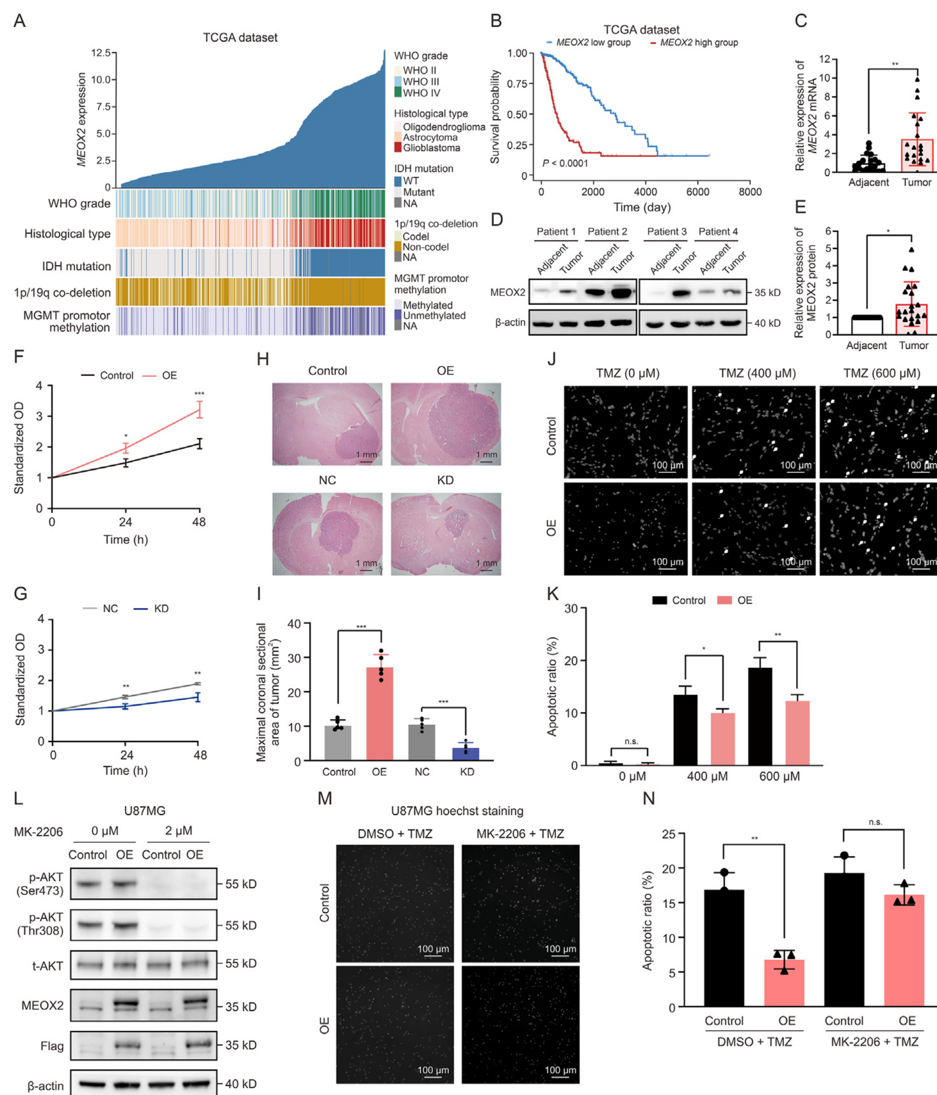

Fig. 1. Mesenchyme homeobox 2 (*MEOX2*) is positively associated with glioma malignancy, and regulates temozolomide (TMZ) chemoresistance. (A) Correlation between *MEOX2* expression and glioma histological features and prognostic biomarkers in The Cancer Genome Atlas (TCGA) dataset ($n = 681$). (B) Kaplan-Meier survival analysis using clinical information from the TCGA dataset. Patients are divided into low and high *MEOX2* groups by median expression level ($n = 681$). (C) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of messenger RNA (mRNA) expression in paired glioblastoma (GBM) tissues. Results are shown as mean ± standard deviation (SD) ($n = 20$). (D) Western blots of representative 4 pairs of GBM and adjacent tissues. (E) Quantifications of MEOX2 expression in 21 pairs of GBM and peritumor tissues (mean ± SD). (F, G) Cell Counting Kit-8 (CCK-8) assay showing that *MEOX2* overexpression (OE) increased cell numbers compared with control (F), and *MEOX2* knockdown (KD) decreased cell numbers in KNS89 cells (G) ($n = 3$). (H, I) Hematoxylin and eosin (H&E) staining of intracranial xenograft tumor in nude mice with KNS89 cells (H) and the maximal coronal sectional area of tumors (I) ($n = 5$ per group). (J, K) Image (J) and quantification (K) of Hoechst staining showing that *MEOX2* OE decreased TMZ-induced apoptosis of KNS89 cells (400 or 600 μM, 48 h) ($n = 3$). (L) Western blots indicated that phosphorylation of protein kinase B (AKT) at both Ser473 and Thr308 were inhibited in *MEOX2* OE/control cells by 2 μM MK-2206 in U87MG cells. (M, N) Hoechst staining (M) and quantification (N) of *MEOX2* OE/control cells treated by combination of TMZ and vehicle (dimethyl sulfoxide (DMSO)) or TMZ and an AKT inhibitor MK-2206 showed that TMZ resistance effect of *MEOX2* OE was antagonized by MK-2206 ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.0001$, based on paired Student's *t*-test. n.s.: not significant. WHO: World Health Organization; IDH: isocitrate dehydrogenase; MGMT: methylguanine DNA methyltransferase; WT: wildtype; NA: not available; NC: negative control; p-AKT: phospho-AKT; t-AKT: total AKT.

the tumor and the Ki67 index. Reciprocally, *MEOX2* KD decreased the growth of glioma xenografts (Figs. 1H, 1I, and S6).

We determined whether *MEOX2* was involved in cell death/survival under basal and chemotherapeutic conditions. *MEOX2* OE decreased the apoptotic rate of glioma cells by TMZ treatment (400 and 600 μM) indicated by Hoechst staining. Conversely, *MEOX2* KD increased the tendency of TMZ-induced cell death (particularly by 600 μM TMZ) (Figs. 1J, K, and S7).

Next, we investigated the molecular mechanisms by which *MEOX2* promotes glioma malignancy using transcriptomics of glioma cells infected with lentiviruses expressing either *MEOX2* OE or KD. The results showed that AKT signaling was tightly regulated by *MEOX2* (Fig. S8). Ectopic activation of AKT signaling occurs in various cancer types, including gliomas, and confers tumor growth and chemoresistance. Therefore, we treated *MEOX2* OE/control cells with the AKT inhibitor, MK-2206, whereas *MEOX2* KD/negative control (NC) cells were treated with the AKT agonist, SC-79. Results indicated that phosphorylation of AKT kinase at both Ser473 and Thr308 was inhibited in *MEOX2* OE/control cells by MK-2206 (2 μM) and overactivated in *MEOX2* KD/NC cells by SC-79 (5 μM) in U87MG and KNS89 cells, respectively (Figs. 1L and S9). MK-2206 abolished the proliferation, migration, and invasion of *MEOX2* OE cells,

whereas SC-79 restored the reduced proliferation, migration, and invasion of *MEOX2* KD cells (Fig. S10). Similar effects were observed in KNS89 cells (Fig. S11). Hoechst staining showed that the TMZ resistance effect of *MEOX2* OE was reversed by MK-2206 (Figs. 1M, 1N, and S12), whereas the pro-apoptotic effect of *MEOX2* KD was counteracted by SC-79 (Figs. S12 and S13). These results indicated that *MEOX2* enhanced glioma resistance to TMZ by activating AKT signaling.

Despite fundamental and clinical advancements in glioma treatment, glioma chemotherapy has improved slightly over the past few decades. One of the leading reasons for this is molecular heterogeneity within tumors, which contributes to chemoresistance in gliomas. Therefore, the development of precise therapeutic approaches to overcome chemotherapeutic resistance remains a pressing question. In this study, we used bioinformatics and experimental approaches to demonstrate that *MEOX2* is a protumor gene that enhances glioma growth and chemoresistance. We propose that *MEOX2* is linked to tumor malignancy and acts as a novel prognostic predictor in glioma and that *MEOX2* may promote glioma growth and TMZ resistance.

In conclusion, our study demonstrates a strong association between *MEOX2* and glioma malignancy, indicating that *MEOX2* positively regulates glioma growth and chemosensitivity through AKT signaling. Our findings not only provide novel insights into the functional roles of *MEOX2* in glioma but also offer a promising target for future chemotherapeutic strategies.

Ethics statement

This work was approved by the Institutional Review Board of West China Hospital of Sichuan University, China. All patients provided written informed consent. The animal work was done in accordance with the Animal Care and Use Committee guidelines of West China Hospital, Sichuan University, China (Approval No.: 2021786-A).

CRediT author statement

Tengfei Li: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Funding acquisition, Writing - Original draft preparation; **Kaijun Sun** and **Wanchun Yang:** Data curation, Formal analysis, Investigation; **Meiling Zhang:** Formal analysis; **Wentao Feng**, **Siliang Chen**, **Mingrong Zuo**, and **Qiuyun Yuan:** Data curation; **Yanhui Liu** and **Mina Chen:** Conceptualization, Funding acquisition, Writing - Reviewing and Editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.12.002.

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