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Long noncoding RNA just proximal to X-inactive specific transcript facilitates aerobic glycolysis and temozolomide chemoresistance by promoting stability of PDK1 mRNA in an m6A-dependent manner in glioblastoma multiforme cells

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Abstract

Improving the chemotherapy resistance of temozolomide (TMZ) is of great significance in the treatment of glioblastoma multiforme (GBM). Long non-coding RNA just proximal to the X-inactive specific transcript (JPX) has been proven to be involved in cancer progression. However, the intrinsic significance and molecular mechanism by which JPX orchestrates GBM progression and TMZ chemotherapy resistance remain poorly understood. Here, JPX was found to be significantly elevated in GBM tissues and cell lines, and patients with high expressions of JPX showed significantly worse prognoses. Functional experiments revealed its carcinogenic roles in GBM cell proliferation, TMZ chemoresistance, anti-apoptosis, DNA damage repair, and aerobic glycolysis. Mechanistically, JPX formed a complex with phosphoinositide dependent kinase-1 (PDK1) messenger RNA (mRNA) and promoted its stability and expression. Furthermore, an RNA immunoprecipitation (RIP) experiment showed that JPX interacted with N6-methyladenosine (m6A) demethylase FTO alpha-ketoglutarate dependent dioxygenase (FTO) and enhanced FTO-mediated PDK1 mRNA demethylation. JPX exerted its GBM-promotion effects through the FTO/PDK1 axis. Taken together, these findings reveal the key role of JPX in promoting GBM aerobic glycolysis and TMZ chemoresistance in an m6A-dependent manner. Thus, it comprises a promising novel therapeutic target for GBM chemotherapy.

KEYWORDS
aerobic glycolysis, glioblastoma, just proximal to X-inactive, m6A methylation modification, TMZ chemoresistance

1 | INTRODUCTION

Glioblastoma multiforme (GBM) is a heterogeneous brain malignancy that has an extremely poor prognosis; its survival time is only 8-15 months.[1] The etiology of GBM is very complex and it is considered one of the most difficult tumors to cure. Temozolomide (TMZ), an oral alkylating agent, remains the first-line therapeutic drug for GBM and astrocytoma, especially for advanced or unresectable GBM. However, treatments with TMZ usually fail due to inherent or acquired TMZ resistance.[2] Therefore, elucidating the molecular mechanism of drug resistance in TMZ chemotherapy can provide potential targets and improvement measures for GBM.

Long non-coding RNAs (lncRNAs) represent a novel spectrum of RNAs that are greater than 200 nucleotides in length.[3] Recently, a plethora of lncRNAs have been screened that participate in the epigenetic signatures of many malignancies.[4] It has been suggested that lncRNAs could function as tumor oncogenes or tumor suppressors due to their ability to account for the modulation of key genes involved in cancer initiation or progression, as well as in chemotherapy resistance.[5] For instance, the lncRNA HOXB-AS3 promotes acute myeloid leukemia abnormal differentiation and progression.[6] Additionally, myocardial infarction related transcript (MIAT) promotes the progression of leukemia through miR-495.[7] Similarly, lncRNA growth arrest-specific transcript 5 (GAS5) facilitates lymphocytic leukemia tumorigenesis and metastasis through miR-222.[8] Recently, JPX has been reported to facilitate tumorigenesis and chemotherapy resistance by regulating mitogen-activated protein kinase (MAPK) pathways.[9] However, the intrinsic key role and/or underlying mechanisms of JPX in GBM remain elusive.

One remarkable feature of cancer cells is that, even under aerobic conditions, they give priority to community glucose and convert it into lactic acid; this is known as the Wahlberg effect.[10] This effect occurs because obtaining sufficient amounts of nucleotides, proteins, and lipids through enhanced aerobic glycolysis is conducive to the rapid proliferation and infinite division of cancer cells.[10] The cause of the Warburg effect is not clear, but it is generally considered a complex process involving the up- or downregulation of many rate-limiting enzymes.[11] Therefore, targeting the Warburg effect has become an important development direction in antitumor therapy, such as hypoxia inducible factor 1 (HIF-1).[12] However, the mechanisms underlying the initiation and promotion of the Warburg effect in GBM are yet to be elucidated.

RNA modification is an important part of epigenetic regulation. As one of the most conservative RNA modifications, the N6-methyladenosine (M6A) modification is the methylation of messenger RNA (mRNA) N-6 adenosine base. The M6A modification is widespread in mammalian transcript modification.[13] Unlike other mRNA modifications, but similarly to DNA and histone modifications, the M6A modification is reversible, and its process plays a key function in modulating the maturation, stability, and translation of precursor mRNA.[14] The process of M6A modification is mostly manipulated by methyltransferases (including METTL3, METTL14 and WTAP) and demethylases (including FTO and ALKBH5).[14] There is increasing evidence that M6A gene modification and its subsequent epigenetic changes are linked to the occurrence, progression, and therapy resistance of malignant tumors.[15] For instance, targeting YTHDF2, which is a RNA M6A reader, has been shown to mitigate the progression of cancer stem cells in AML.[16] Moreover, METTL14 facilitates leukemia genesis via the M6A modification of HIF-1.[17] However, the role and underlying mechanisms for M6A modification in GBM remain unknown.

The current study found that JPX was significantly elevated in GBM cells. Functional experiments confirmed its oncogenic role in GBM, including proliferation, TMZ chemoresistance, anti-apoptosis, DNA damage repair, and aerobic glycolysis. Mechanistically, JPX interacted with phosphoinositide dependent kinase-1 (PDK1) mRNA and promoted its stability. A RNA immunoprecipitation (RIP) showed that JPX interacted with FTO and enhanced FTO-mediated PDK1 m6 modulation. JPX exerted its GBM-promotion effects through FTO/PDK1. This study not only identified a new target for JPX, but also shed light on the modulation of glucose metabolism and TMZ chemoresistance in GBM.

2 | MATERIALS AND METHODS

2.1 | Cell culture

The human glioblastoma cell lines U251, LN229, U87, SHG-44, and LN18 were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). Cell lines were cultivated in RPMI-1640 medium (Gibco) with 10% fetal bovine serum (FBS) (Gibco) and 100 U/ml penicillin at 37°C in a humidified incubator of 5% CO_2.

2.2 | Construction and transfection of plasmids

JPX siRNA (si-JPX) and negative control siRNA (si-NC) were synthesized from Genepharma. The pcDNA3.1-PDK1 (PDK1) and pcDNA3.1-control (vector) were obtained from GeneCopoeia. Cell transfection was conducted with Lipofectamine 2000 (Invitrogen). All operations are carried out in accordance with the manufacturer's instructions.

2.3 | Quantitative real-time PCR

Total RNAs were isolated from cultured cells with TRIzol reagent (Invitrogen). Then, the cDNA was synthesized and real-time PCR were conducted using the SYBR Green qPCR Master Mix (Thermo Scientific). The used primers were provided as follows: JPX, 5′-TGCAGTCAGAAGGGAGCAAT-3′ (forward), 5′-CACCGTCATCAGGCTGTCTT-3′ (reverse); GAPDH, 5′-ATGATGACATCAAGAAGGTGGTG-3′ (forward), 5′-CCATGAGGTCCACCACCCTGTTG-3′ (reverse), PDK1, 5′-CTGTGATACGGATCAGAAACCG-3′ (forward),

5′-TCCACCAAACAATAAAGAGTGCT-3′ (reverse). GAPDH was employed as an endogenous control.

2.4 | CCK-8 assay

A CCK-8 assay (Beyotime) was performed to access the cell proliferation curves of GBM cells. The cells (3000 cells per well) were harvested into 96-well plates, which were then incubated at 37°C in a humidified incubator of 5% CO_2 for 1-5 days. At every fixed point in time, the incubated cells were treated with CCK-8 solution (10 ml/well) for 2 h. The detection of absorbance was performed with an OD value at 450 nm. Three independent experiments were carried out to detect cell proliferation.

2.5 | Apoptosis assay

An apoptosis staining kit was used (BD Biosciences), following which tumor cell apoptosis was detected by flow cytometry. First, the tumor cells and supernatants were collected, following which they were centrifuged at 250 g for 5 minutes. The cells were then washed via PBS, and the washed cells were placed in a binding buffer and mixed with AnnexinV-PE and 7AAD. Finally, the tumor cells were transferred to the flow tube and incubated at room temperature, in the dark, for 20 minutes. In the analysis stage, flow cytometry (FC500MPL, Beckman Coulter) was used for analysis.

2.6 | Western blot assay

After the tumor cells were lysed, they were treated with RIPA buffer and the protein level was calculated using a GBMA analysis kit (Pierce). The cell lysate was separated by a SDS-PAGE step, following which the electrophoretic product was transferred to the PVDF membrane. The PVDF membrane was then sealed in the buffer with the first antibody, following which it was incubated for 2 h. The PVDF membrane was incubated with the second antibody solution overnight and finally the protein expression was detected using an EasyBlot ECL kit (Songjiang Sangong). Antibodies to PDK1 and β-actin were provided by ABCAM (Pudong).

2.7 | Subcellular fractionation

The transfected U251 and LN229 cells were washed in PBS, and then placed on ice for 10 minutes with 500 μL of buffer (10 mM Hepes, 5 mM EDTA, 10 mM NaCl, 0.5 mM $MgCl_2$, 1 mM $KH_2 PO_4$, 5 mM $NaHCO_3$, and 1 mM $CaCl_2$). Then, 50 μL of 2.5 M sucrose was added. The mixture was centrifuged at 6300 g at 4°C for 5 minutes and then centrifuged with TSE buffer at 4°C at 1000 g for 5 minutes. Thus, the supernatant was obtained. After transferring the supernatant, the supernatant was centrifuged for 30 minutes at 21900 g.

2.8 | Glucose uptake, lactate, and ATP assay

The detected cells were inoculated to a 96-well plate, and the cell culture medium and cell supernatant were collected to determine glucose consumption and lactic acid production. Glucose uptake level was determined using a glucose uptake analysis kit (BioVision). Lactic acid production was determined using a lactic acid determination kit II (BioVision). The amount of ATP that was generated was determined using an ATP colorimetric analysis kit (BioVision). All operations were carried out in accordance with the manufacturer's instructions.

2.9 | Extracellular acidification rate and oxygen consumption rate assays

Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using a SeaHorseXFe96 extracellular flux analyzer (Sea Horse Bioscience). A hippocampal XF glycolysis stress test kit was used to determine the ECAR and a hippocampal XF cell Mito stress test kit was used to determine the OCR. All operations were carried out in accordance with the manufacturer's instructions. First, 2×10^4 tumor cells were inoculated into the cell culture plate. After the completion of the baseline assessment, glucose, oligomycin (inhibitor of oxidative phosphorylation), and 2-DG (inhibitor of glycolysis) were added at the recommended and established time points for ECAR detection. During the detection of OCR, FCCP, oligomycin (a reversible inhibitor of oxidative phosphorylation), rotenone (an inhibitor of mitochondrial complex), and Antimycin A (RoTE/AA; an inhibitor of mitochondrial complex III), were added at the recommended and established time points. All the data were analyzed using hippocampal XF-96Wave software. In terms of the display units used, the OCR was expressed in PMOS/min and ECAR was expressed in MPH/min.

2.10 | RNA immunoprecipitation and methylated RIP assays

RNA immunoprecipitation (RIP) was detected using a Magna RNA binding protein immunoprecipitation kit (RIP, Merck MilliPore) and methylated RIP (MeRIP) was detected using a Magna methylation RNA immunoprecipitation kit M6A kit (MERIP, Merck MilliPore). All experimental operations were carried out in accordance with the manufacturer's instructions.

2.11 | Luciferase reporter assay

To construct a luciferase reporter gene system, the pGL3 plasmid was used as a vector. The PDK1 promoter region was cloned into the pGL3 blank plasmid by molecular cloning. The luciferase activity was then determined through a double luciferase report analysis

system after 48 hours. Subsequently, the knockdown plasmid of JPX was transfected at the same time, and its effect on the activity of luciferase activity was observed.

2.12 | Statistical analysis

Three independent experiments were carried out. Statistical analyses were conducted using GraphPad 8.0 (GraphPad, Inc); they are expressed here as mean ± SD. Significant differences were determined by a two-tailed Student's t test or one-way analysis of variance (ANOVA), where $P < .05$ was considered to be statistically significant.

3 | RESULTS

3.1 | lncRNA JPX is overexpressed in GBM and is tightly associated with survival rates of patients with GBM

Data from the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/index.html) revealed that JPX was markedly upregulated in Glioblastoma (GBM) tissues compared to normal tissue (Figure 1A). The expressions of just proximal to X-inactive (JPX) in four different GBM cell lines and normal astrocyte NHA cells were explored with quantitative real-time PCR (qRT-PCR), revealing that JPX was markedly elevated in GBM cells compared to in normal cells (Figure 1B). Moreover, publicly available data from the GEPIA database were used to explore the relationship between the

JPX level and the Overall Survival (OS) of GBM patients; the results indicated that higher JPX levels were closely associated with poor OS, highlighting the significance of JPX in monitoring the prognosis of GBM (Figure 1C).

3.2 | JPX promotes tumor progression and TMZ chemoresistance in GBM cells

JPX-depleted GBM cells were established to identify the biological function of JPX (Figure 2A). The CCK-8 assay results showed that JPX knockdown attenuated the cell growth of GBM cells (Figure 2B,C). Moreover, relatively low IC50 values were observed in JPX-depleted GBM cells compared to si-NC (Figure 2D,E). Apoptosis analysis revealed that JPX-depleted GBM cells underwent more apoptosis than si-NC (Figure 2F). Furthermore, in JPX-depleted groups, EdU-positive cells decreased significantly compared to those in si-NC (Figure 2G). Comet electrophoresis revealed that cells with obvious DNA damage were also higher in JPX-depleted groups than that of si-NC (Figure 2H). Collectively, these data indicate that JPX knockdown enhanced TMZ sensitivity, apoptosis, and DNA damage. Furthermore, they suggest that it inhibited proliferation and growth in GBM cells.

3.3 | JPX knockdown dampens aerobic glycolysis in GBM cells

Initially, JPX knockdown significantly reduced the levels of glucose uptake (Figure 3A), lactic acid production (Figure 3B), and ATP generation in GBM cells (Figure 3C). In addition, JPX knockdown exerted
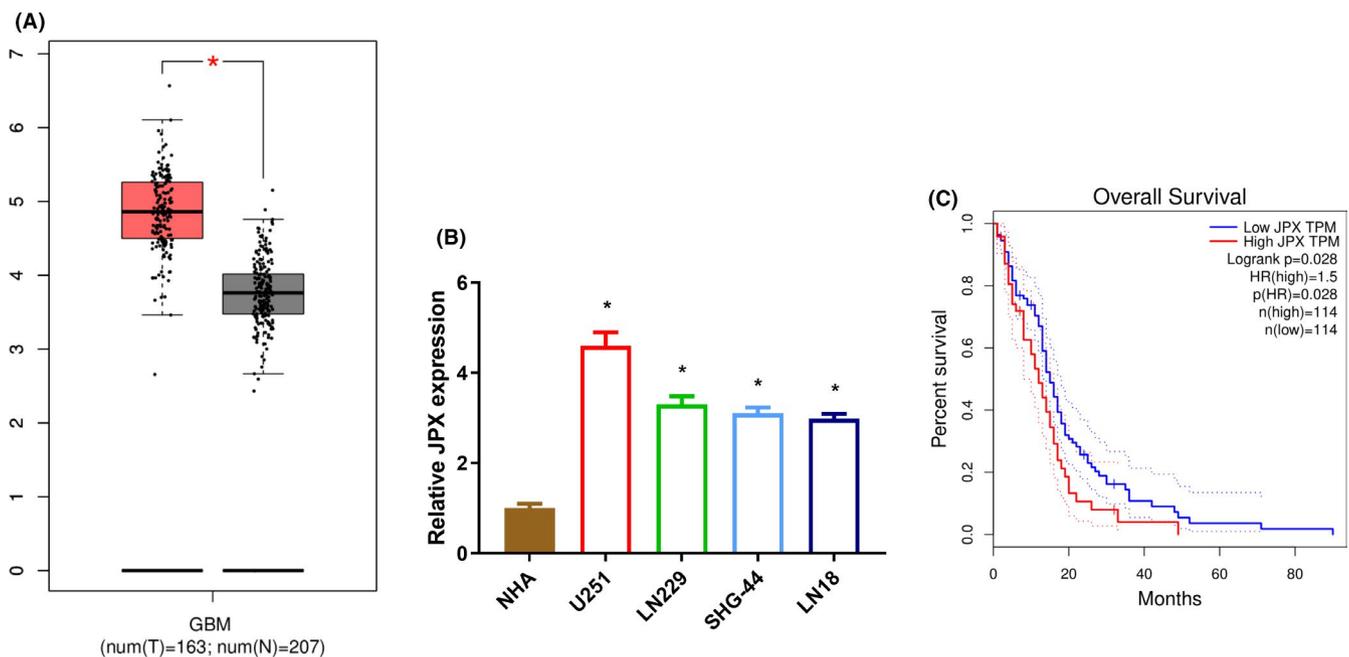

FIGURE 1 LncRNA JPX is overexpressed in GBM and tightly associated with survival rates of patients with GBM. A, Relative expression of JPX in GBM and normal tissues, as analyzed from GEPIA database. B, Expression of JPX in GBM cell lines and normal NHA cell lines, as determined by qRT-PCR. C, Analysis of the correlation between JPX level and overall survival of GBM patients from GEPIA database, using the Kaplan-Meier plotter method; *P <.05 compared with the negative control
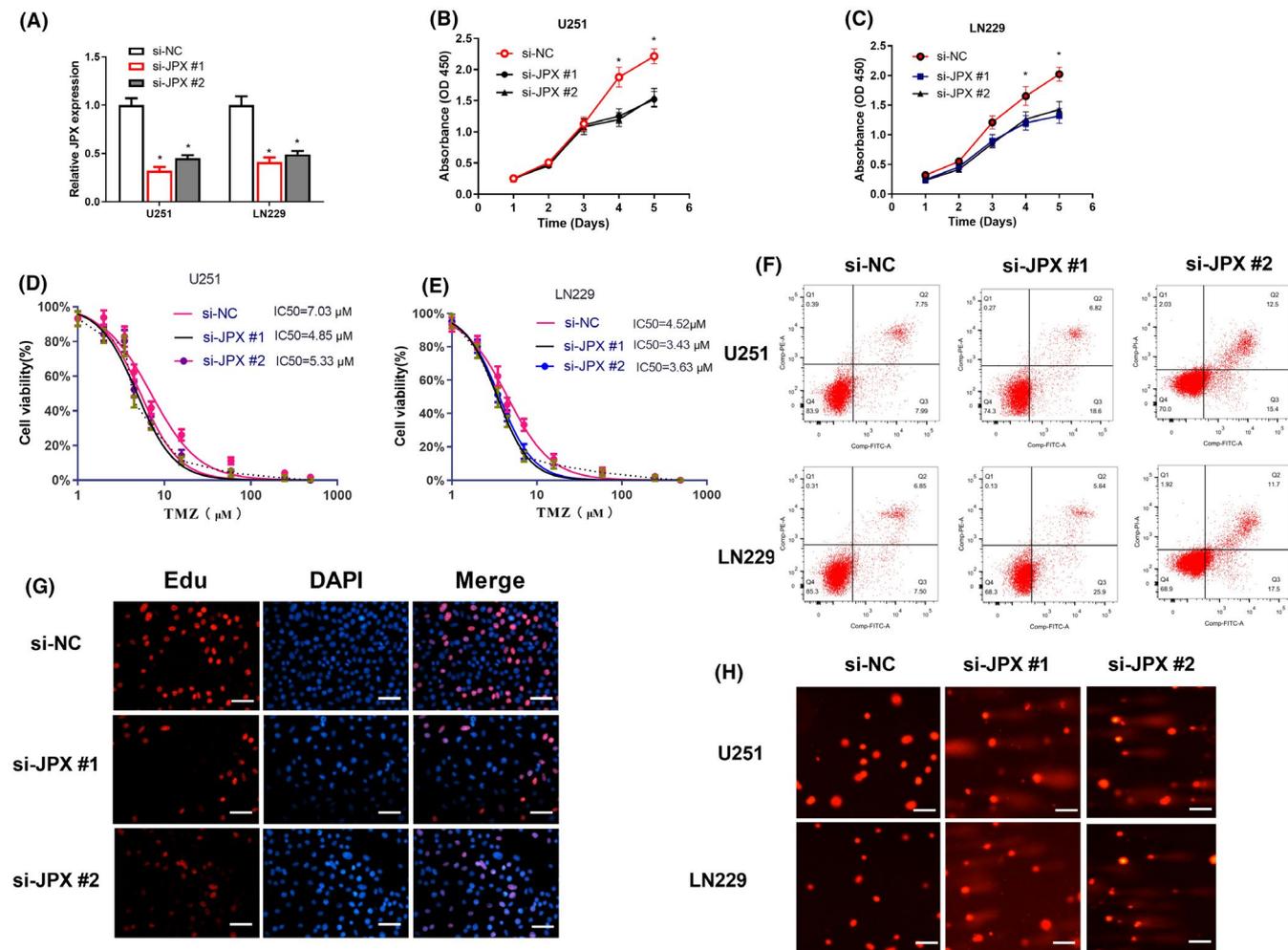
FIGURE 2 JPX promotes tumor progression and TMZ chemoresistance in GBM cells. A, Relative expression of JPX transfected with negative control (NC), siRNA (si-NC), or JPX siRNA (si-JPX) in GBM cells, as calculated with GAPDH employed as an endogenous control. B, C, Cell proliferation of GBM cells, as detected by CCK-8 assay. D, E, The IC50 of cells as detected by IC50 curve. F, Cell apoptosis rate of GBM cells, as detected by Annexin-V FITC methods. G, EDU positive rate of cells, as detected by EDU detection analysis. H, DNA damage of GBM cells, as detected via comet assays. Three independent experiments were carried out; *$P < .05$ compared with the negative control

a diminished ECAR in GBM cells, which is a marker of the overall flux of glycolysis (Figure 3D,E). Moreover, the OCR, which is a marker of mitochondrial function and oxidative phosphorylation, was significantly increased in JPX regarding the knockdown of GBM cells (Figure 3F,G). Taken together, these data strongly demonstrate that JPX facilitated aerobic glucose metabolism in GBM cells.

3.4 | JPX interacts with PDK1 mRNA and maintains its stability

JPX was mainly distributed in the cytoplasm (Figure 4A), suggesting that it played a function in post-transcriptional modulation. To identify the RNA interacting with JPX, a MS2-binding protein (MS2bp)-based RIP assay was conducted; it was performed via GFP antibody immunoprecipitation. PDK1 acts as a limiting enzyme of glycolysis. It is not clear whether there was any interaction between JPX and PDK1 mRNA. The MS2-RIP assay results confirmed that JPX

significantly enriched PDK1 mRNA, compared to the beads in the U251 and LN229 cells (Figure 4B). Furthermore, the RNA pull-down assay confirmed that JPX could enrich PDK1 mRNA (Figure 4C).

JPX knockdown significantly inhibited the expression of PDK1 in both mRNA and protein levels in GBM cells (Figure 4D,E). The knockdown of JPX did not impact the transcription of the PDK1 promoter, suggesting that JPX coordinated PDK1 expression through post-transcriptional modulation (Figure 4F). JPX knockdown diminished the half-life of PDK1 mRNA in GBM cells (Figure 4G). Taken together, these data reveal that JPX facilitated PDK1 expression via maintaining the stability of PDK1 mRNA.

3.5 | JPX modulates PDK1 expression in an RNA demethyltransferase FTO-dependent manner

M6A modification has been reported to modulate RNA stability, including for mRNAs. Here, PDK1 mRNA was considerably enriched
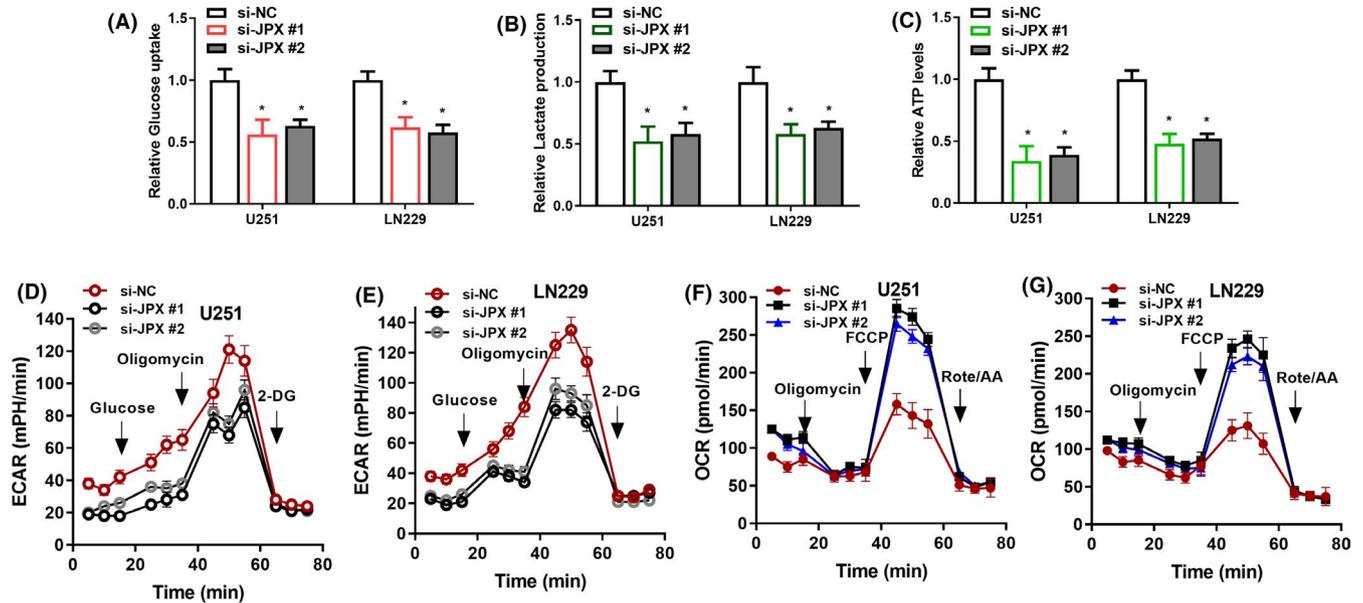
FIGURE 3 JPX knockdown dampens the aerobic glycolysis in GBM cells. A, Glucose uptake of GBM cells. B, Lactate production of GBM cells. C, ATP generation of GBM cells. D, E, ECAR of GBM cells as detected via a Seahorse Bioscience XFp analyzer. F, G, OCR of GBM cells as detected via a Seahorse Bioscience XFp analyzer. Three independent experiments were carried out; *P <.05 compared with the negative control

by M6A antibodies compared to IgG (Figure 5A). Moreover, the Me-RIP assay revealed that JPX knockdown substantially upregulated the M6A methylation of PDK1 mRNA (Figure 5B). These data demonstrate that JPX negatively modified PDK1 mRNA M6A modifications.

FTO alpha-ketoglutarate dependent dioxygenase (FTO) and AlkB homolog 5, RNA demethylase (ALKBH5) function as the main m6A de-methyltransferases. To investigate whether FTO or ALKBH5 elicited effects on JPX-mediated PDK1 m6A modification, interactions between JPX and FTO or ALKBH5 were investigated by RIP. The results exhibited that FTO antibodies, but not ALKBH5, could noticeably enrich JPX (Figure 5C). Furthermore, it was revealed that FTO could interact with PDK1 mRNA. However, JPX knockdown significantly decreased the interaction of FTO with PDK1 mRNA (Figure 5D). Additionally, in the mRNA stability experiment, in line with the results of si-JPX, FTO knockdown facilitated PDK1 degradation, whereas FTO overexpression restored PDK1 degradation induced by the JPX knockdown (Figure 5E). Taken together, these data demonstrate that JPX positively regulated PDK1 mRNA m6A modification in an FTO-dependent manner.

3.6 | JPX facilitates GBM progression and TMZ chemoresistance via modulating PDK1

To further confirm the function of JPX/FTO/PDK1 axis in GBM progression and TMZ chemoresistance, rescue experiments were performed. First, PDK1 protein levels were inhibited after JPX knockdown and PDK1 overexpression could rescue PDK1 expression (Figure 6A). Moreover, in the CCK-8 assays, PDK1 overexpression could partly rescue the proliferation inhibition induced by JPX

knockdown in GBM cells (Figure 6B,C). Similarly, PDK1 overexpression partly rescued the TMZ chemosensitization effect induced by JPX knockdown (Figure 6D). Furthermore, PDK1 overexpression neutralized the inhibition of glucose uptake and lactate production induced by JPX knockdown (Figure 6E,F). Consistently, ECAR analysis indicated that the inhibition of the glycolytic process, induced by JPX knockdown, could be partly recovered by PDK1 overexpression (Figures 4H, 6G). Collectively, these results suggest that the JPX/FTO/PDK1 axis facilitated progression and TMZ chemoresistance through aerobic glycolysis modulation in GBM cells.

4 | DISCUSSION

TMZ-based chemotherapy is a therapeutic cornerstone for GBM patients. However, TMZ chemoresistance is still the major obstacle in GBM treatment. The detailed mechanisms of GBM chemoresistance need to be urgently revealed to improve TMZ efficiency. This study aimed to identify a chemoresistance promotor and the mechanisms underlying the initiation of TMZ chemoresistance in GBM.

An accumulating amount of evidence has highlighted the crucial roles of lncRNAs in modulating the initiation programs and progressions of cancer, such as self-renewal and metastasis, as well as chemoresistance. In nasopharyngeal carcinoma (NPC), LncRNA ANCR fosters tumor proliferation and chemoresistance by inhibiting PTEN.[18] In cervical cancer, HOTAIR overexpression leads to HIF-1α-promoting mediated chemoresistance.[19] Recently, JPX has been reported to elevate myeloma cell growth and treatment resistance. However, the role and regulatory mechanisms of JPX in GBM remain underinvestigated. The present study demonstrates that JPX levels were enhanced in GBM cells. Functionally, JPX knockdown inhibited
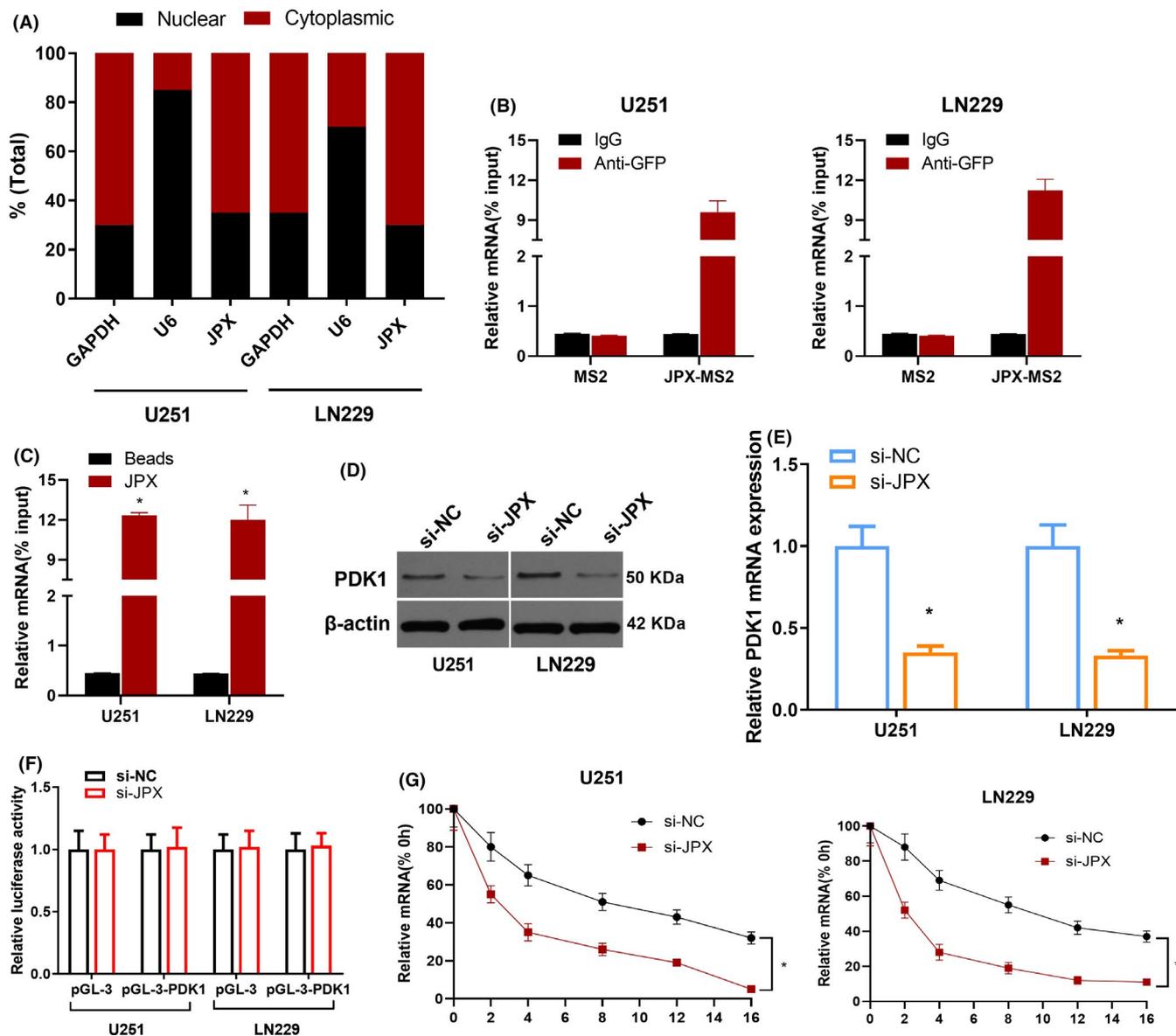
FIGURE 4 JPX interacts with PDK1 mRNA and promotes its stability. A, JPX distribution in U251 and LN229 cells, as measured by subcellular fractionation plus qRT-PCR analysis. B, MS2-RIP RT-PCR, which was used to measure JPX associated RNA in U251 and LN229 cells. C, Pull-down PDK1 mRNA level with biotin-labeled JPX, as tested by qRT-PCR in U251 and LN229 cells. D, Western blot-indicated PDK1 protein levels in control and JPX-knockdown GBM cells. E, qRT-PCR analysis of PDK1 expression in control and JPX-knockdown GBM cells. F, Luciferase reporter plasmid containing PDK1 promoter region or empty vector after transfection into GBM cells. Luciferase activity was measured after 48 h. G, PDK1 mRNA levels following treatment of GBM cells with a-amanitin (50 mM). Three independent experiments were carried out; *P <.05 compared with the negative control

cell proliferation and resistance to TMZ. Furthermore, JPX promoted GBM growth and TMZ chemoresistance in a PDK1-dependent manner. Thus, for the first time this study indicates that JPX facilitates GBM progression and TMZ chemoresistance.

It has been suggested that lncRNAs account for interactions with RBPs and enhance the RBP-mediated stability of target mRNAs.[20] For instance, CASC9 has been shown to interact with RBP CPSF3, which promotes TGF-β mRNA stability, thus contributing to tumor progression and metastasis in colorectal cancer (CRC).[21] In addition, LINC01093 has been shown to directly interact with RBP IGF2BP1, which interrupts the interaction of IGF2BP1 with GLI1 mRNA, resulting in degradation of GLI1 mRNA and the subsequent regression

of HCC.[22] Moreover, the LncRNA-assisted stabilization of transcripts (LAST) has been suggested to maintain CCND1 mRNA stability via binding to CNBP, through recognizing CCND1 mRNA.[23] In the present study, JPX was first shown to directly promote PDK1 mRNA stability and its subsequent overexpression. These findings provide sufficient proof that JPX affected TZM chemoresistance by directly stabilizing PDK1 mRNA in GBM.

Unlike normal cells, cancer cells exert aberrant glucose metabolism with high aerobic glycolysis (known as the Warburg effect). This is characterized by an enhanced glucose uptake and the production of excessive lactic acid.[11,24] Emerging evidence has revealed that the excessive aerobic glycolysis in cancer is highly correlated
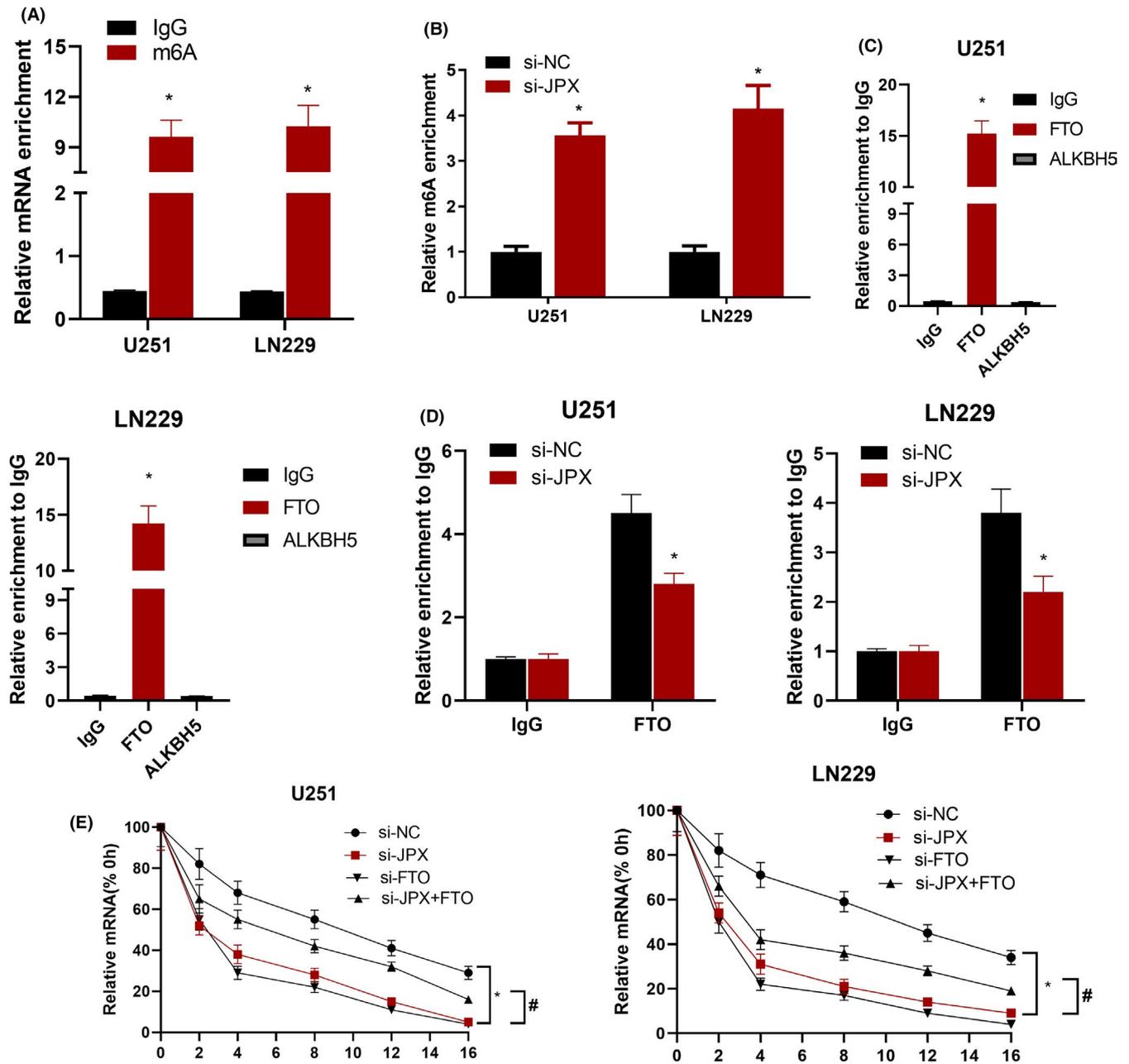
FIGURE 5 JPX modulated PDK1 expression in a RNA de-methyltransferase FTO-dependent manner. A, MeRIP assay conducted to measure the m6A levels of PDK1 mRNA in GBM cells. B, MeRIP assay conducted to detect the m6A levels of PDK1 mRNA in control and JPX-knockdown cells. C, RIP assays of IgG, FTO, or ALKBH5 antibodies conducted in GBM cells; enriched JPX was detected via qRT-PCR. D, RIP assays of IgG or FTO antibodies conducted for control and JPX-knockdown GBM cells. E, FTO knockdown facilitated PDK1 degradation in concert with si-JPX and FTO overexpression restored the degradation of PDK1 mRNA, mediated by JPX knockdown. Three independent experiments were carried out; *P <.05 compared with the negative control

with therapeutic resistance, including chemoresistance, due to the enhanced antioxidant capacity ascribed to accumulation of pyruvate and lactate. Therefore, aerobic glycolysis remains a potential therapeutic target for cancer.[10,25,26] However, whether and how lncRNAs are involved in aerobic glycolysis and TMZ chemoresistance in GBM remains ill-defined. Previously, PDK1 has been found to be elevated in several human malignancies; it has also been linked with poor prognoses.[27] A recent study reported that targeting PDK1 dampened AML cell growth, rendering PDK1 a potential therapeutic

strategy.[28] Nevertheless, the upstream regulating factors of PDK1 in TMZ chemoresistance in GBM remain elusive. In the current study, JPX knockdown was found to inhibit PDK1 mRNA stability and expression, accompanied by decreased glucose uptake, lactate production, ATP flux, and ECAR. This demonstrates the pivotal role of PDK1 in glucose metabolism and TMZ chemoresistance in GBM. Specifically, Me-RIP assays and rescue experiments confirmed that JPX mediates aerobic glycolysis by modulating the FTO/PDK1 axis. This first discovery of the JPX/FTO/PDK1 axis provides a *bona fide*
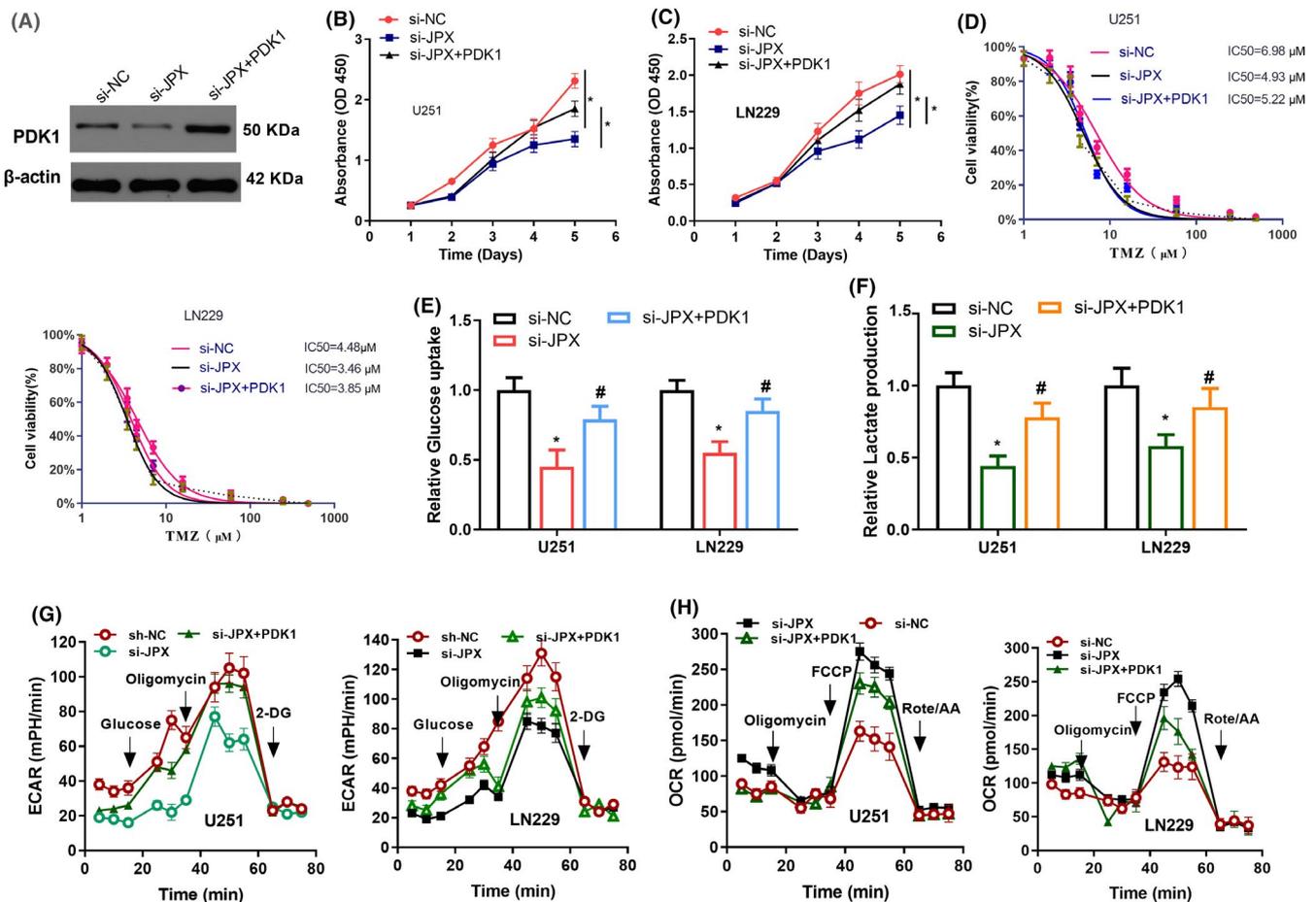
FIGURE 6 JPX facilitates GBM progression and TMZ chemoresistance via modulation of PDK1. A, Western blot assay revealing the PDK1 protein levels in GBM cells. B, C, PDK1 overexpression partly neutralized the inhibitory effects of JPX knockdown on the proliferation of GBM cells. D, TMZ chemosensitization effects of JPX knockdown on GBM cells. E, F, Effect of JPX knockdown on the inhibition of glucose uptake and lactate levels in GBM cells. G, H, Inhibitory effects of JPX knockdown on the glycolytic process in GBM cells, as reflected by ECAR analysis. Three independent experiments were carried out; *P <.05 compared with the negative control, and #P <.05 compared with the si-JPX group

strategy for therapeutic target screening targeting glucose metabolism in GBM.

In recent years, RNA epigenetics and its regulation has become a new field of cancer research. The M6A modification serves as one of the most conservative and critical mRNA modifications in human malignancies.[15,29] More and more studies have shown that the three main regulatory factors of M6A, "writer," "eraser," and "reader," determine the occurrence and development of tumors. As the first found M6a "eraser," FTO has been shown to be involved not only in fat regulation, but also in the progression of a variety of tumors.[30] A previous study found that the function of FTO polymorphism was mostly related to the risk of the initiation of cancers, such as thyroid, colon, liver, pancreatic, and breast cancers.[31] However, a recent study has addressed whether FTO exerts an oncogenic role through mRNA demethylation.[29] A growing body of research has indicated that FTO levels were elevated in AML, and that FTO knockdown accounted for the antitumor phenotype. Mechanistically, FTO has been suggested to stabilize the ASB2 and RARA mRNA transcripts with the loss of M6A,[32] revealing a potential therapeutic target of

FTO for cancer therapy.[32] The current study proposes that JPX maintained the stability of PDK1 mRNA in an FTO-dependent manner in GBM. The results of this study emphasize the oncogenic function of FTO, demonstrating the association between FTO-mediated M6A demethylation and the TMZ chemotherapy resistance of GBM.

In conclusion, it was revealed that the JPX/FTO/PDK1 axis can facilitate aerobic glycolysis in GBM cells, which was shown to be correlated with the sensitivity of GBM cells to TMZ. These findings provide valuable information for understanding aerobic glycolysis regulation and raise the potential that blocking the JPX/FTO/PDK1 axis could serve as a promising strategy for mitigating the efficacy of TMZ in GBM.

ETHICS APPROVAL AND CONSENT TO PARTICIPATION

The animal experiments were approved by the Animal Management and Use Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Hubei, China. Patient consent was obtained regarding data extraction and tissue sample sampling analysis of patients with glioma.

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