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Research article

FLOT1, stabilized by WTAP/IGF2BP2 mediated N6-methyladenosine modification, predicts poor prognosis and promotes growth and invasion in gliomas

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ABSTRACT

The expression, function, and mechanism of FLOT1 (flotillin-1) remains unknown in gliomas. Here, the expression and clinical value of FLOT1 in gliomas was bioinformatically and experimentally analyzed via online omics data and local tissues. Moreover, the effects of FLOT1 depletion on cell proliferation and invasion were also detected. Besides, the underlying roles of N6-methyladenosine modification (m6A) in FLOT1 upregulation was further explored. The results demonstrated that FLOT1 was significantly upregulated in gliomas and positively correlated with advanced progression and poor prognosis of patients. FLOT1 silencing notably suppressed the cell proliferation and invasion in gliomas. The expression of WTAP and IGF2BP2was positively correlated with FLOT1 expression and served as the writer and reader of FLOT1 m6A, respectively, which stabilized FLOT1 mRNA and maintained its upregulation in gliomas. Lastly, ectopic expression of FLOT1 could notably restore the inhibitory effects caused by WTAP and IGF2BP2 depletion in glioma cells. Collectively, our results originally confirmed the upregulation and oncogenic roles of FLOT1, and revealed that WTAP/IGF2BP2 mediated m6A contributed to the upregulation of FLOT1 in gliomas, highlighting the promising application of WTAP/IGF2BP2/FLOT1 axis in target treatment of gliomas.

1. Introduction

Gliomas stand for the most common and malignant types of brain tumors [1]. The unsatisfactory clinical outcomes of gliomas patients are largely due to the insufficient understanding of the pathological mechanisms, which prevents the efficient progression in clinical treatment of gliomas [1,2]. Therefore, the identification of critical genes driving glioma progression and elucidation of the underlying mechanisms are of great importance in exploring more effective therapeutic targets and ultimately enhancing treatment outcomes.

Flotillin family proteins, namely FLOT1 and FLOT2, serve as markers of lipid rafts and play a crucial role in tumorigenesis by coordinating cell communication processes such as adhesion, actin cytoskeleton reorganizations, endocytosis, phagocytosis, and

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signaling transduction [3]. Flotillin proteins' upregulation shows a positive correlation with advanced tumor stage and unfavorable prognosis in nasopharyngeal carcinoma, breast cancer, lung cancer, and gastrointestinal tumors [4–6]. Upregulation of flotillins induces oncogenic signaling pathways that promote tumor proliferation, facilitate metastasis by accelerating the cell cycle, suppressing cell apoptosis, and initiating epithelial-mesenchymal transition (EMT) through PI3K/AKT, TGF-β, and NF-κB pathways [4,6–10]. Several preclinical and clinical studies have provided evidence of small molecule inhibitors' potential in cancer treatment targeting kinases such as PI3K/AKT [11,12]. Since tumor growth-promoting signals' interactions and complementarity leads to drug resistance in single-target inhibitors of PI3K/AKT [11,12]. Identifying common upstream regulatory factors of various survival signals as potential intervention targets for cancer can lead to stronger and more stable anti-cancer efficacy. As lipid rafts serve as a platform for signal transduction [3,4], targeting lipid raft-associated proteins can lead to greater anti-tumor efficacy by deactivating multiple pro-tumor signaling pathways. These findings suggest the potential and promise of flotillin proteins in tumor targeted therapy. Consequently, our recent study unveils upregulation, oncogenic functions, and underlying mechanism of FLOT2 in gliomas [13]. However, there is still uncertainty regarding FLOT1's expression, function, and mechanism in gliomas.

N6-methyladenosine (m6A) is a widespread post-transcriptional modification that plays a vital role in regulating mRNA stability, translation, alternative splicing, and subcellular localization [14]. The catalysis of m6A involves writers (METTL3, METTL14, METTL16, and WTAP), recognition by readers (IGF2BPs and YTHDFs), and removal by erasers (FTO, ALKBH5) [14]. Dysregulation of m6A modification corrupts transcriptome and proteome regulation, thus regulating various biological processes, including carcinogenesis [14]. For instance, m6A stabilizes tumor-driving genes like c-Myc, AKT, and PD-L1 which promote tumor progression and immune escape [15–17]. Therefore, m6A is considered a primary cause of aberrant gene expression in cancers. A recent study provided evidence that m6A may regulate flotillin expression in cancers by demonstrating its critical function in regulating FLOT2 expression in ovarian granulosa cells [18].

In this study, the expression and function of FLOT1 in gliomas was explored. Moreover, the underlying mechanism of FLOT1 upregulation was further investigated. The results demonstrated that FLOT1 was upregulated and promoted cell proliferation and invasion in gliomas. Notably, WTAP and IGF2BP2 could mediate the m6A of FLOT1 mRNA and subsequently maintain its stability to boost malignant progression of gliomas. Therefore, our study revealed the upregulation and oncogenic roles of FLOT1, and the underlying mechanism of FLOT1 upregulation in gliomas, highlighting the promising application of FLOT1 as a target for gliomas treatment.

2. Methods and materials

2.1. Cell lines, small interring RNAs (siRNAs), and plasmids

Gliomas cell lines, U251, T98G, U87, and U118, were obtained from ATCC (American type culture collection) and were cultured with DMEM (VivaCell, Shanghai, China) medium plus 10% fetal bovine serum (FBS, VivaCell, Shanghai, China) and 1 × streptomycin and penicillin (NCM, Suzhou, China) in a humidified cell incubator at 37 °C with 5% CO_2. SiRNAs (small interring RNAs), targeting WTAP, IGF2BP1, IGF2BP2, IGF2BP3, and FLOT1, and siNC (negative control) were purchased from Sangon Biotech (Shanghai) Inc (Shanghai, China). The sequences of siRNAs were listed in Supplementary Table S1. pENTER-FLOT1 expression plasmid and control plasmid were obtained WZ Bioscience Inc (Shandong, China). SiRNAs and plasmids and were introduced into gliomas cells with Lipo8000™ reagent (Beyotime, Shanghai, China) following the manufacturer's instruction and a published study [19]. Briefly, 10 μl siRNA (20 μM) and 10 μl Lipo8000™ reagent were added into 250 μl of serum-free DMEM. After mixing at room temperature for 20 min, the mixture was added to a pre-seeded 6-well plate with cells. After 6 h, the medium was replaced with fresh complete culture medium.

2.2. Online analysis

We performed bioinformatic analysis of FLOT1 expression and its prognostic value as previously described [20]. The RNA-sequence data of gliomas at Level 3 and their paired clinical data were downloaded from UCSC Xena from The Cancer Genome Atlas (TCGA) website. This data was utilized to investigate the expression, clinical associations, and prognostic value of FLOT1, and the correlations between FLOT1 and m6A regulators, in gliomas through the use of R (version 3.6.3). To perform the expression, correlation, survival analyses, and chart production, various R packages, such as DESeq2, Stats, Survival Analysis, and ggplot2 were utilized.

2.3. Tissue samples

We obtained ninety tissue samples, comprising of seventy primary tumor tissues and twenty adjacent tumor tissues, from the department of neurosurgery at Xiangya Hospital, Central South University, and properly prepared them for protein and RNA extraction as well as Immunohistochemical (IHC) assay, as previously described [13]. To adhere to ethical guidelines, the study was performed in compliance with the Declaration of Helsinki, and the use of tissues was approved by the Ethical Committee of Xiangya Hospital of Central South University (No: 201907786) alongside obtaining informed consents from the patients who underwent surgery.

2.4. IHC assay

We analyzed the levels of FLOT1 in gliomas and adjacent normal tissues using an IHC assay, following our prior methods [13]. We

used 1:100 dilution of rabbit *anti*-FLOT1 polyclonal antibody (Abcam, Cambridge, UK). To perform a semi-quantitative analysis, we utilized staining intensity and area, as previously described [13].

2.5. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

We performed qRT-PCR according to previous protocols [13]. Briefly, we extracted total RNAs from tissues and cells using Trizol™ reagent (SIMGEN, Hangzhou, China) following the manufacturer's guidelines, then reversed them to cDNA by using Hifair® III 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) (YEASEN, Shanghai, China). Relative expression levels of FLOT1, WTAP, and IGF2BP2 were determined using Hieff® qPCR SYBR Green Master Mix (No Rox) (YEASEN, Shanghai, China), with GAPDH serving as the control. The primer sequences can be found in Supplementary Table S1.

2.6. MeRIP-qPCR

The experiment to detect m6A modification of FLOT1 utilized the BersinBio™ Methylated RNA Immunoprecipitation (MeRIP) Kit from BersinBio (Guangzhou, China), following the provided instructions. Specifically, 100 μg of fragmented total RNA was incubated with 4 μg of *anti*-m6A antibody/immunoglobulin G (IgG) (Abclonal; Wuhan, China) in immunoprecipitation (IP) buffer at 4 °C for 4 h, in the presence of protease and RNase inhibitors. Then, 30 μl of protein A/G magnetic beads were added to the solution containing M6A/IgG and RNA mixture and incubated by rotating the mixture at 4 °C for 1 h. The precipitated RNAs were eluted and purified using phenol/chloroform/isoamyl alcohol (25:24:1, Solabio; Beijing, China) extraction. The eluted RNAs were then reversely transcribed into cDNA to perform quantitative polymerase chain reaction (qRT-PCR) assays, with the primer sequences provided in Supplementary Table S1.

2.7. RNA immunoprecipitation (RIP)

The Magna RIP RNA-Binding Protein Immunoprecipitation Kit from Millipore (MA, USA) was used to conduct RNA immunoprecipitation (RIP) following the manufacturer's instructions. Briefly, IGF2BP2 (Proteintech; IL, USA) or IgG antibodies were conjugated to magnetic beads at room temperature for 30 min. The beads were then incubated with cell lysate at 4 °C overnight. After proteinase K digestion and washing, the bound RNAs were purified using phenol/chloroform/isoamyl alcohol (125 : 24 : 1, Solabio; Beijing, China) extraction. The relative interaction between IGF2BP2 and FLOT1 mRNA was detected using quantitative polymerase chain reaction (qRT-PCR).

2.8. T7 Biotin Labeled RNA synthesis and RNA pulldown

The biotin-labeled RNA FLOT1 mRNA probes were obtained using Ribo™ RNAmax-T7 Biotin Labeled RNA Synthesis Kit (RiboBio, Guangzhou, China) following the manufacturer's instructions. Subsequently, RNA immunoprecipitation (RNA pulldown) was performed using the BersinBio™ RNA pulldown Kit (BersinBio; Guangzhou, China). Briefly, the biotin-labeled RNA probes were conjugated to streptavidin-coated magnetic beads at room temperature for 30 min. Then, the RNA-conjugated magnetic beads were incubated with nucleic acid-free cell lysates to capture binding proteins at room temperature for 2 h. Lastly, the bound proteins were eluted and analyzed via Western blot analysis.

2.8.1. RNA stability assays

Glioma cells were treated with actinomycin D (10 μg/mL; MCE; NJ, USA) for 0, 2, and 4 h. Total RNA was isolated, and the relative expression of FLOT1 was analyzed using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The mRNA half-life was estimated using linear regression analysis.

2.9. Western blot (WB)

Western blot analysis was performed following a previously described protocol [13]. Briefly, total proteins were obtained from RIPA buffer (NCM Biotech; Suzhou, China)-treated cell lysates through centrifugation at 4 °C. The denatured proteins were separated via 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk and incubated with the appropriate primary antibodies, including rabbit *anti*-FLOT1 (Abcam; Cambridge, UK), rabbit *anti*-IGF2BP2, mouse *anti*-WTAP (Proteintech; IL, USA), and rabbit *anti*-GAPDH (Bioworld; Nanjing, China). Subsequently, the protein amounts were visualized using horseradish peroxidase (HRP)-conjugated secondary antibodies (anti-rabbit or anti-mouse IgG) and chemiluminescent HRP substrate (EpiZyme; Shanghai, China).

2.10. Luciferase reporter assay

The study utilized the dual-luciferase reporter system assay, described in previous reference [21]. To summarize the process, the predicted m6A sites of FLOT1 sequences were cloned into pmirGLO reporter plasmids, which were then co-transfected with siRNAs into glioma cells. After 48 h, the luciferase activities of the cell lysate were measured using the Steady-Glo® Luciferase Assay System

(Promega, WI, USA). The ratio of firefly luciferase activity to Renilla luciferase activity was used as a control to indicate the relative expression activity.

2.11. CCK-8 assay

The study utilized the CCK-8 assay to measure cell growth as previously description [13]. Briefly, cells were seeded at a density of 1×10^4 cells per well in a 96-well plate. CCK-8 reagent (MCE, NJ, USA) was added to the medium and incubated for 5 days. Following a 1-h incubation period, the absorbance at 450 nm was measured using a spectrophotometer (BioTek, WI, USA). Growth curves were plotted based on the absorbance readings taken each day.

2.12. Transwell invasion assay

Transwell assay was performed as previously described [13]. Briefly, 2.5×10^4 cells were seeded onto the upper chamber of a transwell device (Costar, ME, USA), while 750 μl of DMEM medium with 5% FBS was added to the lower well. After 16 h, cells were fixed with methanol and stained using 0.5% crystal violet. The number of invasive cells on the upper side of the membrane were photographed and manually counted under an inverted microscope (Leica, Solms, Germany).

2.13. Statistical analysis

Statistical analysis and charts were analyzed and plotted using Graphpad Prism 8.0 software. Student t-test or Fisher exact test was applied in comparisons between the two groups. $P < 0.05$ were considered to be statistically significant.
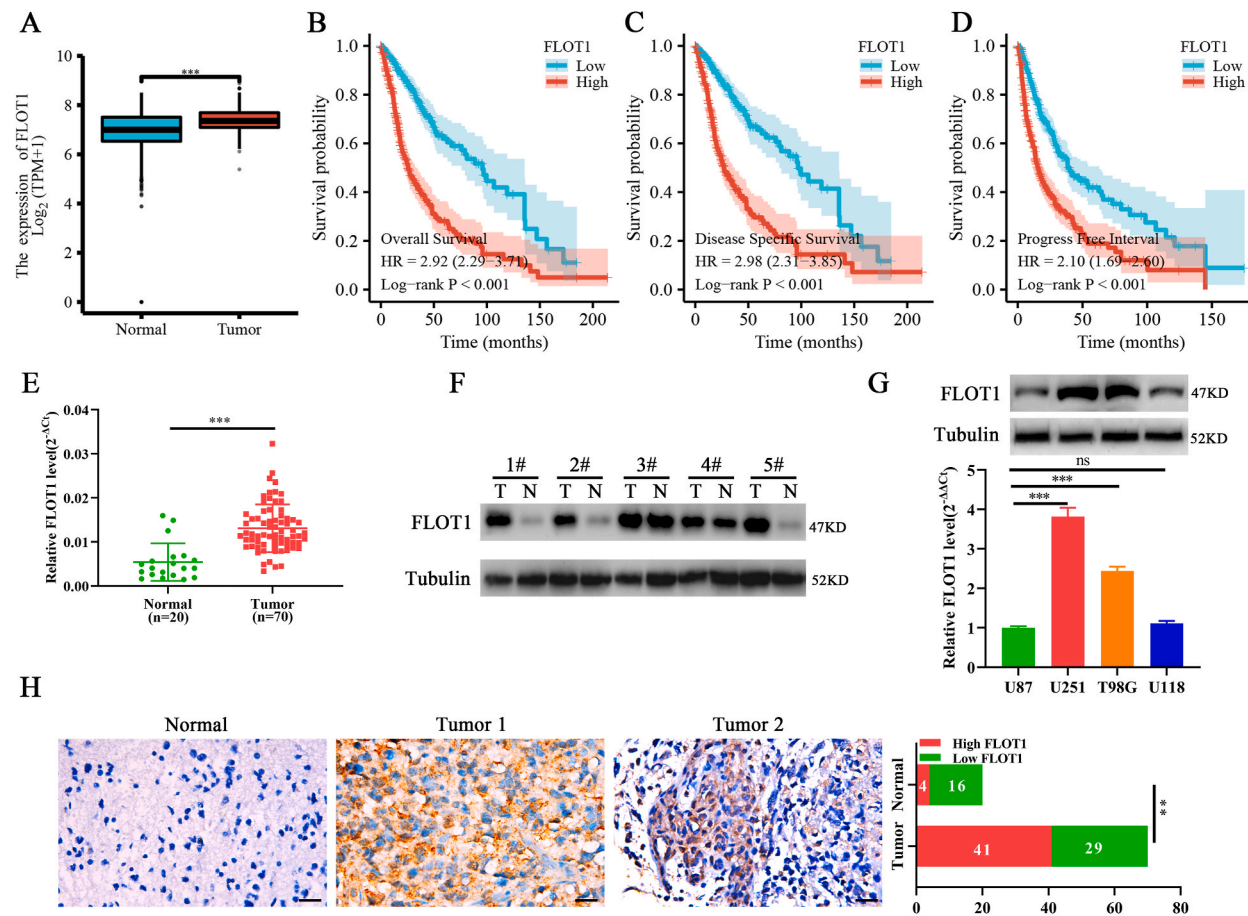

Fig. 1. FLOT1 is upregulated and predicts poor prognosis in gliomas. (A) The TCGA data indicating the upregulation of FLOT1 expression in gliomas tissues. (B–D) The TCGA data indicating the prognostic value of FLOT1 in gliomas including OS, DSS, and PFI. (E) RT-qPCR and (F) WB results indicating the expression of FLOT1 in local gliomas tissues. (G) RT-qPCR and WB results indicating the expression of FLOT1 in gliomas cells. (H) The representative pictures of FLOT1 staining in gliomas and normal tissues. Magnification 200 ×, Scale bar 50 μm **, $P < 0.01$; ***, $P < 0.001$; **ns**, no significant difference.

3. Results

FLOT1 is upregulated, positively associated with malignant progression, and predicts poor prognosis in gliomas.

Firstly, we analyzed the expression of FLOT1 based on TCGA data. FLOT1 was significantly upregulated in gliomas and positively correlated with the malignant characteristics such as WHO grade, IDH status, and 1p/19p chromosome co-deletion (Fig.1A and Supplementary Fig. S1A). Moreover, Gliomas patients with high FLOT1 expression suffered poor prognosis including overall survival (OS), disease specific survival (DSS), and progress free interval survival (PFI) (Fig. 1B–D). Moreover, the univariate and multivariate Cox regression analyses demonstrated that FLOT1 was an independent risk factor for prognosis of gliomas patients (Supplementary Fig. S1A and 1B). Furthermore, FLOT1 upregulation in gliomas tissues and cells was further validated by the results of qRT-PCR, Western blot, and IHC assays (Fig. 1E–H). Besides, IHC data showed that FLOT1 upregulation was positively correlated with WHO grade and Ki-67 intensity (Table 1). Therefore, these results prove that FLOT1 is upregulated, positively correlates with advanced progression, and serves as an independent risk factor for prognosis in gliomas.

3.1. FLOT1 knockdown impairs the growth and invasion of gliomas cells

Next, the expression of FLOT1 in U251 and T98G cells were depleted by transfection of siFLOT1s to analyze the functions of FLOT1 in gliomas. The qRT-PCR and Western blot results (Fig. 2A and B) validated the knockdown efficacy of FLOT1 in glioma cells. Then, the effects of FLOT1 depletion on growth and invasion were tested by CCK-8 and transwell invasion assays. The results demonstrated that FLOT1 silencing significantly inhibited cell growth and invasion supporting by decreased OD_{450nm} value and invasive cells (Fig. 2C and D). Therefore, these findings revealed that FLOT1 could promote cell proliferation and invasion in gliomas.

3.2. WTAP writes the M6A of FLOT1 mRNA and enhances its stability in gliomas

M6A modification plays vital role in regulation of genes expression via modulating stability of substrate mRNAs [14]. We investigated whether m6A related mechanism was involved in regulation of FLOT1 expression in gliomas. Several candidate m6A sites of FLOT1 mRNA were predicted by SRAMP database (Supplementary Table S2). Indeed, the MeRIP-PCR results demonstrated that FLOT1 mRNA was significantly enriched by m6A antibody in gliomas cells (Fig. 3A). Next, the writers mediated m6A of FLOT1 were explored. The correlations of M6A writers, including METTL3, METTL14, METTL16, and WTAP, with FLOT1 in gliomas were analyzed. Among m6A writer genes, only WTAP positively correlated with FLOT1, suggesting WTAP mediated the m6A of FLOT1 mRNA (Supplementary Fig. |S2A). Accordingly, WTAP silencing markedly downregulated the expression of FLOT1 (Fig. 3B and C) in gliomas cells. Moreover, WTAP knockdown significantly promoted turnover of FLOT1 mRNA in gliomas supporting by the results of actinomycin D chase experiment (Fig. 3D). Consistently, dual-luciferase reporter assays indicated that WTAP depletion substantially inhibited luciferase activity of FLOT1 reporter construct but not empty vector (Fig. 3F). Thus, these results showed that WTAP could stabilize FLOT1 mRNA in an m6A-dependent manner.

Table 1
Correlation of FLOT1 expression with clinicopathologic features in glioma tissues (Fisher's exact test, n = 70).

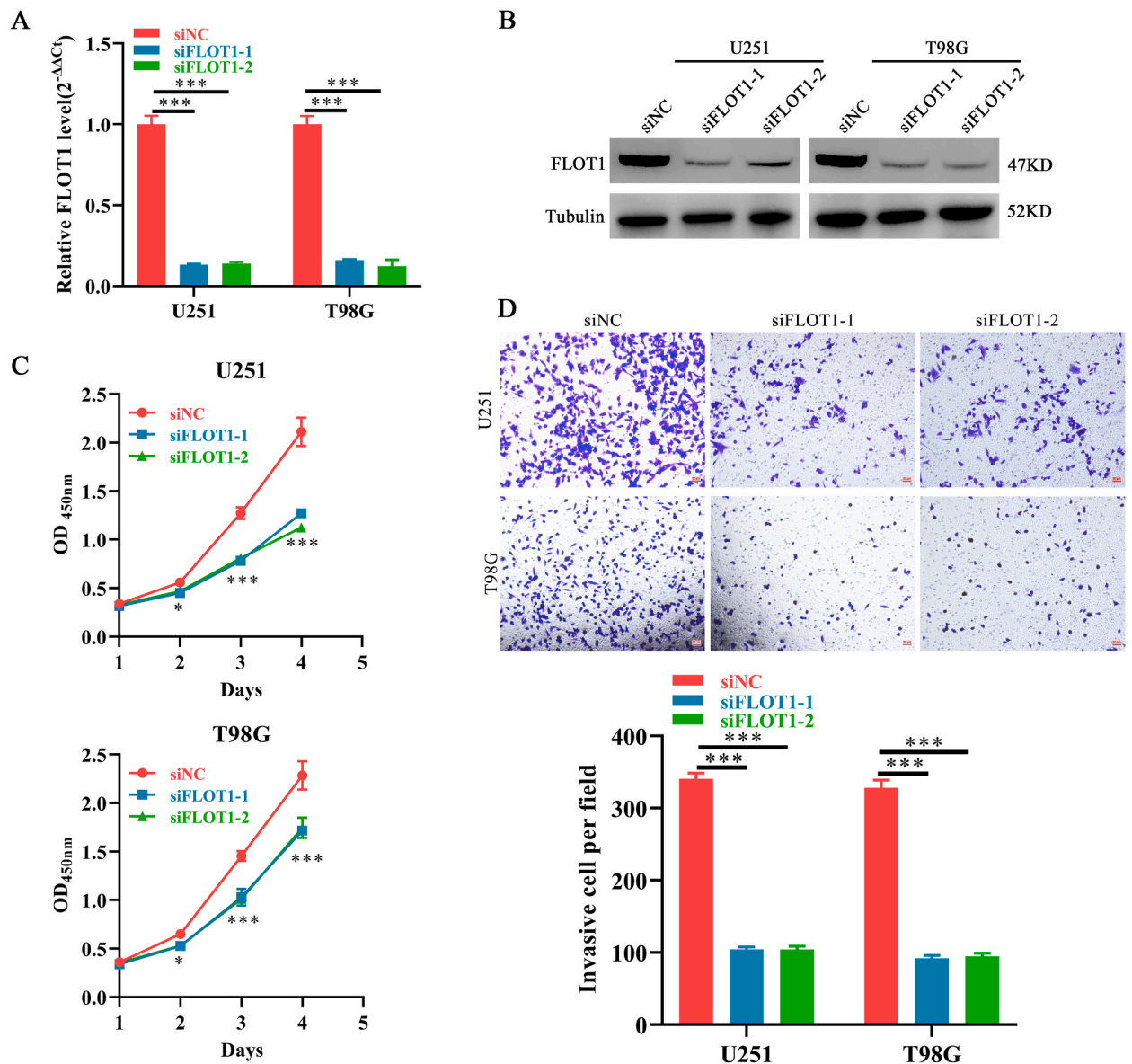| Variables	N	FLOT1 expression		P value
		High	Low	
Age				
≤45	34	18	16	0.4672
>45	36	23	13	
Sex				
Male	42	24	18	0.8087
Female	28	17	11	
Ki67 intensity				
≤15%	18	17	1	**0.0002**
>15%	52	24	28	
IDH mutation				
Positive	34	18	16	0.4672
Negative	36	23	13	
p53 staining				
Positive	56	32	24	0.7651
Negative	14	9	5	
MGMT staining				
Positive	52	33	19	0.1767
Negative	18	8	10	
WHO stage				
I–II	33	7	26	**<0.0001**
III–IV	37	34	3	

Fig. 2. FLOT1 knockdown inhibits cell growth and invasion in gliomas. (A) RT-qPCR and WB results indicating the mRNA and protein level of FLOT1 in U251 and T98G cells transfected with siFLOT1s. CCK-8 (C) and Transwell (D) assay showing the effects of FLOT1 depletion on the growth and invasion of U251 and T98G cells. Magnification 100 ×, Scale bar 50 μm*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

3.3. IGF2BP2 recognizes FLOT1 m6A modification in gliomas

Next, the m6A reader that interpreted the m6A modification of FLOT1 was identified. The oncogenic roles of IGF2BP family proteins, one vital class of m6A reader proteins, have been revealed in gliomas [22]. Thus, the correlation between IGF2BP genes and FLOT1 was analyzed. The expression of FLOT1 was positively correlated with all IGF2BP genes in gliomas (Supplementary Fig. S2A). Thus, IGF2BP genes were depleted and their effects on expression of FLOT1 were detected in gliomas cells. Only IGF2BP2, but not IGF2BP1 and IGF2BP3 (Supplementary Fig. S2B and S2C), knockdown markedly downregulated FLOT1 in gliomas cells (Fig. 3G and H). Consistently, the results of actinomycin D chase experiment demonstrated that IGF2BP2 silencing promoted degradation of FLOT1 mRNA in gliomas (Fig. 3I and J). Dual-luciferase reporter assays showed IGF2BP2 depletion significantly decreased luciferase activity of FLOT1 reporter construct but not empty vector (Fig. 3K). RIP-qPCR results demonstrated that FLOT1 mRNA was significantly enriched by IGF2BP2 antibody in gliomas cells (Fig. 3L). Lastly, RNA pulldown assay results indicated that biotin-labeled FLOT1 mRNA probes, but not control anti-sense probes, specifically precipitated IGF2BP2 (Fig. 3H) in gliomas. Therefore, our findings revealed that the m6A reader IGF2BP2 interpreted WTAP-mediated FLOT1 m6A modification and maintained its stability in gliomas.
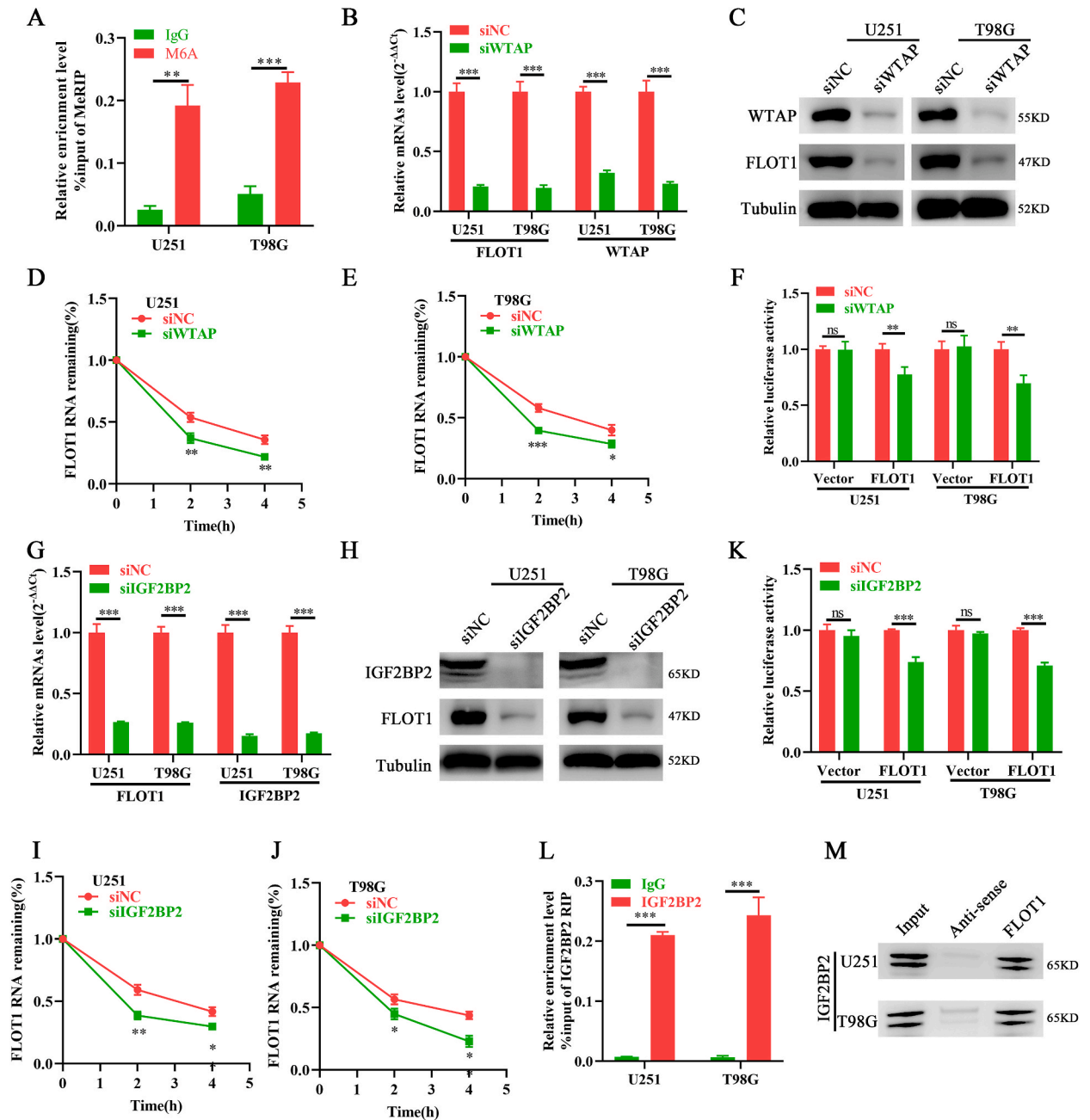
Fig. 3. WTAP and IGF2BP2 mediates the M6A modification of FLOT1 and maintains its stability in gliomas. (A) MeRIP-qPCR indicating the m6a enrichment of FLOT1 transcripts in U251 and T98G cells. (B) RT-qPCR and (C) WB results indicating the effects of WTAP depletion on the level of FLOT1 transcript and protein in U251 and T98G cells. (D) and (E) RT-qPCR indicating the stability of FLOT1 mRNA in U251 and T98G cells upon actinomycin D (10 μg/mL) treatment. (F) Relative luciferase activity of U251 and T98G cells transfected with siNC and siWTAP. (G) RT-qPCR and (H) WB results indicating the effects of IGF2BP2 depletion on the level of FLOT1 transcript and protein in U251 and T98G cells. (I) and (J) RT-qPCR indicating the stability of FLOT1 mRNA in U251 and T98G cells upon actinomycin D (10 μg/mL) treatment. (K) Relative luciferase activity of U251 and T98G cells transfected with siNC and siWTAP. (L) RIP-qPCR indicating the enrichment of FLOT1 transcripts by IGF2BP2 immunoprecipitation in U251 and T98G cells. (M) RNA pulldown assay showing the direct interaction of FLOT1 transcripts with the IGF2BP2 protein. **NS** stands for no significant difference. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; **ns**, no significant difference.

3.4. FLOT1 is an effector of the oncogenic role of WTAP and IGF2BP2 in gliomas

Previous studies revealed the oncogenic roles of IGF2BP2 and WTAP in gliomas [23,24]. In this study, the role of FLOT1 in the oncogenic roles of IGF2BP2 and WTAP in gliomas was explored. The level of FLOT1 was restored in gliomas cells with IGF2BP2 and

WTAP depletion, respectively, via ectopic expression of FLOT1 (Fig.4A). Then, the proliferation and invasion of gliomas cells was investigated. The results of CCK-8 and transwell invasion assay (Fig. 4B and C) indicated that ectopic FLOT1 expression markedly restored the growth and invasion abilities of gliomas cells with IGF2BP2 and WTAP knockdown, respectively. Therefore, our results present that FLOT1, at least partially, mediated the oncogenic roles of IGF2BP2 and WTAP in gliomas.

4. Discussion

The aberrant expression and oncogenic roles of FLOT1 have been revealed in multiple cancers [4], however, its expression pattern, underlying mechanism, and functions in gliomas remains elusive. In this study, we confirmed the upregulation, prognostic value, and oncogenic roles of FLOT1 in gliomas. Importantly, we proved that WTAP and IGF2BP2 respectively mediated and interpreted the m6A modification of FLOT1 mRNA, which stabilized FLOT1 mRNA and maintain the upregulation of FLOT1 in gliomas.
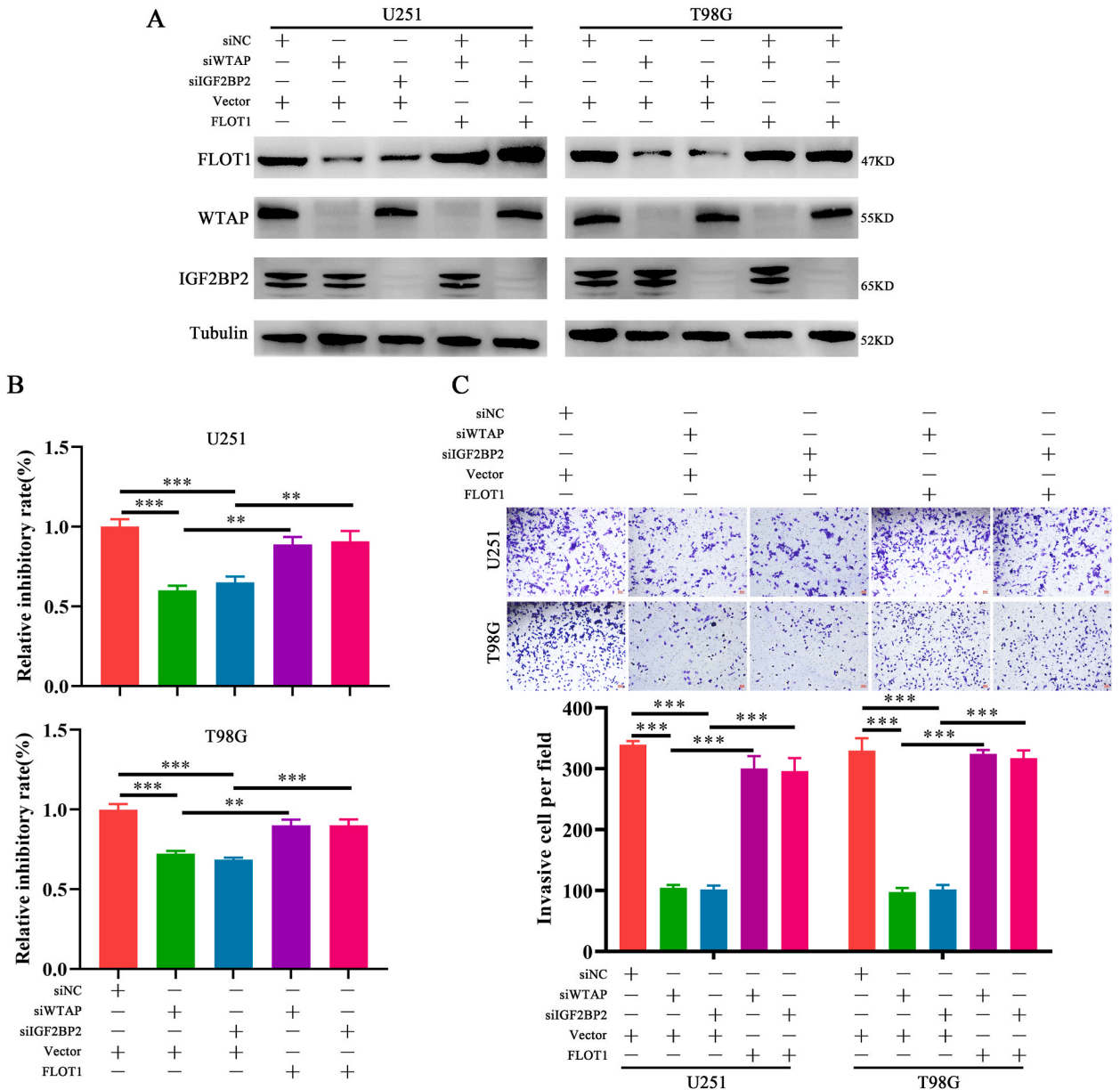

Fig. 4. Ectopic expression of FLOT1 restores the effects of WTAP and IGF2BP2 depletion in gliomas. (A) WB results indicating the level of FLOT1, WTAP and IGF2BP2 protein in U251 and T98G cells co-transfected with siWTAP/siIGF2BP2 and FLOT1 plasmids. (B) CCK-8 assay and (C) Transwell assay indicating the growth and invasion of U251 and T98G cells co-transfected with siWTAP/siIGF2BP2 and FLOT1 plasmids. **, $P < 0.01$; ***, $P < 0.001$.

FLOT1 is upregulated and serves as an oncogene in cancers such as endometrial, cervical, renal, breast, lung, nasopharyngeal, and esophageal cancer [7,10,25–29]. FLOT1 upregulation promotes cancer cell proliferation, anchorage-independent growth, migration and invasion, therapy resistance, and growth and metastasis of xenograft tumors through activating the downstream oncogenic regulators including ERK, AKT, Snail, TGF-β, and NF-κB [7,10,29,30]. Moreover, FLOT1 serves as a risky predictor for advanced tumor status like lymph node metastasis and poor overall and disease-free survivals [7,26,31,32]. In line with these studies, our findings confirmed the upregulation and oncogenic functions of FLOT1 in gliomas, providing the foundation for future in-depth researches.

Studies have shown that M6A regulators play important roles in the malignant progression of gliomas, including death resistance, stemness maintenance, and treatment resistance, by regulating the stability, translational activity, and alternative splicing of different RNA substrates, such as mRNAs and lncRNAs [33–36]. As one of writers, upregulation of WTAP positively correlates with advanced pathological stages and poor prognosis and promotes progression in a plethora of cancers via regulating the m6A status and expression of substrates [21,37,38]. For example, WTAP is highly expressed and serves as an independent risky predictor for prognosis in hepatocellular carcinoma (HCC) [33]. WTAP upregulation promotes proliferation and tumor growth of HCC cells by catalyzing the m6A of ETS1 and maintaining its stability and translation [39]. WTAP mediated m6A modification also contribute to the upregulation of oncogenes, including TNFAIP3 [40], EIF3C [41], lncRNA DIAPH1-AS1 [21], and ENO1 [42], to enhances the chemotherapy resistance, metastasis, and glycolysis in bladder cancer, prostate, nasopharyngeal carcinoma, and breast cancer, respectively. WTAP is highly expressed and positively correlates with tumor grade and poor postoperative survival of patients in gliomas as well [24,43]. In this study, we further revealed the oncogenic function of WTAP in glioma and identified FLOT1 as a new substrate which at least partially mediated the role of WTAP in glioma.

The regulatory effect of m6A depends on the synergistic action of writers and readers, where the m6A information encoded by writers is recognized and executed by readers to ultimately exert biological effects [14,44]. Research has shown that aberrance of m6A readers play important roles in the malignant progression of tumors, including gliomas. For example, YTHDF2 is a classical oncogene and contributes to stemness in gliomas via stabilizing Myc and VEGFA [45]. IGF2BP1 activates YAP/TAZ signaling to promote malignant progression of gliomas via stabilizing m6A-YAP mRNA [46]. As for IGF2BP2, its aberrant upregulation and oncogenic function has also been widely revealed in gliomas. Through binding and regulating the stability or activity of the IGF2, let-7, OIP5-AS1 and lncRNA DANCR, IGF2BP2 promotes gliomas progression via preserving cancer stem cells, regulating oxidative phosphorylation level, fostering chemoresistance, and boosting blood-tumor barrier permeability [23,47–49]. Here, our findings validated the oncogenic roles of IGF2BP2 in gliomas and identified a novel mechanism that IGF2BP2 read the m6A-FLOT1 mRNA, which subsequently stabilized FLOT1 to exert oncogenic functions in gliomas.

5. Conclusion

In conclusion, in this study we confirmed the upregulation and its oncogenic functions of FLOT1. Mechanistically, WTAP and IGF2BP2 mediated m6A modification stabilized FLOT1 and maintained its upregulation in gliomas, highlighting the potential application of WTAP/IGF2BP2/FLOT1 axis in target treatment of gliomas.

Author contribution statement

Tao Song: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Zhongxu Hu: Performed the experiments. Chong Zeng: Performed the experiments; Analyzed and interpreted the data. Haijun Luo: Analyzed and interpreted the data. Jie Liu: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix ASupplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e16280.

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