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High-intensity focused ultrasound inhibits lncRNA MAFA-AS1 -modulated stability of HIF-1 mRNA in an m6A-dependent manner to suppress aerobic glycolysis and adriamycin resistance in ovarian cancer

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► ***Original article***

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ABSTRACT

Background: High-intensity focused ultrasound (HIFU) represents a therapeutic medical procedure that operates by inducing ablation and mechanical disruption. Despite its established efficacy, its potential impact on tumor chemotherapy remains uncertain. Long noncoding RNA (lncRNA) MAFA-AS1 facilitated cancer cell proliferation and fostering drug resistance, but the precise significance and functional implications of MAFA-AS1 in the context of ovarian cancer (OC) remain largely unexplored. The objective of this experiment is to further investigate the potential of HIFU in inhibiting the chemotherapy resistance mechanism of OC. ***Materials and Methods:*** Five types of human ovarian cancer cells were employed in this study. There were four different groups, namely MAFA-AS1 siRNA, si-NC, pcDNA3.1-HIF-1 (hypoxia-inducible factor-1), and pcDNA3.1-control. Quantitative real-time PCR, cell proliferation assay, apoptosis assay, western blot assay, subcellular fractionation, aerobic glycolysis analysis, RNA immunoprecipitation (RIP), and luciferase reporter assay were conducted for experimentation and validation. ***Results:*** Significant upregulation of MAFA-AS1 in OC cells was observed. Through loss-of-function experiments based on HIFU, we unraveled its oncogenic functions in OC. MAFA-AS1 was discovered to bind to HIF-1 mRNA, thereby enhancing its expressed stability. Further investigations revealed an interaction between MAFA-AS1 and fat mass and obesity-associated protein (FTO), positively modulating HIF-1 mRNA stability in an FTO -dependent manner. Importantly, MAFA-AS1 exerts its effects on OC by acting through HIF-1. ***Conclusion:*** The study underscores the role of MAFA-AS1 in promoting aerobic glycolysis and chemical resistance in OC by up-regulating HIF-1 expression, suggesting that targeting MAFA-AS1 holds promise as a therapeutic strategy for OC patients undergoing chemotherapy.

INTRODUCTION

Little improvement has been made in improving the overall survival rate of ovarian cancer (OC) over the last three decades, positioning it as the most lethal among all gynecological malignancies [1]. In cases of advanced or metastatic OC, chemotherapy remains the primary therapeutic method. However, a significant proportion of patients either inherently develop resistance to chemotherapy or acquire it during treatment, resulting in limited efficacy. Consequently, it becomes imperative to elucidate the molecular mechanisms that underlie chemotherapy resistance. Such insights carry the potential to reveal novel targets for augmenting OC therapies.

High-intensity focused ultrasound (HIFU) stands out as a promising medical technique for ablating or mechanically disrupting targeted lesions. Besides its well-documented effects on tissue and mechanical disruption, emerging evidence indicates that HIFU may have the potential to enhance immune responses against carcinogenic conditions [2]. Notably, HIFU is emerging as a promising approach for the treatment of recurrent ovarian, cervical, metastatic pelvic, endometrial, and rectal cancers [3]. Consequently, a comprehensive investigation is warranted to explore HIFU's potential influence on these facets of ovarian cancer advancement.

Long non-coding RNAs (lncRNAs) are characterized by their lack protein-coding capability [4]. Recently identified subtypes of lncRNAs have been implicated in the regulatory networks of hematopoietic malignancies [5]. Accumulating evidence underscores the dual roles played by numerous lncRNAs, serving as both tumor suppressors and oncogenes. These lncRNAs intricately modulate genomic expressions pivotal for cancer development, progression, and chemotherapy resistance [6-8]. Additionally, studies related to MAFA-AS1 have also been carried out. For example, MAFA-AS1 has been identified as a prognostic biomarker associated with poor overall survival (OS) and

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disease-free survival (DFS) in patients with hepatocellular carcinoma (HCC) [9]. Furthermore, lncRNA MAFG-AS1 has been implicated in promoting cell proliferation, migration, and epithelial-mesenchymal transition (EMT) through the miR-3196/STRN4 axis in drug-resistant cells of liver cancer [10]. MAFA-AS1 has been shown to promote myeloma growth and confer chemical tolerance via the JNK and p38 MAPK pathways [11]. However, the intrinsic significance of MAFA-AS1 in the progression of OC remains predominantly unexplored.

Under aerobic conditions, cancer cells exhibit the Warburg effect, wherein they avidly uptake glucose and convert it to lactate [12]. The proliferation of cancer cell necessitates abundant nucleotides, proteins, and lipids, all sourced from glucose-associated metabolic pathways [12]. The initiation of Warburg effect relies on various rate-limiting enzymes, that steer the metabolic alterations observed in cancer cells [13]. Targeting the Warburg effect has emerged as a significant anti-tumor strategy, illustrated by exploration of hypoxia-inducible factor-1 (HIF-1) as a potential therapeutic target in JAK2V617F-positive neoplasms [14]. However, the precise mechanisms driving the initiation and promotion of processes associated with the Warburg effect in OC remain incompletely comprehended.

N6-methyladenosine (m6A) modification is a prevalent internal alteration of mRNA in mammalian cells, exhibiting a broad distribution and abundant occurrence in transcripts [15]. Unlike other mRNA modifications, m6A alteration is a dynamically reversible process [16]. Predominantly controlled by methyltransferases such as METTL3, METTL14, and WTAP, and demethylases like fat mass, obesity-associated protein (FTO) and alkB homolog 5 (ALKBH5), the m6A modification involves the addition or removal of the methyl group from mRNA's adenosine base, thereby governing m6A modification [16]. There are also related literature indicating the involvement of HIF in the regulatory modification of m6A, influencing the cancer-related activities [17, 18]. Mounting evidence highlights the substantial contribution of m6A modification to various human malignancies [19]. For instance, selectively targeting the RNA m6A reader YTH domain family proteins 2 (YTHDF2) has been shown to impair cancerous stem cells in acute myeloid leukemia (AML) [20]. Additionally, the mRNA m6A modification facilitated by methyltransferase like-14 (METTL14) has been implicated in fostering leukemia development, contributing to its onset [21]. However, the role and mechanisms of m6A modification in OC remain incompletely understood, necessitating further investigation to unveil their significance in the disease.

Building upon existing evidence, our hypothesis posits that HIFU has the potential to inhibit the stability of *HIF-1* mRNA, a process regulated by lncRNA MAFA-AS1. This interference is postulated to result in the suppression of drug resistance in OC. The primary aim of our experiment is to elucidate the role of HIFU in the treatment of OC, thereby providing an experimental basis for new treatment strategies for OC. This study represents the first investigation into the potential therapeutic impact of HIFU on ovarian cancer (OC) chemotherapy resistance through the modulation of lncRNA MAFA-AS1 and HIF-1 mRNA stability. By elucidating the intricate molecular interactions underlying HIFU-mediated disruption of chemoresistance mechanisms, our findings offer novel insights into the therapeutic potential of HIFU in OC management. Moreover, the identification of MAFA-AS1 as a key regulator of HIF-1 mRNA stability adds a new dimension to our understanding of OC pathogenesis and provides a basis for the development of targeted therapeutic strategies aimed at overcoming chemoresistance in OC patients.

MATERIALS AND METHODS

Survival rate

The association between lncRNA MAFA-AS1 and overall survival in OC was analyzed using the gene expression profile interactive analysis (GEPIA) database (http://gepia.cancer-pku.cn/).

Cell culture and HIFU treatment

Human OC cell lines, namely OVCAR3 (TCHu228), ES-2 (TCHu111), and SKOV3 (TCHu185), were purchased from the Cell Resources Center, Shanghai Academy of Life Sciences, Chinese Academy of Sciences (Shanghai, China). The human OC cell line A2780 (AW-CCH152) and normal ovarian epithelial cell line IOSE80 (AW-CNH242) were obtained from the Abiowell Biotechnology (Changsha, China). All cell lines were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; Solarbio, Beijing, China) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS; Solarbio, Beijing, China) at 37°C with 5% CO_2. The authentication of cell lines was verified through Short Tandem Repeat (STR) profiling.

For HIFU treatment, cells (2×10^6 cells) were loaded into a 15 mL centrifuge tube. The probe was encased with a sterile membrane and submerged approximately 1 cm below the liquid level for 60 seconds. Pulses were administered at an Isotropy Spatial Averaged Level (ISAL) of 589 W/cm^2, with a pulse duration of 500 milliseconds. All procedures were meticulously conducted within a biosafety cabinet (Heraeus Herasafe, Thermo Fisher scientific, Waltham, MA, USA).

Plasmids construction and transfection

MAFA-AS1 siRNA (5'-AUAUAUGCACAUCGUUUUC

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UC-3' and 5'- GAAAACGAUGUGCAUAUAUCA-3'), si-NC (5'-AUAGCUCCUAGUUAUGAGGAC-3' and 5'-CCUCAUAACUAGGAGCUAUGC-3'), vector pcDNA3.1-HIF-1 (HIF-1, NM_001028720.5), and pcDNA3.1-control (V79020, Invitrogen, California, CA, USA) were obtained from Genepharma (Shanghai, China) and GeneCopoeia (Rockville, MD, USA). The siRNA and vector transfection were facilitated using Lipofectamine 2000 (Invitrogen, Camarillo, CA, USA). The specific operations were conducted following the instructions provided with the kit.

Quantitative real-time polymerase chain reaction (PCR)

Trizol reagent, acquired from Absin biotech (Shanghai, China), was used for ribonucleic acid (RNA) isolation. The qRT-PCR assay was performed with SYBR Green qPCR Master Mix (Solarbio, Beijing, China). Using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference gene, the relative gene expression was identified according to the $2^{-\Delta\Delta Ct}$ method. qPCR was run on an ABI 7500 device (Applied Bio-systems, Waltham, MA, USA). The sequences of primers used are the following: MAFA-AS1 5'-AGGACTGACTGAGCAACT-3' and 5'-AAGCAGGATGGAGATGGA-3'; GAPDH 5'-ATGATGACATCAAGAAGGTGGTG-3' and 5'-CCATGAGGTCCACCACCCTGTTG-3'; HIF-1 5'- GAAC-GTCGAAAAGAAAAGTCTCG-3' and 5'- CCTTATCAA-GATGCGAACTCACA-3'. U6 5'-CTCGCTTCGGCAGCACA-3' and 5'-AACGCTTCACGAATTTGCGT-3'.

Cell viability assay

The OC cell growth curves were assessed using the Cell Counting Kit-8 (CCK-8) assay. Briefly, 2000 cells per well were incubated for 1-5 days, followed by treatment with 10μl of CCK-8 (HY-K0301-100T, MedChemExpress, Shanghai, China) for 2 hours. Cell viability was determined by measuring the Optical Density (OD) at 450 nm. The experiment was replicated thrice to ensure reliability. A microplate reader (HW-400G, Shanghai Huxi, Shanghai, China) was utilized to determine the OD at a wavelength of 450 nm. The proliferation rate was computed using the following formula: cell proliferation rate (%) = (OD treatment group - OD Blank) / (OD control group - OD Blank) × 100%.

Drug sensitivity assay

Cell sensitivity to ADR (adriamycin) was evaluated through a CCK-8 assay, and the IC_{50} value was determined, representing the ADR concentration causing a 50% decrease in absorbance compared with the control. Briefly, transfected or un-transfected cells were seeded into each well of a 96-well plate at 2×10^3 per well overnight. Subsequently, they were incubated with various concentrations (1, 2, 3.64, 4.31, 6.31, 15.85, 59.38, 233.57 and 479.25 μM) of ADR (Sigma-Adrich, St. Louis, MO, USA) for 24 h, followed by HIFU treatment. After that, cell viability was assessed using a CCK-8 assay according to the manufacturer's instructions.

Apoptosis assay

Cell apoptosis was assessed by flow cytometry using BD Biosciences apoptosis staining kit (BD Biosciences, San Jose, CA, USA). The collected cells were centrifuged for 5 minutes at 1500 rpm, stained with Annexin V-phycoerythrin (PE) and fluorescein isothiocyanate isomer I (FITC). After staining, the cells were incubated light-free at room temperature for 20 minutes, and then examined using a flow cytometer.

Western blot assay

For cell harvesting and lysis, a radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) was used under cold conditions. The protein concentrations were subsequently determined using the Bradford protein assay kit from Beyotime (Shanghai, China). The proteins were then separated via 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Solarbio, Beijing, China) and then transferred onto polyvinylidene fluoride (PVDF; Solarbio, Beijing, China) membranes. Following this, the membranes were blocked with 5% non-fat milk powder (Solarbio, Beijing, China) at room temperature for 1.5 hours, after which they were incubated with antibodies specific to HIF-1 (diluted at 1:2000, ab1, Abcam, Cambridge, UK) and β-actin (diluted at 1:2000, ab6276, Abcam, Cambridge, UK) at 4°C overnight. Subsequently, Western blots were washed three times with Tris Buffered Saline Tween (Solarbio, Beijing, China) and then incubated with a secondary antibody (diluted at 1:2000, ab205719, Abcam, Cambridge, UK) for 1.5 hours. Further washing with Phosphate Buffer Saline Tween-20 was carried out. The signal was visualized using an enhanced chemiluminescence assay (EMD Millipore, Billerica, MA, USA). β-actin served as the internal control, and the semi-quantitative analysis of band intensity was performed using ImageJ software (version 1.52r; National Institutes of Health, Bethesda, MD, USA).

Subcellular fractionation

Nucleus and cytoplasm isolation from OC cells was conducted following the PARIS Kit (Invitrogen, Carlsbad, CA, USA) guidelines for subcellular localization of MAFA-AS1. Subsequently, qRT-PCR analysis was performed to assess the levels of GAPDH, MAFA-AS1, and U6.

Aerobic glycolysis analysis

OC cells were cultured in a 96-well plate, and media supernatants along with cells were collected for the measurement of glucose consumption, lactate

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production, and adenosine triphosphate (ATP) generation. The assays employed the Glucose Uptake Assay kit, lactate assay kit II, and ATP Colorimetric Assay kit, following the manufacturer's instructions (Biovision, Milpitas, CA, USA).

For the assessment of extracellular acidification (ECAR) and oxygen consumption rate (OCR) assay, the Seahorse XF Glycolysis Stress Test Kit (Seahorse Bioscience, Billerica, MA, USA) and Seahorse XF Cell Mito Stress Test Kit (Seahorse Bioscience, Billerica, MA, USA) were employed in accordance with the provided guidelines. Briefly, the indicated cells were allowed to grow in stress test medium in Seahorse XF 96 tissue cell culture plates. Sequential injections of glucose, oligomycin, as well as 2-Deoxy-D-glucose (2-DG) were performed for ECAR measurement. To analyze OCR, the assay employed oligomycin, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), rotenone and Rote/AA. The Seahorse XF Report Generator was used for ECAR and OCR analysis.

RNA immunoprecipitation (RIP)

The RIP assay was performed using the Magna RIP kit (Sigma-Aldrich, St. Louis, MO, USA). Cell lysates were subjected to an 8-hour incubation with magnetic beads conjugated with antibodies targeting IGF2BP2 (ab117809, 1:100, Abcam, Cambridge, UK), METTL3 (ab240595, 1:100, Abcam, Cambridge, UK), or IgG (ab205718, 1:200, Abcam, Cambridge, UK). The input (cell lysates) served as a positive control. Following immunoprecipitation, the isolated RNA was examined for enrichment levels of MAFA-AS1 and HIF-1.

For mRNA extraction, mRNA fragments were incubated overnight with anti-m6A-conjugated magnetic beads using the Magna MeRIP™ m6A kit (Sigma-Aldrich, St. Louis, MO, USA) in accordance with the provided protocols. Immunoprecipitation of m6A-modified mRNAs was conducted, and the levels of associated HIF-1 were quantified.

Luciferase reporter assay

The luciferase reporter vectors were prepared by BersinBio (Guangzhou, China). In brief, the HIF-1 promoter region was PCR amplified, and the sequences were inserted into the pGL3 plasmid to create luciferase reporter vectors. OC cells with MAFA-AS1 knockdown were transfected with either pGL3 or pGL3-HIF-1 along with pRL-TK. Luciferase activity was measured after 48 hours using Dual-Luciferase® Reporter Assay System (Promega, Madison city, WI, USA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). The data were presented as mean ± SD. Differences were determined using Student's *t*-test or ANOVA, followed by post hoc tests (Tukey's/ Bonferroni's) as appropriate. A p-value less than 0.05 was considered statistically significant.

RESULTS

HIFU inhibits MAFA-AS1 expression, cell viability and promotes chemoresistance and apoptosis in OC cells

The study initially investigated the expression of MAFA-AS1 in four different OC cell lines and a normal cell line. Analysis of OC patients showed that those with low MAFA-AS1 expression exhibited significantly better outcome (figure 1A, $p<0.05$). Furthermore, the data of figure 1B revealed a significant increase in MAFA-AS1 content in OC cells compared to IOSE80 cells ($p<0.05$). HIFU treatment significantly inhibited the expression of MAFA-AS1 in two OC cells (SKOV3 and ES-2; figure 1C, $p<0.05$). Subsequent CCK-8 assays demonstrated that the MAFA-AS1 downregulation by HIFU treatment led to a notable reduction in cell viability in OC cells (figure 1DE, $p<0.05$). Moreover, MAFA-AS1-downregulated OC cells exhibited lower IC_{50} values compared to the control groups (figure 1FG), indicating increased sensitivity to the chemotherapeutic agent (ADR). Flow cytometry analysis and quantitative analysis of cell apoptosis following HIFU treatment for 48 hours revealed a higher percentage of apoptotic or dead cells in the MAFA-AS1-downregulated groups compared to the control groups (figure 1HI, $p<0.01$).

HIFU inhibits the aerobic glycolysis in OC cells

To explore the role of HIFU in regulating aerobic glycolysis in OC cells, we investigated its effects on key parameters of aerobic glycolysis. Our observations revealed that HIFU treatment in OC cells significantly reduced glucose uptake, lactate generation, and ATP generation (figure 2A-C, $p<0.05$). Furthermore, HIFU treatment induced a decrease in ECAR, indicating a reduction in glycolytic flux in the cells (figure 2D, E, 2F, G). These findings provide strong evidence that HIFU treatment plays a regulatory role in the aerobic glycolysis of OC cells.

MAFA-AS1 promotes HIF-1 mRNA stability

To investigate the mechanism underlying the role of MAFA-AS1 in the malignant phenotype of OC cells, several experimental assays were conducted. Firstly, the subcellular localization of MAFA-AS1 was examined and found its predominant localized in the cytoplasm (figure 3A), suggesting the potential involvement of MAFA-AS1 in post-transcriptional regulation. A luciferase reporter assay was utilized to verify the interaction between MAFA-AS1 and HIF-1. Interestingly, si-MAFA-AS1 did not make a considerably effect on the activity of HIF-1 (figure 3B).

To further explore potential binding partners of MAFA-AS1, the RIP assay was conducted using MS2bp

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as a bait. A plasmid containing full-length MAFA-AS1 with MS2 binding sites (MS2bs) was co-transfected with an MS2bp-GFP expression plasmid into OC cells. Subsequent RIP analysis revealed the enrichment of HIF-1 mRNA in the MAFA-AS1 RIP fraction compared to control groups, confirming the specific binding between MAFA-AS1 and HIF-1 mRNA (figure 3C, $p<0.05$). Moreover, MAFA-AS1 downregulation significantly reduced HIF-1 expression in OC cells (figure 3DE, $p<0.05$). Besides, MAFA-AS1 knockdown promoted the degradation of HIF-1 mRNA (figure 3FG, $p<0.05$). Taken together, these findings indicate that MAFA-AS1 upregulates HIF-1 expression in OC cells by enhancing the stability of *HIF-1* mRNA at the post-transcriptional level.

MAFA-AS1 upregulates HIF-1 through association with FTO

The results demonstrated that *HIF-1* mRNA was significantly enriched by the m6A antibody compared to the IgG control (figure 4A, $p<0.05$), indicating the presence of m6A modification on HIF-1 mRNA. Furthermore, our study analyzed the influence of MAFA-AS1 knockdown on m6A methylation status of *HIF-1* mRNA in OC cells. Interestingly, it was observed that downregulation of MAFA-AS1 markedly increased the m6A methylation of *HIF-1* mRNA (figure 4B, $p<0.05$). These findings suggest that MAFA-AS1 negatively regulates the m6A modification of HIF-1 mRNA, potentially contributing to *HIF-1* mRNA stability.

To investigate the involvement of m6A de-methyltransferases FTO and ALKBH5 in the MAFA-AS1-mediated regulation of *HIF-1* mRNA m6A modification, a RIP assay was conducted using antibodies specific to FTO and ALKBH5. As shown in figure 4C, D, the FTO antibody, but not the ALKBH5 antibody, significantly enriched MAFA-AS1 transcripts ($p<0.05$), indicating a potential interaction between MAFA-AS1 and FTO. To further confirm this interaction, we assessed the binding of *FTO* to *HIF-1* mRNA in the context of MAFA-AS1 knockdown. The results showed that knockdown of MAFA-AS1 significantly decreased the binding of *FTO* to *HIF-1* mRNA (figure 4E, F, $p<0.05$), suggesting that the presence of MAFA-AS1 is required for the association between *FTO* and *HIF-1* mRNA. Moreover, we investigated the functional relevance of the MAFA-AS1 and FTO interaction by examining the effect of FTO overexpression on si-MAFA-AS1-induced down-regulation and degradation of HIF-1 (figure 4G, H, $p<0.05$). Taken together, these findings suggest that MAFA-AS1 positively regulates the m6A modification of *HIF-1* mRNA through its association with FTO.

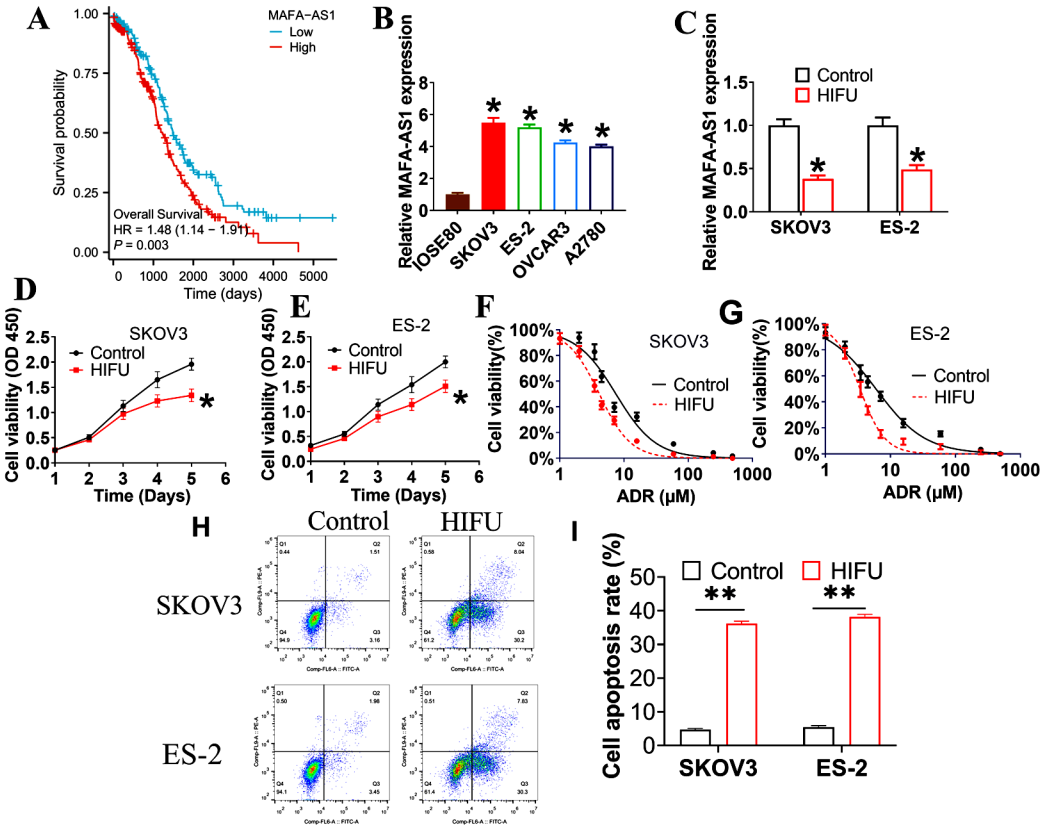

Figure 1. HIFU inhibits MAFA-AS1 expression, cell viability and promotes chemoresistance and apoptosis in OC cells. **(A)** Overall survival of patients with OC between high and low MAFA-AS1 expression. **(B)** The expression levels of MAFA-AS1 were quantified using qRT-PCR in the different cells. **(C)** MAFA-AS1 expression was measured in OC cells after HIFU treatment. **(D-E)** The proliferation of control and MAFA-AS1-downregulated OC cells was assessed using the CCK-8 assay. **(F-G)** The IC50 values of control and MAFA-AS1-downregulated OC cells were assessed using a CCK-8 assay and IC50 curve. **(H-I)** The cell apoptosis of OC was measured using Annexin-V FITC methods and quantitative analysis. *$p<0.05$ or **$p<0.01$ vs. IOSE80 cells or control group.

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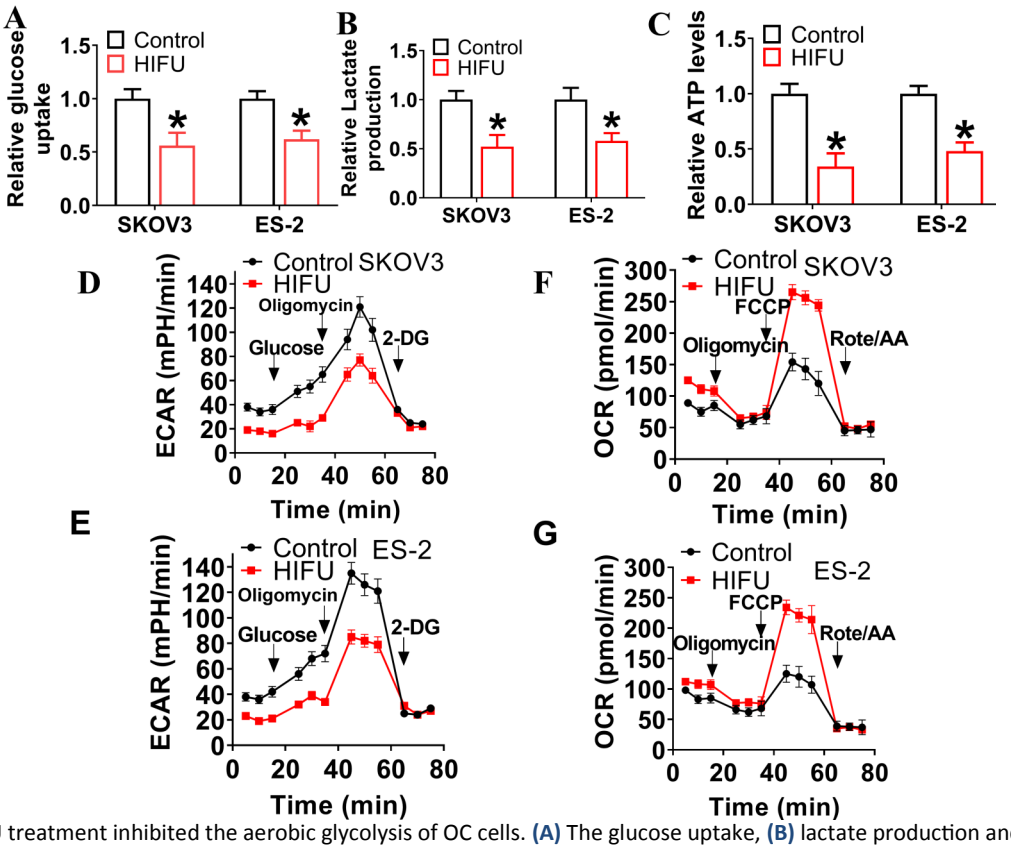

Figure 2. HIFU treatment inhibited the aerobic glycolysis of OC cells. **(A)** The glucose uptake, **(B)** lactate production and **(C)** the ATP levels of OC cells was measured. **(D, E)** ECAR of OC cells was assessed. (F, G) The alterations in OCR, indicating glycolytic activity, were monitored in OC cells. *p<0.05 vs. control group.

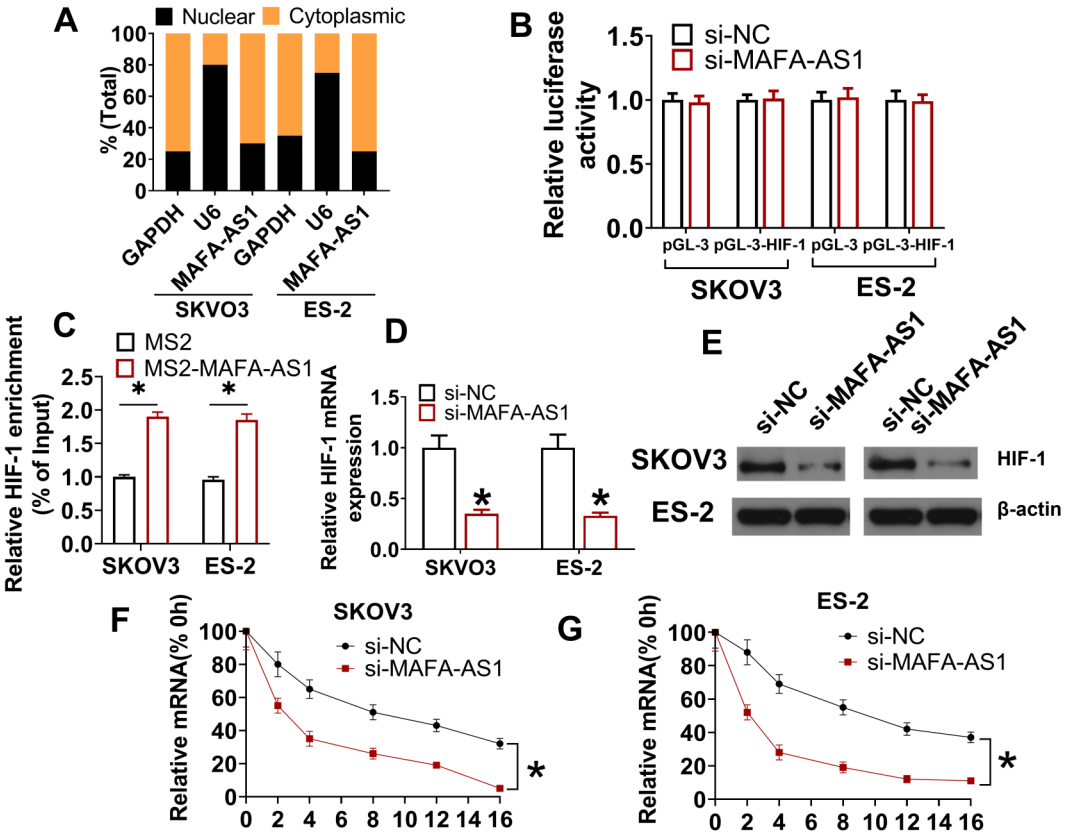

Figure 3. MAFA-AS1 associates with HIF-1 mRNA and promotes its stability. **(A)** MAFA-AS1 localization was assessed in SKOV3 and ES-2 cells using subcellular fractionation and qRT-PCR analysis. **(B)** Luciferase reporter assay was performed to detect interaction MAFA-AS1, and HIF-1 in ES-2 cells. **(C)** The direct interaction was verified by MS2-RIP assays between MAFA-AS1 and HIF-1 in ES-2 cells. **(D-E)** Down-regulated levels of HIF-1 mRNA were detected by qRT-PCR and western blot assay. **(F-G)** MAFA-AS1 knockdown promoted the degradation of HIF-1 mRNA in OC cells. *p<0.05 vs. MS2 or si-NC group.

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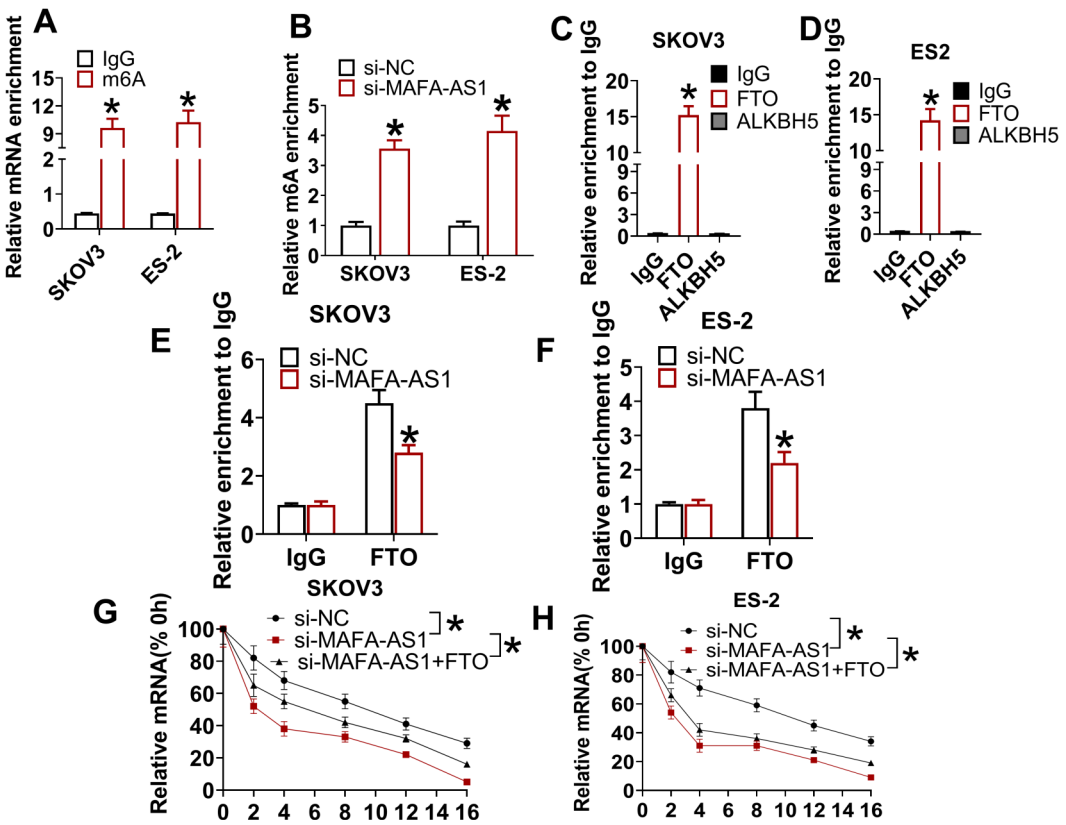

Figure 4. MAFA-AS1 upregulates HIF-1 through FTO. **(A)** The m6A modification of HIF-1 mRNA was detected in SKOV3 and ES-2 cells using MeRIP assay. **(B)** MeRIP assay was conducted to investigate the m6A modification of HIF-1 mRNA in cells with and without MAFA-AS1 downregulation. **(C, D)** The RIP assay was performed in SKOV3 and ES-2 cells to detect pull-down MAFA-AS1, followed by quantification analysis. **(E, F)** The RIP assay was performed in control and MAFA-AS1-downregulated OC cells. **(G, H)** The degradation of HIF-1 mRNA mediated by MAFA-AS1 knockdown was rescued by overexpression of FTO. *p<0.05 vs. IgG or si-NC group.

MAFA-AS1/FTO/HIF-1 axis facilitates OC proliferation and chemoresistance

In the rescue experiments, our aim was to further confirm the roles of the MAFA -AS1/FTO/HIF-1 axis in OC chemoresistance. Firstly, to evaluate the impact on cell proliferation, cell proliferation assays were performed. MAFA-AS1 knockdown suppressed cell proliferation in OC cells, and this effect was partially rescued by *HIF-1* mRNA overexpression (figure 5A, B, p<0.05). Furthermore, we investigated the chemo-sensitization effect of MAFA-AS1 knockdown and its potential rescue by *HIF-1* mRNA overexpression. The results demonstrated that MAFA -AS1 knockdown sensitized OC cells to chemotherapy, but *HIF-1* mRNA overexpression partially rescued this effect, indicating that HIF-1 contributes to the chemoresistance phenotype (figure 5C, D).

Next, to evaluate the impact on aerobic glycolysis, glucose uptake assay, ECAR assay and OCR assay were conducted. MAFA-AS1 knockdown suppressed glucose uptake in OC cells, and this effect was partially rescued by *HIF-1* mRNA overexpression (figure 5E, F, p<0.05, and 5G, H).

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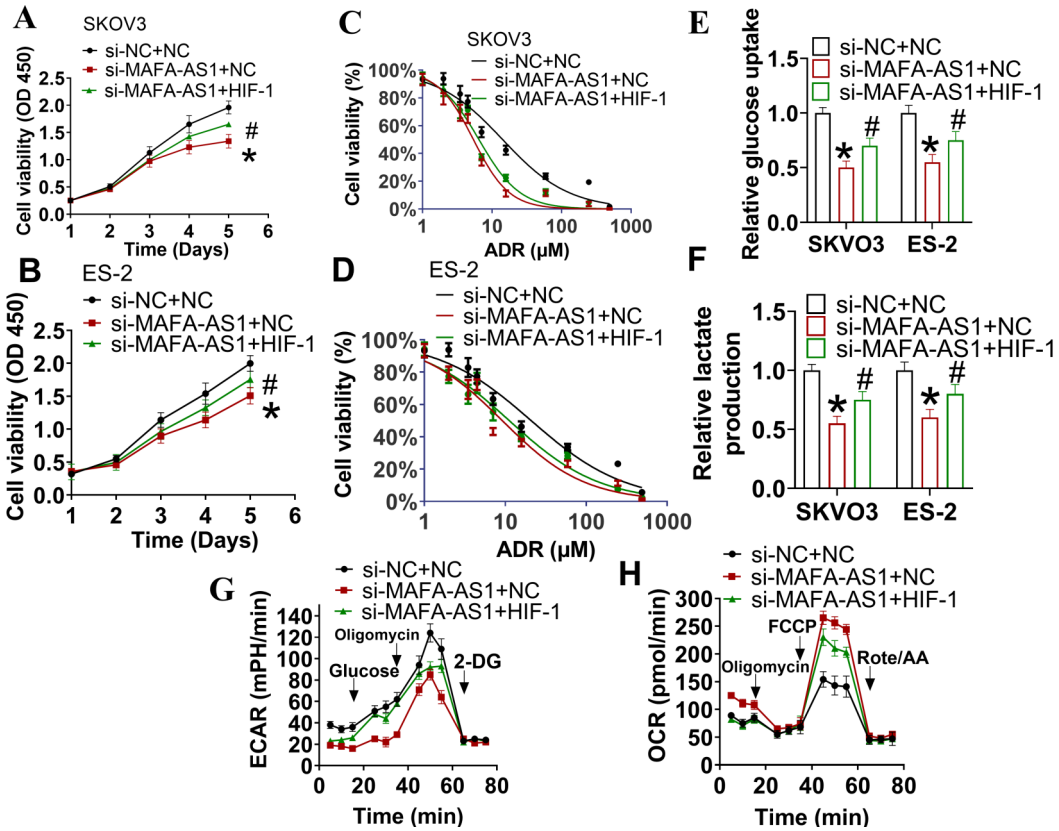

Figure 5. MAFA-AS1/FTO/HIF-1 axis facilitates OC proliferation and chemoresistance. **(A, B)** Cell ability was assessed using MTT assay over the indicated days. **(C, D)** The chemo-sensitization effects of MAFA-AS1 knockdown in the OC cells were partly reversed by HIF-1 mRNA overexpression. **(E, F)** Glucose uptake was performed among the different groups. **(G, H)** ECAR and OCR were carried out to test the metabolism situation in OC cells. *p<0.05 vs. si-NC+NC group; #p<0.05 vs. si-MAFA-AS1+NC group.

DISCUSSION

Chemotherapy resistance is a significant challenge in OC therapy, emphasizing the need to delve into the intricate mechanisms underlying this phenomenon to enhance the efficacy of therapeutic strategies. Our investigation aimed to uncover the mechanisms involved in OC chemoresistance, with the goal of identifying a potential biomarker for chemoresistance. HIFU is a non-invasive technology studied for tumor treatment. Studies have assessed its feasibility and efficacy in treating OC [3, 22]. In our study, we expanded on the findings, revealing that HIFU reduced the expression of lncRNA MAFA-AS1. Furthermore, HIFU treatment lessened OC cell chemoresistance *in vitro*, indicating its potential to overcome OC chemoresistance. The potential impact of HIFU treatment on OC chemoresistance might be linked to the downregulation of MAFA-AS1. In another study, it was discovered that HIF-1 could positively regulate the expression of WTAP under hypoxic conditions, thereby enhancing the Warburg effect within cells and influencing the development and progression of OC [23]. Further research is essential to comprehensively reveal the molecular pathways underlying the interaction among HIFU, MAFA-AS1, and OC chemoresistance. Uncovering these mechanisms holds the potential to enhance the efficacy of chemotherapy for OC patients.

Indeed, emerging research underscores the vital roles of lncRNAs in various cancer-related processes, encompassing proliferation, migration, metastasis, and notably, chemoresistance. In nasopharyngeal carcinoma, for instance, the anti-differentiation non-coding RNA (lncRNA DANCR or ANCR) has been shown to stimulate tumor growth and confer chemotherapy resistance by suppressing phosphatase and tensin homolog (PTEN) expression. This highlights the intricate and diverse regulatory functions that lncRNAs can exert in the context of cancer, with potential implications for therapeutic strategies [24]. Recent studies have placed significant emphasis on the newly identified and conserved lncRNA MAFA-AS1. The heightened expression of MAFA-AS1 in various carcinomas underscores its substantial role in the regulation of cancer-related processes [9, 10, 25]. This suggests that MAFA-AS1 may play a crucial role in the molecular pathways associated with cancer development and progression. However, its exact functions and molecular mechanisms in OC remain unclear. Our findings indicate an elevation of MAFA-AS1 in OC cells. The knockdown of MAFA-AS1 hampers proliferation and reduces chemotherapy resistance, suggesting a role for MAFA-AS1 in promoting OC growth and elevating chemoresistance through m6A modifications. This

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study serves as initial evidence of MAFA-AS1 driving OC progression and chemoresistance. Additionally, reports have suggested that lncRNAs can interact with RNA-binding proteins (RBPs) to modulate the stability of target mRNAs, adding another layer of complexity to the regulatory mechanisms involved [26]. In our study, the verification of MAFA-AS1's direct promotion of HIF-1 expression and mRNA stability provides strong evidence confirming MAFA-AS1's role in OC chemoresistance through the stabilization of *HIF-1* mRNA. This finding adds valuable insights into the molecular mechanisms underlying OC progression and resistance to chemotherapy.

Cancer cells commonly exhibit abnormal metabolism, a phenomenon known as the Warburg effect. This metabolic shift is characterized by increased rates of aerobic glycolysis, wherein cancer cells preferentially utilize glycolysis for energy production even in the presence of oxygen. This metabolic alteration involves heightened glucose uptake and an increase in lactic acid production, contributing to the unique energy demands and characteristics of cancer cells [13, 27]. There is growing evidence linking heightened aerobic glycolysis in malignancies to chemoresistance. This association is attributed to the increased endogenous antioxidant capacity facilitated by the accumulation of pyruvate and lactate within cancer cells. Consequently, targeting aerobic glycolysis has emerged as a promising approach for cancer therapy, aiming to disrupt the metabolic adaptations that contribute to the resistance of cancer cells to chemotherapy [12, 28, 29]. The role of lncRNAs in aerobic glycolysis and chemoresistance of OC remains still uncertain. On the other hand, HIF-1α has been reportedly linked to chemoresistance of OC cells. For instance, IL-6 induces the nuclear translocation of HIF-1α, exacerbating chemoresistance in OC cells [30]. Autophagy induced by HIF-1α contributes to cisplatin resistance in OC cells [31]. Besides, several studies indicated the important relationship between metabolic reprogramming and chemoresistance in cancer progression. IDH2 stabilizes HIF-1α-mediated metabolic reprogramming and enhances chemoresistance in urothelial cancer [32]. The activation of reactive oxygen species/phosphatidyl-inositol 3-kinase/serine-threonine kinase (ROS/PI3K/Akt) and Wnt/β-catenin signaling pathways promotes HIF-1α-mediated metabolic reprogramming, contributing to 5-fluorouracil resistance in colorectal cancer [33]. Despite advancements, the exact upstream HIF-1 regulation, and its OC chemoresistance role remain unclear.

RNA epigenetics is an expanding domain within cancer research. Amongst the alterations observed in mRNA, the m6A modification stands out as a pivotal factor in human malignancies [19, 34]. Mounting evidence reveals enzymes regulating m6A "writing," "erasing," and "reading" impact cancer initiation and progress. FTO was the first "eraser" enzyme identified for m6A, and it has been associated with the progression of various types of cancer [35]. Previous research on FTO's cancer connection focused on analyzing its risk for the different types of cancer [36]. Recently, the oncogenic role of FTO has been elucidated, primarily through its engagement in mRNA demethylation processes. This understanding has shed light on FTO's regulatory mechanisms in cancer development [34]. For example, Wu *et al.* [37] reported that m6A methylation regulator FTO promotes oxidative stress and induces cell apoptosis in OC. FTO also implicates m6A RNA modifications in the regulation of cyclic AMP signaling involved in stemness and OC initiation [38]. These findings provide insights into the role of FTO in OC and its potential as a therapeutic target. In our study, the knockdown of MAFA-AS1 hindered HIF-1 expression and aerobic glycolysis, affirming the significance of HIF-1 in glycolysis and chemoresistance of OC. Our study confirms the role of MAFA-AS1 in mediating glycolysis via the FTO/HIF-1 pathway. This enriches understanding and offers potential for future OC glycolysis-targeted therapies.

Despite the insightful findings, the current study has some inherent limitations. The experiments were conducted *in vitro* rather than *in vivo*. Additionally, the precise and complex regulation behind the suppression of the aerobic glycolysis and chemotherapy resistance of ovarian cancer by high-intensity focused ultrasound inhibiting the stability of *HIF-1* mRNA regulated by lncRNA MAFA-AS1, necessitates further in-depth exploration.

CONCLUSION

Our study highlights the importance of the MAFA-AS1/FTO/HIF-1 axis in the aerobic glycolysis of OC cells, impacting chemotherapy sensitivity. This finding offers fresh insights into OC chemoresistance and underscores the potential of targeting the MAFA-AS1/FTO/HIF-1 axis to enhance OC chemotherapy efficacy of HIFU treatment. This research has significant implications for the development of novel therapeutic approaches in the treatment of OC.

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