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Original Article

eIF3a mediates HIF1α-dependent glycolytic metabolism in hepatocellular carcinoma cells through translational regulation

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Abstract: eIF3a is the largest subunit of eIF3 complex and is a key player in translational control. Recently eIF3a is recognized as a proto-oncogene, which is overexpressed and connected to tumorigenesis of many cancers. However, the mechanistic roles of eIF3a during the tumorigenesis remain largely elusive. Here, we report that depletion of eIF3a significantly reduced HIF1α protein level and cellular glycolysis ability. Mechanistically, we found that eIF3a regulates HIF1α protein synthesis through internal ribosomal entry site (IRES)-dependent translation. Importantly, through analyses of our own sample collection, we found that eIF3a is overexpressed in hepatocellular carcinoma (HCC) tissues, and a high level of eIF3a predicts poor prognosis of HCC patients. TCGA analyses further confirmed that eIF3a is coincident with an elevated activity of HIF1α pathway genes. Collectively, we identify eIF3a as a regulator for glycolysis through HIF1α IRES-dependent translational regulation, which may be a potential therapeutic target for HCC.

Keywords: eIF3a, HIF1α, glycolysis, HCC

Introduction

Translational control is one of the key regulations of gene expression, with four major steps including initiation, elongation, termination, and ribosome recycling [1, 2]. Among them, the initiation step is rate-limiting and highly regulated [3]. In eukaryotes, the eukaryotic translation initiation factors (eIFs) play major roles in this process with at least 12 members [4]. Among these known eIFs, eIF3 complex, composed of 13 subunits from eIF3a to eIF3m, is the largest and most complex one [5]. Although some of these subunits are considered as core subunits of the eIF3 complex [6, 7], various subcomplexes of eIF3 with different subunits have been proven to exist [8-10]. Furthermore, diverse eIF3 subunits have different expression in tumors and were considered to have oncogenic or tumor suppressor functions [11]. For example, eIF3a, the largest subunit of eIF3, which has been reported to play roles in a number of cellular, physiological, and pathological processes, including translation initiation, cell cycle, differentiation, fibrosis, carcinogenesis, and DNA synthesis and repair [12], has been revealed potential oncogenic roles in cancer occurrence, metastasis, prognosis, and therapeutic response [12]. Importantly, eIF3a is found to be overexpressed in the carcinomas of breast, cervix [13], esophagus [14], lung [15], stomach [16], colon [17], ovary [18], urinary bladder [19], oral cavity [20], and pancreas [21]. These findings may indicate that high levels of eIF3a in tumors may be due to overexpression of the entire eIF3 complex to induce a more efficient translation initiation rate. Although this is a common feature in cancer, this possibility was not specifically examined in those tumors. Another possibility is that high levels of eIF3a regulate specific mRNAs translation. Consistent with this hypothesis, translation of hepatitis C virus RNA occurs through essential interactions between eIF3a and a

structured internal ribosome entry site (IRES) element in the viral genome [22-24]. Recently, it has found that eIF3a bound to replication protein A2 (RPA2) IRES element and inhibited its activity [25]. IRES-dependent mechanism was served as an alternative mechanism to support the translation of specific mRNAs [26]. Currently, only a few eukaryotic mRNAs have been proved to contain IRES. However, whether eIF3a has regulatory effect on all these IRES containing mRNAs is still unknown.

Hypoxia-inducible factor 1α (HIF1α) is a key transcription factor, which activates target genes involved in glycolysis and is essential for tumor cells to maintain high demand of glycolytic metabolites in keeping rapid proliferation, as well as metastasis and angiogenesis [27, 28]. The protein level of HIF1α is strictly regulated at post-transcriptional levels [29]. While oxygen-dependent hydroxylation of HIF1α by prolyl hydroxylases and subsequent ubiquitylation by von Hippel Lindau protein (VHL) have been regarded as the primary mechanism in controlling HIF1α protein level, emerging evidences indicate that HIF1α is also subjected to translational regulation [30-32]. Along this line, it has been reported that the HIF1α translation can be regulated at IRES-dependent mechanism [30], however, the exact mechanisms are not clear. Considering eif3a is found to be overexpressed in many tumors and has been shown to regulate IRES activity of certain eukaryotic mRNA, it is of great significance to investigate whether eif3a can regulate HIF1α through IRES-dependent translation.

Cancer cells in general possess enhanced protein synthesis and glycolytic capability to support the growth demand [33, 34]. However, whether and how these two steps are mechanistically connected are not clear. Here, we found that eIF3a is essential for the IRES-dependent HIF1α translation. Inhibition of eIF3a by RNAi approach in Huh-7 and HepG2, two HCC (Hepatocellular carcinoma) derived cancer cell lines, led to significantly reduced HIF1α protein and consequently drastically attenuated cellular glycolytic ability. Significantly, we found that eIF3a is overexpressed in HCC (Hepatocellular carcinoma) tissues, and high levels of eIF3a positively correlate with elevated glycolytic activities and predict poor prognosis for HCC patients, supporting our mechanistic study. As HCC is the third cause of cancer-related mortality worldwide, mainly due to its high rate of metastasis and recurrence [35], our findings not only uncovered eIF3a as a regulator for glycolysis through HIF1α IRES-dependent translational regulation, but also indicate intervention of eIF3a related translational control may confer therapeutic advantage in HCC cancers with high levels of HIF1α and glycolytic activities.

Materials and methods

Cells and transfections

Huh-7 and HepG2 (human hepatocellular carcinoma) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone) supplemented with 10% fetal bovine serum (FBS, Gibco). All cell lines were originally acquired from ATCC. Plasmid transfections were performed using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol, and luciferase assay or western blot assays were performed 48 h after transfection. siRNA transfection was performed using RNAiMAX (Invitrogen), according to the manufacturer's protocol. Cells were collected at 72 hours post siRNA transfection for following analyses. For hypoxic conditions, cells were incubated with 200 μmol/L cobalt chloride ($CoCl_2$) for 20 hours.

Plasmids and siRNA

siRNA oligonucleotides against eIF3a gene were purchased from Genepharma Inc. (eIF-3a siRNA #1: 5'-CCAUGAUAUUGCCCAGCAA-3', eIF3a siRNA #2: 5'-GCAGAUGGUCUUAGAUA-UA-3'). Human eIF3a cDNA was cloned from the HeLa cell line. The siRNA-resistant FLAG-eIF3a vector was constructed by mutation of the eIF3a region (522-540 bp) to CCACGACATCG-CTCAACAG, which had no impact on the encoded protein sequence. The HIF1α overexpression plasmid was constructed by inserting the HIF1α open reading frame, isolated by PCR from human cDNA, into pRevTet-On. IRES reporter constructs pRF, pRhifF and pRHCVF were a generous gift from Dr Gregory Goodall.

Western blot

Western blot analysis was performed using the following antibodies: anti-eIF3a (Cell Signaling Technology 3411); anti-HIF1α (Cell Signaling Technology 3716); and anti-β-Actin (Proteintech 60008-1-Ig).

RT-qPCR

Total RNA was isolated using TRIzol Reagent (Invitrogen) according to manufacturer's protocol. cDNA was synthesized with PrimeScript RT reagent kit (Takara Bio) containing random primers using 1 μg of extracted RNA per sample. RT-qPCR was performed using SYBR Green Master Mix (Roche) with the Roche LightCycler 480 Instrument II system. β-Actin was used as an internal control for normalization. Amplifications were carried out using the following primers: HIF1α forward 5'-TCATCCATGTGACCATGAGG-3' and reverse 5'-CGGCTAGTTAGGGTACACTTC-3'; eIF3a forward 5'-GTGGGAGTCTTACAGGCAGT-3' and reverse 5'-GGTTATGGTGGCGCTGAATC-3'; GLUT1 forward 5'-CTTGGCTCCCTGCAGTTTG-3' and reverse 5'-GGACCCATGTCTGGTTGTAG-3'; GLUT 2 forward 5'-CTGCATCATTCTTTGGTGGG-3' and reverse 5'-CCATCAAGAGAGCTCCAACT-3'; GLUT 3 forward 5'-GAATTAGATTACAGCGATGGG-3' and reverse 5'-GTGTTGTAGCCAAATTGGA-3'; PGK1 forward 5'-CTTCCAGGAGCTCCAAACTG-3' and reverse 5'-CTGTGGGGGTATTTGAATGG-3'; PDK1 forward 5'-GAAGCAGTTCCTGGACTTCG-3' and reverse 5'-ACCAATTGAACGGATGGTGT-3'; LDHA forward 5'-CTTCTAAAGGAAGAACAGACCC-3' and reverse 5'-CAAGAGCAAGTTCATCTGCC-3'; eIF3a forward 5'-GTGGGAGTCTTACAGGCAGT-3' and reverse 5'-GGTTATGGTGGCGCTGAATC-3'; β-Actin forward 5'-CCCTGGAGAAGAGCTACGAG-3' and reverse 5'-CGTACAGGTCTTTGCGGATG-3'.

Measurement of cellular glucose uptake

For glucose uptake assays, cells were incubated in the culture medium with 100 μM 2-NBDG (Peptide Institute) for 1 hrs, as previously described [36]. Fluorescence was measured by fluorescence-activated cell sorting (FACS) using Canto Flow Cytometer (Becton Dickinson). Values were calculated as the mean fluorescence intensities of the indicated samples.

Real-time measurement of the extracellular acidification rate (ECAR)

ECAR was performed according to the manufacturer's guidelines by Seahorse XF96 extracellular Flux analyser (Agilent). In brief, 20,000 cells per well were seeded in 96-well multiplates, and then incubated in a 5% CO_2 incubator at 37°C. The next day, the culture medium was removed and replaced by XF glycolytic stress test medium (powdered DMEM, 143 mM NaCl, 3 mg/l phenol red, 2 mM L-glutamine (Invitrogen), pH 7.3-7.4 at 37°C) and incubated for 1 h in a non-CO_2 incubator at 37°C. ECAR values were measured after injections of 10 mM glucose (port A), 1 μM oligomycin (port B) and 50 mM 2-DG (port C). All additives were purchased from Sigma-Aldrich unless indicated otherwise. For all experiments, three biological replicates were performed and within each biological replicate at least three technical replicates were used.

Luciferase assay

Luciferase reporter constructs were transfected for 48 hours and the firefly and renilla luciferase activities were measured using the Dual Luciferease kit (Promega) according to the manufacturer's instructions.

HCC tissue microarrays and IHC

A total of 106 formalin-fixed paraffin embedded HCC tissues (containing tumor and paratumor compartments) were collected from consecutive patients with HCC who underwent curative resection from 2006 to 2007 at the Liver Cancer Institute of Fudan University (Shanghai, China). Histopathological diagnoses were based on World Health Organization criteria. Ethical approval was obtained from the research ethics committee of Fudan University affiliated Zhongshan Hospital, and written informed consent was obtained from each patient. Among the 106 patients, 91 cases were male, 15 cases were female. The age distribution was from 27 to 80 years old. Tumor size ranged from 1 cm to 20 cm. Follow-up data were summarized at the end of May 2013, with a median follow-up of 54 months. Tissue microarrays (TMAs) were constructed as previously described [37] and immunohistochemistry (IHC) was performed as described elsewhere [38, 39]. IHC analysis was performed using the following antibodies: anti-eIF3a (Cell Signaling Technology 3411).

Statistical analysis

Statistical analysis was performed with SPSS software (19.0; SPSS, Inc., Chicago, IL). Values are presented as mean ± standard deviation (SD). The Student t test was used for comparisons between groups. Pearson's correlation

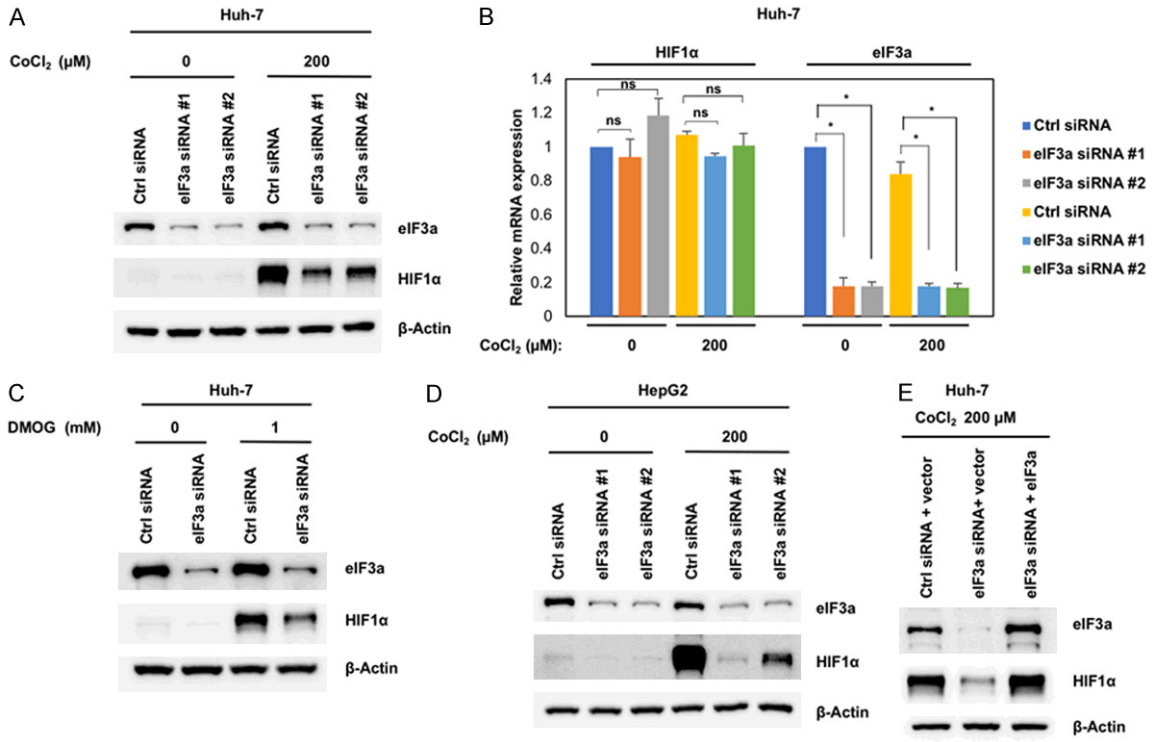

Figure 1. eIF3a depletion led to decreased HIF1α protein level in cancer cells. A. WB analyses of HIF1α, eIF3a and β-Actin (as control) in the indicated Huh-7 cells under $CoCl_2$ treatment. B. RT-qPCR analyses of HIF1α in the control and eIF3a KD Huh-7 cells under the indicated treatment and data are represented as mean ± SD from three biological replicates. *$P < 0.05$, T test. C. WB analyses of HIF1α and eIF3a in the control and eIF3a KD Huh-7 cells under the treatment of PHD inhibitor DMOG. β-Actin was used as control. D. WB analyses of HIF1α, eIF3a and β-Actin (as control) in the indicated HepG2 cells under $CoCl_2$ treatment. E. WB analyses of HIF1α, eIF3a and β-Actin (as control) in the control, eIF3a KD and eIF3a KD Huh-7cells with the indicated rescuing construct under $CoCl_2$ treatment.

analyses were performed for the mRNA levels of *eIF3a* and selected HIF1α target genes. Overall survival rates were analyzed using Kaplan-Meier's method and the log-rank test. P < 0.05 was considered statistically significant.

Results

eIF3a depletion led to decreased HIF1α protein level in cancer cells

To investigate whether eIF3a regulates HIF1α translation, RNAi approach was performed in a human HCC cell line, Huh-7. Notably, we found that eIF3a knock down (KD) using two independent siRNAs significantly decreased HIF1α protein level, under both normoxic condition and $CoCl_2$ treatment, which inhibits O_2-dependent PHD activity and mimic hypoxia condition, in Huh-7 cells (**Figure 1A**). In addition, we examined HIF1α mRNA levels in the control and eIF3a KD Huh-7 cells. As shown in **Figure 1B**, the HIF1α mRNA level was not affected by eIF3a depletion under either normoxic or hypoxic conditions. Since oxygen-dependent hydroxylation of HIF1α by prolyl hydroxylases and subsequent ubiquitylation by von Hippel Lindau protein (VHL) have been considered as the primary mechanism in controlling HIF1α protein level [29], we wanted to clarify whether HIF1α protein is reduced by eIF3a-KD via the ubiquitin proteasome degradation, however, the prolyl hydroxylase inhibitor dimethyloxalylglycine (DMOG) did not restored HIF1α protein level in eIF3a KD cells to a comparable level in the control Huh-7 cells, suggesting that eIF3a regulation of HIF1α is not at the level of protein destabilization (**Figure 1C**). Such eIF3a and HIF1α connection was further verified in another HCC cell line, HepG2 (**Figure 1D**). Thus, the reduction of HIF1α protein is likely occurred at translational steps upon eIF3a inhibition. Importantly, such deceased was largely rescued by reintroduction of a RNAi-resistant eIF3a in the eIF3a KD cells, excluding potential off-target effect of the RNAi approach (**Figure 1E**).

eIF3a depletion led to decreased cellular glycolytic activity in HCC cells

Next, we wanted to further determine the cellular effect of eIF3a depletion in HCC cells. As mentioned in the introduction, HIF1α is a key transcription factor to activate target genes involved in glycolysis, which is required by tumor cells to maintain high demand of glycolytic metabolites in keeping fast proliferation, as well as metastasis and angiogenesis [27, 28], we examined the expression of glycolysis genes in the control and eIF3a KD Huh-7 cells. As shown in **Figure 2A**, in consistent with the reduced HIF1α protein level, eIF3a depletion led to significantly reduced mRNA expression of most of the glycolysis genes targeted by HIF1α. To further examine the phenotype, glucose uptake was measured using a fluorescent glucose analog, 2-NBDG, by flow cytometric analysis, which showed a significant decrease of glucose uptake in the eIF3a KD Huh-7 cells (**Figure 2B**), consistent with the downregulation of GLUT expression. To confirm the metabolic alterations upon eIF3a depletion, we monitored live cells using Seahorse. Compared with the control Huh-7 cells, eIF3a KD led to a significant decrease of the extracellular acidification rate (ECAR), an index of glycolytic activity (**Figure 2C**). In addition to Huh-7 cells, we also found significant reduction of glycolytic activity in HepG2 cells when eIF3a was knocked down (**Figure 2D**), suggesting such regulation is likely general but not Huh-7 specific.

eIF3a regulates HIF1α protein translation through IRES-dependent mechanism

We then wondered what could be the mechanism of eIF3a in regulating HIF1α translation. It has been reported that HIF1α translation could be regulated through both conventional cap-dependent and IRES-dependent mechanisms [30, 40, 41]. Since mTOR-S6K-4EBP1 pathway is essential in activation of global cap-dependent translation, we treated the control and eIF3a KD Huh-7 cells with a well-established mTOR inhibitor, Torin 1, to suppress cap-dependent translation. However, Torin 1 treatment did not affect the change of HIF1α protein level between control and eIF3a KD cells (**Figure 3A**), suggesting that the eIF3a regulation of HIF1α translation is not cap-dependent. We next turned to the possibility of IRES-dependent translational regulation. To do so, we took the advantage of the commonly used HIF1α IRES-dependent bicistronic reporter construct (**Figure 3B**), with which the ratio of firefly over renilla luciferase activity reflects the level of IRES-dependent translation. Supporting an IRES-dependent mechanism, we found that the reporter gene with HIF1α IRES showed significant lower activities in the eIF3a KD Huh-7 and HepG2 cells compared with control cells, respectively (**Figure 3C** and **3D**), consistent with the changes observed for the endogenous proteins (**Figure 1A** and **1D**). Such effect could also be observed for a Hepatitis C virus (HCV) IRES reporter, a well-known eIF3a target (**Figure 3C** and **3D**) [22-24].

Finally, and importantly, restoring HIF1α expression level through a non-IRES containing HIF1α construct in eIF3a Huh-7 KD cells reversed the effect of eIF3a depletion on glycolysis regulation (**Figure 3E** and **3F**), indicating that eIF3a influences cellular glycolysis state through its regulation of the IRES-dependent HIF1α translation.

Elevated eIF3a expression and its correlation with HIF1α activities in the aggressive type of liver cancer

As global metabolic programing to glycolysis is a hallmark of tumorigenesis, and HIF1α activation has been demonstrated to play a crucial role by activating glycolytic genes during this process, we next wondered whether the regulation of HIF1α by eIF3a is also involved in HCC progression [27, 28]. To assess eIF3a expression in HCC, we first examined eIF3a expression by IHC in 106 HCC tissues and matched para-tumor tissues. Such exercise revealed significantly elevated eIF3a protein in 50.9% of the cancer samples compared to paired para-cancer tissues (**Figure 4A**). In contrast, only 12.3% of the HCC cancer samples showed attenuate levels of eIF3a protein compared to the paired para-tumor tissue (**Figure 4A**). Next, we analyzed the prognostic implication of eIF3a expression in 106 HCC patients. Importantly, we found that patients with high eIF3a expression had significantly worse prognosis than those with low eIF3a expression (**Figure 4B**). These results indicate that eIF3a is likely involved in the tumorigenesis and progression of the aggressive type of HCC. Finally, we turned back to TCGA database and used the mRNA levels of HIF1α target genes as the surrogates

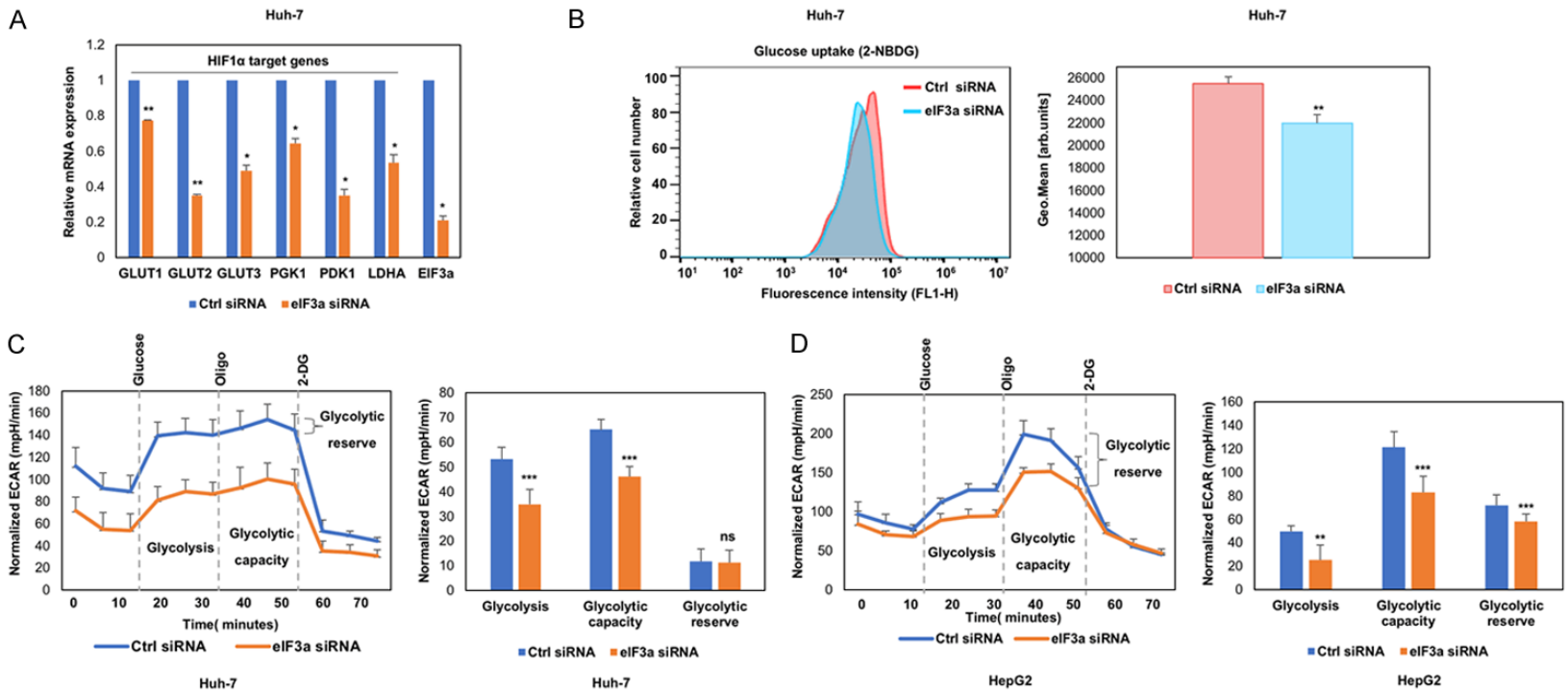

Figure 2. eIF3a depletion led to decreased cellular glycolytic activity in HCC cells. A. RT-qPCR analyses of the indicated genes in the control and eIF3a KD Huh-7 cells and data are represented as mean ± SD from three biological replicates. **P* < 0.05; ***P* < 0.01, T test. B. Glucose uptake analyses using analog 2-NBDG for the indicated Huh-7 cells. 2-NBDG incorporation was determined by flow cytometry. The representative histograms of triplicate samples (left) and geometrical mean fluorescence intensities (right) are shown. ***P* < 0.01, T test. C, D. Left, kinetic ECAR response of indicated cells to glucose (10 mM), oligomycin (1 μM) and 2-DG (50 mM). Right, quantification of glycolysis, glycolysis capacity and glycolytic reserve of indicated Huh-7 and HepG2 cells, respectively. All data are represented as mean ± SD from three biological replicates. ***P* < 0.01; ****P* < 0.001, T test.

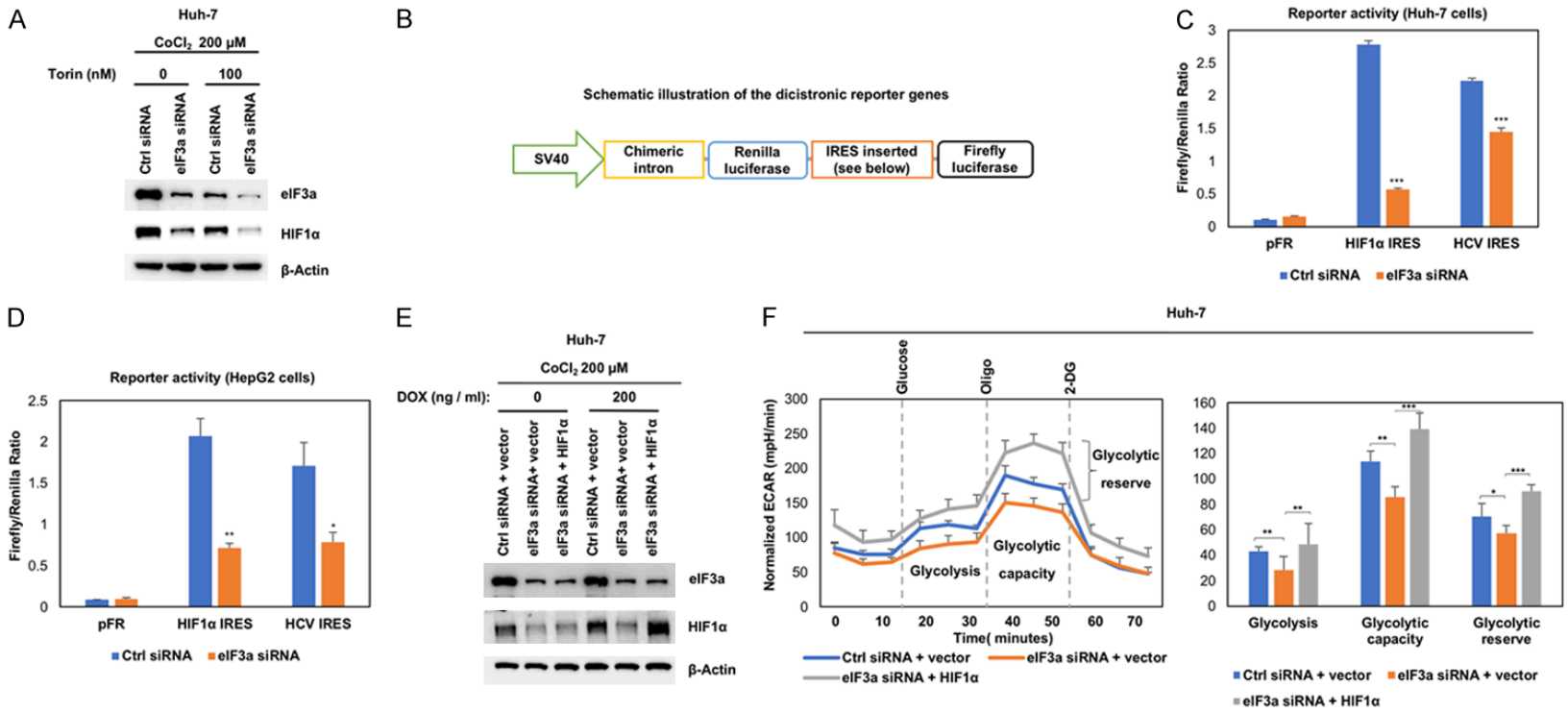

Figure 3. eIF3a regulates HIF1α protein translation through IRES-dependent mechanism. A. WB analyses of HIF1α, eIF3a and β-Actin (as control) in the control and eIF3a KD Huh-7 cells under the indicated treatments. B. Schematic representation of the bicistronic reporter constructs used in the study. C, D. Reporter gene analyses of indicated IRESes activities in the control and eIF3a KD Huh-7 and HepG2 cells, respectively. The ratios of firefly luciferase activities over renilla luciferase activities were calculated and presented as mean ± SD from three biological replicates. **P* < 0.05; ***P* < 0.01; ****P* < 0.001, T test. E. WB analyses of HIF1α, eIF3a and β-Actin (as control) in the control and eIF3a KD Huh-7 cells stably carrying the vector control or the inducible FLAG-HIF1α (non-IRES) expression construct under indicated treatments. F. Left, kinetic ECAR response of indicated cells to glucose (10 mM), oligomycin (1 μM) and 2-DG (50 mM). Right, quantification of glycolysis, glycolysis capacity and glycolytic reserve of indicated Huh-7 cells. All data are represented as mean ± SD from three biological replicates. **P* < 0.05; ***P* < 0.01; ****P* < 0.001, T test.

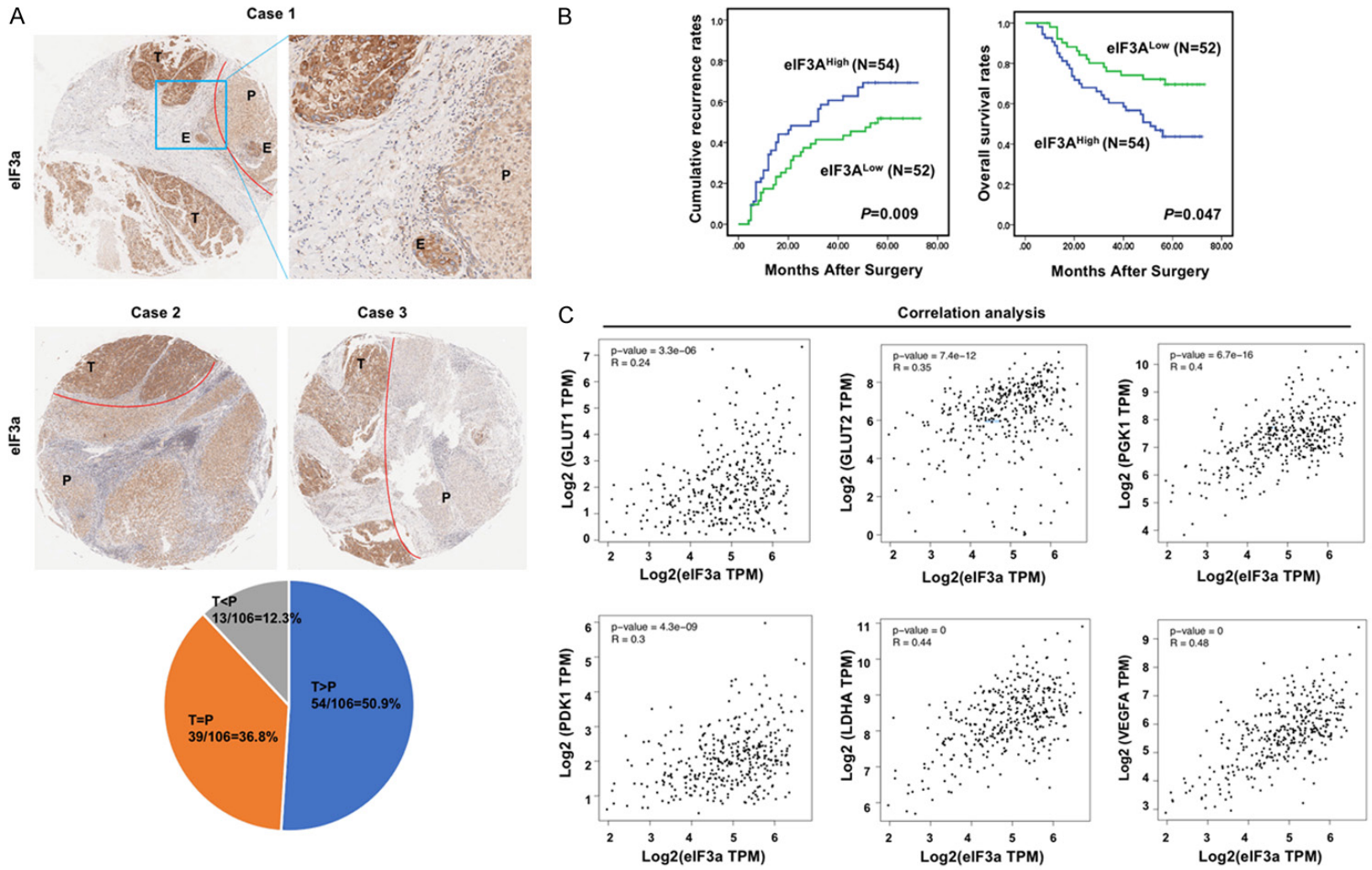

Figure 4. Elevated eIF3a expression and its correlation with HIF1α activities in the aggressive type of liver cancer. A. IHC analyses of eIF3a levels in 106 HCC tissues. T, tumor, E, cancer embolus and P, paratumor as control. Three representative cases were shown in the upper; the IHC staining intensities of all 106 cases were quantified and shown in the bottom. B. Prognostic analyses of eIF3a expression in 106 HCC patients. C. Pearson correlation analyses of the mRNA levels of eIF3a and indicated HIF1α target genes (Data from TCGA). The correlation coefficient (R) and *P* value (by two-sided t-test) were indicated. TPM represents transcripts per million.

of HIF1α activities due to stability issue of HIF1α protein during sample preparation. Consistent with the idea that eIF3a promotes HIF1α translation leading to enhanced glycolytic activity, we found strong correlations at the mRNA levels between eIF3a and 5 well-defined HIF1α target genes, such as *GLUT1*, *GLUT2*, *PGK1*, *PDK1* and *LDHA* again supporting our mechanistic findings (**Figure 4C**). In addition, we also found strong correlations at the mRNA levels between eIF3a and *VEGF, a* well-defined HIF1α target gene, related to angiogenesis [42].

Discussion

Previously, eIF3a was regarded primarily as a translation initiation factor for mRNA translation [43]. A number of recent studies suggest that eIF3a may have many regulatory roles in cellular, physiological, and pathological processes including cell cycle, differentiation, fibrosis, carcinogenesis, and DNA synthesis and repair [12]. Several specific mRNAs had been demonstrated to be regulated by eIF3a, and these mRNAs include RRM2, a-tubulin, p27kip, XPA, XPC, and RPA [18, 44-47]. A more recent study using genome-wide approach had identified a total of 375 transcripts regulated by eIF3a through direct interactions, and most of the transcripts are involved in cell cycle, differentiation, apoptosis and growth, via 5' UTR [48]. All these findings suggest that eIF3a is playing important roles in regulating specific sets of mRNAs, and its malfunction may lead to deregulation of protein synthesis and tumorigenesis. Our finding of eIF3a as a mammalian translation initiation factor, mediating glycolysis metabolism via IRES-dependent HIF1α translation, shed new light on the physiological activities regulated by eIF3a. As HIF1α is a well-established oncogene, especially under hypoxic condition and when cancer cells need to meet high glycolysis demands [27, 28], our work now provide new mechanistic understanding of eIF3a involvement in tumorigenesis through promoting IRES-dependent HIF1α translation. In addition, although most genes are regulated by cap-dependent translation mechanism, some stress-activated genes are regulated by IRES-dependent translation mechanism. Considering previous report and our work have shown eif3a has regulatory effects on IRES-dependent translation, other IRES containing mRNAs, such as CCND1 [49], ESR1 [50], may also subject to a similar regulation by eIF3a. And it will be an interesting question for future investigation.

Clinical researches have revealed that genes encoding translation initiation factors are frequently gained or lost in human tumors and thus are aberrantly expressed during cancer progression [51]. eIF3a is the largest subunit of eIF3 complex, and is widely and extensively investigated. The previous findings suggest that eIF3a is potentially a proto-oncogene and may play an important role in tumorigenesis. For example, eIF3a is found to be overexpressed in the carcinomas of breast [52], cervix [13], esophagus [14], lung [15], stomach [16], colon [17], ovary [18], urinary bladder [19], oral cavity [20], and pancreas [21]. These findings indicate that high level of eIF3a in cancer cells may promote a more efficient translation initiation rate. Although this is a common feature in cancer, the exact downstream events have not been fully explored. In our study, we found that eIF3a is significantly up-regulated in HCC tissues compared to the adjacent normal tissues, and patients with higher eIF3a expression had significantly worse prognosis, indicating eIF3a can be a potential prognostic factor of HCC. Importantly, we found depletion of eIF3a in Huh-7 cells, a well-established HCC cells, significantly decreased HIF1α protein level as well as glucose uptake and cellular glycolytic activity. We also found strong correlations in HCC at the mRNA levels between eIF3a and glycolysis genes regulated by HIF1α from TCGA database, further supporting our mechanistic findings. As many solid tumors utilize HIF1α to transcriptionally activate glycolysis pathway to generate biomaterials and energy in order to meet their rapid growth, investigating the regulatory axis of eIF3a-HIF1α-glycolysis is crucial and worthwhile. Moreover, while effective targeted therapeutics for liver cancer are still lacking, our findings highlight eIF3a related translationally regulatory node as a promising target of therapeutic intervention for liver or other cancers with elevated glycolysis state.

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Disclosure of conflict of interest

None.

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