Eukaryotic translation initiation factor 5A2 promotes metabolic reprogramming in hepatocellular carcinoma cells

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Abstract

Reprogramming of intracellular metabolism is common in liver cancer cells. Understanding the mechanisms of cell metabolic reprogramming may present a new basis for liver cancer treatment. In our previous study, we reported that a novel oncogene eukaryotic translation initiation factor 5A2 (EIF5A2) promotes tumorigenesis under hypoxic condition. Here, we aim to investigate the role of EIF5A2 in cell metabolic reprogramming during hepatocellular carcinoma (HCC) development. In this study, we reported that the messenger RNA (mRNA) level of EIF5A2 was upregulated in 59 of 105 (56.2%) HCC clinical samples (P = 0.015), and EIF5A2 overexpression was significantly associated with shorter survival time of patients with HCC (P = 0.021). Ectopic expression of EIF5A2 in HCC cell lines significantly promoted cell growth and accelerated glucose utilization and lipogenesis rates. The high rates of glucose uptake and lactate secretion conferred by EIF5A2 revealed an abnormal activity of aerobic glycolysis in HCC cells. Several key enzymes involved in glycolysis including glucose transporter type 1 and 2, hexokinase 2, phosphofructokinase liver type, glyceraldehyde 3-phosphate dehydrogenase, pyruvate kinase M2 isoform, phosphoglycerate mutase 1 and lactate dehydrogenase A were upregulated by overexpression of EIF5A2. Moreover, EIF5A2 showed positive correlations with FASN and ACSS2, two key enzymes involved in the fatty acid de novo biosynthetic pathway, at both protein and mRNA levels in HCC. These results indicated that EIF5A2 may regulate fatty acid de novo biosynthesis by increasing the uptake of acetate. In conclusion, our findings demonstrate that EIF5A2 has a critical role in HCC cell metabolic reprogramming and may serve as a prominent novel therapeutic target for liver cancer treatment.
Uncontrolled cell proliferation accompanied with disorder of cell death and tissue homeostasis. In human cancers, this is partly c-MYC dependent (26). Another study demonstrated that expression of oncogenes such as Kras and AKT (20,21), tumor suppressor genes (22) and related signalling pathways, and some the so-called onco-metabolites also give feedback to and modulate the signalling pathways (23,24). These studies revealed that the abnormal expression of oncogenes or tumor suppressor genes could not only influence the phenotypes of cancer but also regulate the metabolic network in order to help the cancer cells to adapt adverse microenvironment (20,25). However, the reciprocal regulations between metabolic pathways and cellular signaling pathways are far more complicated and still need more broaden investigation.

Although the function of EIF5A2 has been widely studied, its effects on cancer metabolism have not been explored. In fact, the biochemical functions of EIF5A family have been documented in the studies on plants and yeast more than 20 years ago. For example, TaeIF5A1 in Tamarix androssowii was reported to mediate abiotic stress tolerance, the mechanism of which includes increasing protein synthesis and preventing membrane damage (26). Another study demonstrated that EIF5A1 and EIF5A2 in Saccharomyces cerevisiae are encoded by two highly homologous genes TIF51A and TIF51B, respectively (27). TIF51A was found to only express under aerobic conditions and TIF51B was only expressed under anaerobic conditions, indicating that the two genes are structurally homologous but functionally distinct. Furthermore, our previous study also confirmed that the expression of EIF5A2 could be induced under hypoxic condition (13). In addition, we found that the overexpression of EIF5A2 could regulate glucose metabolism and amino acid metabolism in normal liver cell LO2 (28). Since EIF5A2 could promote cell growth both in the presence and in the absence of oxygen, which associated with the various ways of biosynthesis in cancer cells, it suggested that EIF5A2 may play a role in the reprogramming of cancer metabolism. In the present study, we focused on elucidating the role of EIF5A2 for liver cancer cell metabolism, aiming at providing a framework for the metabolic function of oncogene EIF5A2 in HCC, as well as presenting novel insights into potential new therapeutic target for liver cancer treatment.

Materials and methods

HCC clinical samples

All of the HCC specimens (tumor and adjacent non-tumor tissues) were collected immediately after surgical resection at the Cancer Center of Sun Yat-Sen University (Guangzhou, China) from 1999 to 2009. Tumor stages were categorized according to the tumor–node–metastasis (TNM) system by American Joint Committee on Cancer (29). Clinical information was available from 98 of 105 patients. For the protein expression studies, 10 pairs of primary HCC samples were collected from the Department of Pathology, Sun Yat-Sen University (Guangzhou, China) for immunohistochemistry and Western blot analysis.

Cell lines

HCC cell lines BEL7402, PLC8024, MHCC97L, MHCC97H, Huh7, Hep3B and an immortalized human liver cell line LO2 was obtained from the Institute of Virology of the Chinese Academy of Medical Sciences (Beijing, China).
Plasmids and reagents
The lentiviral expression plasmid reconstructed with full-length EIF5A2 (LV105-EIF5A2) and empty vector LV105 were obtained from GeneCopeia (Rockville, MD). Lentivirus were produced using the plasmids and were applied to infect HCC cells, according to the manufacturer’s instructions. Mouse anti-EIF5A2 was obtained from Abcam (Cambridge, UK). Other antibodies used in this paper were obtained from Cell Signaling Technology (Danvers, MA) [Supplementary Table 1, available at Carcinogenesis Online]. Short hairpin RNAs targeting EIF5A2 were obtained from Sigma–Aldrich (St Louis, MO).

RNA extraction and quantitative real-time PCR
Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA), and reverse transcription polymerase chain reaction was performed using PrimeScriptTM RT Master Mix (Takara, Japan) according to the manufacturer’s instructions. For quantitative real-time polymerase chain reaction (qPCR), the complementary DNA was amplified using SYBR Green (Applied Biosystems, Carlsbad, CA) and an ABI PRISM 7900 Sequence Detector. All of the primers used in this study were synthesized by Tech Dragon (Hong Kong) [Supplementary Table 2, available at Carcinogenesis Online].

Cell proliferation assay
Cell viability was determined using Cell Proliferation Kit II (XTT; Roche Diagnostics Co., Indianapolis, IN). The frequencies of individual cells that have synthesized DNA were measured using APC bromodeoxyuridine (BrdU) Flow Kit (BD Biosciences, San Diego, CA).

Fluorescence in situ hybridization
A bacterial artificial chromosome clone containing EIF5A2 probe was labeled with Cy3 by nick translation (Life Technologies, Inc.). Then the labeled probe was hybridized to metaphase chromosomes of the cell lines used in this study as described previously [9]. Metaphase chromosomes were counterstained by 4’,6-diamidino-2-phenylindole after hybridization process. Twenty randomly selected metaphase spreads from each cell line were counted and the average copy number of EIF5A2 gene was calculated.

Glucose and lactate detection
Glucose detection kit (glucose oxidase–peroxidase method; Rongsheng, Shanghai, China) and lactate detection kits [Jiancheng, Nanjing, China] were used to determine the concentrations of glucose and lactate in culture medium. Cells were seeded in six-well plates, and the medium was replaced by fresh complete culture medium the next day. After 24 h of incubation, the medium was collected and centrifuged at 4000g for 5 min at 4°C, then the supernatants were used for metabolite detection immediately.

BODIPY® 558/568 C12 staining
A fatty acid probe BODIPY® 558/568 C12 [4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a diazacyclopenta-3-dodecanonic acid, Thermo Fisher Scientific, Waltham, MA] was used to trace the fatty acid synthesis. Cells were seeded on glass coverslips in six-well plates and cultured in complete medium with 0.5 μM BODIPY® 558/568 C12 for 24 h before observation.

Oil red O staining
The Oil Red O stock solution was prepared by completely dissolving 0.5 mg of Oil Red O powder (Sigma–Aldrich) in 100 mL of isopropanol and kept at 4°C. The working solution was freshly prepared every time before use by adding 6.0 mL of stock solution to 4.0 mL of autoclaved MilliQ water. The mixture was filtered through a 0.22 μm filter to remove any precipitates. Cells were seeded on glass coverslips till reaching the confluence of 80% in six-well plates and incubated at 37°C in a 5% carbon dioxide incubator. The cell layers were fixed with 4% paraformaldehyde and incubated in freshly prepared Oil Red O working solution at room temperature for ~50–60 min with gentle shaking. Then, the working solution was removed, and the coverslips were washed and transferred onto clean slides and photos were taken using microscopy under ×200 and ×400.

UPLC/LTQ-Orbitrap XL MS analysis of intracellular metabolites
The sample separation was injected onto a Waters HILIC column accelerated by an Accela liquid chromatographic system (Thermo Fisher Scientific). Mass spectrometric (MS) analysis was performed using an LTQ-Orbitrap XL MS (Thermo Fisher Scientific) fitted with an electrospray source operated in both positive and negative ion modes. The mobile phase consisted of A [0.1% formic acid in acetonitrile:water (vol/vol), 35:65] containing 10 mM ammonium acetate and B (acetonitrile). The gradient program started from 10% B in 5 min and held for 5 min, then increased to 95% B in 8 min and held for 4 min followed by re-equilibration in 8 min. The samples were separated on Zorbax RRHD C18 column (2.1 × 100 mm, 1.8 μm). All data were required with scanning mass range from m/z 100 to 1000.

Subsequently, the differentiating ions were introduced into the database (Metlin and HMDB) and authentic standards for metabolite identification. Heat map was generated by MeV software (http://mev.tm4.org/Welcome).

NMR analysis of acetate in cell culture medium
The cell culture medium was lyophilized and added with 100 μL acetonitrile:water (vol/vol, 60:40). The supernatant was collected by centrifugation at 14,000g for 20 min, then was dried under the gentle nitrogen stream at room temperature prior to nuclear magnetic resonance (NMR) analysis. The mixture solution containing 500 μL of 100% deuterium oxide (heavy water) and 50 μL phosphate buffer solution (0.2 M disodium phosphate/0.2 M monosodium phosphate, pH 7.4) was used to redissolve the residue and minimize the variation in pH. A concentration of 0.3 mM 2,2-dimethyl-2-silapentane-sulfonic acid was used as an internal reference standard at δ 0.0. Then proton NMR measurements were performed on a Varian NMR system 500 MHz spectrometer equipped with triple resonance probe. A conventional presaturation pulse sequence for solvent suppression based on the one-dimensional version of NOESY pulse sequence known as NOEPR (Nuclear Overhauser Effect spectroscopy with Presaturation, delay-90-11-90-tm-90-acquisition) was used. A total of 256 scans with spectral width of 5 kHz were collected for all NMR spectra. All the signals were zero filled to 16 k before regular Fourier transformation.

UPLC–MS/MS analysis
Two hundred microliter of cold solvent mixture [ACN:MeOH:H2O, 40:40:20, vol/vol/vol] was added to the dried residue. The mixture was vigorously vortexed for 30 s and stored at −20°C for 1 h, then centrifuged at 14,000 g for 15 min at 4°C. The 150 μL supernatant of each sample was transferred to a new glass tube for ultra performance liquid chromatography (UPLC–MS)/MS analysis. The analysis was performed on a TSG Quantum Ultra triple quadrupole MS (Thermo Fisher Scientific) via an electrospray interface, operating in negative ionization mode and configuring in selective reaction monitoring mode. The metabolite separation was performed using an ACQUITY UPLC® BEH Amide (1.7 μm, 100 × 2.1 mm) column (Waters, Ireland). The mobile phases were consisted of acetonitrile (A) and 20 mM ammonium formate and 20 mM ammonium hydroxide in solvent mixture (water:acetonitrile, 95:5, vol/vol) (B). The gradient elution program initiated from 80% A; decreased to 65% A in 4 min; to 60% A in 6 min; to 55% A in 8 min; to 50% A in 10 min; and held for 13 min with a flow rate of 0.3 mL/min.

MS conditions were optimized for each metabolite using reference standard. The liquid chromatography–MS/MS data were acquired and processed with LCQuanTM software version 2.5.6 (Thermo Fisher Scientific).

Statistical analysis
All statistical analyses were performed with SPSS for Windows, version 16.0 (SPSS Inc., Chicago, IL). All of the experiments were repeated for at least three times, and the data were expressed as means ± SD. The correlation between EIF5A2 expression and clinical–pathological characteristics of HCC patients was analyzed by the Pearson χ2 test. Paired Student’s t-test was used to assess the statistical significance between two pre-selected groups. Statistically significant difference was declared if P < 0.05. Survival curves were generated according to the Kaplan–Meier method, and statistical analysis was performed using log-rank test.
Results

EIF5A2 is frequently overexpressed in HCC patients
Expression of EIF5A2 between tumor and paired non-tumorous surrounding tissues in 105 HCC cases was compared by qPCR. Overexpression of EIF5A2 (defined as a >2.0-fold increase) was detected in 59 of 105 (56.2%) HCC samples ($P = 0.015$), compared with paired non-tumor tissues (Figure 1A). Western blot analysis showed that EIF5A2 was obviously upregulated in HCC tumor tissues (Figure 1B) as well as in HCC cell lines (Figure 1C). Expression level of EIF5A2 in HCC specimens was also investigated by immunohistochemistry with a monoclonal antibody against EIF5A2. The results confirmed the upregulation of EIF5A2 in HCC tumor tissues compared with paired adjacent non-tumor tissues (Figure 1D).

The correlation of EIF5A2 overexpression with HCC clinicopathological features was analyzed statistically using the qPCR data from 105 pairs of informative HCC clinical samples. The results found that EIF5A2 overexpression was positively associated with hepatitis B surface antigen ($P = 0.048$), differentiation ($P = 0.009$) and tumor stage ($P = 0.001$) (Table 1). Kaplan–Meier analysis showed that EIF5A2 overexpression was significantly associated with shorter survival time of patients with HCC ($P = 0.021$; Figure 1E). These findings showed that EIF5A2 may play an important role in HCC development.

EIF5A2 promotes cell proliferation in HCC cells
To study the role of EIF5A2 in HCC tumorigenesis, we established BEL7402 and PLC8024 cells with EIF5A2 stable knock-down (BEL7402/PLC8024-sh-1/-3) as well as LO2 and MHCC97H cells showing no or less endogenous EIF5A2 transcripts with EIF5A2 stable overexpression (LO2/MHCC97H-EIF5A2) by lentiviral transduction. Empty vector-transfected (LO2/MHCC97H-vec) or non-template short hairpin RNA-transfected (BEL7402/PLC8024-ctrl) cells were used as controls, respectively. The transduction efficiency was confirmed using western blot analysis (Figure 2A). XTT assays showed that the cell growth rates in EIF5A2-repressing PLC8024 and BEL7402 were significantly decreased compared with the control cells ($P < 0.001$, Figure 2B). Consistently, cell growth rates were also significantly increased in EIF5A2-overexpressing LO2 and MHCC97H cells compared with the control cells ($P < 0.001$, Figure 2B). Since the amplification of EIF5A2 occurs frequently in solid tumors such as ovarian cancer (9), esophageal squamous cell carcinoma (13) and non-small cell lung cancer (15), FISH was performed to detect the genomic copy number alteration of EIF5A2 in liver cell lines used in this study. The results showed that copy number-gain of EIF5A2 was observed in PLC8024, BEL7402 and MHCC97H but not in LO2 cells (Supplementary Figure 1, available at Carcinogenesis Online).

To further study the effect of EIF5A2 on cell proliferation, BrdU incorporation assays were applied to investigate the cell proliferative abilities in EIF5A2-repressing BEL7402 cells.
The results revealed that the percentage of BrdU-incorporated cells was decreased after EIF5A2 was knocked down (Figure 2C). In addition, western blot analysis showed that several key factors related to cell cycle checkpoints such as cyclin D1 and cyclin E were downregulated, whereas p53 and p21 were upregulated after depletion of EIF5A2 in HCC cells (Figure 2D). Since c-MYC plays a significant role in cell cycle progression, the expression level of c-MYC in the stable cell lines were also detected by western blot analysis. The results showed that the expression levels of several key enzymes in the glycolysis pathway and tricarboxylic acid cycle were detected by western blot analysis. The results showed that the expression levels of several key enzymes in the glycogen pathways were positively correlated with the expression level of EIF5A2 protein (Figure 3E).

**EIF5A2 reprograms lipid biosynthesis in HCC cells**

As EIF5A2 could elevate Cit, while Cit is the main metabolite to generate acetyl-CoA, which is the primary material of fatty acid de novo synthesis, we hypothesized that EIF5A2 may regulate lipid biosynthesis. So firstly, we compared the metabolic changes between EIF5A2-overexpressing LO2 and control cells by non-targeted metabolomics analysis and found that the profiling of lipid biosynthesis showed the most obvious changes (Supplementary Figure 4, available at Carcinogenesis Online). The metabolic profiling showed that in LO2 cells with EIF5A2 overexpression, the concentration of lyso-phosphatidylcholine (lysoPC), lyso-phosphatidylethanolamine (lysoPE) and phosphatidylinositol (PI) were obviously decreased, whereas the concentration of phosphatidylcholine (PC) was significantly increased (Supplementary Figure 2, available at Carcinogenesis Online). Thus, the lipid profiling supported our notion that EIF5A2 promotes lipogenesis for tumor growth.

To further investigate the metabolic alteration in glucose utilization, we traced the incorporation of individual glucose carbons in EIF5A2-repressing PLC8024 and control cells using [U-13C6]-glucose in glucose-free Dulbecco's modified Eagle's medium D5030 (Supplementary Figure 3, available at Carcinogenesis Online). Stable isotope labeling-assisted metabolomics using liquid chromatography-MS/MS revealed that the concentrations of several intermediate metabolites in glycolysis and tricarboxylic acid cycle, including glucose-6-phosphate (G6P), citrate (Cit) and malate (Mal), were decreased in PLC8024 cells after depletion of EIF5A2 (Figure 3D). In contrast, these metabolites were increased in EIF5A2-overexpressing LO2 cells at different extents (Figure 3D).

To further confirm the metabolic changes, the protein levels of the key enzymes in glycolysis pathway and tricarboxylic acid cycle were detected by western blot analysis. The results showed that the expression levels of several key enzymes in the glycolysis pathways were positively correlated with the expression level of EIF5A2 protein (Figure 3E).

**EIF5A2 regulates glucose metabolism by promoting aerobic glycolysis in HCC**

As EIF5A2 could mediate abiotic stress tolerance in plants and its expression could also be induced under hypoxia condition, we hypothesized that EIF5A2 might play an important role in cellular metabolic reprogramming of liver cancer cells. Firstly, to evaluate whether the glucose metabolism was affected by EIF5A2, glucose uptake rates in the established cell lines were measured by Colorimetry. The results showed that the knockdown of EIF5A2 could significantly decrease glucose uptake rate in both BEL7402 and PLC8024 cells (P < 0.01), whereas overexpression of EIF5A2 could significantly increase glucose uptake rate in LO2 cells (P < 0.001). The glucose uptake rate in MHCC97H cells showed no obvious change (Figure 3A). Since lactate is the end product of aerobic glycolysis, the lactate concentrations in cell culture medium secreted by cells were also tested by colorimetry. The results showed that cells secreted less lactate after EIF5A2 was silenced, whereas more lactate was produced when EIF5A2 was overexpressed (Figure 3B).

To identify potential affected genes of the glycolysis pathway, we checked the transcription levels of several key enzymes in the glycolysis pathway by qPCR. Our results showed that the mRNA levels of enzymes including glucose transporter type 1 and 2 (GLUT1/2), hexokinase 2 (HK2), phosphofructokinase liver type (PFKL), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase A (LDHA) were significantly decreased in EIF5A2-repressing PLC8024 cells compared with the control cells (Figure 3C). In contrast, the mRNA levels of enzymes such as HK2 and PFKL were significantly elevated in EIF5A2-overexpressing LO2 cells compared with the control cells (Figure 3C).

Then, we checked the FASN and ACS2 protein expression in EIF5A2-repressing and -overexpressing clones. The results showed that the protein expression levels of FASN and ACS2 were obviously decreased after depletion of EIF5A2 in PLC8024
and BEL7402. In contrast, ectopic expression of EIF5A2 in LO2 and MHCC97H increased the expression of FASN and ACSS2 (Figure 4C). The mRNA levels of EIF5A2, FASN and ACSS2 were also examined using qPCR, and the results were consistent with the western blot results (Figure 4C).

To illustrate the impact of EIF5A2 on lipid biosynthesis, a fluorescent BODIPY lipid probe was applied to detect cellular fatty acids. After incubation with BODIPY probe 558/568 C12, fluorescence signal in PLC8024 cells with EIF5A2 knockdown was significantly decreased, compared with the control cells (Figure 4D).
Figure 3. Regulation of glucose metabolism by EIF5A2 in HCC cells. (A) Glucose uptake rates of the EIF5A2-repressing or -overexpressing clones were measured by colorimetry using glucose detection kit. The results has been normalized with the cell number of each sample. Data shown are the mean ± SD of three independent experiments. **P < 0.01, ***P < 0.001 by Student’s t-test. (B) Lactate concentration in culture medium secreted by EIF5A2-repressing or -overexpressing clones were detected by colorimetry using lactate detection kit. The results has been normalized with the total protein amount of each sample. Data are the mean ± SD of three independent experiments. **P < 0.01 by Student’s t-test. (C) Regulated key enzymes of glycolysis pathway in the EIF5A2-repressing PLC8024 clones and EIF5A2-overexpressing LO2 clones were detected by qPCR. *P < 0.05, **P < 0.01 by Student’s t-test. (D) Altered metabolites in the EIF5A2-repressing PLC8024 clones and EIF5A2-overexpressing LO2 clones were analyzed by liquid chromatography-MS/MS. Cells were fed with [U-13C6]-glucose (25 mM) in glucose-free culture medium D5030 for 8 h. The synthesis levels of metabolites in glycolysis and tricarboxylic acid cycle generated from [U-13C6]-glucose with significant alteration are shown here. **P < 0.01 by Student’s t-test. (E) Deregulation of the key enzymes in glycolysis and tricarboxylic acid cycle were detected by western Blot. β-Actin was used as internal control.
Inverse results were observed in EIF5A2-overexpressing LO2 cells (Figure 4D). Furthermore, Oil Red O staining showed that lipid droplets in EIF5A2-repressing PLC8024 cells were much less and smaller than those in control PLC8024 cells. In contrast, lipid droplets in EIF5A2-overexpressing LO2 cells were much more and larger than those in control LO2 cells (Figure 4E). These findings strongly suggested that EIF5A2 could promote metabolic process of fatty acid and lipid biosynthesis via upregulation of FASN and ACSS2 in HCC.
ACSS2 is known as the only enzyme involved in acetate/acetyl-CoA metabolism, which is responsible for providing cytoplasmic acetyl-CoA for fatty acid de novo synthesis, especially in short supply of glucose. We thus cultured cells with different concentrations of sodium acetate and glucose and detected the expression levels of ACSS2 and FASN by western blot (Figure 5A). The results showed that protein levels of ACSS2 and FASN in EIF5A2-repressing PLC8024 cells were significantly reduced than those in control PLC8024 cells when given 25 mM of glucose. The difference was more significant when given 10 mM of glucose, suggesting that EIF5A2 could regulate acetate/acetyl-CoA metabolism in HCC cells. We also compared the acetate consumption rate between EIF5A2-repressing PLC8024 and their control cells measured by NMR spectroscopy, and the data further confirmed

Figure 5. EIF5A2 enhances fatty acid de novo biosynthesis via FASN and ACSS2. (A) Expression levels of ACSS2 and FASN in EIF5A2-repressing PLC8024 clones with different concentrations of glucose and acetate were detected by western blot. (B) Relative abundance of acetate in culture medium was detected using NMR. The peak indicated by the arrow showed the position of acetate in samples. The data were normalized with the internal standard 2,2-dimethyl-2-silapentane-5-sulfonic acid and represented using bar chart in the right panel. (C) The mRNA levels of EIF5A2, FASN and ACSS2 were determined in 105 pairs of HCC clinical samples by qPCR. The Delta C of the three genes was compared between non-tumor and tumor tissues. (D) Positive correlations were detected among EIF5A2, FASN and ACSS2 relative expression in 105 clinical HCC cases, respectively. *P < 0.0001 by the Pearson χ² test.
that the cells with EIF5A2 knockdown consumed less acetate under the same culturing condition (Figure 5B).

The correlation of EIF5A2 expression with FASN and ACSS2 were also studied by qPCR in 105 randomly selected HCC clinical samples. The results showed that the mRNA levels of FASN, ACSS2 and EIF5A2 were all upregulated in HCC tumor samples compared with paired adjacent non-tumor samples (Figure 5C). Furthermore, the relative expression levels between EIF5A2 and FASN, EIF5A2 and ACSS2, as well as FASN and ACSS2 were significantly positively correlated (Figure 5D).

Discussion

The overexpression of EIF5A2 has been widely reported in many solid tumors, including HCC. Its oncogenic functions such as promoting cell proliferation, tumorigenesis, metastasis and angiogenesis implied its important role in the development and progression of human cancers. Although the function of EIF5A2 in tumorigenesis has been well investigated, there is little study clarifying the role of human EIF5A2 in cell proliferation from the metabolic perspective. In the present study, we confirmed that EIF5A2 could regulate cell proliferation in several human liver cell lines. Results from BrdU incorporation assay supported that EIF5A2 could increase the glucose uptake rates and lactate secretion rates even without given treatment such as serum depletion, indicating that EIF5A2 plays an important role in the cell proliferation process.

A critical question was then raised: How EIF5A2 participate in the process of cell proliferation? It has been well-known that the rapid cell growth requires a high metabolic rate to provide necessary energy and intermediate metabolites for the maintenance of diverse metabolic activities. And in previous studies, many oncogenes and tumor suppressor genes such as c-MYC and p53 have been reported to be associated with the reprogramming of cellular metabolism (30,31). From this respective, it is quite possible that EIF5A2 may regulate cell proliferation through reprogramming cellular metabolism. So we further investigated the metabolic changes resulted from the overexpression and knocking down of EIF5A2 in liver cell line model.

Previous studies have reported that the lack of nutrients is responsible for the occurrence of oncogenic cell cycles. In the tumor microenvironment, the rapid growth of tumor cells and the poor vascularization in tumor tissues leads to nutrient deprivation, limited oxygen supply and limited adenosine triphosphate production. To adapt to the suboptimal growth condition and satisfy the needs of proliferative tumor cells, the cellular metabolic network was reprogrammed, such as the switch of glucose utilization from tricarboxylic acid cycle to aerobic glycolysis (32,33), and some less used pathways were activated, such as pentose phosphate pathway (34,35). Our results found that the overexpression of EIF5A2 could increase the glucose uptake rates and lactate secretion rates of liver cell lines, whereas the depletion of EIF5A2 showed the opposite effects, strongly suggesting that EIF5A2 might participate in metabolic reprogramming in liver cancer cells.

In addition, we examined key enzymes involved in the glycolysis pathway in EIF5A2-repressing and EIF5A2-overexpressing HCC cell lines at both mRNA and protein expression levels. We found that GLUT1/2, HK1/2, GAPDH, pyruvate kinase M2 isofrom (PKM2) and LDHA were positively correlated with the expression level of EIF5A2. GLUT1 and GLUT2 are the two main isoforms of glucose transporters that function as transporting the blood glucose into cell cytoplasm for glycolysis process (36). The overexpression of GLUTs enable the cells to uptake more glucose for high rate of metabolic activities. HK is the first rate-limiting enzyme in glycolysis and was frequently upregulated in cancer cells (37). PKM2 is another rate-limiting enzyme that catalyzes the last step of glycolysis and produces pyruvate. The overexpression and high enzymatic activity of PKM2 is benefit for the rapid proliferation of cancer cells (38,39). LDH is a well-studied enzyme that converts pyruvate into lactate for glucose fermentation. Its dominant isofom LDHA was overexpressed in many human cancers and LDHA inhibition was considered to be a promising approach for anticancer therapy (40,41). Our study found that the mRNA levels of the above-mentioned enzymes were significantly associated with the expression level of EIF5A2 in HCC cell lines with forced or silenced EIF5A2 expression.

The mechanisms how EIF5A2 to regulate transcription of these metabolic genes is still unclear. Our recent study found that EIF5A2 could translocate from the cytoplasm to the nucleus, bind to the promoter region of HIF1α and upregulate its expression even under normoxic condition (15). The dysregulation and overexpression of HIF1α have been heavily implicated in cancer biology, specifically in areas of energy metabolism. Thus, it is reasonable to speculate that EIF5A2 may induce mRNA of metabolic genes via HIF1α signaling. The protein levels of several key enzymes in glycolysis pathway were also assessed in these cell lines, but the differences were not so obvious. Although the enzyme activity measurement has not been performed yet, it is possible that EIF5A2 may regulate one or more enzymes by controlling their activity instead of protein expression levels. Similarly, the metabolic flux analysis using [U-13C1]-glucose as a metabolic tracer indicated that the glucose consumption process was repressed by the silence of EIF5A2 and activated by the overexpression of EIF5A2.

On the other hand, the alteration of fatty acid biosynthesis and lipogenesis was also remarkable. Lipids are necessary components of cellular membrane, including glycerophospholipids such as PC and PE, and other lipids such as sterols, sphingolipids and lyso-phospholipids (19). In the aggressive cancers, de novo lipogenesis might promote membrane lipid saturation. In our study, we found an obvious increase of Cit in cell lysosPCs and lysosPEs with one fatty acid residue were downregulated, whereas PCs and PEs with two fatty acid residues were enhanced in LO2-EIF5A2 cells compared with LO2-vec cells, indicating that EIF5A2 could promote the de novo lipogenesis to meet the cell proliferation and may contribute to tumorigenesis. The BODIPY® 558/568 C12 staining and Oil Red O staining showed that the number and size of the lipid droplets in cells were positively correlated with the EIF5A2 expression levels in cells. To further explore the alteration of lipid and fatty acid metabolism regulated by EIF5A2, we also looked into the expression levels of key enzymes in fatty acid de novo synthesis in the EIF5A2-repressing and EIF5A2-overexpressing HCC cell lines. We found that both mRNA and protein levels of two enzymes of the fatty acid metabolic pathway, FASN and ACSS2, were consistent with EIF5A2 in the EIF5A2-repressing and -overexpressing HCC cell lines. The different consumption rates of acetate in the EIF5A2-repressing PLC8024 cells also supported our hypothesis that EIF5A2 could participate in lipid synthesis reprogramming via regulation of ACSS2.

In agreement with these observations, we could reach a conclusion that the oncogenic candidate EIF5A2 could promote cell proliferation and trigger cellular metabolic reprogramming in liver cancer cells, including glucose metabolism and fatty acid biosynthesis. EIF5A2 might be used as a new prognostic or predictive markers and a new target for future therapeutic interventions in HCC patients.
Supplementary material
Supplementary Figures 1–4 and Supplementary Tables 1–2 can be found at http://carcin.oxfordjournals.org/

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