Decreased expression of acetyl-CoA synthase 2 promotes metastasis and predicts poor prognosis in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most malignant cancers and has high morbidity and mortality rates in China.1 Surgery is considered one of the best treatment options for patients with this disease,2,3 although metastasis greatly limits the number of patients who are surgical candidates. Not only do many patients diagnosed with HCC show metastasis, but many postoperative patients also suffer from metastasis after undergoing curative resection.4 Therefore, metastasis is a major issue in the treatment of HCC, and elucidating its underlying mechanism is a key research topic.

Hypoxia is a common phenomenon that occurs in solid tumors, especially after tumor growth.5 Hypoxia can result in many changes in the genomic expression of tumor cells, allowing cells to adapt to harsh environments that may be associated with metastasis and poor prognosis.6–9 According to many reports, a series of changes caused by hypoxia is regulated by hypoxia-inducible factors (HIFs), namely, HIF-1α and HIF-2α. Hypoxia-inducible factor-2α is more common in chronic hypoxia than HIF-1α and is more specifically expressed in endothelial cells and hepatocytes.10 It has been reported that both the expression level and acetylation status of HIF-2α are precisely adjusted in hypoxia.11 We previously reported that HIF-2α can regulate CDCP1 (CUB domain containing protein 1) to promote PKCδ (protein kinase Cδ)-mediated migration in HCC.12 Acetyl-CoA synthase 2 (ACSS2) is an enzyme that converts acetate to acetyl-CoA, which is an important intermediate metabolite.13–14 Acetyl-CoA synthase 2 also plays a key role in the acetylation of HIF-2α, which can provide the acetyl group for acetylation.15–16 Recent studies on ACSS2 have been carried out for a variety of cancers, and there are controversies regarding the effect of ACSS2 on HCC and other cancers.17–19

Materials and Methods

Cell culture and transfection. The MHCC97H, MHCC97L, and HCCLM3 cell lines were obtained from the Liver Cancer Institute of Fudan University (Shanghai, China), and the other HCC cell lines were purchased from ATCC (American Type Culture Collection Manassas, VA, USA). The LO2 cell line was obtained from the Chinese Academy of Sciences, Shanghai, China. ACSS2 gene expression was silenced with ACSS2 siRNA, purchased from Suzhou GenePharma China (Suzhou, China) and synthesized using the following probes: ACSS2-
Acetyl-CoA synthase 2 (ACSS2) expression in hepatocellular carcinoma (HCC) cell lines and patients. (a) Expression of ACSS2 in four HCC cell lines was detected by Western blot analysis. The data showed that a higher degree of malignancy was associated with lower ACSS2 protein expression in the HCC cell lines. The LO2 cell line, which was derived from normal liver tissue, was used as a control. The LM3 and MHCC97H cell lines are highly metastatic compared with the MHCC97L cell line, whereas the Hep3B cell line shows almost no metastatic ability. All cell lines were maintained in normoxic conditions. (b) ACSS2 expression was also detected in 12 pairs of human HCC carcinoma (c) and non-carcinoma (N) tissues by Western blot analysis. Non-carcinoma tissues showed higher expression. (c) Another 13 pairs of human HCC and non-carcinoma tissues were assessed by RT-PCR, and a similar result was obtained. Average (Avg.) expression of ACSS2 mRNA in the carcinoma and non-carcinoma tissues is shown. Data are shown as the mean ± SD of three independent experiments. Error bars represent SD. *P < 0.05; **P < 0.01.

Fig. 1. Acetyl-CoA synthase 2 (ACSS2) expression in hepatocellular carcinoma (HCC) cell lines and patients. (a) Expression of ACSS2 in four HCC cell lines was detected by Western blot analysis. The data showed that a higher degree of malignancy was associated with lower ACSS2 protein expression in the HCC cell lines. The LO2 cell line, which was derived from normal liver tissue, was used as a control. The LM3 and MHCC97H cell lines are highly metastatic compared with the MHCC97L cell line, whereas the Hep3B cell line shows almost no metastatic ability. All cell lines were maintained in normoxic conditions. (b) ACSS2 expression was also detected in 12 pairs of human HCC carcinoma (c) and non-carcinoma (N) tissues by Western blot analysis. Non-carcinoma tissues showed higher expression. (c) Another 13 pairs of human HCC and non-carcinoma tissues were assessed by RT-PCR, and a similar result was obtained. Average (Avg.) expression of ACSS2 mRNA in the carcinoma and non-carcinoma tissues is shown. Data are shown as the mean ± SD of three independent experiments. Error bars represent SD. *P < 0.05; **P < 0.01.
medium with DMEM containing 0.5% serum to begin the experiment. A 10-μL tip was used to induce scratches with equal widths. The cells were then maintained at 37°C in a 5% CO₂ air incubator, and the distances between both sides of the scratch at 0, 3, 6, 9, 12, and 24 h after scratching were recorded. We chose three random sites to calculate the mean and standard deviation. Images were collected at 0 and 24 h.

**Western blot analysis and antibodies.** Protein from human tissue samples and cells was quantified by the BCA method (Thermo Fisher Scientific, Waltham, MA, USA) and separated by SDS-PAGE. The protein was then transferred to membranes (Millipore, Billerica, MA, USA). After blocking with 5% skimmed milk powder, the membrane was incubated with anti-ACSS2 rabbit polyclonal antibody (1:1000; Abcam, HongKong, China), anti-HIF-2α rabbit polyclonal antibody (1:1000, NB100-122; Novus Biologicals, Littleton, Colorado, USA), anti-transforming growth factor-α (TGFα) rabbit mAb (1:1000, ab208156; Abcam), anti-cyclinD1 rabbit mAb (1:5000, ab134175; Abcam), anti-E-cadherin rabbit polyclonal antibody (1:1000, ab15148; Abcam), anti-N-cadherin rabbit polyclonal antibody (1:500, ab18203; Abcam), anti-Snail rabbit mAb (1:1000, #3876; Cell Signaling Technology, Danvers, MA, USA), anti-vimentin rabbit mAb (1:1000, ab92547; Abcam), and anti-β-actin mouse mAb (1:1000, sc-47778; Santa Cruz Biotechnology, CA, USA). After incubating overnight at 4°C, the membrane was further probed with anti-mouse (1:4000, sc2005; Santa Cruz Biotechnology) or anti-rabbit IgG (1:4000, sc2004; Santa Cruz Biotechnology).

![Fig. 2. Downregulation of acetyl-CoA synthase 2 (ACSS2) increased the invasion and migration of hepatocellular carcinoma (HCC) cells in hypoxic conditions. (a) siRNA knockdown was verified by Western blot analysis in MHCC97H cells. Grayscale analysis of the three replicates is shown as a histogram. ACSS2 expression was normalized to β-actin. (b,c) Transwell invasion experiment and scratch test showed that downregulation of ACSS2 resulted in increased invasiveness and migration of HCC cells in hypoxic conditions. Data are shown as mean ± SD of three independent experiments. Error bars represent SD. *P < 0.05; **P < 0.01. NC, normal control.](image-url)
Real-time PCR. RNA from human tissue samples and cells was extracted with TRIzol (Invitrogen). Then 2 μg total RNA was reverse transcribed into cDNA using a transcription First Strand cDNA Synthesis Kit (Invitrogen, Paisley, UK) according to the manufacturer’s instructions. Next, quantitative real-time PCR using a Bio-Rad (Hercules, CA, USA) Laboratories CFX96 system was carried out to analyze the amount of cDNA produced in the above transcription step. The Power SYBR Green Master Mix used in the RT-PCR was purchased from Roche (Basel, Switzerland). We determined the expression in a sample using three separate experiments with triplicate wells per experiment. The primer sequences used were as follows: ACSS2 forward, 5'-GGATTCAGCTGAGTCTTC-3'; ACSS2 reverse, 5'-CATGCCACCAAGTCAATC-3'; phosphoglycerate kinase 1 PGK1 (Phosphoglycerate kinase 1) forward, 5'-CGCTGCTGCTTGAAG-3'; PGK1 reverse, 5'-CTGCTGCTGCTTAACTC-3'; vascular endothelial growth factor VEGF (Vascular endothelial growth factor) forward, 5'-GGCCAAGCATAGGAGGAT-3'; VEGF reverse, 5'-ACGCTCAGGAGCTATACCG-3'; glucose transporter 1 GLUT1 (Glucose transporter 1) forward, 5'-GGGCGTTCGTTGGG-3'; GLUT1 reverse, 5'-CCAGCTGTTGATGTTGCGG-3'; TGFα forward, 5'-CGCTCCTGGTATGTTGGG-3'; TGFα reverse, 5'-TGGGAATCTGGGCACTTATT-3'; cyclinD1 forward, 5'-GCA-TGTTCGTTGGCCTCTAAG-3'; and cyclinD1 reverse, 5'-TTCAATGAAATCGTGCGGG-3'.

Immunohistochemistry. All HCC patient paraffin blocks used for this assay were approved by the Tianjin Medical University Cancer Institute and Hospital and were confirmed histologically by HE staining. After blocking with 3% BSA, the tissues were incubated with anti-ACSS2 antibody (1:100, ab66038; Abcam), anti-HIF-2α antibody (1:250, NB100-122; Novus Biologicals) and anti-TGFα antibody (1:1000, ab208156; Abcam) for 30 min and then transferred to 4°C overnight. The next day, after incubating with a goat anti-rabbit secondary antibody PV-6000 Kit (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 1 h at 37°C, the tissues were stained with DAB and hematoxylin and then dehydrated and dried before observing. Slides with the primary antibodies omitted were used as negative controls. The staining results were estimated by one researcher who was blind to the clinicopathologic staging and outcomes of all patients during observation. Five fields were selected for an average.

Immunoprecipitation. Hypoxia-inducible factor-2α protein was immunoprecipitated from a 35-mm plate of HCC cells.
Whole-cell extracts were incubated with protein A beads (16-156; Millipore) and normal rabbit IgG (2729S; Cell Signaling Technology) to clear non-specific binding. We then incubated the supernatants with 2 μL anti-HIF-2α (NB100-122; Novus Biologicals) antibody or normal rabbit IgG (2729S; Cell Signaling Technology) for 3 h before mixing with protein A beads. After binding overnight, the supernatant was removed by centrifugation, and the immunoprecipitated proteins were eluted and detected by WB.

Statistical analysis. The results and data were analyzed by spss 18.0 software (SPSS, Chicago, IL, USA) and expressed as mean ± SD. Differences between two groups were evaluated with t-tests, and P < 0.05 was considered statistically significant. Some of the WB images were used for grayscale analysis, and the resulting histograms represent the average of three replicates. The $\chi^2$-test was used to explore the relationship between two variables. Survival curves (overall survival [OS] and disease-free survival [DFS]) of 110 patients were calculated by Kaplan–Meier analysis.

Results

Acetyl-CoA synthase 2 expression in HCC cell lines and patients. To elucidate the effect of ACSS2 on HCC, four HCC
Table 2. Clinical characteristics and laboratory parameters of patients with hepatocellular carcinoma according to ACSS2 expression level

| Parameter       | n   | ACSS2 low expression, n = 56 | ACSS2 high expression, n = 54 | P-value |
|-----------------|-----|------------------------------|------------------------------|---------|
| Age, years      |     |                              |                              |         |
| <50             | 35  | 21                           | 14                           | 0.193   |
| ≥50             | 75  | 35                           | 40                           |         |
| Gender          |     |                              |                              |         |
| Male            | 98  | 48                           | 50                           | 0.247   |
| Female          | 12  | 8                            | 4                            |         |
| Tumor size, cm  |     |                              |                              |         |
| <5              | 52  | 25                           | 27                           | 0.574   |
| ≥5              | 58  | 31                           | 27                           |         |
| Tumor number    |     |                              |                              |         |
| Single          | 76  | 35                           | 41                           | 0.128   |
| Multiple        | 34  | 21                           | 13                           |         |
| Satellite lesions |    |                              |                              |         |
| No              | 59  | 21                           | 38                           | 0.001*  |
| Yes             | 51  | 35                           | 16                           |         |
| Portal vein invasion |    |                              |                              |         |
| No              | 58  | 21                           | 37                           | 0.001*  |
| Yes             | 52  | 35                           | 17                           |         |
| Microvascular invasion |    |                              |                              |         |
| No              | 35  | 10                           | 25                           | 0.001*  |
| Yes             | 75  | 46                           | 29                           |         |
| Cirrhosis       |     |                              |                              |         |
| No              | 22  | 5                            | 17                           | 0.003*  |
| Yes             | 88  | 51                           | 37                           |         |
| Clinical stage  |     |                              |                              |         |
| Stage I and II  | 63  | 22                           | 41                           | <0.001* |
| Stage III and IV | 47  | 34                           | 13                           |         |
| Histological grade |    |                              |                              |         |
| Well            | 92  | 38                           | 44                           | 0.101   |
| Poor            | 28  | 18                           | 10                           |         |
| HIF-2α expression |     |                              |                              |         |
| Low             | 60  | 27                           | 33                           | 0.174   |
| High            | 50  | 29                           | 21                           |         |
| TGFβ2 expression |     |                              |                              |         |
| Low             | 65  | 28                           | 37                           | 0.048*  |
| High            | 45  | 28                           | 17                           |         |

HIF-2α, hypoxia-inducible factor-2α; TGFβ2, transforming growth factor 2α. *P ≤ 0.05.

cell lines and the LO2 cell line were selected for the detection of ACSS2 expression. We initiated the culture of the five cell lines at the same time and harvested the cells when they were in the logarithmic growth phase. Western blot results revealed that the MHCC97L and Hep3B cells showed higher ACSS2 expression than the more malignant MHCC97H and LM3 cell lines (Fig. S1). The LO2 cell line, which was derived from normal liver tissue, also showed high ACSS2 expression (Fig. 1a). We then detected ACSS2 expression in C and NC tissues of 25 patients by WB and RT-PCR; patient background characteristics are shown in Table 1. The WB data showed that NC tissues exhibited higher ACSS2 expression (Fig. 1b). Similar results were found in the RT-PCR data of another 13 pairs of human HCC samples (Fig. 1c). These results indicate that ACSS2 may be negatively correlated with the malignancy of HCC.

Acetyl-CoA synthase 2 knockdown promoted the invasion and migration ability of HCC cells under hypoxic conditions. We used siRNA technology to knock down ACSS2 gene expression and explored its influence on the invasion and migration ability of MHCC97H cells. Knockdown was verified (Fig. 2a), and Transwell experiments were then carried out to detect the invasion ability of the cells. A hypoxic environment was induced by adding CoCl2 (400 μmol/mL) to the medium. The results revealed that the siACSS2 group showed higher invasion ability in the hypoxic environment than the control group (Fig. 2b). The migration test showed that knockdown of ACSS2 enhanced the migration ability of MHCC97H cells under hypoxic conditions (Fig. 2c). Taken together, these results suggest that downregulation of ACSS2 expression may result in increased metastases under hypoxia.

Knockdown of ACSS2 promoted epithelial–mesenchymal transition of HCC cells without HIF-2α elevation under hypoxia. Epithelial–mesenchymal transition (EMT) is described as the process by which epithelial cells convert into individual migratory cells. The EMT phenomenon is also considered an important step in cancer metastasis. Our siRNA experiments found that more spindle-shaped cells were recognized in the hypoxia-si group under hypoxic conditions than in other groups (Fig. 3a). We then examined the relevant indicators of EMT in siACSS2 cells and negative control (NC) cells. The results showed that there were more N-cadherin, vimentin, and Snail and less E-cadherin expression in siACSS2 cells compared to NC cells during hypoxia, which indicated that EMT was promoted in siACSS2 cells under hypoxic conditions (Fig. 3b). Notably, this phenomenon did not occur in normoxic conditions (Fig. 3b). Due to the different results obtained between the hypoxic and normoxic conditions, we speculated that some hypoxia-induced molecules might lead to these differences. We next detected HIF-2α expression in siACSS2 cells and NC cells under hypoxia and normoxia. The data showed that HIF-2α expression was markedly higher under hypoxic conditions. However, there was no difference in HIF-2α protein expression levels between the siACSS2 group and the NC group (Fig. 3c), which indicated that the total amount of HIF-2α protein was not the driving factor affecting the EMT process after ACSS2 knockdown.

Acetylation of HIF-2α affected the EMT process. Epigenetic modifications, including methylation and acetylation, have great impact on protein function. Hypoxia-inducible factor-2α also undergoes these post-translational modifications. Acetylation and deacetylation modifications of HIF-2α even surpass the role of expression regulation, which in turn affects the expression of the downstream molecules of HIF-2α activity. We examined the downstream molecules of HIF-2α by RT-PCR. The result revealed increased expression levels of some molecules downstream of HIF-2α, including TGFβ2 and cyclinD1, after ACSS2 knockdown, during hypoxia (Fig. 4a). However, these changes did not occur under normoxia. We also detected some molecules that were regulated by HIF-1α, or both HIF-1α and HIF-2α, and there were no differences between the hypoxic and normoxic conditions after ACSS2 knockdown (Fig. 4a). Next, we selected TGFβ2 for further verification by WB, and the data revealed a similar result (Fig. 4b). Then we used immunohistochemistry (IHC) to investigate the relationship between ACSS2 and TGFβ2 and HIF-2α.
in HCC samples, which indicated that the correlation between ACSS2 expression and TGF-α expression had statistical significance (Table 2, Fig. S2). However, there was no similar statistical significance between the expression of ACSS2 and HIF-2α (Table 2, Fig. S2). Moreover, to ensure the acetylation status of HIF-2α, we undertook immunoprecipitation on the NC and ACSS2-knockdown groups. The data indicated significant downregulation of the acetylation levels of HIF-2α protein in the ACSS2-knockdown group (Fig. 4c), which confirmed the influence of ACSS2 on HIF-2α acetylation in other studies.\(^{(15,16)}\) Taken together, these results suggest that deacetylation of HIF-2α occurred under hypoxia after ACSS2 knockdown, which enhanced the activity of HIF-2α and promoted the EMT process.

**Low expression of ACSS2 is associated with poor prognosis and progression in HCC patients.** The cell biology experiments above showed that the metastatic potential of HCC cells increased following knockdown of ACSS2 expression. Next, we undertook IHC assays using a cohort of 110 HCC patients (2010–2015). Staining intensity was divided into four levels, as shown in Figure 5(a): negative, weak, moderate, and strong staining. We defined the first two images as the low-expression group and the other two images as the high-expression group (Fig. 5a). We analyzed the clinical pathology characteristics of 110 patients by ACSS2 expression level (Table 2) and found that ACSS2 expression was related to satellite lesions, cirrhosis, vascular invasion, and poor clinical stage. The correlation of ACSS2 expression and clinical stage was shown intuitively with a histogram (Fig. 5b). We then undertook survival analysis of the cases. Kaplan–Meier analysis revealed that low ACSS2 expression was associated with poor prognosis, which was reflected in both OS and DFS (Fig. 5c). The difference between high expression and low expression groups was statistically significant (Table 3). We recently obtained three pairs of special specimens: two specimens of PR and their corresponding N; the other specimen included C, PVTT, and N tissue. We detected ACSS2 expression in these specimens and found that the PR tissue showed lower ACSS2 expression than the corresponding non-carcinoma tissue (N), which was similar to its primary tumor (C).

Discussion

Acetyl-CoA synthase 2 is one isoform of the acetyl-CoA synthetase family, which consists of ACSS1, ACSS2, and ACSS3. Acetyl-CoA synthase 2 is localized in cytoplasm and nuclei, whereas the other two isoforms are located in mitochondria.\(^{(31)}\) Acetyl-CoA synthase 2 has been a key research topic in recent years.
Table 3. Analysis of prognostic factors influencing overall survival (OS) and disease-free survival (DFS) in patients with hepatocellular carcinoma

|                      | OS       | DFS      |
|----------------------|----------|----------|
|                      | n   | $\chi^2$-test | P-value | n   | $\chi^2$-test | P-value |
| Age, years           |      |          |         |      |          |         |
| <50                  | 35  | 3.494    | 0.062   | 35  | 1.502    | 0.220   |
| ≥50                  | 75  |          |         | 75  |          |         |
| Gender               |      |          |         |      |          |         |
| Male                 | 98  | 0.660    | 0.416   | 98  | 0.637    | 0.425   |
| Female               | 12  | 12       |         | 12  |         |         |
| Tumor size, cm       |      |          |         |      |          |         |
| <5                   | 52  | 4.621    | 0.032*  | 52  | 1.304    | 0.253   |
| ≥5                   | 58  |          |         | 58  |          |         |
| Tumor number         |      |          |         |      |          |         |
| Single               | 76  | 11.437   | 0.001*  | 76  | 11.948   | 0.001*  |
| Multiple             | 34  | 34       |         | 34  |         |         |
| Satellite lesions    |      |          |         |      |          |         |
| No                   | 59  | 20.775   | <0.001* | 59  | 17.669   | <0.001* |
| Yes                  | 51  |          |         | 51  |          |         |
| Portal vein invasion |      |          |         |      |          |         |
| No                   | 58  | 12.616   | <0.001* | 58  | 14.445   | <0.001* |
| Yes                  | 52  |          |         | 52  |          |         |
| Microvascular invasion|     |          |         |      |          |         |
| No                   | 35  | 13.368   | <0.001* | 35  | 10.825   | 0.010*  |
| Yes                  | 75  |          |         | 75  |          |         |
| Clinical stage       |      |          |         |      |          |         |
| Stage I and II       | 63  | 41.424   | <0.001* | 63  | 26.147   | <0.001* |
| Stage III and IV     | 47  | 47       |         | 47  |         |         |
| Histological grade   |      |          |         |      |          |         |
| Well                 | 82  | 0.535    | 0.465   | 82  | 0.062    | 0.804   |
| Poor                 | 28  |          |         | 28  |          |         |
| HIF-2α expression    |      |          |         |      |          |         |
| Low expression       | 60  | 7.063    | 0.008*  | 60  | 1.998    | 0.158   |
| High expression      | 50  | 50       |         | 50  |          |         |
| TGFβ expression      |      |          |         |      |          |         |
| Low expression       | 65  | 4.294    | 0.038*  | 65  | 0.841    | 0.359   |
| High expression      | 45  | 45       |         | 45  |          |         |
| ACSS2 expression     |      |          |         |      |          |         |
| Low expression       | 56  | 9.980    | 0.002*  | 56  | 7.048    | 0.008*  |
| High expression      | 54  | 54       |         | 54  |          |         |

HIF-2α, hypoxia-inducible factor-2α; TGFβ, transforming growth factor β; *P ≤ 0.05.

years with emphases on two areas. The first area is the relationship between ACSS2 and energy metabolism. It has been reported that acetate is an important source of energy for many tumors, and ACSS2 plays a key role in this energy supply process.\(^{(13,14,31,32)}\) In addition, ACSS2 is indispensable for the acetylation of certain molecules,\(^{(33)}\) such as HIF-2α.\(^{(15,16)}\) However, there are some controversies surrounding the effect of ACSS2 in tumors. Some scientists have recognized ACSS2 as a cancer-promoting factor,\(^{(13,14,32)}\) whereas others have reported opposing results, in that the lack of ACSS2 expression predicts poor prognosis in patients,\(^{(34,35)}\) especially in cancer of the digestive system. The theory of ACSS2 promoting tumors is mainly found in breast cancer and glioma, which emphasizes the role of ACSS2 in producing acetyl-CoA; the opposite opinion on ACSS2 in digestive system cancer is mainly from the IHC of clinical samples. Our data indicate that decreased expression of ACSS2 may promote HCC metastasis and predict poor prognosis. In contrast to mammary glands, our data indicate that normal liver tissue and normal liver cells show high ACSS2 expression, and the gradual loss of ACSS2 indicates the occurrence and progression of HCC.

Our invasion and migration experiments showed that there were significant changes after ACSS2 knockdown under hypoxia. We thus speculated that the impact of ACSS2 on invasion and metastasis might be executed through the HIF family, which is considered to play an important role in inducing EMT and metastasis.\(^{(36–38)}\) Previous studies have reported that the acetate/ACSS2 switch could regulate HIF-2α stress signaling\(^{(15)}\) and that HIF-2α is more common in chronic hypoxia.\(^{(10)}\) Therefore, we focused on both the amount and the activity of HIF-2α. We found that ACSS2 knockdown reduced the acetylation levels of HIF-2α and enhanced the expression levels of its downstream molecules. However, ACSS2 knockdown had no effect on the amount of HIF-2α protein under hypoxia. We then identified changes in EMT status after ACSS2 knockdown and HIF-2α deacetylation. All the results reported above indicate that ACSS2 knockdown deacetylates HIF-2α, which enhances the activity of HIF-2α and induces EMT. Our IHC experiment of clinical specimens also proved that low expression of ACSS2 was related to many metastasis indicators, such as satellite lesions and vascular invasion.

Taken together, our study revealed that ACSS2 plays an important role in the acetylation process of HIF-2α protein and therefore effectively modulates the activity of HIF-2α under hypoxic conditions, which has great impacts on the prognosis of HCC patients.

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Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Expression of N-cadherin and E-cadherin in hepatocellular carcinoma cell lines.

Fig. S2. Representative images of hypoxia-inducible factor-2α (HIF-2α) and transforming growth factor-α (TGF-α) expression in a cohort of 110 human hepatocellular carcinoma samples.