Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. SET and MYND domain-containing protein 3 (SMYD3) has been shown to promote the progression of various types of human cancers, including liver cancer; however, the detailed molecular mechanism is still largely unknown. Here, we report that SMYD3 expression in HCC is an independent prognostic factor for survival and promotes the proliferation and migration of HCC cells. We observed that SMYD3 upregulated sphingosine-1-phosphate receptor 1 (S1PR1) promoter activity by methylating histone 3 (H3K4me3). S1PR1 was expressed at high levels in HCC samples, and high S1PR1 expression was associated with shorter survival. S1PR1 expression was also positively correlated with SMYD3 expression in HCC samples. We confirmed that SMYD3 promotes HCC cell growth and migration in vitro and in vivo by upregulating S1PR1 expression. Further investigations revealed that SMYD3 affects critical signaling pathways associated with the progression of HCC through S1PR1. These findings strongly suggest that SMYD3 has a crucial function in HCC progression that is partially mediated by histone methylation at the downstream gene S1PR1, which affects key signaling pathways associated with carcinogenesis and the progression of HCC.
apoptosis [13, 20]. However, the specific mechanisms contributing to the carcinogenesis and progression of HCC remain unclear, and the target genes of SMYD3 that are essential for transcriptional deregulation must be further characterized. In addition, correlations between SMYD3 expression and clinical features in patients with HCC have not been fully described.

Therefore, our study aimed to investigate the correlations between SMYD3 expression and both pathological features and clinical characteristics of patients with HCC and to search for potential downstream target genes of SMYD3 to elucidate the possible mechanism by which SMYD3 drives hepatocarcinogenesis.

RESULTS
SMYD3 was overexpressed and associated with aggressive behaviors in HCC tissues

We first investigated the relative expression of SMYD3 in HCC tissues from 16 patients using reverse transcription-polymerase chain reaction (RT-PCR). Our analysis of SMYD3 mRNA levels revealed significantly higher expression in 75% (12/16) of HCC tissues than in matched noncancerous tissues (Fig. 1A). A subsequent quantitative real-time polymerase chain reaction (qRT-PCR) analysis of another cohort of 80 pairs of HCC samples confirmed this positive relationship (62.5%, 50/80) (Supplementary Fig. 1), and the expression levels of SMYD3 were remarkably higher in the tumor specimens than those in matched noncancerous tissues (Fig. 1B). We performed immunohistochemistry (IHC) on microarrays containing paired HCC and noncancerous tissues from 148 patients to discern the relationship between the expression of the SMYD3 protein and HCC. The IHC scores were in accordance with the staining intensity (range 0 to 12). Obviously, the SMYD3 protein was also expressed at high levels in HCC samples (Fig. 1C). Furthermore, patients with HCC overexpressing SMYD3 had more aggressive microvascular invasion (ratio: 43/94 vs. 15/54, \( p = 0.031 \)) and a more advanced pTNM stage (ratio: 52/94 vs. 19/35, \( p = 0.018 \)) than patients with low levels of SMYD3 (Fig. 1D). In addition, the Kaplan-Meier method showed that patients with high SMYD3 expression had a significantly worse prognosis than those with low SMYD3 expression (\( p = 0.02 \)) (Fig. 1E). We detected SMYD3 expression in HCC cell lines using qRT-PCR and western blotting to elucidate the role of SMYD3 in HCC. SMYD3 was expressed at higher levels in multiple HCC cell lines (SK-Hep-1, Huh-7, PLC/PRF/
5, Hep3B, SMMC-7721, and HepG2.2.15) than in LO2 cells (Fig. 1F, G and Supplementary Fig. 2).

Overexpression of SMYD3 facilitated the proliferation, colony formation, and migration of HCC cells

Overexpression of SMYD3 was induced by stably transfecting a plasmid containing the cDNA into cells with low SMYD3 expression (Huh-7 and SK-Hep-1), and knockdown of SMYD3 (sh-SMYD3) was achieved by stably transfecting an shRNA into Hep3B cells with high SMYD3 expression to evaluate the effects of SMYD3 on cell proliferation and migration. The cell lines with ectopic SMYD3 expression exhibited significantly increased cell growth, while cells with SMYD3 knockdown showed reduced cell growth (Fig. 2A). Colony formation assays validated the results described above; the number of colonies was obviously increased and decreased in the SMYD3 overexpression and knockdown groups, respectively, compared with the control group (Fig. 2B). A flow cytometry analysis was performed to evaluate whether SMYD3 affects HCC cells by altering the cell cycle profile and apoptosis. As shown in Fig. 2C, more cells were distributed in the G1 phase after SMYD3 knockdown, suggesting that knockdown of SMYD3 induced cell cycle arrest in the G1/S phase. In addition, apoptosis assays also revealed that knockdown of SMYD3 exerted an apoptosis-inducing effect on HCC cells. Moreover, wound-healing and transwell assays indicated that the upregulation of SMYD3 enhanced the migration capacities of SK-Hep1 cells, while the knockdown of SMYD3 markedly reduced the migration of Hep3B cells (Fig. 2D, E). Based on these results, SMYD3 overexpression plays an important role in promoting the proliferation, colony formation, and migration of HCC cells.

SMYD3 potentially regulated the expression of S1PR1

We analyzed the mRNA expression levels in Huh7-SMYD3 cells and SK-Hep1-SMYD3 cells and compared them with those in Huh7-Ctrl and SK-Hep1-Ctrl cells, respectively, by performing a microarray analysis to further explore the SMYD3-mediated mechanisms regulating the expression of downstream genes to promote oncogenesis. As shown in Fig. 3A, the microarray results revealed that 189 mRNAs were upregulated and 183 mRNAs were downregulated by SMYD3 in SK-Hep1 cells, and 219 mRNAs were upregulated and 194 mRNAs were downregulated by SMYD3 in Huh7 cells (≥2-fold). After intersecting the two datasets, 85 genes were selected for further study (Supplementary Table 1).

Next, we performed chromatin immunoprecipitation sequencing (ChIP-seq) to profile the genome-wide occupancy of SMYD3 in Huh7 cells transfected with Ctrl or SMYD3 vectors. As shown in Fig. 3B, the overall distribution of SMYD3 occupancy throughout the genome was increased upon the overexpression of SMYD3. Furthermore, SMYD3 binding peaks showed significant central enrichment and increased at their TSS regions (−3 kb to +3 kb) upon the overexpression of SMYD3 compared to Ctrl cells (Fig. 3C, D). SMYD3 binding was enriched around the TSSs of the target genes, which prompted us to analyze the SMYD3 coverage around the TSS regions of the 11 overlapping genes (−3 kb to +3 kb) from the ChIP-seq list, we identified 11 overlapping genes (Fig. 3E), and S1PR1, RIN3, and RTKN2 were located at the 1 kb promoter region (−1 kb to the TSS) (Fig. 3F, Supplementary Fig. 3 and 4). As shown in the ChIP landscape, SMYD3 binding peaks around the TSS regions of S1PR1 were higher in SMYD3-overexpressing cells. Then, we analyzed the relative mRNA expression levels of these 11 genes in HepG2/L02, SMMC-7721/Chang live, SK-Hep1-SMYD3/Sk-Hep1-Ctrl, and Huh7-SMYD3/Huh7-Ctrl cells. The expression levels of S1PR1, MXRA8, and RTKN2, especially S1PR1, were significantly upregulated in HCC and SMYD3-overexpressing cell lines (Fig. 3G).

Based on these results, we selected S1PR1 as the candidate gene for subsequent experiments. qRT-PCR and western blotting were performed to confirm whether SMYD3 regulates S1PR1
expression, and the results showed that SMYD3 overexpression upregulated the S1P1R1 mRNA and protein, whereas knockdown of SMYD3 produced the opposite outcomes (Fig. 3H, I). These results strongly support the concept that S1P1R1 is regulated by SMYD3 in HCC cells.

**SMYD3 regulated S1P1R1 via histone lysine methylation of the S1P1R1 promoter**

Based on previous studies showing that the SET domain of SMYD3, a histone lysine methyltransferase, affects downstream gene expression [20, 21], we hypothesized that SMYD3 mediated lysine methylation of the S1P1R1 promoter sequence to regulate S1P1R1 expression in HCC cells. We investigated the specific DNA sequence that bound the motifs of SMYD3 to verify this assumption and identified three CCCTCC motifs in the S1P1R1 promoter region (Fig. 4A) [20, 22]. Luciferase assays revealed increased luciferase activity in SMYD3-transfected cells in a dose-dependent manner, while promoter activities decreased in response to SMYD3 knockdown in HCC cells (Fig. 4B). Then, we mutated the three SMYD3-binding sites in a site-directed mutagenesis. Compared with the control wild-type sequence, mutations at sites 2 and 3 reduced the luciferase activity, especially when site 3 was mutated (Fig. 4C, left panel). Furthermore, Huh7 cells transfected with the SMYD3 overexpression vector showed increased luciferase activity, whereas the same cells with mutations in SMYD3-binding sites showed reduced activity (Fig. 4C, right panel). Moreover, we carried out ChIP-PCR with antibodies recognizing SMYD3 and H3K4me3 at the above 3 sites (Fig. 4D).

As shown in Fig. 4E, the S1P1R1 promoter was indeed occupied by the SMYD3 protein, and this binding was enhanced in the presence of ectopically expressed SMYD3. Correspondingly, knockdown of SMYD3 led to its downregulation at the S1P1R1 promoter region (Fig. 4F). We concluded that SMYD3 indirectly affected S1P1R1 transcription by mediating the methylation of the S1P1R1 promoter at site 3 in HCC cells. These results support our hypothesis that SMYD3 regulates S1P1R1 expression by mediating histone lysine methylation at the S1P1R1 promoter.

**SMYD3 regulated the downstream signaling pathway of S1P1R1**

We performed IHC experiments on paired tissues from 148 patients with HCC to detect the differential expression of S1P1R1 between HCC and adjacent normal tissues and found abundantly higher IHC scores in HCC tissues than in the paired normal tissues (Fig. 5A, B). Furthermore, higher S1P1R1 expression was correlated with shorter survival (p = 0.022) (Fig. 5C). All these results support the hypothesis that S1P1R1 overexpression indicates a poor prognosis for patients with HCC. Moreover, S1P1R1 expression was positively correlated with SMYD3 expression, consistent with our previous results showing that S1P1R1 is the downstream effector of SMYD3 (Fig. 5D).

Recent studies have suggested that the activation of S1P1R1 is involved in regulating various aggressive biological phenotypes of tumors by modulating its downstream signaling pathways [23–25]. We investigated changes in the expression of constituents of those signaling pathways, including the AKT, STAT3, and MAPK pathways, using western blotting to validate whether the downstream signaling pathways of SMYD3 were regulated by S1P1R1. Overexpression of SMYD3 or S1P1R1 increased the levels of phosphorylated AKT, STAT3, and Erk1/2, while knockdown of
SMYD3 or S1PR1 reduced p-AKT, p-STAT3, and p-Erk1/2 levels. Furthermore, knockdown of S1PR1 ablated the increases in the levels of p-AKT, p-STAT3, and p-Erk1/2 induced by SMYD3, and when cells with SMYD3 knockdown were transfected with pGL-S1PR1-WT, pGL-S1PR1-MT1, pGL-S1PR1-MT2, pGL-S1PR1-MT3, and SMYD3 (pcDNA3.1-SMYD3) (left panel) and Huh7-Ctrl and Huh7-SMYD3 cells were cotransfected with pGL-S1PR1-WT, MT1, MT2 and MT3 (right panel). The promoter activities were measured by performing luciferase reporter assays. The examined primer position for ChIP-PCR assays at the S1PR1 locus was indicated. E, F The ChIP assay was performed in SK-Hep1-Ctrl and SK-Hep1-SMYD3 cells (E) and Hep3B-sh-NC and Hep3B-sh-SMYD3 (F) cells using specific antibodies. The immunoprecipitated DNA was measured with real-time PCR using the primers listed above. Data are presented as the means ± SD of three separate experiments, each performed in triplicate. *P < 0.05; **P < 0.01.

**Discussion**

SMYD3 was originally described as an H3K4 methyltransferase that regulates the transcriptional activities of downstream genes involved in cancer progression [20]. Furthermore, a large amount of evidence indicates critical roles for SMYD3 in the proliferation, invasion, and migration of different tumor cells [13, 14, 26–28]. We investigated venous invasion, pTNM stage, and overall survival in HCC samples from a SMYD-positive group and SMYD3-negative group to confirm the correlations between SMYD3 expression and the clinical characteristics and pathological parameters in patients with HCC. The results confirmed that SMYD3 overexpression is a risk factor in patients with HCC, consistent with other studies [29–31]. We established SMYD3-overexpressing and SMYD3-knockdown vectors and transfected them into HCC cells to investigate changes in biological phenotypes, such as cell proliferation, migration, and colony formation. SMYD3 dramatically facilitated the development and

**Fig. 4** SMYD3 regulated S1PR1 via methylation of the S1PR1 promoter. A The specific SMYD3 DNA binding site (−CCCTCC−) in the S1PR1 promoter. B Luciferase assays with the S1PR1 promoter were performed in HCC cells transfected with different amounts of pcDNA-SMYD3 vector in SK-Hep1 cells or cells with stable overexpression (Huh7 cells) or knockdown (Hep3B cells) of SMYD3. C SK-Hep1 cells were cotransfected with pGL-S1PR1-WT, pGL-S1PR1-MT1, pGL-S1PR1-MT2, pGL-S1PR1-MT3, and SMYD3 (pcDNA3.1-SMYD3) (left panel) and Huh7-Ctrl and Huh7-SMYD3 cells were cotransfected with pGL-S1PR1-WT, MT1, MT2 and MT3 (right panel). The promoter activities were measured by performing luciferase reporter assays. D The examined primer position for ChIP-PCR assays at the S1PR1 locus was indicated. E, F The ChIP assay was performed in SK-Hep1-Ctrl and SK-Hep1-SMYD3 cells (E) and Hep3B-sh-NC and Hep3B-sh-SMYD3 (F) cells using specific antibodies. The immunoprecipitated DNA was measured with real-time PCR using the primers listed above. Data are presented as the means ± SD of three separate experiments, each performed in triplicate. *P < 0.05; **P < 0.01.
progression of HCC, and we concluded that SMYD3 is involved in the aggressive behaviors of HCC and plays a crucial role in determining the prognosis of patients.

SMYD3 methylates not only histone H3 at lysine 4 but also histone H3 at lysine 9, histone H4 at lysine 5, and histone H4 at lysine 20 [13, 18, 32]; these histone lysine methylation events mediated by the SET domain of SMYD3 exert significant effects on cancer development and progression. As shown in previous studies, aberrant histone lysine methylation, including monomethylation, dimethylation, and trimethylation, is often associated with promoters and enhancers of oncogenes in cancer cells [33], which provides strong evidence supporting the hypothesis that SMYD3 promotes carcinogenesis via histone lysine methylation. In addition, the SET domain has been shown to catalyze the trimethylation of H3K4, which is associated with increased transcription of target genes [34, 35]. Based on these studies, we used ChIP-seq to investigate the genes that are highly overexpressed in HCC and combined those results with SMYD3 target genes to explore whether H3K4me3 of downstream genes is a mechanism by which SMYD3 promotes the carcinogenesis of HCC. From the identified genes, we selected S1PR1 for further study due to its high expression in HCC.

Some studies have reported a critical role for S1PR1 in cancer biology [23, 36, 37]. However, the mechanism by which SMYD3 mediates S1PR1 expression has not been elucidated. We verified that the expression and function of S1PR1 in HCC were regulated by SMYD3. The results implied that S1PR1 is the downstream gene of SMYD3. Furthermore, ChIP-PCR indicated that a critical site in the S1PR1 promoter exhibited a high level of H3K4me3 when targeted by SMYD3. At the same time, luciferase assays revealed that mutating these sites reduced the activity of SYMD3. Therefore, SMYD3 methylates lysines in histones at the S1PR1 promoter to regulate S1PR1 expression.

We performed western blotting to detect the levels of p-AKT, p-STAT3, and p-Erk1/2 in different treatment groups compared with those in the control group and to determine whether the downstream signaling pathways of S1PR1 are regulated by SMYD3. As expected, signaling pathways mediated by S1PR1 were upregulated by SMYD3 overexpression, whereas SMYD3 knockdown downregulated their levels. In vivo experiments were conducted to compare the tumor size in the treatment groups and the control group. According to the results, S1PR1 is involved in SMYD3-related HCC progression.

Collectively, these studies support the hypothesis that SMYD3 plays a crucial function in HCC progression, partially by mediating histone methylation at the downstream gene S1PR1, which affects critical signaling pathways associated with the carcinogenesis and progression of HCC.

**PATIENTS AND METHODS**

**Patient samples**

Eighty fresh HCC tissues and corresponding adjacent noncancerous liver tissues (more than 2 cm from the margin) were obtained from patients undergoing liver resection at Sun-Yat-Sen Memorial Hospital of Sun-Yat-Sen Medical College, South China University of Technology, and Sun-Yat-Sen University, from January 2010 to January 2013. The median age of the patients was 59.6 years (range, 33-76 years). The study protocol was approved by the institutional review board (IRB) of Sun-Yat-Sen Memorial Hospital of Sun-Yat-Sen University, and written informed consent was obtained from all patients.
Sen University between January 2008 and December 2012. Another 148 pairs of paraffin-embedded HCC samples and adjacent noncancerous tissues were obtained from January 1999 to December 2007. Eleven fresh normal liver samples and 10 paraffin-embedded normal liver samples were obtained from the specimen bank of the Department of Hepatobiliary Surgery at Sun-Yat-Sen Memorial Hospital. The use of clinical specimens was approved by the Ethical Institutional Review Board of the Sun-Yat-Sen Memorial Hospital.

**HCC cell lines**

HEK293T cells and the hepatoma cell lines HepG2, Huh7, SK-hep1, PLC/PRF/5 (derived from HBV-infected liver), and Hep3B (derived from HBV-infected liver) were purchased from the Chinese Academy of Sciences Cell Bank of Type Culture Collection (CBTCCCAS). LO2, SMMC-7721, and HepG2.2.15 (HepG2-derivative with the integration of the HBV genome) cells were purchased from BioHermes. All cell lines had guaranteed authenticity through short tandem repeat profiling and comparison to DNA profiles of known cell lines. Cells were cultured in high-glucose Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated at 37 °C in an atmosphere containing 5% CO2.

**RNA extraction, reverse transcription, and quantitative real-time polymerase chain reaction (qRT-PCR)**

Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). Complementary DNA was

---

**Fig. 6** SMYD3 promoted HCC progression via S1PR1. CCK8 (A), colony formation (B), cell cycle (C), and apoptosis (D) assays were performed to evaluate cell proliferation and migration in response to the overexpression or knockdown of SMYD3 combined with the overexpression or knockdown of S1PR1 in Huh7, SK-Hep1, and Hep3B cells. Data are presented as the means ± SD of three separate experiments, each of which was performed in triplicate. E Nude mice were subcutaneously inoculated with Huh7 cells transfected with control, SMYD3 overexpression, SMYD3 shRNA, S1PR1 overexpression, and SMYD3 shRNA combined with S1PR1 overexpression vectors. Photographs show mice bearing subcutaneous tumors from each group (upper panel) or the dissected tumors (lower panel) (n = 5). F Tumor volumes were measured and recorded every 3 days, and tumor growth curves were created for each group. G The weight of the local tumors from each group was measured. H Western blots showing SMYD3, S1PR1, p-AKT, AKT, p-STAT3, STAT3, p-Erk1/2, and Erk1/2 expression levels in Huh7 tumors. Bar = 50 μm. The image is representative of two experiments. *P < 0.05; **P < 0.01.
synthesized from total RNA using reverse transcriptase and random primers. Quantitative real-time PCR was performed in the GoTaq® qPCR System (Promega, Beijing, China). The gene-specific primers are as follows: SMYD3, 5′-GGATGGAGCACCTTCAGAATC-3′; S1PR1, 5′-GCCAGCGACCA GCCAGTGGAC-3′; β-actin, 5′-CAGTTGGAGGAGACAAGGAAG-3′; GAPDH, 5′-GGCATCGACCCCTGATTAAC-3′; and 5′-GGCATCGGTGAGAATTTG-3′.

**Construction of tissue microarrays and IHC**

Tissue microarray blocks consisting of 148 paired HCC samples and 10 normal liver samples were constructed using a tissue microarray (Quik-Path, UNITMA). IHC was performed to detect the expression of the SMYD3 and S1PR1 proteins in the tissue microarray blocks using IHC kits (Biohao Biotechnology, Guangzhou). The immunostaining was assessed by counting the total and positively stained cells at a magnification of ×400 in at least randomly selected 10 fields. The staining intensity and extent of the stained area were graded using the semiquantitative scoring system described below. For the staining intensity of the cytoplasm, no staining = 0; weak staining = 1; moderate staining = 2; and strong staining = 3; for the extent of stained cells, 0% = 0, 1 to 20% = 1, 21 to 50% = 2, 51 to 80% = 3, and 81 to 100% = 4. The final immunoreactive score was determined by multiplying the intensity score by the extent of stained cells (range 0 to 12) [40].

**Stable overexpression or knockdown of SMYD3 and S1PR1 in HCC Cells**

The open reading frames of SMYD3 and S1PR1 were cloned into the retroviral vector pMSCV-eGFP-Puro according to the manufacturer’s instructions. The shRNAs targeting SMYD3 and S1PR1 were inserted into the lentiviral vector pGCl-eGFP-Hygromycin (GV54); both constructs were generated by Genechem Company. The shRNA sequences of SMYD3 and S1PR1 are as follows: sh-SMYD3, 5′-GGATGGAGGACCCCTGAGGAT-3′ and sh-S1PR1, 5′-CTGGCTCAAGACCGTAATTAT-3′. Production and purification of the lentivirus and construction of the stably infected cell lines were performed as previously described [41].

**Proliferation assay**

The MTT assay was conducted using a cell proliferation kit according to the manufacturer’s instructions. Briefly, 400 HCC cells in 200 μL of complete culture medium were added to each well of a 96-well plate and cultured at 37 °C in an atmosphere containing 5% CO2 for 2-3 weeks until colonies formed. Then, colonies were fixed with 10% gels for 2-3 weeks until colonies formed. Then, colonies were fixed with 10% 37% formaldehyde and visualized by performing crystal violet staining. Colonies containing more than 10 cells were counted under a microscope. All conditions were analyzed in triplicated and independently tested three times.

**Western blot analysis**

Cells were lysed, and proteins were collected and separated by SDS-PAGE on 10% gels. The separated proteins were then transferred onto polyvinylidene fluoride membranes (Millipore), blocked with TBST containing 5% nonfat dry milk for 2 h, and incubated with antibodies against SMYD3, S1PR1, Erk, p-Erk, GAPDH (all from Santa Cruz Biotechnology) AKT, p-AKT, Stat3, and p-Stat3 (Cell Signaling Technology) overnight at 4 °C. The GAPDH antibody was used as an internal control. Then, membranes were washed 3 times with TBST and incubated with a horseradish peroxidase-conjugated secondary antibody (Biohao Biotechnology) before the protein bands were visualized with an ECL plus western blot detection kit (Beyotime Biotechnology). Quantitative increases in protein phosphorylation were evaluated by performing a densitometry analysis of the ratio of phosphorylated protein/total protein of the treated cells. All western blots were repeated 2-3 times, and the mean changes in the treated groups compared with the non-treated cells are shown under the gels in the figures.

**Microarray analysis**

A cell mRNA microarray was constructed according to the instructions from Affymetrix Inc. and was analyzed at the Gene ChIP Analysis Center at Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University. Changes greater than two-fold compared to the control were considered significant.

**Luciferase assays**

The 5′-flanking region (nucleotides −604 to +5) of S1PR1 was cloned into the pGL3-Basic vector (Promega) by Genearay Biotech. The mutant constructs MT1, MT2, and MT3 were generated using a Site-Directed Mutagenesis Kit (Clontech) and sequences were verified. The wild-type and mutant fragments were inserted into a pmirGLO Dual-Luciferase vector (Promega) downstream of the luciferase gene, and the resulting constructs were designated pGL-S1PR1-WT, pGL-S1PR1-MT1, pGL-S1PR1-MT2, and pGL-S1PR1-MT3. Cells were infected with the indicated vector and then transfected with the indicated luciferase constructs, as described in the corresponding figure legend. Cell transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. According to the manufacturer’s protocol, the cells were assayed 24 h after transfection to determine both firefly and Renilla luciferase activities using a luciferase assay kit (Promega). All transfections were performed in triplicate.

**Chromatin immunoprecipitation**

The cells were grown in three 100-mm culture dishes to 80% confluency. Then, they were fixed with formaldehyde, and the ChIP protocol was performed using a SimpleChIP® Enzymatic Chromatin IP Kit (Agarose Beads) from Cell Signaling Technology according to their instructions. For sonication, 14 cycles of 30 s ON and 30 s OFF were conducted to obtain the required fragment sizes. The ChIP products were analyzed by Ribobio for ChIP-seq or PCR analysis for ChIP-PCR. RNA-seq data are deposited in the Sequence Read Archive (SRA) database and are available through the accession PRJNA699769. The primers for the S1PR1 promoter are listed below: a, F 5′-CACTTTGCAAGTAGAGCACGGAGG-3′; R 5′-AGGGATCCGAGAAGAGAAGAAGAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGA4.

The wound-healing test was conducted as follows: 5 × 10^5 cells in high-glucose DMEM supplemented with 10% FBS were seeded into 6-well plates and incubated overnight at 37 °C in an atmosphere containing 5% CO2. Pipette tips were used to scratch the monolayers, which plates and incubated overnight at 37 °C in an atmosphere containing 5% CO2. Pipette tips were used to scratch the monolayers, which
Animal studies
For the tumor formation assay, 4-week-old to 5-week-old BALB/c athymic nude mice were used and randomly assigned to individual experimental groups. The animal care and experimental protocols were approved by the Animal Research Committee of Sun Yat-sen University. The animal experiments were performed in accordance with established guidelines. In total, 1 × 10^5 cells were suspended in 200 μl of serum-free DMEM and injected into the right flanks of the mice. The tumor volume was measured every 3 days using calipers. The tumor volume was calculated using the following formula: \( V = (\text{width}^2 \times \text{length}) \times 0.5 \). The tumor samples were collected from the mice, and protein expression was analyzed using western blotting.

Statistical analysis
Statistical analyses were performed using SPSS version 19.0 software. Continuous data are reported as the means ± SD, and the significance of differences was compared using Student's t-tests, Fisher's exact tests, chi-square tests and one-way ANOVA with Bonferroni post hoc corrections, as appropriate. The cumulative survival probability was evaluated using the Kaplan-Meier method, and differences were assessed using log-rank tests. \( P < 0.05 \) was considered statistically significant.

REFERENCES
1. National Cancer Institute. Surveillance Research Program, National Cancer Institute. Fast stats: an interactive tool for access to SEER cancer statistics. http://surveillance.cancer.gov/. April 15, 2020.
2. Alqahtani A, Khan Z, Alloghi A, Said Ahmed TS, Ashraf M, Hammoda DM. Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina. 2019;55:526.
3. Huang B, Tian ZF, Li GF, Fan Y, Yin HY, Li Y, et al. LINC01352 is an advanced-stage prognostic biomarker and metastatic oncogene in hepatocellular carcinoma. Cancer Biomark. 2019;26:31–9.
4. Huang P, Xu Q, Yan Y, Lu H, Zou B, et al. HbX/Eralpha complex-mediated LINC01352 downregulation promotes HBV-related hepatocellular carcinoma via the miR-135b-APC axis. Oncogene. 2020;39:774–89.
5. Mak LY, Wong DK, Pollicino T, Raimondo G, Hollinger FB, Yuen MF. Occult hepatitis B infection and hepatocellular carcinoma: Epidemiology, virology, hepatocarcinogenesis and clinical significance. J Hepatol. 2020;73:952–64.
6. Vieira FO, Costa-Pinheiro P, Almeida-Rios D, Graça I, Monteiro-Reis S, Simões-Sousa S, et al. Smyd3 contributes to a more aggressive phenotype of prostate cancer and targets Cyclin D2 through H4K20me3. Oncotarget. 2015;6:13644–57.
7. Fenizia C, Bottino C, Corbetta S, Fittipaldi R, Floris P, Gaudenzi G, et al. SMYD3 promotes the epithelial-mesenchymal transition in breast cancer. Nucleic Acids Res. 2019;47:1278–93.
8. Li B, Pan R, Zhou C, Dai J, Mao Y, Chen M, et al. SMYD3 promoter hypomethylation is associated with the risk of colorectal cancer. Future Oncol. 2018;14:1825–34.
9. Jiang Y, Lue S, Che X, Xia N, Li Q, Feng W. Overexpression of SMYD3 in ovarian cancer is associated with ovarian cancer proliferation and apoptosis via methylation H3K4 and H4K20. J Cancer. 2019;10:4072–84.
10. Sarris ME, Mouflous P, Haroniti A, Giakountis A, Talianidis I. Smyd3 is a transcriptional potentiator of multiple cancer-promoting genes and required for liver and colon cancer development. Cancer Cell. 2016;29:354–66.
11. Tracy C, Warren JS, Sulkil M, Wang L, Garcia J, Makaju A, et al. The Smyd family of Methyltransferases: role in cardiac and skeletal muscle physiology and pathology. Curr Opin Physiol. 2018;1:140–52.
12. Sirinupong N, Brunzelle J, Doko E, Yang Z. Structural insights into the autoinhibition and posttranslational activation of histone methyltransferase Smyd3. J Mol Biol. 2011;406:149–59.
13. Chen LB, Xu JY, Yang Z, Wang GB. Silencing SMYD3 in hepatoma demethylates RIZ1 promoter induces apoptosis and inhibits cell proliferation and migration. World J Gastroenterol. 2007;13:5718–24.
14. Wang SZ, Luo XG, Shen J, Zou JN, Lu YH, Xi T. Knockdown of SMYD3 by RNA interference inhibits cervical carcinoma cell growth and invasion in vitro. BMB Rep. 2008;41:294–9.
15. Zhou Z, Jiang H, Tu Y, Wu X, Zhang J, Hu Z, et al. ANKH1 is required for SMYD3 to promote tumor metastasis in hepatocellular carcinoma. J Exp Clin Cancer Res. 2019;38:18.
16. Mazur PK, Reynoird N, Khati P, Jansen PW, Wilkinson AW, Liu S, et al. SMYD3 links lysine methylation of MAPK2 to Ras-driven cancer. Nature. 2014;510:283–7.
17. Yoshioka Y, Suzuki T, Matsuoka Y, Nakaido M, Tsurita G, Simone C, et al. SMYD3-mediated lysine methylation in the PH domain is critical for activation of AKT1. Oncotarget. 2016;7:75023–37.
ACKNOWLEDGEMENTS
This work was supported by the National Natural Science Foundation of China (No. 81702404 and 81672405), the Natural Science Foundation of Guangdong Province, China (No. 2017A030313536), and the Guangzhou Science Research Project (No. 201607010225, TQG20190002 and CKPTTH11800001).

AUTHOR CONTRIBUTIONS
PH, HZ, WL, and JX designed the research; HZ, PH, ZZ, RZ, YY, and HY performed the research and interpreted results; PH, ZZ, and JX wrote the manuscript; LL, JX, and PH reviewed the manuscript.

ETHICS STATEMENT
The use of clinical specimens was approved by the Ethical Institutional Review Board of the Sun-Yat-Sen Memorial Hospital. The animal care and experimental protocols were approved by the Animal Research Committee of Sun Yat-sen University.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41419-021-04009-8.

Correspondence and requests for materials should be addressed to L.L., JX, W.L. or P.H.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021, corrected publication 2021