Molecular Mechanism of KDM5B Development in Hepatocellular Carcinoma

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Abstract

Objective: To investigate the mechanism of cell cyclin-dependent kinase (KDM5B), a key enzyme driving all cell cycle transitions, promoting HCC progression and metastasis. **Methods:** The expression of KDM5B in normal liver, HCC and its adjacent tissues was analyzed by RT-PCR and IHC. Lentivirus transfection method was used to construct stable cell lines with KDM5B overexpression and down-regulation, and the role of KDM5B in HCC migration and invasion was detected at cell level and animal level. Western blotting and Transwell experiments were performed to verify the effect of KDM5B and/or CCR2 inhibitors on HCC progression and metastasis by using liver orthotopic transplantation tumor model and immunofluorescence methods. **Results:** RT-PCR showed that the expression level of KDM5B in HCC was significantly higher than that in adjacent tissues, and the increase of KDM5B was relatively significant. Upregulation of KDM5B in nude mouse liver orthotopic transplantation tumor model can promote the incidence of lung metastasis and shorten the survival time of nude mice, whereas upregulation of KDM5B can reduce the incidence of lung metastasis and prolong the survival time of nude mice. **Conclusion:** This study clarified the expression of KDM5B in HCC and its function in promoting HCC migration, invasion and metastasis. The molecular mechanism of KDM5B promoting HCC metastasis was revealed, providing a potential therapeutic target for HCC.

Keywords

Liver Cancer, Cyclin-Dependent Kinase (KDM5B), Pathology, Molecular Mechanism

1. Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related deaths and the sixth most new cases globally, with the World Health Organiza-

tion predicting that more than 1 million HCC deaths will occur worldwide by 2030 [1]. The main causes of HCC include HBV/HCV infection, aflatoxin exposure, excessive alcohol intake and non-alcoholic fatty liver disease [2]. Although the current therapeutic strategies for HCC include surgical resection, radiotherapy, chemotherapy and liver transplantation, most HCC patients have advanced disease, and relapse and distant metastasis limit the therapeutic strategies for advanced HCC. It has been reported that the recurrence rate of HCC after treatment is as high as 70%, with most recurrence occurring within 1 - 3 years after clinical resection [3]. High postoperative recurrence and metastasis lead to limited treatment for advanced patients, which is the main reason for poor prognosis of HCC [4]. However, the exact mechanism of HCC recurrence and metastasis remains unclear. To study the key molecular mechanisms of HCC metastasis is of great significance for finding new therapeutic methods and improving prognosis.

Cyclin-dependent kinase KDM5B is a key enzyme that drives all cell cycle transitions, regulating basic cell activities primarily by regulating cell cycle and transcription, and its activity is strictly controlled to ensure the success of cell division [5]. However, abnormal activation of KDM5B associated with cell cycle regulation is widespread in human tumors. Some recent studies have shown that KDM5B can drive and maintain the growth of tumor cells, especially in tumors driven by its abnormally regulated transcription factors, such as MYC-driven HCC [6]. In view of the fact that KDM5B controls the vital process of tumor cell survival and proliferation, this type of abnormally regulated KDM5B has become a hot spot in tumor research. For example, KDM5B is widely expressed in a variety of tumors, and its elevated expression usually indicates poor prognosis [7]. In addition, the KDM5B inhibitor (palbociclib) can be used for the treatment of postmenopausal estrogen receptor (ER) positive/human epidermal growth factor receptor 2 (HER2) negative breast cancer and has been approved by the FDA [8]. Therefore, abnormally regulated KDM5B may be an effective target for tumor therapy. We hypothesized that KDM5B might be involved in the development of HCC. Therefore, this study tested this hypothesis by conducting in vitro and *in vivo* experiments.

2. Expression of KDM5B in Human HCC Tissues and Its Relationship with Prognosis

Tissue specimen

This study was approved by the hospital Ethics Committee. The HCC patients in this study came from two batches, including 35 HCC patients who underwent radical surgery at Haikou People's Hospital and 30 HCC patients who underwent radical surgery at Hainan Provincial People's Hospital between 2019 and 2020. Preoperative clinical diagnosis of HCC was based on the criteria of the American Association for the Study of Liver Diseases. All patients signed informed consent forms. In addition, 15 normal liver tissues and 18 matched HCC

and para-cancer fresh tissues were collected. 15 pairs of normal liver tissues, liver cancer tissues and para-cancer tissues were stored in liquid nitrogen for the extraction of corresponding RNA and protein, and all tissues were stained by HE.

Participants who met the following criteria were included in the study:

1) Patients diagnosed with hepatocellular carcinoma according to the diagnosis and treatment norms of primary liver cancer, including pathological and clinical diagnosis.

2) Hepatocellular carcinoma was diagnosed in the pathological section of selected specimens after hepatocellular carcinoma resection.

3) Except for specimens with pathological diagnosis of hepatic adenocarcinoma, hepatobiliary duct carcinoma, and metastatic liver cancer.

4) All cases were collected before surgery without any treatment.

Participants were surveyed about their age, sex, and Body Mass Index (BMI) smoking and alcohol consumption. Appropriate amount of cancer tissue and paracancer tissue (normal liver tissue 2 - 5 cm away from the tumor) removed during the operation were collected and immediately frozen in liquid nitrogen for preservation. All patients in this study were fully aware of the purpose of the experimental study and signed informed consent. At the same time, this experiment was approved by the Ethics Committee of our hospital and carried out in accordance with the Declaration of Helsinki.

Using R language limma "pack" (http://www.bioconductor.org/packages/release/bioc/html/limma.html) variance analysis GEO database (https://www.ncbi.nlm.nih.gov/gds) liver cancer gene expression chips GSE45267, GSE62232 and GSE117361, And through GEPIA TCGA database (https://portal.gdc.cancer.gov) (http://gepia2.cancer-pku.cn/#index) difference analysis LIHC significant difference of hepatocellular carcinoma (HCC) gene expression data, The names of human transcription factors were obtained from Cistrome (http://cistrome.org), the intersection of significantly different genes and transcription factors was used to draw Venn diagram, and the key transcription factor was determined as KDM5B (difference analysis threshold: $logFC > 0.5$, $P < 0.05$) based on existing literatures.

3. Methods

3.1. RT-PCR

3.1.1. Tissue RNA Extraction (Takara Kit)

(1) Steel ball, EP tube, gun head soaked in 0.1% DEPC water overnight.

(2) Fresh liver tissue (tumor tissue, para-cancer tissue and normal tissue) was extracted from liquid nitrogen and clipped

Appropriate size was placed into sterile 2 ml EP tube, and the remaining tissues were stored in liquid nitrogen for later use.

(3) Put steel balls into the 2 ml EP tube and add 600 μl Buffer RL (50xDTT solution has been added).

To the final concentration of 2%, the tissue homogenizer grinds the tissue (50 r/s, 5 min).

(4) The ground tissue was transferred to 1.5 ml EP tube and centrifuged at 12,000 rpm at 4˚C for 5 min.

(5) The supernatant of the centrifuged tissue homogenate was transferred to the gDNA Eraser Spin Column and placed in 2 ml

In the collection tube, centrifuge at 12,000 rpm at 4˚C for 1 min.

(6) Discard the gDNA Eraser Spin Column and retain the filtrate in the collection tube.

(7) Formulation of 70% ethanol.

(8) Add 70% ethanol (equal volume) to liquid (6), blow and mix well.

(9) The liquid in (8) was added to the RNA Spin column placed on a 2 ml collection tube at 12,000 rpm at 4˚C.

Centrifuge for 1min and discard the filtrate.

(10) 500 μl Buffer RWA was added to the above RNA Spin column and centrifugated at 12,000 rpm at 4˚C for 30 s.

Discard the filtrate.

(11) 600 μl Buffer RWB was added to the above RNA Spin column and centrifuged at 12,000 rpm at 4˚C for 30 s.

Discard the filtrate.

Repeat step (11).

(13) The RNA Spin column was re-placed on a 2 ml collection tube and centrifuged at 12,000 rpm at 4˚C for 2 min.

(14) Add RNA Spin column (membrane center) placed on 1.5 ml EP tube (sterile without enzyme)

50 - 200 μl RNase free dH$_2$O or 0.1% DEPC water was left at room temperature for 5 min and centrifuged at 12,000 rpm at 4˚C for 2 min eluting RNA.

(15) To increase the amount of RNA, the RNA eluted in step (14) can be readded to the RNA Spin

In the center of the column membrane, stand at room temperature for 5 min, and centrifuge at 12,000 rpm at 4˚C for 2 min.

(16) The RNA concentration and purity of 2 μl RNA samples were determined by spectrophotometer.

3.1.2. cDNA Synthesis (Takara's cDNA Synthesis Kit)

(1) Prepare reaction system on ice for subsequent reverse transcription procedure according to the following parameters (total volume 20 μl):

5 × PrimeScript RT Master Mix 4 μl

RNA * (20 μl reaction system can use up to 1000 ng of RNA)

RNase Free dH$_2$O complement to 20 μl

(2) The reagent was centrifuged, mixed, and transcribed on the reverse transcriptionist. The reaction procedure was: 37˚C 15 min → 5 s at 85˚C → 10 min at 4˚C. After the reaction, the product was stored in the refrigerator at −20˚C or at −80˚C for a long time.

3.1.3. PCR Reaction

(1) Primer sequence was designed by Primer Premier software and synthesized by Takara Company.

(2) Prepare PCR reaction solution (prepared on ice) according to the following components:

2 × SYBR Green qPCR Master Mix	10 μl
Forward Primer	0.8 μl
Reverse Primer	0.8 μl
c DNA	2 μl
RNase Free dH$_2$O	6.4 μl

(3) Set PCR reaction procedure: predenaturation 95˚C 30 s; PCR reaction: 95˚C for 5 s, 60˚C for 30 s, 40 cycles Ring; Analysis of dissolution curve.

(4) Result analysis: GAPDH was used as the internal reference gene and 2-Δ was used.

The ΔCT formula calculated the mRNA expression of the target gene.

Horizontal, all samples are set with 3 multiple holes.

3.2. Immunohistochemical Staining

3.2.1. Dyeing

(1) Baking at 65˚C: tissue slice baking for 30 min, tissue chip baking for 1 h.

(2) Dewaxing xylene I and II for 10 min each; 100%, 100%, 95%, 90%, 85%, 80%, 75%.

The concentration gradient of alcohol treatment is 5 min; the slices were washed in PBS for 5 min.

(3) Antigen repair: Select the appropriate antigen repair solution according to the antibody instructions, high-pressure repair for 2 min, and natural cooling.

After that, wash with PBS for 3 min × 3 times.

(4) Peroxidase was blocked by 3% hydrogen peroxide for 10 min.

(5) Wash with PBS for 3 min × 3 times.

(6) Goat serum was blocked for 30 min.

(7) Incubation of the primary antibody overnight: Dilute the primary antibody according to the antibody instructions, drop the serum, add an appropriate amount of the primary antibody, and put it in a wet box.

Overnight incubation with primary resistance in 4˚C refrigerator.

(8) The slices were taken out the next morning, rewarmed at room temperature for 30 min, washed with PBS for 3 min × 3 times.

(9) According to the primary resistance, the corresponding secondary resistance was added, incubated at room temperature for 30 min, and washed with PBS for 5 min × 3 times.

(10) Horseradish peroxidase was washed for 15 min with PBS for 3 min × 3 times.

(11) DAB color development: Observe the tissue color, stop the color development when it is dyed light brown, and rinse under tap water for 2 - 4 min.

(12) Hematoxylin staining for 2 min and rinsing under tap water for 2 - 4

min.

(13) The slices were successively placed in 75%, 80%, 85%, 90%, 95%, 100% and 100% concentration gradient alcohol.

After dehydration for 5 min respectively, xylene II and I were transparent for 10 min respectively.

(14) Neutral gum sealing sheet: seal the sheet at the fume hood after the slice is dried naturally.

(15) After the slices were dried, scan the slices with a scanning instrument to observe the staining.

3.2.2. Interpretation of Dyeing Results

The staining results were interpreted by two pathologists independently. Scoring criteria for staining intensity: 0 (negative), 1 (weak positive), 2 (moderate positive) or 3 (strong positive). Staining range scale: 0 (none), 1 (1% - 25%), 2 (26% - 50%), 3 (51% - 75%) or 4 (76% - 100%). Score = staining intensity score × staining range score. If the score is 4 - 12 points, the stain is positive; If the score is 0 - 3, the stain is negative.

3.3. Statistical Analysis

Statistical analysis was performed with SPSS 22.0 (SPSS Inc. Chicago, IL). Data are expressed as mean ± standard deviation. Cancer tissues and adjacent tissues were examined by Student's t tests, and differences between groups were compared by two-way analysis of variance (ANOVA). Kaplan-Meier curve and log-rank test were used for survival analysis. $P < 0.05$ was considered statistically significant.

4. Results

4.1. Expression of KDM5B Family Genes in HCC

Firstly, we analyzed the mRNA expression of 21 genes of KDM5B kinase family in 15 matched HCC and paracancer tissues. We found that KDM5B can be detected in both HCC and paracancer tissues. There was no significant difference in mRNA expression between matched HCC and adjacent tissues.

4.2. Overexpression of KDM5B Is Associated with Poor Prognosis in HCC Patients

First, we examined the correlation between the expression of KDM5B and clinicopathological parameters of HCC patients in a sample group. Correlation analysis showed that the up-regulation of KDM5B expression was positively correlated with the number of tumors, tumor size, capsule deletion, microtubule invasion, tumor differentiation, and TNM stage in HCC patients. Second, we verified this result in another group and reached a similar conclusion: high expression of KDM5B was positively correlated with tumor number, tumor size, microtubule invasion, tumor differentiation, and TNM stage in HCC patients. In

addition, in two independent cohorts, Kaplan-Meier analysis showed that patients with increased KDM5B expression had shorter overall survival and higher recurrence rates.

5. Discussion

In this part of the study, we first detected the mRNA expression of various kinases in the KDM5B family in 15 matched HCC and paracancer tissues. The results showed that the expression level of KDM5B in HCC tissues was significantly higher than that in para-carcinoma tissues. In addition, we found that among the significantly upregulated KDM5B in HCC tissues, the upregulated KDM5B expression was relatively the most significant in comparison with paracancerous tissues. KDM5B is one of the important kinases in the KDM5B family, which is mainly involved in transcriptional regulation. Studies have found that KDM5B plays an important role in the occurrence and development of various tumors through different mechanisms, so we further studied its expression in liver cancer. We detected KDM5B mRNA expression in 15 normal liver tissues and 18 matched HCC and paracancer tissues. It was found that the mRNA expression level of KDM5B in HCC tissues was significantly increased compared with paired para-cancerous tissues and normal liver tissues. The IHC results of the two groups showed that the protein expression of KDM5B in HCC tissues was significantly higher than that in adjacent tissues, and the expression of KDM5B was mainly located in the nucleus. Therefore, both mRNA and protein expression levels of KDM5B are up-regulated in human HCC tissues. However, the mechanism of KDM5B in the occurrence and development of HCC remains unclear, and further studies are needed.

6. KDM5B Promotes the Invasion and Metastasis of HCC Cells

In the first part of the experiment, we found the mRNA and protein table of KDM5B in human HCC tissues. The level of reach increased significantly. The mRNA and protein expressions of KDM5B in HCC patients with recurrence and metastasis were still significantly higher than those in the corresponding non-recurrence and non-metastasis groups. Similarly, the expression of KDM5B in HCC metastasis tissues was significantly higher than that in primary HCC tissues and paracancer tissues. Correlation analysis showed that the expression of KDM5B was significantly correlated with tumor number, tumor size, microtubule invasion, tumor differentiation degree, and TNM stage in HCC patients. Survival analysis found that HCC patients with elevated KDM5B expression had a higher recurrence rate and shorter survival time. After clarifying the expression of KDM5B in HCC, we conducted functional tests in this part of the experiment The expression of KDM5B was detected in hepatocellular carcinoma cell lines, and the effects of KDM5B on invasion and metastasis of hepatocellular carcinoma cell lines were verified at both cellular and animal levels.

7. Materials

7.1. Human HCC Cell Lines

Human immoribile cell HL7702 was purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Human HCC cell lines (HCCLM6, HCCLM3, MHCC97L, MHCC97H) were donated from Zhongshan Hospital of Fudan University. Human HCC cell lines (PLC/PRF/5, Hep3B, SNU354, HepG2, Huh-6, Huh-7, and SNU368) were purchased from the U.S. ATCC cell bank. The above cell lines were cultured in DMEM (Dulbecco's modified Eagle's medium) plus 10% fetal bovine serum, 100 μg/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 incubator at 37˚C.

7.2. Experimental Animals

The animal experiments involved in this study were approved by the Animal Committee of Haikou People's Hospital. BABL/C nude mice (male, 6 - 8 weeks old) were purchased from Guangdong Medical Laboratory Animal Center [License No. SCXK (Guangdong) 2018-0002].

8. Methods

8.1. Cell Culture and Treatment

1) Preparation of media; 2) Cell resuscitation; 3) Cell fluid exchange; 4) Cell passage; 5) Cell freezing.

8.2. Cell RNA Extraction (Takara Kit)

EP tube and gun head soaked in 0.1% DEPC water overnight; Cells with planks to be extracted for RNA were removed, and the adherent cells were washed twice with sterile pre-cooled PBS (PBS was gently added along the side wall to avoid cell shedding); Buffer RL of 350 μl lysate was added into each cell pore (50xDTT solution was added to the final concentration of 2%), then transferred to 1.5 ml EP tube after blowing and mixing, and left for 2 min at room temperature. The cell lysate mixture in the EP tube was transferred to the gDNA Eraser Spin Column placed on the 2 ml collection tube. Centrifuge at 12,000 rpm at 4˚C for 1min; The gDNA Eraser Spin Column was discarded and the filtrate in the collection tube was retained. Formulation of 70% ethanol; Add 70% ethanol (equal volume) to the medium liquid, blow and mix well; 500 μl Buffer RWA was added into the above RNA Spin column, centrifuged at 12,000 rpm at 4˚C for 30 s, and the filtrate was discarded. 600 μl Buffer RWB was added into the above RNA Spin column, centrifuged at 12,000 rpm at 4˚C for 30 s, and the filtrate was discarded. The RNA Spin column was re-placed on a 2 ml collection tube and centrifuged at 12,000 rpm at 4˚C for 2 min. 50 - 200 μl was added to the center of the membrane of RNA Spin column placed in 1.5 ml EP tube (sterile without enzyme). RNase free dH_2O or 0.1% DEPC water, standing at room temperature for 5 min, centrifuge at 12,000 rpm at 4˚C for 2 min to elute RNA; If you need to

increase the amount of RNA.

cDNA synthesis

Ditto part I

PCR Reaction

Ditto part I

Extraction of total cell protein; Protein immunoblotting; Hematoxylin and eosin (HE) staining; hematoxylin and Eosin (HE) staining; The design and packaging of lentivirus was completed by Shanghai Jikai Gene Chemical Technology Co., LTD. Construction of stable cell line; Luciferase labeled cell lines were constructed.

8.3. Transswell

Migration Experiment and Invasion experiment

Construction of orthotopic tumor model in nude mice; live imaging of nude mice.

8.4. Statistical Analysis

The experimental data were statistically analyzed by SPSS 21.0 software. Student's t test was used for quantitative variables, and Chi-square test was used for categorical variables. $P < 0.05$ was considered statistically significant.

9. Results

KDM5B expression was increased in HCC cells

By RT-qPCR and Western blotting, we detected KDM5B in normal liver tissues, liver immortem cells HL7702, and HCC cell lines mRNA and eggs in Hep3B, PLC/PRF/5, SNU354, Huh6, HepG2, SNU368, Huh7, MHCC97L, HCCLM6, HCCLM3, and MHCC97H White expression situation. The results showed that compared with normal liver tissues and liver HL7702 cells, KDM5B was highly expressed in HCC cell lines except Hep3B cells.

In vitro experiments, overexpression of KDM5B promoted the migration and invasion ability of HCC cells, while downregulation of KDM5B inhibited the migration and invasion ability of HCC cells. Transwell migration and invasion experimental results showed that overexpression of KDM5B significantly increased the migration and invasion ability of Hep3B cells. However, down-regulating KDM5B expression significantly inhibited the migration and invasion ability of MHCC97H cells.

In vivo experiments, overexpression of KDM5B promoted the migration and invasion ability of HCC cells, while downregulation of KDM5B inhibited the migration and invasion ability of HCC cells To further verify the effect of overexpression and downregulation of KDM5B on HCC cell lines *in vivo*, We transfected luciferase gene into four stable cell lines Hep3B-control, Hep3B-KDM5B, MHCC97H-shcontrol and MHCC97H-shKDM5B, and constructed a nude mouse model of liver implantation *in situ*. It was found that overexpression of KDM5B

significantly enhanced the biological signal intensity of Hep3B cells in nude mice in the liver *in situ* implantation model, promoted the incidence of lung metastasis in nude mice, and significantly increased the number of lung metastasis lesions in nude mice, but reduced the survival time of nude mice. On the contrary, after down-regulating the expression of KDM5B, the biological signal intensity of MHCC97H cells in nude mice in the liver in situ implantation model was significantly reduced, the incidence of lung metastasis and the number of lung metastatic lesions in nude mice were significantly reduced, and the survival time of nude mice was correspondingly prolonged.

10. Discussion

Previous literature has reported that KDM5B is abnormally expressed in various tumors and is associated with tumor metastasis and poor prognosis [9]. In patients with microsatellite-stable (MSS) metastatic colon cancer, up-regulation of KDM5B significantly shortened the survival of patients with stage II-IV colon cancer. Moreover, the expression of KDM5BmRNA was positively correlated with the expression level of immune evasion related genes in tumors, and negatively correlated with the infiltration of CD8$^+$ T cells in tumor sites [10]. In lung cancer, KDM5B can promote cell proliferation and metastasis, and its inhibitors can inhibit cell proliferation, colony formation and cell cycle progression in lung cancer, and induce cell apoptosis [11]. Similarly, in ovarian cancer and osteosarcoma cells, small interfering RNA or KDM5B inhibitors can effectively inhibit their proliferation and metastasis and induce cell apoptosis [12]. In cervical cancer, the expression level of KDM5B is highly correlated with disease stage, pathological grade, deep interstitial infiltration, tumor size and lymph node metastasis, and the elimination of KDM5B by specific siRNA can inhibit the proliferation of cervical cancer cells in vitro and tumorgenesis *in vivo* [13]. Therefore, the expression of KDM5B is closely related to the proliferation and metastasis of tumor cells. However, overexpression of KDM5B is significantly associated with poor prognosis in HCC, but there are few studies on the role of KDM5B in HCC metastasis and its mechanism. In this part of the experiment, we mainly studied the function of KDM5B in HCC. Firstly, we detected the mRNA and protein expression of KDM5B in normal liver tissues, liver immortable cells HL7702 and HCC cell lines by RT-qPCR and Western blotting methods. It was found that except Hep3B cells, KDM5B was highly expressed in hepatoma cell lines. In addition, the expression level of KDM5B in HCC cell line was relatively low in Hep3B cell line and relatively high in MHCC97H cell line. Therefore, a stable transmissible cell line was constructed by infecting these two cell lines with lentivirus. Transwell migration and invasion assay results showed that overexpression of KDM5B significantly increased the migration and invasion ability of Hep3B cells. However, down-regulating the expression of KDM5B significantly inhibited the migration and invasion ability of MHCC97H cells [14]. In order to further verify the effect of overexpression and downregulation of KDM5B on

liver cancer cell lines, we further verified the function of KDM5B in animal models, that is, constructed a nude mouse model of liver orthotopic transplantation of tumor. It was found that overexpression of KDM5B significantly enhanced the metastasis ability of Hep3B cells, while downregulation of KDM5B significantly reduced the metastasis ability of MHCC97H cells. Based on the above studies, we found that KDM5B can promote the occurrence and development of liver cancer at both cellular and animal levels. However, at present, the specific mechanism of how KDM5B promotes the occurrence and development of HCC has not been fully clarified [15]. Therefore, in addition to the expression and function of KDM5B in HCC, further relevant studies are needed to find the key target genes of KDM5B in promoting HCC progression, so as to clarify the specific molecular mechanism of KDM5B in promoting HCC metastasis.

Fund Project

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Llovet, J.M., Zucman-Rossi, J., Pikarsky, E., Sangro, B., Schwartz, M., Sherman, M. and Gores, G. (2016) Hepatocellular Carcinoma. *Nature Reviews Disease Primers*, **2**, Article No. 16018. https://doi.org/10.1038/nrdp.2016.18

[2] Forner, A., Reig, M. and Bruix, J. (2018) Hepatocellular Carcinoma. *Lancet*, **391**, 1301-1314. https://doi.org/10.1016/S0140-6736(18)30010-2

[3] Yang, J.D., Hainaut, P., Gores, G.J., Amadou, A., Plymoth, A. and Roberts, L.R. (2019) A Global View of Hepatocellular Carcinoma: Trends, Risk, Prevention and Management. *Nature Reviews Gastroenterology & Hepatology*, **16**, 589-604. https://doi.org/10.1038/s41575-019-0186-y

[4] Zhu, A.X., Duda, D.G., Sahani, D.V., Jain, R.K. (2011) HCC and Angiogenesis: Possible Targets and Future Directions. *Nature Reviews Clinical Oncology*, **8**, 292-301. https://doi.org/10.1038/nrclinonc.2011.30

[5] Villanueva, A. (2019) Hepatocellular Carcinoma. *The New England Journal of Medicine*, **380**, 1450-1462. https://doi.org/10.1056/NEJMra1713263

[6] Yang, W.X., Pan, Y.Y. and You, C.G. (2019) CDK1, CCNB1, CDC20, BUB1, MAD2L1, MCM3, BUB1B, MCM2, and RFC4 May Be Potential Therapeutic Targets for Hepatocellular Carcinoma Using Integrated Bioinformatic Analysis. *BioMed Research International*, **2019**, Article ID: 1245072. https://doi.org/10.1155/2019/1245072

[7] Giordano, S and Columbano, A. (2014) Met as a Therapeutic Target in HCC: Facts and Hopes. *Journal of Hepatology*, **60**, 442-452. https://doi.org/10.1016/j.jhep.2013.09.009

[8] Llovet, J.M., Montal, R., Sia, D. and Finn, R.S. (2018) Molecular Therapies and Precision Medicine for Hepatocellular Carcinoma. *Nature Reviews Clinical Oncology*, **15**, 599-616. https://doi.org/10.1038/s41571-018-0073-4

[9] Wang, D., Han, S., Peng, R., Jiao, C., Wang, X., Yang, X., Yang, R. and Li, X. (2016) Depletion of Histone Demethylase KDM5B Inhibits Cell Proliferation of Hepatocellular Carcinoma by Regulation of Cell Cycle Checkpoint Proteins p15 and p27. *Journal of Experimental & Clinical Cancer Research*, **35**, Article No. 37. https://doi.org/10.1186/s13046-016-0311-5

[10] Bamodu, O.A., Huang, W.C., Lee, W.H., Wu, A., Wang, L.S., Hsiao, M., Yeh, C.T. and Chao, T.Y. (2016) Aberrant KDM5B Expression Promotes Aggressive Breast Cancer through MALAT1 Overexpression and Downregulation of hsa-miR-448, *BMC Cancer*, **16**, Article No. 160. https://doi.org/10.1186/s12885-016-2108-5

[11] Gong, J., Yan, S., Yu, H., Zhang, W. and Zhang, D. (2018) Increased Expression of Lysine-Specific Demethylase 5B (KDM5B) Promotes Tumor Cell Growth in Hep3B Cells and is an Independent Prognostic Factor in Patients with Hepatocellular Carcinoma. *Medical Science Monitor*, **24**, 7586-7594. https://doi.org/10.12659/MSM.910844

[12] Guo, J.C., Yang, Y.J., Zhang, J.Q., Guo, M., Xiang, L., Yu, S.F., Ping, H. and Zhuo, L. (2019) microRNA-448 Inhibits Stemness Maintenance and Self-Renewal of Hepatocellular Carcinoma Stem Cells through the MAGEA6-Mediated AMPK Signaling Pathway. *Journal of Cellular Physiology*, **234**, 23461-23474. https://doi.org/10.1002/jcp.28915

[13] Pu, Y., Xiang, J. and Zhang, J. (2020) KDM5B-Mediated microRNA-448 Up-Regulation Restrains Papillary Thyroid Cancer Cell Progression and Slows Down Tumor Growth via TGIF1 Repression. *Life Sciences*, **250**, 117519. https://doi.org/10.1016/j.lfs.2020.117519

[14] Liu, J., Eckert, M.A., Harada, B.T., Liu, S.M., Lu, Z., Yu, K., Tienda, S.M., Chryplewicz, A., Zhu, A.C., Yang, Y., Huang, J.T., Chen, S.M., Xu, Z.G., Leng, X.H., Yu, X.C., Cao, J., Zhang, Z., Liu, J., Lengyel, E. and He, C. (2018) m(6)A mRNA Methylation Regulates AKT Activity to Promote the Proliferation and Tumorigenicity of Endometrial Cancer. *Nature Cell Biology*, **20**, 1074-1083. https://doi.org/10.1038/s41556-018-0174-4

[15] Zhou, Y., Yin, Z., Hou, B., Yu, M., Chen, R., Jin, H. and Jian, Z. (2019) Expression Profiles and Prognostic Significance of RNA N6-Methyladenosine-Related Genes in Patients with Hepatocellular Carcinoma: Evidence from Independent Datasets. *Cancer Management and Research*, **11**, 3921-3931. https://doi.org/10.2147/CMAR.S191565