

# Research Progress on the Role of Enzymes Involved in Histone Methylation in Hepatocellular Carcinoma

**Zhiqian Wang, Hongliang Dai, Huizhao Su, Shihui Lai, Luo Dai, Xiaomeng Liu, Yan Wang, Gege Shu, Bo Tang\*, Yang Li\***

Department of Hepatobiliary Surgery and Medical Oncology, The First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China

**Abstract:** Abnormal histone modification plays an important role in the occurrence and development of hepatocellular carcinoma (HCC). As an important histone modification pattern, histone methylation is closely related to the occurrence and development of HCC. In recent years, a large number of studies have shown that abnormal histone methylation is involved in the proliferation, invasion, and metastasis of HCC and is related to the prognosis of HCC patients. Enzymes associated with histone methylation play an important role in the histone methylation process. Exploration of the relationship between histone methylation and HCC, and in-depth studies on the pathogenesis of the disease would contribute to the finding of tumor markers and the development of targeted drugs, which is of great significance for the diagnosis, treatment, and prognosis of patients with HCC. This review summarizes the research progress regarding the enzymes involved in histone methylation in HCC.

**Keywords:** Histone methylase, Histone demethylase, Hepatocellular carcinoma

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## \*CORRESPONDING AUTHORS

Bo Tang  
E-mail: dr\_sntangbo@163.com  
Yang Li  
E-mail: young88818@163.com

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## 1. Introduction

Epigenetic modifications include DNA methylation, chromatin remodeling, noncoding RNA regulation, and histone modification. There are five histones, namely, H1, H2A, H2B, H3, and H4. In the nucleus of eukaryotes, a histone octamer composed of two molecules of each of the four histones (H2A, H2B, H3, and H4) is surrounded by 146 base pairs, which together constitute the basic functional unit of chromatin, that is, nucleosome<sup>[1]</sup>. Histone modifications, including methylation, ubiquitination, acetylation and phosphorylation, represent an important way of epigenetic modification that regulates gene expression, and plays an important role in maintaining the stability of chromatin structure. As one of the most stable histone modifications, histone methylation participates in many pivotal biological processes and plays an important role in the occurrence and development of tumors<sup>[2]</sup>. Histone methylation is a covalent modification of histone arginine and lysine. Arginine can be mono-methylated or di-methylated, while lysine can be mono-methylated, di-methylated, or tri-methylated<sup>[3]</sup>. Histone methylation and demethylation are a dynamic and reversible process, regulated by different enzymes. Abnormal histone methylation status has a potent impact on the occurrence and development of tumors<sup>[4]</sup>.

As one of the most common malignant tumors, hepatocellular carcinoma (HCC) is difficult to diagnose in the early stage, easy to relapse and metastasize, and having extremely poor prognosis. Despite of the progress in the research about histone

methylation in HCC, its specific molecular mechanism is still unclear. Some studies have shown that histone methylation plays an important role in the occurrence and development of liver cancer. This paper reviews the research status quo regarding the enzymes involved in histone methylation in HCC.

## 2. Histone methyltransferase (HMT) and HCC

HMT can be divided into protein arginine methyltransferase (PRMT) and histone lysine methyltransferase (HKMT). PRMT can be divided into asymmetric and symmetric dimethylated transferases according to whether they can catalyze dimethylation symmetrically. The former includes PRMT1-4, PRMT6, and PRMT8, and the latter includes PRMT5, PRMT7, and PRMT9<sup>[5]</sup>. Among them, PRMT7 belongs to type III enzyme and histone is its only catalytic substrate<sup>[6]</sup>. PRMTs generally use glycine-and-arginine-rich (GAR) domains as substrates to catalyze the transfer of methyl groups from *S*-adenosyl-L-methionine to GAR, except PRMT4<sup>[7]</sup>.

HKMT can be divided into SET domain containing lysine methyltransferase (SETD), euchromatin HKMT (EHMTs), H<sub>3</sub>K<sub>79</sub> methylated HKMTDOTIL (disruptor of telomeric silencing 1-like), etc.<sup>[8]</sup> Members of the SETD family contain a Rubis-sub-bind domain with 100 – 300 residues and a RuBisCo LSMT C-terminal, which is essential for the N-terminal tail binding of SETD proteins to histones H3 and H4<sup>[9]</sup>. G9a, located in autosome chr6p21.31, is also an SET domain-containing HMT, and G9a belongs to SUV39H1 family and mainly catalyzes the dimethylation of lysine 9 to mediate histone H3 methylation (H<sub>3</sub>K<sub>9</sub>me<sub>2</sub>)<sup>[10]</sup>. In addition, it was reported that G9a catalyzes the methylation modification of lysine 27 on histone H3<sup>[11]</sup>.

### 2.1. Histone arginine methylase and HCC

A large number of studies have shown that abnormal histone methylation is closely related to HCC progression. In mammals, histone arginine methylation usually occurs on certain amino acid residues, including arginine residues 2, 8, 17, and 26 of histone H3 (H3R2, H3R8, H3R17, and H3R26) and arginine residue 3 of histone H4 (H4R3)<sup>[12]</sup>. Jiang *et al.* found that PRMT9 is highly expressed in HCC, promoting invasion and metastasis of HCC by inducing epithelial-mesenchymal transition (EMT) and activating PI3K/AKT signaling pathway; therefore, high expression of PRMT9 can be used as a prognostic marker and therapeutic target<sup>[13]</sup>. The previous studies have shown that PRMT9 of human being, like PRMT3 of nematodes, may catalyze arginine methylation of histone H2A<sup>[14]</sup>. PRMT5 is highly expressed in many digestive system tumors and is considered to be an oncogene<sup>[15]</sup>. Zhang *et al.* found that PRMT5 can promote the proliferation of HCC cells by targeting  $\beta$ -catenin to regulate the expression of

downstream effector cyclin D1, playing an important role in the occurrence of liver cancer<sup>[16]</sup>. PRMT5 can limit the hepatitis B virus replication by inhibiting the transcription of covalently closed circular DNA and interfering with pregenomic RNA envelope, thereby delaying the development of viral hepatitis B and resultant liver cancer<sup>[17]</sup>. Wei *et al.* found that PRMT1 was highly expressed in HCC cells, thereby activating EMT and transforming growth factor signaling, and promoting the proliferation, invasion, and metastasis of HCC cells<sup>[18]</sup>. The development of drugs or inhibitors targeting PRMT can provide new therapeutic options for HCC patients.

### 2.2. Histone lysine methylase and HCC

EZH2, also known as KMT6A, is a lysine methyltransferase that mainly catalyzes H<sub>3</sub>K<sub>27</sub>me<sub>3</sub> and plays an important role in the development of HCC<sup>[19]</sup>. EZH2 maintains the H<sub>3</sub>K<sub>27</sub>me<sub>3</sub> status at the miR-22-3p promoter to inhibit its transcription, and in turn promotes the expression of galectin-9, accelerating the proliferation, invasion, and metastasis of HCC cells. Xiao *et al.*<sup>[20]</sup> found that EZH2 directly increased H<sub>3</sub>K<sub>27</sub>me<sub>3</sub> at the CD274 and IRF1 promoters, and further inhibited the expression of programmed death ligand-1 (PD-L1) in HCC cells, suggesting EZH2 as a potential therapeutic target for combination of immunotherapy.

SETD1A is significantly associated with the enrichment and expression of H3K4me3 to regulate gene transcription. Silencing *SETD1A* could inhibit the proliferation, invasion, and metastasis of HCC cells. Overexpression of SETD1A is closely related to the poor prognosis of HCC patients<sup>[21]</sup>. Wu *et al.* found that SETD1A can promote the chemoresistance of HCC cells to sorafenib by activating Yes-associated protein (YAP), and reduce the proliferation inhibition and cell death induced by sorafenib, representing an important potential target to overcome the chemoresistance of HCC<sup>[22]</sup>. SETD3 was overexpressed in HCC, and knockout of SETD3 can significantly inhibit the proliferation of HCC cells<sup>[23]</sup>. Xu *et al.* found that SETD3 increased the DNA methylation level of doublecortin-like kinase 1 (DCLK1) promoter, inhibited its transcription, and as a result, prevented metastasis of HCC through DCLK1/PI3K/MMP-2 signaling pathway<sup>[24]</sup>. Li *et al.* found that knockout of SETD4 can reduce the phosphorylation of AKT without affecting ERK phosphorylation, enhancing chemosensitivity of HepG2 cells to sorafenib<sup>[25]</sup>.

SETD7 plays a key role in the regulation of cell cycle. SETD7 promotes the proliferation of HCC cells and is also closely related to metastasis, recurrence, and TNM staging of HCC patients, representing a novel and independent prognostic factor for HCC patients<sup>[26]</sup>. SETD7 can cause the methylation of histone H3K4 and promote the proliferation, invasion, and metastasis of HCC cells by regulating the post-translational modification of E2F transcription factor 1<sup>[27]</sup>. EHMT2 is highly expressed in HCC and promotes the

occurrence and development of HCC by silencing tumor suppressor gene *RARRES3*<sup>[28]</sup>. DOT1L is the only HMT lacking SET domain and plays an important role in diverse biological development. However, its role in HCC has not been reported. At present, the development of its inhibitors for use as anti-cancer therapy is a research hotspot.

### 3. Histone demethylase and HCC

Histone demethylase is divided into histone arginine demethylase and histone lysine demethylase. The existence of histone arginine demethylase has always been controversial. JMJD6 and peptidylarginine deiminase 4 (PADI4) are arginine methyltransferases that have been identified<sup>[29]</sup>. However, these two enzymes are not arginine demethylase in a true sense of the word. Weddy *et al.*<sup>[30]</sup> showed that JMJD6 is a lysine hydroxylase. Other studies showed that JMJD6 could not demethylate the arginine residues of histones H4 and H3<sup>[31]</sup>. Silencing *JMJD6* does not affect the methylation of H4R3. PADI4 can catalyze the deimination of histone arginine and turn it into citrulline. In this way, it can prevent the methylation of arginine and regulate the expression of endogenous pS2 gene stimulated by estradiol, thus affecting the structure and function of chromatin<sup>[32]</sup>.

Histone lysine demethylases can be divided into two families: Lysine specific demethylase (LSD) and Jumonji domain containing (JMJD). LSD1 can specifically remove the mono- and di-methylation modification on lysine 4 and 9 of histone H3<sup>[33]</sup>, while JMJD family can remove the trimethylation modification of lysine<sup>[34]</sup>. In general, methylation on lysine 9, 20, and 27 of histone H3 is associated with gene silencing, while methylation on lysine 4, 36, and 79 of histone H3 is associated with gene activation<sup>[35]</sup>. Under the regulation of lysine methyltransferase (KMT) and lysine demethylase (KDM), the methylation status of lysine residues in histone remains stable, and the stability of genome is maintained<sup>[36]</sup>.

#### 3.1. Histone arginine demethylase and HCC

JMJD6, as the first identified PRMT, has been studied in breast cancer, oral cancer, lung adenocarcinoma, ovarian cancer, colon cancer, HCC, and other cancers<sup>[31]</sup>. Studies have shown that JMJD6 has carcinogenic properties, but there is no direct evidence indicating that JMJD6 can remove the methylation of histone arginine, so it remains a controversial issue that needs to be solved. Wan *et al.* showed that JMJD6 is highly expressed in HCC and promotes the expression of CDK4 by directly targeting the promoter of cyclin CDK4, thus promoting the proliferation of HCC cells and accelerating the progression of HCC<sup>[37]</sup>.

Chang *et al.* showed that PADI4 expression was increased in breast cancer, HCC, esophageal squamous cell carcinoma, colorectal adenocarcinoma, renal cell carcinoma, ovarian adenocarcinoma, endometrial cancer, bladder cancer, and other malignant tumor tissues, but

almost no expression was found in benign tissues, especially in liver cancer tissues, and low expression was found in serum of HCC patients<sup>[38]</sup>. A previous study has shown that the growth of liver tumors of mice with PADI4 knockout would significantly slow down, indicating the importance of PADI4 in the development of tumor; therefore, its specific mechanism needs to be further studied<sup>[39]</sup>.

### 3.2. Histone lysine demethylase and HCC

#### 3.2.1. LSD1 and HCC

LSD1, also known as KDM1A, is the first identified histone demethylase. LSD1 promotes the activation of  $\beta$ -catenin and regulates the expression of Lgr5 (leucine-rich repeat-containing G-protein-coupled receptor 5) by directly regulating the level of mono-methylation and dimethylation in the promoter of histone H3K4, thus promoting the tumorigenicity and chemotherapy resistance of HCC<sup>[40]</sup>. Huang *et al.* showed that inhibition of LSD1 attenuated Wnt/catenin signaling pathway and enhanced the drug sensitivity of HCC cells to sorafenib<sup>[41]</sup>. LSD1 plays an important role in regulating the growth and cycle progression of HCC cells. Knockout of LSD1 can inhibit the growth of hepatoma cells, reduce the number of S phase cells, and increase the levels of H3K4me1/2 and H3K9me1/2<sup>[42]</sup>. Wu *et al.* showed that inhibition of LSD1 could enhance the drug sensitivity to regorafenib, promote the cytotoxicity and apoptosis of HCC cells, and inhibits the proliferation of hepatoma cells<sup>[43]</sup>. These results indicate that LSD1 is closely related to the HCC occurrence and development. Thus, targeted drug therapy toward LSD1 is expected to improve the treatment of HCC in the future.

#### 3.2.2. JMJD family and HCC

Dong *et al.* found that KDM4D plays an important role in stellate cell activation and liver fibrosis by regulating toll like receptor signaling pathway<sup>[44]</sup>. KDM4D is highly expressed in liver cancer stem cell like cells (LCSC), and by activating Wnt/ $\beta$ -catenin and Notch signaling pathways, it decreases the level of H3K9me3 in promoter region, promotes the transcription of EpCAM and Sox9, and enhances the expression of EpCAM and Sox9, thus promoting the self-renewal of LCSC and accelerating the development of liver cancer<sup>[45]</sup>. KDM5B is overexpressed in a variety of cancers and regulates the expression of oncogenes and tumor suppressor genes by regulating the demethylation of lysine 4 of histone H3<sup>[46]</sup>. KDM5B is upregulated in HCC and plays an important role in the proliferation of HCC cells. Silencing *KDM5B* promotes the expression of cyclins p15 and p27 by increasing the trimethylation of histone H3K4, thus preventing cell cycle progression in G1/S phase and inhibiting the proliferation of hepatoma cells<sup>[47]</sup>. Tang *et al.* found that JMJD3 mediates the trimethylation of lysine 27 of histone H3, which promotes the reversible progression of EMT and

the invasion and metastasis of HCC by upregulating the expression of slug protein<sup>[48]</sup>. This suggests that JMJD3 is a key factor in stemness and metastatic behavior of HCC. A previous study also showed that JMJD3 and EZH2 play an important role in the proliferation of hepatocytes<sup>[49]</sup>. Inhibition of its enzymatic activity can regulate the methylation of lysine 27 of histone H3, and then affect the differentiation and regeneration of hepatocytes.

#### 4. Clinical applications of histone methylation in HCC

To sum up the preceding sections, **Table 1** presents the enzymes involved in histone methylation and their relationship with HCC. **Figure 1**, on the other hand, portrays the sites of histone methylation and demethylation under the context of HCC.

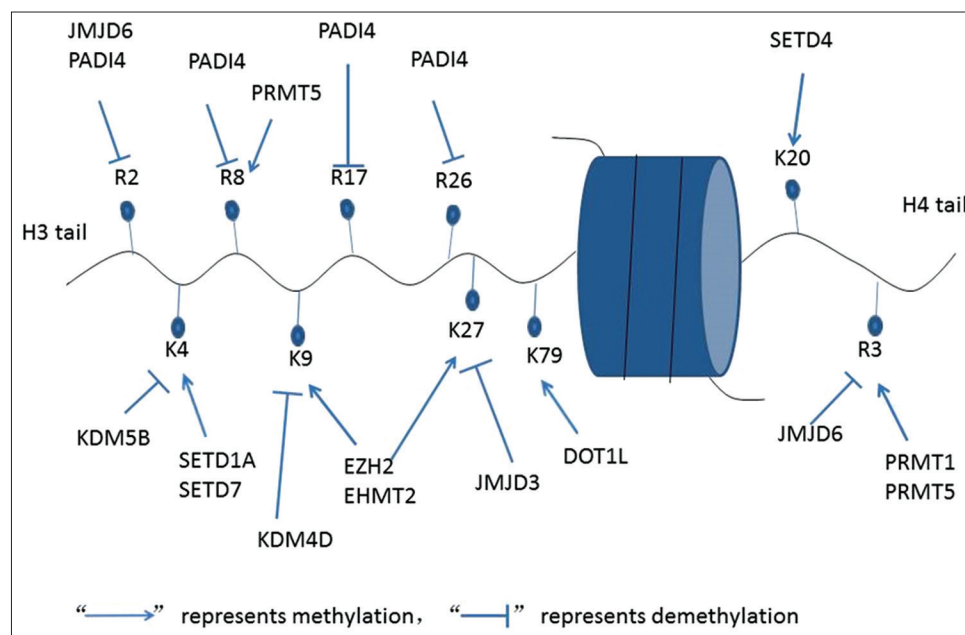
The inhibitors of histone methylation-related enzymes have been under development in recent years, although the relevant research is still at the infancy stage. Due to the low activity, poor selectivity, drug toxicity, and other reasons, their application in clinical practice still requires further verification through research at molecular level and clinical trials. Mann *et al.* showed that peptides based on histone H<sub>4</sub> can inhibit PRMT1, providing a new scheme for the development of specific inhibitors of PRMT1 in structure<sup>[50]</sup>. At the cellular level, UNC0638 could specifically inhibit EHMT2 and reduce the level of H<sub>3</sub>K<sub>9</sub>me<sub>2</sub> on the gene promoter. UNC0638 is stable in cells with high cell titer and low cytotoxicity<sup>[51]</sup>. BIX-01294 is the first identified effective small molecule inhibitor of EHMT2<sup>[52]</sup> that can down regulate the expression of survivin and promote the apoptosis of hepatoma cells<sup>[53]</sup>. Studies have shown that the expression of ras related

GTPase C (RRAGC) in HCC cells treated with BIX-01294 was increased, and EHMT2 was the key regulator of stress response gene expression in HCC cells<sup>[54]</sup>. UNC 0646 and UNC 0631 could reduce the level of H<sub>3</sub>K<sub>9</sub>me<sub>2</sub> by inhibiting EHMT2, with high activity *in vitro* and low cytotoxicity<sup>[55]</sup>. Studies by Gu *et al.* showed that nano diamond (ND)-mediated UNC0646 can enhance its inhibitory effect on HCC *in vitro*, and increase its targeted release to prolong the drug half-life time under the guidance of ND system<sup>[56]</sup>. This curative effectiveness was also seen *in vivo* in an orthotopic HCC mouse model. Continuing to develop drugs targeting EHMT2 can provide new therapeutic options for HCC patients.

#### 5. Summary

To date, a large number of abnormal histone modifications have been identified as independent prognostic factors for a variety of human cancers. Histone methylation regulates gene transcription, participates in DNA damage repair, and DNA replication and is closely related to cell mitosis and cell cycle progression. It plays an important role in the occurrence, development, and metastasis of HCC. The occurrence and development of liver cancer are a multi-gene and multi-step process<sup>[57]</sup>. The main reason for poor prognosis of liver cancer patients is that the disease can easily relapse, metastasize to other parts such as lungs, intestine, and other adjacent organs. In addition, most patients are at the intermediate or terminal stage once diagnosed as early diagnosis of HCC is difficult. Hence, it is crucial to identify new and specific tumor markers for early diagnosis of HCC.

At present, many breakthroughs have been made in the study about the role of histone methylation in HCC, but



**Figure 1.** Actions of enzymes involved in histone methylation and demethylation



Table 1. Relationship between histone methylation and HCC

Type of modification	Enzyme	Alias	Targeted locus	Relationship with HCC	References
Arginine methylation	PRMT1	-	H <sub>4</sub> R <sub>3</sub>	Enzyme expression increases, promoting the proliferation, invasion and metastasis of hepatoma cells by activating EMT and TGF signaling pathways	[18]
	PRMT5	-	H <sub>3</sub> R <sub>8</sub> , H <sub>4</sub> R <sub>3</sub>	Enzyme expression increases, promoting the proliferation of HCC cells through targeting $\beta$ -catenin and regulating the expression of cyclin D1.	[16]
	PRMT9	-	May be H2A	Enzyme expression increases, promoting invasion, and metastasis of hepatoma cells by activating PI3K/AKT signaling pathway	[14]
Lysine methylation	EZH2	KMT6	H <sub>3</sub> K <sub>9</sub> , H <sub>3</sub> K <sub>27</sub>	Enzyme expression increases, promoting the proliferation, invasion, and metastasis of HCC cells through miR-22-3p/galectin-9 axis	[19]
	SETD1A	KMT2F	H <sub>3</sub> K <sub>4</sub>	Enzyme expression increases; high expression of the enzyme is closely related to the prognosis of patients with liver cancer and is a potential therapeutic target	[22]
	SETD3	-	To be determined	Enzyme expression increases, promoting the proliferation, and metastasis of HCC through influencing DCLK1/PI3K/MMP-2 signaling pathway	[24]
	SETD4	-	H <sub>4</sub> K <sub>20</sub>	Silencing <i>SETD4</i> can enhance the sensitivity of HepG2 cells to sorafenib, but the specific mechanism is unknown	[25]
	SETD7	KMT7, SETD7/9	H <sub>3</sub> K <sub>4</sub>	Enzyme expression increases, regulating cell cycle and promoting proliferation, invasion, and metastasis of HCC cells by regulating E2F transcription factor 1	[26,27]
	EHMT2	KMT1C, G9a	H <sub>3</sub> K <sub>9</sub>	Enzyme expression increases, promoting HCC progression by silencing <i>RARRES3</i>	[28]
	DOT1L	KMT4	H <sub>3</sub> K <sub>79</sub>	No relevant research available	-
Arginine demethylation	JMJD6	-	H <sub>3</sub> R <sub>2</sub> , H <sub>4</sub> R <sub>3</sub>	Enzyme expression increases, promoting the expression of CDK4 by targeting its promoter, thus promoting the proliferation of hepatoma cells	[37]
	PADI4	-	H <sub>3</sub> R <sub>2</sub> , H <sub>3</sub> R <sub>8</sub> , H <sub>3</sub> R <sub>17</sub> , H <sub>3</sub> R <sub>26</sub>	Enzyme expression increases in liver cancer tissues but remains low in serum of patients with liver cancer; justification behind this contradiction is unclear	[38]
Lysine demethylation	LSD1	KDM1A	H <sub>3</sub> K <sub>4</sub> , H <sub>3</sub> K <sub>9</sub>	Enzyme expression increases; by activating $\beta$ -catenin and regulating Lgr5, high enzyme expression promotes chemoresistance in HCC, and is a potentially important drug target	[40]
	KDM4D	JMJD2D	H <sub>3</sub> K <sub>9</sub>	Enzyme expression increases, thereby activating Wnt/ $\beta$ -catenin and Notch signaling pathway, promoting the expression of EpCAM and Sox 9, and accelerating the progression of liver cancer	[45]
	KDM5B	JARID1B, PLU1	H <sub>3</sub> K <sub>4</sub>	Enzyme expression increases, thereby inhibiting the expression of p15 and p27, regulating cell cycle, and promoting the proliferation of HCC cells	[47]
	JMJD3	KDM6B	H <sub>3</sub> K <sub>27</sub>	Enzyme expression increases, thereby upregulating slug expression and promoting EMT, invasion, and metastasis	[48]

PRMT1, protein arginine methyltransferase 1; PRMT5, protein arginine methyltransferase 5; PRMT 9, protein arginine methyltransferase 9; EZH2, enhancer of Zeste homolog 2; SETD1A, SET domain containing (lysine methyltransferase) 1A; SETD3, SET domain containing (lysine methyltransferase) 3; SETD4, SET domain containing (lysine methyltransferase) 4; SETD7, SET domain containing (lysine methyltransferase) 7; EHMT2, euchromatin histone lysine methyltransferase 2; DOT1L, disruptor of telomeric silencing 1-like; JMJD6, jumonji domain containing protein 6; PADI4, peptidylarginine deiminase 4; LSD1, lysine-specific demethylase 1; KDM4D, lysine demethylase 4D; KDM5B, lysine demethylase 5B; JMJD3, jumonji domain containing protein 3

the specific molecular mechanism is still unclear. Exploring the relationship between histone methylation and HCC is helpful to clarify the pathogenesis of HCC and develops new diagnostic markers, prognostic markers, and targeted drugs which may directly improve the current approaches to early diagnosis, prognosis, and treatment of HCC.

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## Conflict of interest

None of the authors has any potential conflicts to disclose.

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