Metadherin–PRMT5 complex enhances the metastasis of hepatocellular carcinoma through the WNT–β-catenin signaling pathway

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Abstract

Accumulating data suggest that metadherin (MTDH) may function as an oncogene. Our previous study showed that MTDH promotes hepatocellular carcinoma (HCC) metastasis via the epithelial-mesenchymal transition. In this study, we aim to further elucidate how MTDH promotes HCC metastasis. Using Co-immunoprecipitation (co-IP) and mass spectrometry, we found that MTDH can specifically bind to protein arginine methyltransferase 5 (PRMT5). Further functional assays revealed that PRMT5 overexpression promoted the proliferation and motility of HCC cells and that knockout of PRMT5 impeded the effect of MTDH. The immunohistochemistry assay/tissue microarray results showed that when MTDH was overexpressed in HCC cells, PRMT5 translocated from the nucleus to the cytoplasm, with the subsequent translocation of β-catenin from the cytoplasm to the nucleus and upregulation of the WNT–β-catenin signaling pathway. Further in vivo experiments suggested that PRMT5 and β-catenin played a pivotal role in MTDH-mediated HCC metastasis. We therefore concluded that the MTDH–PRMT5 complex promotes HCC metastasis by regulating the WNT–β-catenin signaling pathway.

Introduction

Surgical resection and liver transplantation are curative interventions for hepatocellular carcinoma (HCC). However, high recurrence rates after surgical intervention compromise the outcome of patients with HCC (1). This predicament highlights the need for a better understanding of the molecular mechanisms underlying HCC invasion and metastasis.

Accumulating data have suggested that metadherin (MTDH) may function as an oncogene. MTDH overexpression is observed in several tumor types, such as HCC, breast cancer, malignant glioma and so forth (2–6), and is associated with a poor clinical outcome. MTDH plays key roles in carcinogenesis (7,8), angiogenesis (9), metastasis (2,10) and chemo resistance (11,12). For instance, Lee et al. (13) revealed that MTDH promotes tumor progression through the phosphorylation of PI3K/Akt; moreover, MTDH enhances the expression of angiogenic factors, such as hypoxia-inducible factor 1-α (9), and matrix metalloprotease-9 (5), and promotes the transformation of endothelial cells to carcinoma-associated fibroblasts (14).

Our previous study demonstrated that MTDH promotes HCC metastasis via the epithelial-mesenchymal transition (EMT) (6). MTDH inhibition in HCC cells leads to the downregulation of N-cadherin and Snail, and the upregulation of E-cadherin. Furthermore, increased cytoplasmic β-catenin and reduced nuclear β-catenin have also been observed when MTDH was
subjected to affinity purification. The purified protein complex was 2D-LC-MS/MS analysis. 2D-LC-MS/MS analyses were performed using a Nano Acuity UPLC system (Waters, Milford, MA) coupled to a LTQ Orbitrap XL mass spectrometer (Thermo Electron, Bremen, Germany), equipped with an online nanoelectrospray ion source (Michrom Biosources, Auburn, CA). A BEH100 C18 reverse phase (75-mm inner diameter, 20 cm) capillary column was used in this study. Under 19-kV accelerating voltage, MS was performed in reflection mode with an m/z range of 400–2000 Da. All MS/MS data were identified using SEQUEST (v.28; BioWorks 3.3 software package; Thermo Electron) against the Human International Protein Index (IPI) database (IPI human v3.45 FASTA with 71,983 entries). The search results were checked by examination of the Xcorr (cross-correlation) and the Dcn (delta normalized correlation) scores.

Glutathione S-transferase pull down

GST pull down assay was applied as described previously (16). The glutathione S-transferase (GST)-MTDH protein GST-MTDH (aa 49–69) was generated by PCR amplification of the DNA fragment encoding amino acid residues 49–69 of human MTDH. The pulled down mixture was analyzed by SDS–PAGE.

Luciferase reporter assay

293 T cells were seeded 24 h before transfection in 24 wells plates at 50–60% confluence. The TCF1/TCF7 promoter reporter constructs were co-transfected along with MTDH, PRMT5 and β-catenin or vector using Lipofectamine 3000 (Invitrogen). After 24 and 36 h, luciferase activity was assessed using the Dual-Luciferase Reporter reagent (Promega) following the manufacturer’s instructions. Renilla luciferase was used for normalization.

Metastasis assays in vivo

Male athymic BALB/c nude mice were purchased from Shanghai Institute of Material Medicine, Chinese Academy of Science, and were raised in specific pathogen-free conditions. Four cell lines (mock-luc, MTDH-PRMT5-luc, MTDH/PRMT5/shβ-catenin-luc or shβ-catenin-luc) were created using LMS cells. 2 × 107 cells were injected in the tail vein of nude mice. The pulmonary metastases (bioluminescence) were detected using an IVIS live-imaging system (Caliper IVIS Lumina XR) every week for 4 weeks. β-Luciferin (150 μl) was injected intravenously before detection. After 4 weeks, the mice were killed and the total number of metastases was counted. Six mice per group were used in each experiment, and animal care and experimental protocols were conducted in accordance with guidelines established by the Shanghai Medical Experimental Animal Care Commission.

Results

PRMT5 is identified to be closely associated with MTDH

To identify MTDH associated proteins in HCC, we affinity-purified MTDH-interacting proteins in MTDH-overexpressing HepG2 cells (MTDH) and their parental counterparts (Control) using co-IP. The purified protein complex was resolved on SDS–PAGE and silver stained, and a new 72-kDa band was observed (Figure 1A). The band was then retrieved and analyzed by MS. A large number of proteins were identified (Table 1), among which PRMT5 had the highest unique peptide score.

PRMT5 belongs to the family of methyltransferases, which catalyze arginine methylation (17). PRMT5 is associated with resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and silver stained, then the bands of interest were retrieved and analyzed by mass spectrometry (MS): The gel pieces were digested with trypsin, then vacuum-dried and resuspended in acetonitrile for MS analysis. 2D-LC-MS/MS analyses were performed using a Nano Acuity UPLC system (Waters, Milford, MA) coupled to a LTQ Orbitrap XL mass spectrometer (Thermo Electron, Bremen, Germany), equipped with an online nanoelectrospray ion source (Michrom Biosources, Auburn, CA). A BEH100 C18 reverse phase (75-mm inner diameter, 20 cm) capillary column was used in this study. Under 19-kV accelerating voltage, MS was performed in reflection mode with an m/z range of 400–2000 Da. All MS/MS data were identified using SEQUEST (v.28; BioWorks 3.3 software package; Thermo Electron) against the Human International Protein Index (IPI) database (IPI human v3.45 FASTA with 71,983 entries). The search results were checked by examination of the Xcorr (cross-correlation) and the Dcn (delta normalized correlation) scores.

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PRMT5 belongs to the family of methyltransferases, which catalyze arginine methylation (17). PRMT5 is associated with
multiple benign and malignant cellular processes and has emerged as a pivotal molecule in tumorigenesis and metastasis (18–21). We therefore selected PRMT5 for further investigation. We performed western blot analysis to compare the expression levels of PRMT5 in one hepatic cell line (L02) and four HCC cell lines with different metastatic potentials (HepG2, Huh7, 97L and LM3) (Figure 1B). The results showed that PRMT5 expression was low in the normal hepatic cell line (L02) and low metastatic HCC cell lines (HepG2 and Huh7), but high in the high metastatic HCC cell lines (97L and LM3). We then treated LM3 with a lentivirus targeting PRMT5 to generate an LM3-knockdown (LM3-KO) cell line in which PRMT5 was stably downregulated compared with that in the parental cell lines. Furthermore, we generated a 97L-overexpressing (97L-OE) cell line in which the level of PRMT5 expression was higher than that in the parental 97L cell line (Figure 1C and D). Proliferation assays using CCK-8 revealed that the PRMT5 knockdown resulted in a significant decrease in the proliferation rate of the HCC cells and vice versa (Figure 1E). We also performed two-chamber migration assays to compare in vitro motility between the parental and interfered HCC cells, and the results suggested that PRMT5 enhanced the motility of HCC cells (Figure 1F). These abilities of PRMT5 were reminiscent of those of MTDH observed in our previous study (6).

To verify the connection between MTDH and PRMT5, we generated an MTDH-overexpressing (MTDH) cell line that expressed a higher level of MTDH than that in the parental cell line, and then, we treated the MTDH cells with a lentivirus targeting PRMT5 to generate MTDH-shPRMT5, in which PRMT5 expression was higher than that in the parental 97L cell line (Figure 1C and D). Proliferation assays using CCK-8 revealed that the PRMT5 knockdown resulted in a significant decrease in the proliferation rate of the HCC cells and vice versa (Figure 1E). We also performed two-chamber migration assays to compare in vitro motility between the parental and interfered HCC cells, and the results suggested that PRMT5 enhanced the motility of HCC cells (Figure 1F). These abilities of PRMT5 were reminiscent of those of MTDH observed in our previous study (6).

Figure 1. PRMT5 is closely associated with MTDH. (A) The SDS-PAGE and silver staining of the purified protein complexes extracted from MTDH-overexpressing and control cells. (B) Western blot analysis of PRMT5 in hepatic and HCC cell lines. (C, D) Efficiency of PRMT5 knockdown (KO) and overexpression (OE) according to western blot (C) and qRT-PCR (D) analysis. (E) Proliferation assays reveals that PRMT5 knockdown results in a significant decrease in the HCC cell proliferation rate and vice versa. (F, G) The results of the two-chamber migration assay show that the number of PRMT5-KO-treated cells that migrated through the transwell was far fewer than that of the cells in the control group. In addition, more PRMT5-OE-treated cells than control cells migrated through the transwell (P < 0.05).
was stably downregulated compared with that in the parental cell lines (Figure 2A). MTDH overexpression led to a marked increase in the proliferation rate; however, when PRMT5 was knocked down, the proliferation rate returned to normal (Figure 2B). The two-chamber migration assays also showed that PRMT5 knockdown hampered the motility of the HCC cells, even when MTDH was overexpressed (Figure 2C). These results suggest that the function of MTDH is mediated by PRMT5.

A single domain in the 49–69 region of MTDH is essential for PRMT5 binding. To further explore the interaction between MTDH and PRMT5, we performed co-IP assays with either an MTDH (Figure 3A) or a PRMT5 (Figure 3B) antibody, and the co-eluted proteins were detected by western blot assays with either a PRMT5 or an MTDH antibody. Both proteins were detected in the co-eluted protein complex, which confirmed that PRMT5 was closely associated with MTDH.

MTDH is a single-pass transmembrane protein (22) that can be divided into three truncations (Figure 3C): the 1–48 domain is the extracellular region, the 49–69 domain is the transmembrane region, and the 70–582 domain in the cytoplasmic region. To identify the region in MTDH that mediated the interaction between MTDH and PRMT5, we first generated a series of Myc-epitope-tagged truncations of MTDH. We co-expressed full-length Flag-PRMT5 and Myc-MTDH in 293 T cells, and co-IP assays were performed with Flag and Myc antibodies. Notably, the full-length protein and the 49–69 domain of MTDH were bound to PRMT5, whereas the 1–48 and 70–582 domains were not (Figure 3D), suggesting that the amino acid residue 49–69 was critical for PRMT5 binding.

To further determine whether PRMT5 directly bound to the 49–69 domain of MTDH, we expressed GST-MTDH and Flag-PRMT5 proteins in Escherichia coli and purified these proteins for in vitro binding assays. Co-IP assays were performed using a GST antibody, and the co-eluted Flag-PRMT5 was detected with a PRMT5 antibody (Figure 3E). Indeed, PRMT5 could bind to both the full length protein and the 49–69 domain of MTDH,

Table 1. MS analysis of MTDH-associated proteins

| Protein name (annotation)                      | Unique peptides |
|-----------------------------------------------|-----------------|
| PRMT5 (protein arginine N-methyltransferase 5) | 20              |
| HSPA9 (cDNA FLJ19077, highly similar to Stress-70 protein, mitochondrial) | 20              |
| RPN1 (dolichyl-diphosphooligosaccharide-protein glycosyltransferase subunit 1 precursor) | 18              |
| HSPA5 (78-kDa glucose-regulated protein)      | 17              |
| AGPS (alkyldihydroxyacetonephosphate synthase, peroxisomal) | 7               |
| GTPBP1 (GTP-binding protein 1)                | 4               |
| ATAD3B (isoform 1 of ATPase family AAA domain-containing protein 3B) | 3               |
| KIAA00020 (Pumilio-domain-containing protein KIAA00020) | 3               |

Proteins with highest score of unique peptide are listed.

Figure 2. PRMT5 knockdown impedes the effect of MTDH. (A) Efficiency of MTDH overexpression (MTDH) and PRMT5 knockdown in MTDH-OE (MTDH-shPRMT5) according to western blot. (B) Proliferation assays reveal that PRMT5 knockdown inhibits the proliferation rate of MTDH cells. (C) The results of the two-chamber migration assay show that fewer MTDH-shPRMT5-treated cells than control MTDH cells migrated through the transwell (*P < 0.05).
suggesting that PRMT5 formed a complex by directly interacting with the 49–69 domain of MTDH.

**PRMT5 overexpression activates the WNT–β-catenin signaling pathway**

To find the downstream targets of PRMT5, we performed RNA-seq assays to identify differentially expressed genes in 97L-Vector VS 97L-PRMT5-OE cells (Figure 4A). We observed that overexpression of PRMT5 resulted in the upregulation of several downstream molecules of the WNT pathway (LEF1, Cyclin D1 and TCF1/TCF7) (23). Further quantitative real-time PCR and western blot analysis confirmed that the expression levels of these proteins were higher when PRMT5 was overexpressed and lower when PRMT5 was knocked down (Figure 4B and C), indicating that a high level of PRMT5 may activate the WNT signaling pathway.

Our previous study showed that β-catenin is closely associated with MTDH in HCC (6). β-Catenin is a crucial factor in the canonical WNT pathway (23–25). We therefore explored the role of β-catenin in MTDH/PRMT5-mediated HCC metastasis. We generated a cell line (MTDH-PRMT5) that expressed higher levels of both MTDH and PRMT5 than those in the parental cell line, and after that, we treated the cells with a lentivirus targeting β-catenin to generate MTDH-PRMT5-shβ-catenin, in which β-catenin was stably downregulated. Luciferase reporter assay was performed on these cell lines to test the effect of MTDH/PRMT5/β-catenin on TCF1/TCF7 transcriptional activity. The results revealed that MTDH and PRMT5 markedly enhanced the activity of TCF1/TCF7, and the activity was impeded when β-catenin was suppressed (Figure 4D).

The proliferation rate and two-chamber migration assays showed that β-catenin knockdown suppressed the growth rate (Figure 4E) and motility (Figure 4F) of MTDH-PRMT5 cell lines, indicating that β-catenin mediates the function of MTDH and PRMT5 in HCC cells.
Figure 4. PRMT5 overexpression activates the WNT–β-catenin signaling pathway. (A) RNA-seq analyses showed that PRMT5 overexpression in 97-H cells resulted in the upregulation of a group of WNT signaling target genes. (B) qRT-PCR analysis of LEF1, Cyclin D1 and TCF1/TCF7 in PRMT5-KO and PRMT5-OE cells compared with their parental counterparts. (C) Western blot results of LEF1, Cyclin D1 and TCF1/TCF7 in the four groups of cells. (D) TCF1/TCF7 promoter driven luciferase reporter assay on MOCK, MTDH-PRMT5, MTDH/PRMT5/shβ-catenin, and shβ-catenin cells. (E) Proliferation assays reveal that β-catenin knockdown inhibits the proliferation rate of MTDH-PRMT5 cells. (F) The results of the two-chamber migration assay show that fewer MTDH-PRMT5-shβ-catenin treated cells than control MTDH-PRMT5 cells migrated through the transwell. (G) Representative bioluminescent images of mice that received mock-luc, MTDH-PRMT5-luc, MTDH/PRMT5/shβ-catenin-luc, or shβ-catenin-luc at day 28 after transfer. Data are bioluminescent signal of each group, representing the pulmonary metastases. (H) Number of pulmonary metastases is significantly larger in the MTDH-PRMT5 group (*P < 0.05).
To explore the effect of MTDH/PRMT5/β-catenin on HCC metastasis, we injected four cell lines (mock, MTDH-PRMT5, MTDH-PRMT5-shβ-catenin or shβ-catenin) in the tail vein of nude mice to create pulmonary metastases. The bioluminescence was detected every week for 4 weeks. After 4 weeks, the mice were killed and the total number of metastases was counted. The results also showed that β-catenin knockdown reduced lung metastases of parental MTDH-PRMT5 cell lines (Figure 4G and H).

PRMT5 and β-catenin competitively bind to MTDH.

To further explore the relationship between PRMT5, β-catenin and MTDH in HCC, we assessed their expression levels in serial sections from the same tissue microarray of 323 patients with HCC (Figure 5A). The IHC results showed that the PRMT5 expression levels in most of the tumor tissues were significantly higher than that in the corresponding adjacent non-tumorous tissues, with PRMT5High accounting for 53.3% (172 of 323) of all patients. The Pearson chi-square test indicated that PRMT5 expression was positively correlated with age, gender, liver cirrhosis, serum alpha fetoprotein (AFP), tumor diameter, Edmonson grade, TNM stage and MTDH expression (Supplementary Table 2, available at Carcinogenesis Online).

PRMT5 was localized to both the nuclear and cytoplasmic compartments. However, the distribution varied among patients, with PRMT5Nuclear accounting for 36.8% (119 of 323) of all the patients. The Pearson chi-square test also showed a close correlation between PRMT5 and age, serum AFP, microvascular invasion, Edmonson grade, MTDH expression (Supplementary Table 3, available at Carcinogenesis Online). Our former study indicated that β-catenin translocation from the membrane to the
nucleus could be induced by MTDH overexpression in HCC (6). We therefore compared the distribution of PRMT5 and β-catenin in the tissue microarray. The results showed that when PRMT5 was localized to the nucleus, β-catenin was localized to the membrane, and vice versa (P < 0.001) (Figure 5A, Supplementary Table 3, available at Carcinogenesis Online).

We then stably overexpressed MTDH in HepG2 and HuH7 cells, and separated the cell extracts into nuclear and cytoplasmic fractions. As evidenced by western blot (Figure 5B), MTDH overexpression in HCC cells resulted in an increase in PRMT5 and a decrease in β-Catenin in the cytoplasm, with a corresponding decrease in PRMT5 and increase in β-catenin in the nucleus. In addition, the inhibition of PRMT5 led to increased cytoplasmic β-catenin and decreased nuclear β-catenin (Figure 5C). These results confirmed that MTDH overexpression led to the translocation of PRMT5 from the nucleus to the membrane, and of β-catenin from the membrane to the nucleus.

The earlier results suggested that PRMT5 and β-catenin competitively bind to the same domain on MTDH. To confirm our hypothesis, we co-expressed full-length HA-β-catenin and either Myc-MTDH or Myc-49–69-MTDH in 293 T cells, and co-IP assays were carried out with an HA antibody, and the co-eluted proteins were visualized by western blot assay with a Myc antibody. The results showed that both the full-length protein and the 49–69 domain of MTDH bound to β-catenin (Figure 5D).

To determine whether PRMT5, PRMT5 and β-catenin formed functional ternary complexes, we co-expressed Myc-MTDH and HA-β-catenin together with an increasing amount of Flag-PRMT5 in 293 T cells, and performed co-IP assays with Flag antibody. The co-eluted proteins were visualized by western blot with either Flag or HA antibody (Figure 5E). MTDH Flag antibody. The co-eluted proteins were visualized by western blot with either Flag or HA antibody (Figure 5E). MTDH readily co-immunoprecipitated both PRMT5 and β-catenin. Strikingly, increasing the amount of PRMT5 caused MTDH to immuno-precipitate more PRMT5 and less β-catenin, suggesting that PRMT5 decreased the assembly of the MTDH and β-catenin complex. These results suggest that PRMT5 and β-catenin may competitively bind to the same domain on MTDH.

**Discussion**

Herein, we identified the MTDH–PRMT5 complex as an enhancer of HCC metastasis via the WNT–β-catenin signaling pathway. We demonstrated that PRMT5 and β-catenin may competitively bind to the same domain on MTDH. When MTDH is overexpressed, MTDH preferentially recruits PRMT5, and the redundant β-catenin translocates to the nucleus, and participates in the WNT–β-catenin signaling pathway. PRMT5 knockdown hampers the effect of MTDH on the proliferation and motility of the HCC cells, and inhibition of β-catenin disrupts the effect of both MTDH and PRMT5.

PRMT5 is an important modulator of tumor cell function, and PRMT5 dysregulation is observed in multiple malignancies (26–28). Powers et al. (26) revealed that high levels of PRMT5, with corresponding arginine methylation of the tumor suppressor programmed cell death 4, correlate with a worse outcome in patients with breast cancer by Chen et al. (29) demonstrated that PRMT5 plays a key role in a TGFβ1–PRMT5–MEP50-axis-mediated EMT in lung cancer and breast cancer. Recently, PRMT5 was confirmed to have an oncogenic function in the context of leukemia/lymphoma (28) and has been recognized as a promising target for leukemia therapy (28,30). Through co-IP and MS, we demonstrated that PRMT5 is closely associated with MTDH. Functional assays revealed that the overexpression of PRMT5 promoted HCC cell proliferation and motility, an association that we previously observed with high MTDH levels (6). We also found that when PRMT5 was inhibited, MTDH overexpression promoted neither the proliferation nor motility of HCC cells, implying that MTDH functioned only in the presence of PRMT5. These results highlight the significance of PRMT5 in MTDH-mediated regulation.

PRMT5 has been linked to the remodeling of chromatin structure via arginine methylation of histone proteins H4 and H3, thereby modifying gene transcription, DNA repair and RNA processing (28,31,32). For instance, several studies have demonstrated that PRMT5 is required in the regulation of the P53 gene via arginine methylation (28,33–35). Pal et al. (18) found that PRMT5 inhibited tumor suppressor gene tumorigenicity 7 and non-metastatic 23 through H3R8 methylation and H3K9 deacetylation. PRMT5 also forms a complex with the ajuba LIM protein and Snail proteins and suppressed the expression of E-cadherin (16).

In our study, we provide considerable evidence showing that in addition to epigenetic regulation of histone methylation, PRMT5 effects the location of β-catenin in HCC through spatial interactions. Using co-IP and IHC analysis of serial sections of HCC samples, we confirmed that PRMT5 and β-catenin competitively bind to the same domain on MTDH. Furthermore, using reciprocal co-IP, we revealed that PRMT5 and β-catenin share the same binding site in the 49–69 region of MTDH. The translocation of β-catenin from the cytoplasm to the nucleus after MTDH overexpression was also confirmed and was consistent with the observations of our previous report (6). In addition, the competitive binding and translocation of β-catenin depended on the expression levels of MTDH and PRMT5 in the cells (Figure 5F).

In this study, we also demonstrated that PRMT5 may promote the proliferation and motility of HCC cells by activating the canonical WNT pathway. Nuclear β-catenin can bind to high-mobility group box proteins, which contains the T-cell factor/lymphoid enhancer factor (TCF/LEF), and thereby activate the target genes of the WNT signaling pathway (23). Accumulating evidence shows that the activation of WNT/β-catenin-mediated signaling plays a key role in the development of HCC (6,24,36,37). Yoo et al. (24) showed that the ERK42/44-mediated activation of WNT/β-catenin signaling upregulates TCF/LEF, which is the ultimate initiator of the WNT pathway. Tsao et al. (37) revealed that WNT/β-catenin signaling plays a significant role in the SOX1-mediated development of HCC. These findings show that WNT/β-catenin signaling is pivotal in the progression of HCC. LEF1, Cyclin D1 and TCF1/TCF7 are central mediators of the WNT–β-catenin pathway (23,38,39). Notably, we have shown that these proteins are upregulated and activated when PRMT5 is overexpressed, indicating that a high level of PRMT5 may activate the WNT–β-catenin signaling pathway (Figure 5F).

β-Catenin disrupted the effect of both MTDH and PRMT5, which suggests that β-catenin plays a pivotal role in MTDH/PRMT5-mediated HCC metastasis.

WNT/β-catenin signaling also induces EMT via the translocation of β-catenin from the cytoplasm to the nucleus, where β-catenin contributes to enhancing the expression of several EMT-inducing transcription factors (40,41). This outcome might explain our former findings whereby MTDH may promote the EMT of HCC cells via the WNT–β-catenin signaling pathway (6).

Nevertheless, as MTDH is a transmembrane protein that interacts with multiple proteins (2,10,42,43), β-catenin is unlikely to be the only factor affected by the MTDH–PRMT5 complex. The spatial characterizations of the binding motifs in MTDH and PRMT5/β-catenin need further investigation.
In summary, our findings indicate that PRMTs and β-catenin may competitively bind to the same domain on MTDH. Thus, the MTDH–PRMT5 complex may promote HCC metastasis by regulating the WNT–β-catenin signaling pathway. This dynamic may provide new targets for therapy and markers for HCC diagnosis.

Supplementary material
Supplementary data are available at Carcinogenesis online.

Funding
National Natural Science Funds of China (nos. 81402376, 81572296, 81502028, 31601114); Shanghai Hospital Development Center (SHDC12015104); National Key Research and Development Program (2016YFC0902400).

Conflict of Interest Statement: The authors declare that they do not have anything to disclose regarding funding from industries or conflict of interest with respect to this manuscript.

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