Genes & Diseases (2023) 10, 2622–2638

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

FULL LENGTH ARTICLE

PRMT1 promotes the proliferation and metastasis of gastric cancer cells by recruiting MLXIP for the transcriptional activation of the β-catenin pathway

Feng Wang [a,c], Shitong Chen [a,c], Shihan Peng [a,c], Xujun Zhou [a,c], Houyi Tang [a,c], Hanghua Liang [a,c], Xi Zhong [a,c], He Yang [a,c], Xiaoxue Ke [a,c,*], MuHan Lü [b,], Hongjuan Cui [a,c,d,*]**

[a] *State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China*
[b] *Department of Gastroenterology, The Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Jiangyang District, Luzhou, Sichuan 646000, China*
[c] *Cancer Center, Medical Research Institute, Southwest University, Chongqing 400716, China*
[d] *Jinfeng Laboratory, Chongqing 401329, China*

Received 23 June 2022; accepted 3 February 2023
Available online 28 March 2023

KEYWORDS
β-catenin signaling pathway;
Gastric cancer;
PRMT1;
Transcriptional regulation;
Ubiquitination

Abstract Protein arginine methyltransferase 1 (PRMT1), a type I PRMT, is overexpressed in gastric cancer (GC) cells. To elucidate the function of PRMT1 in GC, PRMT1 expression in HGC-27 and MKN-45 cells was knocked down by short hairpin RNA (shRNA) or inhibited by PRMT1 inhibitors (AMI-1 or DCLX069), which resulted in inhibition of GC cell proliferation, migration, invasion, and tumorigenesis *in vitro* and *in vivo*. MLX-interacting protein (MLXIP) and Kinectin 1 (KTN1) were identified as PRMT1-binding proteins. PRMT1 recruited MLXIP to the promoter of β-catenin, which induced β-catenin transcription and activated the β-catenin signaling pathway, promoting GC cell migration and metastasis. Furthermore, KTN1 inhibited the K48-linked ubiquitination of PRMT1 by decreasing the interaction between TRIM48 and PRMT1. Collectively, our findings reveal a mechanism by which PRMT1 promotes cell proliferation and metastasis mediated by the β-catenin signaling pathway.

* Corresponding author. State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China.
** Corresponding author.
E-mail addresses: kexiaoxue@126.com (X. Ke), 52721759@qq.com (M. Lü), hcui@swu.edu.cn (H. Cui).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2023.02.006

Introduction

Gastric cancer (GC) is a common malignancy, with more than 1 million cases diagnosed per year worldwide.[1] As the fifth most common cancer, GC is associated with high mortality, and the median survival is less than one year because GC is frequently diagnosed at an advanced stage.[2] Many factors induce GC development, including agedness, cigarette smoking, *Helicobacter pylori* infection, alcohol consumption, and previous gastric surgery.[3] As the strongest risk factor, *H. pylori* encodes an oncoprotein named cytotoxin-associated gene A (CagA), which affects the expression of signaling proteins.[4] In addition to the aforementioned factors, gene mutation is an important factor associated with GC tumorigenesis. Almost 40% of GC patients carry mutations in the CDH1 or CTNNA1 gene, and other candidate genes, including TP53, FBXO24, and DOT1L, exhibit multisite mutations.[5] Furthermore, changes in HER2 gene expression can lead to the occurrence of GC. As a member of the human epidermal growth factor receptor (EGFR) family, HER2 regulates cell proliferation, differentiation, and survival.[6] Trastuzumab, which is a humanized monoclonal antibody against HER2, has been used for the treatment of advanced GC and, when combined with chemotherapy, can extend overall survival.[7] However, for most GC patients, the underlying genetic alteration is unknown. Identifying the genes associated with GC tumorigenesis may provide therapeutic targets for gene therapy.

Arginine methylation, which was first described in the late 1960s and early 1970s, is a common posttranslational modification that regulates multiple cellular processes.[8] The enzymes that induce arginine methylation are named protein arginine methyltransferases (PRMTs). All nine annotated mammalian PRMTs are classified into three types based on their different functions. All PRMTs catalyze the generation of monomethylarginine (MMA).[9] Based on MMA type, type I enzymes (PRMT1, PRMT2, PRMT3, PRMT4, PRMT6, and PRMT8) methylate the same nitrogen with a second methyl group, forming asymmetric dimethylarginine (ADMA), whereas type II enzymes (PRMT5 and PRMT9) induce the methylation of a guanidine nitrogen, forming symmetric dimethylarginine (SDMA), and the type III enzyme (PRMT7) only catalyzes the formation of MMA.[10]

PRMT dysregulation has been associated with cancers. According to 949 PRMT-related publications, among all nine PRMTs, PRMT1, PRMT4, and PRMT5 are the most highly expressed in cancers.[11] These three PRMTs promote tumorigenesis through arginine-methylation-mediated control of histone or nonhistone activity and thus regulate oncogene or signaling pathway expression, RNA transport and splicing, or the DNA damage response.[12]

Our previous study revealed that PRMT4 promotes GC cell proliferation through the transcriptional activation and methylation of Notch 2.[13] Here, we report that PRMT1 is highly expressed in GC cells and indicates a poor prognosis for GC patients. As a type I PRMT, PRMT1 has received some attention because of its function in tumorigenesis and development. In 2004, Robert G Roeder and his colleagues found that PRMT1, CARM1, and p300 acted cooperatively in the transcriptional activation of p53.[14] Subsequently, some studies reported that PRMT1 knockdown activates the p53 signaling pathway [15]and that the PRMT1-p53 pathway controls epicardial EMT and invasion.[16] Additionally, PRMT1 can participate in ubiquitination, promoting breast cancer metastasis by regulating the stability of EZH2.[17] In acute megakaryocytic leukemia, PRMT1 controls RNA splicing by inhibiting the degradation of RBM15 by CNOT4.[18] In colorectal cancer, PRMT1 enhances the EGFR signaling pathway through methylation of SMARCA4 and NONO.[19,20] In this study, we identified multiple PRMT1-binding proteins and revealed that PRMT1 forms a transcriptional complex with MLX-interaction protein (MLXIP), which activates the expression of β-catenin by binding to its promoter. Furthermore, we found that Kinectin 1 (KTN1) stabilizes PRMT1 protein expression by binding with PRMT1 and inhibiting the ubiquitination of PRMT1 by tripartite motif-containing 48 (TRIM48). This study not only reveals the mechanism by which PRMT1 promotes cell proliferation and metastasis *in vivo* and *in vitro* through transcriptional activation of the β-catenin signaling pathway but also explains the process of PRMT1 ubiquitination in GC cells. These findings may lead to a novel strategy for GC targeted therapy.

Materials and methods

Cell culture and cell proliferation assay

The GES-1 gastric tissue cell line, BGC-832, HGC-27, MGC-803, MKN-45, and SGC-7901 gastric cancer cell lines and HEK293FT human embryonic renal cell line were purchased from American Type Culture Collection (ATCC, Beijing, China). GES-1 and the gastric cancer cell lines were cultured in Park Memorial Institute-1660 (RPMI-1640, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco, 10099141) and 1% penicillin and streptomycin (P/S). The HEK293FT cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% FBS (Gibco, 10099141), 1% P/S, 4 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids (MEM), and 0.5 mg/mL G418. All the cell lines were cultured in 5% CO_2 at 37 °C in a humidified incubator. Two PRMT1 inhibitors, AMI-1 (HY-18962) and DCLX069 (HY-122096), were purchased from MCE. Cell proliferation analyses were performed as previously described.[21]

Transfection and infection

Human PRMT1 shRNA, KTN1 shRNA, TRIM48 shRNA, and MLXIP shRNA were purchased from BGI. The shPRMT1, shKTN1, shTRIM48, and shMLXIP sequences (Table 1) were inserted into a pLKO.1 vector. The constructed plasmid was combined with 0.6 μg of pLP1, pLP2, and pLP/VSVG plasmids and transfected into HEK293FT cells using Lipofectamine 2000 transfection agent. After culturing for 48 h, the supernatant was collected.

The virus-containing supernatant was supplemented with 4 μg/mL polybrene and used for infection by incubation for 24 h. After two rounds of infection, the cells were cultured with 2 mg/mL puromycin for stable cell line selection. After two days of selection, the stable cell lines were harvested for subsequent experiments.

Immunoprecipitation (IP) and mass spectrometry (MS)

IP analyses were performed as previously described.[13] The PRMT1-binding proteins were identified by liquid chromatography-tandem mass spectrometry (Thermo Scientific, Shanghai Applied Protein Technology, Shanghai, China).

Western blot (WB) assay and antibodies

For the WB assays, cells were harvested and lysed with RIPA lysis buffer containing protease inhibitor cocktail for 30 min. Then, the samples were centrifuged for 15 min (12,000 g, 4 °C), and the supernatant was collected. After boiling, the proteins in the supernatant were separated by 10% SDS—PAGE and transferred to a PVDF membrane. The PVDF membrane was blocked with 5% fat-free milk for 2 h at room temperature and then incubated with primary antibodies (Table 2) overnight at 4 °C. The following day, after washing three times with TBST buffer, the membrane was incubated with secondary antibodies for 2 h at room temperature. The signals were detected by enhanced chemiluminescence (ECL) reagent (Clinx Science, Shanghai, China) and visualized by WB detection instruments (Clinx Science, Shanghai, China).

Chromatin immunoprecipitation (ChIP) assay

According to a CUT & RUN assay (86,652, CST) protocol, ChIP analyses were performed with ChIP qRT—PCR primers (Table 3).

Quantitative real-time PCR (qRT—PCR)

Briefly, cells were harvested and lysed with TRIzol. Then, total RNA was extracted according to the Life Technology instructions, and cDNA was thus obtained. qRT—PCR was performed based on a qRT—PCR assay (E096-01 B, Novoprotein) protocol using qRT—PCR primers (Table 4).

Proximity ligation assay (PLA)

After transient transfection, 3×10^4 cells were grown on coverslips in 24-well plates in preparation for a PLA. The cells were fixed in 4% paraformaldehyde for 20 min and permeabilized in 0.3% Triton X-100 for 15 min at room temperature. Then, after blocking in 5% goat serum for 1 h at room temperature, the coverslips were incubated with primary antibodies overnight at 4 °C. After washing with wash buffer A three times, the coverslips were incubated with PLA probes (DUO92001 and DUO92005, Sigma) for 1 h at 37 °C. Then, the coverslips were incubated with ligation-ligase solution (DUO92008, Sigma) for 30 min at 37 °C and

Table 1 List of shRNA primers used in this study.

Name	Sequence (5′-3′)	
shPRMT1-1#	F:CCGGGTGTTCCAGTATCTCTGATTACTCGAGTAATCAGAGATACTGGAACACTTTTTG	R:AATTCAAAAAGTGTTCCAGTATCTCTGATTACTCGAGTAATCAGAGATAC TGGAACAC
shPRMT1-2#	F:CCGGTGAGCGTTCCTAGGCGGTTTCCTCGAGGAAACCGCCTAGGAACGCTCATTTTTG	R:AATTCAAAAATGAGCGTTCCTAGGCGGTTTCCTCGAGGAAACCGCCT AGGAACGCTCA
shMLXIP-1#	F:CCGGGAGATTGTGATCCGGAGTATCTCGAGATACTCCGGATCACAATCTCTTTTTG	R:AATTCAAAAAGAGATTGTGATCCGGGAGTATCTCGAGATACTCCCGG ATCACAATCTC
shMLXIP-2#	F:CCGGGTGTCCTTGGTGTTGAAGAATCTCGAGATTCTTCAACACCAAGGACACTTTTTG	R:AATTCAAAAAGTGTCCTTGGTGTTGAAGAATCTCGAGATTCTTCAAC ACCAAGGACAC
shKTN1-1#	F:CCGGCGTGATCGTTTAACAAGTAAACTCGAGTTTACTTGTTAAACGATCACGTTTTTG	R:AATTCAAAAACGTGATCGTTTAACAAGTAAACTCGAGTTTACTTGTT AAACGATCACG
shKTN1-2#	F:CCGGGCCAAATTAAAGCCTTATTTACTCGAGTAAATAAGGCTTTAATTTGGCTTTTTG	R:AATTCAAAAAGCCAAATTAAAGCCTTATTTACTCGAGTAAATAAGG CTTTAATTTGGC
shTRIM48-1#	F:CCGGACTTCCTCTCAGACCTATCTTCTCGAGAAGATAGGTCTGAGAGGAAGTTTTTTG	R:AATTCAAAAAACTTCCTCTCAGACCTATCTTCTCGAGAAGATAG GTCTGAGAGGAAGT
shTRIM48-2#	F:CCGGCATCTGCATGAACTACTTCATCTCGAGATGAAGTAGTTCATGCAGATGTTTTTG	R:AATTCAAAAACATCTGCATGAACTACTTCATCTCGAGATGAAGT AGTTCATGCAGATG

Table 2 List of antibodies.

Antibody	Company	Product Code
PRMT1	Abcam	Ab190892
His-Tag	Abcam	Ab5000
MLXIP	Abcam	Ab176688
TRIM48	Bioss Antibodies	BS-16741 R
Myc-Tag (Mouse)	Cell Signaling Technology	2276
Myc-Tag (Rabbit)	Cell Signaling Technology	3946
Flag-Tag (Mouse)	Cell Signaling Technology	8146
Flag-Tag (Rabbit)	Cell Signaling Technology	14,793
HA-Tag	Cell Signaling Technology	3724
β-Catenin	Cell Signaling Technology	8480
β-Catenin Activated Targets Antibody Sampler Kit	Cell Signaling Technology	8655 T
Tubulin	Proteintech	66,031-1-lg
KTN1	Proteintech	19841-1-AP

amplification-polymer solution (DUO92008, Sigma) for 100 min at 37 °C. Finally, the signal was captured by confocal microscopy (FluoView FV1000, Olympus, Japan) after nuclear and tubulin staining.

Wound-healing assay

A straight line was scraped with 10-μL pipette tips in cells grown in 6-well plates. After the plates were washed twice with PBS, the cells were incubated in an FBS-free medium. Microscope photographs of the scratch made in the HGC-27 cells were taken at 0 h, 12 h, and 24 h, and photographs of the MKN-45 cells were taken at 0 h, 30 h, and 60 h. The wound width at 0 h was set to be 100% in each group. A wound width analysis was performed with Photoshop and GraphPad 5.0 software.

Migration and invasion assays *in vitro*

For the *in vitro* migration assay, 8×10^4 cells in 200 μL of FBS-free medium were plated in a 24-well plate cell culture insert, and RPMI-1640 medium containing 10% FBS was added to the bottom of the insert. After incubation for 36 h, the cells were fixed in 4% paraformaldehyde for 20 min and then stained with crystal violet blue for 20 min. After washing three times with PBS, the cells on the upper surface of the insert were removed with a cotton swab. Then, the polyester (PET) insert membrane was exfoliated and examined with a microscope. For the invasion assay, 50 μL of Matrigel was added to each insert before the cells were plated. Then, the insert was incubated at 37 °C in a humidified incubator until the Matrigel had solidified. Then, the invasion assay was performed following the same protocol used for the migration assay.

Table 3 List of ChIP qRT-PCR primers used in this study.

β-Catenin promoter primer	Sequence (5′-3′)	
Amplicon A	F:TCGTGAGTGGGGACAGAAG	R:GCAGCCCAGTCTCACAGC
Amplicon B	F:TGACAAGTGGAACCAGATAAAA	R:TGTCCCCACTCACGAAGG
Amplicon C	F:CAGACGGCAGTTGGCATT	R:GTTACCTCTGTGCTTTTATCCC

Table 4 List of qRT-PCR primers used in this study.

Gene Name	Sequence (5′-3′)	
PRMT1	F:AGGTGGACATCTATACCGTCAA	R:CGTAGTCATTCCGCTTCACTT
MLXIP	F:GCTCTTCGAGTGCATGACTTT	R:TCTCTCCACTGTAGCTTCAGG
β-Catenin	F:CATCTACACAGTTTGATGCTGCT	R:GCAGTTTTGTCAGTTCAGGGA
CCND1	F:CAATGACCCCGCACGATTTC	R:CATGGAGGGCGGATTGGAA
MYC	F:GTCAAGAGGCGAACACACAAC	R:TTGGACGGACAGGATGTATGC
TCF1	F:AACACCTCAACAAGGGCACTC	R:CCCCACTTGAAACGGTTCCT
PPARδ	F:GCCTCTATCGTCAACAAGGAC	R:GCAATGAATAGGGCCAGGTC
c-JUN	F:TCCAAGTGCCGAAAAAGGAAG	R:CGAGTTCTGAGCTTTCAAGGT
MET	F:GGTTCACTGCATATTCTCCCC	R:ACCATCTTTCGTTTCCTTTAGCC
GAPDH	F:ACGGATTTGGTCGTATTGGG	R:CGCTCCTGGAAGATGGTGAT

Subcutaneous xenograft and metastasis assay *in vivo*

For an *in vivo* subcutaneous xenograft assay, NOD/SCID female mice (4 weeks old) (Cavens, Jiangsu, China) were subcutaneously injected in both flanks with 4×10^6 cells. One month after the cell injection, the mice were euthanized, and tumors were weighed and photographed.

For an *in vivo* metastasis assay, 4×10^6 cells were injected via the tail vein. After 6 weeks, the mice were euthanized, and lung tissues were collected and immobilized in 4% paraformaldehyde before hematoxylin and eosin staining. Macroscopic metastases were quantified by counting lesions in all lobes in the lung of each mouse.

Animal experiments were approved by the IACUC of Southwest University and carried out in conformity with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006).

Statistical analysis

All experiments were carried out in triplicate, and statistical parameters, including the sample size and significance analysis, are specified in the figure legends. Data from at least three experiments were the basis for calculating the mean ± standard deviation. Two-tailed Student's *t*-test was performed for paired samples following a normal distribution with different but similar SD values. All data analyses were performed with GraphPad Prism 5 software. A P value < 0.05 was considered statistically significant.

Results

PRMT1 is highly expressed in gastric cancer and is associated with a poor prognosis

To measure the expression of PRMT1 in GC, we used the Oncomine database in our analysis. Specifically, the data from four datasets (Derrico Gastric Statistics, Wang Gastric Statistics, Cho Gastric Statistics, and Chen Gastric Statistics) indicated that PRMT1 was overexpressed in GC tissues (Fig. 1A–D). Consistent with these data, an analysis of The Cancer Genome Atlas (TCGA) database enabled us to confirm that PRMT1 expression levels were increased in GC tissues compared with normal tissues (Fig. 1E). Additionally, we found that the expression of PRMT1 gradually increased, becoming higher than that in normal tissue in grade 3 GC (Fig. 1F), which suggested that PRMT1 may be correlated with GC malignancy. Moreover, PRMT1 expression was examined via WB assay with a normal gastric tissue cell line (the GES-1 cell line) and five GC cell lines (the BGC-823, HGC-27, MGC-823, MKN-45, and SGC-7901 cell lines), and PRMT1 expression was found to be increased in the GC cell lines (Fig. 1G). Kaplan–Meier survival analysis suggested that PRMT1 overexpression predicted shorter overall survival in GC patients ($P < 0.001$; Fig. 1H). As a previous study reported that PRMT1 was predominantly expressed in the nucleus,[22] immunofluorescence assays were carried out to confirm this reported result. As shown in Figure 1I, the immunofluorescence results revealed that PRMT1 was expressed in both the cytoplasm and nucleus. Moreover, the expression levels of PRMT1 in normal tissues were lower than those in GC tissues, in which PRMT1 was highly expressed. Data from six normal tissues and thirty-six GC tissues are shown in Figure 1J and Figure S1A. The results of these assays strongly suggest that PRMT1 may be a GC oncogene.

PRMT1 promotes cell proliferation and tumor growth in gastric cancer

To address the role played by PRMT1 in GC, we used short hairpin RNA (shRNA) technology and generated stable PRMT1-silenced GC cells. As shown in Figure 2A, expression of either shRNA vector led to depleted PRMT1 expression (shGFP was used as the negative control). Next, cell proliferation was measured by the MTT assay with control and PRMT1-silenced cells, and the results indicated that the proliferation rate was slowed significantly compared with that of the shGFP-expressing cells (Fig. 2B). In addition, we found that overexpressed PRMT1 could promote the proliferation of GES1 cells (Fig. S1B). To our surprise, our MTT assay results contradicted those of Min Xu, who reported that cell proliferation was promoted after PRMT1 expression was knocked down.[23] Therefore, to validate our results, two PRMT1 inhibitors (AMI-1 and DCLX069) were employed to measure the influence of PRMT1 on GC cell proliferation. Cell proliferation was inhibited in a dose-dependent manner after treatment with either PRMT1 inhibitor (Fig. 2C, D). Consistent with these findings, BrdU staining assays showed that the percentage of BrdU-positive cells was reduced in the PRMT1-depleted groups (Fig. 2E) and PRMT1-inhibited groups (Fig. 2F).

Next, the tumorigenic potential of GC cells expressing either shGFP or shPRMT1 *in vitro* was examined by a soft agar assay. Negative control cells formed a greater number of and larger colonies than PRMT1-silenced cells (Fig. 2G). As shown in Figure 2H and I, the tumor growth ability of GC cells *in vivo* was inhibited in cells with PRMT1 down-regulation. Collectively, our results confirmed that PRMT1 is essential for cell proliferation and tumorigenesis in GC.

PRMT1 enhances gastric cancer cell migration and invasion *in vitro* and metastasis *in vivo*

To further delineate the functions of PRMT1 in GC, we systematically measured the effect of PRMT1 on cell migration and metastasis *in vitro* and *in vivo*. First, wound-healing assays were performed after the PRMT1 expression was knocked down. As shown in Figure 3A and B and Figure S1C and D, PRMT1 depletion inhibited wound healing in HGC-27 and MKN-45 cell monolayers. In addition, the wound healing rate was markedly decreased by treatment with the PRMT1 inhibitors (Fig. 3C, D; Fig. S1E, F). After determining the influence of PRMT1 on wound healing, we assessed the effect of PRMT1 expression on cell migration and invasion *in vitro* by performing Transwell assays and determined that the cell migration and invasion rates were reduced after PRMT1 expression or activity was inhibited *in vitro* (Fig. 3E–H; Fig. S1G–J). Finally, we evaluated the effect of PRMT1 on lung metastasis after tail vein injection

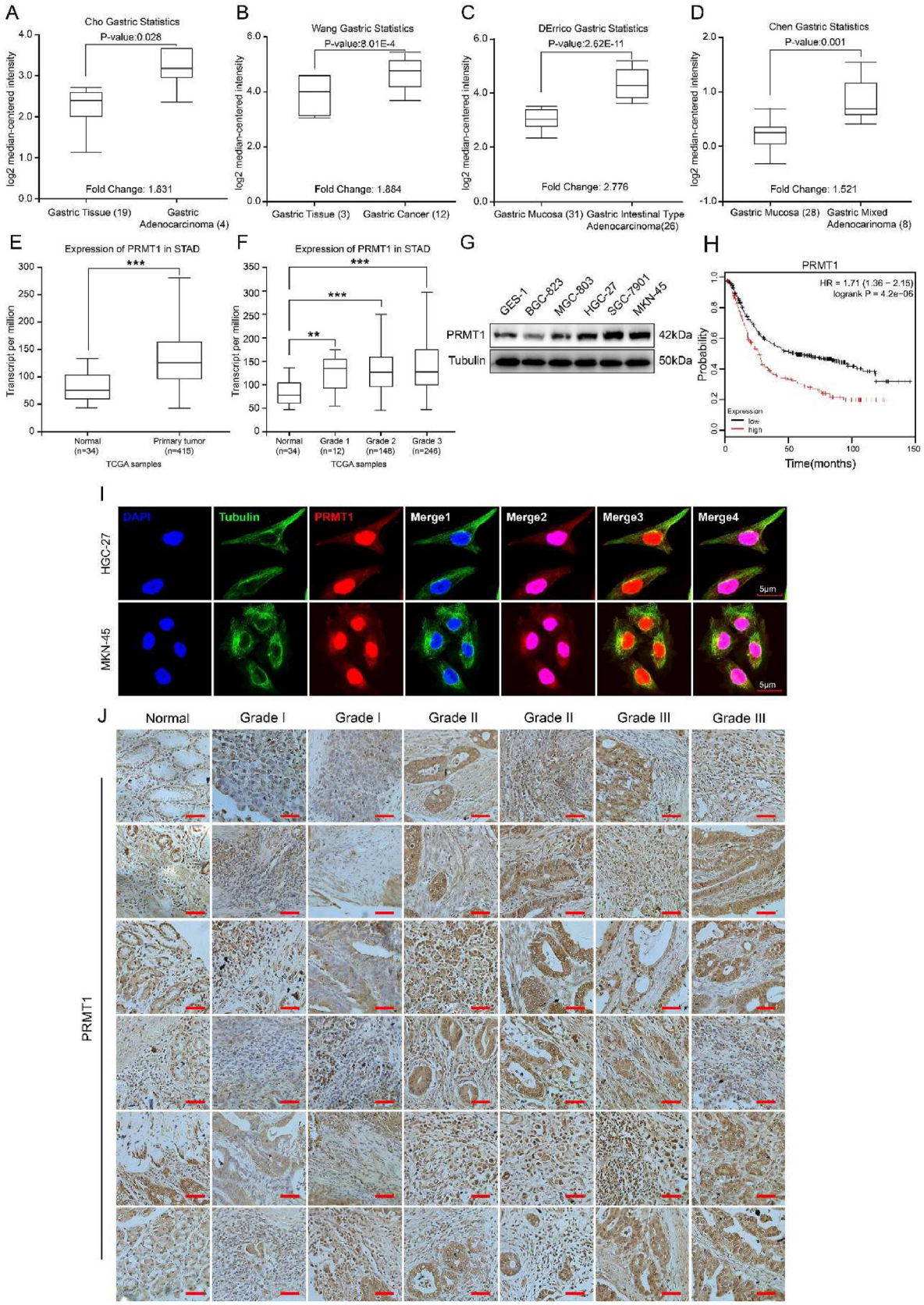

Figure 1 PRMT1 is highly expressed in gastric cancer (GC) and is associated with a poor prognosis. **(A—D)** Box plots showing PRMT1 expression levels in gastric tissue and gastric adenocarcinoma (A), gastric tissue and gastric cancer (B), gastric mucosa and gastric intestinal-type adenocarcinoma (C), and gastric mucosa and gastric mixed adenocarcinoma (D) in the Oncomine database with the indicated log-rank test *P* values. **(E, F)** Box plots showing PRMT1 expression levels in normal tissue and primary tumors (E) and

in vivo. One month after inoculation, mouse lungs were collected and stained with hematoxylin and eosin. As shown in Figure 3I, J and Figure S1K, compared with those in the control group, the mice injected with PRMT1-silenced cells formed fewer and smaller lung metastatic nodules. In summary, we concluded that PRMT1 promoted the metastasis of GC cells *in vitro* and *in vivo*.

PRMT1 activates the β-catenin signaling pathway mediated by the transcriptional activation of β-catenin

PRMT1 has been reported to only regulate p53 activity in breast cancer via the EGFR and Wnt signaling pathways mediated through its histone methylation sites and nonhistone substrates.[15,24] To gain further insight into the mechanism by which PRMT1 regulates cell proliferation and metastasis, we analyzed the genes and signaling pathways that were correlated with the expression of PRMT1. The GEPIA 2.0 database revealed that the expression of β-catenin and its target genes was positively correlated with PRMT1 (Fig. 4A). Following this lead, we determined the expression levels of these genes in control and PRMT1 silenced cells by performing qRT—PCR and WB assays. As shown in Figure 4B—E, we found that both the mRNA and protein expression levels of β-catenin and its target genes were down-regulated in PRMT1-depleted cells compared with control cells, suggesting that β-catenin signaling pathway activation was inhibited after PRMT1 expression was knocked down.

Transcriptional regulation is an important function of PRMTs, and PRMTs activate or suppress target gene transcription by methylating histones or transcription factors.[25,26] Considering the results from previous studies, we hypothesized that PRMT1 may transcriptionally regulate β-catenin. Hence, chromatin immunoprecipitation (ChIP) assays were performed after the Flag-PRMT1 protein was expressed in HGC-27 and MKN-45 cells, and the assays confirmed that PRMT1 binds directly to the β-catenin promoter (Fig. 4F—H). Overall, our results suggest that PRMT1 promotes β-catenin signaling pathway activation by up-regulating β-catenin transcription.

PRTM1 interacts with and recruits MLXIP to the β-catenin promoter

As a transcriptional cofactor, PRMT1 participates in cellular physiological activities by methylating transcription factors or histone substrates. PRMT1 has been reported to promote the proliferation, migration, and invasion of colorectal cancer cells by inducing R251 methylation of the NONO protein.[19] Additionally, PRMT1 promotes breast cancer cell metastasis by methylating EZH2 and regulating EZH2 stability.[17] In this study, to identify PRMT1-binding proteins, we first precipitated the PRMT1 protein in GC cells, followed by MS assay, and the results showed that MLX-interacting protein (MLXIP/MondoA) is a PRMT1-binding protein (Fig. S2A).

In addition, reciprocal co-IP with GC cell lines (Fig. 5A, B) showed that MLXIP interacted with PRMT1. Complementarily, a PLA revealed that PRMT1 binds to MLXIP in the nucleus (Fig. 5C; Fig. S2B, C).

Notably, we found that like the expression level of PRMT1, the expression levels of β-catenin and its target genes were positively correlated with MLXIP expression (Fig. S2D). To delineate the functions of MLXIP in the regulation of β-catenin activity, qRT—PCR and WB assays were used to examine the activity of the β-catenin signaling pathway in MLXIP-depleted cells. The results of the qRT—PCR and WB assays showed that the β-catenin signaling pathway was inhibited in MLXIP-silenced cells (Fig. 5D—F). Therefore, ChIP assays were performed, and they confirmed that MLXIP binds to the β-catenin promoter in the same region as PRMT1 (Fig. 5G, H).

Then, to identify the role played by PRMT1 in the PRMT1-MLXIP complex-mediated regulation of β-catenin activity, PRMT1 expression in MYC-MLXIP-expressing HEK293FT cells was knocked down by shRNA or inhibited with PRMT1 inhibitors, and ChIP assays were performed with an anti-MYC antibody. The ChIP assays demonstrated that the binding between MLXIP and the β-catenin promoter was significantly weaker in the PRMT1-down-regulated and PRMT1 activity-inhibited cells (Fig. 5I, J). These results suggest that PRMT1 recruits MLXIP to the β-catenin promoter by binding with MLXIP in the nucleus.

KTN1 binds with PRMT1 to stabilize PRMT1 protein expression

The MS results suggested that kinectin (KTN1) may be a PRMT1-binding protein (Fig. S2A). To confirm this finding, endogenous PRMT1 and KTN1 proteins were immunoprecipitated with PRMT1 or KTN1 antibodies. The results of the co-IP assay revealed that KTN1 binds with PRMT1 (Fig. 6A, B). Furthermore, we carried out a PLA after transient transfection of HGC-27 and MKN-45 cells with the MYC-tagged KTN1 protein. The PLA results showed that, in almost all cases, KTN1 binds with PRMT1 in the cytoplasm (Fig. 6C, D).

Similarly, the expression of β-catenin and its target genes was also positively correlated with KTN1 expression, as indicated by an analysis of the GEPIA 2.0 database (Fig. S2E). Considering our previous results, we speculated that KTN1 may influence the expression or function of PRMT1. Therefore, KTN1 expression was knocked down in GC cells, and a WB assay demonstrated that PRMT1 expression was decreased in the KTN1-down-regulated cells

normal tissue at different grades of the stomach adenocarcinoma (STAD) dataset (F) in the TCGA database. **(G)** The expression of PRMT1 in a normal gastric tissue cell line (the GES-1 cell line) and five GC cell lines (the BGC-823, HGC-27, MGC-823, MKN-45, and SGC-7901 cell lines) was examined by Western blot assay. **(H)** Overall survival curve analysis of progression-free survival of GC patients based on data obtained through the Kaplan—Meier plotter tool with the indicated log-rank test *P* values. **(I)** Immunofluorescence assays were performed to determine the subcellular localization of PRMT1. **(J)** IHC for PRMT1 expression in six normal tissues and thirty-six gastric cancer tissues. Scale bar = 10 μm $^{**}P < 0.01$, $^{***}P < 0.001$.

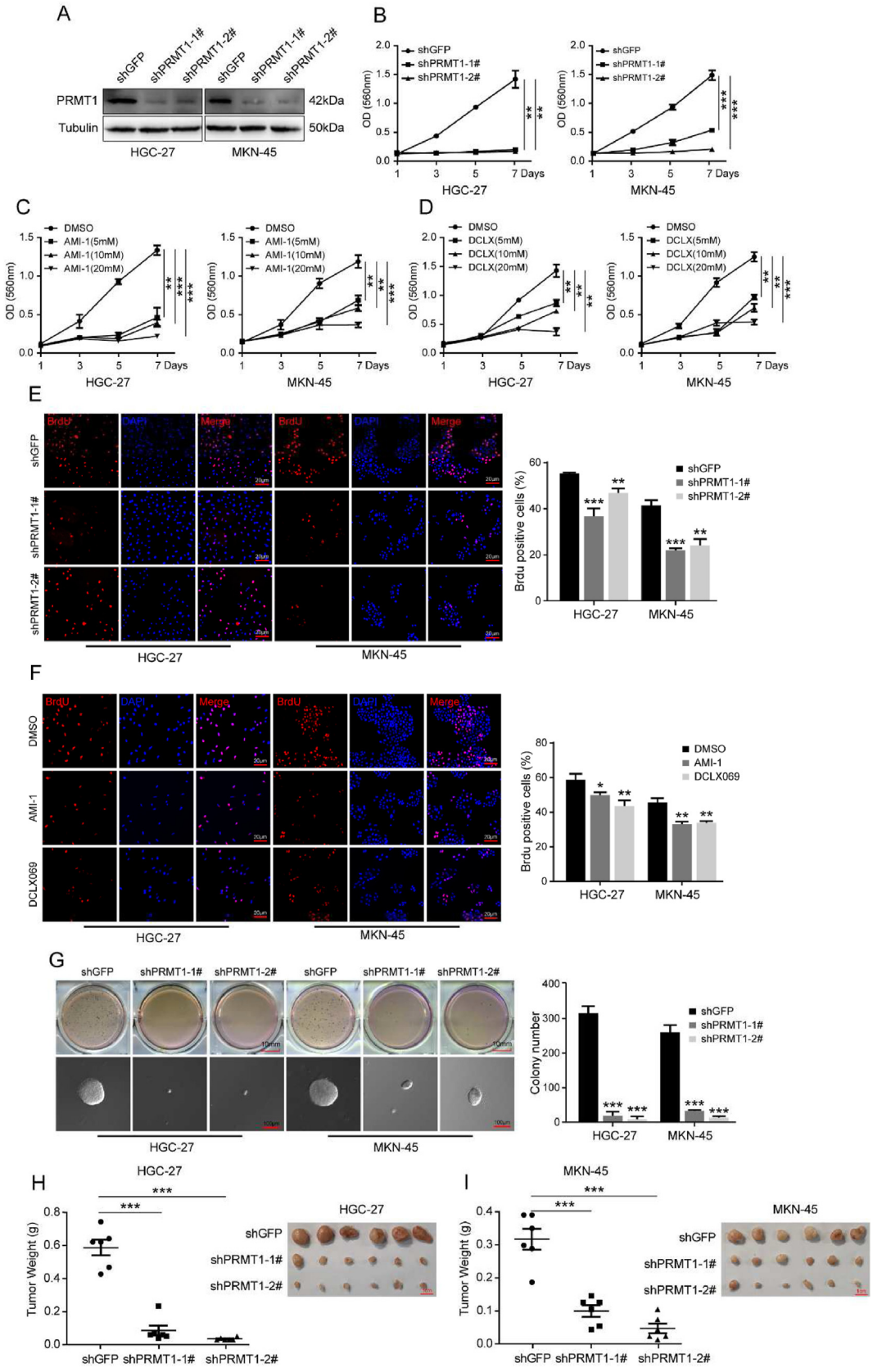

Figure 2 Cell proliferation and tumor growth were inhibited after PRMT1 expression was inhibited. **(A)** The expression of PRMT1 was examined through Western blot assays performed after PRMT1 expression was knocked down in HGC-27 and MKN-45 cells. Short hairpin (sh) GFP was used as the negative control, and tubulin was used as the loading control. **(B—D)** The proliferation rate of control and PRMT1-silenced cells was measured by MTT assay (B) after dimethyl sulfoxide (DMSO) and AMI-1 (a PRMT1 inhibitor)

(Fig. 6E). In addition, we examined the ubiquitination of PRMT1 in KTN1-silenced cells and found that the PRMT1 ubiquitination levels were enhanced in the KTN1-knockdown cells (Fig. 6F). In addition to a ubiquitination assay, KTN1-silenced cells were treated with MG132 to inhibit protein degradation, and PRMT1 protein expression was restored after MG132 treatment (Fig. 6G). To identify the contribution of KTN1 to PRMT1 degradation, we transfected HEK293FT cells with MYC-KTN1 and treated these cells with cycloheximide (CHX). WB assays were performed to monitor the changes in the half-life of PRMT1, and the turnover rate of PRMT1 was found to increase with KTN1 overexpression (Fig. 6H; Fig. S2F). Taken together, these results indicate that KTN1 inhibits the ubiquitination of PRMT1 by binding with PRMT1.

KTN1 inhibits the TRIM48-ubiquitination-mediated degradation of PRMT1

To further explore the mechanism by which KTN1 inhibits PRMT1 degradation, we individually replaced the lysine (K) residues K6, K11, K27, K29, K33, K48, or K63 with arginine resides in HA-UB expression plasmids and expressed these mutants with a Flag-PRMT1 expression plasmid in KTN1-silenced HEK293FT cells. An *in vivo* ubiquitination assay revealed that the degree of PRMT1 polyubiquitination was decreased in UB-K48R-expressing cells, implying that polyubiquitination of PRMT1, which was inhibited by KTN1, was established through K48 linkages (Fig. 7A). In a previous report, Atsushi Matsuzawa and his colleagues revealed that TRIM48 promoted K48-linked ubiquitination of PRMT1[27]; hence, we hypothesized that TRIM48 and KTN1 play antagonistic roles in PRMT1 ubiquitination. To test whether TRIM48 is critical for KTN1-mediated PRMT1 degradation, we first knocked down KTN1 expression, TRIM48 expression, or both in GC cells. As shown in Figure 7B, WB results indicated that PRMT1 expression was restored after TRIM48 expression was knocked down in KTN1-silenced cells, suggesting that TRIM48 was essential for PRMT1 ubiquitination in KTN1-depleted cells. Additionally, as shown in Figure 7C, PRMT1 expression was restored in both KTN1- and TRIM48-overexpressing cells compared with TRIM48 overexpressing cells. Flag-PRMT1 and His-TRIM48 were expressed in control or KTN1-overexpressing GC cells, and therefore, we performed a PLA, and the plots showed significantly decreased binding in KTN1-overexpressing cells compared with the control cells (Fig. 7D, E). Furthermore, the MYC-KTN1 protein was expressed in Flag-PRMT1-overexpressing HEK293FT cells in a concentration-dependent manner, and a co-IP assay was performed with these cells and an anti-Flag antibody. As shown in Figure 7F, TRIM48-PRMT1 binding was weakened with KTN1 overexpression in a dose-dependent manner. In summary, our results demonstrated that KTN1 stabilized PRMT1 protein expression by inhibiting the interaction between TRIM48 and PRMT1.

Discussion

Protein arginine methyltransferases (PRMTs) have been reported to play oncogenic roles in multiple human cancers, including GC.[11,28,29] Our previous study demonstrated that CARM1/PRMT4, type I PRMTs, promoted GC cell proliferation through the transcriptional activation and methylation of the Notch 2 signaling pathway.[13] Another type I PRMT, PRMT1 has been reported to function in pancreatic cancer, colorectal cancer, breast cancer, and leukemia,[19,30–32] but the role played by PRMT1 in GC has not been fully characterized. In this study, we found that PRMT1 is highly expressed in GC and associated with poor prognosis, implying that PRMT1 may play an oncogenic role in GC.

In 2018, Min Xu's group found that PRMT1 inhibits the proliferation of GC cells but promotes GC cell migration and invasion.[23] Activating invasion and metastasis pathways and evading growth suppressors are two hallmarks of cancer,[33] and surprisingly, PRMT1 exhibited the opposite effects on cell proliferation and metastasis. To confirm the function of PRMT1 in GC cell proliferation, we knocked down PRMT1 expression with shRNA, performed MTT and BrdU assays, and found that cell proliferation was inhibited by PRMT1 silencing. Notably, shRNA may target genes in addition to PRMT1, which may have led to different cell proliferation assay results. Two PRMT1 inhibitors (AMI-1 and DCLX069) were used to treat GC cells, and the results obtained with the inhibitors were the same as those obtained through shRNA treatment. To examine the change in cell mobility after the expression or activity of PRMT1 was inhibited, Transwell assays were performed *in vitro*, and lung metastasis assays were performed *in vivo*. The results demonstrated that cell mobility decreased *in vitro* and *in vivo* regardless of whether PRMT1 expression or activity was abrogated. Overall, we reveal that PRMT1 promotes the proliferation and mobility of GC cells.

The Wnt/β-catenin signaling pathway, also known as the canonical Wnt signaling pathway, participates in diverse physiological processes, such as proliferation, metastasis, apoptosis, and differentiation.[34] The Wnt/β-catenin signaling pathway has been established to promote the development and progression of various types of cancers.[35,36] Thus, studying the mechanisms by which the Wnt/β-catenin signaling pathway is regulated may provide new targets for clinical therapy. As the core component of this signaling pathway, β-catenin has attracted considerable attention. In recent years, phosphorylation and ubiquitination have been identified as processes leading to the degradation of β-catenin. Protein kinase Cδ phosphorylates β-catenin at S715 and then promotes its degradation by enhancing the TRIM33-β-catenin interaction.[37] Adenomatous polyposis coli has been

treatment (C), and in cells after DMSO and DCLX069 (a PRMT1 inhibitor) treatment (D). **(E, F)** BrdU staining assays were performed after PRMT1 expression down-regulation (E) and after treatment with PRMT1 inhibitors (F). Scale bar = 20 μm. The quantification of BrdU-positive cells (right panel) is presented. **(G)** Soft agar assays were performed with PRMT1-depleted cells. The scale bar above represents 10 mm, and the scale bar below represents 100 μm. The colony quantification (right panel) results are presented. **(H, I)** Subcutaneous xenografts were generated after PRMT1 expression knockdown in HGC-27 cells (H) and MKN-45 cells (I). Scale bar = 1 cm $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

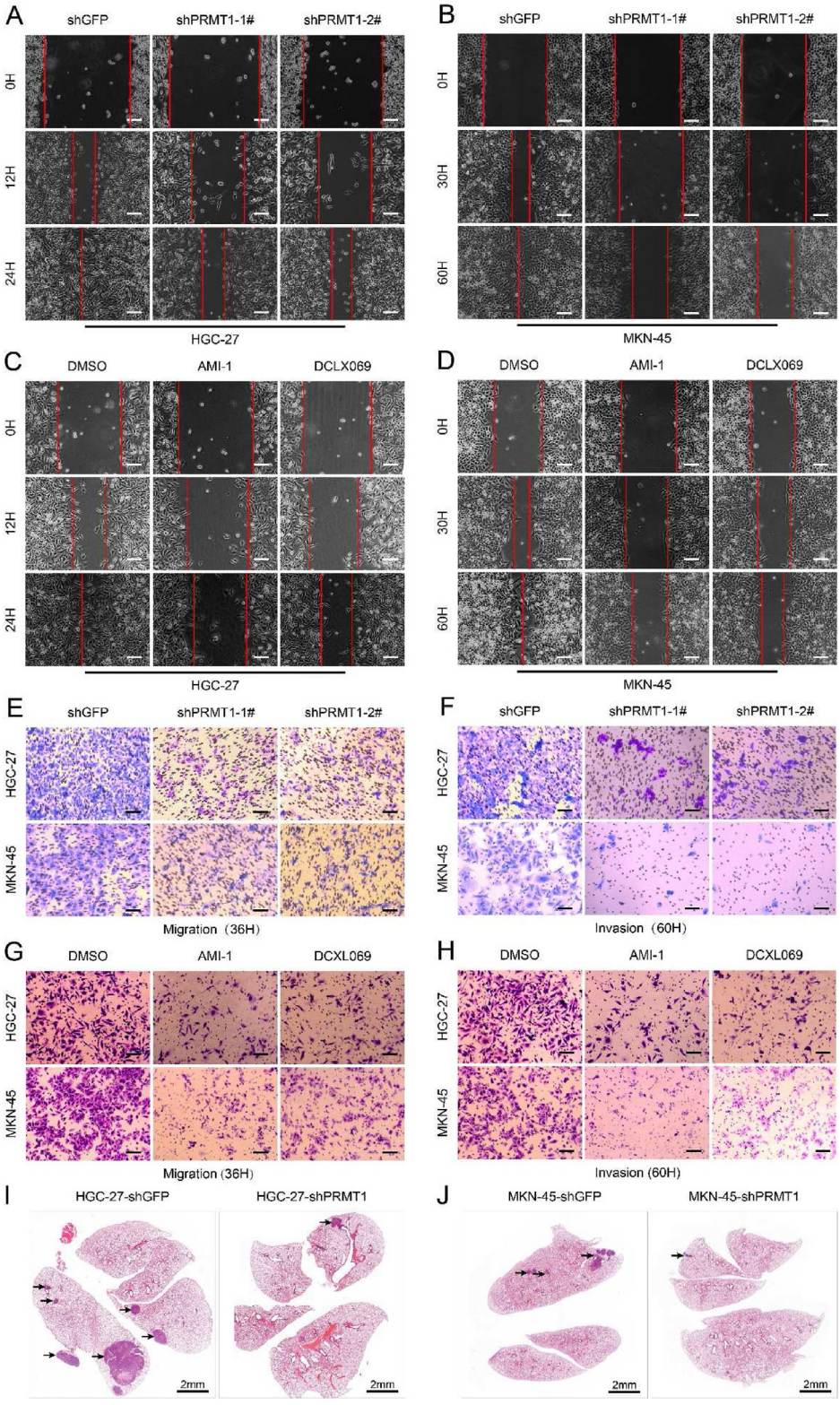

Figure 3 PRMT1 promotes cell migration and invasion *in vitro* and *in vivo*. **(A—D)** Wound healing assays were performed after PRMT1 expression was knocked down in HGC-27 cells (A) and MKN-45 cells (B) and after cells were treated with AMI-1 and DCLX069 (C, D, respectively). **(E—H)** Transwell assays were performed with PRMT1 expression down-regulated in GC cells (E, F) treated with PRMT1 inhibitors (G, H), to measure cell migration and invasion *in vitro*. **(I, J)** GC cells with stably expressing PRMT1 knockdown vector or control cells were injected into nude mice via the tail vein. After one month, lung tissues were removed and stained with hematoxylin and eosin. Scale bar = 2 mm.

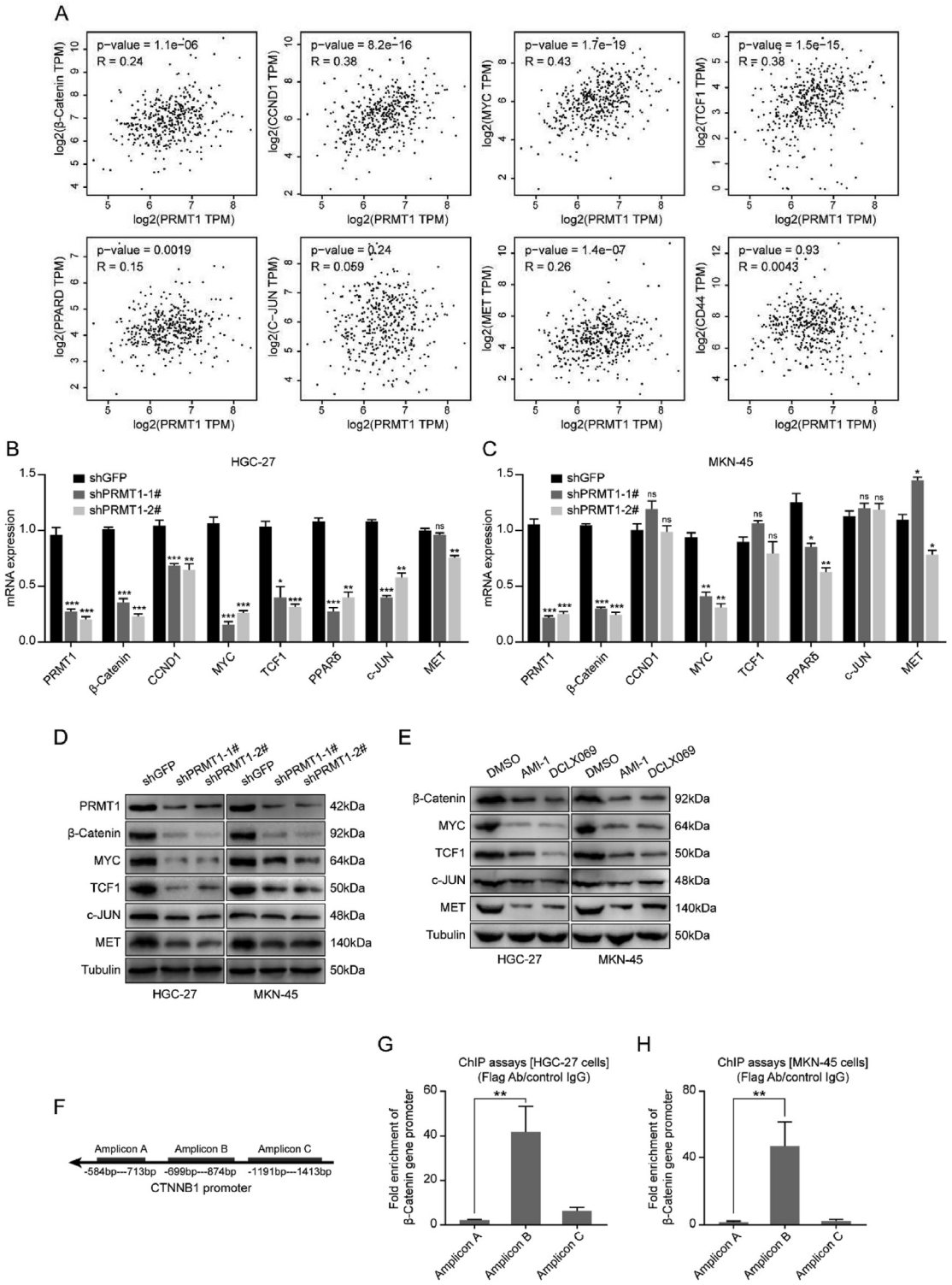

Figure 4 PRMT1 actives the β-catenin signaling pathway through its transcriptional regulatory function. **(A)** The relationships between PRMT1 and genes expressed downstream in the β-catenin signaling pathway were detected using the GEPIA 2.0 database. **(B, C)** The mRNA expression levels of β-catenin and its target genes were examined in PRMT1-silenced cells. **(D)** The protein expression levels of β-catenin and its target genes were examined after PRMT1 down-regulation. **(E)** The protein expression levels of β-catenin and its target genes were examined after being treated with PRMT1 inhibitors. **(F)** Schematic representation showing the β-catenin promoter with amplicon regions. **(G, H)** Chromatin immunoprecipitation assays were performed using an anti-Flag antibody with HGC-27 cells (G) and MKN-45 (H) cells after transient transfection with a Flag-PRMT1 overexpression plasmid, followed by qRT—PCR with a total of 3 primers targeting the β-catenin promoter. Rabbit IgG was used as the negative control. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

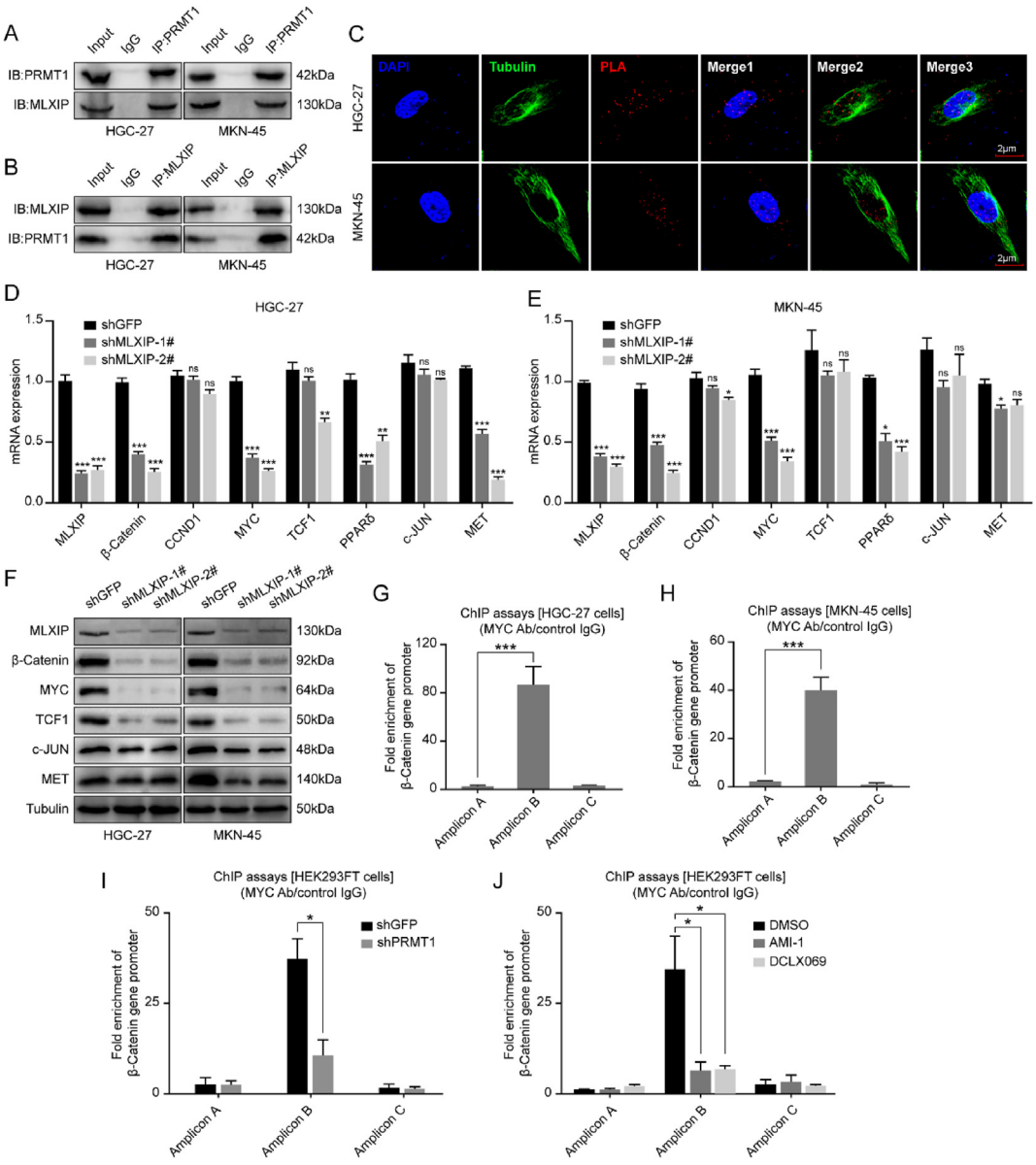

Figure 5 PRMT1 recruits MLXIP to the β-catenin promoter by binding with MLXIP. **(A, B)** Co-immunoprecipitation was performed using anti-PRMT1 and anti-MLXIP antibodies with HGC-27 and MKN-45 cells, and samples were analyzed using anti-PRMT1 and anti-MLXIP antibodies and Western blot assays. **(C)** A proximity ligation assay was performed using anti-MYC and anti-PRMT1 antibodies after the MYC-MLXIP protein was expressed in HGC-27 and MKN-45 cells. Nuclei were stained with DAPI (blue), and tubulin was stained to identify the cytoplasm (green). Scale bar = 2 μm. **(D, E)** mRNA expression levels of MLXIP, β-catenin and β-catenin target genes were determined after MLXIP expression was knocked down in GC cells. **(F)** Protein expression levels of MLXIP, β-catenin and β-catenin target genes were examined in MLXIP-silenced GC cells. **(G, H)** ChIP assays were performed using an anti-MYC antibody after MYC-MLXIP protein was expressed in GC cells. **(I, J)** Chromatin immunoprecipitation assays were performed with MYC-MLXIP-expressing HEK293FT cells after PRMT1 expression was inhibited with short hairpin RNA (shRNA) (I) or PRMT1 inhibitors (J). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

reported to promote β-catenin capture, phosphorylation, and ubiquitylation in a β-TrCP-dependent manner.[38] To date, however, little is known about the transcriptional regulation of β-catenin. In this study, we performed database analyses and found a positive association between the expression of PRMT1 and the β-catenin signaling pathway. Then, qRT—PCR and WB assays indicated that the β-catenin signaling pathway was inhibited in PRMT1-silenced cells. Based on the ChIP assay results, we also found that PRMT1 binds to the β-catenin promoter.

Although PRMT1 has been reported to bind to the β-catenin promoter in pancreatic cancer[32] and is known to be a transcriptional cofactor, the transcription factor involved in this process is unknown. To identify the transcriptional complex in which PRMT1 participates and that binds to the β-catenin promoter, co-IP and MS assays were performed to

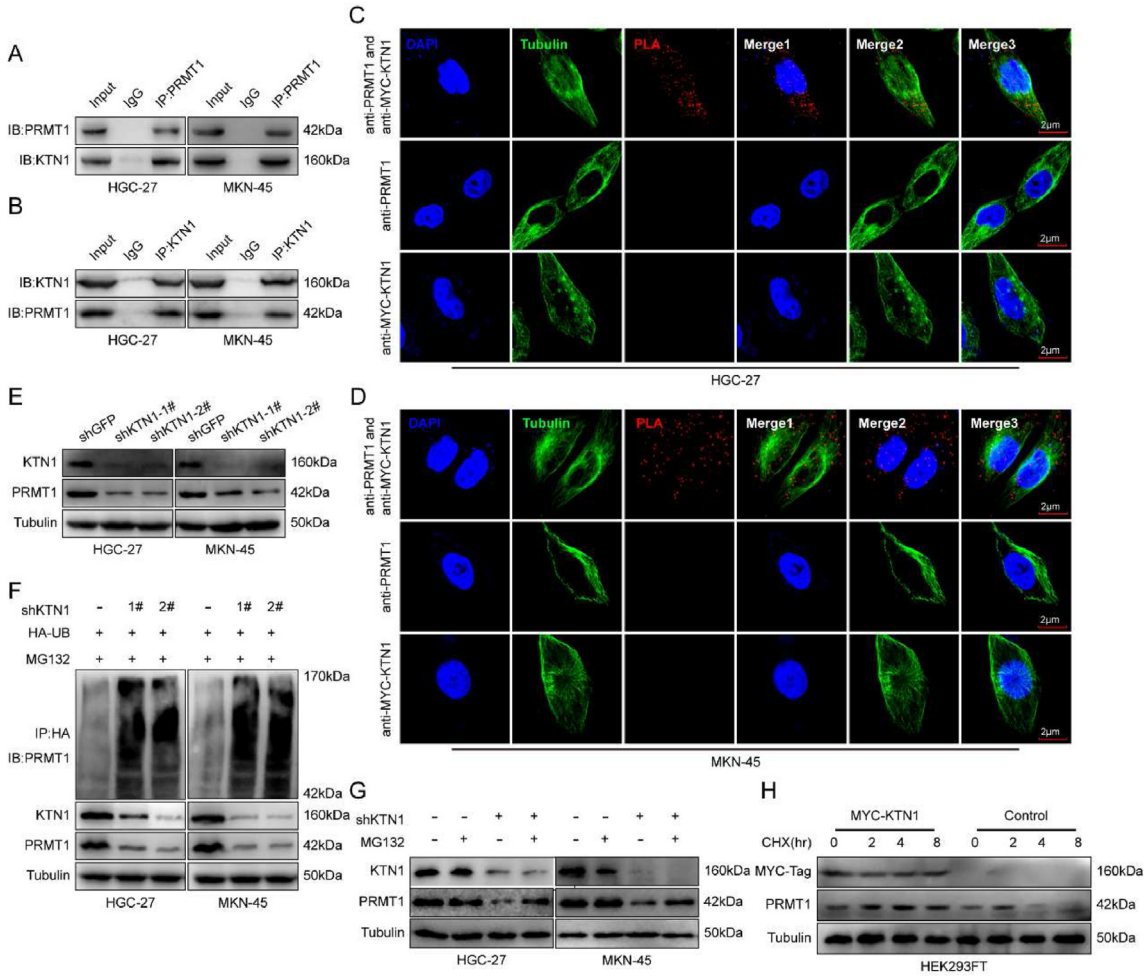

Figure 6 KTN1 inhibits PRMT1 ubiquitination. **(A, B)** Co-immunoprecipitation was performed in HGC-27 and MKN-45 cells using anti-PRMT1 and anti-KTN1 antibodies, and the samples were analyzed by Western blotting using anti-PRMT1 and anti-KTN1 antibodies. **(C, D)** After expressing the MYC-KTN1 protein in HGC-27 cells (C) and MKN-45 (D) cells, a proximity ligation assay was performed with anti-MYC and anti-PRMT1 antibodies. Nuclei were stained with DAPI (blue), and tubulin was stained to identify the cytoplasm (green). Scale bar = 2 μm. **(E)** The expression levels of KTN1 and PRMT1 were ascertained in control and KTN1-silenced cells by Western blot assay. **(F)** Cells expressing KTN1 or not were transfected with a hemagglutinin-ubiquitin (HA-UB) plasmid and treated with MG132 for 8 h. Then, ubiquitinated PRMT1 proteins were pulled down using an anti-HA antibody and immunoblotted with an anti-PRMT1 antibody. **(G)** After KTN1 expression was knocked down, the cells were treated with MG132 for 8 h, and the expression of PRMT1 was determined by Western blot assay. Tubulin was used as the loading control. **(H)** HEK293FT cells were transfected with the indicated plasmid. Then, the cells were treated with cycloheximide (CHX) for the indicated times and immunoblotted with anti-PRMT1 and anti-MYC antibodies.

identify PRMT1-binding proteins, and multiple proteins were identified, including kinectin 1 (KTN1) and MLX-interacting protein (MLXIP) (Fig. S2A). Among these PRMT1-binding proteins, we chose to focus on MLXIP because of its transcriptional regulatory function.[39,40] MLXIP (also called MondoA), a c-Myc-related transcription factor, has been shown to interact with TXNIP and affect glucose uptake and tumorigenesis.[41–43] The data from the GEPIA 2.0 database revealed that the expression of β-catenin and its target genes was correlated with MLXIP expression; hence, we speculated that PRMT1 and MLXIP may form a transcriptional complex at the β-catenin promoter. Therefore, PLA and ChIP assays were performed, and the results indicated that PRMT1 recruited MLXIP to the β-catenin promoter and activated the expression of β-catenin. Thus, we identified β-catenin as a target gene of the PRMT1-MLXIP complex. More downstream genes or signaling pathways of this complex await discovery via ChIP-sequencing (ChIP-seq) assays or other technology-based analyses.

Interestingly, we found that KTN1 expression was positively correlated with β-catenin and its target genes. Because PRMT1 interacts with KTN1 primarily in the cytoplasm, we excluded the possibility that KTN1 may be involved in the transcriptional activation of β-catenin. As a kinectin, KTN1 has been reported to promote hepatocellular carcinoma progression and the growth of triple-negative breast cancer.[44,45] In 2021, Zhenhua Ding and his colleagues made a breakthrough in their study on KTN1 function. They found that KTN1 stabilized the protein expression level of EGFR by decreasing its ubiquitination,[46] suggesting that the

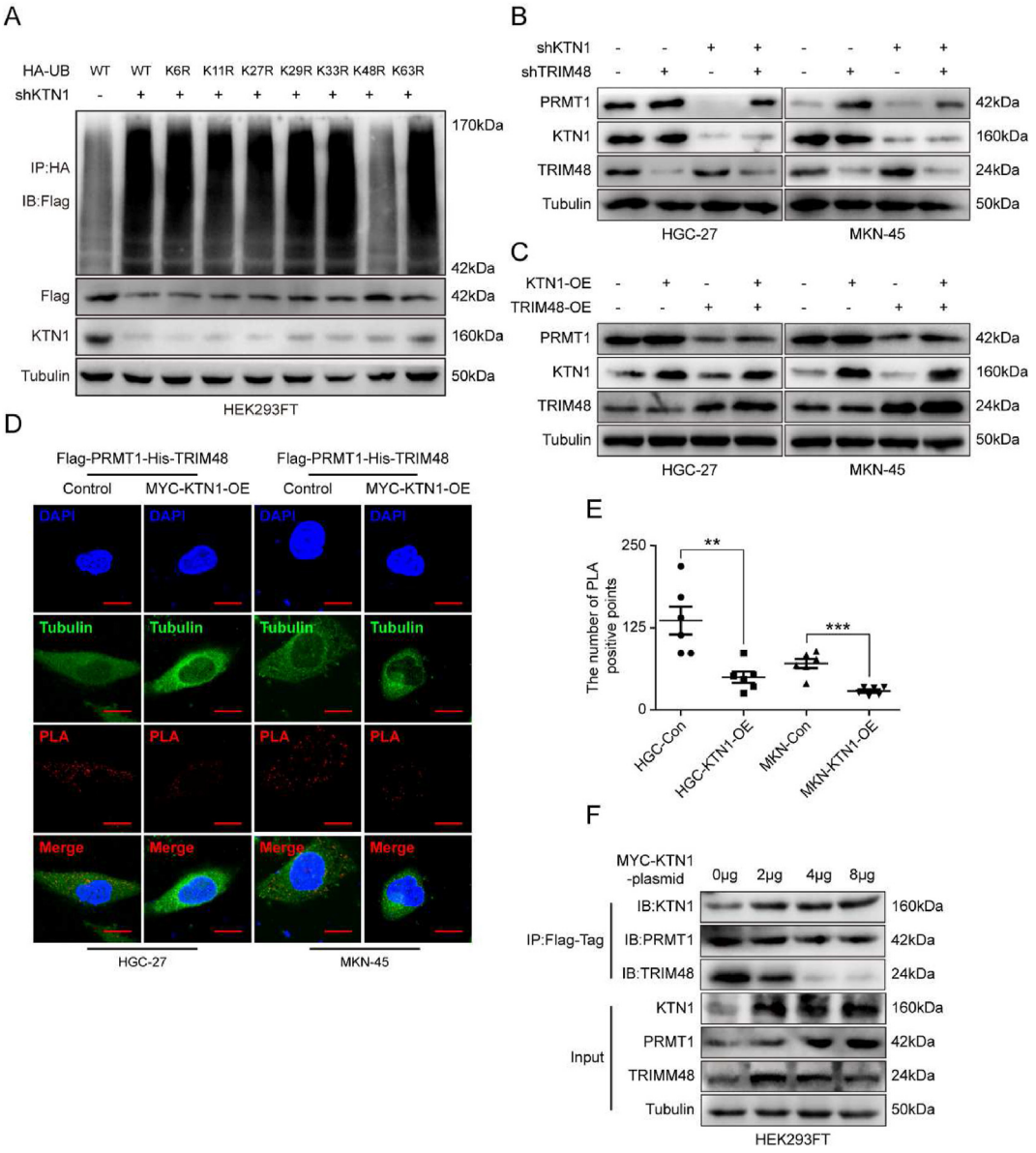

Figure 7 KTN1 inhibits the interaction between PRMT1 and TRIM48. **(A)** HEK293FT cells with or without KTN1 down-regulation were transfected with a Flag-PRMT1 plasmid and the indicated mutated HA-UB plasmids or wild-type HA-UB plasmid. Then, ubiquitinated PRMT1 proteins were pulled down with an anti-HA antibody and immunoblotted with an anti-Flag antibody. **(B)** TRIM48 expression was knocked down in control or KTN1-depleted cells, and the expression of PRMT1 was then examined by Western blot assay. **(C)** The expression of PRMT1 was examined after TRIM48 was overexpressed in control or KTN1-overexpressing cells. **(D)** HGC-27 and MKN-45 cells expressing Flag-PRMT1 and His-TRIM48 proteins were transiently transfected with or without MYC-KTN1, and then a proximity ligation assay was performed with anti-Flag and anti-His antibodies. Scale bar $=$ 5 μm. **(E)** The histogram demonstrates the quantification of PLA-identified signaling, which is shown in Figure 7D. **(F)** After transfecting the Flag-PRMT1 plasmid and different doses of the MYC-KTN1 plasmid into HEK293FT cells, the Flag-PRMT1 protein was pulled down using an anti-Flag antibody and immunoblotted with the indicated antibodies to determine the degree of interaction between PRMT1 and TRIM48. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

MALAT1-KTN1-EGFR axis promoted the development of cutaneous squamous cell carcinoma.[47] These results revealed that KTN1 participated in the regulation of protein degradation; hence, we focused on whether KTN1 regulates the protein expression of PRMT1. First, PRMT1 expression was examined after KTN1 expression was down-regulated, and we found that PRMT1 expression was decreased. Additionally, ubiquitination and protein half-life assays suggested that KTN1 up-regulated PRMT1 expression by inhibiting K48-linked ubiquitination. In 2017, a group from Tohoku reported that tripartite motif-containing 48 (TRIM48) promotes ASK1 activation through ubiquitination-dependent degradation of PRMT1.[27] In contrast to the inhibitory effect of KTN1, TRIM48 enhanced PRMT1 K48-linked ubiquitination,

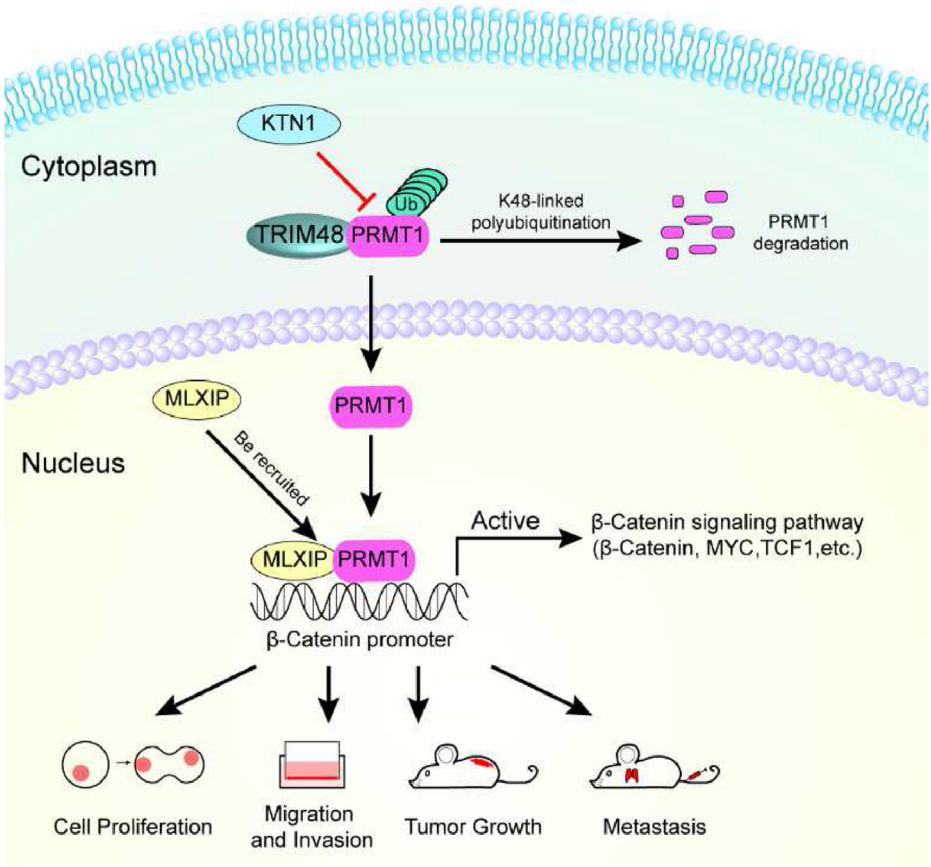

Figure 8 Schematic diagram showing PRMT1-enhanced gastric cancer metastasis mediated by the β-catenin signaling pathway *in vitro* and *in vivo*. In the cytoplasm, KTN1 inhibits K48-linked polyubiquitination of PRMT1 by decreasing the interaction between TRIM48 and PRMT1. Then, in the nucleus, PRMT1 recruits MLXIP to the β-catenin promoter and transcriptionally activates the β-catenin signaling pathway. PRMT1 promotes gastric cancer cell proliferation, migration, and invasion of *in vitro* and tumor growth and metastasis *in vivo*.

implying that KTN1 and TRIM48 may play opposite roles in the ubiquitination of PRMT1. Therefore, we knocked down TRIM48 expression in KTN1-silenced cells and found that PRMT1 expression was restored. In addition, TRIM48 was overexpressed in KTN1-overexpressing cells, demonstrating that the expression of PRMT1 was decreased in TRIM48-and KTN1-overexpressing cells, in contrast to its expression in cells when only KTN1 expression was up-regulated. An IP assay and PLA revealed that KTN1 inhibited TRIM48 binding to PRMT1. In this study, our findings strongly supported the idea that KTN1 and TRIM48 play opposite roles in PRMT1 degradation, but the site of PRMT1 ubiquitination affected by KTN1 and TRIM48 remains unclear.

In summary, we present evidence indicating an essential function for PRMT1 in GC cell proliferation and mobility. PRMT1 up-regulates β-catenin transcription by forming a transcriptional complex with MLXIP at the β-catenin promoter. Additionally, KTN1 is a PRMT1-binding protein and facilitates PRMT1 escape from ubiquitin-mediated degradation induced by TRIM48 (Fig. 8).

Conflict of interests

The authors declare that there is no conflict of interests.

Funding

This study was supported by the National Natural Science Foundation of China (No. 82203339), the Postdoctoral Research Foundation of China (No. 2021M692679), the Chongqing Postdoctoral Science Foundation (China) (No. 7820100607), the Natural Science Foundation Project of Chongqing (China) (No. cstc2021jcyj-bsh0067 and cstc2022ycjh-bgzxm0145).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.02.006.

Abbreviations

ADMA	asymmetric dimethylarginine
ChIP	chromatin immunoprecipitation
CHX	cycloheximide
GC	gastric cancer
IP	immunoprecipitation
KTN1	kinectin 1

MLXIP MLX-interacting protein
MMA monomethylarginine
MS mass spectrometry
MTT methyl thiazolyl tetrazolium
PLA proximity ligation assay
PRMT1 protein arginine methyltransferase 1
qRT—PCR quantitative real-time polymerase chain reaction
SDMA symmetric dimethylarginine
shRNA short hairpin RNA
TRIM48 tripartite motif-containing 48
WB Western blot

References

1. Choi S, Park S, Kim H, et al. Gastric cancer: mechanisms, biomarkers, and therapeutic approaches. *Biomedicines*. 2022; 10(3):543.
2. Smyth EC, Nilsson M, Grabsch HI, et al. Gastric cancer. *Lancet*. 2020;396(10251):635—648.
3. Yaghoobi M, McNabb-Baltar J, Bijarchi R, et al. What is the quantitative risk of gastric cancer in the first-degree relatives of patients? A meta-analysis. *World J Gastroenterol*. 2017; 23(13):2435—2442.
4. Hatakeyama M. Malignant *Helicobacter pylori*-associated diseases: gastric cancer and MALT lymphoma. *Adv Exp Med Biol*. 2019;1149:135—149.
5. Majewski IJ, Kluijt I, Cats A, et al. An α-E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. *J Pathol*. 2013; 229(4):621—629.
6. Akiyama T, Sudo C, Ogawara H, et al. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science*. 1986;232(4758):1644—1646.
7. Van Cutsem E, Sagaert X, Topal B, et al. Gastric cancer. *Lancet*. 2016;388(10060):2654—2664.
8. Lorton BM, Shechter D. Cellular consequences of arginine methylation. *Cell Mol Life Sci*. 2019;76(15):2933—2956.
9. Blanc RS, Richard S. Arginine methylation: the coming of age. *Mol Cell*. 2017;65(1):8—24.
10. Bedford MT, Clarke SG. Protein arginine methylation in mammals: who, what, and why. *Mol Cell*. 2009;33(1):1—13.
11. Kim H, Ronai ZA. PRMT5 function and targeting in cancer. *Cell Stress*. 2020;4(8):199—215.
12. Jarrold J, Davies CC. PRMTs and arginine methylation: cancer's best-kept secret? *Trends Mol Med*. 2019;25(11):993—1009.
13. Wang F, Zhang J, Tang H, et al. Nup54-induced CARM1 nuclear importation promotes gastric cancer cell proliferation and tumorigenesis through transcriptional activation and methylation of Notch 2. *Oncogene*. 2022;41(2):246—259.
14. An W, Kim J, Roeder RG. Ordered cooperative functions of PRMT1, p300, and CARM1 in transcriptional activation by p53. *Cell*. 2004;117(6):735—748.
15. Liu LM, Tang Q, Hu X, et al. Arginine methyltransferase PRMT1 regulates p53 activity in breast cancer. *Life*. 2021;11(8):789.
16. Jackson-Weaver O, Ungvijanpunya N, Yuan Y, et al. PRMT1-p53 pathway controls epicardial EMT and invasion. *Cell Rep*. 2020; 31(10):107739.
17. Li Z, Wang D, Lu J, et al. Methylation of EZH2 by PRMT1 regulates its stability and promotes breast cancer metastasis. *Cell Death Differ*. 2020;27(12):3226—3242.
18. Zhang L, Tran NT, Su H, et al. Cross-talk between PRMT1-mediated methylation and ubiquitylation on RBM15 controls RNA splicing. *Elife*. 2015;4:e07938.
19. Yin XK, Wang YL, Wang F, et al. PRMT1 enhances oncogenic arginine methylation of NONO in colorectal cancer. *Oncogene*. 2021;40(7):1375—1389.
20. Yao B, Gui T, Zeng X, et al. PRMT1-mediated H4R3me2a recruits SMARCA4 to promote colorectal cancer progression by enhancing EGFR signaling. *Genome Med*. 2021;13(1):58.
21. Wang F, Zhang D, Mao J, et al. Morusin inhibits cell proliferation and tumor growth by down-regulating c-Myc in human gastric cancer. *Oncotarget*. 2017;8(34):57187—57200.
22. Altan B, Yokobori T, Ide M, et al. Nuclear PRMT1 expression is associated with poor prognosis and chemosensitivity in gastric cancer patients. *Gastric Cancer*. 2016;19(3):789—797.
23. Zhang Y, Wang D, Zhang M, et al. Protein arginine methyltransferase 1 coordinates the epithelial-mesenchymal transition/proliferation dichotomy in gastric cancer cells. *Exp Cell Res*. 2018;362(1):43—50.
24. Suresh S, Huard S, Brisson A, et al. PRMT1 regulates EGFR and Wnt signaling pathways and is a promising target for combinatorial treatment of breast cancer. *Cancers*. 2022; 14(2):306.
25. Yang Y, Bedford MT. Protein arginine methyltransferases and cancer. *Nat Rev Cancer*. 2013;13(1):37—50.
26. Zhao Q, Rank G, Tan YT, et al. PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. *Nat Struct Mol Biol*. 2009;16(3): 304—311.
27. Hirata Y, Katagiri K, Nagaoka K, et al. TRIM48 promotes ASK1 activation and cell death through ubiquitination-dependent degradation of the ASK1-negative regulator PRMT1. *Cell Rep*. 2017;21(9):2447—2457.
28. Huang T, Yang Y, Song X, et al. PRMT6 methylation of RCC1 regulates mitosis, tumorigenicity, and radiation response of glioblastoma stem cells. *Mol Cell*. 2021;81(6):1276—1291. e9.
29. Hernandez SJ, Dolivo DM, Dominko T. PRMT8 demonstrates variant-specific expression in cancer cells and correlates with patient survival in breast, ovarian and gastric cancer. *Oncol Lett*. 2017;13(3):1983—1989.
30. He X, Zhu Y, Lin YC, et al. PRMT1-mediated FLT3 arginine methylation promotes maintenance of FLT3-ITD^{+} acute myeloid leukemia. *Blood*. 2019;134(6):548—560.
31. Li Z, Wang D, Wang W, et al. Macrophages-stimulated PRMT1-mediated EZH2 methylation promotes breast cancer metastasis. *Biochem Biophys Res Commun*. 2020;533(4):679—684.
32. Song C, Chen T, He L, et al. PRMT1 promotes pancreatic cancer growth and predicts poor prognosis. *Cell Oncol*. 2020;43(1): 51—62.
33. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646—674.
34. Cheng X, Xu X, Chen D, et al. Therapeutic potential of targeting the Wnt/β-catenin signaling pathway in colorectal cancer. *Biomed Pharmacother*. 2019;110:473—481.
35. Gajos-Michniewicz A, Czyz M. WNT signaling in melanoma. *Int J Mol Sci*. 2020;21(14):4852.
36. Wei CY, Zhu MX, Yang YW, et al. Downregulation of RNF128 activates Wnt/β-catenin signaling to induce cellular EMT and stemness via CD44 and CTTN ubiquitination in melanoma. *J Hematol Oncol*. 2019;12(1):21.
37. Xue J, Chen Y, Wu Y, et al. Tumour suppressor TRIM33 targets nuclear β-catenin degradation. *Nat Commun*. 2015;6:6156.
38. Ranes M, Zaleska M, Sakalas S, et al. Reconstitution of the destruction complex defines roles of AXIN polymers and APC in β-catenin capture, phosphorylation, and ubiquitylation. *Mol Cell*. 2021;81(16):3246—3261. e11.
39. Richards P, Ourabah S, Montagne J, et al. MondoA/ChREBP: the usual suspects of transcriptional glucose sensing; Implication in pathophysiology. *Metabolism*. 2017;70: 133—151.
40. Mejhert N, Kuruvilla L, Gabriel KR, et al. Partitioning of MLX-family transcription factors to lipid droplets regulates metabolic gene expression. *Mol Cell*. 2020;77(6):1251—1264.e9.

41. Lu Y, Li Y, Liu Q, et al. MondoA-thioredoxin-interacting protein axis maintains regulatory T-cell identity and function in colorectal cancer microenvironment. *Gastroenterology*. 2021;161(2):575—591.e16.
42. Wilde BR, Ayer DE. Interactions between Myc and MondoA transcription factors in metabolism and tumourigenesis. *Br J Cancer*. 2015;113(11):1529—1533.
43. Carroll PA, Diolaiti D, McFerrin L, et al. Deregulated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. *Cancer Cell*. 2015;27(2):271—285.
44. Pan J, Chao NX, Zhang YY, et al. Upregulating *KTN1* promotes hepatocellular carcinoma progression. *J Cancer*. 2021;12(16):4791—4809.
45. Gao L, Chen S, Hong M, et al. Kinectin 1 promotes the growth of triple-negative breast cancer via directly co-activating NF-kappaB/p65 and enhancing its transcriptional activity. *Signal Transduct Targeted Ther*. 2021;6(1):250.
46. Ma J, Ma S, Zhang Y, et al. Kinectin1 depletion promotes EGFR degradation via the ubiquitin-proteosome system in cutaneous squamous cell carcinoma. *Cell Death Dis*. 2021;12(11):995.
47. Zhang Y, Gao L, Ma S, et al. MALAT1-KTN1-EGFR regulatory axis promotes the development of cutaneous squamous cell carcinoma. *Cell Death Differ*. 2019;26(10):2061—2073.