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PRMT6 promotes colorectal cancer progress via activating MYC signaling

Xin Zhang[1], Mingxin Jin[1], Yali Chu[1], Fengjun Liu[1], Hui Qu[1] and Cheng Chen[1*]

Abstract

Colorectal cancer (CRC) remains a major global health challenge, with high rates of incidence and mortality. This study investigates the role of protein arginine methyltransferase 6 (PRMT6) as an oncogene in CRC and its mechanistic involvement in tumor progression. We found that PRMT6 is significantly overexpressed in CRC tissues compared to adjacent normal tissues and is associated with poorer patient survival. Functional assays demonstrated that PRMT6 promotes CRC cell proliferation, migration, and invasion. Mechanistically, PRMT6 enhances MYC signaling by stabilizing c-MYC through mono-methylation at arginine 371, which inhibits c-MYC poly-ubiquitination and subsequent degradation. This post-translational modification is crucial for PRMT6-induced cancer cell proliferation. Xenograft models further validated that PRMT6 knockdown results in reduced tumor growth and decreased c-MYC levels. Our findings highlight PRMT6 as a key regulator of c-MYC stability and CRC progression, suggesting that targeting PRMT6 or its effects on c-MYC could offer a promising strategy for CRC treatment.

Keywords Colorectal cancer, PRMT6, c-MYC

Introduction

Colorectal cancer ranks as the third most common cancer globally and the second leading cause of cancer-related deaths. There were an estimated 1.8 million new cases and approximately 881,000 deaths worldwide attributed to colorectal cancer [1]. Treatment modalities include surgical resection of tumors, chemotherapy, radiation therapy, and targeted therapy [2]. However, relapse and drug resistance in patients with advanced colorectal cancer have always been difficult in clinical treatment [3, 4]. In-depth understanding of the molecular mechanism of colorectal cancer occurrence and development may be helpful for the clinical treatment of advanced colorectal cancer patients [5].

PRMT6 is a protein arginine methyltransferase and a member of the PRMT family. It primarily catalyzes the mono-methylation and asymmetric di-methylation of arginine residues, influencing various cellular processes such as transcription, translation, and signal transduction [6]. PRMT6 is significant in cancer, affecting cell behavior including growth, migration, invasion, apoptosis, and drug resistance, making it a potential target for anti-tumor therapies across different cancer types [6–8]. However, the role of PRMT6 in cancer varies. In prostate cancer, overexpression of PRMT6 can inhibit cell migration and invasion by upregulating TSP-1 and downregulating MMPs [9, 10]. In ovarian cancer, PRMT6 expression correlates with drug metabolism-related proteins G6PD and GSTP1, potentially leading to drug resistance [11]. In colorectal cancer, PRMT6 promotes tumor progression by epigenetically suppressing the expression

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of CDKN2B and CCNG1 [12]. These examples show that the role of PRMT6 in cancer is complex and diverse, with its specific mechanisms and potential therapeutic value needing further research and exploration.

The MYC gene, which consists of three paralogs - C-MYC, N-MYC, and L-MYC, is one of the most frequently deregulated driver genes in human cancer [13]. MYC proteins belong to the superfamily of basic helix-loop-helix leucine zipper (bHLHLZ) DNA binding proteins and function as transcriptional modulators [14]. They regulate genes involved in various cellular processes such as cell growth, cell cycle, differentiation, apoptosis, angiogenesis, metabolism, DNA repair, protein translation, immune response, and stem cell formation [15, 16]. Due to its high prevalence of deregulation and its causal role in cancer formation, maintenance, and progression, targeting MYC is considered an attractive strategy for cancer treatment [15]. However, MYC is considered difficult to drug due to its structural features, including a largely disordered structure lacking a suitable binding pocket for low molecular weight compounds, as well as its lack of catalytic activity and nuclear localization, making it challenging to target with large molecules like monoclonal antibodies [17, 18]. Post-translational modifications of MYC play a crucial role in its function as a therapeutic target in drug development. These modifications can impact MYC's stability, subcellular localization, protein-protein interactions, and transcriptional activity, ultimately influencing its oncogenic potential and response to targeted therapies [19–21]. Understanding and targeting these post-translational modifications of MYC are essential for developing effective drugs that can modulate MYC activity and combat cancer progression [22, 23].

Here, we firstly identified PRMT6 as an oncogene in colorectal cancer, and patients with higher PRTM6 expression had worse overall survival time. Also, we demonstrated that PRMT6 positively regulated colorectal cancer proliferation in vitro. For mechanism, we revealed that PRMT6 overexpression activated MYC signaling via suppressing MYC poly-ubiquitination level. Furthermore, PRMT6 mono-methylated MYC at arginine 371, which was responsible for inhibiting MYC poly-ubiquitination level. Besides, PRMT6-mediated cancer cell proliferation relied on MYC R371 mono-methylation. In vivo experiments, we proved that silencing of PRMT6 obviously suppressed tumor proliferation and intratumoral c-MYC expression. Collectively, these findings further confirmed the biological function of PRMT6 in colorectal cancer and might provide an effective clinical treatment strategy for MYC-driven colorectal cancer.

Methods and material

Cell lines

RKO and SW48 cancer cell lines were acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA). RKO cells were cultured in Minimum Essential Medium (Gibco, Cat. No. 11095080), while SW48 cells were cultured in L-15 Medium (Gibco, Cat. No. 11415064). All cells were maintained at 37 °C in a 95% air and 5% CO2 atmosphere.

Agents and antibodies

Antibodies targeting PRMT6 (Cat. No. 14641), β-Actin (Cat. No. 4970), Flag-tag (Cat. No. 14793), HA-tag (Cat. No. 3724), CDK4 (Cat. No. 12790), GLUT1 (Cat. No. 73015), His-tag (Cat. No. 12698), c-MYC (Cat. No. 13987), Mono-Methyl Arginine (Cat. No. 8711), Asymmetric Di-Methyl Arginine (Cat. No. 13522), and Ki-67 (Cat. No. 9449) were sourced from Cell Signaling Technology (CST). Cycloheximide (HY-12320), MG-132 (HY-13259), Chloroquine (HY-17589 A), EPZ020411 (HY-12970), PRMT6-IN-3, and SGC6870 (HY-126300) were acquired from MedChemExpress.

CCK8 assays

The proliferation capacity of SW48 and RKO cancer cells was assessed using the Cell Counting Kit-8 (C0037, Beyotime) following the manufacturer's protocol.

Trans-well assays

100 μL matrigel was added into the upper chamber for invasion assays. 40,000-100,000 SW48 or RKO cancer cells suspended in 100 μL of serum-free medium were added to the upper chamber. The lower chamber was filled with conditioned medium to promote migration. The transwell plate was then incubated at 37 °C in a CO2 incubator for approximately 12–16 h. After incubation, non-migrated cells in the upper chamber were removed with a cotton swab. Cells that migrated to the lower surface of the membrane were fixed with 4% paraformaldehyde and stained with 1% crystal violet. Finally, the stained cells were examined and counted under a microscope to evaluate migration capability.

Cell viability assay

Cell viability was detected by CellTiter-Lumi™ Luminescent Cell Viability Assay Kit (C0065M, Beyotime) following the manufacturer's protocol.

EdU assays

EdU assays were performed using BeyoClick™ EdU Cell Proliferation Kit with Alexa Fluor 594 (C0078S, Beyotime).

Western blot and immunoprecipitation assays

Cells were lysed with NP-40 lysis buffer containing protease and phosphatase inhibitors. Protein concentrations were quantified using a BCA assay. Protein samples were combined with SDS sample buffer and heated at 95 °C for 5 min. Equal amounts of protein were loaded into each well, and SDS-PAGE was performed, running the gel at 80 V for the stacking phase and 120 V for the resolving phase. Proteins were then transferred to a PVDF membrane at 100 V for 2 h. The membrane was blocked with 5% non-fat milk in TBST for 1 h at room temperature to minimize non-specific binding. It was then incubated overnight at 4 °C with a primary antibody diluted in blocking buffer. Afterward, the membrane was treated with an HRP-conjugated secondary antibody diluted in blocking buffer for 1 h at room temperature. Chemiluminescence was detected using an ECL substrate.

For immunoprecipitation, the protein supernatant was incubated with magnetic beads for 1–2 h to reduce non-specific interactions. The beads were separated by centrifugation or a magnetic rack, and the supernatant was collected. A specific antibody was added to the supernatant and incubated overnight at 4 °C with rotation. Magnetic beads were then added and incubated at 4 °C with rotation for an additional 1–2 h. After washing the beads 3–5 times with NP-40 lysis buffer (5 min per wash), bound proteins were eluted using SDS sample buffer and heating at 95 °C for 5 min before proceeding with Western blot analysis.

Immunohistochemistry (IHC)

Tissue samples were preserved in formalin and embedded in paraffin. Sections with a thickness of 4–5 μm were prepared using a microtome and underwent deparaffinization, rehydration, antigen retrieval, blocking, antibody incubation, and imaging. IRS scoring system was used to quantify the IHC experiments. The immunohistochemistry (IHC) results were evaluated by two independent experts using the following criteria: Intensity Scoring—0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining; Extent Scoring—0 for 0% positive cells, 1 for 1–25% positive cells, 2 for 26–50% positive cells, 3 for 51–75% positive cells, and 4 for 76–100% positive cells. The IHC score was determined by multiplying the Intensity Scoring by the Extent Scoring. When the IHC score is less than 7, it is regarded as low expression, whereas a score of 7 or above is considered high expression.

In vivo xenograft experiments

Female nude mice, aged six to eight weeks, were acquired from GemPharmatech. Subcutaneous injections of pretreated cancer cells (5 × 10^5) were administered to the mice's backs. Tumor development was observed at the site of injection, with measurements of tumor volume and morphological changes recorded. Tumor volume was determined using the formula: 0.5 × major axis × minor axis^2. After 18 days, the mice were humanely euthanized, and tumor tissues were collected for further analysis.

Statistical analysis

Bioinformatics analyses were carried out using the TCGA database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga), the GEO database (https://www.ncbi.nlm.nih.gov), and the GEPIA tool (http://gepia.cancer-pku.cn/detail.php?gene=ERBB2). For statistical evaluation, GSEA software (Version 4.3.3) and GraphPad Prism (Version 9.0) were employed. Comparisons between two groups were performed using Student's t-test, while ANOVA was used for multiple groups. A significance level of $P<0.05$ was considered indicative of statistical significance.

Results

PRMT6 functioned as an oncogene in colorectal cancer

To elucidate the biological function of PRMT6 in colorectal cancer, we first analyzed its expression using data from the TCGA and GEO databases. The results indicated that PRMT6 is significantly upregulated in colorectal cancer tissues compared to adjacent normal tissues (Fig. 1A-C). Additionally, ROC curve analysis demonstrated that PRMT6 served as a promising marker for distinguishing between tumor and non-tumor colorectal tissues (Fig. 1D). The GEPIA database further confirmed that PRMT6 was overexpressed in multiple types of cancer (Figure S1A). To further validate the role of PRMT6 in colorectal cancer, we examined its expression levels in colorectal cancer tissues and adjacent normal tissues using western blot (7 pairs) and IHC assays (69 pairs). The results showed that intratumoral PRMT6 expression was significantly higher compared to normal tissues (Fig. 1E-G). Furthermore, patients with higher T and clinical stages exhibited elevated PRMT6 expression (Fig. 1H-J). PRMT6 expression showed no correlation with patients' age or gender (Figure S2B and S2C). We then categorized colorectal cancer patients into two groups based on PRMT6 expression levels. Analysis of data from GSE36864 and our own clinical cohort revealed that patients with higher PRMT6 expression had worse overall survival (Fig. 1K, L). Interestingly, similar trends were observed in multiple types of cancers (Figure S1D). Overall, these findings suggested that PRMT6 might function as an oncogene in colorectal cancer.

PRMT6 positively regulated the proliferation, migration and invasion ability of colorectal cancer cell in vitro

To test our hypothesis, we constructed SW48 and RKO cell lines stably expressing either an empty vector or

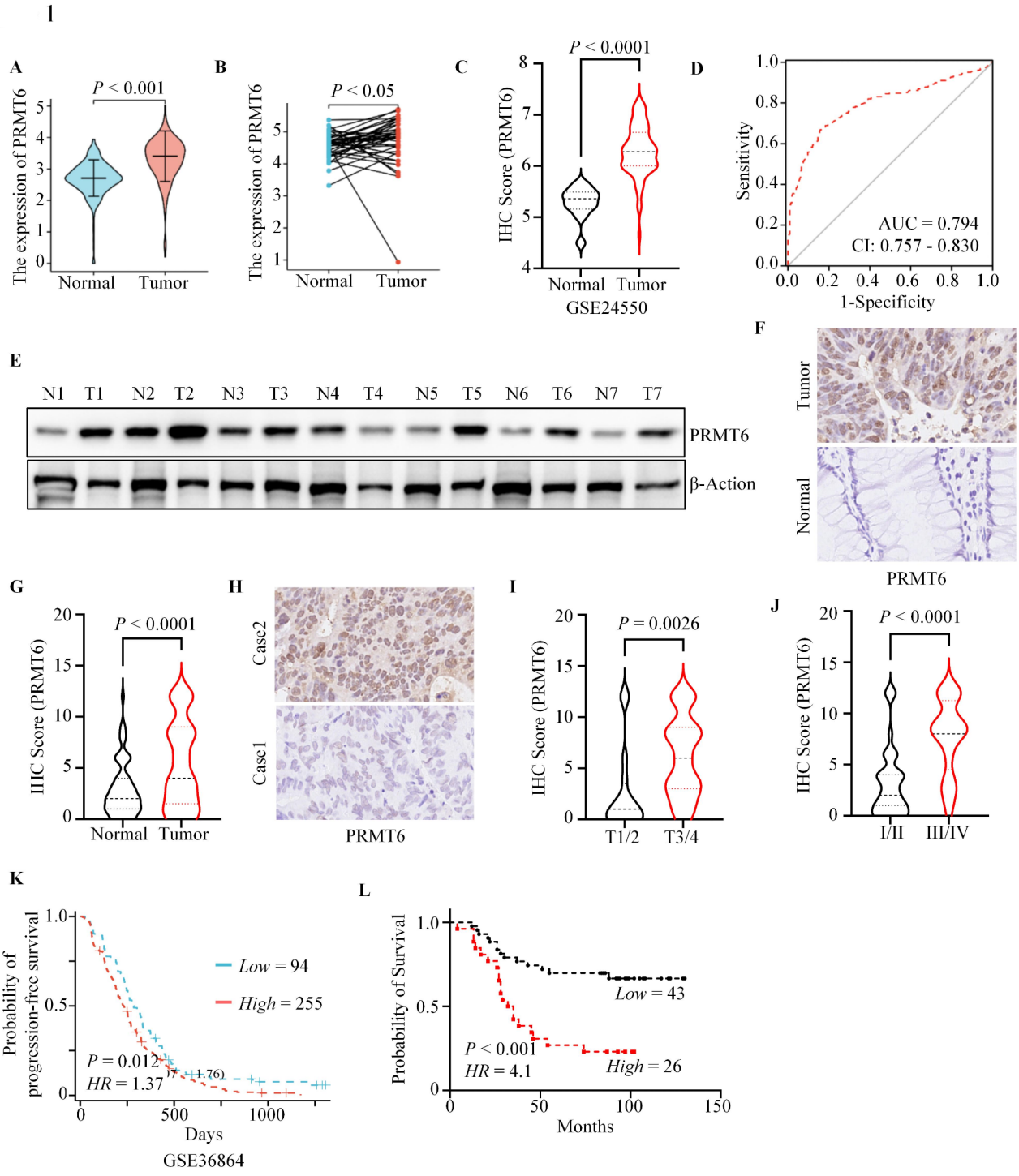

Fig. 1 PRMT6 functions as an oncogene in colorectal cancer. **A**-**C**. Expression levels of PRMT6 were compared between colorectal cancer and adjacent normal tissues using the TCGA and GSE24550 datasets. **D.** ROC curve analysis was used to evaluate the potential of PRMT6 as a diagnostic marker for distinguishing colorectal tumor tissues from normal tissues. **E.** Western blot analysis was performed to evaluate PRMT6 expression levels in colorectal cancer tissues compared to adjacent normal tissues. **F**, **H.** Representative images of immunohistochemistry (IHC) staining showing PRMT6 expression in colorectal cancer tissues and adjacent normal tissues.**G.** Statistical analysis comparing PRMT6 expression levels between colorectal cancer tissues and adjacent normal tissues. **I**, **J.** Statistical analysis of PRMT6 expression levels in patients categorized by T stage and clinical stage. **K**, **L.** Kaplan-Meier survival analysis of patients from the GSE36864 dataset, stratified by PRMT6 expression levels. Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

flag-tagged PRMT6 using lentiviral transduction. Western blot analysis confirmed the successful expression of PRMT6 (Fig. 2A). Next, we assessed the proliferation ability of vector and PRMT6-overexpressing cancer cells. CCK8 assays showed that overexpression of PRMT6 significantly promoted cancer cell proliferation (Fig. 2B). Cells overexpressing PRMT6 exhibited higher cellular ATP levels than vector controls (Fig. 2C) and demonstrated more active DNA replication (Fig. 2D, E). Metastasis was a hallmark of advanced malignant tumors, so we also evaluated the effect of PRMT6 on cancer cell metastasis. Transwell assays demonstrated that PRMT6-overexpressing cells had enhanced migration and invasion capabilities compared to vector controls (Fig. 2F, G).

Subsequently, we knocked down PRMT6 expression in SW48 and RKO cancer cells. Western blot analysis confirmed effective knockdown (Fig. 3A). Our data consistently demonstrated that silencing PRMT6 significantly inhibited cancer cell proliferation, DNA replication, and cellular ATP levels (Fig. 3B-E). Transwell assays further showed that cells with reduced PRMT6 expression exhibited lower migration and invasion abilities than control shNC cells (Fig. 3F, G). In summary, these findings demonstrated that PRMT6 positively regulated the proliferation, migration, and invasion capabilities of colorectal cancer cells.

PRMT6 positively activated MYC signaling via stabilizing c-MYC

To elucidate the mechanism underlying PRMT6-mediated colorectal cancer progression, we first conducted Gene Set Enrichment Analysis (GSEA) using 2 TCGA and 10 GEO datasets. FDR < 0.25 and $P < 0.05$ were considered to indicate statistical significance. Finally, analysis from all datasets indicated that MYC signaling was more active in tumors with higher PRMT6 expression, which inspired us to investigate whether PRMT6 could regulate MYC signaling (Fig. 4A and Figure S2A). Western blot analysis showed that overexpression of PRMT6 significantly increased c-MYC expression (Figure S2B), while knockdown of PRMT6 led to a significant decrease in the expression of c-MYC and the downstream genes expression of MYC signaling (CDK4 and GLUT1) (Fig. 4B). Additionally, we transfected SW48 cells with either wild-type PRMT6 (WT) or an enzyme activity-deficient mutant (PRMT6 KLA) and treated the cells with DMSO or the PRMT6 inhibitor PRMT6-IN-3 (Fig. 4C and Figure S2C). We then ectopically expressed either PRMT6 WT or the KLA mutant in SW48 cells stably expressing shPRMT6-3'UTR. Western blot analysis and CCK8 assays demonstrated that overexpression of PRMT6 WT, but not the KLA mutant, restored c-MYC signaling activation and cancer cell proliferation that had been inhibited by PRMT6 silencing (Figure S2D and 2E). The results

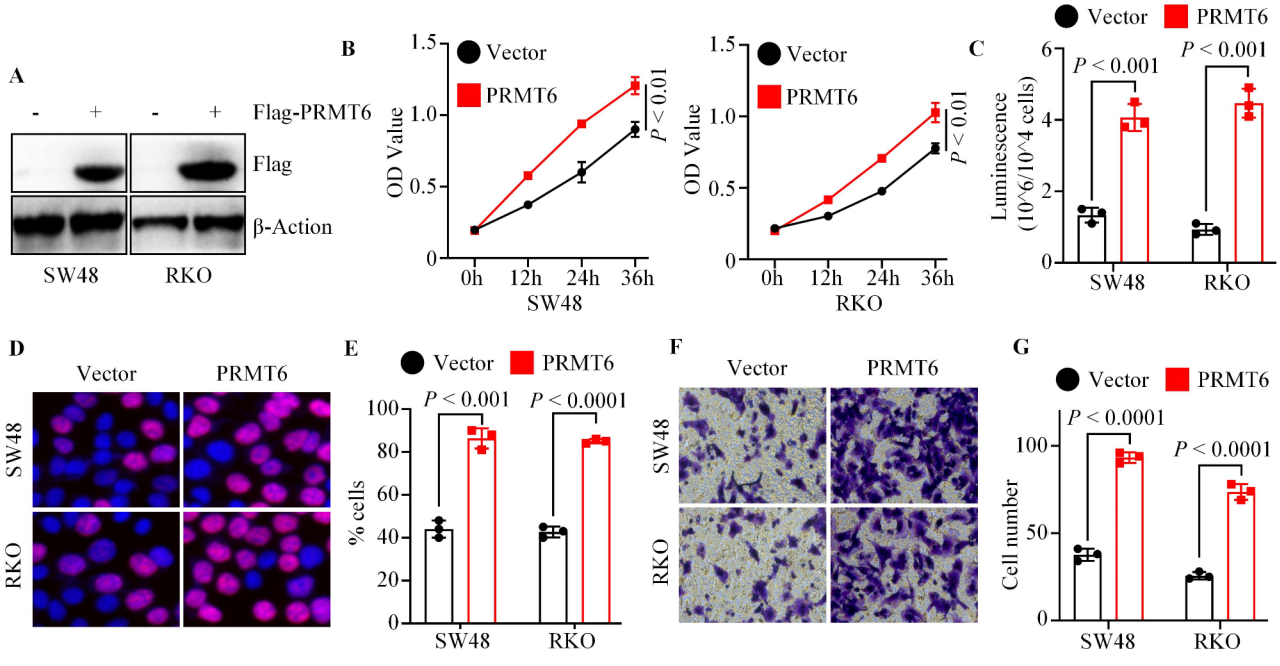

Fig. 2 PRMT6 overexpression promotes cancer cell proliferation, migration, and invasion. (**A**) Western blot analysis was performed to confirm the successful construction of colorectal cancer cells stably expressing either vector or PRMT6. **(B)** CCK8 assays were conducted to evaluate the proliferation ability of colorectal cancer cells stably expressing either vector or PRMT6. **(C)** Cell viability assays were used to assess the intracellular ATP levels in colorectal cancer cells stably expressing either vector or PRMT6. **D**, **E.** BrdU assays were performed to evaluate the DNA replication ability of colorectal cancer cells stably expressing either vector or PRMT6. **F**, **G.** Transwell assays were conducted to assess the migration and invasion abilities of colorectal cancer cells stably expressing either vector or PRMT6.Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

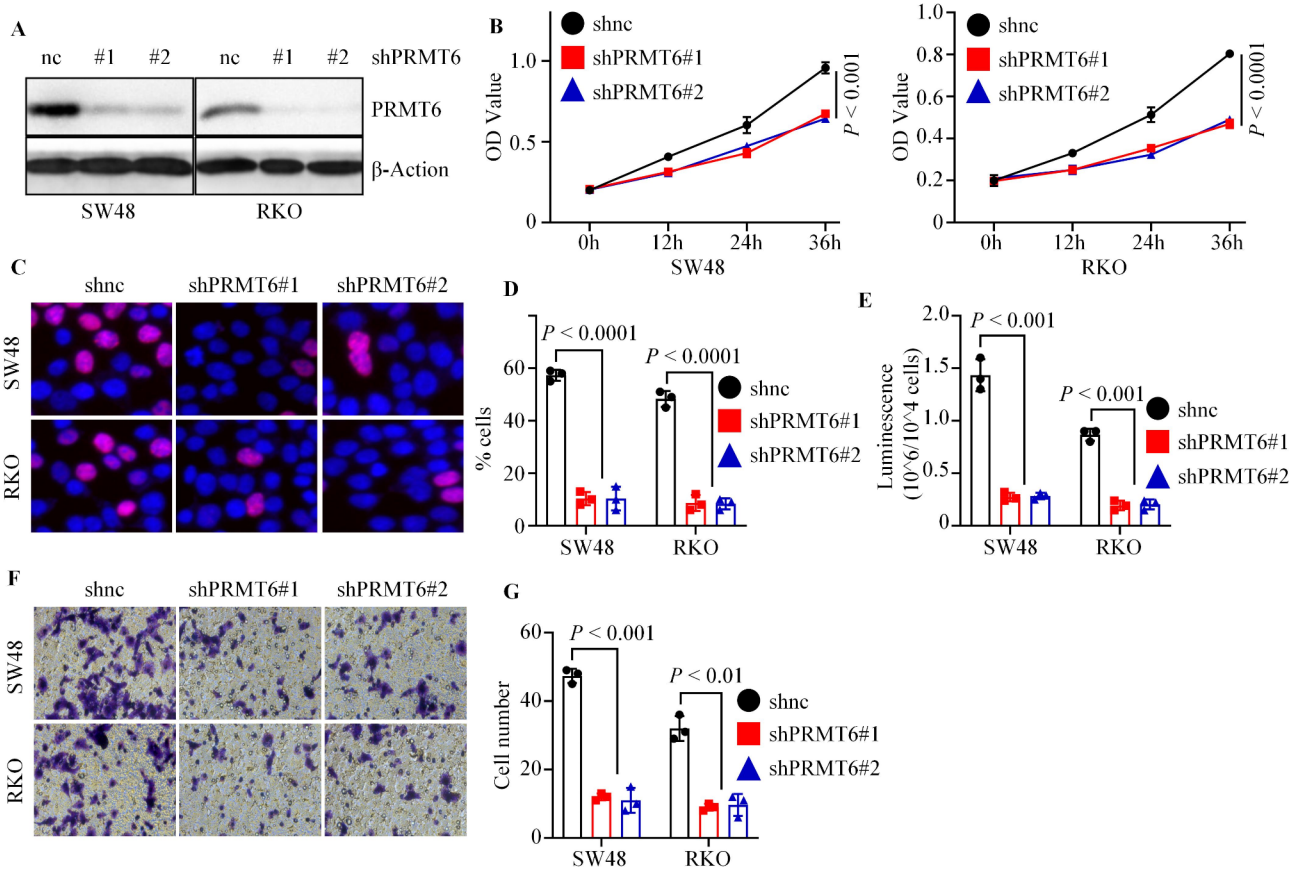

Fig. 3 Silencing PRMT6 significantly inhibits cancer cell proliferation, migration, and invasion. (**A**) Western blot analysis was performed to confirm the successful construction of colorectal cancer cells stably expressing either shNC or shPRMT6. **(B)** CCK8 assays were conducted to evaluate the proliferation ability of colorectal cancer cells stably expressing either shNC or shPRMT6. **(C)** Cell viability assays were used to assess the intracellular ATP levels in colorectal cancer cells stably expressing either shNC or shPRMT6. **D**, **E.** BrdU assays were performed to evaluate the DNA replication ability of colorectal cancer cells stably expressing either shNC or shPRMT6. **F**, **G.** Transwell assays were conducted to assess the migration and invasion abilities of colorectal cancer cells stably expressing either shNC or shPRMT6. Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

demonstrated that PRMT6 enzyme activity was essential for PRMT6-mediated activation of MYC signaling. However, RT-PCR analysis revealed that PRMT6 did not affect the mRNA expression of c-MYC (Figures S3A and S3B). We further investigated the post-transcriptional regulation of c-MYC by PRMT6 using cycloheximide (CHX) to inhibit endogenous c-MYC synthesis. Western blot analysis showed that knockdown of PRMT6 accelerated the degradation rate of c-MYC (Fig. 4D and E). These findings suggested that PRMT6 regulated c-MYC expression at the post-transcriptional level. Since the autophagy-lysosome pathway and the ubiquitin-proteasome pathway were the two main mechanisms for protein degradation, we treated vector and PRMT6-overexpressing cells with chloroquine (CQ), an autophagy-lysosome pathway inhibitor, and MG132, a ubiquitin-proteasome pathway inhibitor. The results indicated that MG132, but not CQ, abrogated PRMT6-induced c-MYC expression (Fig. 4F). Subsequently, we assessed the polyubiquitin levels of endogenous c-MYC in shNC and shPRMT6 cells. Silencing PRMT6 significantly increased the polyubiquitin level of c-MYC (Fig. 4G). In a cohort of 69 colorectal tumor cases, we evaluated the levels of intratumoral PRMT6, c-MYC, and the percentage of intratumoral cancer cells positive for Ki-67. Statistical analysis showed a positive correlation between PRMT6 expression and both c-MYC expression and the number of Ki-67-positive cancer cells (Fig. 4H and I, and Figure S3C). c-MYC expression showed no correlation with patients' age or gender (Figure S3D and 3E). Patients with higher T and clinical stages exhibited elevated c-MYC expression (Figure S3F and 3G). Analysis of GEPIA database revealed that c-MYC was significantly upregulated in colorectal cancer tissues compared to adjacent normal tissues (Figure S3H). Collectively, our data revealed that PRMT6 acted

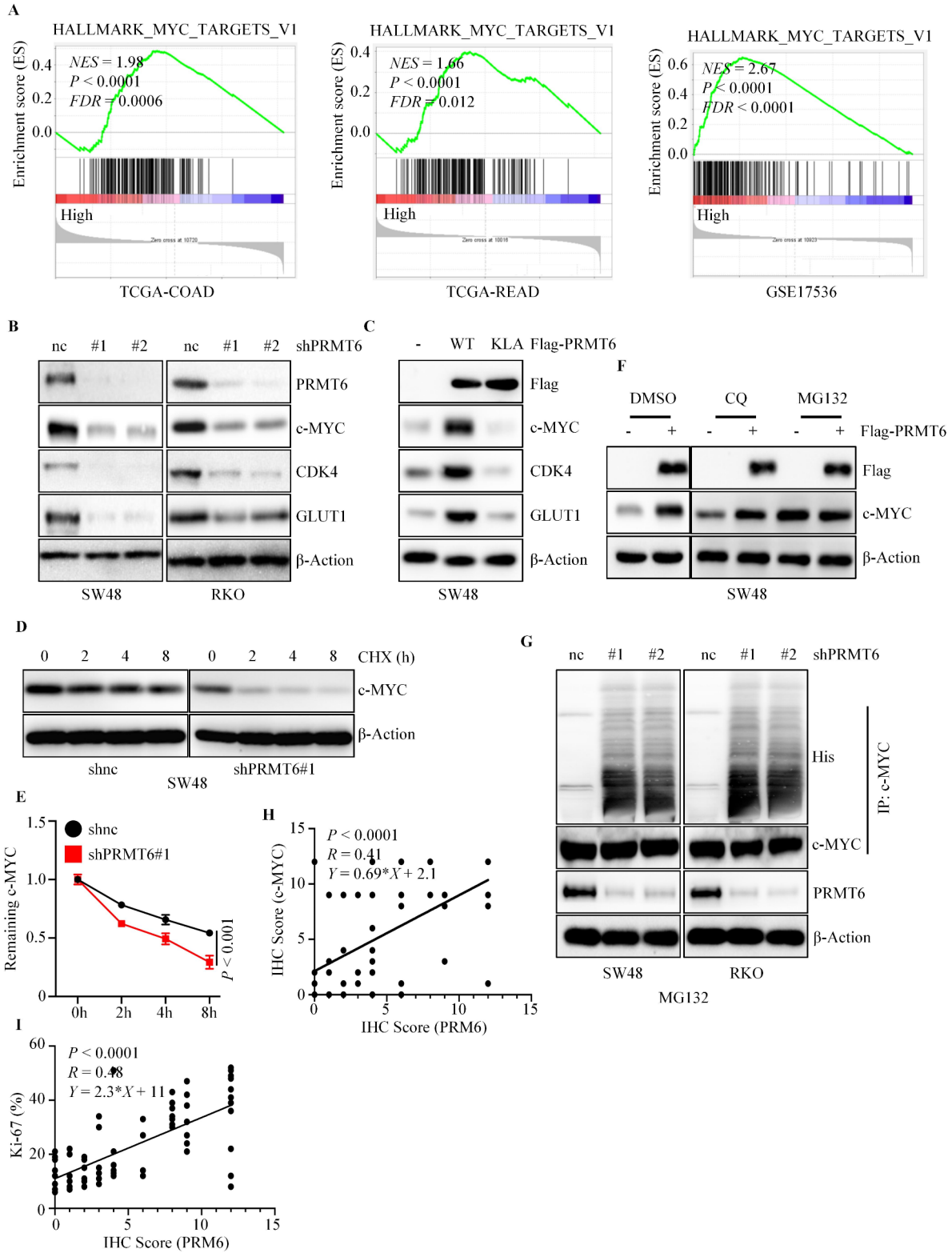

Fig. 4 (See legend on next page.)

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Fig. 4 PRMT6 positively regulates MYC signaling by blocking the poly-ubiquitination of c-MYC. (**A**) GSEA analysis was performed to assess the correlation between PRMT6 expression levels and MYC signaling activation. **(B)** Western blot analysis was conducted to detect the expression of identified proteins in shNC and shPRMT6 cancer cells. **(C)** Western blot analysis was performed to detect the expression of identified proteins in cancer cells ectopically expressing vector, PRMT6, or the PRMT6 KLA mutant. **D**, **E.** Cycloheximide (CHX) was applied to inhibit the synthesis of endogenous c-MYC, and Western blot analysis was conducted to assess the degradation rate of c-MYC. **F.** Western blot analysis was performed to detect the expression of identified proteins in different pre-treated cancer cells. **G.** Western blot and immunoprecipitation assays were conducted to assess the poly-ubiquitination levels of c-MYC in shNC and shPRMT6 cancer cells. **H**, **I.** Scatter plots were generated to assess the relationship between PRMT6 expression levels and c-MYC expression levels (H), or between PRMT6 expression levels and the ratio of Ki-67 positive cells (I). Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

as an activator of MYC signaling by blocking the polyubiquitination of c-MYC.

PRMT6 mono-methylated c-MYC at arginine 371

After browsing the GENEMANIA database, we identified a potential interaction between PRMT6 and c-MYC (Figure S4A). This interaction was confirmed by immunoprecipitation assays (Fig. 5A and Figure S4B). PRMT6 was an arginine methyltransferase that primarily catalyzes the mono-methylation and asymmetric di-methylation of arginine residues. We hypothesized that PRMT6 could methylate c-MYC. Western blot analysis revealed that c-MYC exhibited lower levels of mono-methylation, but not asymmetrical di-methylation, in shPRMT6 cells compared to shNC cells (Fig. 5B). Next, we transfected SW48 cancer cells with either wild-type PRMT6 (WT) or the enzyme activity-deficient mutant PRMT6 KLA. MG132 was used to inhibit the degradation of endogenous c-MYC. Western blot analysis showed that enzyme activity is necessary for PRMT6-mediated c-MYC methylation (Fig. 5C). Consistently, inhibitors of PRMT6 enzyme activity significantly suppressed c-MYC methylation (Fig. 5D). In vitro methylation assays further confirmed that PRMT6 can mono-methylate c-MYC (Fig. 5E). Using GPS-MSP software, we identified four potential mono-methylation sites on c-MYC (R5, R346, R355, and R371). We constructed c-MYC mutants by replacing these arginine residues with lysine. Interestingly, we found that only the R371K mutant abrogated PRMT6-mediated c-MYC methylation (Fig. 5F). We used the CRISPR-Cas9 system and lentivirus to generate colorectal cancer cells stably expressing either c-MYC WT or c-MYC R371K (Figure S4C, S4D). Western blot analysis confirmed that PRMT6 overexpression increased the mono-methylation level of c-MYC in c-MYC WT cells but not in c-MYC R371K mutant cells (Fig. 5G). Notably, the c-MYC R371K mutant exhibited higher polyubiquitin levels than c-MYC WT (Fig. 5H). PRMT6 overexpression inhibited the polyubiquitination of c-MYC in c-MYC WT cells but not in c-MYC R371K cells (Fig. 5H). Of note, the analysis of c-MYC sequences across various species revealed that arginine at position 371 was conserved. In summary, our data demonstrated that PRMT6 negatively regulated the polyubiquitination of c-MYC by mono-methylating c-MYC at R371.

c-MYC R371 mono-methylation was essential for PRMT6-mediated colorectal cancer cell proliferation

To further elucidate the relationship between PRMT6 signaling and MYC signaling, we constructed SW48 cancer cells stably expressing Vector + c-MYC WT, PRMT6 + c-MYC WT, Vector + c-MYC R371K, or PRMT6 + c-MYC R371K. Western blot analysis confirmed successful construction (Fig. 6A). The results demonstrated that PRMT6 overexpression upregulated the expression levels of c-MYC, CDK4, and GLUT1 in c-MYC WT cells, but not in c-MYC R371K cells (Fig. 6A). CCK8 and BrdU assays revealed that the c-MYC R371K mutant abolished PRMT6-mediated cancer cell proliferation (Fig. 6B-D). Additionally, PRMT6 overexpression increased intracellular ATP levels in c-MYC WT cells, but not in c-MYC R371K cells (Fig. 6E). We further generated SW48 cancer cells stably expressing shNC + c-MYC WT, shNC + c-MYC WT, shPRMT6#1 + c-MYC R371K, or shPRMT6#1 + c-MYC R371K using lentivirus (Fig. 6F). Loss of PRMT6 decreased intracellular c-MYC, CDK4, and GLUT1 expression levels in c-MYC WT cells, but not in c-MYC R371K cells (Fig. 6F). Consistently, silencing PRMT6 significantly inhibited cell proliferation and intracellular ATP levels in c-MYC WT cells, but not in c-MYC R371K cells (Fig. 6G-J). Notably, c-MYC R371K cells exhibited lower expression levels of c-MYC, CDK4, and GLUT1, as well as reduced proliferation ability and intracellular ATP levels compared to c-MYC WT cells (Fig. 6A-J). In summary, these findings demonstrate that mono-methylation of c-MYC at R371 is essential for c-MYC or PRMT6-mediated colorectal cancer cell proliferation.

Silencing of PRMT6 significantly suppressed MYC signaling and tumor proliferation in vivo

To further verify the oncogenic function of PRMT6 in colorectal cancer, we conducted an in vivo xenograft experiment. We subcutaneously injected SW48 cells stably expressing either shNC or shPRMT6 into the backs of 10-week-old female mice. Tumor volumes were measured every three days. After 18 days, all mice were sacrificed, and tumors were isolated for further analysis. The results showed that tumors from the shPRMT6 group had a lower growth rate and weight compared to those from the shNC group (Fig. 7A-C). We also recorded

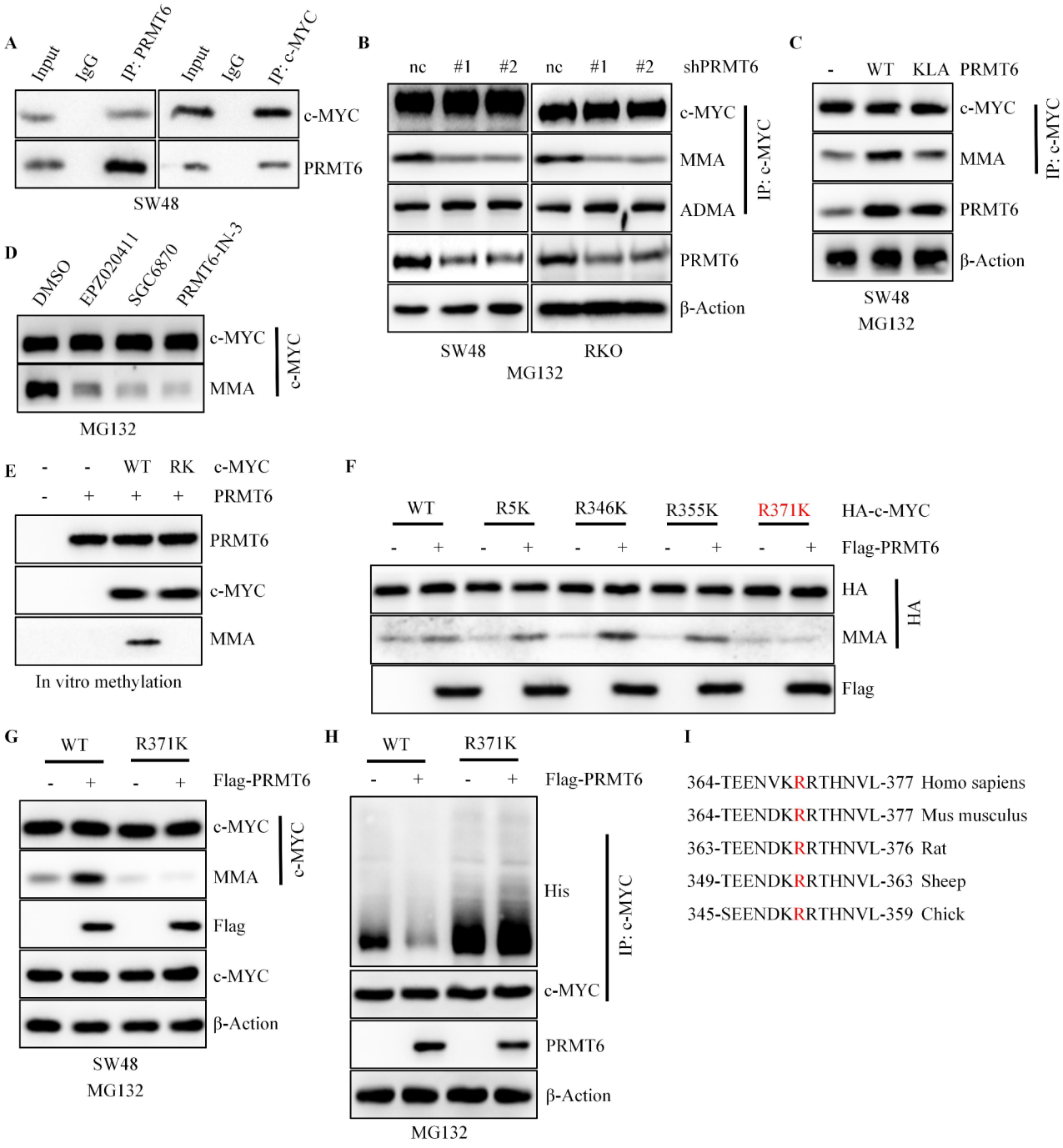

Fig. 5 c-MYC Arg371 is mono-methylated by PRMT6. (**A**) Western blot and immunoprecipitation assays were performed to confirm the binding between PRMT6 and c-MYC. **(B)** Western blot and immunoprecipitation assays were conducted to assess the levels of mono-methylation (MMA) and asymmetric di-methylation (ADMA) of c-MYC in shNC and shPRMT6 cancer cells. **(C)** Western blot and immunoprecipitation assays were used to evaluate the MMA level of c-MYC in cancer cells ectopically expressing vector, PRMT6, or the PRMT6 KLA mutant. **(D)** Western blot and immunoprecipitation assays were performed to assess the MMA level of c-MYC in cancer cells treated with DMSO or PRMT6 inhibitors. **(E)** In vitro methylation assays confirmed that PRMT6 can directly mono-methylate c-MYC. **(F)** c-MYC KR mutants were transfected into cancer cells ectopically expressing either vector or PRMT6. Western blot and immunoprecipitation assays were performed to assess the MMA levels of c-MYC mutants. **(G)** Western blot and immunoprecipitation assays were conducted to evaluate the MMA levels of c-MYC in different cancer cells. **(H)** Western blot and immunoprecipitation assays were performed to assess the poly-ubiquitination levels of c-MYC WT and c-MYC R371K in cancer cells ectopically expressing either vector or PRMT6. **(I)** The amino acid sequence of c-MYC was analyzed for conservation across various species. Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

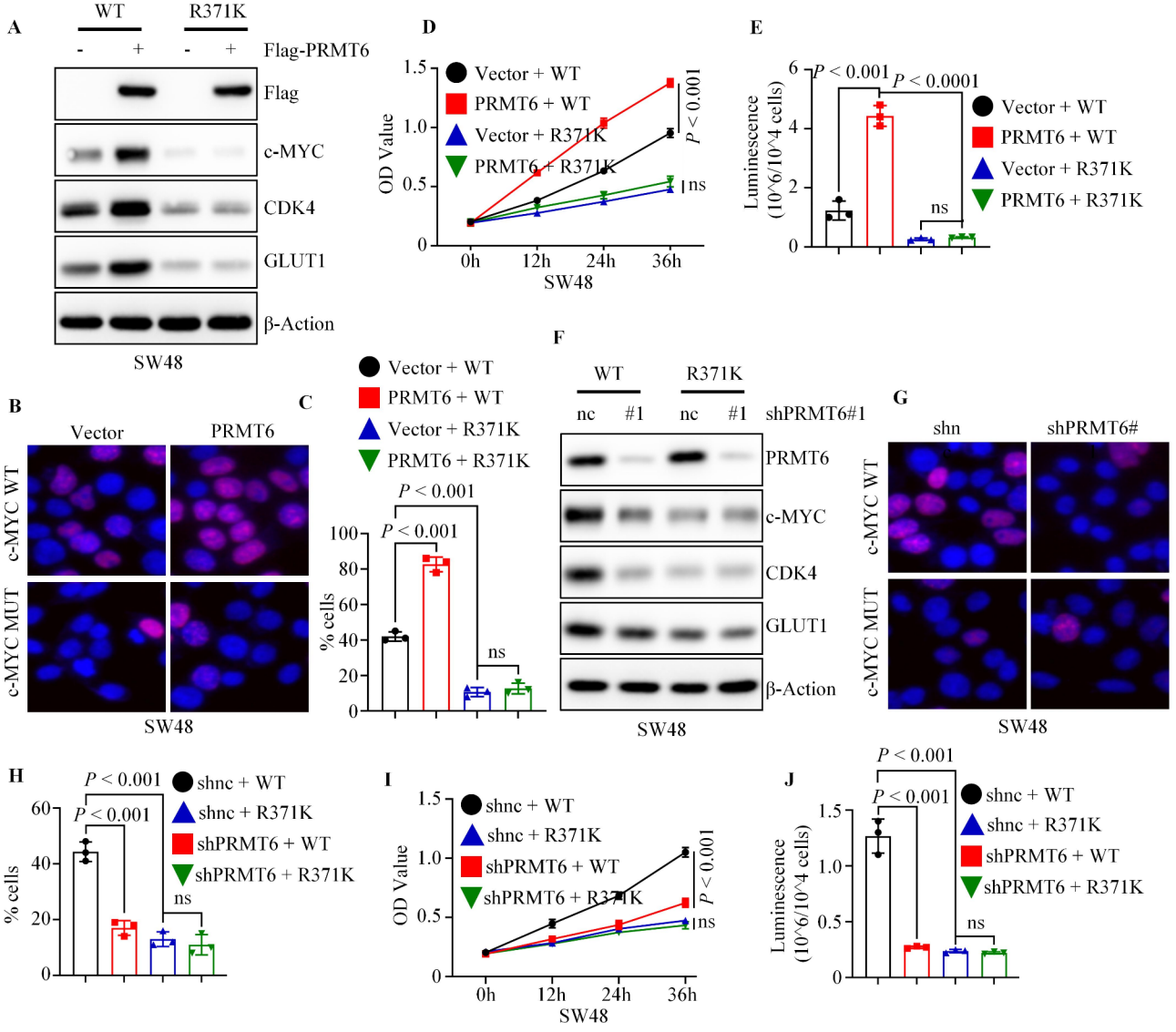

Fig. 6 PRMT6-mediated cancer cell proliferation depends on c-MYC R371 mono-methylation. **A**, **F.** Western blot analysis was performed to assess the expression levels of c-MYC, CDK4, and GLUT1 in different pre-treated cancer cells. **B**, **C**, **G**, **H.** BrdU assays were conducted to evaluate DNA replication ability in different pre-treated cancer cells. **D**, **I.** CCK8 assays were performed to detect the proliferation ability in different pre-treated cancer cells. **E**, **J.** Cell viability assays were carried out to assess intracellular ATP levels in different pre-treated cancer cells. Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

the overall survival time of the mice. Consistently, mice with shPRMT6 tumors had longer overall survival times than those with shNC tumors (Fig. 7D). Next, we performed immunohistochemistry to assess the intratumoral expression levels of PRMT6, c-MYC, and Ki-67. The results indicated that knockdown of PRMT6 significantly inhibited the expression of c-MYC and reduced the proportion of Ki-67-positive cells (Fig. 7E). Western blot analysis further confirmed that silencing PRMT6 markedly decreased the expression of c-MYC and Ki-67 (Fig. 7F). Overall, our data suggested that PRMT6 might be an effective target for colorectal cancer treatment.

Discussion

In this study, we explored the role of PRMT6 in colorectal cancer and uncovered its potential as an oncogene. Our findings show that PRMT6 was significantly overexpressed in colorectal cancer tissues compared to adjacent normal tissues, and higher PRMT6 expression correlates with poorer overall survival in patients. The oncogenic role of PRMT6 in colorectal cancer was further confirmed through a series of in vitro and in vivo experiments. We demonstrated that PRMT6 positively regulated cancer cell proliferation, migration, and invasion. Notably, PRMT6 was found to activate MYC signaling

Figure 7

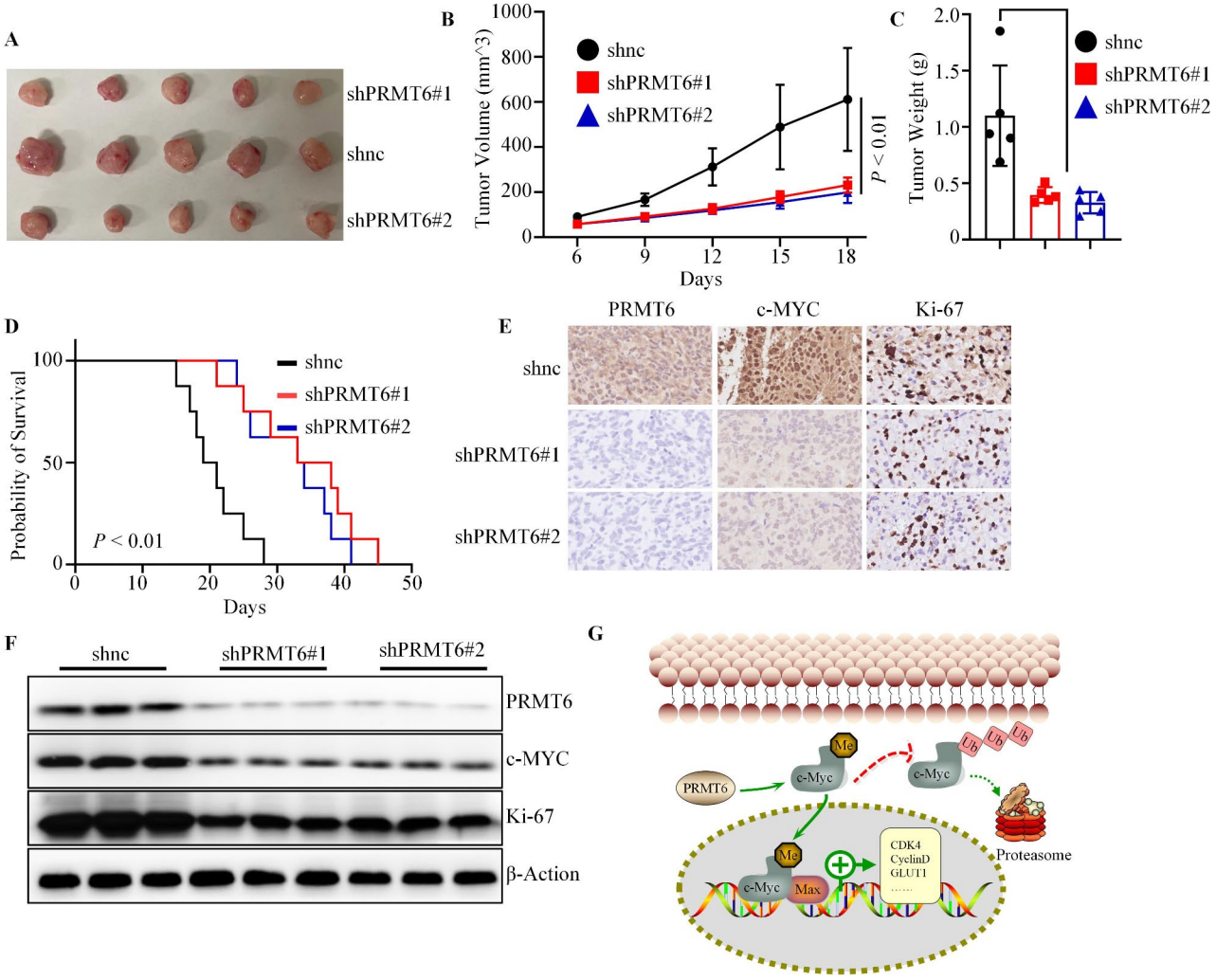

Fig. 7 Knockdown of PRMT6 significantly inhibits colorectal tumor proliferation in vivo. (**A**) Representative images of tumors from mice injected with SW48 cells stably expressing shNC or shPRMT6. **(B)** Tumor growth curves showing the growth rates of tumors derived from mice injected with shNC or shPRMT6 cells. **(C)** Scatter plot of tumor weights from mice injected with shNC or shPRMT6 cells. **(D)** Kaplan-Meier survival curves for mice bearing tumors from the three experimental groups. **(E)** Representative images of immunohistochemistry (IHC) assays showing PRMT6, c-MYC, and Ki-67 expression in tumors. **(F)** Western blot analysis to detect the expression levels of intratumoral PRMT6, c-MYC, and Ki-67. **(G)** Working model: PRMT6 mono-methylated c-MYC at arginine 371 site, which suppressed the poly-ubiquitin level of c-MYC, and then promoted colorectal cancer progress. Student's t-test was employed for statistical analysis, and all Western blot experiments were performed in triplicate

by stabilizing c-MYC. This was mediated by the mono-methylation of c-MYC at arginine 371, a modification that we identified as crucial for PRMT6-mediated oncogenic activity. The mono-methylation of c-MYC at R371 by PRMT6 not only enhanced c-MYC stability but also significantly promotes colorectal cancer cell proliferation. In vivo xenograft experiments reinforced the importance of PRMT6 in colorectal cancer. Tumors expressing shPRMT6 exhibited significantly reduced tumor growth and prolonged overall survival compared to control mice. Furthermore, knockdown of PRMT6 led to a marked decrease in intratumoral c-MYC expression and a lower proportion of Ki-67 positive cells, indicating reduced proliferation within the tumor microenvironment. This finding added a new layer of complexity to the regulation of MYC, suggesting that arginine methylation can be a pivotal determinant of MYC protein stability and activity. Besides, these insights underscored the potential of targeting PRMT6 or the specific methylation of MYC as a therapeutic strategy in MYC-driven cancers.

Furthermore, our results suggested that PRMT6 expression could serve as a prognostic marker for colorectal cancer, helping to identify patients with more aggressive disease and poorer outcomes. Monitoring PRMT6 levels could guide treatment decisions and facilitate the development of personalized therapeutic

approaches. Given the challenges associated with directly targeting MYC due to its structural features, targeting its regulatory mechanisms offers a promising alternative [17, 24]. our study provided a promising alternative by focusing on the regulation of c-MYC through PRMT6-mediated methylation. Targeting PRMT6 or the specific methylation of c-MYC could offer a novel approach to disrupting MYC signaling in colorectal cancer, thereby addressing the critical issue of drug resistance and relapse in advanced-stage patients.

However, the complexity of PRMT6's role in cancer warranted further investigation. While we had established its oncogenic role in colorectal cancer, PRMT6 had been reported to exhibit varying functions in different cancer types. Understanding the context-dependent roles of PRMT6 would be crucial for developing effective and specific therapeutic interventions. Additionally, the potential off-target effects and toxicity of PRMT6 inhibitors must be carefully evaluated in preclinical and clinical settings (an effective approach: targeting the interaction between PRMT6 and MYC).

In conclusion, our study underscored the critical role of PRMT6 in colorectal cancer progression through its regulation of c-MYC stability and activity. These findings not only deepened our understanding of the molecular mechanisms driving colorectal cancer but also opened new avenues for the development of targeted therapies aimed at improving outcomes for patients with MYC-driven cancers.

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions
ZX and CC conceived the study, drafted and revised the manuscript. ZX and JMX performed the experiments and analyzed the data. CYL assisted with data collection and analysis. LFJ and QH supervised the research.

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Data availability
All data and materials used in the study were available in the manuscript.

Declarations

Ethical approval and consent to participate
The current study was approved by the Ethical Review Committee of Shandong University (No.KYLL-2021(ZM)-209). All patients who participated in the current study provided signed informed consent.

Consent for publication
All authors approved the final manuscript draft for publication.

Competing interests
Authors declared no conflict of interest.

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References

1. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467–80.
2. Shin AE, Giancotti FG, Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol Sci. 2023;44(4):222–36.
3. Brenner H, Kloor M, Pox CP. Colorectal cancer. Lancet. 2014;383(9927):1490–502.
4. Li J, Ma X, Chakravarti D, Shalapour S, DePinho RA. Genetic and biological hallmarks of colorectal cancer. Genes Dev. 2021;35(11–12):787–820.
5. Aghapour SA, Torabizadeh M, Bahreiny SS, Saki N, Far MAJ, Yousefi-Avarvand A, et al. Investigating the dynamic interplay between Cellular Immunity and Tumor cells in the Fight Against Cancer: an updated Comprehensive Review. Iran J Blood Cancer. 2024;16(2):84–101.
6. Chen Z, Gan J, Wei Z, Zhang M, Du Y, Xu C, et al. The emerging role of PRMT6 in Cancer. Front Oncol. 2022;12:841381.
7. Huang T, Yang Y, Song X, Wan X, Wu B, Sastry N, et al. PRMT6 methylation of RCC1 regulates mitosis, tumorigenicity, and radiation response of glioblastoma stem cells. Mol Cell. 2021;81(6):1276–e919.
8. Sun M, Li L, Niu Y, Wang Y, Yan Q, Xie F, et al. PRMT6 promotes tumorigenicity and cisplatin response of lung cancer through triggering 6PGD/ENO1 mediated cell metabolism. Acta Pharm Sin B. 2023;13(1):157–73.
9. Michaud-Levesque J, Richard S. Thrombospondin-1 is a transcriptional repression target of PRMT6. J Biol Chem. 2009;284(32):21338–46.
10. Kim NH, Kim SN, Seo DW, Han JW, Kim YK. PRMT6 overexpression upregulates TSP-1 and downregulates MMPs: its implication in motility and invasion. Biochem Biophys Res Commun. 2013;432(1):60–5.
11. Feng Q, Li X, Sun W, Sun M, Li Z, Sheng H, et al. Targeting G6PD reverses paclitaxel resistance in ovarian cancer by suppressing GSTP1. Biochem Pharmacol. 2020;178:114092.
12. Chen Y, Liang W, Du J, Ma J, Liang R, Tao M. PRMT6 functionally associates with PRMT5 to promote colorectal cancer progression through epigenetically repressing the expression of CDKN2B and CCNG1. Exp Cell Res. 2023;422(1):113413.
13. Baluapuri A, Wolf E, Eilers M. Target gene-independent functions of MYC oncoproteins. Nat Rev Mol Cell Biol. 2020;21(5):255–67.
14. Amjadi-Moheb F, Paniri A, Akhavan-Niaki H. Insights into the links between MYC and 3D chromatin structure and Epigenetics Regulation: implications for Cancer Therapy. Cancer Res. 2021;81(8):1925–36.
15. Chen H, Liu H, Qing G. Targeting oncogenic myc as a strategy for cancer treatment. Signal Transduct Target Ther. 2018;3:5.
16. Lourenco C, Resetca D, Redel C, Lin P, MacDonald AS, Ciaccio R, et al. MYC protein interactors in gene transcription and cancer. Nat Rev Cancer. 2021;21(9):579–91.
17. Llombart V, Mansour MR. Therapeutic targeting of undruggable MYC. EBioMedicine. 2022;75:103756.
18. Das SK, Lewis BA, Levens D. MYC: a complex problem. Trends Cell Biol. 2023;33(3):235–46.
19. Hann SR. Role of post-translational modifications in regulating c-Myc proteolysis, transcriptional activity and biological function. Semin Cancer Biol. 2006;16(4):288–302.
20. Wolpaw AJ, Dang CV. MYC-induced metabolic stress and tumorigenesis. Biochim Biophys Acta Rev Cancer. 2018;1870(1):43–50.
21. Vervoorts J, Luscher-Firzlaff J, Luscher B. The ins and outs of MYC regulation by posttranslational mechanisms. J Biol Chem. 2006;281(46):34725–9.
22. Aghaei M, Khademi R, Far MAJ, Bahreiny SS, Mahdizade AH, Amirrajab N. Genetic variants of dectin-1 and their antifungal immunity impact in hematologic malignancies: a comprehensive systematic review. Curr Res Transl Med. 2024;72(4):103460.
23. Aghaei M, Khademi R, Bahreiny SS, Saki N. The need to establish and recognize the field of clinical laboratory science (CLS) as an essential field in advancing clinical goals. Health Sci Rep. 2024;7(8):e70008.

24. Saki N, Haybar H, Aghaei M, Subject. Motivation can be suppressed, but scientific ability cannot and should not be ignored. J Transl Med. 2023;21(1):520.

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