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PRMT1 promotes immune escape in hepatocellular carcinoma by regulating arginine methylation modification of MYC protein

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ABSTRACT

Arginine methyltransferase 1 (PRMT1) is widely recognized as an oncogene in various cancers. However, its specific role and underlying mechanisms in hepatocellular carcinoma (HCC) remain insufficiently understood. This study investigated the function of PRMT1 in HCC development and immune evasion. A comprehensive approach combining database analysis (including TCGA, The Human Protein Atlas, Kaplan-Meier Plotter, and TIMER2.0), molecular techniques (such as RT-qPCR, Western blot analysis, and co-immunoprecipitation), cell-based assays (including MTT, colony formation, transwell, and T cell killing assays), and *in vivo* models was employed to explore PRMT1's role in HCC. The findings revealed a marked upregulation of PRMT1 in both HCC clinical samples and cell lines. Depletion of PRMT1 inhibited cell proliferation and immune evasion while reducing cell migration and invasion. Mechanistically, PRMT1 was shown to interact with MYC, facilitating its arginine methylation and enhancing its protein stability. Moreover, re-expression of MYC significantly reversed the anti-tumour effects associated with PRMT1 depletion. *In vivo* experiments further corroborated these results. Collectively, PRMT1 promotes HCC progression and immune escape by mediating ADMA methylation of MYC, thereby regulating its stability and expression.

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Arginine methylation; PRMT1; MYC; hepatocellular carcinoma; immune escape

Introduction

Hepatocellular carcinoma (HCC) is a prevalent and highly fatal malignancy with a significant incidence and mortality rate [1]. Although the 5-year survival rate for patients with early-stage HCC treated with surgery ranges from 50–80%, the recurrence rate within 5 years can reach as high as 70% [2]. In contrast, the 5-year survival rate for patients with advanced HCC requiring systemic treatment is less than 16% [3]. Despite notable progress in HCC therapies over the past decade, many patients with solid tumours still fail to benefit. This limitation is partly due to the multi-step, heterogeneous nature of HCC development, which involves both genetic and epigenetic modifications driving malignant transformation [4]. Additionally, immune evasion by tumour cells contributes to the challenging prognosis, high recurrence, and early metastasis of HCC, making complete eradication of the disease challenging [5]. With advances in cancer treatment, molecular targeted therapy has emerged as a promising approach for managing advanced HCC [6]. Therefore, a deeper understanding of HCC mechanisms is essential for identifying novel molecular targets for effective treatment.

Arginine methylation, the addition of methyl groups to the nitrogen atom of arginine in peptides, is a widespread post-translational modification (PTM) in eukaryotes, regulating numerous biological processes [7]. Protein arginine methyltransferases (PRMTs) mediate the methylation of arginine residues, playing key roles in maintaining tissue homeostasis and disease phenotypes through processes such as transcription, RNA processing, signal transduction, and DNA damage responses [8,9]. PRMT1, a type I PRMT family member located on chromosome 19q13.3,

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produces NG Asymmetric Dimethylarginine (ADMA) and is encoded by a gene comprising 12 exons and 11 introns [10]. Notably, PRMT1 is highly expressed in various cancers, and its elevated expression correlates with poor prognosis across multiple cancer types [11]. Consequently, the oncogenic potential of PRMT1 has garnered significant attention.

PRMT1 methylates numerous substrates involved in critical biological functions essential for cellular homeostasis. Consequently, dysregulation of PRMT1 activity can contribute to the onset and progression of cancer. Several studies have demonstrated that PRMT1 plays a pivotal role in the progression and prognosis of various solid tumours, with its absence significantly inhibiting the proliferation of cancer cells. For instance, PRMT1 regulates the expression of key genes involved in the cell cycle, RNA metabolism, DNA replication, and DNA damage response in pancreatic tumours, thereby supporting tumour growth [12]. In colorectal cancer (CRC), PRMT1 is highly expressed in clinical specimens and promotes tumour development by mediating the arginine methylation of R251-NONO. Moreover, elevated PRMT1 expression in CRC is associated with poor prognosis in patients with locally advanced disease [13]. In clear cell renal cell carcinoma (ccRCC), upregulated PRMT1 expression correlates with adverse pathological features and poor prognosis. Silencing PRMT1 induces G1 cell cycle arrest and suppresses ccRCC cell growth through the LCN2/p-AKT/p-RB signalling axis [14]. These findings underscore the importance of PRMT1 in cancer progression, suggesting that targeting its key mechanisms may offer potential for developing novel anti-cancer therapies.

Beyond its role in cancer, arginine methylation is a critical modulator of the immune response. PRMT1, as a key regulatory factor in liver tumourigenesis, warrants further investigation, particularly in HCC. PRMT1 deficiency leads to reduced expression of Hnf4α, which accelerates liver cell proliferation and increases liver size by 33% [15]. Preliminary studies have revealed that PRMT1 is upregulated in HCC tissues and cells, promoting tumour growth and metastasis [16]. Furthermore, an increase in PRMT1 expression in clinical HCC samples is associated with poor prognosis and recurrence. PRMT1 facilitates HCC progression and metastasis both *in vitro* and *in vivo* through activation of the STAT3 signalling pathway [17]. However, the precise mechanisms underlying PRMT1's role in HCC development remain inadequately explored and require further investigation.

This study elucidated the role and mechanisms of PRMT1 in HCC progression and immune escape. PRMT1 was highly expressed in HCC, with a significant correlation with patient prognosis. Silencing PRMT1 significantly inhibited HCC cell proliferation, migration, invasion, and immune evasion. Mechanistic analysis revealed that PRMT1 mediates the ADMA methylation of MYC, regulating its protein expression and stability, thereby accelerating HCC progression and immune escape. Thus, PRMT1 represents a promising prognostic marker and therapeutic target for HCC.

Materials and methods

Database analysis

PRMT1 expression in liver cancer and its association with staging were analysed using the TCGA database. The HUMAN PROTEIN ATLAS database was utilized to evaluate PRMT1 protein expression in both tumour and normal tissues. The Kaplan-Meier Plotter database assessed the relationship between PRMT1 levels and prognosis. The TIMER2.0 database was employed to explore the correlation between PRMT1 expression and the infiltration of four types of tumour-infiltrating immune cells (TIICs) in liver cancer. Additionally, the CAMOIP database's immune infiltration module was used to further evaluate the correlation between immune cell infiltration and PRMT1 expression.

Clinical sample collection

A total of 20 pairs of human HCC tissues and corresponding adjacent non-tumour tissue samples were collected from patients undergoing surgery at the North China University of Science and Technology Affiliated Hospital. All samples were obtained with informed written consent from the patients, and the use of human specimens was

approved by the hospital's Institutional Ethics Committee.

Cell culture

THLE-2, Hep3B, and Huh7 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, under standard conditions (37°C and 5% CO_2). All cell lines were purchased from the typical culture bank of the Chinese Academy of Sciences (Shanghai).

Cell transfection

The transfection reagents, including various sequences and vectors, were constructed by RiboBio (Guangzhou).

PRMT1 knockdown and MYC overexpression cells were generated using siRNA (siPRMT1 1#: GGCACCUCUUCAAGGACAAGG; siPRMT1 2#: GCCTACTTCAACATCGAGT) or plasmid vectors. For transfection, cells were seeded in six-well plates at appropriate densities. siRNA or vectors were transfected into HCC cells using Lipofectamine 3000 (Invitrogen) according to the manufacturer's instructions. After 48 hours, transfection efficiency was evaluated.

Stable PRMT1 knockdown cells were established using lentiviral vectors containing the shPRMT1 sequence (shPRMT1 1# TOP: GCAACTCCATGTTTCATAA, BOTTOM: TTATGAAACATGGAGTTGC; shPRMT1 2# TOP: CCATCGACCTGGACTTCAA, BOTTOM: TTGAAGTCCAGGTCGATGG). Lentivirus was produced by co-transfecting HEK293T cells with expression and packaging plasmids (psPAX2 and pMD2.G). After 48 hours, lentivirus was collected and used to infect HCC cells. Stable transfected cells were selected for 15 days using 2 μg/ml puromycin.

RT-qPCR

Total RNA was extracted from cells and tissues using the Qiagen RNeasy mini kit (Qiagen) and reverse transcribed into cDNA with the iScript cDNA synthesis kit (Bio-Rad). PCR amplification was performed using specific primers (PRMT1-F: 5'-CTTTGACTCCTACGCACACTT-3,' PRMT1-R: 5'-GTGCCGGTTATGAAACATGGA-3;' MYC-F: 5'-GGATTCCCGCCTCAGAATAAC-3,' MYC-R: 5'-GTGGGTGTGGGTTGTTCAGG-3;' GAPDH-F: 5'-GAAGGTGAAGGTCGGAG-3;' GAPDH-R: 5'-GAAGATGGTGATGGGATTTC-3') and Real SYBR Mixture (CoWin Bioscience) on an ABI PRISM 7500 instrument (Thermo Fisher, USA). Gene expression was quantified using the $2^{-\Delta\Delta Ct}$ method, with GAPDH as the reference.

Western blotting and co-immunoprecipitation (Co-IP)

Total protein lysates were extracted from cells using RIPA buffer, supplemented with protease and phosphatase inhibitors. After quantification, equal amounts of protein were separated by SDS-PAGE and transferred to a PVDF membrane (Millipore). The membrane was blocked with 3% BSA and incubated overnight at 4°C with anti-PRMT1, anti-MYC, anti-ADMA, or anti-GAPDH antibodies. The membrane was then incubated with a horseradish peroxidase-conjugated secondary antibody (Abcam), and protein bands were visualized using enhanced chemiluminescence reagent (Millipore) in an imaging system.

For immunoprecipitation, 0.5 to 1 mg of cell lysate was incubated overnight at 4°C with anti-PRMT1 or anti-MYC antibody. Protein G agarose beads (Roche, Switzerland) were added, and incubation continued for an additional 4 hours. The protein-antibody complex bound to the beads was eluted three times using cell lysis buffer. Standard Western blotting techniques were used to detect associated binding proteins.

MTT assay

Cell proliferation was assessed using an MTT assay. Cells were seeded in 96-well plates, and at 0, 24, 48, and 72 hours, 20 μL of MTT (Sigma, 5 mg/mL) was added to each well. After 4 hours of incubation, 200 μL of DMSO (Solarbio) was added to dissolve the formazan crystals, and optical density (OD) was measured at 490 nm.

Cell colony assay

Colony formation assays were performed to evaluate the clonogenic ability of cells. A total of 1000 cells were plated onto 6 cm plates and incubated at 37°C for 14 days. When visible colonies formed, cells were washed twice with PBS, fixed with 4% paraformaldehyde, and stained with crystal violet. Colonies were imaged and quantified using a microscope (Olympus).

Transwell assay

Cell migration and invasion were evaluated using a transwell chamber (8.0 μm pore size, Corning) with or without Matrigel (BD Biosciences). The serum-free medium containing cell suspensions was inoculated into the upper chamber at a density of 5×10^4. The lower chamber was filled with medium supplemented with 10% FBS to serve as a chemoattractant. After 24 hours of incubation, cells that did not migrate or invade into the lower chamber were removed with a cotton swab. Migrated or invaded cells were then fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Leagene). Cells were subsequently imaged and quantified in randomly selected fields of view using a microscope.

T-cell killing assay

For T cell cytotoxicity assays, tumour cells were co-cultured with T cells in 12-well plates for 24 hours. The supernatant was collected, and lactate dehydrogenase (LDH) activity was measured using the LDH Assay Kit (Beyotime) to quantify the killing effect of T cells on tumour cells.

Cycloheximide (CHX) treatment

To assess the effect of PRMT1 silencing on MYC protein stability, cells were treated with 100 μg/mL cycloheximide (Sigma Aldrich) for 0, 25, 50, 75, and 100 minutes. Protein was extracted using RIPA buffer at each specified time point. MYC protein stability was evaluated through standard western blotting procedures.

Animal experiments

All animal experiments were approved by the North China University of Science and Technology Affiliated Hospital Institutional Animal Care and Use Committee and conducted in compliance with animal care guidelines. Female BALB/c nude mice (6–8 weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. After one week of acclimation, the mice were randomly divided into two groups of six mice each. Hep3B cells transfected with shNC or shPRMT1 (5×10^6) were subcutaneously injected into the right flank of the mice. Tumour volume was measured every 4 days using the formula: V (mm^3) = 1/2 × long diameter (L) × short diameter $(W)^2$. After 4 weeks, the mice were euthanized, and tumours were collected and weighed. The SignalStay Boost IHC kit (Cell Signalling Technology) was used to assess the expression of MYC and Ki67 in tumour tissues.

Statistical analysis

Experimental data are presented as mean ± SEM and analysed using GraphPad Prism 8 software. Differences between two groups were compared using a Student's t-test. A p-value of < 0.05 was considered statistically significant.

Results

PRMT1 is highly expressed in HCC

Epigenetic alterations are implicated in the progression of various diseases, with arginine methylation being a common PTM of proteins. Dysregulated expression of PRMT1 has been linked to the development of several cancers. To further investigate the role of PRMT1 in HCC, PRMT1 expression was analysed in HCC using the TCGA database. The results showed a significant upregulation of PRMT1 in HCC (Figure 1a). Analysis of its relationship with patient staging revealed that higher PRMT1 levels were associated with advanced stages of the disease (Figure 1b). Additionally, analysis of PRMT1 protein expression in tumour tissues from patients with liver cancer *via* the database confirmed a substantial increase in PRMT1 expression

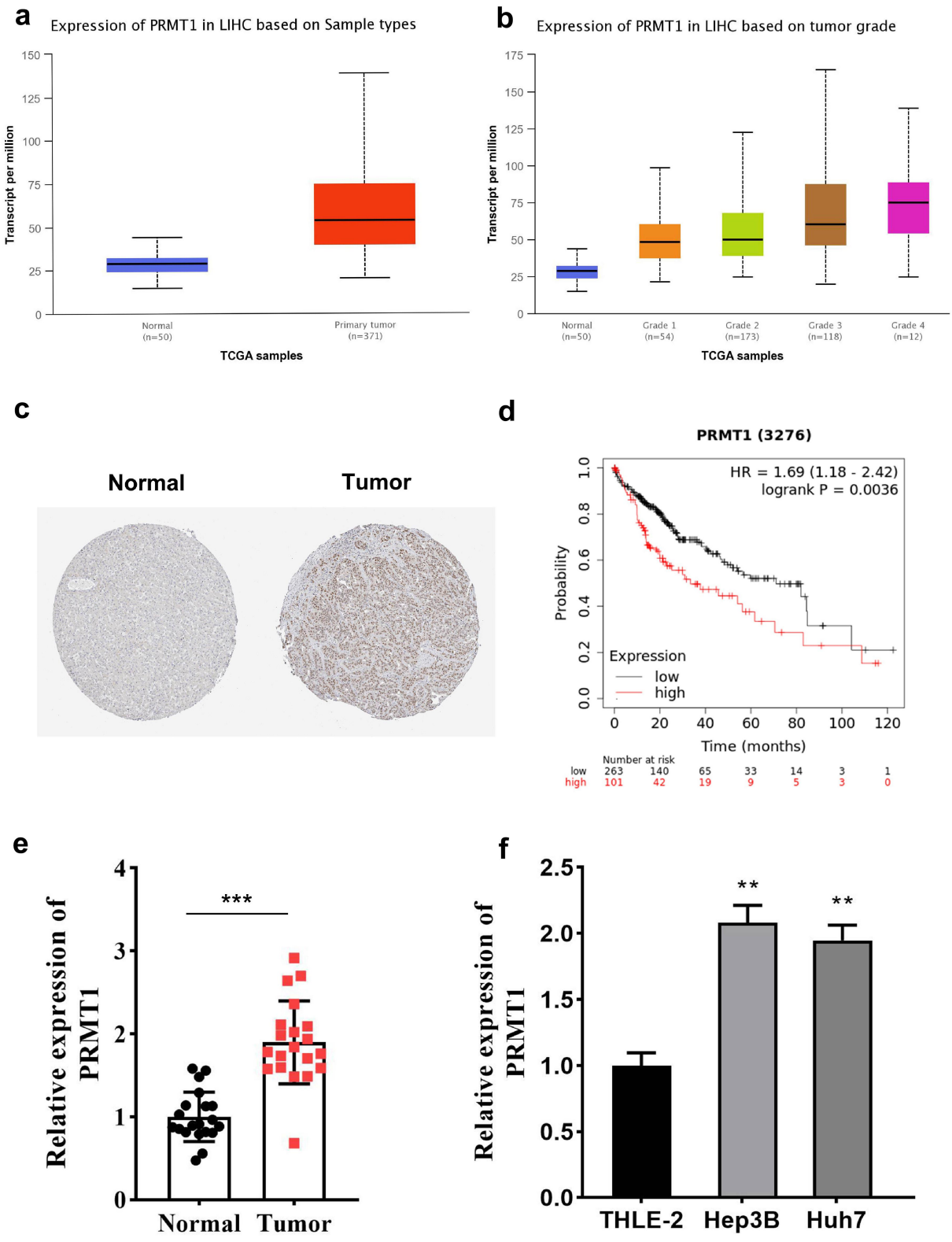

Figure 1. Expression of PRMT1 in HCC and its relationship with prognosis. (a). TCGA analysis showing PRMT1 expression in liver cancer. (b). TCGA analysis correlating PRMT1 expression with the stage of patients with liver cancer. (c). Human protein atlas database analysis of PRMT1 protein expression in liver cancer and normal tissues. (d). Kaplan-Meier Plotter analysis of PRMT1 expression and its association with prognosis. (e). Measurement of PRMT1 expression in clinical tissue samples from patients with HCC. (f). Examination of PRMT1 expression in normal hepatocytes and HCC cells.

(Figure 1c). Kaplan-Meier survival analysis revealed that patients with elevated PRMT1 levels had markedly lower survival rates compared to those with lower PRMT1 expression (Figure 1d). To further validate these findings, clinical samples were collected, confirming a significant increase in PRMT1 expression in HCC tumour tissues (Figure 1e). Additionally, upregulation of PRMT1 expression was observed in HCC cell lines, including Hep3B and Huh7 (Figure 1f). These results collectively suggest that PRMT1 is highly expressed in HCC and may play a role in the malignant progression of the disease.

PRMT1 deficiency restrains the malignant process in HCC cells

To elucidate the biological function of PRMT1 in HCC, loss-of-function experiments were conducted. siPRMT1#1 and siPRMT1#2 were used to silence PRMT1 expression in HCC cells, and knockdown efficiency was confirmed by RT-qPCR and Western blot analysis. Both mRNA (Figure 2a) and protein (Figure 2b) levels of PRMT1 were significantly reduced in Hep3B and Huh7 cells. Subsequently, the impact of PRMT1 silencing on cell growth, migration, and invasion was assessed. MTT and colony formation assays demonstrated that PRMT1 knockdown significantly inhibited cell proliferation (Figure 2c) and colony formation ability (Figure 2d) in both Hep3B and Huh7 cells. Moreover, transwell assays showed that PRMT1 depletion notably suppressed cell migration (Figure 2e) and invasion (Figure 2f). Conversely, stable overexpression of PRMT1 promoted cell growth, migration, and invasion in HCC cells (Additional file 1). These results collectively indicate that PRMT1 is involved in the malignant progression of Hep3B and Huh7 cells.

Loss of PRMT1 arrests immune escape in HCC cells

Recent studies have highlighted immune escape as a crucial adaptive mechanism by which tumours evade detection by the body's immune system, largely driven by epigenetic regulatory mechanisms within tumour cells [18]. However, further investigation is required to determine whether PRMT1 plays a role in regulating immune escape in HCC. To explore the relationship between PRMT1 expression and immune cell infiltration, the CAMOIP database was utilized. The analysis revealed that PRMT1 expression was correlated with the infiltration of various immune cells, including B cells, CD4+ T cells, CD8+ T cells, macrophages, natural killer (NK) cells, regulatory T cells (Tregs), and neutrophils. Among these, macrophages and Tregs exhibited the strongest association with PRMT1 expression (Figure 3a). Furthermore, the link between PRMT1 expression and immune subtypes was assessed, indicating that low PRMT1 expression was associated with enhanced immune responses and lymphocyte activation (Figure 3b). Additionally, the prognostic significance of PRMT1 expression and immune cell infiltration was investigated in HCC. The results indicated that low PRMT1 expression combined with high CD8+ T cell infiltration was associated with significantly improved survival rates in patients with HCC (Figure 3c). The TIMER 2.0 database further confirmed that PRMT1 expression in HCC was negatively correlated with the infiltration of immune cells, including B cells, CD8+ T cells, macrophages, and NK cells (Figure 3d-g). Collectively, these results suggest that PRMT1 plays a critical role in shaping the tumour immune microenvironment in HCC. To further investigate the role of PRMT1 in immune evasion, HCC cells transfected with si-PRMT1, oe-PRMT1, or control constructs were co-cultured with activated CD8+ T cells, and T cell-mediated cytotoxicity was assessed using an LDH release assay. The results revealed that PRMT1-deficient Hep3B and Huh7 cells showed significantly increased LDH activity, indicating enhanced T cell-mediated cytotoxicity and reduced immune suppression compared to control cells (Figure 4a). In contrast, overexpression of PRMT1 in tumour cells exacerbated immune suppression and reduced T cell-mediated killing (Figure 4b). These results strongly suggest that PRMT1 is involved in the regulation of immune evasion in HCC cells.

PRMT1 mediates protein stability of MYC through arginine methylation

To elucidate the mechanisms underlying PRMT1's regulation of cell proliferation and metastasis, genes and signalling pathways linked to PRMT1

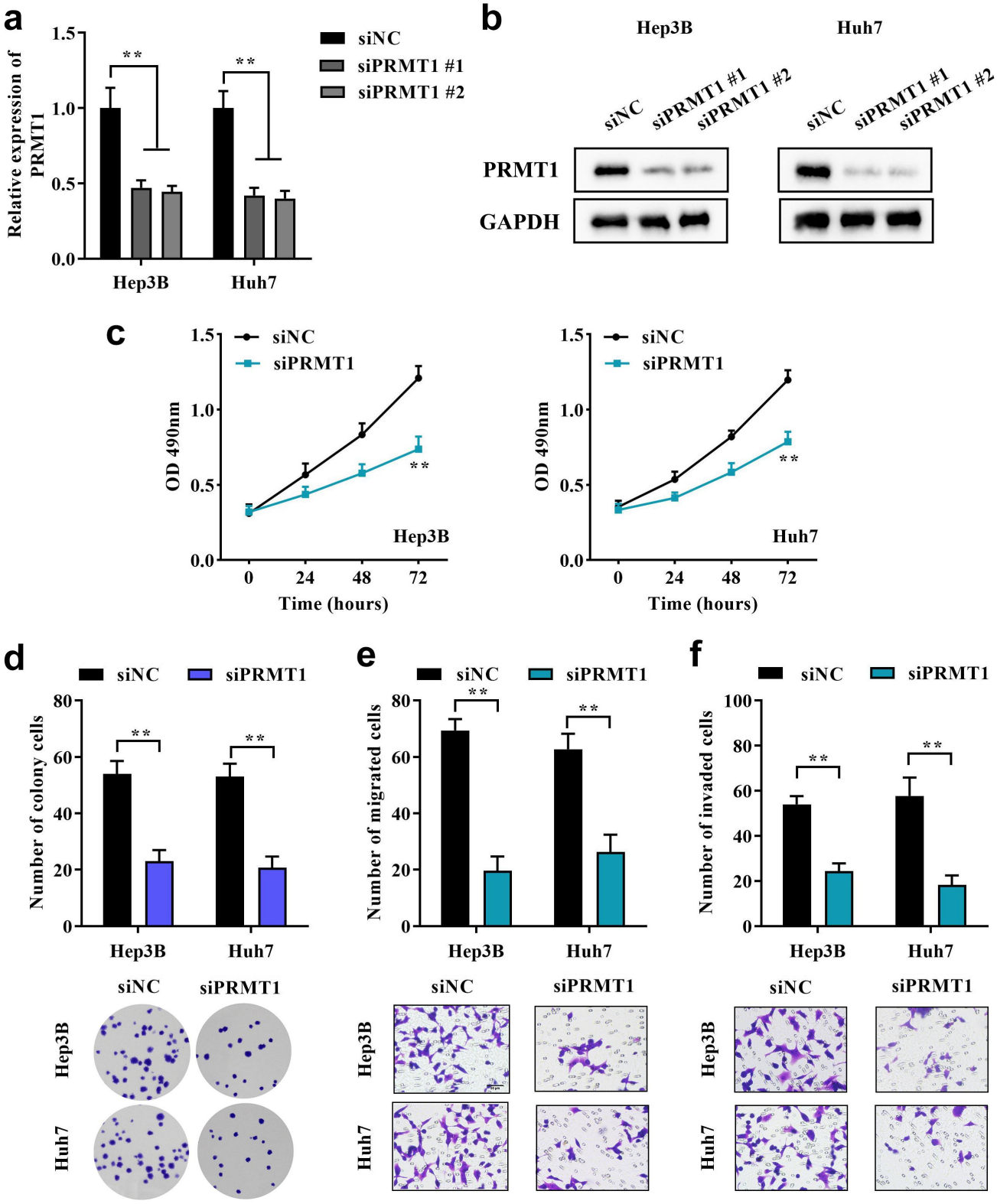

Figure 2. Knockdown of PRMT1 inhibited the proliferation, migration, and invasion of HCC cells. (a). RT-qPCR analysis of transfection efficiency. (b). Western blot analysis of protein expression after PRMT1 knockdown. (c). CCK-8 assay to evaluate cell viability. (d). Colony formation assay to assess cell proliferation. (e). Transwell assay for cell migration. (f). Transwell assay for cell invasion.

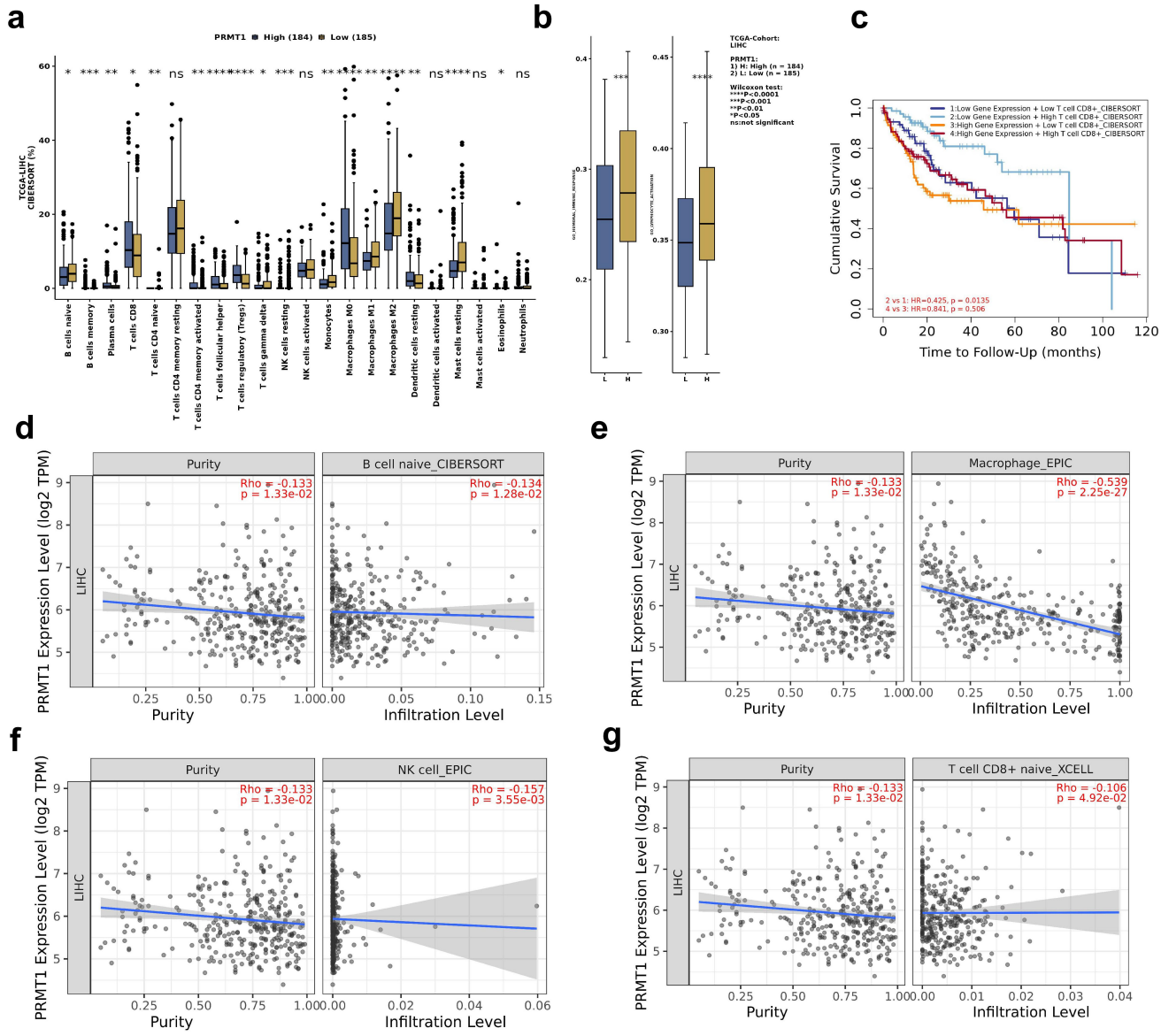

Figure 3. Correlation between PRMT1 and tumour-infiltrating immune cells in HCC. (a). Correlation between PRMT1 expression and immune infiltration in HCC. (b). Correlation between PRMT1 expression and the abundance of immune cell subtypes. (c). Kaplan-Meier survival curves of patients with HCC exhibiting high or low CD8 levels. (d-g). Correlation analysis of PRMT1 with B cells, CD8+ T cells, macrophages, and natural killer (NK) cells in liver cancer using the TIMER2.0 database.

expression were screened. Notably, PRMT1 regulates MYC protein levels by enhancing transcription and stabilizing the protein [19]. Based on these findings, analysis using the GEPIA2.0 database revealed a positive correlation between PRMT1 expression and MYC, along with its downstream genes, in HCC (Additional file 2). Furthermore, patients with HCC exhibited elevated MYC expression compared to non-HCC cases (Additional file 2), indicating that the PRMT1-MYC axis may play a pivotal role in HCC.

MYC, a proto-oncogene implicated in tumourigenesis, is involved in critical pathways regulating cell growth and survival. This study aimed to determine whether MYC is regulated by PRMT1-dependent arginine methylation in HCC [20]. Co-immunoprecipitation (Co-IP) experiments first confirmed the direct binding of PRMT1 to MYC in Hep3B and Huh7 cells. After immunoprecipitation of PRMT1, MYC protein was detected in both cell lines *via* Western blot analysis, indicating that PRMT1 interacts with endogenous MYC

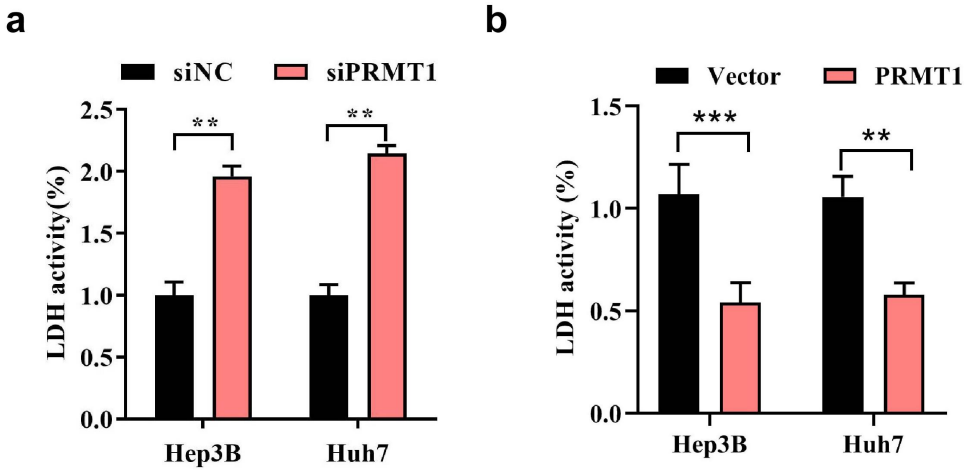

Figure 4. T cell killing assay.

(Figure 5a). Previous studies have established PRMT1 as the primary arginine methyltransferase responsible for ADMA formation. To investigate whether PRMT1 mediates MYC arginine methylation, MYC was extracted from the two HCC cell lines and analysed for methylation using ADMA antibodies. The appearance of MYC ADMA signals in both cell lines (Figure 5b) confirmed that PRMT1 methylates MYC. To further explore the role of PRMT1, two shPRMT1 constructs

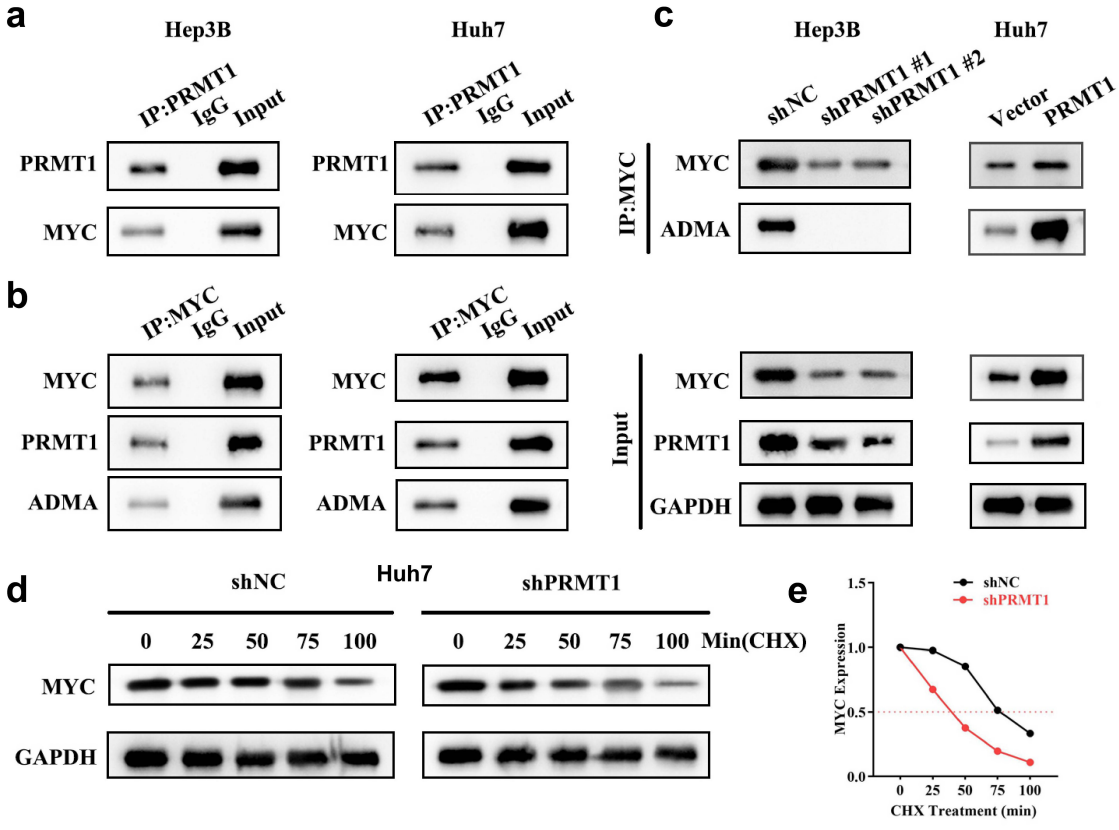

Figure 5. PRMT1 affects MYC expression and protein stability through arginine methylation modification. (a). Immunoprecipitation of PRMT1 protein from Hep3B and Huh7 cells, followed by Western blot analysis to detect its interaction with MYC. (b). Immunoprecipitation of MYC protein from Hep3B and Huh7 cells, followed by Western blot analysis to assess its interaction with PRMT1 and ADMA signaling. (c). Immunoprecipitation of MYC protein from stable HCC cells, followed by Western blot analysis to measure ADMA levels after PRMT1 knockdown or overexpression. (d-e). CHX treatment to assess the stability of MYC protein.

(shPRMT1 #1 and shPRMT1 #2) were used to silence PRMT1 expression in Hep3B cells. Silencing PRMT1 resulted in decreased endogenous MYC ADMA levels, while PRMT1 overexpression increased the MYC ADMA signal in cell lysates (Figure 5c). A search for potential PRMT1-mediated methylation sites identified two arginine residues (R299 and R346) (Additional file 3). To assess their roles in PRMT1-mediated methylation, R-to-K MYC mutants were co-expressed with PRMT1 in HEK293T cells, and ADMA levels were measured. No significant changes in ADMA levels were observed in the R299K mutant, but a reduction in ADMA levels was detected in the R346K mutant (Additional file 3). To further investigate the impact of R346 on MYC protein expression, plasmids expressing MYC-WT, MYC-R346K, and a PRMT1 overexpression vector were transfected into MYC knockdown HCC cells, and Western blot analysis was performed. The results demonstrated that the R346K mutation reduced the ability of PRMT1 to enhance MYC expression, suggesting that MYC-WT is more likely to be targeted and asymmetrically dimethylated by PRMT1 compared to MYC-R346K mutants (Additional file 3). Finally, to assess the effect of PRMT1 on MYC protein stability, CHX treatment was applied. The results confirmed that MYC expression was significantly reduced in PRMT1-deficient cells compared to control cells as the treatment time increased (Figure 5d,e). Collectively, these results indicate that PRMT1 regulates MYC expression and stability through arginine methylation.

Inadequacy of PRMT1 represses malignant processes and immune escape in HCC cells through MYC

The aforementioned results confirmed a positive correlation between PRMT1 and MYC expression. To further investigate the role of the PRMT1-MYC axis in HCC progression and immune escape, rescue experiments were conducted. MYC was successfully overexpressed in Hep3B and Huh7 cells through transfection (Figure 6a,b). Following co-transfection of siPRMT1 and the MYC vector, a series of functional assays were performed. Notably, cell proliferation (Figure 6c) and colony formation (Figure 6d) were significantly enhanced in MYC-overexpressing cells compared to those with PRMT1 silencing alone. Similarly, the number of migrating (Figure 6e) and invading (Figure 6f) cells was markedly increased in cells co-transfected with siPRMT1 and the MYC vector. Moreover, immune escape was notably augmented in cells overexpressing MYC (Figure 6g). These results confirm that PRMT1 regulates the proliferation, migration, invasion, and immune escape of HCC cells through MYC.

Inadequacy of PRMT1 limits tumour formation in nude mice in vivo

Building on these *in vitro* results, the role of PRMT1 in HCC progression and immune escape was further explored using *in vivo* models. Stable PRMT1 knockdown cells were subcutaneously injected into nude mice. Tumour growth monitoring revealed that PRMT1 silencing significantly inhibited tumour growth, as evidenced by smaller tumour volume and lighter tumour weight (Figure 7a-c). Additionally, immunohistochemistry (IHC) analysis demonstrated reduced expression of MYC (Figure 7d) and Ki67 (Figure 7e) in PRMT1-deficient xenograft tumours. Together, these data suggest that PRMT1 regulates HCC cell growth *via* MYC.

Discussion

HCC is one of the most prevalent cancers and a leading cause of global mortality [21]. Extensive evidence suggests that poor prognosis and low five-year survival rates in cancer are linked to the aberrant expression of specific genes and the activation of key signalling pathways. Despite significant advances in HCC diagnosis and treatment in recent decades, prognosis remains disappointing due to an incomplete understanding of its pathogenesis [22]. In this context, targeted therapies and immunotherapies have introduced new treatment possibilities, but patients with advanced HCC continue to face substantial therapeutic challenges [23]. Thus, in-depth investigation of the mechanisms underlying HCC development is crucial for identifying new potential therapeutic targets to improve clinical outcomes.

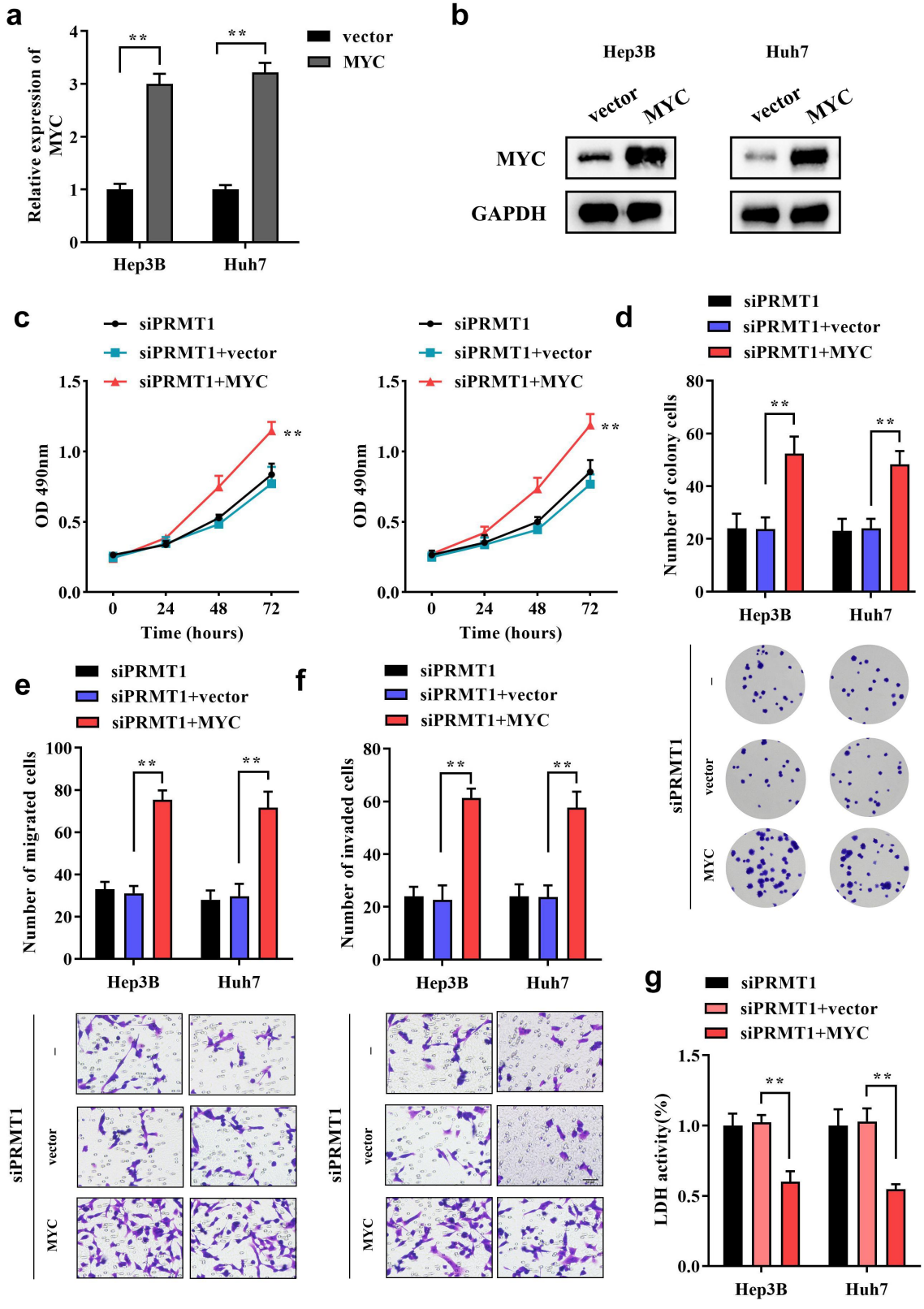

Figure 6. PRMT1 affects the proliferation, migration, invasion, and immune escape of HCC cells through MYC. (a). RT-qPCR analysis to assess transfection efficiency. (b). Western blot analysis of MYC protein expression. (c). CCK-8 assay to evaluate cell viability. (d). Colony formation assay to assess cell proliferation. (e). Transwell migration assay. (f). Transwell invasion assay. (g). T cell killing assay.

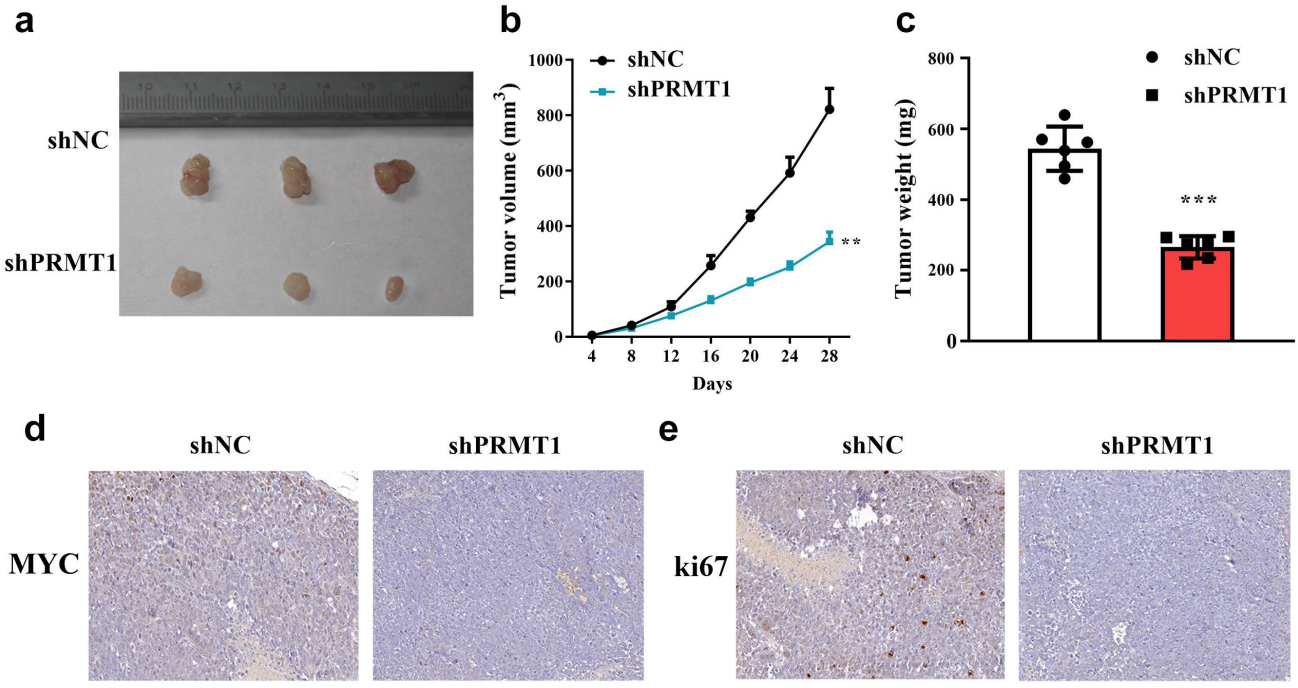

Figure 7. PRMT1 affects xenograft tumour formation *in vivo*. (a). Representative image of the tumour. (b-c). Measurement of tumour volume and weight. (d). IHC staining to assess MYC protein expression. (e). IHC staining to evaluate Ki67 protein expression.

PRMTs have emerged as promising therapeutic targets for anti-cancer therapies [24]. As a major type I PRMT, PRMT1 is frequently overexpressed in various cancers, where it regulates signalling pathways and protein-protein interactions by depositing ADMA modifications on different substrates. PRMT1-mediated ADMA modifications are involved in numerous cellular functions and physiological/pathological conditions, including cancer-related processes such as tumourigenesis, metastasis, proliferation, survival, and differentiation [25]. For instance, PRMT1 catalyses the methylation of FLT3-ITD at arginine 972/973, promoting leukaemia cell growth in an FLT3 methylation-dependent manner [26]. In breast cancer, PRMT1 levels are elevated, where it represses EZH2 expression through methylation of R342 on EZH2, thereby enhancing epithelial-to-mesenchymal transition (EMT), invasion, and metastasis [27]. Furthermore, R342-EZH2 methylation promotes cell cycle progression by inhibiting the transcription of P16 and P21 [28]. PRMT1-mediated R236-PHGDH methylation also enhances serine biosynthesis, mitigates oxidative stress, and promotes HCC growth both *in vitro* and *in vivo* [29]. These studies underscore the critical role of arginine methylation dysregulation in cancer progression and highlight PRMT1 as a promising target. However, research on PRMT1 in HCC remains limited, and further detailed exploration of its underlying mechanisms is urgently needed.

Consistent with previous studies, database and clinical analyses in this study confirmed that PRMT1 expression is significantly elevated in HCC, and higher expression levels correlate with poorer patient prognosis. Functional assays demonstrated that PRMT1 loss not only restricted cell proliferation but also reduced cell migration and invasion. Moreover, PRMT1 has been identified as an immunomodulator involved in immune escape in various cancers. For instance, PRMT1 inhibits the cGAS/STING pathway through R133-cGAS methylation, promoting PD-L1 expression and facilitating immune escape [30]. Additionally, PRMT1 prevents necrotic apoptosis in CRC *via* R486-RIP3 methylation, thereby enhancing immune escape in colon cancer [31]. PRMT1 also plays a key role in modulating hepatic immune responses in HCC, with targets including TRAF6, NF-κB, PPARγ, c-Myc, STAT3, FOXOs, and β-catenin, all of which are involved in

regulating immune cells [32]. These findings underscore the potential of PRMT1 as a target for cancer immunotherapy. In line with these reports, our study found a significant correlation between PRMT1 expression and the abundance of TIICs such as CD8+ T cells, macrophages, NK cells, and Tregs. Furthermore, PRMT1 silencing diminished immune escape in HCC cells. However, the precise mechanisms by which PRMT1 mediates immune escape in HCC require further investigation.

The MYC gene, located on chromosome 8q24.21, is one of the most frequently dysregulated driver genes in human cancers, including HCC, where MYC is overexpressed in more than 50% of cases [33]. c-Myc overexpression is commonly observed in HCC, and its amplification is strongly linked to more aggressive malignancy and worse prognosis [34]. PTMs of MYC are critical for regulating its biological functions and protein stability [35]. For example, MYC promotes WDR4 transcription, and high WDR4 levels increase m7G methylation, subsequently enhancing CCNB1 expression in HCC, thereby regulating EMT, cell cycle, and apoptosis [36]. Additionally, lncRNA PVT1 enhances the proliferation, migration, and invasion of hepatitis B virus-positive HCC cells by modulating histone methylation at the c-Myc promoter [37]. SMYD2 inhibits the interaction between c-Myc and FBW7 by methylating c-Myc, thus reprogramming glutamine metabolism *via* the c-Myc/GLS1 axis and promoting HCC cell proliferation [38]. However, the role of arginine methylation in the regulation of MYC and its impact on HCC progression remain poorly understood. Notably, our study revealed that PRMT1 mediates the arginine methylation of MYC, regulating both its expression levels and protein stability. Furthermore, PRMT1 silencing hindered the malignant progression and immune escape of HCC cells through MYC. These findings were further corroborated by *in vivo* data.

The findings presented above have raised several important questions that warrant further investigation. Arginine methylation regulates various signal transduction pathways, and more research is needed to fully elucidate the molecular mechanisms by which PRMT1-mediated methylation influences MYC function. Although PRMT1 expression correlates with immune cell infiltration within the tumour microenvironment, its potential as a target for immunotherapy in HCC warrants further exploration in future studies. Previous research has shown that the PRMT1-MYC signalling axis contributes to olaparib resistance [19], suggesting that the role of MYC methylation in chemotherapy resistance should be further examined. The significance of the PRMT1-MYC network in drug resistance and the development of targeted treatment strategies also requires additional investigation.

Conclusion

In conclusion, this study confirmed that PRMT1 is significantly upregulated in HCC. Loss of PRMT1 impedes malignant cellular processes and immune escape by mediating arginine methylation of MYC. Moreover, elevated PRMT1 levels are strongly associated with poorer prognosis in patients with HCC, positioning PRMT1 as a potential molecular marker for prognosis and a promising therapeutic target.

Author contributions

Han Zhou designed the study and drafted the manuscript. Yang Wang collected and processed data, performed statistical analysis, and conducted the experiments. Dan Wang analysed and interpreted the data. Mei Zhang and Kaidi Wang assisted with data collection and contributed to the experimental work. Chunhui Liu supervised and revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Data availability statement

The datasets used in the current study are available from the corresponding authors upon reasonable request.

Ethics statement

This study was approved by the Ethics Committee of North China University of Science and Technology Affiliated

Hospital. All participants provided written informed consent at the time of recruitment, and all experiments involving human tissue specimens were conducted in accordance with the Declaration of Helsinki. Animal studies were performed in compliance with the ARRIVE guidelines.

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