Protein arginine methyltransferase 5 is implicated in the aggressiveness of human hepatocellular carcinoma and controls the invasive activity of cancer cells

JU-YEON JEON[1,7*], JEE SAN LEE[1*], EUN-RAN PARK[1], YAN NAN SHEN[1,6], MI-YEUN KIM[1], HYUN-JIN SHIN[1], HYUN-YOO JOO[1], EUNG-HO CHO[2], SUN MI MOON[2], UI SUP SHIN[2], SUN HOO PARK[3], CHUL JU HAN[4], DONG WOOK CHOI[5], MAN BOCK GU[7], SANG-BUM KIM[2] and KEE-HO LEE[1,8]

[1]Division of Radiation Cancer Research; Departments of [2]Surgery, [3]Pathology and [4]Internal Medicine, Korea Institute of Radiological and Medical Sciences, Seoul 01812; [5]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Republic of Korea; [6]Key Laboratory of Radiobiology, School of Public Health, Jilin University, Changchun, Jilin 130012, P.R. China; [7]Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841; [8]Department of Biotechnology, College of Natural Sciences, Seoul Women's University, Seoul 01797, Republic of Korea

Received November 13, 2017; Accepted April 3, 2018

DOI: 10.3892/or.2018.6402

Abstract. Protein arginine methyltransferase 5 (PRMT5) is a protein that catalyzes transfer of methyl groups to the arginine residues of proteins and is involved in diverse cellular and biological responses. While the participation of PRMT5 in cancer progression has been increasingly documented, its association with the invasive phenotype currently remains poorly understood. In the present study, we revealed that PRMT5 is overexpressed in human hepatocellular carcinoma (HCC) and in colon cancer and its depletion leads to the suppression of cell invasive activity via the reduction of the expression of MMP-2. Real-time quantitative RT-PCR analysis of 120 HCC patient tissues revealed the overexpression of PRMT5 in HCC and the association of PRMT5 with aggressive clinicopathological parameters, such as poorer differentiation (P=0.004), more frequent hepatic vein invasion (P=0.019), larger tumor size (P=0.011) and higher α-fetoprotein levels (P=0.020). Similarly to the data obtained with HCC, overexpression of PRMT5 was also displayed in colon cancer tissues, compared to matched non-tumor regions. Consistent with the significant association of the overexpression of PRMT5 with hepatic vein invasion in patient specimens, PRMT5 depletion via siRNA transfection led to a marked reduction in the invasion rate in both HCC and colon cancer cells. Reduced invasion associated with PRMT5 depletion was accompanied by a decrease in the expression of MMP-2. Collectively, our results indicated that PRMT5 overexpression in HCC and colon cancer cells contributed to their acquisition of aggressive characteristics, such as invasiveness, thus presenting a promising therapeutic target for the treatment of these diseases.

Correspondence to: Dr Kee-Ho Lee, Division of Radiation Cancer Research, Korea Institute of Radiological and Medical Sciences, 215-4 Gongneung-Dong, Nowon-Ku, Seoul 01812, Republic of Korea
E-mail: khlee@kirams.re.kr

Dr Sang-Bum Kim, Department of Surgery, Korea Institute of Radiological and Medical Sciences, 215-4 Gongneung-Dong, Nowon-Ku, Seoul 01812, Republic of Korea
E-mail: lull2@kirams.re.kr

*Contributed equally

Abbreviations: PRMT5, protein arginine methyltransferase 5; HCC, human hepatocellular carcinoma; MMP, matrix metalloproteinase; AFP, α-fetoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase

Key words: PRMT5, hepatocellular carcinoma, colon cancer, invasion, α-fetoprotein, differentiation

Introduction

Hepatocellular carcinoma (HCC) is the sixth major type of cancer and the third most common cause of cancer-related deaths worldwide, mainly associated with hepatitis B and C virus infections (1). The global incidence of HCC is increasing, with surgical resection and liver transplantation being the current major treatment strategies in patients diagnosed in the early stages (2). The overall survival rate of patients diagnosed with HCC is <10% due to high recurrence and metastasis rates and failure of early detection. While the cellular and molecular mechanisms underlying HCC have been extensively studied over the last decades, the pathogenesis process of this type of tumor remains poorly understood.

PRMT5, a type II protein arginine methyltransferase, is a 72 kDa protein that catalyzes methylation of arginine residues in various substrate proteins, including chromatin-associated proteins and transcriptional factors (3). The function of PRMT5 as a methyltransferase has been extensively studied

in relation to cancer. PRMT5 acts as cancer-inducing gene by promoting cell proliferation (4-8), inhibiting transcription of tumor suppressor genes (4,9-13) and inducing metastatic predisposition via epithelial-mesenchymal transition (14). The symmetric dimethylation of the arginine residues of histones H3 and H4 by PRMT5 triggers the modification of the chromatin structure and alterations in the expression patterns of diverse genes (3,15-17). Additionally, PRMT5 induces transcriptional inhibition by directly methylating the tumor suppressor proteins p53 and E2F1, thus bestowing advantages for cancer cell survival (9,13). Recent studies have demonstrated that PRMT5 suppresses the expression of E-cadherin, the hallmark of EMT transition, through interactions with Ajuba and the E-cadherin transcription factor Snail (6,14).

PRMT5 has been extensively characterized in relation to various types of cancer and its emerging role as a potential oncoprotein is of significant clinical interest. However, the expression of PRMT5 in relation to invasion phenotypes in HCC and colon cancer has rarely been documented. Earlier invasion studies using siRNAs were conducted under conditions of decreased proliferation and increased cell death, which potentially contributed to the decrease in the invasion activity of cancer cells (17-20). Using HCC microarray datasets, we have evaluated several molecular markers differentially expressed in HCC (21-23). In the present study, we revealed that PRMT5 was overexpressed in HCC and colorectal cancer tissues and its depletion suppressed the invasion of cancer cell lines without affecting the colony survival. Consistent with its correlation with the invasive phenotype, the overexpression of PRMT5 in HCC patient tissues was associated with aggressive clinicopathological parameters, including poorer differentiation and greater invasion, tumor size and α-fetoprotein level.

Materials and methods

Patients and tissue samples. HCC and colon cancer tissues were collected from surgically resected patient specimens who underwent surgery between April 1992 and December 2004, and between March 2014 and August 2014, respectively, at the Korea Cancer Center Hospital. A total of 120 HCC (including 33 pair-matched samples) and 10 pair-matched colon cancer samples were used. This study was approved by the Institutional Review Board, Korea Cancer Center Hospital. Written informed patient consent was either waived for HCC or obtained for colon cancer tissues.

Cell culture and gene silencing via siRNA transfection. Huh7 (from the Japanese Cancer Research Resources Bank), SW480 (from the American Type Culture Collection) and SNU-709 cells (from the Korean Cell Line Bank) were cultured in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (w/v) antibiotics. The cells were transfected with control or PRMT5 siRNA at a concentration of 10 nM using Lipofectamine RNAiMAX reagent (13778-150; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. The following human PRMT5 siRNA oligonucleotide sequences were used: PRMT5 siRNA#1, 5'-UGUGACUAUAGUAAGAG GAUUGCAGUG-3' and PRMT5 siRNA#2, 5'-AGGGACUGG AAUACGCUAAUUGUGGGA-3'.

RNA extraction and cDNA synthesis. Total RNA was extracted from cells using the RNeasy Mini kit (cat. no. 304-150; GeneAll Biotechnology Co., Ltd., Seoul, Korea). The concentration and quality of total RNA were assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 260 and 280 nm. For cDNA synthesis, total RNA was reverse-transcribed using the iScript cDNA synthesis kit (cat. no. 170-8891; Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol.

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Semi-quantitative RT-PCR was performed using the Maxime PCR PreMix kit (i-StarTaq; Intron Biotechnology, Seongnam, Korea) and the following primer sequences: PRMT5, 5'-CTCCTACCTCCAATACCTGG-3' (sense) and 5'-CATTCC CTCATGTCTGATGA-3' (antisense); matrix metalloproteinase-2 (MMP-2), 5'-ATCTTTGCTGGAGACAAATTC-3' (sense) and 5'-AACTTCACGCTCTTCAGACTT-3' (antisense); β-actin, 5'-GGACTTCGAGCAAGAGATGG-3' (sense) and 5'-AGC ACTGTGTTGGCGTACAG-3' (antisense); β2-microglobulin, 5'-GTGCTCGCGCTACTCTCTCT-3' (sense) and 5'-CGGCAG GCATACTCATCTTT-3' (antisense).

Quantitative real-time RT-PCR. Quantitative real-time RT-PCR was performed using the iQTM SYBR® Green Supermix (cat. no. EBT-1801) and CFX96 real-time RT-PCR detection system (both from Bio-Rad Laboratories). The following primers were used: PRMT5#1, 5'-TTTCCCATCCTCTTCCCTATTAAG-3' (sense) and 5'-CCCACTCATACCACACCTTC-3' (antisense); PRMT5#2, 5'-CCGGCTACTTTGAGACTGG-3' (sense) and 5'-TTTGGCCTTCACGTACCG-3' (antisense); β2-microglobulin, 5'-AAGGACTGGTCTTTCTATCTCTTGTA-3' (sense) and 5'-ACTATCTTGGGCTGTGACAAAGTC-3' (antisense). Relative gene expression was analyzed using the comparative threshold cycle (2-ΔΔC(t)) method.

Protein extraction and western blot analysis. Total cell lysates was lysed with TNN buffer [120 mM NaCl, 40 mM Tris-HCl, pH 8.0, 0.5% (w/v) NP-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 100 mM sodium fluoride, 5 mM EDTA] containing protease inhibitor (P3100-010; GenDEPOT, Katy, TX, USA) and quantified using Bio-Rad protein assay based on the Bradford method (cat. no. 500-0006; Bio-Rad Laboratories). Equivalent amounts of protein were electrophoresed via 10% (w/v) SDS-PAGE and transferred onto nitrocellulose membrane (cat. no. 10600002; GE Healthcare Life Sciences, NJ, USA). The membrane was subsequently placed in TBST buffer containing 5% (w/v) skim milk and blocked at room temperature for 1 h, followed by treatment with primary antibodies, including MMP-2 (diluted to 1:2,000; cat. no. sc-10736), β-actin (diluted to 1:3,000; cat. no. sc-47778) and PRMT5 (diluted to 1:3,000; cat. no. sc-376937) (all from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) for 2 h and further treatment with horseradish peroxidase-conjugated secondary antibody (diluted to 1:3,000; cat. no. A120-101P and ct. no. A90-116P; Bethyl Laboratories, Montgomery, TX, USA) for 1 h. Target proteins were detected using a chemiluminescence kit (cat. no. sc-204806; Santa Cruz Biotechnology, Inc.).

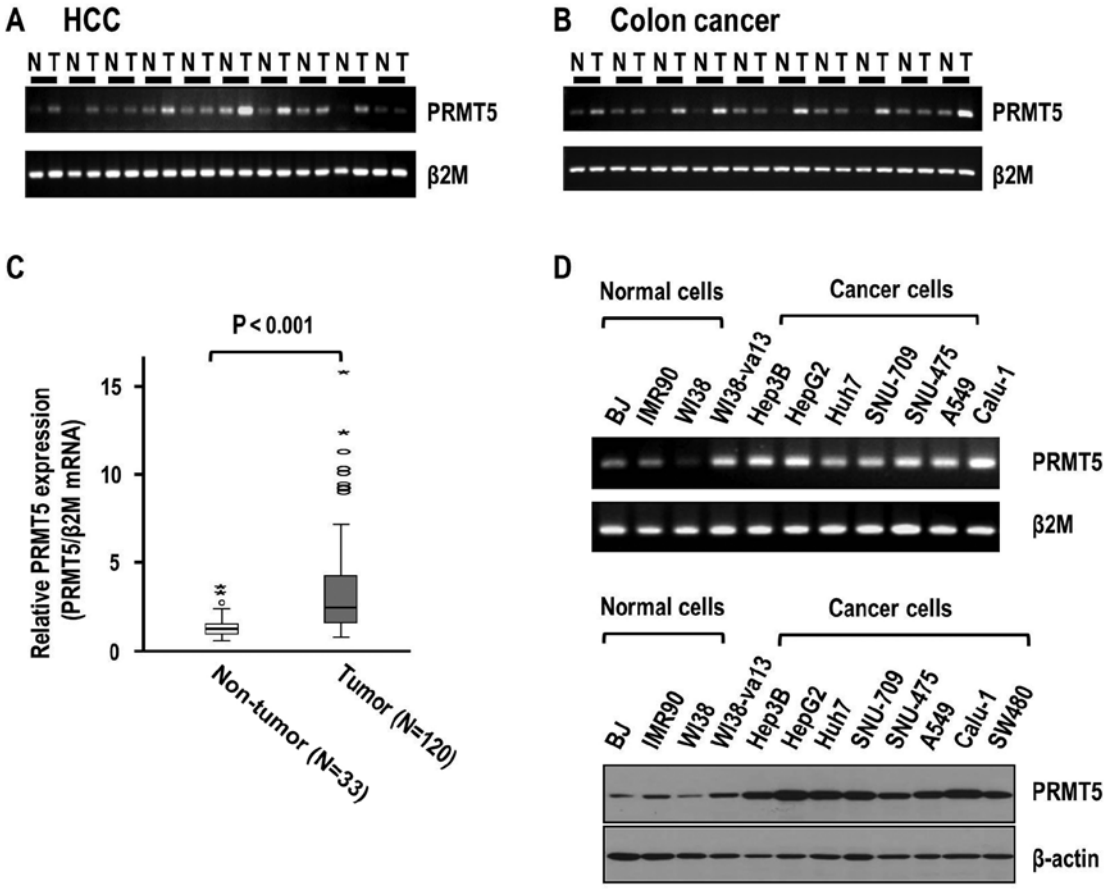

Figure 1. PRMT5 expression is increased in HCC and colon cancer tissues. (A and B) PRMT5 expression in (A) HCC and (B) colon cancer tissues was determined in pair-matched tumor (T) and corresponding adjacent non-tumor tissues (N) using semi-quantitative RT-PCR with β-2 microglobulin (β2M) as an internal control. (C) Relative levels of PRMT5/β2M mRNA in HCC and adjacent non-tumor liver tissues was quantitated via real-time RT-PCR. P-value was calculated by Mann-Whitney U test. (D) Semi-quantitative RT-PCR and western blot analysis of PRMT5 expression in normal and cancer cell lines. PRMT5, protein arginine methyltransferase 5; HCC, human hepatocellular carcinoma.

Matrigel invasion assay. The precoated filter chamber (6.5 mm in diameter, 8 μm pore size) of a polycarbonate Transwell membrane (Corning Inc. Corning NY, USA) was coated with Matrigel. Cells ($2x10^4$) suspended in serum-free medium were added into the upper compartment of the chamber and FBS [10% (w/v)] medium placed in the bottom of the chamber as a chemoattractant. After 24 and 32 h of incubation, the cells were fixed, stained with Hemacolor solution (cat. no. 111674; Merck Millipore, Darmstadt, Germany), visualized under the microscope and quantified by counting four different fields. All experiments were performed in triplicate.

Colony formation assay. After 24 h of transfection, the cells were seeded at a density of 1,000 cells/plate on a 60-mm culture dish. Subsequently, the cells were incubated for 10-13 days, fixed with 3.7% (w/v) formalin for 15 min, washed with distilled water and stained with 0.5% (w/v) crystal violet for 30 min at room temperature.

Statistical analysis. Statistical analysis was performed using the SPSS software (version 23.0; IBM Corporation, Armonk, NY, USA). The Mann-Whitney U test was applied to compare the expression of PRMT5 in non-tumor and tumor tissues. For comparisons of clinicopathological parameters according to low and high expression of PRMT5, the Chi-square or the Fisher's exact test were used as deemed appropriate. P<0.05 was considered to indicate a statistically significant difference.

Results

PRMT5 is overexpressed in human HCC and colon cancer tissues. To ascertain whether PRMT5 is associated with human cancer, we assessed PRMT5 mRNA levels in surgically removed frozen HCC and colon cancer tissue specimens. Semi-quantitative RT-PCR analyses revealed higher expression of PRMT5 in HCC than that in the corresponding adjacent liver tissues (Fig. 1A). Similar to the HCC tissues, colon cancer tissues exhibited higher expression of PRMT5 compared to non-tumor tissues (Fig. 1B). To further validate the overexpression of PRMT5, we performed quantitative real-time RT-PCR analysis using 120 HCC tissues (n=120) (Table I) and normalized expression to that in normal liver tissues, that were obtained from metastatic cancers with no background fibrosis and cirrhosis. Consistently, real-time RT-PCR data demonstrated greater expression of PRMT5 in HCC than in adjacent liver tissues (P<0.001) (Fig. 1C). The mean increase in PRMT5 expression in adjacent liver (n=33) and tumor tissues (n=120), was 3.58-fold (median, 2.47-fold). Our results clearly indicated that PRMT5 was significantly overexpressed in human HCC and colon cancer tissues.

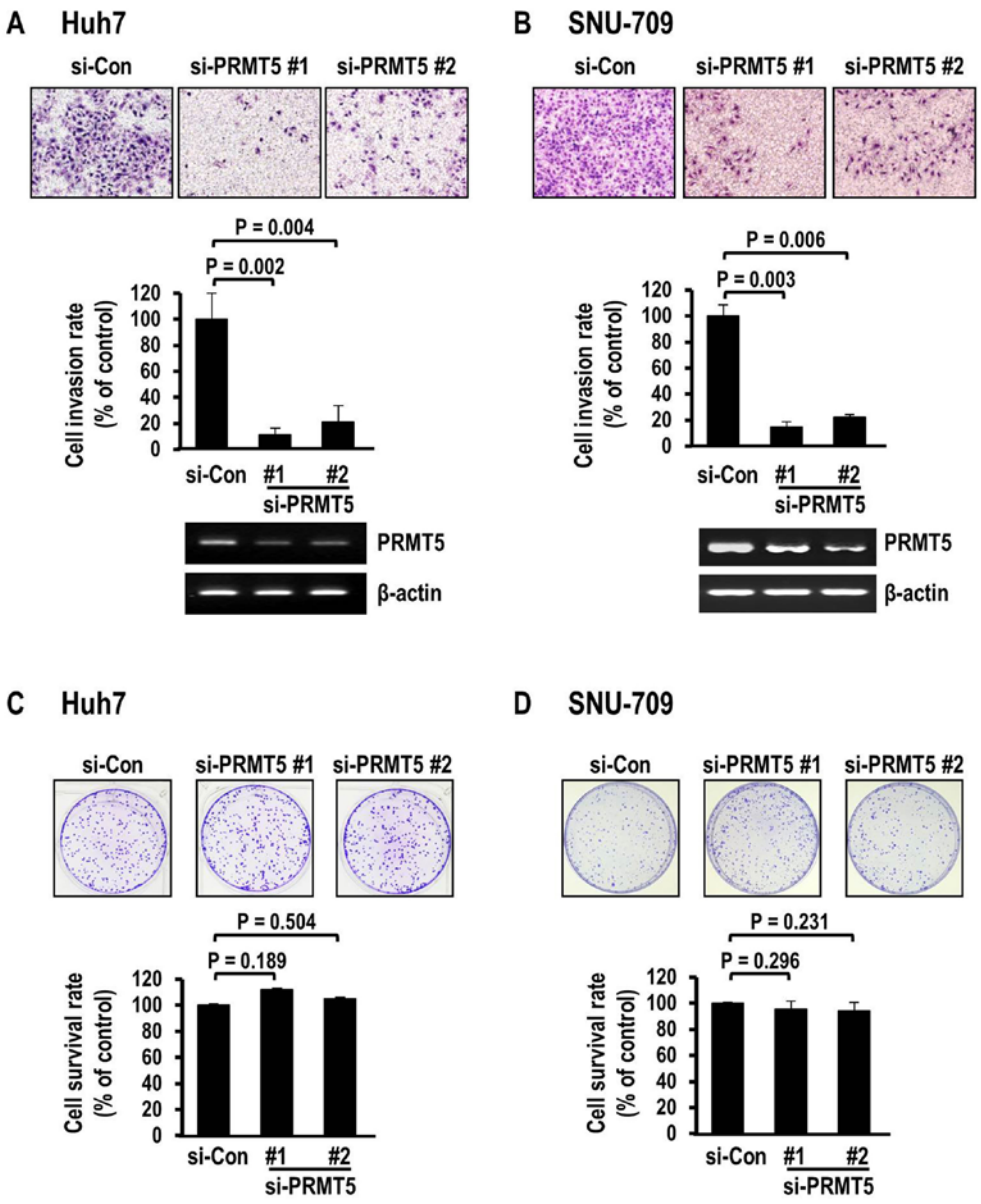

Figure 2. PRMT5 depletion induces a decrease in HCC cell invasion. (A and B) Huh7 and SNU-709 HCC cells transfected with PRMT5 or control siRNA invading the Matrigel chamber were visualized with crystal violet staining under a microscope (x100 mgnification) and counted. (C and D) Colony survival was analyzed with low-density seeding of siRNA-transfected cells used for (A) and (B) and visualized with crystal violet staining. The relative cell invasion (A and B) and colony survival (C and D) following PRMT5 siRNA transfection were calculated by dividing by values obtained from cells transfected with control siRNA. Bars represents mean ± SD from triplicate experiments and Student's t-test was used for statistical analysis. PRMT5, protein arginine methyltransferase 5; HCC, human hepatocellular carcinoma

PRMT5 overexpression is associated with invasion, differentiation, tumor size and α-fetoprotein levels. To further determine the clinicopathological correlations of PRMT5, patients were divided into low (n=68) and high (n=52) PRMT5 expression groups according to mRNA levels. Based on a 2.0-fold criterion in terms of the expression of PRMT5, the high expression group was significantly associated with higher α-fetoprotein (AFP; P=0.020), larger tumor size (P=0.011), poorer differentiation (P=0.004) and more frequent microscopic hepatic invasion (P=0.019), compared to the low expression group (Table II). The association of PRMT5 with AFP was significant, irrespective of the cut-off criterion (Table III). Our results collectively indicated that the overexpression of PRMT5 contributed to the aggressive phenotype of HCC. To further evaluate the significance of PRMT5 in human cancer, we examined PRMT5 expression patterns in other normal and cancer cell lines. Expression of PRMT5 in normal fibroblast cell strains (BJ, IMR90 and WI38) was clearly lower than that in various cancer cell lines, including HCC (Hep3B, HepG2, Huh7, SNU-709 and SNU-475), lung (A549 and Calu-1) and colon cancer (SW480) (Fig. 1D). Notably, WI38-va13 cells transformed with SV-40 exhibited higher PRMT5 expression, compared to parental WI38 cells.

PRMT5 depletion induces a decrease in HCC invasion and the expression of matrix metalloproteinase (MMP)-2. The clinicopathological correlation of PRMT5 overexpression with hepatic invasion in HCC tissues prompted us to examine the effect of PRMT5 depletion on HCC invasion. Depletion of PRMT5 achieved by transfection with specific siRNAs against

Table I. Demographic and pathological data of patients.

Variables	Classification	Distribution
Sex	Male/female	101/19
Age	Years, mean ± SD (range)	52.9±10.64 (25-77)
Etiology	Non B non C/B/C	21/93/6
AST	IU/l, mean ± SD (range)	53.1±34.6 (15-182)
ALT	IU/l, mean ± SD (range)	46.0±25.1 (8-141)
AFP	ng/ml, mean ± SD (range)	9852.8±66103.9 (1-690,400)
Child-Pugh score	A/B/C	99/9/0
Tumor size	cm, mean ± SD (range)	5.9±3.6 (1.2-18.5)
Number of tumors	Single/Multiple	86/20
Grade[a]	1/2/3/4	14/67/36/1
Fibrosis	No/Yes	40/70
Cirrhosis	No/Yes	64/46
Microscopic hepatic vein invasion	No/Yes	94/14

[a]Edmonson-Steiner histological grade. AST, aspartate aminotransferase; ALT, alanine transaminase; AFP, α2-fetoprotein.

coding sequences led to a marked decrease in the invasion of the Huh7 HCC cells, as determined using the Matrigel invasion assay (Fig. 2A). Suppression with two different siRNAs exerted similar significant effects on invasion (siRNA#1, P=0.002; siRNA#2, P=0.004), compared to the control siRNA, with decreases of 88.6 and 78.8%, respectively. Decreased invasion induced by PRMT5 depletion was consistently observed in another HCC cell line, SNU-709 (Fig. 2B). Similar to the Huh7 cells, the SNU-709 cells exhibited severely decreased invasion rates of 85.2% (siRNA#1) and 77.8% (siRNA#2), respectively, clearly demonstrating that PRMT5 depletion markedly attenuated the invasive activity of HCC cells. The PRMT5-mediated decrease in invasion may be attributable to alterations in proliferation and apoptosis. In fact, the PRMT family has been shown to be involved in protein arginine methylation during cell death and proliferation (17-20). Accordingly, we performed colony survival analysis with a view to observe phenotypic changes, including cellular proliferation and death. Notably, in contrast to the data obtained from the invasion analysis, PRMT5 depletion did not affect colony survival in either cell line. Specifically, PRMT5 siRNA (#1 and #2)-transfected Huh7 (Fig. 2C) and SNU-709 cells (Fig. 2D) did not exhibit differences in colony survival, compared to corresponding cells transfected with control siRNA. These findings excluded the possibility that the suppression of invasion mediated by PRMT5 is due to alterations in cell proliferation or death. Actually, the siRNAs used in the present study recognized the coding sequences of several isoforms (siRNA#1, isoforms 1-5; siRNA#2, isoforms 1, 2, 4 and 6) (Fig. 3C). To further determine whether the PRMT5-controlled invasion was associated with matrix metalloproteinase-2 (MMP-2), a protease that cleavages extracellular matrix and promotes cancer cell invasion (24-26), we evaluated the expression of MMP-2 under conditions of PRMT5 depletion in HCC cancer cell lines. Notably, MMP-2 mRNA as well as MMP-2 protein levels were suppressed in PRMT5-depleted Huh7 cancer cells (Fig. 3A). Consistently, the expression of MMP-2 in SNU-709 cells was decreased upon PRMT5 knockdown (Fig. 3B). Our results indicated that PRMT5 depletion-mediated decrease in cell invasion occured through the reduction of the expression of MMP-2 in the HCC cells.

PRMT5 depletion weakens the invasion of colon cancer cells accompanied by decreased expression of MMP-2. Our finding that PRMT5 depletion induced a decrease in the invasive activity of HCC further raised the question of whether PRMT5 exerts similar effects on colon cancer cell invasion. To resolve this issue, we examined the invasive activity of human colon cancer cell lines depleted of PRMT5. Similar to the results obtained with HCC cells, SW480 colon cancer cells exhibited decreased invasion following the knockdown of PRMT5 (Fig. 4A). Transfection with two different PRMT5 siRNAs (#1 and #2) induced significant decreases in the invasion rate (80.2 and 79.8%, respectively). However, the clonogenic survival of colon cancer cells was not affected (Fig. 4B) while MMP-2 levels were consistently decreased upon PRMT5 depletion (Fig. 4C). Collectively, the results clearly indicated that PRMT5 depletion weakened the invasive activity of both colon cancer and HCC cell types through the inhibitory effects on MMP-2 expression and activity. Furthermore, PRMT5 contributed to the aggressive characteristics of human cancer cells by promoting their invasive activity.

Discussion

Extensive analysis of PRMT5 in relation to human cancer has revealed its significant overexpression in various types of cancer, including breast (27) and gastric cancer (28), lymphoma (5,7,29) leukemia (7,30) and prostate cancer (31-33). Earlier clinicopathological analyses demonstrated that higher expression of PRMT5 was correlated with advanced tumor grade, presence of lymph node metastasis and poor prognosis (19,34,35). Consistent with previous studies by Zhang *et al* (17,18) and Shimizu *et al* (19), PRMT5 was significantly overexpressed in our HCC patient tissue samples,

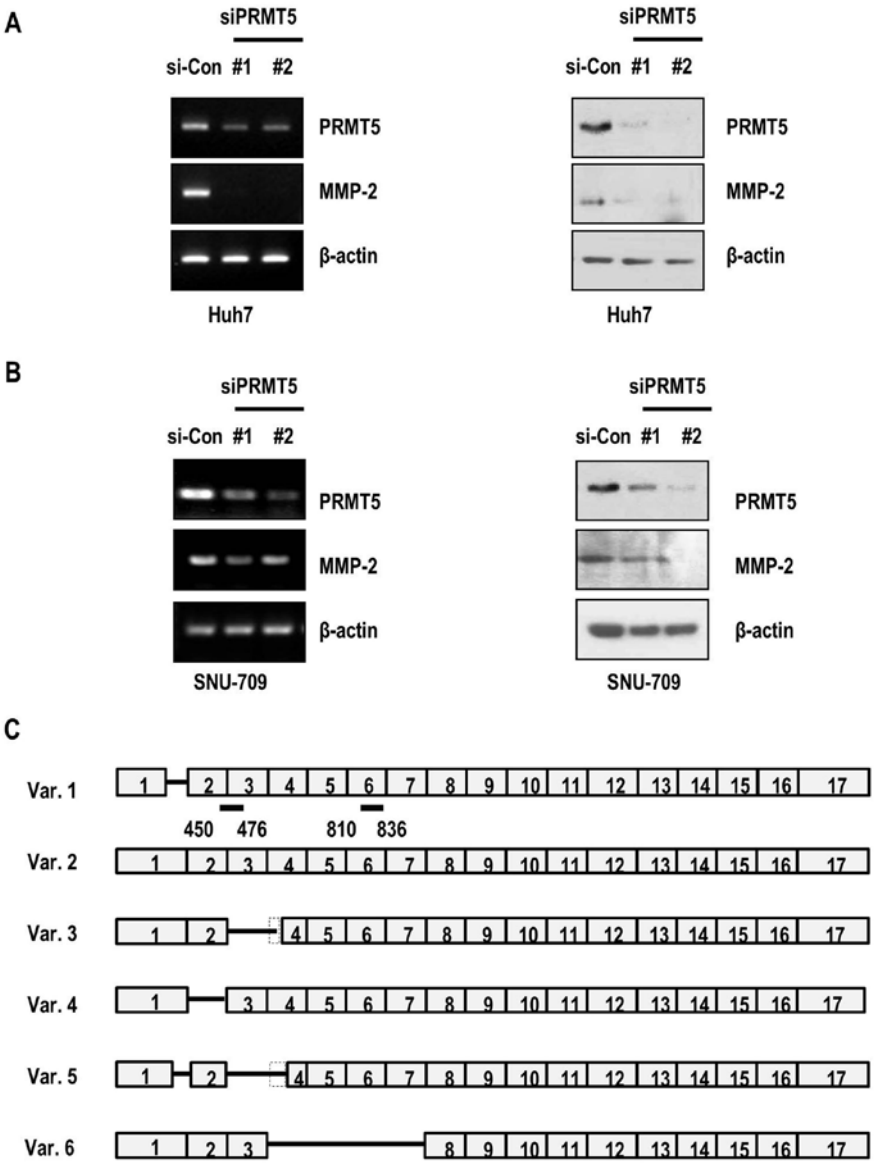

Figure 3. PRMT5 depletion induces suppression of the expression of MMP-2. (A and B) Semi-quantitative RT-PCR analysis of MMP-2 mRNA in (A) Huh7 and in (B) SNU-709 cells. Secreted MMP-2 protein levels in culture medium were determined by western blot analysis. (C) Schematic diagram demonstrating exon and intron organization in six variants of PRMT5 registered in the NCBI database. PRMT5 is composed of 12 exons that are marked in numbers in the box. Filled bars on the mRNA sequences of variant 1 indicate the locations of PRMT5 siRNA. siRNA#1 and #2 recognize the positions 450-476 between exons 2 and 3 and the 810-836 position in exon 6, respectively. PRMT5, protein arginine methyltransferase 5; HCC, human hepatocellular carcinoma; MMP-2, matrix metalloproteinase-2.

confirming the validity of these findings. Notably, our clinicopathological findings indicated the association among higher expression of PRMT5 with more frequent presence of microscopic hepatic invasion and higher AFP levels. To our knowledge, this is the first study to report such a correlation and our results further highlight the importance of PRMT5 as a potential HCC biomarker.

In addition to *in vivo* studies, several *in vitro* experiments have revealed that depletion of PRMT5 induced a decrease in cancer cell survival (36) and proliferation (19,28,34,36-38). While these phenotypes have been observed in various cancer cell lines, the exact molecular mechanisms remain to be established. In the present study, we used two different siRNAs to deplete the expression of PRMT5 in two HCC cell lines and one colon cancer cell line. Consistently, the knockdown of PRMT5 led to a significant decrease in the invasiveness of cancer cells, based on data from the invasion assay and the expression patterns of MMP-2. These results were in accordance with the previous finding that the depletion of PRMT5 modulated the expression of E-cadherin through interactions with Ajuba, to regulate the transcription factor Snail (6). However, earlier invasion analyses were performed under conditions whereby the PRMT5 siRNAs used induced a decrease in cell proliferation and/or survival (17,18,38). Decreased proliferation and survival can affect the invasion of cancer cells, thus reducing cell activity. Notably, in our experiments, PRMT5 depletion-mediated decrease in invasion activity was achieved with no adverse effects on colony survival. This discrepancy may be attributed to differences in the efficacy of siRNA or the rate of decrease in the expression of PRMT5. Indeed, the PRMT5 siRNAs used depleted isoforms 1-5 (siRNA#1) and isoforms 1, 2, 4 and 6 (siRNA#2) (Fig. 3C).

Table II. Correlations between the expression of PRMT5 and the clinicopathological parameters.

Variables	PRMT5 expression <2 fold	PRMT5 expression ≥2 fold	P-value[a]
Sex			
Male	60	41	0.163
Female	8	11	
HBsAg			
Negative	18	9	0.234
Positive	50	43	
AST (U/l)			
<100	64	46	0.724
≥100	4	6	
ALT (U/l)			
<100	65	49	1.000
≥100	3	3	
AFP (ng/ml)			
<20	35	15	0.020[b]
≥20	33	35	
Child-Pugh score			
A	54	44	0.295
B and C	7	2	
Tumor size (cm)			
<5	40	18	0.011[b]
≥5	28	33	
Tumor number			
Solitary	52	34	0.096
Multiple	8	12	
Grade			
1	13	1	0.004[b]
2,3,4	54	50	
Fibrosis			
No	20	20	0.188
Yes	44	26	
Cirrhosis			
No	40	24	0.279
Yes	24	22	
Macroscopic vascular invasion			
No	62	46	0.402
Yes	2	4	
Microscopic hepatic vein invasion			
No	58	36	0.019[b]
Yes	4	10	

[a]Statistically significant P-values were assessed by the Chi-square or the Fisher's exact test. [b]Significant P-values (P<0.05). PRMT5, protein arginine methyltransferase 5; HBsAg, hepatitis B surface antigen. AST, aspartate aminotransferase; ALT, alanine transaminase; AFP, α2-fetoprotein.

Table III. Correlation between the expression of PRMT5 and AFP levels.

Variables	PRMT5 expression <2-fold	PRMT5 expression ≥2-fold	P-value[a]
AFP (ng/ml)			
<20	35	15	0.020[b]
≥20	33	35	
AFP (ng/ml)			
<50	41	20	0.029[b]
≥50	27	30	
AFP (ng/ml)			
<100	49	22	0.002[b]
≥100	19	28	
AFP (ng/ml)			
<200	51	26	0.010[b]
≥200	17	24	
AFP (ng/ml)			
<400	55	29	0.007[b]
≥400	13	21	

[a]Statistically significant P-values were assessed by the Chi-square or the Fisher's exact test. [b]Significant P-values (P<0.05). AFP, α-fetoprotein; PRMT5, protein arginine methyltransferase 5.

Collectively with data from comprehensive earlier studies using human cancer patient tissues, our present results highlighted the utility of PRMT5 as a biomarker for various human cancer types, including HCC. The finding that depletion of PRMT5 induced a marked decrease in cancer cell invasive activity further supported its potential use as an anticancer therapeutic target. Further research is warranted to clarify the mechanisms by which PRMT5 regulates cancer cell invasion activity, including the substrates involved.

Acknowledgements

The biospecimens and data used in the present study were provided by the Radiation Tissue Resources Bank of Korea Cancer Center Hospital (TB-2016-04-C/P20).

Funding

The present study was supported by grants from the National Research Foundation of Korea (nos. 2012M3A9B6055346, 2017M3A9A8033561 and 2017R1A2B4008805) and the Korea Institute of Radiological and Medical Sciences (nos. 50531-2018 and 50542-2018) funded by the Ministry of Science, ICT and Future Planning, Republic of Korea.

Availability of data and materials

The data and materials used in the present study are available from the corresponding authors upon reasonable request.

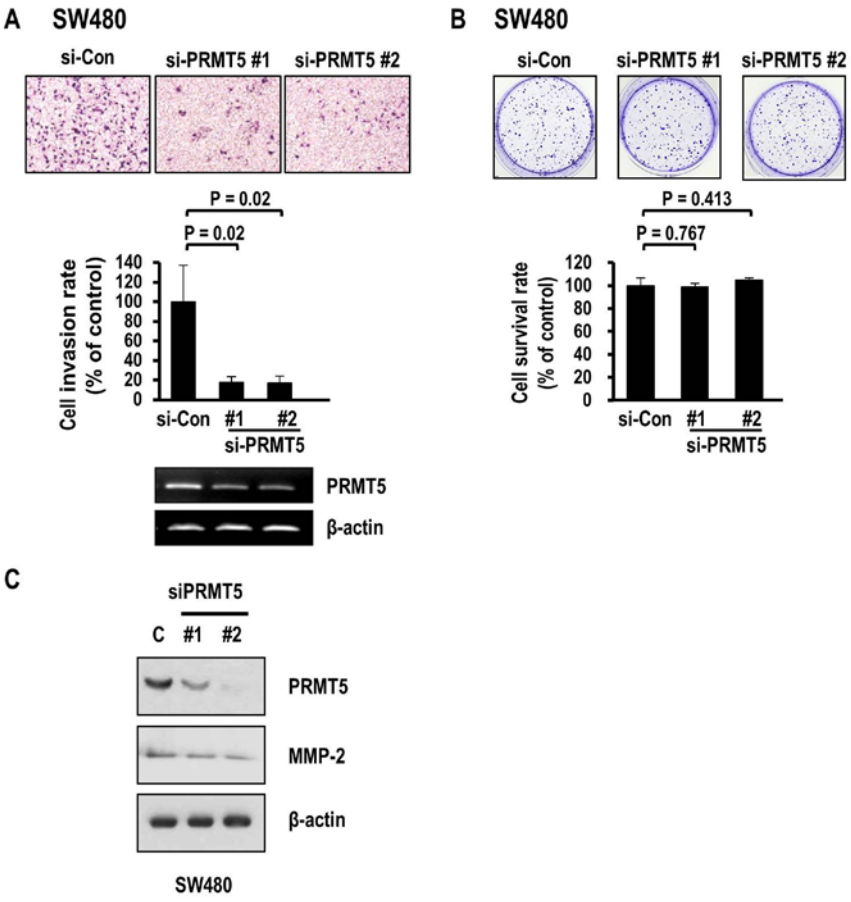

Figure 4. PRMT5 depletion weakens the invasive activity of colon cancer cells accompanied by decreased expression of MMP-2. (A and B) Cell invasion and colony survival were determined in SW480 colon cancer cells transfected with PRMT5-specific (#1 and #2) or control siRNA. Relative invasion and survival rates of PRMT5 siRNA-transfected cells were evaluated, compared with cells transfected with control siRNA. Bars represents mean ± SD from triplicate experiments and Student's t-test was used for statistical analysis. (C) MMP-2 protein level was assessed in siRNA-transfected cells. PRMT5, protein arginine methyltransferase 5; HCC, human hepatocellular carcinoma; MMP-2, matrix metalloproteinase-2.

Authors' contributions

SBM, MBG and KHL designed and guided the study. JYJ, ERP, YNS and MYK performed the experiments. JYJ, JSL and KHL wrote the paper. JYJ and JSL performed statistical analysis. HJS and HYJ reviewed and edited manuscript. EHC, SMM, USS, SHP, CJH, DWC and SBK provided tissues and generated clinical data. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Korea Cancer Center Hospital. Patient consent was either waived for liver or obtained for colon cancer tissues.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
2. Karaman B, Battal B, Sari S and Verim S: Hepatocellular carcinoma review: Current treatment, and evidence-based medicine. World J Gastroenterol 20: 18059-18060, 2014.
3. Wolf SS: The protein arginine methyltransferase family: An update about function, new perspectives and the physiological role in humans. Cell Mol Life Sci 66: 2109-2121, 2009.
4. Pal S, Vishwanath SN, Erdjument-Bromage H, Tempst P and Sif S: Human SWI/SNF-associated PRMT5 methylates histone H3 arginine 8 and negatively regulates expression of ST7 and NM23 tumor suppressor genes. Mol Cell Biol 24: 9630-9645, 2004.
5. Pal S, Baiocchi RA, Byrd JC, Grever MR, Jacob ST and Sif S: Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma. EMBO J 26: 3558-3569, 2007.
6. Hou Z, Peng H, Ayyanathan K, Yan KP, Langer EM, Longmore GD and Rauscher FJ III: The LIM protein AJUBA recruits protein arginine methyltransferase 5 to mediate SNAIL-dependent transcriptional repression. Mol Cell Biol 28: 3198-3207, 2008.
7. Wang L, Pal S and Sif S: Protein arginine methyltransferase 5 suppresses the transcription of the RB family of tumor suppressors in leukemia and lymphoma cells. Mol Cell Biol 28: 6262-6277, 2008.
8. Stopa N, Krebs JE and Shechter D: The PRMT5 arginine methyltransferase: Many roles in development, cancer and beyond. Cell Mol Life Sci 72: 2041-2059, 2015.
9. Jansson M, Durant ST, Cho EC, Sheahan S, Edelmann M, Kessler B and La Thangue NB: Arginine methylation regulates the p53 response. Nat Cell Biol 10: 1431-1439, 2008.

10. Guo Z, Zheng L, Xu H, Dai H, Zhou M, Pascua MR, Chen QM and Shen B: Methylation of FEN1 suppresses nearby phosphorylation and facilitates PCNA binding. Nat Chem Biol 6: 766-773, 2010.
11. He W, Ma X, Yang X, Zhao Y, Qiu J and Hang H: A role for the arginine methylation of Rad9 in checkpoint control and cellular sensitivity to DNA damage. Nucleic Acids Res 39: 4719-4727, 2011.
12. Bandyopadhyay S, Harris DP, Adams GN, Lause GE, McHugh A, Tillmaand EG, Money A, Willard B, Fox PL and Dicorleto PE: HOXA9 methylation by PRMT5 is essential for endothelial cell expression of leukocyte adhesion molecules. Mol Cell Biol 32: 1202-1213, 2012.
13. Cho EC, Zheng S, Munro S, Liu G, Carr SM, Moehlenbrink J, Lu YC, Stimson L, Khan O, Konietzny R, *et al*: Arginine methylation controls growth regulation by E2F-1. EMBO J 31: 1785-1797, 2012.
14. Kumar B, Yadav A, Brown NV, Zhao S, Cipolla MJ, Wakely PE, Schmitt AC, Baiocchi RA, Teknos TN, Old M, *et al*: Nuclear PRMT5, cyclin D1 and IL-6 are associated with poor outcome in oropharyngeal squamous cell carcinoma patients and is inversely associated with p16-status. Oncotarget 8: 14847-14859, 2017.
15. Tanaka H, Hoshikawa Y, Oh-hara T, Koike S, Naito M, Noda T, Arai H, Tsuruo T and Fujita N: PRMT5, a novel TRAIL receptor-binding protein, inhibits TRAIL-induced apoptosis via nuclear factor-kappaB activation. Mol Cancer Res 7: 557-569, 2009.
16. Nicholas C, Yang J, Peters SB, Bill MA, Baiocchi RA, Yan F, Sïf S, Tae S, Gaudio E, Wu X, *et al*: PRMT5 is upregulated in malignant and metastatic melanoma and regulates expression of MITF and p27(Kip1.). PLoS One 8: e74710, 2013.
17. Zhang B, Dong S, Zhu R, Hu C, Hou J, Li Y, Zhao Q, Shao X, Bu Q, Li H, *et al*: Targeting protein arginine methyltransferase 5 inhibits colorectal cancer growth by decreasing arginine methylation of eIF4E and FGFR3. Oncotarget 6: 22799-22811, 2015.
18. Zhang B, Dong S, Li Z, Lu L, Zhang S, Chen X, Cen X and Wu Y: Targeting protein arginine methyltransferase 5 inhibits human hepatocellular carcinoma growth via the downregulation of beta-catenin. J Transl Med 13: 349, 2015.
19. Shimizu D, Kanda M, Sugimoto H, Shibata M, Tanaka H, Takami H, Iwata N, Hayashi M, Tanaka C, Kobayashi D, *et al*: The protein arginine methyltransferase 5 promotes malignant phenotype of hepatocellular carcinoma cells and is associated with adverse patient outcomes after curative hepatectomy. Int J Oncol 50: 381-386, 2017.
20. Prabhu L, Wei H, Chen L, Demir Ö, Sandusky G, Sun E, Wang J, Mo J, Zeng L, Fishel M, *et al*: Adapting AlphaLISA high throughput screen to discover a novel small-molecule inhibitor targeting protein arginine methyltransferase 5 in pancreatic and colorectal cancers. Oncotarget 8: 39963-39977, 2017.
21. Kim BY, Suh KS, Lee JG, Woo SR, Park IC, Park SH, Han CJ, Kim SB, Jeong SH, Yeom YI, *et al*: Integrated analysis of prognostic gene expression profiles from hepatitis B virus-positive hepatocellular carcinoma and adjacent liver tissue. Ann Surg Oncol 19 (Suppl 3): S328-S338, 2012.
22. Kim BY, Choi DW, Woo SR, Park ER, Lee JG, Kim SH, Koo I, Park SH, Han CJ, Kim SB, *et al*: Recurrence-associated pathways in hepatitis B virus-positive hepatocellular carcinoma. BMC Genomics 16: 279, 2015.
23. Park ER, Kim SB, Lee JS, Kim YH, Lee DH, Cho EH, Park SH, Han CJ, Kim BY, Choi DW, *et al*: The mitochondrial hinge protein, UQCRH, is a novel prognostic factor for hepatocellular carcinoma. Cancer Med 6: 749-760, 2017.
24. Seftor RE, Seftor EA, Gehlsen KR, Stetler-Stevenson WG, Brown PD, Ruoslahti E and Hendrix MJ: Role of the alpha v beta 3 integrin in human melanoma cell invasion. Proc Natl Acad Sci USA 89: 1557-1561, 1992.
25. Brooks PC, Strömblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP and Cheresh DA: Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell 85: 683-693, 1996.
26. Muschel RJ and Yong J: The Gelatinases, MMP-2 and MMP-9-Implications for invasion and metastasis. In: Proteases and Their Inhibitors in Cancer Metastasis. Foidart JM and Muschel RJ (eds). Springer Netherlands, Dordrecht, pp39-52, 2002.
27. Wu Y, Wang Z, Zhang J and Ling R: Elevated expression of protein arginine methyltransferase 5 predicts the poor prognosis of breast cancer. Tumour Biol 39: 1010428317695917, 2017.
28. Kanda M, Shimizu D, Fujii T, Tanaka H, Shibata M, Iwata N, Hayashi M, Kobayashi D, Tanaka C, Yamada S, *et al*: Protein arginine methyltransferase 5 is associated with malignant phenotype and peritoneal metastasis in gastric cancer. Int J Oncol 49: 1195-1202, 2016.
29. Chung J, Karkhanis V, Tae S, Yan F, Smith P, Ayers LW, Agostinelli C, Pileri S, Denis GV, Baiocchi RA, *et al*: Protein arginine methyltransferase 5 (PRMT5) inhibition induces lymphoma cell death through reactivation of the retinoblastoma tumor suppressor pathway and polycomb repressor complex 2 (PRC2) silencing. J Biol Chem 288: 35534-35547, 2013.
30. Jin Y, Zhou J, Xu F, Jin B, Cui L, Wang Y, Du X, Li J, Li P, Ren R, *et al*: Targeting methyltransferase PRMT5 eliminates leukemia stem cells in chronic myelogenous leukemia. J Clin Invest 126: 3961-3980, 2016.
31. Gu Z, Li Y, Lee P, Liu T, Wan C and Wang Z: Protein arginine methyltransferase 5 functions in opposite ways in the cytoplasm and nucleus of prostate cancer cells. PLoS One 7: e44033, 2012.
32. Zhang HT, Zhang D, Zha ZG and Hu CD: Transcriptional activation of PRMT5 by NF-Y is required for cell growth and negatively regulated by the PKC/c-Fos signaling in prostate cancer cells. Biochim Biophys Acta 1839: 1330-1340, 2014.
33. Deng X, Shao G, Zhang HT, Li C, Zhang D, Cheng L, Elzey BD, Pili R, Ratliff TL, Huang J, *et al*: Protein arginine methyltransferase 5 functions as an epigenetic activator of the androgen receptor to promote prostate cancer cell growth. Oncogene 36: 1223-1231, 2017.
34. Bao X, Zhao S, Liu T, Liu Y, Liu Y and Yang X: Overexpression of PRMT5 promotes tumor cell growth and is associated with poor disease prognosis in epithelial ovarian cancer. J Histochem Cytochem 61: 206-217, 2013.
35. Shilo K, Wu X, Sharma S, Welliver M, Duan W, Villalona-Calero M, Fukuoka J, Sif S, Baiocchi R, Hitchcock CL, *et al*: Cellular localization of protein arginine methyltransferase-5 correlates with grade of lung tumors. Diagn Pathol 8: 201, 2013.
36. Chen H, Lorton B, Gupta V and Shechter D: A TGFβ-PRMT5-MEP50 axis regulates cancer cell invasion through histone H3 and H4 arginine methylation coupled transcriptional activation and repression. Oncogene 36: 373-386, 2017.
37. Chen D, Zeng S, Huang M, Xu H, Liang L and Yang X: Role of protein arginine methyltransferase 5 in inflammation and migration of fibroblast-like synoviocytes in rheumatoid arthritis. J Cell Mol Med 21: 781-790, 2017.
38. Saha K, Fisher ML, Adhikary G, Grun D and Eckert RL: Sulforaphane suppresses PRMT5/MEP50 function in epidermal squamous cell carcinoma leading to reduced tumor formation. Carcinogenesis 38: 827-836, 2017.