PRMT2β, a C-terminal splice variant of PRMT2, inhibits the growth of breast cancer cells

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Abstract. Our previous study reported several alternative splicing variants of arginine N-methyltransferase 2 (PRMT2), which lose different exons in the C-terminals of the wild-type PRMT2 gene. Particularly, due to frame-shifting, PRMT2β encodes a novel amino acid sequence at the C-terminus of the protein, the function of which is not understood. In the present study, we determined the role of PRMT2β in breast cancer cell proliferation, apoptosis and its effect on the Akt signaling pathway. Stable breast cancer MCF7 cell line with lentivirus-mediated PRMT2β overexpression was obtained after selection by puromycin for 2 weeks. The effect of lentivirus-mediated PRMT2β overexpression on breast cancer cellular oncogenic properties was evaluated by MTT, colony formation, cell cycle analysis and apoptosis assays in MCF7 cells. Luciferase activity assay and western blot analysis were performed to characterize the effects of PRMT2β on *cyclin D1* promoter activities and the Akt signaling pathway. Tissue microarray was performed to investigate the association of PRMT2β with breast cancer progression. Lentivirus-mediated PRMT2β overexpression suppressed the cell proliferation and colony formation of breast cancer MCF7 cells. PRMT2β overexpression induced cell cycle arrest and apoptosis of MCF7 cells. Furthermore, PRMT2β was revealed to suppress the transcription activity of the *cyclin D1* promoter, and PRMT2β was also found to inhibit cyclin D1 expression via the suppression of Akt/GSK-3β signaling in breast cancer cells. Clinically, it was revealed that PRMT2β expression was negatively correlated with human epidermal growth factor receptor 2 (HER2) (p=0.033) in breast tumors. Our results revealed that PRMT2β, a novel splice variant of PRMT2, plays potential antitumor effect by suppressing cyclin D1 expression and inhibiting Akt signaling activity. This also opens a new avenue for treating breast cancer.

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Abbreviations: PRMT2, arginine N-methyltransferase 2; AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; AP-1, activator protein-1; Dox, doxycycline hyclate; FBS, fetal bovine serum; GFP, green fluorescent protein

Key words: PRMT2β, antitumor, proliferation, breast cancer

Introduction

Alternative splicing is a sophisticated and ubiquitous nuclear process in eukaryotic cells by which a single gene may yield various mRNAs, leading to products of multiple protein isoforms. In humans, more than 70% of genes are alternatively spliced (1,2), which greatly increases the diversity of proteins that can be encoded by the surprisingly low number of human genes. Alternative splicing is known to play a role in the alteration of the binding properties of proteins, their enzymatic activity, their intracellular localization, or their stability and post-translational modifications (3,4). Recently increasing evidence has revealed that alternative splicing plays an important role in tumorigenesis and tumor progression, and splice variants represent potentially useful targets for the development of novel therapeutic strategies (5,6).

Human protein arginine N-methyltransferase 2 (PRMT2, HRMT1L1) is a protein that belongs to the arginine methyltransferase family and has weak methyltransferase activity on a histone H4 substrate. However, its optimal substrates have not yet been identified (7,8). Subsequent studies have revealed that PRMT2 is clearly involved in a variety of cellular processes, including lung function, the inflammatory response, apoptosis promotion, and Wnt and leptin signaling regulation (9-13), suggesting that PRMT2 has diverse roles in transcriptional regulation through different mechanisms depending on its binding partners.

Our previous study demonstrated that the PRMT2 gene produces several isoforms through complex alternative splicing at the 3' end of the pre-mRNA, and each has a unique C-terminal

sequence (14). Due to the distinct gene structure characteristics, each isoform is therefore predicted to be functionally different. PRMT2β is the most different isoform compared with the wild-type. Sequence analysis revealed that PRMT2β deletes exon 7-9 in its coding sequence, and downstream frame-shifting. As a result, PRMT2β encodes 83 new amino acids at the C-terminus of the protein. We had previously demonstrated the negative effect of PRMT2 on breast cancer cell proliferation *in vitro* and *in vivo*. Moreover, cyclin D1 (CCND1) was found to be downregulated by PRMT2, and PRMT2 was further shown to suppress the estrogen receptor α (ERα) binding affinity to the activator protein-1 (AP-1) site in the *CCND1* promoter through indirect binding with the AP-1 site, resulting in the inhibition of the *CCND1* promoter activity in MCF-7 cells (15). These studies however, neglected to examine the specific functional contributions of PRMT2β to breast cancer progression. In the present study, we revealed that PRMT2β suppressed the cell proliferation and colony formation of breast cancer MCF7 cells, and induced cell cycle arrest and apoptosis of MCF7 cells. PRMT2β was revealed to suppress the transcription activity of the *CCND1* promoter, and PRMT2β was also found to inhibit CCND1 expression via the suppression of Akt/GSK-3β signaling in breast cancer cells.

Materials and methods

Cell culture. MCF7 and 293T cells were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA), and cultured as previously described (14). Ethics approval and consent for the use of the human tissue was confirmed by the Ethics Committee of The First Affiliated Hospital of the University of South China.

Lentiviral vector construction and lentivirus infection. Recombined lentiviral vector *pGC-LV-GV308-PRMT2β* with the variant *PRMT2β* (FJ436411) gene (*LV-Tet-on-PRMT2β*) and *pGC-LV-GV308-PRMT2* (NM_001535) with the wild-type *PRMT2* (*LV-Tet-on-PRMT2*) gene were constructed by the GeneChem Co. (Shanghai, China). The *pGC-LV-GV308* vector was used as a negative control. The packaging plasmid pHelper 1.0 and pHelper 2.0 were purchased from GeneChem Co.

We co-transfected the *pGC-LV-GV308-PRMT2β* or *pGC-LV-GV308-PRMT2* vectors with the pHelper 1.0 and pHelper 2.0 packaging plasmid into 293T cells to generate recombinant lentiviruses. Culture medium was collected 72 h post-transfection, and MCF7 cells were then infected with the aforementioned lentiviruses. A total of $5x10^5$ MCF7 cells were seeded into a 6-well cell plate and further incubated for 12 h to reach 30% confluency, and then infected with LV-Tet-on-PRMT2β (PRMT2β overexpression group) or LV-Tet-on-PRMT2 (PRMT2 overexpression group) for 48 h in the presence of 8 μg/ml of Polybrene (Sigma-Aldrich, St. Louis, MO, USA). Stable cell lines either with the PRMT2β-3Flag or the PRMT2-3Flag were obtained after infection with LV-tet-on-PRMT2β or LV-tet-on-PRMT2 cells which were selected by puromycin for 2 weeks. Western blot analysis was performed to verify the expression of PRMT2β-3Flag or PRMT2-3Flag induced by 5 μg/ml of Dox in the infected MCF7 cells.

Cell proliferation and colony formation assays. For cell proliferation assays, MCF7 cells were seeded in a 96-well plate (2,000 cells/well) and counted using an automated cell counter (Nexcelom Bioscience, Lawrence, MA, USA). For the colony formation assay, the cells were seeded in a 6-well plate (600 cells/well) and maintained for 8 days. The culture medium with or without 5 μg/ml of Dox was changed every 2 days. Each experiment was carried out in triplicate and performed at least twice.

Soft agar colony formation assay. The assay was performed in 6-well plates. A bottom layer of agar (0.6%) with enriched Dulbecco's modified Eagle's medium (DMEM), [final 10% fetal bovine serum (FBS)] was poured first. After the bottom agar solidified, MCF7 cells ($3.0x10^3$) were seeded in top agar (0.3%) with enriched DMEM supplemented with 20% FBS and incubated at 37˚C for 15 days. The culture medium with or without 5 μg/ml of Dox was changed twice weekly. Colonies were visualized by staining for 1 h with 0.005% crystal violet.

Cell cycle analysis by flow cytometry. After transfection, the MCF7 cells were plated in 6-well plates at $2x10^5$ cells/well. Following the designated treatments, the cells were harvested by trypsinization and washed with PBS and fixed in ice-cold 75% ethanol overnight at -20˚C. The fixed cells were washed, and dissolved in RNAse and subsequently incubated at 37˚C for 30 min. Next, the cells were stained with propidium iodide (PI) for 30 min. The DNA content of the cells ($1x10^4$ cells/experimental group) was determined using a BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Transient transfection and luciferase activity assay. Transient gene delivery was carried out to assess the effect of PRMT2β and PRMT2 on the *CCND1* promoter activity in 293T cells as previously described (14). A luciferase assay kit (Promega, Madison, WI, USA) was used to assess the reporter activity according to the manufacturer's instructions. Luciferase activity was normalized using a *Renilla* luciferase internal control.

Western blot analysis. Total cell or tissue lysates were lysed on ice for 30 min. Soluble proteins (20 μg) were probed with anti-PRMT2, anti-Flag and anti-CCND1 antibodies (1:500; Abcam, Cambridge, MA, USA) and anti-p-AKT, anti-AKT, anti-p-GSK-3β and anti-GSK-3β (1:800; Cell Signaling Technology, Danvers MA, USA). Loading variations were normalized against β-actin, which was identified by anti-β-actin monoclonal Ab (1:1,000; Abcam).

Tissue microarray and immunohistochemical analysis. The tissue microarray (BR1503b; US Biomax, Inc., Rockville, MD, USA) consisting of 138 breast malignant tumor cases, 6 normal cases and 6 benign tumor cases was utilized, and was histologically interpretable and analyzed for the correlation with clinicopathological parameters. IHC staining was performed as detailed in our previous study (14). The rabbit polyclonal PRMT2 (1:50; Aviva Systems Biology, Beijing, China) was used.

We developed affinity-purified rabbit polyclonal anti-PRMT2β Abs as a custom service from Boster (Wuhan,

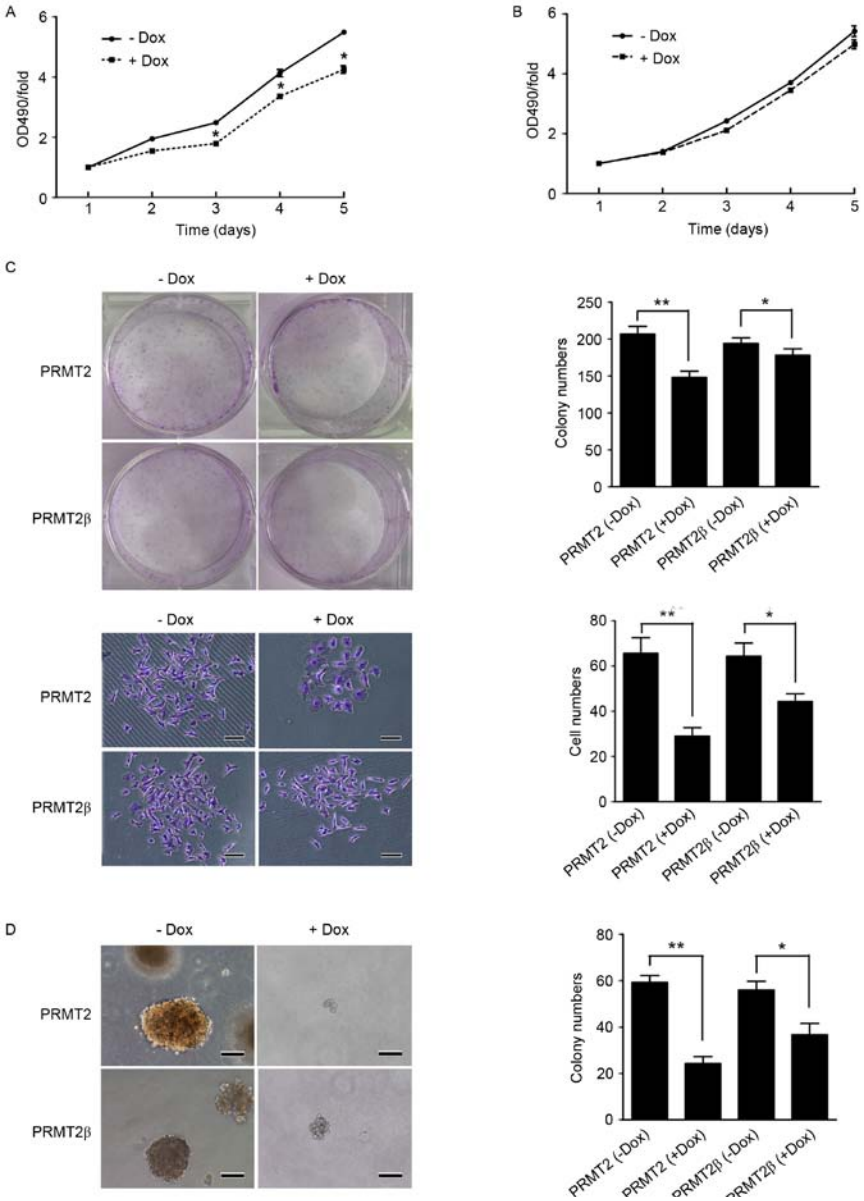

Figure 1. Lentivirus-mediated PRMT2β overexpression inhibits breast cancer MCF7 cell proliferation. (A and B) Growth curves of cells in each group. MTT analysis revealed that lentivirus-mediated PRMT2 and PRMT2β overexpression inhibited breast cancer cell proliferation (n=3); $^{*}p<0.05$. (C) Colony formation assay revealed that lentivirus-mediated PRMT2 and PRMT2β overexpression significantly inhibited breast cancer cell colony formation and the cell numbers of each colony (n=3, $^{*}p<0.05$, $^{**}p<0.01$). Scale bar, 50 μm. (D) Soft agar colony formation assay revealed that lentivirus-mediated PRMT2 and PRMT2β overexpression inhibited breast cancer cell soft agar colony formation (n=3, $^{*}p<0.05$, $^{**}p<0.01$). Scale bar, 200 μm. PRMT2, arginine N-methyltransferase 2; Dox, doxycycline hyclate.

China). The Abs were raised against a synthetic peptide antigen corresponding to the unique C-terminal 15 aa of PRMT2β (HLEMTVDALFGKQCA). The specificity of the Ab was tested in the PRMT2β expression vector-transfected 293T cells that do not express endogenous PRMT2β. Immunofluorescence was used to demonstrate immunoreactive signals only in transfectants with the PRMT2β-expressing vectors, but not in transfectants harboring an empty expression vector.

Statistical analysis. All experiments were performed using 3 replicates and the results are expressed as the mean ± SEM or mean ± SD. Statistical analysis was carried out using SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA). A statistical association between clinicopathological and molecular parameters was tested, using the non-parametrically two-tailed Mann-Whitney U test. p-values <0.05 were considered significant. Spearman's rank correlation coefficients were used to assess the correlation of PRMT2β and PRMT2 expression with clinicopathological parameters.

Results

PRMT2β suppresses the cell proliferation and colony formation of breast cancer MCF7 cells. Our previous study reported that suppression of PRMT2 expression promoted the proliferation of breast cancer cells (15). To address the cellular and molecular mechanisms of the alternatively spliced PRMT2 in breast cancer cells, a tetracycline (doxycycline hyclate, Dox)-inducible lentiviral system was

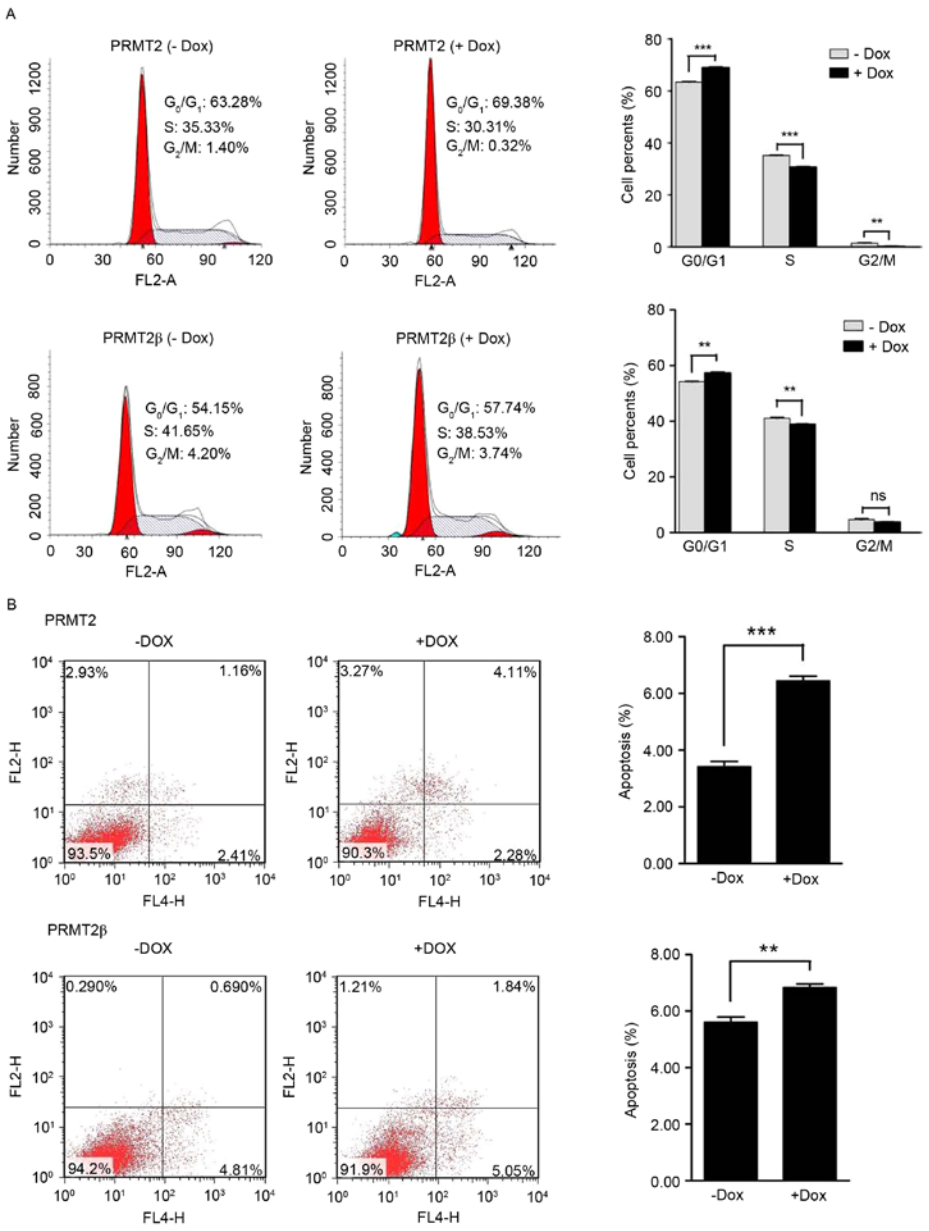

Figure 2. PRMT2β induces cell cycle arrest and apoptosis of MCF7 cells. (A) Detection of the proportion of breast cancer MCF7 cells in the cell cycle stages after overexpression of PRMT2 and PRMT2β, respectively. The proportion of cells in the G_0-G_1 phase of the cell cycle increased and the proportion of cells in the S phase decreased in the PRMT2 and PRMT2β groups (**p<0.01, ***p<0.001). Bar heights represent the mean of the data and bars represent the SEM of the data obtained in 3 independent experiments. (B) Flow cytometric analysis of MCF7 cells in the - Dox and + Dox group. Comparison of the percentage of apoptotic cells between the - Dox and + Dox groups. PRMT2 and PRMT2β overexpression significantly increased the percentage of apoptotic cells when compared with - Dox group (**p<0.01, ***p<0.001). PRMT2, arginine N-methyltransferase 2; Dox, doxycycline hyclate.

established to overexpress PRMT2β or PRMT2, and the *pGC-LV-GV308* vector was used as a negative control. Stable cell lines either with the PRMT2β-3Flag or the PRMT2-3Flag were obtained after selection with puromycin for 2 weeks. Western blot analysis was performed to verify the expression of PRMT2β-3Flag or PRMT2-3Flag induced by Dox in the infected MCF7 cells (data not shown). As shown in Fig. 1A, with treatment of 5 μg/ml of Dox, MCF7 cells carrying lentivirus PRMT2 expression exhibited a markedly decreased proliferative ability compared to those without treatment of 5 μg/ml of Dox at the indicated time-points (p<0.05; Fig. 1A). Whereas, MCF7 cells with lentivirus PRMT2β expression exhibited a weakly decreased proliferative ability compared to those without treatment of 5 μg/ml of Dox (Fig. 1B). The proliferative ability exhibited no difference in the negative control group when treated with or without 5 μg/ml of Dox (data not shown).

Colony formation assay shown in Fig. 1C, with treatment of 5 μg/ml of Dox, revealed that both the number of formed colonies and the cell number of each colony from the MCF7 cells carrying lentivirus PRMT2 expression were markedly decreased compared to the cells without treatment of 5 μg/ml of Dox (p<0.01; Fig. 1C). Whereas, with treatment of Dox, both the number of colonies formed and the cell number of each colony from the MCF7 cells carrying lentivirus PRMT2β expression were weakly decreased compared to the

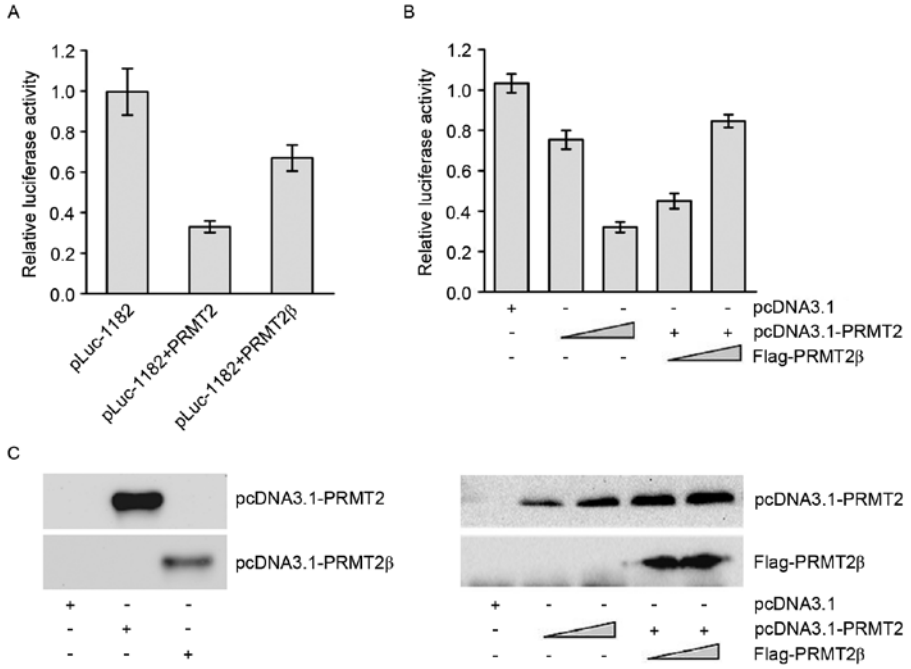

Figure 3. PRMT2β antagonizes the transcription suppressive activity of PRMT2 on the *CCND1* promoter. (A) PRMT2β suppresses the activity of the *CCND1* promoter. 293T cells were transfected with indicated constructs together with a *CCND1* luciferase reporter plasmid (pLuc-1182). Twenty-four hours after transfection, the cells were harvested, and the luciferase activity was assessed and normalized to *Renilla* activity. Each bar represents the mean ± SD for triplicate experiments. (B) The transcription suppression of PRMT2 activity was alleviated by PRMT2β. 293T cells were plated in 24-well plates 1 day before transfection. For transfections, 200 ng of pLuc-1182 reporter plasmid, 100 ng of pcDNA3.1 or 100 ng of pcDNA3.1-PRMT2 constructs plus increasing amounts of pcDNA3.1-PRMT2β (0, 10, or 100 ng) constructs were used. Total plasmid DNAs were made up to 400 ng with pcDNA3.1 empty vectors in each transfection. The cells were collected 24 h after transfection. The luciferase activity was assessed and normalized to *Renilla* activity. Each bar represents the mean ± SD for triplicate experiments. (C) The protein expression of pcDNA3.1-PRMT2 and pcDNA3.1-PRMT2β was confirmed using western blotting with anti-PRMT2 or anti-FLAG. PRMT2, arginine N-methyltransferase 2

cells without treatment of 5 μg/ml of Dox ($p<0.05$; Fig. 1C). Meanwhile, with soft agar colony formation assay we found that both the number and the size of formed colonies from the MCF7 cells carrying lentivirus PRMT2β expression were significantly decreased compared to the cells without treatment of 5 μg/ml of Dox ($p<0.05$; Fig. 1D). Similar results were also observed in the cells carrying lentivirus of PRMT2 expression, and the proliferation inhibition effect PRMT2 was shown to be more profound than that of PRMT2β ($p<0.01$; Fig. 1D). These results indicated that PRMT2β suppressed the cell proliferation and colony formation of breast cancer MCF7 cells.

PRMT2β induces cell cycle arrest and apoptosis of MCF7 cells. We then carried out cell cycle analysis to further characterize the suppressive effects of PRMT2β on the cell cycle. MCF7 cell populations in the G_0-G_1 and S phases were 57.74 and 38.53% in the PRMT2β (+ Dox) group, while they were 54.15 and 41.65% in the PRMT2β (- Dox) group. After 3 independent experiments, in the PRMT2β (+ Dox) group, the population of cells in the G_0-G_1 phase increased (vs. - Dox, $p<0.01$; Fig. 2A) and the population of cells in the S phase significantly decreased (vs. - Dox, $p<0.01$; Fig. 2A). Moreover, MCF7 cell populations in the G_0-G_1 and S phases were 69.38% and 30.31% in the PRMT2 (+ Dox) group, while they were 63.28% and 35.33% in the PRMT2 (- Dox) group. After 3 independent experiments, in the PRMT2 (+ Dox) group, the population of cells in the G_0-G_1 phase increased (vs. - Dox, $p<0.001$; Fig. 2A) and the population of cells in the S phase significantly decreased (vs. - Dox, $p<0.001$; Fig. 2A). These results revealed that PRMT2β significantly induced G_0-G_1 phase arrest in MCF7 cells.

We further examined the effect of PRMT2β on apoptosis in MCF7 cells. As shown in Fig. 2B, the percentage of apoptotic cells was 6.89% in the PRMT2β (+ Dox) group, and 5.5% in the PRMT2β (- Dox) group. After 3 independent experiments, in the PRMT2β (+ Dox) group, the apoptosis of the MCF7 cells increased (vs. - Dox, $p<0.01$; Fig. 2B). Furthermore, the percentage of apoptotic MCF7 cells in the PRMT2 (+ Dox) group was 6.39%, and 3.57% in the PRMT2 (- Dox) group. After 3 independent experiments, in the PRMT2 (+ Dox) group, the apoptosis of the MCF7 cells increased (vs. - Dox, $p<0.001$; Fig. 2B). These results revealed that PRMT2β induced the apoptosis in MCF7 cells.

PRMT2β antagonizes the transcription suppressive activity of PRMT2 on the CCND1 promoter. In a previous study, we identified that PRMT2 suppresses the *CCND1* promoter activity through indirect binding with the AP-1 site in the *CCND1* promoter and functions as a transcription coregulator (15). To understand the involvement of PRMT2β in gene transcription regulation, 293T cells were co-transfected with pcDNA3.1-PRMT2 and/or pcDNA3.1-PRMT2β and *CCND1* luciferase reporter (pLuc-1182). The results indicated that transfection of either pcDNA3.1-PRMT2 or pcDNA3.1-PRMT2β resulted in the suppression of the reporter activity (Fig. 3A), and the suppressive activity of pcDNA3.1-PRMT2β was weaker compared with that of pcDNA3.1-PRMT2. Co-transfection of FLAG-PRMT2β with

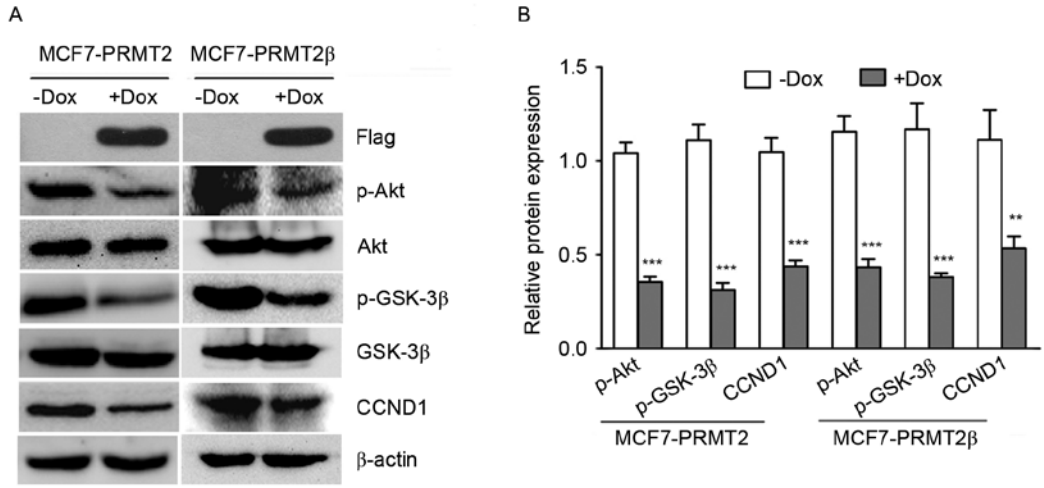

Figure 4. PRMT2β suppresses the expression of CCND1 in breast cancer MCF7 cells. (A) Western blot analysis of MCF7 cells in the - Dox and + Dox groups. Total protein extracts were assessed for total and phosphorylated Akt and GSK3β, and CCND1. The expression of p-Akt, p-GSK3β and CCND1 was decreased by PRMT2β and PRMT2 overexpression, respectively. Actin served as a loading control. (B) Relative protein levels of p-Akt, p-GSK3β and CCND1 normalized to β-actin in MCF7 cells ($^{**}p<0.01$, $^{***}p<0.001$). PRMT2, arginine N-methyltransferase 2; Dox, doxycycline hyclate.

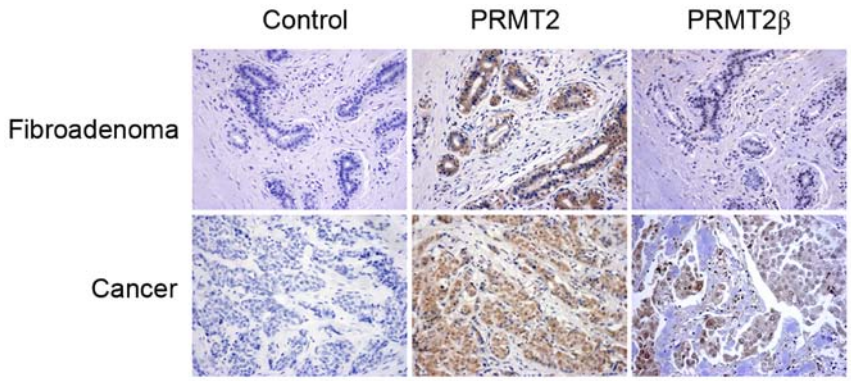

Figure 5. Protein expression of PRMT2β and PRMT2 in breast fibroadenoma and carcinoma tissues (images in original magnification, x200). PRMT2, arginine N-methyltransferase 2.

pcDNA3.1-PRMT2 led to a relieved suppression of the reporter activity by PRMT2 (Fig. 3B), suggesting that PRMT2β could antagonize the transcription suppression activity of PRMT2. The protein expression of pcDNA3.1-PRMT2, pcDNA3.1-PRMT2β and FLAG-PRMT2β was examined by western blotting with anti-PRMT2 and anti-FLAG antibodies (Fig. 3C).

PRMT2β inhibits CCND1 expression via the suppression of Akt/GSK-3β signaling in breast cancer cells. Our previous study reported that PRMT2 suppressed the CCND1 expression in MCF-7 cells partially via the suppression of Akt/GSK-3β signaling. We therefore explored whether PRMT2β was involved in the Akt/GSK-3β/CCND1 axis in MCF7 cells. As shown in Fig. 4, with treatment of 5 μg/ml of Dox, the expression of p-Akt and p-GSK3β in MCF7 cells carrying lentivirus PRMT2β expression was markedly decreased compared to the cells without treatment of 5 μg/ml of Dox. Lentivirus-mediated PRMT2β overexpression also markedly suppressed the expression of CCND1, suggesting that both the overexpression of PRMT2β and PRMT2 suppressed Akt/GSK-3β/CCND1 signaling. These results support the notion that PRMT2β suppressed the CCND1 expression in MCF-7 cells partially via the suppression of Akt/GSK-3β signaling.

Table I. Expression of PRMT2β and PRMT2 in normal breast tissue and tumors.

		PRMT2			PRMT2β		
	n	Neg.	Pos.	P-value	Neg.	Pos.	P-value
Tissue types				0.000			0.096
Normal	6	4	2		2	4	
Benign	6	1	5		3	3	
Malignant	138	6	132		98	40	

Neg., negative; Pos., positive.

PRMT2β expression is negatively correlated with human epidermal growth factor receptor 2 (HER2). To further identify the association of PRMT2β with breast cancer progression, a tissue microarray (BR1503b; US Biomax, Inc.) consisting of

Table II. Correlation of PRMT2β and PRMT2 expression with clinicopathological parameters in breast carcinoma.

		PRMT2					PRMT2β			
	n	-	+	++	+++	P-value	-	+	++	P-value
AR						0.641				0.430
-	95	5	62	25	3		65	25	5	
+	21	1	18	2	0		15	6	0	
++	21	0	11	9	1		16	4	1	
+++	1	0	1	0	0		1	0	0	
ER						0.714				0.844
-	91	5	59	22	5		65	20	6	
+	11	0	10	1	0		8	3	0	
++	10	0	7	3	0		8	2	0	
+++	26	1	15	10	0		16	10	0	
PR						0.039				0.766
-	100	4	72	19	5		71	25	4	
+	15	1	8	6	0		12	3	0	
++	9	0	4	5	0		8	1	0	
+++	14	0	8	6	0		7	5	2	
HER2						0.019				0.033
-	113	3	82	28	0		76	31	6	
+	17	2	7	5	3		15	2	0	
++	7	1	1	3	2		6	1	0	
+++	1	0	1	0	0		1	0	0	
Ki-67						0.400				0.738
-	83	4	57	19	3		59	20	4	
+	19	2	9	8	0		14	4	1	
++	24	0	16	6	2		19	4	1	
+++	12	0	9	3	0		5	7	0	
p53						0.648				0.296
-	50	2	32	16	0		35	13	2	
+	38	1	30	7	0		24	12	2	
++	14	1	7	5	1		10	2	2	
+++	36	2	22	8	4		29	7	0	

AR, androgen receptor; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

138 breast malignant tumor cases, 6 normal cases and 6 benign tumor cases was used. The tissue microarray analysis revealed that the PRMT2β protein expression exhibited a decreased tendency in different tissue types: 66.7% in normal breast tissue, 50.0% in breast benign tumors and 29.0% in breast malignant tumors, although the statistical analysis revealed no significant difference (p=0.096; Table I). Conversely, the percentage of cases with PRMT2 protein expression increased from normal breast tissue to breast malignant tumors: 33.3% in normal breast tissue, 83.3% in breast benign tumors and 95.7% in breast malignant tumors, and the difference was significant (p=0.000; Table I). The immunostainings of breast fibroadenoma and carcinoma tissue of representative cases are shown in Fig. 5. Furthermore, statistical analysis confirmed that the PRMT2β expression was negatively correlated with HER2 (p=0.033; Table II), when analyzed regardless of breast tumor types. PRMT2β expression in breast carcinoma did not exhibit a statistically significant correlation with androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), Ki-67 and the p53 status of the tumor (p>0.05; Table II). PRMT2 expression in breast carcinoma also exhibited a statistically significant correlation with the PR and HER2 status (p=0.039 and 0.019, respectively; Table II).

Discussion

Protein arginine methyltransferases (PRMTs) catalyze the methylation of a variety of protein substrates, and there are 9 PRMTs encoded in mammalian genomes. PRMTs are generally ubiquitously expressed and involved in multiple cellular processes including cell growth (16), differentiation (17), embryogenesis (18), nuclear/cytoplasmic protein shuttling (19), RNA splicing and transport (20,21), and post-transcriptional regulation (22). Dysregulation of PRMTs may probably be

implicated in the pathogenesis of some different diseases, particularly cancer. Accumulating evidence has revealed that overexpression of these enzymes is often associated with various cancers (23-26), which may make some of them viable targets for therapeutic strategies (6). Recently, the existence of alternatively spliced isoforms of PRMTs provides an additional layer of complexity. Specifically, PRMT1, PRMT2, CARM1 and PRMT7 have been shown to have alternative isoforms and most of them play an essential role on the progression and aggressiveness of cancer (24,27-30).

Our previous study found several alternative splicing variants of PRMT2, which are missing different exons in the C-terminals of the wild-type PRMT2 gene, and produce different isoforms. Particularly, due to frame-shifting, PRMT2β loses original structure, and encodes a novel amino acid sequence at the C-terminus of the protein (14). PRMT2 suppressed E2F1 transcriptional activity in an RB-dependent manner (31). PRMT2 also inhibited NF-κB function and promoted cell apoptosis (11). Our previous study found that suppression of PRMT2 expression promoted the cell proliferation of breast cancer MCF7 cells (15). To explore the role of PRMT2β in the cell proliferation, apoptosis and cell cycle in breast cancer cells, stable cell lines with PRMT2β-3Flag or PRMT2-3Flag were established. We found that the expression of PRMT2β suppressed the cell proliferation and colony formation of MCF7 cells. We also found that PRMT2β expression induced cell cycle arrest and apoptosis of MCF7 cells. In agreement with our previous observations, the wild-type PRMT2 also inhibited breast cancer cell growth. Furthermore, the function of PRMT2β was less efficient than that of the wild-type PRMT2. This may be due to the loss of exons 7-10 in PRMT2β, which affects the location of some nuclear receptor-binding interfaces in the C-terminus. However, the mechanism requires further exploration (32).

In a previous study, we identified that PRMT2 suppresses the *CCND1* promoter activity through indirect binding with the AP-1 site in the *CCND1* promoter, and functions as a transcription coregulator (15). To understand the cellular function of PRMT2β, we further investigated the role of PRMT2β in transcription regulation with 293T cells. We found that both PRMT2β and PRMT2 suppressed the activity of the CCND1 promoter, and that the suppressive activity of PRMT2β was weaker compared with that of PRMT2. With coexpression of PRMT2β and PRMT2, the bigger the amount of PRMT2β was, the weaker the inhibition of the CCND1 promoter activity was. We speculated that PRMT2β antagonizes the transcription suppressive activity of PRMT2 by competitively binding to the AP-l site of the *CCND1* promoter, attenuating the transcriptional suppression of PRMT2. It is well known that CCND1 overexpression is a common event in cancer and usually leads to defective modulation at the post-translational level (33,34). Therefore, regulation of the CCND1 protein level is one of the critical aspects in cell proliferation and tumor progression. We had previously demonstrated that miRNA-mediated PRMT2 downregulation promotes CCND1 expression via activation of Akt/GSK-3β/CCND1 signaling in breast cancer cells (15). In the present study, we confirmed that lentivirus-mediated PRMT2β expression also significantly decreased Akt/GSK-3β/CCND1 signaling, consistent with the results of wild-type PRMT2, indicating that PRMT2β functions as another negative modulator of the Akt/GSK-3β/CCND1 axis leading to the suppression of proliferation of breast cancer cells.

The tissue microarray analysis revealed that the PRMT2β protein expression exhibited a decreased expression in breast malignant tissue, although the statistical analysis revealed that this difference was not significant, indicating that PRMT2β plays a role in normal breast development and that the loss of PRMT2β expression may be related with the progression of breast cancer, consistent with the results from a cell model. Further analysis of the tissue microarray data revealed that PRMT2β expression was negatively correlated with HER2 ($p=0.033$), although the molecular mechanism remains unknown. Further investigations are required to clarify the alternative molecular mechanisms of PRMT2β in the progression of breast cancer. In summary, our results revealed that PRMT2β, a novel splice variant of PRMT2, has a potential antitumor effect through the suppression of CCND1 expression and inhibition of Akt signaling activity. This also opens a new avenue for treating breast cancer.

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