

The methylation profiles of PRDM promoters in non–small cell lung cancer

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Background: Non–small cell lung cancer (NSCLC) is one of the leading malignant tumors worldwide. Aberrant gene promoter methylation contributes to NSCLC, and PRDM is a tumor suppressor gene family that possesses histone methyltransferase activity. This study aimed to investigate whether aberrant methylation of PRDM promoter is involved in NSCLC.

Materials and methods: Primary tumor tissues, adjacent nontumorous tissues, and distant lung tissues were collected from 75 NSCLC patients including 52 lung squamous cell carcinoma (LSCC) patients and 23 lung adenocarcinoma patients. The expression of PRDMs was detected by polymerase chain reaction (PCR), Western blot, and immunohistochemical analysis. The methylation of PRDM promoters was detected by methylation-specific PCR. The correlation of methylation and expression of PRDMs with clinicopathological characteristics of patients were analyzed.

Results: mRNA expression of *PRDM2*, *PRDM5*, and *PRDM16* was low or absent in tumor tissues compared to distant lung tissues. The methylation frequencies of *PRDM2*, *PRDM5*, and *PRDM16* in tumor tissues were significantly higher than those in distal lung tissues. In LSCC patients, methylation of *PRDM2* and *PRDM16* was correlated with smoking status and methylation of *PRDM5* was correlated with tumor differentiation.

Conclusion: The expression of *PRDM2*, *PRDM5*, and *PRDM16* is low or absent in NSCLC, and this is mainly due to gene promoter methylation. Smoking may be an important cause of *PRDM2* and *PRDM16* methylation in NSCLC.

Keywords: lung tumor, PR domain zinc finger protein, epigenetics, methylation, gene expression

Introduction

Lung cancer has been a globally important public health problem for decades; it was the seventh leading cause of disease deaths in 1990 and is expected to be the fifth leading cause of disease deaths in 2010.¹ Despite encouraging progress on tumor therapy in the past decades, the prognosis of lung cancer has not been markedly improved. Non–small cell lung cancer (NSCLC) accounts for ~80% of lung cancer cases, and its prognosis is relatively better than small cell lung cancer. Epidemiological data show that the average survival of a NSCLC patient is <8 months if left untreated.² The overall 5-year survival rate for NSCLC is <17% because of the lack of early diagnosis and timely therapy. Cigarette smoking is an important risk factor of lung cancer, and other environmental risk factors for lung cancer include exposure to secondhand tobacco smoking, occupational lung carcinogens, radiation, and air pollution.³

Epigenetic regulation is a process that influences the accessibility of DNA to transcriptional regulatory factors that activate or repress gene expression. Epigenetic

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mechanisms such as aberrant DNA methylation, histone modification, chromatin remodeling, and functional non-coding RNAs have contributed to the pathogenesis of lung cancer, providing additional markers for early detection, monitoring, prognosis, risk assessment, and personalized treatment of lung cancer.^{4,5}

PR domain zinc finger proteins (PRDMs) are evolutionarily conserved zinc finger transcription factors with tissue-specific expression profile and they play key roles during cell differentiation, organ development, and human diseases.^{6,7} PRDMs regulate target gene expression through PR domain-dependent histone modification at gene promoters.⁶ PRDM genes are usually inactivated in hematological malignancies and solid cancers, and the inactivation mechanisms include promoter methylation, homozygous deletions, frameshift mutation, missense mutations, and PR domain deletion.⁸

Currently, the role of PRDMs in lung cancer remains unclear. PRDM1, PRDM2, PRDM14, and PRDM16 have been implicated in lung cancer pathogenesis, but the results were contradictory.⁹⁻¹⁴ We hypothesized that methylation-mediated PRDM inactivation may participate in the pathogenesis of NSCLC. In this study, clinical tissue specimens of primary carcinoma tissue, tumor adjacent tissue, and distant lung tissue were collected from 75 NSCLC patients, and the expression and methylation of PRDMs were analyzed. Our results demonstrated that DNA methylation of PRDM2, PRDM5, and PRDM16 was correlated with the malignancy of NSCLC.

Materials and methods

Patients and tissue specimens

A total of 52 lung squamous cell carcinoma (LSCC) patients and 23 lung adenocarcinoma (LAC) patients were enrolled in this study. The clinical characteristics of the patients are shown in Table 1. All diagnoses were confirmed by radiology combined with histopathology, and staging was according to the criteria provided by the International Association for the Study of Lung Cancer in 2009. Cancerous tissue, paracancerous tissue (<2 cm from primary carcinoma), and distant lung tissue (at least 4 cm away from primary carcinoma) were collected from each patient. Tissue specimens were paraformaldehyde-fixed for the preparation of paraffin-embedded sections or frozen in liquid nitrogen for later use. The study protocols have been approved by the ethics committee of Hunan Provincial People's Hospital, and written informed consent was obtained from all the patients.

Table 1 Clinical characteristics of the patients

Characteristics	Patients with lung squamous cell cancer, n	Patients with lung adenocarcinoma, n
Gender		
Male	43	13
Female	9	10
Age		
<60 years	16	14
≥60 years	36	9
Smoking history		
Yes	40	9
No	12	14
Tumor differentiation		
Well/moderate	32	14
Poor	20	9
Clinical stage		
I	20	13
II	23	7
III	9	3
Lymph node metastasis		
No	3	17
Yes	20	6

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted from the tissues by using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA was synthesized by reverse transcription by using RT kit (Promega Corporation, Fitchburg, WI, USA) according to the manufacturer's instructions. PCR was performed with TaqMasterMix (Promega, Madison, WI, USA). The oligonucleotide sequences of primers were designed with an online software (<http://frodo.wi.mit.edu/>) and synthesized by Takara. The sequences of primers, annealing temperatures, and the length of products are shown in Table 2. After 35 cycles, PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide, then the images were scanned by using ultraviolet (UV) gel imaging system. The expression level of target gene in each sample was calculated relative to that of β -actin.

Methylation-specific PCR (MSP)

Genomic DNA was extracted from the tissues using Universal Genomic DNA Extraction Kit (Takara, Tokyo, Japan), and modified by bisulfite treatment with EZ-DNA methylation kit (Zymo Research, Orange, CA, USA) according to the manufacturer's instructions, then used for MSP. Primer pairs for methylated and unmethylated target gene were designed with online software (<http://www.urogen.org/methprimer/index1.html>) and synthesized by Takara (Japan).

Table 2 Primers used for reverse transcription polymerase chain reaction

Target gene	Upstream primer	Downstream primer	Annealing temperature (°C)	Product length (bp)
<i>PRDM1</i>	CCACCAACAGTGAAGAGGTTAT	GGATTTCTTTCACGCTGTACTC	57.8	486
<i>PRDM2</i>	GCTCAAACAACCTTCTCAAACC	TGCCTTCAGAGTCACTACAATG	56.7	518
<i>PRDM3</i>	AGAAACAGGAGGGAGGGAGA	GCCTTTGGATCTCATGCTGT	60.2	189
<i>PRDM5</i>	GATCAAGTGGGTGCTCACAA	CATTGATAGGGACGCTCACC	58.0	474
<i>PRDM6</i>	GACCAGGATGGGACACTACAG	CACATTTCCAAAGATGCCAGT	58.8	520
<i>PRDM7</i>	GTGGGCAAGGTTTCAGTGAT	GTATGGCAGCAGAGGAGAGG	60.0	846
<i>PRDM8</i>	GTCCAATCGGCCAGAGATAA	CTCCGGGAATTTCTCTTTTC	60.2	680
<i>PRDM10</i>	TGGCCCTGCTATGAATGTAAC	GGGATTGGGATAGTGGTCTGT	60.4	323
<i>PRDM12</i>	CTCGGCATCTTCTCCAAGAC	TGAGTTTCCGTACCACACCA	60.1	318
<i>PRDM13</i>	AATCAGCAGCTGTCCGAGAT	GTCTGTGAAGCAGACGTCCA	59.5	501
<i>PRDM14</i>	AGGTGGGTGGATCACTCAAG	CTGGTTCTGTACCCAGGTT	60.0	225
<i>PRDM15</i>	GGATGACTGCAACTGGATGAT	CTGTTCTTTGGTGGCAACATT	57.8	454
<i>PRDM16</i>	AAATACTGACGGACGTGGAAGT	GACACTGGTCGCATTTGTACTC	59.1	555
<i>PRDM17</i>	TCTGGCTGGACTAGCTTGGT	GTAGGGAAGCCAGACTGCTG	60.0	623
<i>β-Actin</i>	CACGATGGAGGGGCCGGACTCATC	TAAAGACCTCTATGCCAACACAGT	62.9	225

The sequences of primers, annealing temperatures, and the length of products are shown in Table 3. After 35 cycles, PCR products were electrophoresed on 2% agarose gel and stained with ethidium bromide, then the images were scanned by using UV gel imaging system.

Western blot analysis

Tissues were lysed in RIPA lysis buffer, lysate was centrifuged at 15,000×g for 30 minutes at 4°C, the supernatant was collected and protein concentration was measured by BSA method. Fifty microgram protein samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, the membranes were blocked with 5% non-fat dry milk for 1 hour at room temperature and then incubated with specific antibody (Abcam, Cambridge, MA, USA) against *PRDM2* (1:500 dilution), *PRDM5* (1:500 dilution), *PRDM16* (1:500 dilution), or β -actin (1:2,000 dilution) at 4°C overnight. The membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. Finally, the membranes were developed using ECL kit (Pierce, Rockford, IL, USA) and exposed to X-ray film for analysis by Image.plus5.1 software (Media Cybernetics, Rockville, MD, USA).

Immunohistochemistry (IHC)

IHC was performed on paraformaldehyde-fixed and paraffin-embedded tissue sections. Tissue sections (4 μ m thickness) were deparaffinized with xylene and rehydrated with graded alcohol, then treated with antigen retrieval solution

(10 mmol/L sodium citrate buffer, pH 6.0). The sections were incubated with specific antibody (Abcam; 1:150 dilution, omitted in negative control) against *PRDM2*, *PRDM5*, *PRDM16*, or β -actin at 4°C overnight, then incubated with biotinylated secondary antibody (1:1,000 dilution), followed by avidin-biotin-peroxidase complex, according to the manufacturer's instructions. Finally, the sections were incubated with 3',3'-diaminobenzidine and counterstained with Harris' modified hematoxylin. The staining intensity was graded by using a 4-point scale: 0, no staining; 1, light yellow; 2, brown; and 3, dark brown. The proportion of cells stained was assessed by using a 3-point scale: 1, <30% staining; 2, 30%–70% staining; and 3, >70% staining. The combined value of 4-point scale and 3-point scale was assessed as follows: 0–1, negative staining; 2, weakly positive staining; 3, positive staining; and ≥ 4 , strong positive staining.

Statistical analysis

Data are presented as mean \pm standard deviation. All statistical analyses were performed by using SPSS13.0 software (SPSS Inc., Chicago, IL, USA). Student's *t*-test or one-way analysis of variance followed by the Newman–Keuls test was performed to analyze numerical data. Chi-square test was performed to analyze categorical data. $P < 0.05$ was considered significant.

Results

mRNA expression of PRDM family members in lung cancer tissues

The mRNA expression of *PRDM1*, *PRDM2*, *PRDM3*, *PRDM5*, *PRDM6*, *PRDM7*, *PRDM8*, *PRDM10*, *PRDM12*,

Table 3 Primers used for methylation specific-polymerase chain reaction

Name	Target gene	Upstream primers	Downstream primer	Annealing temperature (°C)	Product length (bp)
M-PRDM2	Methylated PRDM2	GGTAGTAAATAAAATTTAGTAGTTGCGT	CCCTAAAACATAAAATCCTACGTA	55.4	206
U-PRDM2	Unmethylated PRDM2	TTAGGGTAGTAAATAAAATTTAGTAGTTGTG	CACCCATAAACTAAAATCCTACATA	56.0	212
M-PRDM5	Methylated PRDM5	CGTTATTCGAGTAGGTAGCGTTAC	CATCTTACGACAAAAAACCAGAT	56.8	101
U-PRDM5	Unmethylated PRDM5	TGTTATTTGAGTAGGTAGTGTATGT	AACATCTTACAAACAAAAAACCAAT	53.8	103
M-PRDM16	Methylated PRDM16	GTAACCGGAGTAAATTAGTTCCG	CGAAAATAAAACGACGACTACG	58.8	106
U-PRDM16	Unmethylated PRDM16	TAGTAAATGGAGTAAATTAGTTTGG	CCAAAAATAAAACAACTACAAT	55.9	109

PRDM13, PRDM14, PRDM15, PRDM16, and PRDM17 was detected by RT-PCR in primary carcinoma tissues, tumor adjacent tissues, and distant lung tissues. The results showed that mRNA expression levels of *PRDM2*, *PRDM5*, and *PRDM16* in primary carcinoma tissues were lower than in tumor adjacent tissues and distant lung tissues, mRNA expression levels of PRDM1, PRDM10, PRDM14, and PRDM15 in primary carcinoma tissues were not different from tumor adjacent tissues and distant lung tissues, while mRNA expression level of PRDM6 was higher in primary carcinoma tissues than in tumor adjacent tissues and distant lung tissues (Figures 1 and 2; Tables 4 and 5). For other PRDMs, we could not detect their mRNA expression (data not shown).

Methylation status of the promoters of PRDM family members in lung cancer tissues

We wondered whether decreased *PRDM2*, *PRDM5*, and *PRDM16* mRNA expression in tumor tissues was due to promoter methylation; hence, we performed MSP and the results are shown in Figures 3 and 4 and Table 6.

In patients with LSCC, *PRDM2* gene methylation (with fully methylated and partially methylated) frequency was 67.3%, 50.0%, and 17.3%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues. In patients with LAC, *PRDM2* gene methylation frequency was 78.3%, 34.8%, and 21.7%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues. In patients with LSCC, *PRDM5* gene methylation was 73.1%, 44.2%, and 21.1%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues. In patients with LAC, *PRDM5* gene methylation frequency was 82.6%, 47.8%, and 17.4%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues. In patients with LSCC, *PRDM16* gene methylation was 80.8%, 40.4%, and 21.2%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues. In patients with LAC, *PRDM16* gene methylation frequency was 82.6%, 52.2%, and 30.4%, respectively, in tumor tissues, adjacent tissues, and distant lung tissues.

Protein expression of PRDM family members in lung cancer tissues

To confirm the results of RT-PCR, we performed Western blot analysis on lung cancer tissues. The results showed that protein expression levels of *PRDM2*, *PRDM5*, and *PRDM16* in primary carcinoma tissues were lower than in tumor adjacent tissues and distant lung tissues (Figure 5). Furthermore, IHC staining showed that protein expression levels

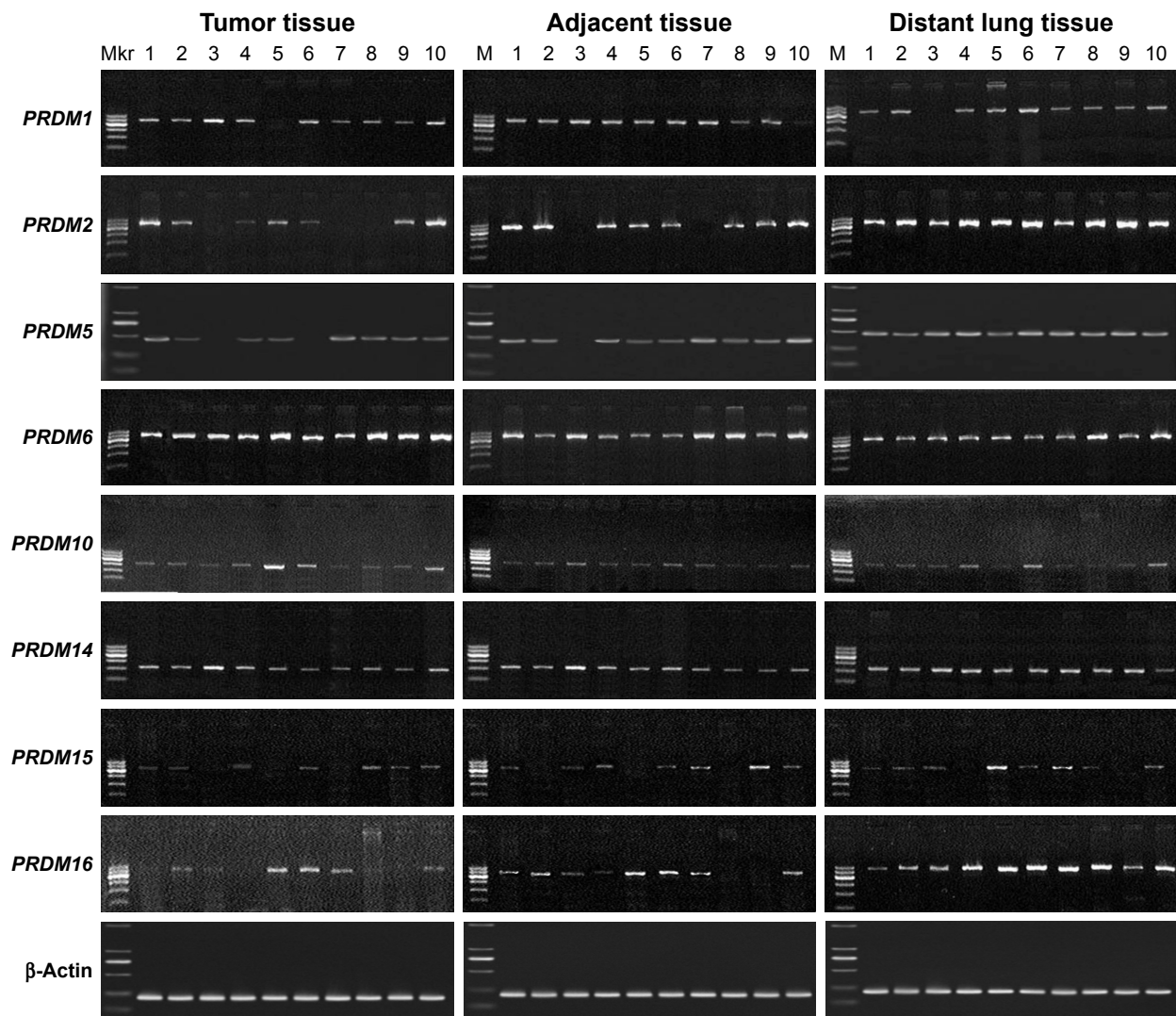


Figure 1 Representative RT-PCR results to detect the expression of PRDM mRNAs in tumor tissues, adjacent tissues, and distant lung tissues of lung squamous cell carcinoma patients.

Note: Lane 1–10: samples from no. 1–10 patients.

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; PRDM, PR domain zinc finger protein; Mkr, marker.

of *PRDM2*, *PRDM5*, and *PRDM16* in primary carcinoma tissues were lower than in tumor adjacent tissues and distant lung tissues (Figure 6; Table 7).

Correlation of *PRDM2*, *PRDM5*, and *PRDM16* methylation and clinical aspects of lung cancer patients

Next we analyzed the correlation of *PRDM2*, *PRDM5*, and *PRDM16* gene methylation status with the age, gender, smoking, tumor differentiation, clinical stage, lymph node metastasis in lung squamous carcinoma, and LAC patients. The results showed that *PRDM2* and *PRDM16* gene methylation in LSCC patients was related to smoking, and methylation status of *PRDM5* gene was associated with tumor differentiation of LSCC (Table 8). However, *PRDM2*, *PRDM5*, and

PRDM16 gene methylation showed no correlation with age, gender, smoking, tumor differentiation, clinical stage, and lymph node metastasis in LAC patients (Table 9).

Discussion

In the past few decades, comprehensive utilization of various treatment methods has significantly improved cancer survival, but the prognosis of patients with lung cancer did not significantly change. Tobacco is still the most important risk factor for lung cancer.³ Because environmental tobacco exposure is a risk factor of lung cancer, the role of epigenetic mechanisms in the pathogenesis of lung cancer has attracted more attention. Tobacco exposure is directly related to gene methylation abnormality and the inactivation of tumor suppressor gene expression.¹⁵ Thus, abnormal methylation plays

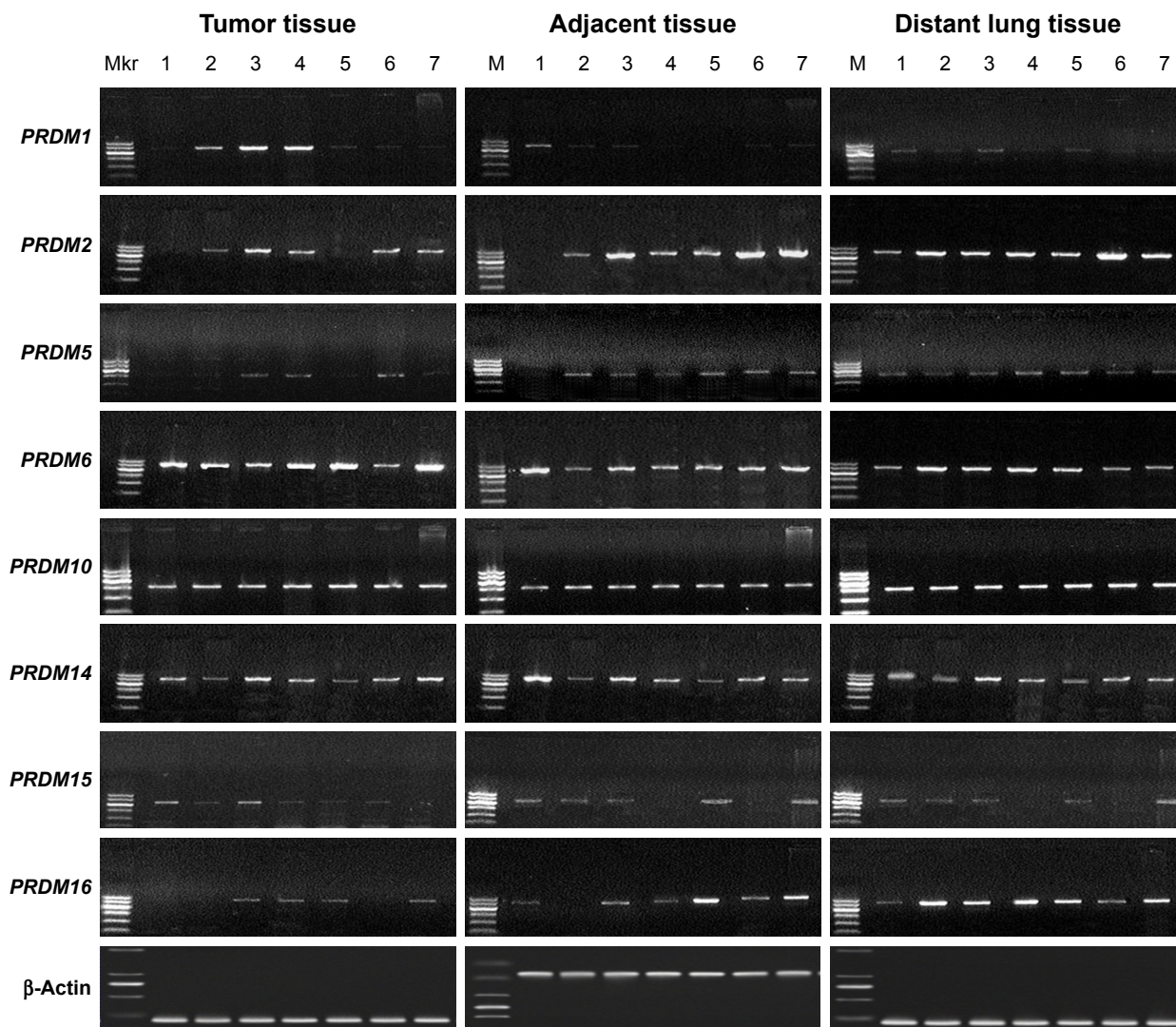


Figure 2 Representative RT-PCR results to detect the expression of PRDM mRNAs in tumor tissues, adjacent tissues, and distant lung tissues of lung adenocarcinoma patients.

Note: Lane 1–7: samples from no. 1–7 patients.

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; PRDM, PR domain zinc finger protein; Mkr, marker.

Table 4 PRDM mRNA levels in lung tissues of lung squamous cell carcinoma patients (n=52)

Gene	Primary carcinoma tissue	Tumor adjacent tissue	Distant lung tissue
PRDM1	0.36±0.14	0.37±0.10	0.34±0.13
PRDM2	0.38±0.15	0.60±0.11 [^]	0.77±0.14 ^{^*}
PRDM5	0.23±0.11	0.41±0.14	0.57±0.16 ^{^*}
PRDM6	0.94±0.18	0.71±0.24 [^]	0.67±0.16 [^]
PRDM10	0.27±0.12	0.24±0.09	0.23±0.10
PRDM14	0.35±0.09	0.37±0.08	0.37±0.07
PRDM15	0.16±0.10	0.14±0.09	0.18±0.11
PRDM16	0.26±0.13	0.52±0.23 [^]	0.67±0.21 ^{^*}

Notes: [^]Comparison with tumor tissue, $P<0.05$; ^{^*}Compared with adjacent tissues, $P<0.05$. Data are presented as mean ± standard deviation.

Abbreviation: PRDM, PR domain zinc finger protein.

Table 5 PRDM mRNA levels in lung tissues of lung adenocarcinoma patients (n=23)

Gene	Primary carcinoma tissue	Tumor adjacent tissue	Distant lung tissue
PRDM1	0.32±0.13	0.28±0.10	0.26±0.10
PRDM2	0.50±0.19	0.72±0.21 [^]	0.89±0.17 ^{^*}
PRDM5	0.21±0.16	0.34±0.13 [^]	0.45±0.09 ^{^*}
PRDM6	0.85±0.14	0.76±0.15 [^]	0.71±0.12 [^]
PRDM10	0.57±0.08	0.54±0.09	0.58±0.06
PRDM14	0.37±0.10	0.39±0.09	0.41±0.06
PRDM15	0.18±0.10	0.16±0.08	0.15±0.09
PRDM16	0.28±0.15	0.47±0.22 [^]	0.64±0.18 ^{^*}

Notes: [^]Comparison with tumor tissue, $P<0.05$; ^{^*}Compared with adjacent tissues, $P<0.05$. Data are presented as mean ± standard deviation.

Abbreviation: PRDM, PR domain zinc finger protein.

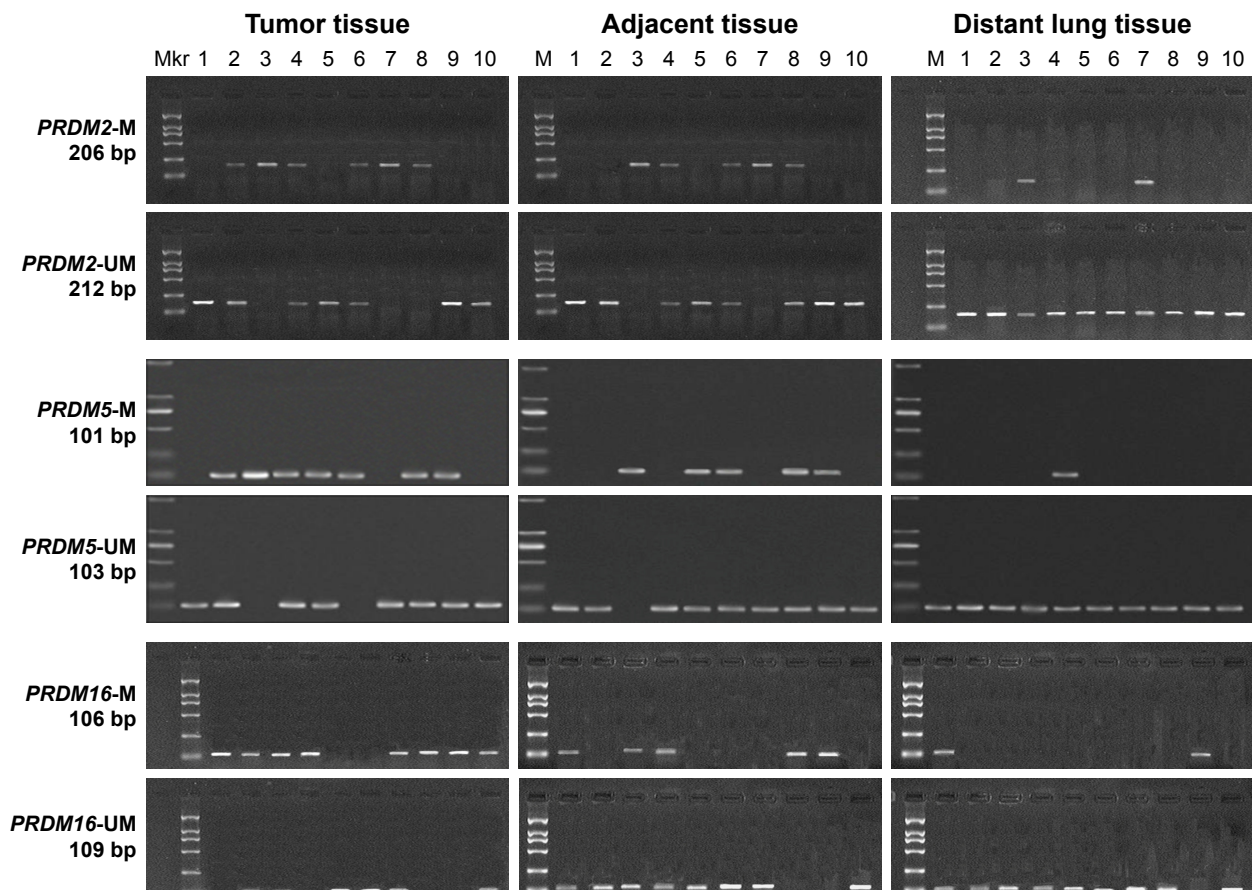


Figure 3 Representative MSP results to detect the methylation of *PRDM2*, *PRDM5*, and *PRDM16* promoters in tumor tissues, adjacent tissues, and distant lung tissues of lung squamous cell carcinoma patients.

Note: Lane 1–10: samples from no. 1–10 patients.

Abbreviation: MSP, methylation-specific polymerase chain reaction; Mkr, marker; M, methylated; UM, unmethylated.

an important role in the pathogenesis of lung cancer and provides a reference for early diagnosis and individualized treatment of lung cancer.¹⁶

At present, 17 PRDM coding genes from the human genome have been identified and named PRDM1 to PRDM17.^{6,7} PRDM is widely involved in the pathogenesis of many kinds of tumors and is an important tumor suppressor gene family.⁸ In this study, we collected tumor tissues and surrounding tissues from patients with NSCLC. RT-PCR, Western blot, and IHC staining showed that *PRDM2*, *PRDM5*, *PRDM16* mRNA, and protein expression levels of distant lung tissues were lower than in cancer tissues and adjacent tissues. These results suggest that *PRDM2*, *PRDM5*, and *PRDM16* expression may be suppressed due to gene hypermethylation in lung cancer. Methylation-specific PCR confirmed that *PRDM2*, *PRDM5*, and *PRDM16* gene methylation frequency of tumor tissues was higher than that of the adjacent tissues.

LSCC is more common in smokers, and abnormal methylation is a common mechanism of smoking-related diseases.¹⁵

Our results showed that in LSCC, *PRDM2* and *PRDM16* gene methylation was correlated with the smoking of the patients, indicating that smoking may be an important cause of *PRDM2* and *PRDM16* gene methylation in squamous cell carcinoma of the lung. Yoon et al reported that the single-nucleotide polymorphism of *PRDM2* gene was associated with the risk of lung cancer.¹² However, we failed to find significant correlation between *PRDM2* and *PRDM16* gene methylation and clinical pathological characteristics of LAC patients. This may be due to the small sample size of this study, but *PRDM2* and *PRDM16* gene methylation may differ between LAC and squamous cell carcinoma of the lung. Further studies are needed to elucidate the mechanism.

Notably, we found that *PRDM5* gene methylation in LSCC was related to tumor differentiation because poorly differentiated squamous cell carcinoma had higher methylation ratio. *PRDM5* may be involved in the regulation of differentiation of LSCC and *PRDM5* gene methylation may help evaluate the prognosis of squamous cell carcinoma of

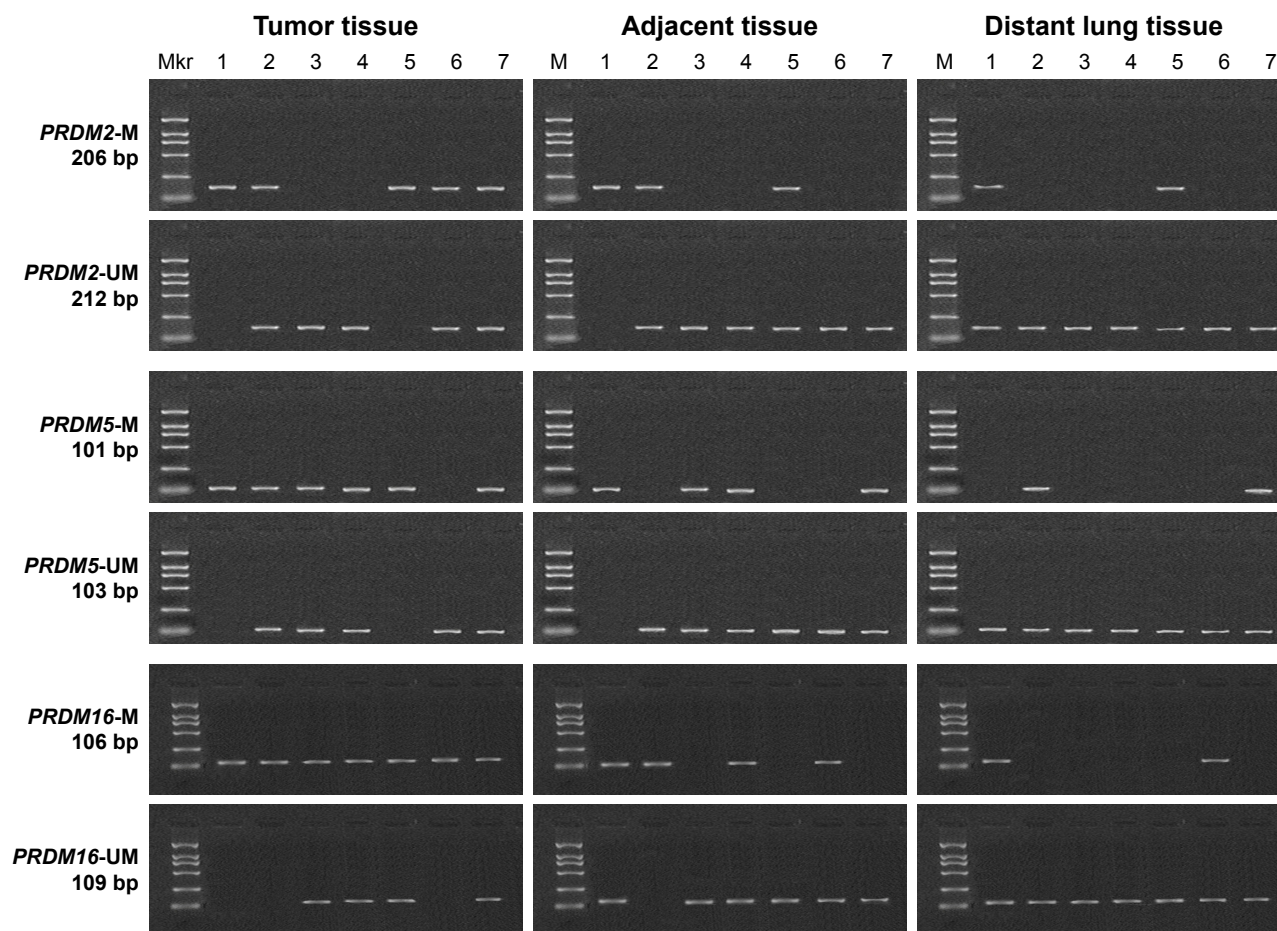


Figure 4 Representative MSP results to detect the methylation of *PRDM2*, *PRDM5*, and *PRDM16* promoters in tumor tissues, adjacent tissues, and distant lung tissues of lung adenocarcinoma patients.

Note: Lane 1–7: samples from no. 1–7 patients.

Abbreviation: MSP, methylation-specific polymerase chain reaction; Mkr, marker; M, methylated; UM, unmethylated.

the lung. The methylation of *PRDM5* resulted in decreased expression of *PRDM5* in nasopharyngeal carcinoma, esophageal cancer, gastric cancer, cervical cancer, colon cancer, and other tumors.^{16–19} However, the expression of *PRDM5*

in lung cancer has not been reported. Our study provided the first evidence that the methylation of *PRDM5* gene is involved in the pathogenesis of lung cancer and is related to tumor differentiation in patients with LSCC. *PRDM5*

Table 6 *PRDM2*, *PRDM5*, and *PRDM16* methylation status in lung tissues

	Lung squamous cell carcinoma, n (%)			Lung adenocarcinoma, n (%)		
	Tumor tissues	Adjacent tissues	Distant lung tissues	Tumor tissues	Adjacent tissues	Distant lung tissues
<i>PRDM2</i>						
Fully methylated	12 (23.1)	8 (15.4)	0 (0)	5 (21.7)	2 (8.7)	0 (0)
Partially methylated	23 (44.2)	18 (34.6)	9 (17.3)	13 (56.5)	6 (26.1)	5 (21.7)
Unmethylated	17 (32.7)	26 (50.0)	43 (82.7)	5 (21.7)	15 (65.2)	18 (78.3)
<i>PRDM5</i>						
Fully methylated	14 (26.9)	8 (15.4)	2 (3.8)	8 (34.8)	4 (17.4)	0 (0)
Partially methylated	24 (46.2)	15 (28.8)	9 (17.3)	11 (47.8)	7 (30.4)	4 (17.4)
Unmethylated	14 (26.9)	29 (55.8)	41 (78.8)	4 (17.4)	12 (52.2)	19 (82.6)
<i>PRDM16</i>						
Fully methylated	18 (34.6)	7 (13.5)	3 (5.8)	7 (30.4)	2 (8.7)	1 (4.3)
Partially methylated	24 (46.2)	14 (26.9)	8 (15.4)	12 (52.2)	10 (43.5)	6 (26.1)
Unmethylated	10 (19.2)	31 (59.6)	41 (78.8)	4 (17.4)	11 (47.8)	16 (69.6)

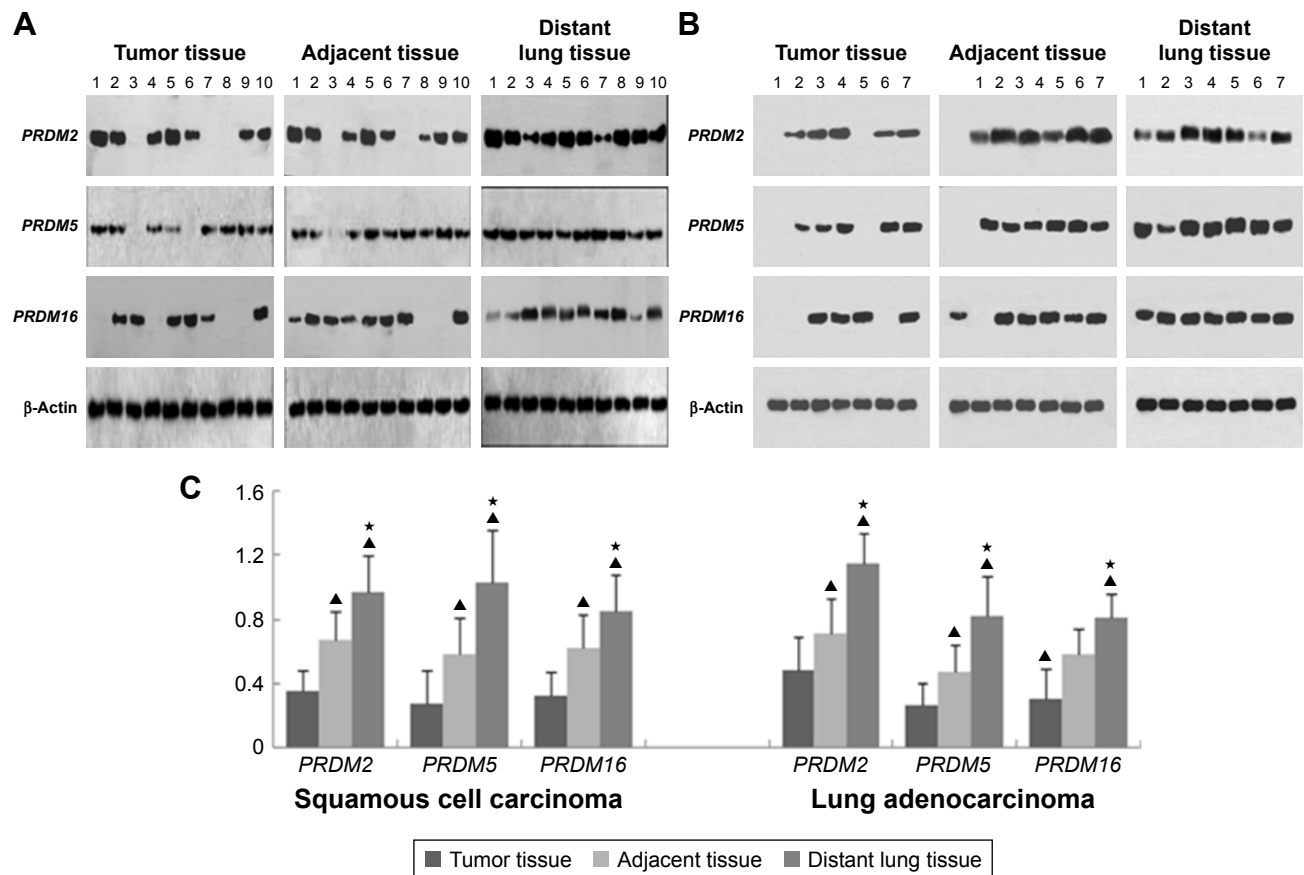


Figure 5 Western blot analysis of PRDM expression in clinical samples.

Notes: (A) Representative blots showing the expression of PRDM proteins in tumor tissues, adjacent tissues, and distant lung tissues of lung squamous cell carcinoma patients. M: marker; lane 1–10: samples from no. 1–10 patients. (B) Representative blots showing the expression of PRDM proteins in tumor tissues, adjacent tissues, and distant lung tissues of lung adenocarcinoma patients. M: marker; lane 1–7: samples from no. 1–7 patients. (C) Densitometry analysis of relative levels of PRDM proteins as shown in A and B. *Comparison with tumor tissue, $P < 0.05$; *Compared with adjacent tissues, $P < 0.05$.

Abbreviation: PRDM, PR domain zinc finger protein.

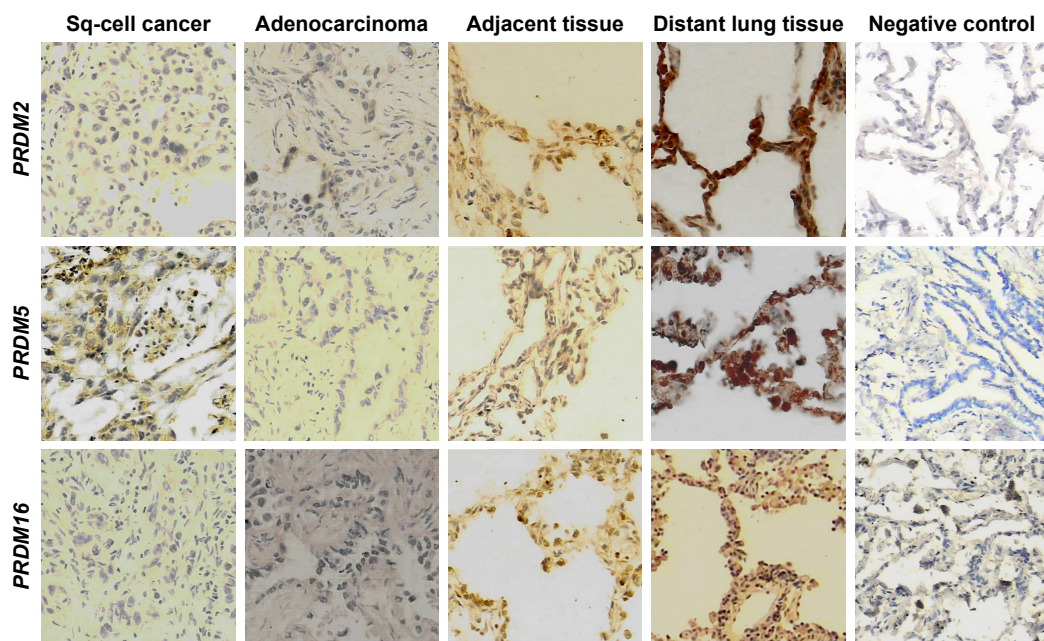


Figure 6 Representative immunohistochemical staining of PRDM2, PRDM5, and PRDM16 in clinical samples.

Note: Magnification: 200×.

Abbreviation: Sq-cell, squamous cell.

Table 7 Immunohistochemical staining of *PRDM2*, *PRDM5*, and *PRDM16* in clinical specimens

	Squamous cell carcinoma, n			Lung adenocarcinoma, n		
	Tumor	Adjacent	Distant	Tumor	Adjacent	Distant
<i>PRDM2</i>						
Negative	15	8	0	7	2	0
Weakly positive	16	8	6	10	5	2
Positive	16	20	16	4	9	6
Strong positive	5	16	30	2	7	15
<i>PRDM5</i>						
Negative	18	10	2	10	5	0
Weakly positive	16	13	7	7	5	2
Positive	16	16	11	5	6	7
Strong positive	2	13	32	1	7	14
<i>PRDM16</i>						
Negative	22	9	3	9	4	1
Weakly positive	18	15	10	9	9	8
Positive	12	24	31	5	9	11
Strong positive	0	4	8	0	1	3

lacks intrinsic histone methyltransferase activity, but it can recruit other histone methyltransferases to the promoter regions and regulate the transcription of tumor-associated genes.²⁰ In addition, *PRDM5* interacts with insulator factors to modulate chromatin organization and regulate target gene transcription.²¹ Further mechanistic studies are needed to understand the role of *PRDM5* in lung cancer.

PRDM16 participates in the regulation of cell differentiation and tissue and organ development.²² Current studies mainly focus on *PRDM16* and blood tumors, and the

results showed that *PRDM16* promoted the occurrence of bone marrow hyperplasia syndrome and leukemia.^{23,24} Man et al reported that *PRDM16* gene copy number increased in osteosarcoma patients.²⁵ Takahata et al reported that *PRDM16* expression increased in gastric cancer cells and promoted tumor development by inhibiting TGF-beta signaling pathway.¹⁴ These findings suggest that *PRDM16* may act as an oncogene to promote tumor. However, a recent study reported that *PRDM16* functions as a tumor suppressor and its highly specific H3 K4 methyltransferase activity is essential for

Table 8 Correlation of *PRDM2*, *PRDM5*, and *PRDM16* methylation with clinical characteristics of lung squamous cell carcinoma

	<i>PRDM2</i> , n			<i>PRDM5</i> , n			<i>PRDM16</i> , n		
	m	un-m	P-value	m	un-m	P-value	m	un-m	P-value
Age									
<60 years	10	6		12	4		14	2	
≥60 years	25	11		26	10		28	8	
Gender									
			>0.05			>0.05			>0.05
Male	29	14		32	11		35	8	
Female	6	3		6	3		7	2	
Smoking									
			<0.05			>0.05			<0.05
Yes	30	10		30	10		36	4	
No	5	7		8	4		6	6	
Tumor differentiation									
			>0.05			<0.05			>0.05
High/middle	20	12		20	12		24	8	
Low	15	5		18	2		18	2	
Clinical stages									
			>0.05			>0.05			>0.05
I	14	9		14	9		17	6	
II	13	7		16	4		18	2	
III	8	1		8	1		7	2	
Lymph node metastasis									
			>0.05			>0.05			>0.05
No	19	13		23	9		24	8	
Yes	16	4		15	5		18	2	

Abbreviations: m, methylated; un-m, unmethylated.

Table 9 Correlation of *PRDM2*, *PRDM5*, and *PRDM16* methylation with clinical characteristics of lung adenocarcinoma

	<i>PRDM2</i> , n			<i>PRDM5</i> , n			<i>PRDM16</i> , n		
	m	un-m	P-value	m	un-m	P-value	m	un-m	P-value
Age									
<60 years	11	3		11	3		11	3	
≥60 years	7	2		8	1		8	1	
Gender			>0.05			>0.05			>0.05
Male	10	3		12	1		12	1	
Female	8	2		7	3		7	3	
Smoking			>0.05			>0.05			>0.05
Yes	8	1		8	1		7	2	
No	10	4		11	3		12	2	
Tumor differentiation			>0.05			>0.05			>0.05
High/middle	10	4		10	4		11	3	
Low	8	1		9	0		8	1	
Clinical stages			>0.05			>0.05			>0.05
I	9	4		10	3		10	3	
II	6	1		6	1		6	1	
III	3	0		3	0		3	0	
Lymph node metastasis			>0.05			>0.05			>0.05
No	13	4		14	3		13	4	
Yes	5	1		5	1		6	0	

Abbreviations: m, methylated; un-m, unmethylated.

suppressing MLL1-rearranged acute leukemia.²⁶ Currently, the expression of *PRDM16* has not been reported in lung cancer patients. Our study is the first to show that *PRDM16* expression is repressed in lung cancer patients, which is related to the methylation of *PRDM16* promoter. These data suggest that *PRDM16* may be a tumor suppressor in lung cancer.

Although we reported that *PRDM2*, *PRDM5*, and *PRDM16* gene methylation was correlated to their low expression levels in lung cancer samples, we could not exclude other mechanisms such as chromosome translocation, microsatellite instability, allelic loss, gene copy number variation, gene mutation, and non-coding RNAs that contribute to the regulation of *PRDM2*, *PRDM5*, and *PRDM16* expression in NSCLC. In addition, the role of *PRDM2*, *PRDM5*, and *PRDM16* in the pathogenesis of NSCLC may be related to the influence of genomic stability due to histone methyltransferase activity of PRDMs. Further studies are needed to provide deep understanding of the role of PRDMs in lung cancer.

In summary, we found that the expression of *PRDM2*, *PRDM5*, and *PRDM16* is low or absent in NSCLC tissues, and this is mainly due to gene promoter methylation. Smoking may be an important cause of *PRDM2* and *PRDM16* methylation in NSCLC. While our data indicate that PRDM status may be helpful for the diagnosis of lung cancer, large-scale studies are needed to evaluate the potential of PRDMs as diagnostic and prognostic markers of lung cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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