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

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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
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.5-7 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.urogene.org/methprimer/index1.html) and synthesized by Takara (Japan).
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, PRDM16, 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 μm 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 ± 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 2 Primers used for reverse transcription polymerase chain reaction

| Target gene | Upstream primer | Downstream primer | Annealing temperature (°C) | Product length (bp) |
|-------------|-----------------|--------------------|-----------------------------|--------------------|
| PRDM1       | CCACCAACAGTGAGGGTTAT | GGATTTCTTCACACCTGACTC | 57.8 | 486 |
| PRDM2       | GCCTCAACACTCTCCAACT | TGCCCTCAGAGTGACACATG | 56.7 | 518 |
| PRDM3       | AGAAACAGGAGGGAGGAAGA | GCTCCTGAGTCTCACCTG | 60.2 | 189 |
| PRDM5       | GCATCAAGGGGGCTGCAAA | CATTGATAGGGACGTCACC | 58.0 | 474 |
| PRDM6       | GACCGAGCTGAGGACACTACG | CACATTTCCAGATGCGAGT | 58.8 | 520 |
| PRDM7       | GTGGAACCTGTCAGTTGACTG | GTATGGGACAGAGGAGAGG | 60.0 | 846 |
| PRDM8       | GTCAATTGGCGGACGATAAA | CGTCGGGAAATTCTCCTTT | 60.2 | 680 |
| PRDM10      | TTGCCCTGCTTGAAGTGTAAC | GGATTTGGGATGTGTTCTG | 60.4 | 323 |
| PRDM12      | CTGGGAGCTCTCCAAAGAC | TGAGTTCCGATAACCACCA | 60.1 | 318 |
| PRDM13      | CATCGAGCTGCTCCGAGAT | GCTGTTGACAGACCTCGCA | 59.5 | 501 |
| PRDM14      | AGGTGGTGATGACACTCAAG | CTGTTCTGTACCCAGGTC | 60.0 | 225 |
| PRDM15      | GATGACTGCAAATCTTGATG | CGTGTTTCTTGGGCAATT | 57.8 | 454 |
| PRDM16      | AAATACTGACAGGAGGCTGAAG | GACACTGTCGACTTTGACTC | 59.1 | 555 |
| PRDM17      | TCTGGCCTGACTAGCTTTGT | GATGGAAGGACACAGACTG | 60.0 | 623 |
| β-Actin     | CACAGATGAGGGGCGCCGACTCATC | TAAAGACCTCTATGCCAACAGT | 62.9 | 225 |
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
PRDM methylation in NSCLC

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; **Comparison 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; **Comparison with adjacent tissues, P<0.05. Data are presented as mean ± standard deviation.

Abbreviation: PRDM, PR domain zinc finger protein.
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.4 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
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. 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 |
|                     |                                     |                           |                     |
| **PRDM2**           |                                     |                           |                     |
| Fully methylated    | 12 (23.1)                           | 8 (15.4)                  | 0 (0)               |
| Partially methylated| 23 (44.2)                           | 18 (34.6)                 | 9 (17.3)            |
| Unmethylated        | 17 (32.7)                           | 26 (50.0)                 | 43 (82.7)           |
| **PRDM5**           |                                     |                           |                     |
| Fully methylated    | 14 (26.9)                           | 8 (15.4)                  | 2 (3.8)             |
| Partially methylated| 24 (46.2)                           | 15 (28.8)                 | 9 (17.3)            |
| Unmethylated        | 14 (26.9)                           | 29 (53.8)                 | 41 (78.8)           |
| **PRDM16**          |                                     |                           |                     |
| Fully methylated    | 18 (34.6)                           | 7 (13.5)                  | 3 (5.8)             |
| Partially methylated| 24 (46.2)                           | 14 (26.9)                 | 8 (15.4)            |
| Unmethylated        | 10 (19.2)                           | 31 (59.6)                 | 41 (78.8)           |
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 |
| **PRDM2**       |        |          |         |        |          |         |
| 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      |
| **PRDM5**       |        |          |         |        |          |         |
| 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      |
| **PRDM16**      |        |          |         |        |          |         |
| 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       |

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

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

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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