で提案されているTSLC1/CADM1に注目した治療法が西南日本同様、東日本の日本海沿岸地域で行うことが妥当かどうかを判断するための生物学的根拠が十分であるとはいえない。

そこで本分担研究は、TSLC1/CADM1 とその関連分子、あるいは TSLC1/CADM1 抑制性 miRNA が東日本の日本海沿岸（秋田県）で発生する ATL においても同様の異常あるいは傾向を示すかを実証するべく、行われている。まず、秋田県内各病院の病理解剖例の参照により、過年度１９例、本年度１例の ATL 症例が同定された。この症例規模は上記の治療法の妥当性検討や病理学的研究一般のために十分な症例数が達成されたと考える。

　病理解剖例を用いた分子病理学的解析として、TSLC1/CADM1 免疫組織化学を２０症例６８病変に行い、８８．２％の病変で TSLC1/CADM1 の強発現を確認した。この結果は、従来の臨床材料および細胞株を用いた検討の結果を裏付ける。同時に TSLC1/CADM1 系については、西南日本 ATL 症例も日本海側東北地方の ATL 症例も同様の傾向であり、TSLC1/CADM1 系に着目した治療戦略が ATL に対して一般性を有することがわかる。また、２例８病変で TSLC1/CADM1 の発現が中等度陽性にとどまる。これらの症例に関しては、化学療法施行前生検検体でのＡＴＬ細胞のTSLC1/CADM1 発現を検討することによって、TSLC1/CADM1 が疾病の経過中に減弱したのか、あるいはこの２症例の病理解剖

例のパラフィンブロックの状態が不良であったのか、検証を要すると考える。

CADM1 の発現を抑制する miR-214/199a-5p と miR-375 に関し、TSLC1/CADM1 発現(+)および(++)の病変の間で、発現量に有意な差はみられなかった。病理検体を用いた検討では、ATL 細胞以外の反応性リンパ球や各種細胞の混入が避けられないため、ATL 細胞に限定した microRNA 測定が困難であった可能性がある。

E．結論

秋田県内病院の 20 例の ATL 病理解剖例につき、免疫組織化学的に TSLC1/CADM1 高発現が検討病変中の 88.2%と極めて高率に見られることが示された。

F．健康危険情報

特になし。

G. 研究発表

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H. 知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得：なし。

2. 実用新案登録：なし

3. その他：なし

III. 研究成果の刊行物一覧

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
なし							

雑誌

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IV. 研究成果の刊行物・別刷

ELSEVIER

Original contribution

Expression of PRMT5 in lung adenocarcinoma and its significance in epithelial-mesenchymal transition ☆,☆☆

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Summary Although protein arginine methyltransferase 5 (PRMT5) has been implicated in various cancers, its expression pattern in lung adenocarcinoma cell lines and tissues has not been elucidated enough. In this study, microarray analysis of 40 non–small-cell lung carcinoma cell lines showed that *PRMT5* was a candidate histone methyltransferase gene that correlated with epithelial-mesenchymal transition. Immunocytochemical analysis of these cell lines indicated that the expression of PRMT5 was localized to the cytoplasm of E-cadherin–low and vimentin-high cell lines, whereas it was predominant in the nucleus and faint in the cytoplasm of E-cadherin–high and vimentin-low cell lines. Immunohistochemical analysis of lung adenocarcinoma cases (n = 130) revealed that the expression of PRMT5 was high in the cytoplasm of 47 cases (36%) and the nuclei of 34 cases (26%). The marked cytoplasmic expression of PRMT5 was frequently observed in high-grade subtypes (1 of 17 low grade, 21 of 81 intermediate grade, and 25 of 32 high grade; *P* < .0001) such as solid adenocarcinoma with the low expression of thyroid transcription factor 1 (the master regulator of lung) and low expression of cytokeratin 7 and E-cadherin (2 markers for bronchial epithelial differentiation), whereas the high nuclear expression of PRMT5 was frequently noted in adenocarcinoma in situ, a low-grade subtype (6 of 17 low grade, 25 of 81 intermediate grade, and 3 of 32 high grade; *P* = .0444). The cytoplasmic expression of PRMT5 correlated with a poor prognosis (*P* = .0089). We herein highlighted the importance of PRMT5 expression, especially its cytoplasmic expression, in the process of epithelial-mesenchymal transition and loss of the bronchial epithelial phenotype of lung adenocarcinoma.

1. Introduction

The loss of E-cadherin, as a criterion standard of epithelial-mesenchymal transition (EMT), has been reported in approximately 10% of resected primary lung adenocarcinoma cases [1], and no effective therapeutic method has yet been established for lung cancer with the features of EMT [2].

We recently demonstrated that lung adenocarcinoma could be classified into 2 groups: a bronchial epithelial phenotype and mesenchymal-like phenotype [3]. The "bronchial epithelial phenotype" represents a group of lung adenocarcinomas that highly expresses bronchial epithelial markers and includes the thyroid transcription factor 1 (TTF-1)–positive terminal respiratory unit type [4] in addition to TTF-1–negative tumors that highly express bronchial epithelial markers such as cytokeratin 7 (CK7) and mucin 1 (MUC1) [3]. Mutations or amplifications in *epidermal growth factor receptor (EGFR)*, *c-met proto-oncogene (MET)*, and *human epidermal growth factor receptor 2 (HER2)* are frequent in the bronchial epithelial phenotype. In contrast, the "mesenchymal-like phenotype" is characterized by the absence of bronchial epithelial markers (TTF-1, MUC1, and CK7), no *EGFR*, *MET*, or *HER2* mutations or amplifications, and presence of the features of EMT, such as the low expression of E-cadherin and high expression of fibroblast growth factor receptor 1 (FGFR1), vimentin, and Zinc finger E-box-binding homeobox 1 (ZEB1) [3]. The absence of *EGFR*, *MET*, or *HER2* mutations or amplifications in the mesenchymal-like phenotype suggests that other genetic or epigenetic abnormalities may play a role in this group of tumors.

The identification of epigenetic regulatory factor mutations including *MLL*, *EZH2*, *ARID1A*, and *DNMT3A* in various tumors has recently been attracting a lot of interest [5-8]. Tumors with these mutations have an undifferentiated, stem cell–like, and EMT phenotype, which suggests that epigenetic mechanisms through histone modifications may be correlated with EMT in tumors. In this study, we focused on protein arginine methyltransferase 5 (PRMT5). PRMT5 catalyzes the symmetrical dimethylation of arginine residues on histone and nonhistone substrates and plays multiple roles in cellular processes, including differentiation, proliferation, apoptosis, and ribosome biogenesis [9-15]. Although the overexpression of PRMT5 has been reported in various cancers including lung cancer [13,16-21], its expression pattern in terms of cytoplasmic and nuclear localization in each histologic subtype of lung adenocarcinoma and its relation to bronchial epithelial markers, *EGFR* status, clinicopathological factors, and prognosis have not yet been elucidated in detail. We herein described the distinct expression pattern of PRMT5 and its significance in malignant progression, especially in EMT.

2. Materials and methods

2.1. Gene expression profile of 40 non–small-cell lung carcinoma (NSCLC) cell lines

We used the microarray analysis data of 40 cell lines. Detailed information is available in the Supplementary methods and our previous studies [3,22].

2.2. Cell lines and medium

We used 6 cell lines (HCC4006, H1650, PC3, A549, H522, and H1651) for Western blotting and immunocytochemistry. HCC4006, H1650, and PC3, harboring *EGFR* mutations, were used as representatives of the bronchial epithelial phenotype, whereas A549, H522, and H1651 with wild-type *EGFR* were used as representatives of the mesenchymal-like phenotype in our previous study [3]. All cell lines were maintained in RPMI 1640 media supplemented with 10% fetal bovine serum and 1% antibiotics in a humidified atmosphere with 5% carbon dioxide and 95% air.

2.3. Antibodies

The antibodies used in this study are summarized in Supplementary Table S1.

2.4. Protein analysis and immunocytochemistry of cell lines

Experimental details of Western blotting and immunocytochemistry are given in the Supplementary methods.

2.5. Tissue microarray sections

We used tissue microarrays (TMAs) that were produced to accommodate primary lung adenocarcinoma tissue core sections collected from patients who had undergone surgical resection at the University of Tokyo Hospital between June 2005, and September 2008. Informed consent was obtained from all patients, and the study was approved by the institutional ethics review committee. The demographic and clinicopathological details of patients and tumors are provided in the Supplementary methods.

2.6. Histopathologic grading of each subtype of lung adenocarcinoma

Each case was classified according to the predominant histopathologic subtype in the invasive lesions on TMA
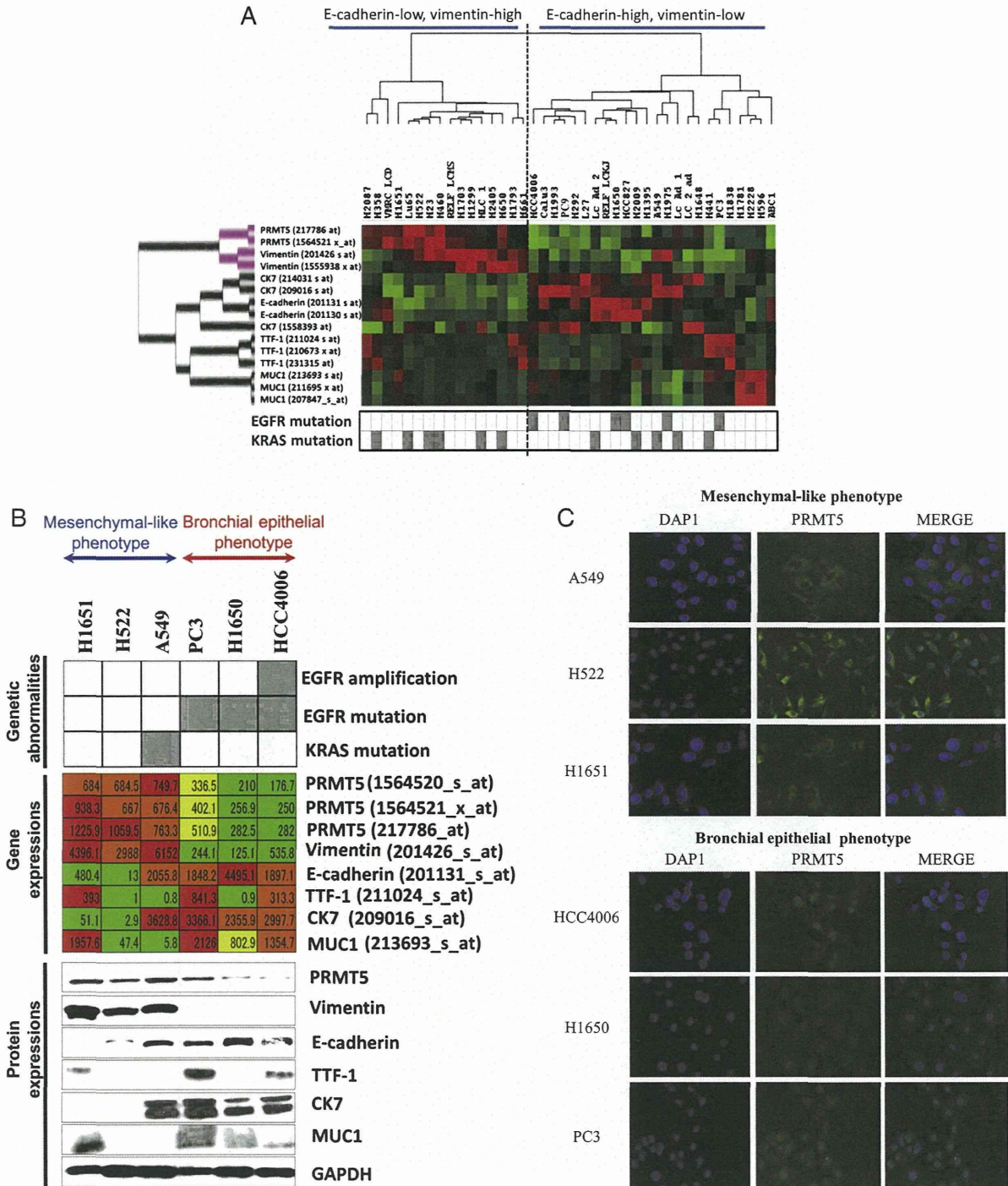
Fig. 1 A, Hierarchical cluster analysis of 40 lung cancer cell lines using *PRMT5*, *vimentin*, *CK7*, *TTF-1*, *E-cadherin*, and *MUC1* gene expression. The genetic statuses of *EGFR* and *KRAS* are shown in the lower panel (the gray box indicates the presence of genetic abnormalities, and the white box shows the absence of genetic abnormalities). B, Genetic statuses of *EGFR* and *KRAS* (upper panel) and gene expression levels (middle panel) and protein expression levels (lower panel) of PRMT5, vimentin, E-cadherin, TTF-1, CK7, and MUC1 in the 6 cell lines. Color indications in the middle lane are as follows: red means more than or equal to 1.5 times the average of each gene expression level; orange, less than 1.5 times the average and more than or equal to the average; yellow, less than the average and more than or equal to half the average; and green, less than half the average. C, PRMT5 immunocytochemical expression in mesenchymal-like phenotype cell lines (A549, H522, and H1651) and bronchial epithelial phenotype cell lines (PC3, H1650, and HCC4006). (Original magnification ×400) (blue, 4′,6-diamidino-2-phenylindole (DAPI) for nuclear staining; green, PRMT5; merge, mixed nuclear and PRMT5 view). Gene and protein expression of PRMT5 in cell lines.
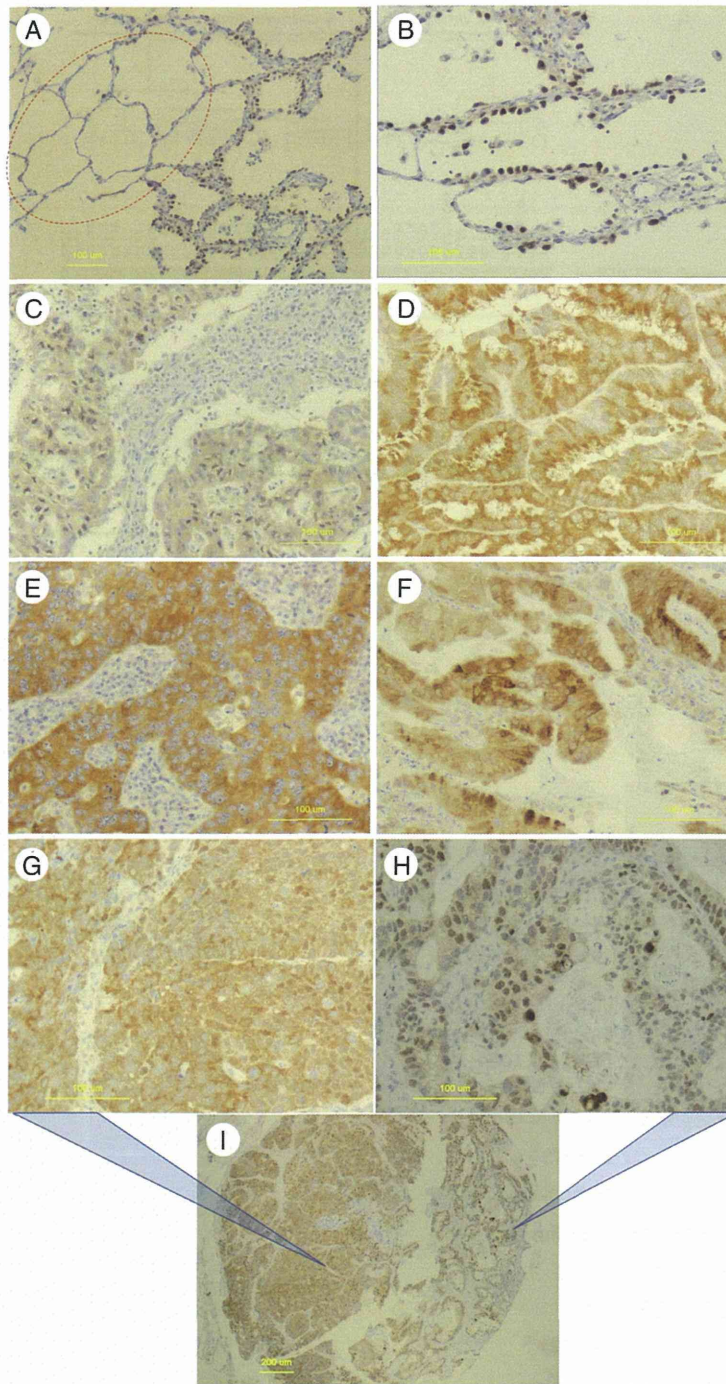
Fig. 2 PRMT5 expression in primary lung adenocarcinoma. A, The well-differentiated adenocarcinoma component (lepidic growth component) was positive for PRMT5 (right side), whereas the expression of PRMT5 was not observed in normal lung alveolar epithelia (surrounded by a red dotted line). B, A magnified view of the lepidic growth component showing the nuclear expression of PRMT5. C, Moderately differentiated adenocarcinoma (acinar and papillary adenocarcinoma) sometimes showed the nuclear expression of PRMT5. D, Moderately differentiated adenocarcinoma (acinar and papillary adenocarcinoma) sometimes showed the cytoplasmic expression of PRMT5. E, Poorly differentiated adenocarcinoma (solid adenocarcinoma) frequently showed the cytoplasmic expression of PRMT5. F, Invasive mucinous adenocarcinoma also frequently showed the cytoplasmic expression of PRMT5. G to I, Heterogeneous PRMT5 expression in lung adenocarcinoma. I shows a low-power field of invasive adenocarcinoma with mixed subtypes. The left side shows solid adenocarcinoma components, and right side shows acinar adenocarcinoma and lepidic growth components. G shows a high-power field of solid adenocarcinoma components, which showed the cytoplasmic expression of PRMT5. H shows a high-power field of acinar adenocarcinoma and lepidic growth components, which showed the nuclear expression of PRMT5. Original magnification ×100 (A), ×200 (B-H), ×40 (I).

sections by 2 pathologists (D. M. and A. G.). Each histologic subtype of lung adenocarcinoma was classified into 3 grades, by referring to the histopathologic grading described previously by Yoshizawa et al [23] with a slight modification. Detailed information on histopathologic subtyping and grading is provided in the Supplementary methods.

2.7. Immunohistochemistry and evaluation

Formalin-fixed, paraffin-embedded tumor specimens were analyzed by immunohistochemistry using antibodies to PRMT5, E-cadherin, CK7, MUC1, and TTF-1. Staining procedures and evaluation methods are given in the Supplementary methods.

2.8. Bioinformatic analyses and statistics

Details are shown in the Supplementary methods.

3. Results

3.1. *PRMT5*, a candidate gene involved in EMT, among histone methyltransferases and demethylases, depending on oligonucleotide array analysis of 40 cell lines.

We extracted expression profile data for histone methyltransferases and demethylases and examined the relative expression levels of these genes in the 40 lung cancer cell lines examined to identify histone methyltransferases and demethylases that correlated with EMT. The comprehensive data set for the expression profiles of these genes is shown in Supplementary Table S2. We then calculated the correlation coefficients of vimentin and E-cadherin for each gene and selected the genes that met the following requirements: (correlation coefficient with vimentin − correlation coefficient with E-cadherin) × ½ > 0.3 or < −0.3. The results are shown in Supplementary Table S3. We focused on *PRMT5* as the best suitable candidate correlated with EMT. We performed hierarchical cluster analysis of the 40 NSCLC cell lines, based on *PRMT5*, *TTF-1*, *MUC1*, *CK7*, *E-cadherin*, and *vimentin* gene expression. We found that *PRMT5* was correlated with *vimentin* and highly expressed in cell lines that expressed high levels of *vimentin* and low levels of *E-cadherin* and the other bronchial epithelial markers (*TTF-1*, *CK7*, and *MUC1*) (Fig. 1A). *EGFR* mutations were frequently observed in *PRMT5*-low cell lines, whereas *v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS)* mutations appeared in both *PRMT5*-high and *PRMT5*-low cell lines (Fig. 1A).

3.2. Protein expression of PRMT5 in mesenchymal-like and bronchial epithelial phenotype cell lines by Western blotting and immunocytochemistry

We performed Western blot analysis using 6 lung adenocarcinoma cell lines that contained 3 mesenchymal-like phenotypes: H522, H1651, and A549 and 3 bronchial epithelial phenotypes: HCC4006, H1650, and PC3, to compare the protein expression of PRMT5 between the 2 phenotypes. Fig. 1B summarizes the following: (i) the genetic status of *EGFR* and *KRAS* (upper panel); (ii) gene expression levels of *PRMT5*, vimentin, E-cadherin, TTF-1, CK7, and *MUC1* (middle panel); and (iii) protein expression levels of *PRMT5*, vimentin, E-cadherin, TTF-1, CK7, and *MUC1* (lower panel) in the 6 cell lines. The expression of the PRMT5 protein was higher in mesenchymal-like cell lines in which the expression of vimentin was high and that of bronchial epithelial markers was low.

We then performed immunocytochemical analysis using these 6 cell lines and found that PRMT5 expression was predominant in the cytoplasm in the mesenchymal-like phenotype, whereas it was predominant in the nucleus but faint in the cytoplasm in the bronchial epithelial phenotype with *EGFR* mutations (Fig. 1C). This result suggests that the cytoplasmic expression of PRMT5 may be associated with EMT and/or wild-type *EGFR*.

3.3. Immunohistochemical expression of PRMT5 in primary lung adenocarcinoma tissues

We used TMA sections of primary lung adenocarcinoma cases (n = 130) to examine the immunohistochemical expression patterns of PRMT5. Forty-three of 130 cases showed the high cytoplasmic expression and low nuclear expression of PRMT5, 30 showed the low cytoplasmic expression and high nuclear expression, and 53 showed low

Table 1   Correlations between PRMT5 expression levels in the cytoplasm (C) and nucleus (N) and histopathologic subtypes of primary lung adenocarcinomas

Subtypes		C+	C−
Lepidic growth component	N+	0	6
	N−	1	10
Acinar adenocarcinoma component	N+	0	4
	N−	9	12
Papillary adenocarcinoma component	N+	1	20
	N−	11	24
Solid adenocarcinoma component	N+	3	0
	N−	20	6
Invasive mucinous adenocarcinoma component	N+	0	0
	N−	2	1
	Total	47	83

Table 2   Correlations between PRMT5 expression levels in the cytoplasm (C) and nucleus (N) and histopathologic grades of primary lung adenocarcinomas

Histologic grades	C+	C−	P	N+	N−	P
Low grade	1	16	<.0001	6	11	.0444
Intermediate grade	21	60		25	56	
High grade	25	7		3	29	

cytoplasmic expression and low nuclear expression. Although both cytoplasmic and nuclear expression levels of PRMT5 were high in 4 cases, an inverse correlation was observed between the cytoplasmic and nuclear expression of PRMT5 in 130 cases ($P = .0002$). We then examined the expression levels of PRMT5 in the cytoplasm and nucleus of each histopathologic subtype. Normal alveolar epithelia were negative for PRMT5 (Fig. 2A), whereas the nuclear expression of PRMT5 was high in the well-differentiated adenocarcinoma component, that is, lepidic growth component (6 of 17, 35%), which less frequently showed the high cytoplasmic expression of PRMT5 (1 of 17, 6%) (Fig. 2A and B; Table 1). Moderately differentiated adenocarcinoma components, that is, acinar or papillary adenocarcinomas, showed the high nuclear expression of PRMT5 in 25 of 81 cases (31%) and high cytoplasmic expression of PRMT5 in 21 of 81 cases (26%) (Fig. 2C and D; Table 1). The poorly differentiated adenocarcinoma component, that is, solid adenocarcinoma with mucin, frequently showed the high cytoplasmic expression of PRMT5 (23 of 29, 79%) and less frequently showed the high nuclear expression of PRMT5 (3 of 29, 10%) (Fig. 2E and Table 1). Cytoplasmic predominance was also seen in mucinous adenocarcinoma (2 of 3, 66%) (Fig. 2F and Table 1). PRMT5 sometimes showed heterogeneous staining pattern, typically showing nuclear positive staining in well- to moderately differentiated adenocarcinoma components and cytoplasmic staining in poorly differentiated adenocarcinoma components (Fig. 2G-I).

Histologic progression to higher grade with loss of bronchial epithelial phenotype is involved in the process of EMT in our previous report [2]. Here, we examined correlations between histologic grades (low grade, intermediate grade, and high grade) and PRMT5 expression levels in the cytoplasm and nucleus, respectively (Table 2). The

Table 3   Correlation between the grades of lung adenocarcinoma and groups defined by the PRMT5 expression pattern

Histologic grades	C+ group	C−N+ group	C−N− group	P
Low grade	1	6	10	C+ vs C−N+: <.0001
Intermediate grade	21	24	36	C−N+ vs C−N−: .3780
High grade	25	0	7	C+ vs C−N−: <.0001

Table 4   Correlations between the cytoplasmic expression levels of PRMT5 and (i) clinicopathological factors, (ii) EGFR mutations, and (iii) the expression of bronchial epithelial markers

	Cytoplasmic PRMT5		
	High	Low	P
Pathologic stage [a]			.0887
Stage IA	14	38	
Stage IB-IV	32	45	
T stage			.0680
T1	16	42	
T2,T3,T4	31	41	
Nodal involvement [b]			.8858
Positive	12	21	
Negative	34	56	
Lymphatic invasion			.5064
Positive	12	17	
Negative	35	66	
Vessel invasion			.0376
Positive	24	27	
Negative	23	56	
Pleural invasion			.1489
Positive	26	35	
Negative	21	48	
Dissemination			.2835
Positive	0	2	
Negative	47	81	
Tumor size			.3640
<3 cm	16	22	
>3 cm	31	61	
Pulmonary metastasis			.5916
Positive	4	5	
Negative	43	78	
Smoking index [c]			.7410
<600	21	34	
>600	24	44	
EGFR mutations [d]			.0880
Positive	12	31	
Negative	25	31	
E-cadherin			.0138
High level	42	82	
Low level	5	1	
TTF-1			<.0001
High level	30	78	
Low level	17	5	
CK7			.0140
High level	34	74	
Low level	13	9	
MUC1 (membranous)			.0006
High level	21	62	
Low level	26	21	
MUC1 (depolarized)			.0002
High level	12	3	
Low level	35	80	

[a] Pathologic N factors were not determined for 7 cases.
[b] Six of 7 cases were more than stage IA and included 2 cases of stage IV patients with pleural dissemination.
[c] The smoking index was not determined for 7 cases.
[d] The EGFR genetic status was not determined for 31 cases.

prevalence of high-grade tumors was significantly higher in cases that expressed high levels of PRMT5 in the cytoplasm than in those that expressed low levels in the cytoplasm ($P <$ .0001), whereas the prevalence of low-grade tumors was significantly higher in cases that expressed high levels of PRMT5 in the nucleus than in those that expressed low levels in the nucleus ($P =$ .0444). However, among 4 cases that expressed high levels of PRMT5 in both the cytoplasm and nucleus, 3 (75%) showed high-grade subtype (solid adenocarcinoma), and 1 (25%) showed intermediate-grade subtype (papillary adenocarcinoma), which suggested to us that cytoplasmic PRMT5 expression would be more closely correlated with histologic grades than nuclear PRMT5 expression. Next, we classified 130 cases into 3 groups according to their PRMT5 expression patterns to verify the significance of PRMT5 cytoplasmic expression (Table 3): a C+ group, comprising cases that expressed high levels in the cytoplasm with or without expression in the nucleus (n = 47); C−N+ group, comprising cases that expressed low levels in the cytoplasm and high levels in the nucleus (n = 30); and C−N− group, comprising cases that expressed low levels in both the cytoplasm and nucleus (n = 53). We compared histologic grades and this group classification and showed that grades in the C+ group were significantly higher than those in the C−N+ and C−N− groups ($P <$ .0001); however, no

significant difference was observed between the C−N+ and C−N− groups ($P =$ .3780) (Table 3). These results suggested that the nuclear localization of PRMT5 may not be correlated with the maintenance of a differentiated phenotype, whereas the cytoplasmic localization of PRMT5 appears to be significant in the processes of EMT.

We examined the correlation between the cytoplasmic expression of PRMT5 and clinicopathological factors, *EGFR* status, and bronchial epithelial markers (TTF-1, CK7, MUC1, and E-cadherin) (Table 4). We found that high cytoplasmic PRMT5 expression was correlated with low expression levels of TTF-1, CK7, MUC1 (membranous), and E-cadherin and high expression levels of depolarized MUC1 (Fig. 3A-E; Table 4). The prevalence of wild-type *EGFR* was slightly higher in cases that expressed high levels of PRMT5 in the cytoplasm ($P =$ .0880). Fig. 3F to J shows the typical immunohistochemical expression patterns of TTF-1, CK7, E-cadherin, and MUC1 in lepidic growth components with the high nuclear expression of PRMT5.

3.4. Prognostic significance of PRMT5 expression

Survival curves based on histologic grades are shown in Fig. 4A. Low-grade cases showed the best prognosis (the 5-
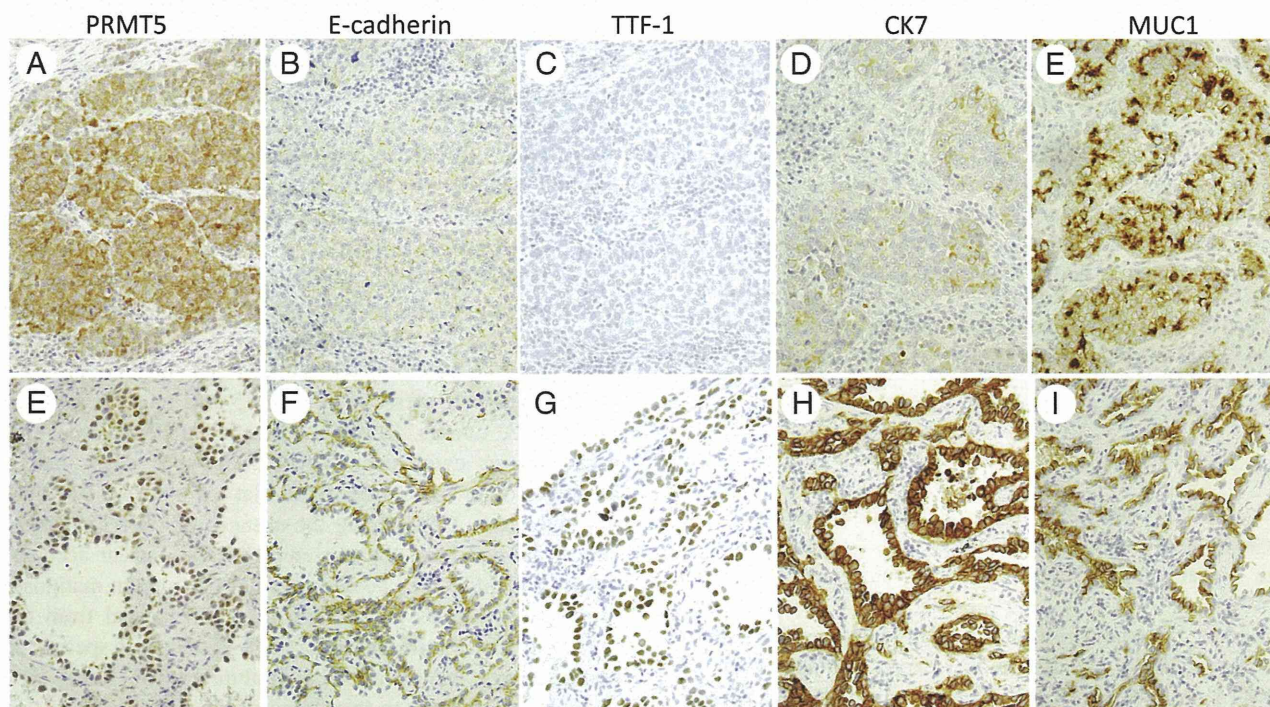

Fig. 3   Immunohistochemical expression of PRMT5, E-cadherin, TTF-1, CK7, and MUC1 in the same tissue samples. A to E, Case of solid adenocarcinoma with mucin (high-grade subtype). A shows the cytoplasmic expression of PRMT5. B shows the low level of E-cadherin expressed in the membrane. C shows the low level of TTF-1 expressed in the nucleus. D shows the low level of CK7 expressed in the membrane and cytoplasm. E shows the low level of membranous MUC1 expressed and the depolarized (cytoplasmic) expression pattern of MUC1. F to J, Case of adenocarcinoma in situ (low-grade subtype). F shows the nuclear expression of PRMT5. G shows the high level of E-cadherin expressed in the membrane. H shows the high level of TTF-1 expressed in the nucleus. I shows the high level of CK7 expressed in the membrane and cytoplasm. J shows the high level of MUC1 expressed in the apical membrane (Original magnification ×200).

A

Patient survival according to histological grades

B

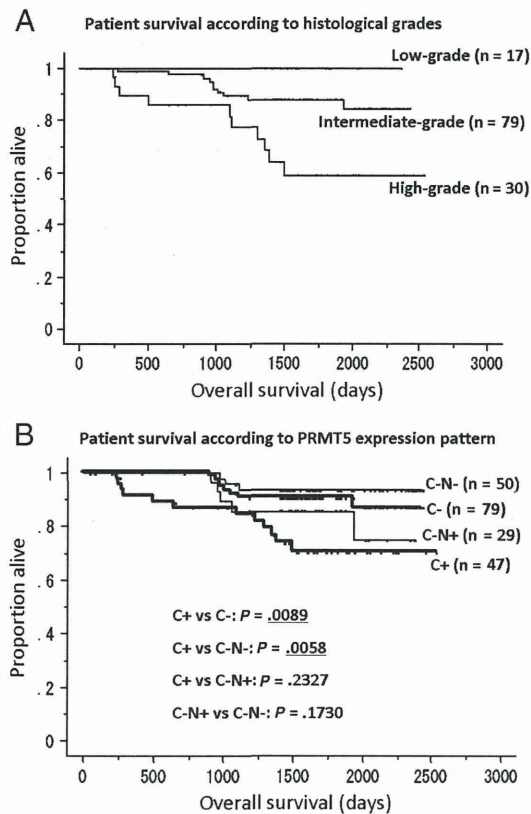Patient survival according to PRMT5 expression pattern

Fig. 4 Overall survival curves according to (A) histologic grades and (B) PRMT5 expression patterns. A, Patient survival curves according to histologic grades. Patients were classified into 3 groups according to their histologic grades: low grade (n = 17), intermediate grade (n = 79), and high grade (n = 30). B, Patient survival curves according to PRMT5 expression patterns. In this figure, the C+ group comprised cases that expressed high levels of PRMT5 in the cytoplasm with or without its expression in the nucleus (n = 47); C− group, cases that expressed low levels of PRMT5 in the cytoplasm with or without its expression in the nucleus (n = 79); C−N+ group, cases that expressed low levels of PRMT5 in the cytoplasm and high levels in the nucleus (n = 29); and C−N− group, cases that expressed low levels of PRMT5 in both the cytoplasm and nucleus (n = 50).

year survival rates = 100%), whereas high-grade cases showed worst prognosis (the 5-year survival rates = 58.9%). The 5-year survival rate of intermediate-grade cases was 87.9%.

Survival curves based on the PRMT5 expression pattern are shown in Fig. 4B. Cases that expressed high levels of PRMT5 in the cytoplasm (C+ group) had significantly poorer survival rates than those that expressed low levels in the cytoplasm (C− group) (P = .0089) (Fig. 4B). We also compared prognoses among the aforementioned groups: the C+ group, C+N− group, and C−N− group. The C+ group had the worst prognosis, whereas the C−N+ group had a slightly poorer prognosis than that of the C−N− group (P = .1730) (Fig. 4B).

4. Discussion

We here demonstrated the high cytoplasmic expression of PRMT5 in mesenchymal-like phenotype cell lines and that high cytoplasmic PRMT5 expression was closely related to the high-grade subtypes of primary lung adenocarcinomas with the loss of E-cadherin and other bronchial epithelial markers (TTF-1, CK7, and MUC1) and a poor prognosis.

Shilo et al [24] recently reported a correlation between cytoplasmic PRMT5 and the histologic high grade in NSCLC, except for lung adenocarcinoma. The frequency of cytoplasmic expression in adenocarcinomas was higher in our study (36%) than in the study of Shilo et al [24] (8%). These discrepancies have been attributed to differences in the evaluation methods used because we set a high value for predominant invasive lesions in histologic grading and the evaluation of PRMT5 expression (as shown in the Supplementary methods). We considered our histologic grading of the TMA cores to be accurate because the results obtained closely correlated with patient prognosis, which was consistent with the findings by Yoshizawa et al [23]. We speculated that the cytoplasmic expression of PRMT5 may contribute to a poor prognosis by promoting high-grade transformation and EMT. However, how cytosolic PRMT5 induces EMT remains unknown. PRMT5 is known to methylate splice some proteins SmD1, SmD3, and SmB/B′ in the cytoplasm, which are involved in premessenger RNA splicing [25]. PRMT5 may affect the expression of some EMT-related genes when this epigenetic cytoplasmic role is considered. EMT has also been associated with the gain of stem cell properties [26], and cytoplasmic PRMT5 of embryonic stem cells is important to maintain pluripotency through the methylation of cytosolic histone H2A during mouse development [27]. This finding also justifies the accumulation of PRMT5 in the cytoplasm during EMT. There was a slightly inverse correlation between *EGFR* mutations and cytoplasmic PRMT5 expression. We speculated that this result will reflect the high frequency of wild-type *EGFR* in lung tumors with EMT features [1].

The nuclear expression of PRMT5 was more frequent in lower grade tumors. However, the nuclear PRMT5-positive cases among cytoplasmic PRMT5-negative cases had a slightly poorer prognosis than that of nuclear PRMT5-negative cases. Considering the absence of PRMT5 expression in normal lung alveolar epithelia, the nuclear accumulation of PRMT5 may be an important first step in malignant progression, and its localization may be changed from the nucleus to the cytoplasm during EMT. Finally, we speculated that epigenetic therapy aimed at inhibiting PRMT5 may be a possible new therapy to treat tumors with EMT features.

Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.humpath.2014.02.013.

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HPV-associated lung cancers: an international pooled analysis

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Abbreviations: AdjPr, adjusted prevalence; CI, confidence interval; FFPE,
formalin fixed paraffin embedded; HPV, human papillomavirus; OR, odds
ratio; SCC, squamous cell carcinoma.

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Human papillomavirus (HPV) is the etiologic risk factor for cervi-
cal cancer. Some studies have suggested an association with a subset
of lung tumors, but the etiologic link has not been firmly estab-
lished. We performed an international pooled analysis of cross-
sectional studies (27 datasets, $n = 3249$ patients) to evaluate HPV
DNA prevalence in lung cancer and to investigate viral presence
according to clinical and demographic characteristics. HPV16/18
were the most commonly detected, but with substantial variation
in viral prevalence between geographic regions. The highest preva-
lence of HPV16/18 was observed in South and Central America,
followed by Asia, North America and Europe (adjusted prevalence
rates = 22, 5, 4 and 3%, respectively). Higher HPV16 prevalence
was noted in each geographic region compared with HPV18, except
in North America. HPV16/18-positive lung cancer was less likely
observed among White race (adjusted odds ratio [OR] = 0.33, 95%
confidence interval [CI] = 0.12–0.90), whereas no associations were
observed with gender, smoking history, age, histology or stage.
Comparisons between tumor and normal lung tissue show that
HPV was more likely to be present in lung cancer rather than nor-
mal lung tissues (OR = 3.86, 95% CI = 2.87–5.19). Among a subset
of patients with HPV16-positive tumors, integration was primar-
ily among female patients (93%, 13/14), while the physical status
in male cases ($N = 14$) was inconsistent. Our findings confirm that
HPV DNA is present in a small fraction of lung tumors, with large
geographic variations. Further comprehensive analysis is needed to
assess whether this association reflects a causal relationship.

Introduction

Lung cancer is the third most common cancer in men and women of
all races and the leading cause of cancer death in the United States and
worldwide. Cigarette smoking is the primary risk factor and accounts
for ~85% of all lung cancer cases. Other less prevalent risk factors are
genetic factors, family history of lung cancer and exposures to radon,
asbestos, arsenic, diesel exhaust and some forms of silica and chro-
mium. Human papillomavirus (HPV) is a small non-enveloped DNA
tumor virus of the family Papillomaviridae and is the established
etiological agent of genital warts, cervical cancer and a proportion of
cancers of the vulva, vagina, penis, anus and oropharynx (1,2). More
than 100 different genotypes have been identified, and types 16 and
18 are the most common oncogenic types, leading to the development
of ~70% of all cervical carcinomas.

In 1979, Syrjänen first hypothesized that certain types of HPV
are responsible for causing cancer in the lung (3). In the last three
decades, the number of reports suggesting an association between
HPV and lung cancer has increased tremendously. A review of HPV
in 2468 lung cancer cases was first published in 2002 and showed
that, using morphological, immunohistochemical and HPV DNA
detection methods (4), ~22% of the cases analyzed contained HPV.
This meta-analysis was updated in 2007 and reported a prevalence of
24.5%, but considerable heterogeneity was observed between stud-
ies (5). In our meta-analysis in 2009 (6), we also identified a wide
variation in the prevalence of HPV in lung cancer tissues, despite the
fact that only studies utilizing PCR-based methods for HPV detection
were included in order to reduce the heterogeneity between studies.
Nevertheless, HPV16 and 18 were the two most common genotypes
detected in lung tumors, and a higher prevalence of HPV16 and 18
was noted in Asian populations compared with European populations.
In 2012, the most recent meta-analysis was published and reported
that the variability of HPV prevalence may be due to differences in
geographical study origin and histological types of lung cancer rather
than the method of HPV detection (7). However, meta-analyses find-
ings to date have limitations because important characteristics such as