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III. 研究成果の刊行物・別刷り

Human Pathology (2014) xx, xxx–xxx

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Original contribution

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

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Received 5 December 2013; revised 16 January 2014; accepted 12 February 2014

Keywords:
PRMT5;
Lung adenocarcinoma;
EMT;
Epigenetics

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.

☆ Competing interests: We have no financial relationships to disclose.
☆☆ Funding/Support: This study was supported in part by the Smoking Research Foundation; and JSPS KAKENHI Grant Number 25460432.
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0046-8177/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.humpath.2014.02.013

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

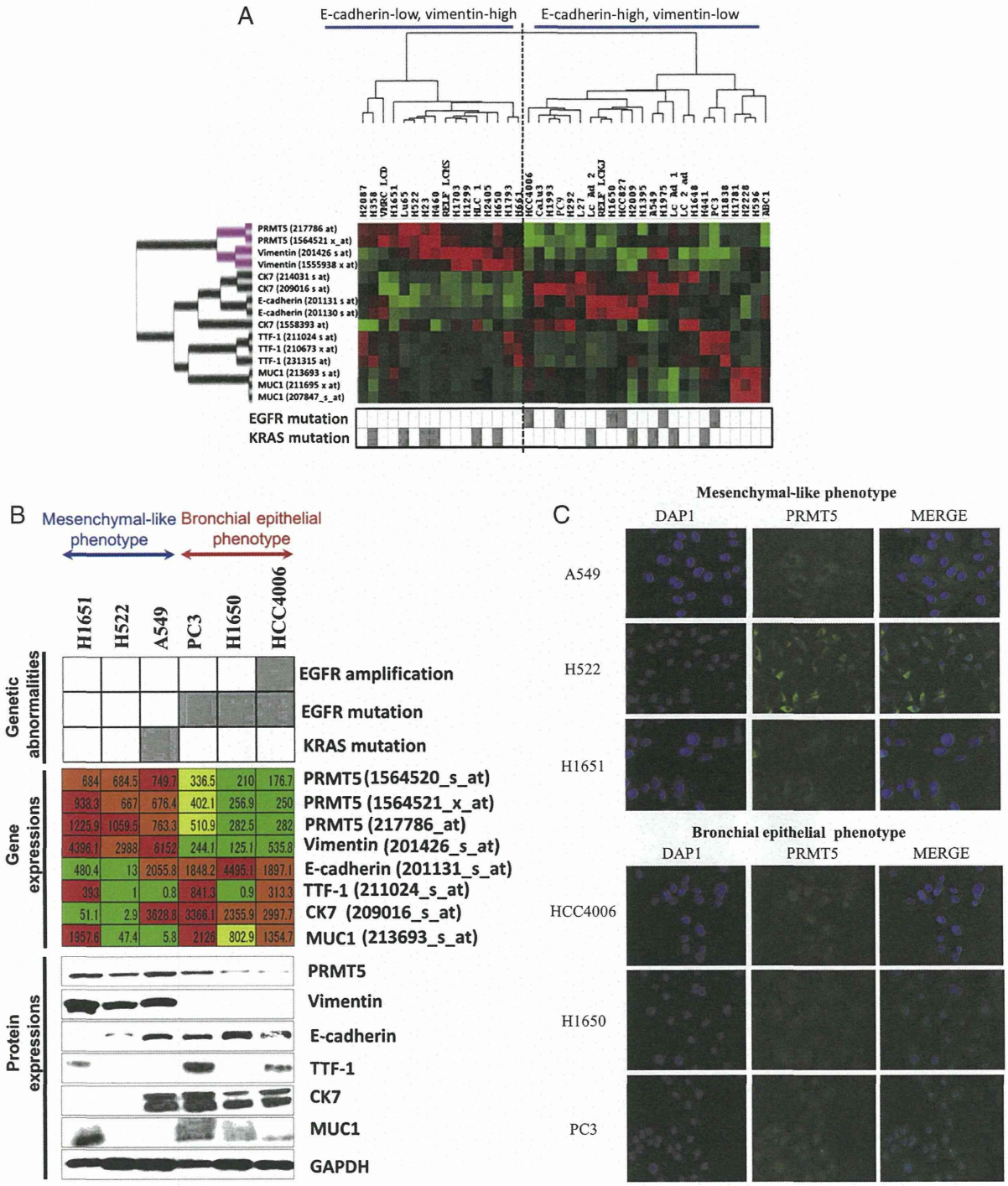

Fig. 1 A, Hierarchical cluster analysis of 40 lung cancer cell lines using *PRMT5*, *vimentin*, *CK7*, *TTF-1*, *E-cadherin*, and *MUC1* gene expression. The genetic statuses of *EGFR* and *KRAS* are shown in the lower panel (the gray box indicates the presence of genetic abnormalities, and the white 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.