論文の内容の要旨
Dissertation Abstract

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

（原発性肺腺癌における PRMT5 の発現パターンと上皮間葉系転換との関連性について）

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Introduction

Lung cancer is considered to be a leading cause of cancer death in many developed countries, including the United States and Japan. Epidermal growth factor receptor (EGFR) inhibitors, as molecular targeted therapies, have a certain therapeutic efficacy for lung cancer patients. However, no effective therapeutic method is established for lung cancer with features of epithelial-mesenchymal transition (EMT), in which EGFR mutations are less frequent.

EMT is a phenomenon of epithelial cells to loose epithelial phenotype and to gain mesenchymal phenotype temporally or permanently. In recent years, identification of mutations of epigenetic regulatory factors in various tumors has been a hot topic. Interestingly, tumors with these mutations showed EMT phenotype, which suggest that epigenetic mechanisms may be correlated with EMT of tumors.

Previously we grouped adenocarcinoma cell lines into bronchial epithelial phenotype which shows high expressions of TTF1, MUC1,and CK7, (epithelial markers), and frequent mutation or amplification of EGFR, MET and HER2, and mesenchyme-like phenotype which shows negative expression of TTF-1, MUC1 and CK7, no mutation or amplification of EGFR, MET and HER2, , and with features of epithelial mesenchymal transition (EMT), such as low E-cadherin and high FGFR1, vimentin, and ZEB1 expression. The absence of mutations or amplifications of EGFR, MET, or HER2 in mesenchyme-like phenotype tumors suggested 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. Tumors with these mutations have an undifferentiated, stem cell-like, and EMT phenotype, which suggests that that epigenetic mechanisms may be correlated with EMT in tumors

In the present study, I focused on Protein arginine methyltransferase 5 (PRMT5) as a candidate gene that correlated with EMT among the histone methyltransferases and demethylases. I used a selection criterion which based on selecting genes with significant correlation with vimentin and inverse correlation with E-Cadherin, as these two proteins are famous markers of EMT. PRMT5 is an arginine methyltransferase which catalyzes the symmetrical dimethylation of arginine residues on histone and non-histone substrates. It plays multiple roles in cellular processes and localizes to both nucleus and cytoplasm. However little is known about the functional differences of cytoplasmic and nuclear localization

Although the overexpression of PRMT5 has been observed in lung cancer, its expression pattern in terms of cytoplasmic and nuclear localization in each histological subtype of lung adenocarcinoma and its relation to bronchial epithelial markers, EGFR status, clinicopathological factors, and prognosis have not yet been elucidated in details.

So, here I described the distinct expression pattern of PRMT5 and its significance in malignant progression, especially in EMT.

Materials and methods

Microarray analysis of lung adenocarcinoma cell lines (n=40) by Affymetrix U133A. Western blot analysis and immunocytochemistry (ICC) using cell lines as follows; Bronchial epithelial phenotype: HCC4006, H1650, PC3. Mesenchyme-like phenotype: A549, H522, H1651. Immunohistochemistry (IHC) of TMA sections of lung adenocarcinoma cases (n=130) resected in Univ. of Tokyo hospital. Primary antibodies: PRMT5:Rabbit polyclonal (SIGMA), E-cadherin :Mouse monoclonal (BD bio), TTF-1:Mouse monoclonal (Dako), CK7 :Mouse monoclonal (Dako), MUC1 :Mouse monoclonal (Novocastra)

Results

Using the microarray data I extracted expression profile data for the histone methyltransferases and demethylases and examined the relative expression levels of these genes in the 40 lung cancer cell lines. Next, we calculated the correlation coefficients of vimentin and E-cadherin for each gene, and selected the genes which met the following requirement (Correlation coefficient with vimentin – correlation coefficient with E-cadherin) x ½ > 0.3, or <-0.3. I focused on PRMT5 as the best suitable candidate correlated with EMT. We performed hierarchical

cluster analysis of 40 lung cancer cell lines, based on the gene expressions of PRMT5, TTF-1, MUC1, CK7, E-cadherin, and vimentin and found that PRMT5 was correlated with vimentin and frequently highly expressed in mesenchyme-like phenotype cell lines which show high vimentin and low bronchial epithelial markers (E-Cadherin, CK7, MUCI, TTF-1). These finding suggested the relation between PRMT5 expression and EMT. Next I performed western blot analysis to confirm the microarray data; the result showed that PRMT5 protein expression was more in the mesenchyme-like cell lines. Using immunocytochemistry we found that the expression of PRMT5 is predominantly cytoplasmic in the mesenchyme-like cell lines (E-cadherin low and vimentin-high cell lines) and nuclear in bronchial epithelial phenotype (E-cadherin-high and vimentin-low cell lines).

Then we used primary lung adenocarcinoma tissue samples (130 cases) to test the protein expression pattern in normal alveolar epithelium, well, moderately, and poorly differentiated tumors. We found that the normal alveolar epithelium is negative for PRMT5 expression; the lepidic growth component which represents a well differentiated adenocarcinoma shows a nuclear pattern of PRMT5 expression, whereas the poorly differentiated adenocarcinoma such as solid adenocarcinoma frequently shows cytoplasmic PRMT5 expression. Moderately differentiated adenocarcinoma components, that is, acinar or papillary adenocarcinomas, showed variable patterns of expression with no nuclear or cytoplasmic predominance.

We also found that the cytoplasmic expression is inversely correlated with the bronchial epithelial markers E-Cadherin, TTF-1 and MUC1 (membranous) and CK7.We could identify significant correlation between cytoplasmic PRMT5 expression and vessel invasion. On the other hand we identified that the higher cytoplasmic PRMT5 expressing cases significantly exhibit poorer prognosis.

Discussion

PRMT5 is known to be located in both the nucleus and cytoplasm. In cytoplasm it interacts and methylate many proteins such as EGFR, DR4, DR5, Sm proteins and regulates cell proliferation and RNA processing. Recently it has been identified to be expressed in the cytoplasm in ES cells to maintain pluripotency through methylation of cytosolic histone H2A during mouse development. Among the other features of EMT, it is known that EMT is related to gain of stem cell properties, so it is obvious that cytoplasmic expression is related to EMT. Here I described the accumulation of PRMT5 in the cytoplasm in the highly invasive adenocarcinoma tumors and its inverse correlation with epithelial markers while significantly correlated with the EMT mark

vimentin. I also found that EGFR-mutated tumors, not significantly, but tended to show low cytoplasmic PRMT5 expressions. Cytoplasmic PRMT5 was reported to methylate EGFR Arg 1175, and abolishment of this methylation enhanced ERK activation, which suggested that cytoplasmic PRMT5 may, conversely, hinder the growth of EGFR mutated tumors. Furthermore cytoplasmic accumulation of PRMT5 is associated with poor survival of patients.

Depending on my results I believe that the cytoplasmic expression of PRMT5 in lung adenocarcinoma is of great importance; as I have used tissue samples in my study, however the mechanism needs further studies.

The mechanism of EMT and cancer invasion is complex and probably not a single molecule would be responsible, but knowing additional information about the molecular mechanism is important to support identifying the full view of cancer invasion and metastasis through EMT in lung adenocarcinoma.

I 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 in lung adenocarcinoma.