The tissue array analysis of the aberrant expression of HLA class I molecules in human non-small cell lung cancer

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Background: Down-regulation or loss of human leukocyte antigen (HLA) class I has been reported in various malignancies in association with poor patient survival. These findings may reflect an escape of tumor cells losing intact HLA class I molecules from HLA class I-restricted, tumor-associated antigen-specific cytotoxic T lymphocytes in recognition as well as in destruction. However, clinical impact of the down-regulation or loss of HLA class I in patients with non-small cell lung cancer (NSCLC) is still unknown. In the present study, we investigated clinical significance of alteration in expression of HLA class I molecules in surgically resected NSCLC using tissue array analysis.

Method: Formalin-fixed paraffin-embedded sections were obtained from 105 NSCLC (60 adenocarcinoma, 37 squamous cell carcinoma, and 6 large cell carcinoma). HLA class I and β2-microglobulin expression were analyzed during tissue array analysis. Immunohistochemical study was performed using anti-HLA class I mAb EMR8-5 and anti-β2-microglobulin mAb BBM1/sc-13565. Correlation between HLA class I or β2-microglobulin expression and clinicopathological parameter was analyzed statistically. Additionally, survival analysis was also performed using Kaplan-Meier’s method.

Result: There was no significant correlation between HLA class I and clinicopathological parameters. On the other hand, significant correlations were observed between β2-microglobulin expression and tumor size (p=0.0003), histological subtype (p=0.0285), nodal status (p=0.0335), and pathological stage (p=0.0335). Loss of β2-microglobulin expression was correlated with poor patient survival (p=0.0121), although HLA class I was not (p=0.8243).

Conclusion: Although clinical impact of HLA alteration was still unclear, β2-microglobulin expression might be involved in cancer progression in human NSCLC possibly through aberration of several molecules which contains β2-microglobulin.

Rtk signal pathways differentially activated in lung adenocarcinoma

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Purpose: To assess the activation patterns of RTK pathways, i.e. protein kinase B (PKB/Akt) and mitogen activated protein kinase (MAPK) pathways, in lung adenocarcinoma as compared with clinico-pathological factors and status of EGFR and KRAS mutations.

Experimental Design: 99 evaluable cases with primary lung adenocarcinomas surgically resected between 1998 and 1999 were selected for this study. Akt and MAPK phosphorylation (pAkt+ and pMAPK) was examined by means of immunohistochemistry. We defined that cases with pAkt+/pMAPK- immunoreactivity to be the “Akt pathway type” and the pAkt-/pMAPK+ pattern to be the “MAPK pathway type.” Both positive and negative controls were prepared using mouse xenografts of PC-3 (pAkt+/MAPK-) and HTB26 (pAkt-/pMAPK+) cell lines. Univariate and multivariate analysis were performed to evaluate the association of these downstream mainly activated pathways and clinical significance. The mutational status of EGFR and KRAS was determined by direct sequencing.

Results: Of the 99 cases, rates of the Akt pathway type and the MAPK pathway type were 32% (n=32) and 29% (n=29), respectively. Cases with immunoreactivity to both of the antibodies were not included in either type. Mutation assay revealed EGFR mutations in 55 cases (55%), and KRAS mutation in 7 cases (7%). The predictive factors for the MAPK pathway type were cumulative smoking and KRAS mutation by univariate analysis. As all the cases with KRAS mutations were strongly positive for pMAPK, KRAS mutation status was mostly a highly predictive factor for the Akt pathway type.

Conclusions: Association between Ras/MAPK pathways activation and KRAS mutations was confirmed in tumor specimens of lung adenocarcinoma. The MAPK pathway type was statistically associated also with cumulative smoking.
identification of DNA methylation markers for NSCLC and adjacent normal lung tissue using HpaII-MspI Methylation chip

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Introduction: Epigenetic alterations in certain genes are now known as at least important as genetic mutation in pathogenesis of cancer. Especially abnormal promoter region methylation in tumor suppressor genes is known to result in gene silencing and therefore loss of gene function. Objective We, the authors, wanted to search for new lung cancer-specific tumor suppressor genes through the study, and to figure out the roll of known tumor suppressor genes of other kinds of cancer for NSCLC as well.

Material and Methods: Cancer tissue & adjacent normal tissue were obtained from 10 patients who diagnosed with NSCLC and underwent surgery in Konyang university hospital in 2005. NotI-M sera Methylation chip was used to analyze DNA methylation on promoter region of 27 genes in tumor tissue & nearby normally-appearing tissue. The rate of methylation was measured and compared for both groups to find out the genes associated with tumorigenesis. Also the patients were grouped by age, gender, history of smoking, and cell type of NSCLC, and compared between groups. And as normal control group we obtained lung tissue from two young patients with pneumothorax during their bullectomies, methylations were examined in the same way and compared with tumor and non-tumor tissue from NSCLC patients.

Result: Among the 27 genes, the higher rate of methylation for tumor tissue than that of non-tumor tissue were observed in 14 genes, and 6 genes is known to result in gene silencing and therefore loss of gene function. Objective We, the authors, wanted to search for new lung cancer-specific tumor suppressor genes through the study, and to figure out the roll of known tumor suppressor genes of other kinds of cancer for NSCLC as well.

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Conclusion: Among the 27 genes, the higher rate of methylation for tumor tissue than that of non-tumor tissue were observed in 14 genes, and 6 genes including AR, HTR1B, CFTR from those 14 genes in normal lung tissue were not methylated, suggesting the possibility of the role for TSG(tumor suppressor gene) of NSCLC.

P3-009

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P3-008

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P3-010

Using immunohistochemistry to evaluate protein expression levels of female sex hormone receptors (ER, PR) and epidermal growth factor receptor family members (EGFR, HER2) in East Asian non-small cell lung cancers (NSCLC)

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Background: The non-smoking East Asian female with lung adenocarcinoma is a phenotype that has been correlated with higher frequencies of EGFR mutations, with corresponding increased tumor response to tyrosine kinase inhibitors. We hypothesize that estrogen receptor (ER) has a role in the biological mechanism underlying this clinical entity, in a combinatorial fashion with other signaling transduction components. Here, we examine the protein expression levels via immunohistochemistry of isofoms of ER, namely ERα, ERβ, progesterone receptor (PR), EGFR and HER2.

Methods: Tissue microarrays (TMA): These were constructed from paraffin-embedded blocks of surgically resected tissues. Each array was targeted to contain replicates of 33 adenocarcinomas and 16 squamous cell carcinomas, of which 26 and 2 had matched normal lung tissues, respectively.

Immunohistochemistry: Commercially available antibodies identifying ERα, ERβ, PR, EGFR and HER2 were used for staining and detection, according to the manufacturers’ protocols. The ER, PR and HER2 analyses used breast invasive ductal carcinomas as positive controls. Extent (% of cells) and intensity of staining were scored on a scale of...