

Abstracts of the 2006th Maruyama Memorial Lectures of the 75th Annual Meeting of the Medical Association of Nippon Medical School

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Abstracts of the 2006th Maruyama Memorial Research Fund Prize Memorial Lecture (I)

Serum Biomarker of Lung Carcinogenesis Induced by Cigarette Smoking

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Background

There are several risk factors for lung cancer, including environmental and occupational factors, and the genetic predisposition of the individual. Cigarette smoking is the major risk factor in lung carcinogenesis; the chances of this malignancy developing is increased 10- to 20-fold in smokers compared with non-smokers. It is known that this causal relationship between smoking and lung cancer is particularly strong for squamous cell carcinoma and small cell carcinoma, but there are also correlations with the development of adenocarcinoma. On the other hand, the genetic make-up of the individual is also thought to play a role because lung cancer develops in less than 20% of smokers. One proposal is the presence of genes linked to enzymatic activity that either has the ability to activate the precursor of a carcinogen contained in cigarette smoke or the ability to inactivate a carcinogen. One candidate for the former is the genetic polymorphism of the *CYP1A1* gene, whereas one candidate for the latter is glutathione-S-transferase μ , but further elucidation is still required.

Recently, the concept of "proteomics" has been proposed, which aims to study an organism's complete complement of proteins. The establishment of a database of the entire human genome and the introduction of mass spectrometry have meant that proteomic analysis has frequently been applied to cancer research. In the study we report here, proteomic analysis identified an abnormality of a serum protein found to be associated with lung cancer in patients with a history of smoking. These results should increase our understanding of the incidence or developmental processes of lung cancer in association with smoking and may help to establish an innovative diagnostic method for identifying patients at high risk. This, in turn, might help in the development of a prophylactic approach to treatment. Furthermore, the study was aimed at understanding the underlying mechanisms involved, which might enable targeted therapies to be developed.

2-D DIGE

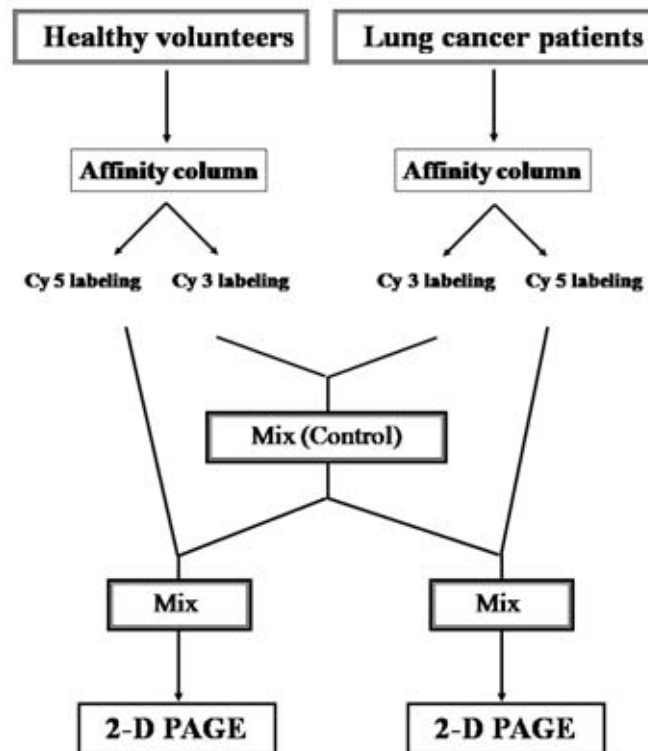


Fig. 1 Schematic diagram of 2D-DIGE used for comparative analysis of protein expression profiles.

Method

We analyzed the sera from a smoker with refractory squamous cell carcinoma of the lung, a smoker (without lung cancer), and a nonsmoker (without lung cancer). First, we used an affinity column (Agilent Technologies, Santa Clara, CA, USA) to separate the serum proteins that occurred with high frequency, including albumin, transferrin, haptoglobin, alpha-1-antitrypsin, IgA, and IgG, from those with a low frequency. By using fractionated samples, we were able to identify 10 to 20 times more protein spots than would have been possible using crude serum; this in turn allowed us to make a more quantitative and comprehensive analysis. Second, the serum proteins that occurred less frequently were subjected to two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). As a platform for 2D-PAGE, we used two-dimensional difference gel electrophoresis (2D-DIGE). In the 2D-DIGE system, multiple protein samples were labeled with different fluorescent dyes, mixed together, and then co-separated in a single gel. We made modifications to this protocol (**Fig. 1**), in which the internal standard sample was first prepared and labeled with Cy3, and the individual samples were labeled with Cy5. These differently-labeled samples were mixed and co-separated in one gel. This meant that all gels contained a common image from the internal control sample and that gel-to-gel variations between samples could be avoided. This enabled us to improve the reproducibility. From about 2,000 kinds of protein expression profiles, we performed statistical analysis to identify a candidate protein that showed a close relation between the expression and posttranslational modification and clinical condition of the cancer.

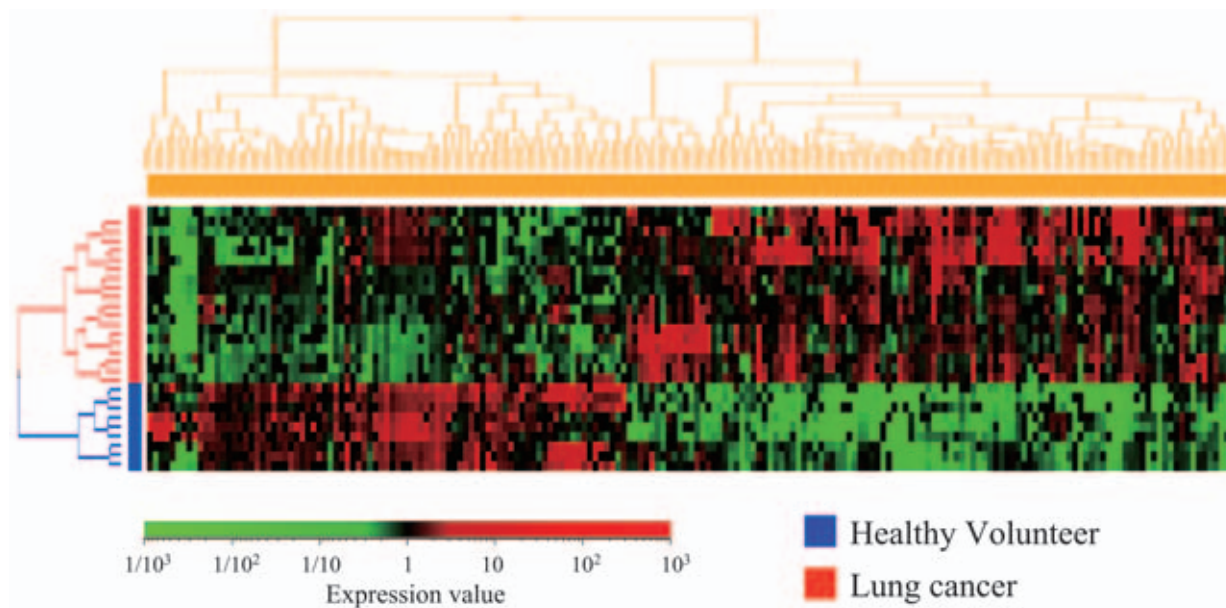


Fig. 2 Hierarchical clustering analysis of the patient with lung cancer and the healthy volunteers using the 262 selected protein spots. Red, high expression; green, low expression. Columns, serum samples; rows, protein spots.

Results

We found 262 protein spots, from a total of 2,260, whose expression in the patient with lung cancer differed significantly (by more than two-fold) from that in healthy volunteers (Student's *t*-test, *p*-value less than 0.01). The classification performance of the 262 selected protein spots was validated by unsupervised classification. Hierarchical clustering analysis revealed a distinct separation between clusters containing the patient with lung cancer and the healthy volunteers (**Fig. 2**). Next, we performed analysis between 2 smokers (one with cancers and one without) and the nonsmokers to examine the effect of cigarette smoking. In the two groups, we recognized a significant difference in the expression of 168 protein spots. Furthermore, for 34 protein spots, there was a significant difference in the analysis between the smoker with lung cancer and the smoker without cancer. The intensity of 20 protein spots among these 34 was up-regulated in the patient with lung cancer. This result suggests that cigarette smoking is responsible for inducing the abnormality of serum proteins seen in lung carcinogenesis. In the future, we intend to identify proteins corresponding to the protein spots of interest by using mass spectrometry.

Conclusion

We detected serum proteins that appear to be associated with smoking-related lung cancer abnormalities. They are candidate proteins for tumor markers of lung cancer, and may help increase understanding of the pathologic processes of carcinogenesis of the lung due to smoking.