further optimization. For example, it may be possible to dissociate protein complexes prior to the immuno-depletion procedure so that albumin-bound proteins are recovered in the flow-through fraction. For better depletion of target proteins, the flow-through fraction could be applied repeatedly to the immuno-affinity column or two immuno-affinity columns could be connected in tandem. These variations are compatible with our current protocol based on 2-D-DIGE. An alternative explanation of the failure of the immuno-affinity procedure to completely deplete the target plasma proteins is that these proteins display aberrant features in lung cancer and are unable to bind to an immuno-affinity column containing antibodies raised against normal plasma proteins. These issues should be considered further when immuno-affinity procedures are applied to disease proteomics.

We found isoform-specific aberrations of plasma protein expression in lung cancer: the protein spots corresponding to 12 gene products were both up- and down-regulated. In contrast, the spots of 28 proteins showed consistent up- or down-regulation. Because the spots of haptoglobin, which showed the largest identified number of spots from a single gene product in our study, were consistently up-regulated in lung cancer, a high number of spots does not always result in inconsistent regulation, suggesting isoform-specific regulation of certain proteins. These isoform-specific aberrations may not be identified by analysis of peptide subsets from complex digests as accomplished using multidimensional protein identification technology, suggesting the importance of analysis of intact plasma proteins. Although isoform-specific alterations could be a potential source of biomarkers, it might be difficult to use such isoforms as biomarkers in a clinical setting if their monitoring requires time-consuming proteomic technology such as the combination of multidimensional chromatography and 2-D-PAGE. More conventional and high-throughput methods will be required for clinical application. Although use of a specific antibody is always an early consideration when developing a high-throughput method, we demonstrated that the results of Western blotting following SDS-PAGE did not always match those from 2-D-PAGE. This discrepancy probably arises from the facts that the antibodies used were not isoform specific and the samples were prepared differently. To solve this problem, a novel technique such as the use of DNA aptamers [28] may be required to generate molecules with high affinity for each protein variant. This issue is a generic problem using 2-D-PAGE, in which various isoforms are visualized, quantified and selected as candidate biomarkers. However, this does not mean that a 2-D-PAGE approach is impractical. We believe that 2-D-PAGE is a powerful tool for providing detailed proteomic information that cannot be obtained from other proteomic methods, and that novel technologies are required to utilize the output of 2-D-PAGE for clinical application.

We are grateful to Dr. Teruhiko Yoshida for his collaboration and to Kano Nishiyama for technical assistance. This work was supported by a grant from the Ministry of Health, Labor and Welfare and by the Program for Promotion of Fundamental Studies at the National Institute of Biomedical Innovation of Japan. Tetsuya Okano and Tatsuhiko Kakisaka are awardees of Research Resident Fellowships from the Foundation for Promotion of Cancer Research (Japan) for the 3rd Term Comprehensive 10-Year Strategy for Cancer Control.

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