Identification of Biomarker and Therapeutic Target by Nano LC-MS/MS

○Hisashi Hirano
(Internatl. Grad. Sch. Arts Sci., Yokohama City Univ.)

The proteomic analysis of plasma and tissues in patients has been a major approach to determining biomarkers essential for early disease diagnoses and drug discoveries. The detection of proteins in the plasma, however, is analytically challenging because the dynamic concentration range of them is extremely wide. We established a novel technique to analyze plasma proteins. In this technique, an originally developed "abundant protein depletion device" and a sequentially linked three-dimensional liquid chromatography-tandem mass spectrometry (3D-LC-MS/MS) system were used (Proteomics 6:6845, 2006). Using this technique, we identified nearly 3,000 low abundant proteins in less than one mL plasma for about three days. Furthermore, by combining this technique with the isobaric tag for relative and quantitative analysis (iTRAQ) reagent, we developed an efficient technique to detect quantitatively disease-associated proteins. By use of various proteomic techniques including those developed in our laboratory, we detected and identified about 40 ovarian cancer-associated proteins (Proteomics 6:5880, 2006). The expression of most of the proteins including annexin IV was regulated at transcriptional level in the cancer tissues. When the gene expression encoding these proteins with the siRNAs was suppressed, the proliferation of the cancer cells was reduced at different levels depending on the protein. However, prohibitin detected as the cancer-associated protein was not regulated at transcriptional level. We found that phosphorylation level of prohibitin changed in the ovarian cancer tissues, suggesting that the expression of this protein is controlled by post-translational modification. These proteins were considered to be candidates of biomarkers or targets of drug discovery.