METABOLOMIC AND PROTEOMIC ANALYSES FOR PHARMACOLOGICAL RESPONSES TO ANTICANCER AGENTS

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The individualization of anticancer chemotherapy based on each patient’s responsiveness to drugs is required to improve cancer chemotherapy. We have applied exploratory genomic, proteomic and metabolomic analyses to discover useful biomarkers that can predict responders/non-responders to drugs or high-risk patients in cancer chemotherapy. Recently, metabolomics has been included as a new “omics” science, joining to genomics, transcriptomics and proteomics as a new field toward the understanding of global system biology. Metabolome is the biochemical phenotype of cellular events after gene expression, transcription and translation. In the present symposium, we report our recent studies on chemosensitivity of human colorectal carcinoma against 3 key anticancer agents, 5-FU, CPT-11 and oxaliplatin. The novel metabolomic approach is useful to clarify the intracellular mechanism of drug action as well as to find out low molecular weight biomarkers for predicting drug responses. We also applied proteomic approach to develop protein biomarkers.

Protein expression was investigated using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) to identify a particular protein markers associated with drug sensitivity. We found the 11.1 kDa protein as a potential biomarker for predicting oxaliplatin sensitivity of human colorectal cancer cells. In an animal model, nude mice xenografted with human colorectal carcinoma showing different chemosensitivity were administered CPT-11. The metabolic perturbation in response to CPT-11 exposure was measured by exploratory metabolomic analysis of serum using the capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) analysis. CE-TOFMS is an ideal tool for quantitative metabolomic analysis that can detect over 1000 charged species. We found that different peak quantity of expression was detected in m/z 166.086 (L-phenylalanine) between low sensitivity and high sensitivity cancers in the serum metabolites by CE-TOFMS analysis. L-phenylalanine might be a low molecular weight biomarker that can reveal sensitivity for cancer against CPT-11 therapy.

5-Fluorouracil (5-FU) is thought to exert anticancer effects via conversion to several active metabolites: fluorodeoxyuridine monophosphate (FdUMP), fluorouridine triphosphate (FUTP), and fluorodeoxyuridine triphosphate (FdUTP). Most of previous studies addressed relationships between chemosensitivity to 5-FU and the gene expression of metabolic enzymes that convert 5-FU to its active metabolites or catabolic enzyme (DPD). However, there is no report focusing on changes in the total intracellular metabolite pool (metabolome) caused by 5-FU, which reflect its anticancer effects directly. CE-TOFMS was applied to elucidate the dynamic change in intracellular metabolism of cancer cells with response to 5-FU exposure. The intracellular level of dUMP, a substrate for thymidylate synthase (TS), remarkably increased after 5-FU exposure, which subsequently led to strikingly increased dUDP and dUTP levels. In contrast, the level of dTMP, a product of TS, decreased after 5-FU exposure. Its depletion caused subsequent depletion of dTDP and dTTP required for DNA synthesis. We also observed the increase in the level of FdUMP.

In summary, 1) we have found a possible protein biomarker to predict chemosensitivity of human colorectal cancers. 2) We also found the difference in serum L-phenylalanine level between nude mice implanted human colorectal carcinoma with low and high sensitivity to CPT-11. 3) By metabolomic analysis, we showed the first evidence quantitatively demonstrating the mode of intracellular 5-FU actions and metabolite kinetics. These approaches can be applied to pharmacodynamic analysis, toxicity assessment, and biomarker analysis.