Metabolic profiling of renal cell carcinoma studied by high-resolution ^{1}H NMR spectroscopy on human serum in combination with multivariate data analysis

H. Gao[1], B. Dong[2], X. Liu[1], Y. Huang[2], and D. Lin[1]

[1]Analytical Chemistry Laboratory, Shanghai Institute of Materia Medica, the Chinese Academy of Sciences, Shanghai, China, People's Republic of, [2]Department of Urologic Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China, People's Republic of

Introduction Human renal cell carcinoma (RCC) is a prevalent malignancy disease well known for its insidious onset, with patients often developing metastases beyond the kidney at the time of diagnosis and the prospects for cure are dismal [1]. A quite favorable prognosis can be obtained when kidney cancer is detected and nephrectomized at an early stage [2]. Thus, early diagnosis for RCC is of importance for timely treatments and patient survival. On the basis of the combination of ^{1}H NMR analysis of the biofluids with multivariate data analysis, a novel scientific field, metabonomics is becoming an emerging technique widely used in disease studies and has been proved to be a rapid non-invasive approach capable of early detecting metabolic changes under different pathophysiological procedures for class discrimination and biomarker identification [3]. In the present study, metabonomics approach was employed to study the metabolic profiling of human RCC serum samples and two objectives were addressed (1) to depict the systemic changes of metabolite composition of early human RCC serum in comparison with those of healthy subjects, (2) to exploit the potential methods for early diagnosis and monitoring prognosis of RCC patients in terms of sample classification on the serum of patients.

Materials and Methods The early RCC blood samples were obtained preoperatively from 16 pT1a RCC patients. The blood samples before and after nephrectomy were obtained preoperatively and postoperatively from the same 28 RCC patients, respectively. Control blood samples were obtained from 55 healthy volunteers. 200 μL serum was mixed with 400 μL of phosphate buffer and centrifuged at 12,000 g for 10 min at 4 °C. Aliquots of 500 μL of the supernatants were moved into the 5 mm tubes for high resolution ^{1}H CPMG spectra, which were recorded on a Varian INOVA 600 NMR spectrometer using CPMG sequence to attenuate the broad protein signals [4]. Spectral processing and data-reduction to 241 integrated regions of equal width of 0.04 ppm were performed using standard routines provided by the VNMR 6.1C software package. The integral segments for each NMR spectrum were normalized and input into SIMCA-P 10.5 for the principal component analysis (PCA). Data were visualized and biomarker were identified from the PCA scores plots and corresponding loading plots [5].

Results Average ^{1}H CPMG spectra of serum samples are shown in Fig. 1A. The PCA scores plots for the PC1 and PC2 from the serum CPMG spectra of early RCC patients and healthy humans, and of RCC patients before nephrectomy, after nephrectomy and healthy humans are shown in Fig. 1B and C, respectively.

Discussion The distinct separation of serum samples of pT1a RCC patients from those of healthy humans (Fig. 1B) suggests that it is possible to diagnose and treat human RCC in the early stages by phlebotomizing and monitoring the serum metabolic signatures. Fig. 1C shows the serum samples of RCC patients before nephrectomy lay in the left, those of the same RCC patients after nephrectomy were situated in the middle, and those of healthy humans were located in the right side of the plot. Interestingly, four samples of the RCC patients after nephrectomy (labeled numbers 1-4) were mixed in the cluster of RCC samples before nephrectomy. Further clinical chemistry analysis after several months showed that three of them (labeled 2, 3 and 4) did have a worse prognosis, while the indubitable progress of patient 1 remains to be further carefully monitored. The PCA loading plots revealed that, compared to the healthy, the early RCC serum had higher levels of lipid, lactate, alanine, pyruvate, glycerol, and unsaturated lipid, but declined levels of acetoacetate, choline, and glucose/glycogen. Such systemic changes of metabolite concentrations were reversed in the serum of RCC patients after nephrectomy. These results suggests that metabonomics offers an efficient and convenient approach to address the tumor biochemistry. Such novel approach may have some potential for early diagnosis and prognosis inspection of malignant diseases on the basis of just a single blood sample.

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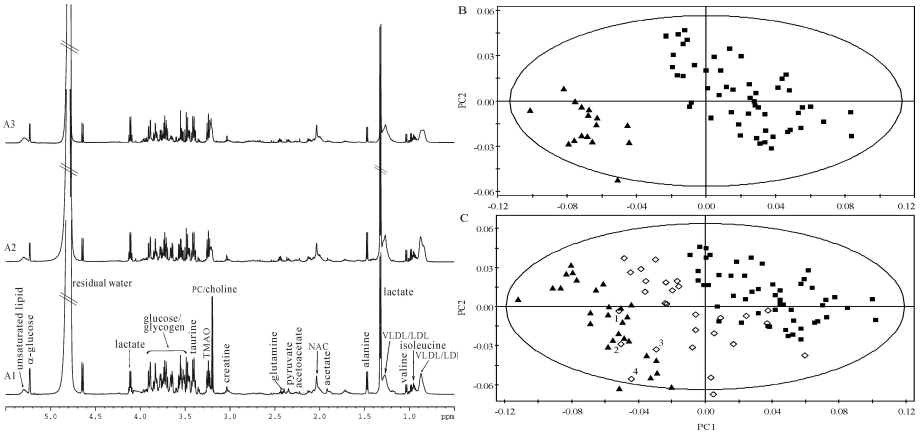

Figure 1 Average spectra of 600 MHz ^{1}H CPMG experiments for serum samples from (A1) healthy humans, (A2) early RCC patients, and (A3) RCC patients after nephrectomy. LDL, low-density lipoproteins; NAC: N-acetylglycoproteins; PC: phosphatedylcholine; TMAO, trimethylamine-N-oxide. B, PCA scores plot of healthy humans (■) and early RCC patients (▲) from PC1 and PC2. C, PCA scores plot of healthy humans (■), RCC patients before nephrectomy (▲) and those after nephrectomy (□) from the PC1 and PC2.

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