行政院國家科學委員會專題研究計畫　期中進度報告

利用表面強化雷射離子化飛行時間質譜儀篩選腎細胞癌診斷及預後標記分子(1/2)

計畫類別：個別型計畫
計畫編號：NSC93-2314-B-002-157-
執行期間：93年08月01日至94年07月31日
執行單位：國立臺灣大學醫學院泌尿科

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報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94年5月31日

Screening diagnostic and prognostic maker of renal cell carcinoma (RCC) by Surface-Enhanced Laser Desorption/Ionization- time of flight (Seldi-tof)

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Introduction

Renal Cell Carcinoma comprises about 3% of all human neoplasm. Like melanoma, in view of the observed spontaneous remissions of advanced renal cancer, immune mechanisms have been suggested to play a role in the eradication of local and metastatic RCC. Nevertheless, the impelling factors of tumor specific immunological reaction remains unclear, suggesting additional RCC associated tumor antigens remain to be exploited.

Seldi-tof is a novel variant of mass spectrometry. It’s various coating of the protein chips, such as hydrophilic, hydrophobic, anionic, cationic or special receptor/ligand, will specifically bind target proteins. The detector will picked up molecular weight signals according to the time-of-flight from the desorption/ionization events.

We have hypothesis some tumor associated molecules may leak into blood stream and eventually vanished after surgical removal to RCC for a certain period of time. When compare pre-operative and more than 2-months post-operative serum paired samples, 5 clusters of polypeptides show an interesting reciprocal profile.

Materials and Methods

Thirty-six paired pre-operative and post-operative RCC serum samples were used as training group, additional serum of non-RCC urological patients and kidney transplantation living donor were used as control group in this project. Ten milliliter of whole blood was draw from patient during radical nephronectomy as pre-operative group, blood from out-patient visit more than 2 months later as post-operative group. The whole blood was left at least 30 minutes for clotting. Serum was collected after 15K centrifugation for 20 minutes, and kept -80C before analysis.

Various kind of Ciphergen protein chip were used for profiling training serum. Among them are H50, IMAC3, SAX, WCX2, and NP20 which represent specific surface coating of hydrophobic, immobilized metal affinity capture, strong anion exchange, and weak cation exchange material respectively. The chips were activated according to manufacturer’s manual. Pre-treatment of 2 times U9 buffer in 4C for 20 minutes, 9 parts of respective binding buffer were used to dilute the urea treated samples and apply to coating spots. Either wash buffer or binding buffer was used to wash off un-binding protein on the coated spots, EAM solution with alpha-cyano-4-hydroxy

cinnamic acid was used to co-crystallize protein with the matrix.
Proteins bound to the chelated metal (through histidine, tryptophan, cysteine, or phosphorylated amino acids) and also hydrophobic and weak cationic surfaces are detected with the ProteinChip Reader. Data will be collected by averaging 50 laser shots with an intensity of 160 and a detector sensitivity of 8. Reproducibility is estimated using two representative serum samples. The CV was estimated for the selected mass peaks.

Bioinformatics and Biostatistics

All spectra will be compiled, and qualified mass peaks (signal-to-noise ratio >5) with mass-to-charge ratios (*m/z*) between 2000 and 150 000 are auto-detected. Peak clusters are completed using second-pass peak selection (signal-to-noise ratio >2, within 0.3% mass window), and estimated peaks are added. The peak intensities were normalized to the total ion current of *m/z* between 2000 and 150 000. All these were performed using ProteinChip Software 3.0 (Ciphergen). The only additional preprocessing step was logarithmic transformation of the peak intensity data. Such a transformation in general reduces the range of intensity data. As a result, the variance of the transformed peak intensity (across multiple samples) tends to be less volatile over the entire length of the spectrum.

Results

We have the first result from 5 RCC patients using hydrophobic H50 protein chip. The pre-operative serum demonstrate two distinctive signals in 8K/4K m/z (Figure 1)

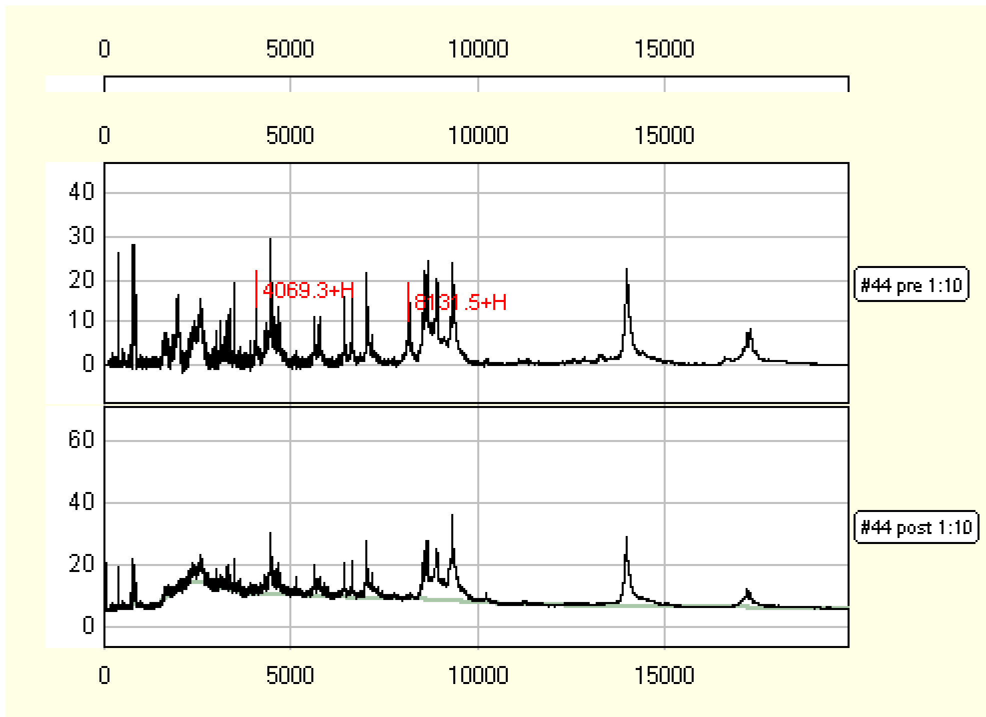

Figure1: Representative histogram compares the pre-operative and post-operative RCC patient's serum. 8K/4K peaks in the pre-operative serum are marked with red m/z.

The WCX2 chip differentiate 5 clusters of polypeptides that efficiently distinguish pre-operative and post-operative serum.

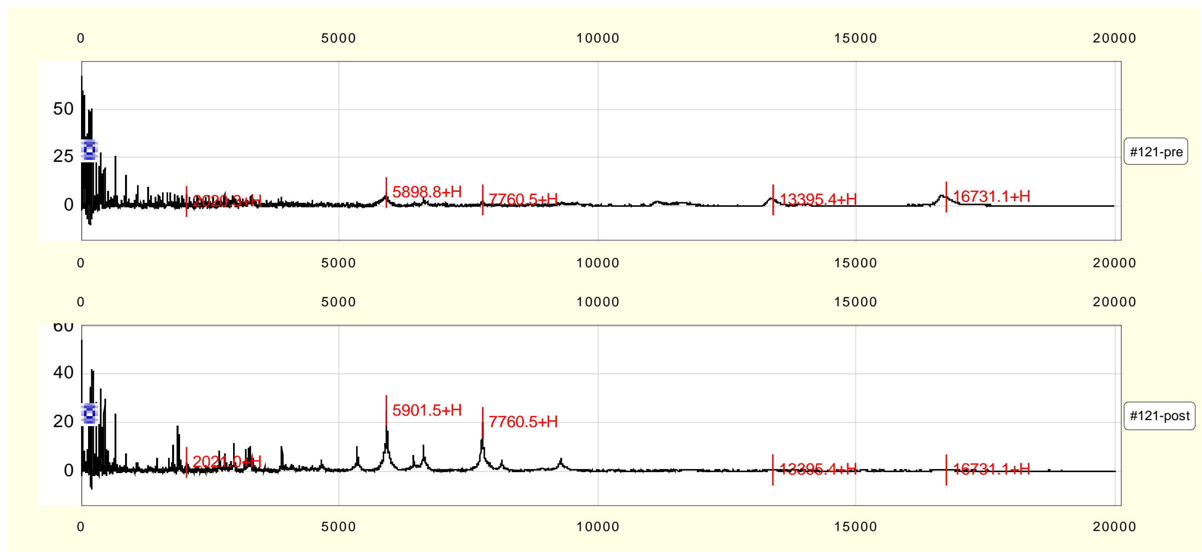

Figure2: Representative histogram compares the pre-operative and post-operative RCC patient's serum. 16.7K, 13.3K, 11K, 7.7K and 5.9K clusters express differently.

Because each paired sample is from the same patient, the genetic background bios can be easily subtracted and minimized the false positive signals. The candidate polypeptides were subjected to analysis.