行政院國家科學委員會專題研究計畫 成果報告

進一步純化並鑑定由弱陽離子蛋白質晶片篩選出腎細胞癌特異性之多胜肽

研究成果報告(精簡版)

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計畫參與人員：碩士級-專任助理：呂政弦

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 12 月 11 日

行政院國家科學委員會補助專題研究計畫 ■ 成 果 報 告
□期中進度報告

（計畫名稱）

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計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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Surface Enhanced Laser-assisted De/ionization Time-Of-Fly (SELDI-TOF) mass spectrometry resolved distinct profile of Renal Cell Carcinoma (RCC) patient's serum

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Introduction:

Renal cell carcinoma (RCC) is a potentially lethal malignancy. Many of the RCC are asymptomatic and are discovered incidentally during routine physical checkup. Therefore, effective screen marker is needed for RCC. Recently, MN/CA9 had been reported as renal cell carcinoma (RCC) marker. Further challenge of MN/CA9 to immunotherapy reveals fare results, which imply additional tumor marker (s) for RCC.

Tumor markers for malignant disease had been very useful either for diagnosis or prognosis. Recently, surface enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS) had been used to screen neoplasms (Kozak et al 2003, Qu et al 2002) as well as alcohol abuse (Nomura et al 2004).

SELDI-TOF is a novel type of mass spectrometry. It uses aluminum chips with special coating surface to capture specific protein of interest (Figure 1A). After wash off non-specific binding protein, the chips is sent to a vacuum chamber and bombarded with laser. The proteins on the aluminum surface is excited with the matrix and fly though the chamber. The detector in SELDI-TOF records protein's mass according to the time-of-fly and expressed as m/z (Figure 1B). The SELDI-TOF technology has been used directly to detect protein fingerprints in complex biological samples, such as urine (Vlahou et al 2001) and serum (Rosty et al 2002).

In this study, we used SELDI technology to compare paired serum samples obtained before and after nephrectomy for RCC patients. After analysis, five clusters of polypeptides with reciprocal behavior had been found.

Materials and methods

We used serum from 36 patients to establish the model. Patients diagnosed with RCC grade I/II were included. For every patient, serum samples collected before nephrectomy were grouped as pre-operative, two months or later in the outpatient clinics, were collected as post-operative. All samples were store at -80oC until analysis.

An aliquot of the stored sera was used for the SELDI-TOF MS analysis. In our study, every ProteinChip were tested, however, a cationic exchanger (WCX2) were chosen for the analysis. TOF mass spectra were generated in a Ciphergen Protein Biology System II by averaging 56 laser shot per spot with an intensity of 115 to 145 and detector sensitivity to 8. All spectra were compiled to be analyzed, and qualified mass peaks (signal-to-noise ratio >20) with mass-to-charge ratios (m/z) between 2000 and 20000 were auto-detected. Peak clusters were completed using second pass peak section (signal-to-noise ratio >2, within 0.3% mass window), and statistical estimated peaks were added. All these were performed using ProteinChip Software 3.0.1 (Ciphergen).

Serum from eight metastatic RCC patients' and 16 non-RCC patients were collected and subjected the same experimental procedure as the model samples.

Results:

We obtained mass spectra for a total of 72 serum samples from SELDI analysis using WCX2 arrays. Figure 2 shows representative spectra of proteins retained on the WCX2 protein chips. The Mass records can be expressed by trace view (Figure 2A) or gel view (Figure 2B). This model samples was analyzed with BioWizard software (Ciphergen) and showed 5 clusters with statistical significance. It was noted that 16.7 kDa and 13.4 kDa peaks, prominent in pre-operative sera, significantly decreased in post-operative sera. On the contrary, lower molecular weight peaks of 7.7 kDa, 5.9 kDa, and 2.0 kDa were prominent in post-operative but very few in the pre-operative sera (Figure 3A).

When compare non-RCC patients sera with the model samples, they had very similar profile with the post-operative sera (Figure 3B, Figure 4). Since the early stage of RCC tumor mass were removed by surgery, post-operative profile should represent tumor free status. We pooled the non-RCC patients' profile to the model samples and analyzed again, the non-RCC profiles closely resemble those post-operative in model samples (Figure 3B, Figure 4).

The purification and identification of these polypeptides revealed possibilities of the followings proteins: alphaB-crystallin, alpha-enolase and triosephosphate isomerase 1. The roles of these proteins deserve further investigation.

Discussions:

The reproducibility of the SELDI spectra on a single chip (intra-assay) and between chips (inter-assay) was reported by Adam et al (2002). They used seven proteins in the range of 3-10 kDa to calculate the coefficient of variance for a pooled normal serum sample. The peak location was 0.02% and 0.03% for intra-assay and inter-assay respectively, and the intra- and inter-assay normalized intensity (peak height) was 12.1% and 20.5% respectively. SELDI seems to be a good screening tool rather than a quantitative instrument.

In this study, five clusters of polypeptides showed significant reciprocal relationship imply strong co-relation with RCC. The higher molecular weight 16.7 kDa and 13.4 kDa clusters may represent tumor associated proteins. However, the lower molecular weight 7.7 kDa, 5.9 kDa, and 2.0 kDa may represent proteins that has been suppressed by the tumor. Although non-RCC patients also expressed these polypeptides, they have the same profile as those of post-operative sera. The results suggest a valuable criteria to differentiate RCC in patient's serum.

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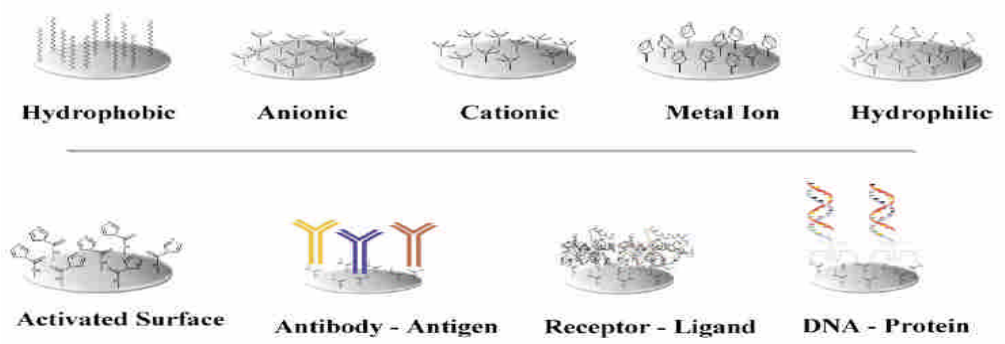
Figure 1 . (A) Various coatings of ProteinChip array are available for sample preparation. The chemically modified surfaces are used to retain proteins based on their specific physical properties. (B) Schematic diagram of the SELDI Ciphergen mass spectrometer.
The metastatic RCC sera showed no different profile between pre- or post-operative samples (data not shown), and the profile similar to post-operative of RCC patients'. Additional marker(s) between non-RCC, post-operative, and metastatic serum are under investigation.

Figure 2: Representative SELDI-tof output of two patients serum with WCX2 ProteinChip in trace view format (A) and gel view format (B).

Figure 3: (A) BioWizard software shows significant profiles of 5 clusters of polypeptides with m/z of 16.7K, 13.4K, 7.7K, 5.9K, and 2.0K. Two reciprocal pre- and post-operative serum display very distinctive profile (B) Pooling of non-RCC patient's serum results reveal similar profile as those of post-operative group.

Figure 4: M/z of #14 patient histogram between surgery

1A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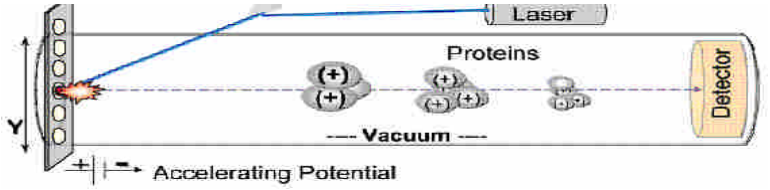
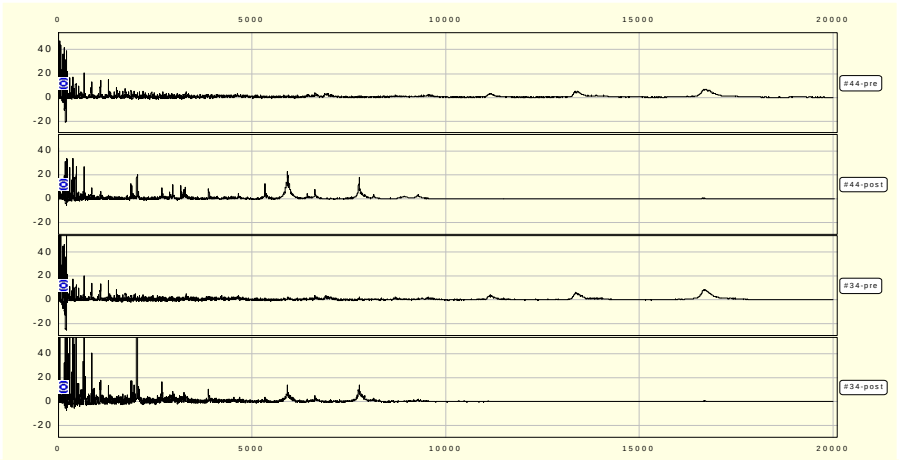
Anionic
Cationic
Metal Ion
Hydrophilic
Activated Surface
Antibody - Antigen
Receptor - Ligand
DNA - Protein

1B


Laser
Proteins
Detector
Y
---- Vacuum ----
Accelerating Potential

2A

A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20000
40
20
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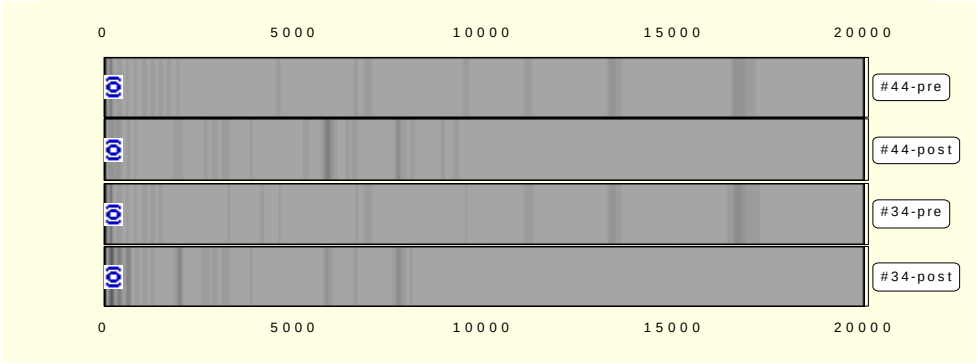
#44-pre
#44-post
#34-pre
#34-post

B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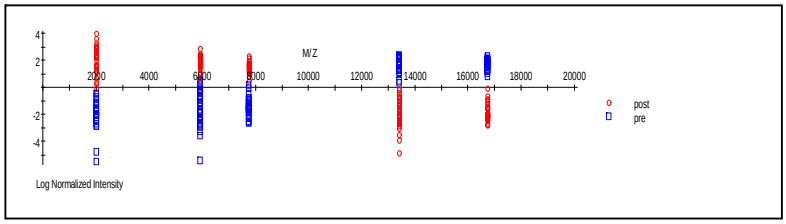
#34-post

3

A



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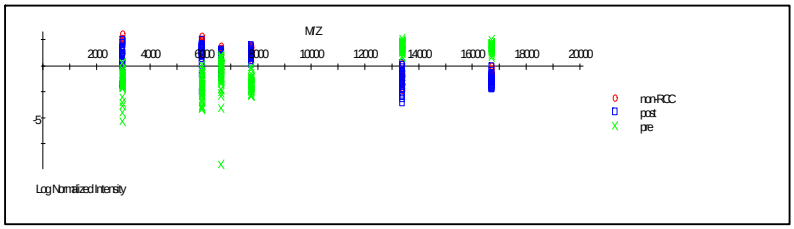

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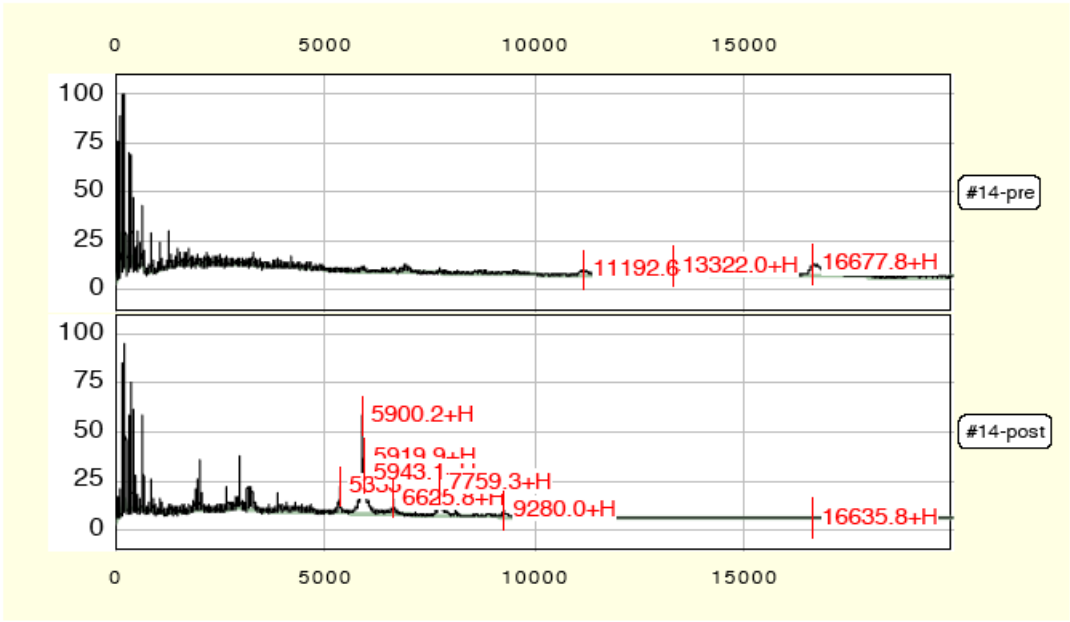

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