

Using SELDI-TOF-MS technology for screening serum markers of hepatic carcinoma in rats ☆

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Abstract

Objective: To identify potential serum markers of hepatic carcinoma in rats through Surface-Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometry (SELDI-TOF-MS) Technology. **Methods:** A rat model of hepatic carcinoma was established. The serum samples of hepatic carcinoma and normal rats were analyzed via SELDI-TOF-MS Technology. The changes of the serum protein fingerprint patterns were observed between the experimental group of hepatic carcinoma and the controls. The analysis was conducted by statistical software-Biomarker Wizard. **Results:** Fifty-six protein peaks in the serums were found. Within m/z 0-20 000, the protein peaks of m/z 1158, 8 835 and 15 302 of hepatic carcinoma serums were obviously higher in the rat models compared with those in the controls ($P < 0.01$). **Conclusion:** Three peaks were considered as potential biomarkers according to the serum protein fingerprint patterns of the hepatic carcinoma group and the control group.

Key words: hepatic carcinoma model; SELDI-TOF-MS; serum marker

INTRODUCTION

Hepatic carcinoma is one of the leading causes of death in the world. The occurrence of hepatic carcinoma in china is especially high with the mortality rate up to 50 percent^[1-2]. The traditional hepatic carcinoma diagnostic marker-alpha-fetoprotein(AFP) doesn't have high specificity and sensitivity, so that researchers have been seeking other more sensitive and specific molecular markers^[3-5]. In recent years, the Surface-Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometry (SELDI-TOF-MS) Technology has provided a useful tool for finding biomarkers of tumors^[6-8]. In this experiment we used SELDI-TOF-MS technology to test the protein expression profile of the serums

of the rat models of hepatic carcinoma, analyzed the differences of protein expression and found three new potential biomarkers of hepatic carcinoma.

MATERIALS AND METHODS

Animal model establishment^[9]

Ninety-five healthy male Wistar rats (from Shanghai Slyke Laboratory Animal Center), 120~150 g, 6 weeks old, were randomly divided into the model and control group, 85 and 10 in each group, respectively. ① the model group: Diethylnitrosamine (DEN) 1% aqueous solution gavage, 70 mg/kg, once a week, for 14 weeks. ② the control group: the same dose of distilled water gavage. Five rats in the model group were sacrificed respectively at 2, 4 and 6 weeks from the second week. Ten rats in the model group were sacrificed respectively at 8, 10, 12, 14, 16, 18, 20 weeks randomly, and one rat in the control group was sacrificed at the same period. Dynamic observation was taken of the gross appearance of the livers, and histological changes between the DEN-treated model rats and control rats. Orbital blood

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was collected, and the upper centrifugal serum was drew after blood coagulation and stored at -70°C . The specimens were separated into two groups according to histological changes.

Protein chip processing

Pretreatment chips and chip testing: $5\ \mu\text{l}$ hydrochloride were added to each well of WCX22 chips at room temperature for 5 min. Then the chips were washed three times with deionized water, liquid discarded. Sodium acetate (pH 6.5) $150\ \mu\text{l}$ was added to each well of WCX22 chips at room temperature for 5 min, liquid discarded (once repeated). $50\ \mu\text{l}$ diluted sample were added to each well of WCX22 chips by greatly shaking on a platform shaker at a speed of 700 rpm for 5 min and incubated at 4°C , liquid discarded. Then the chips were washed three times with $150\ \mu\text{l}$ Combine/elution washed buffer, for 5 min each time, rinsed quickly with $200\ \mu\text{l}$ HEPES (pH 7.0) the last time. $75\ \mu\text{l}$ acetonitrile and $75\ \mu\text{l}$ 1% trifluoroacetic acid were added to SPA by greatly shaking on a platform shaker at a speed of 700 rpm for 5 min until all SPA dissolved, and centrifuged for 1 min. SPA were added to each well of WCX22 chips 2 times ($0.5\ \mu\text{l}/\text{time}$). In the calibrated mass spectrometer with standard protein chip NP20, instrument parameters was set, and the datum with Biomarker Wizard were collected. The computer received the raw data and mapped out protein mass spectrometry quickly and accurately (y axis stands for relatively high-protein peak, x axis stands for mass-to-charge ratio).

Statistical analysis

All spectra datum were collected based on statistical analysis (using Biomarker Wizard). m/z peak of protein expression profiles were compared between the model and the control group by *t* test.

RESULTS

Gross appearance

Liver lesion of the model group can be summarized as three stages: ① the first stage: the first nine weeks, no significant pathological changes in liver; ② the second stage: 10 to 15 weeks, texture hardens, surface roughness, gray-white nodules (inequality of size) located in marginal of the liver; ③ the third stage: 16 to 22 weeks, surface roughness, ranging from the number, size, gray-white cancer nodules, 2 cm in diameter, hemorrhage and necrosis can be visible in the section. Gray-white nodule can be visible in liver of all the 20-week-old rats. Direct adhesion to adjacent tissue was observed in 2 rats of the model group, and lung metastasis can be seen in another 2 rats of the model group (Fig. 1).



Fig. 1 The gross appearance of the livers from DEN-treated rats: the liver from the rat by DEN treated at the 16th week

Histologic changes

The first 7 weeks: non-special damage of liver can be seen; the 8th week: proliferation nodules can be seen; 10th to 12th weeks: cirrhosis can be seen; the 14th week: gray-white nodules can be observed; the 16th week: micronodules can be seen; 22nd week: lung metastases (Fig. 2).

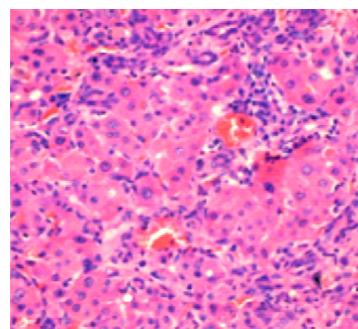


Fig. 2 Histological changes of the liver carcinoma of rats (HE, $\times 200$)

Changes of the serum protein fingerprint patterns between the hepatic carcinoma model and control group

According to histological changes of the liver, serum samples were separated into two groups: ① model group: 32 serum samples of rats with liver carcinoma, ② control group: 10 serum samples of normal rats. Serum samples were then assayed by SELDI-TOF-MS technique, and 56 protein peaks appearing differently in the serums were found. With m/z 0~20 000, the protein peaks of m/z 1158, 8835 and 15302 of hepatic carcinoma serums were obviously higher than those of the normal group ($P < 0.05$, Fig 3~5, Table 1).

DISCUSSION

Hepatic carcinoma is one of the most common malignant tumors in China. The occurrence mechanism remains unclear. The protein expression mass spectrometry of human genes and its changes during the course of the disease are still new fields.^[10-11] The protein mass

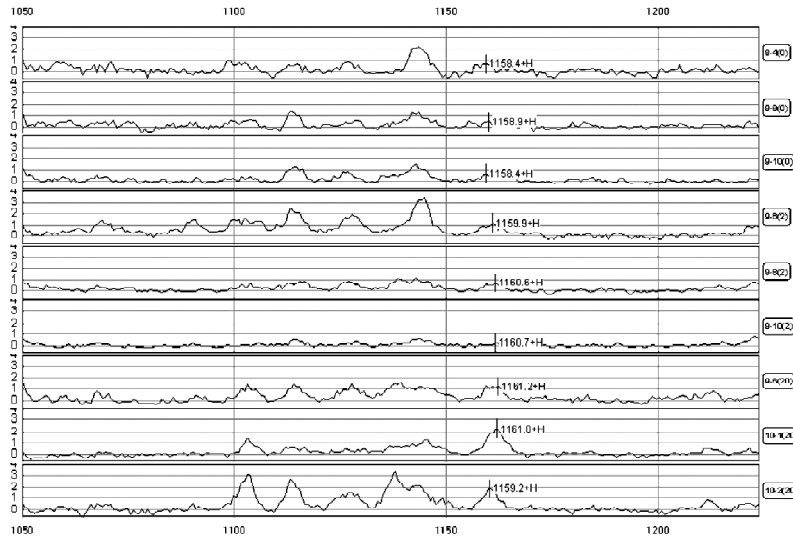


Fig. 3 Serum protein peaks between the model group and control group(m/z 1158 Da S)

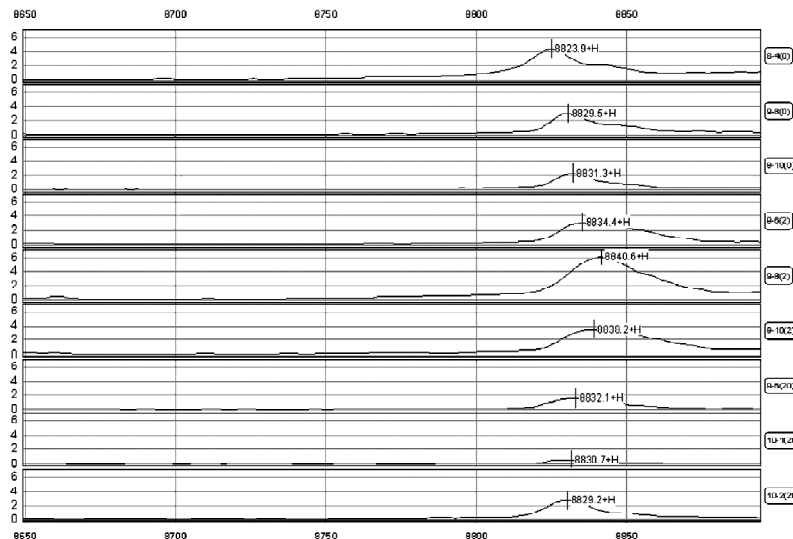


Fig. 4 Serum protein peaks between the model group and control group(m/z 8835Da)

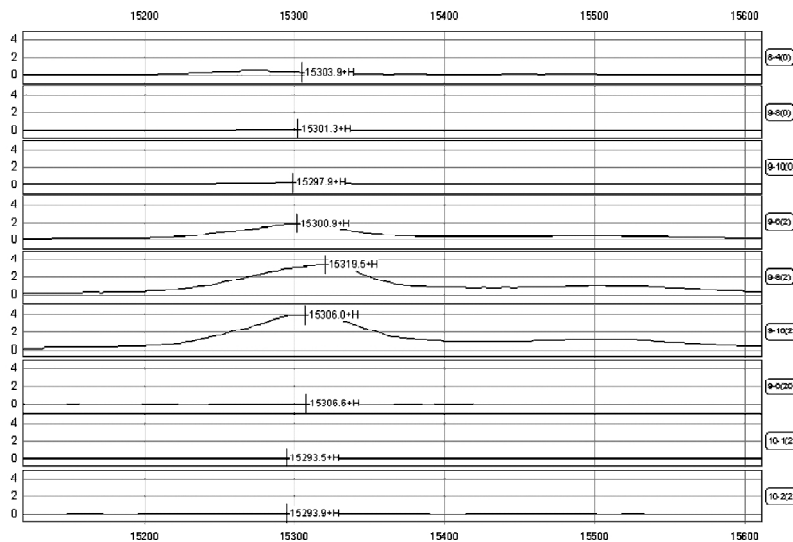


Fig. 5 Serum protein peaks between the model group and control group(m/z 15302Da)

Table 1 Three labeled peaks on the cancer and control groups

m/z	intensity of peak		P-value
	model group	control group	
1158	0.86 ± 0.56	0.60 ± 0.75	0.0081
8835	1.63 ± 1.04	0.91 ± 0.47	0.0120
15302	1.65 ± 0.70	1.29 ± 1.28	0.0128

spectrometry analysis technology has established a good technical platform for us to conduct the research of proteomics in liver disease systemically^[12-14].

SELDI-TOF-MS technology is a proteomics detection technology developed in recent years. Compared to traditional proteomics^[15], it is fast, sensitive and has a high-throughput. To complex biological samples, such as serums, urine and tears, the spotting sample testing can be conducted directly without a primary separation or purification of the samples. It is very suitable for testing the low abundance proteins which are cancer-related in human tissues and body fluids.

Using SELDI-TOF-MS technology for screening serum markers has been applied to a variety of tumors.^[16-18] Some serum marker molecules which have important clinical value in tumor diagnosis, prognosis evaluation, and disease course detection have been screened out. This technology provides a new strategy for the research of the occurrence of tumors, the screening of drug target proteins and markers of early diagnosis.

This experiment detected the changes of the serum protein fingerprint patterns between the hepatic carcinoma models and the normal control group via SELDI-TOF-MS Technology. Fifty-six protein peaks appeared differently in the serums. Three of them are the most distinct. The protein peak of m/z 1158 goes up clearly during the later period of hepatic carcinoma. This is likely related to the prognosis of hepatic carcinoma. The protein peaks of m/z 8835 and m/z 15302 have significant differences during the early period of live cancer. Therefore, it has a certain value of clinical application in the early diagnosis of hepatic carcinoma. However, SELDI-TOF-MS technology can not give the details of the protein such as molecular weight, conformations, biochemical characteristics, which otherwise can be detected with Two-Dimensional Electrophoresis and Two Hybrid Technique^[19]. Meanwhile, in another study we obtained four target molecules which have the diagnostic value through gene chip technology (summarized in another article). Further research and experiment are needed to combine the two results.

References

- [1] Allen J, Venook A. Hepatocellular carcinoma: epidemic and treatment. *Curr Oncol Rep* 2004 ;6:177-83.
- [2] Okuda H. Hepatocellular carcinoma development in cirrhosis. *Best*

Pract Res Clin Gastroenterol 2007;21: 161-73.

- [3] Stein DF, Myaing M. Normalization of markedly elevated alphafetoprotein in a virologic nonresponder with HCV-related cirrhosis. *Dig Dis Sci* 2002; 47: 2686-90.
- [4] Pompili M, Rapaccini GL, Covino M, Pignataro G, Caturelli E, Siena DA, et al. Prognostic factors for survival in patients with compensated cirrhosis and small hepatocellular carcinoma after percutaneous ethanol injection therapy. *Cancer* 2001; 92: 126-35.
- [5] Ma J, Gong Q, Lin M, Xi Y, Wang M, Chen Z, et al. Combined live tumormarkers in detecting primary hepatic carcinoma. *Chin J Surg* 2000; 38:14-6.
- [6] Pawletz CP, Trock B, Pennanen M, Tsangaris T, Magnant C, Liotta LA, et al. Proteomic patterns of nipple aspirate fluids obtained by SELDI-TOF: potential for new biomarkers to aid in the diagnosis of breast cancer. *Dis Markers* 2001; 17:301-7.
- [7] Miguët L, Bogumil R, Decloquement P, Herbrecht R, Potier N, Mauvieux L, et al. Discovery and identification of potential biomarkers in a prospective study of chronic lymphoid malignancies using SELDI-TOF-MS. *J Proteome Res* 2006; 5:2258-69.
- [8] Lin Z, Jenson SD, Lim MS, Elenitoba-Johnson KS. Application of SELDI-TOF mass spectrometry for the identification of differentially expressed proteins in transformed follicular lymphoma. *Mod Pathol* 2004; 17:670-8.
- [9] Jiang YC, Dong QN, Xiao BL, Li SQ. Studies on Hepatic carcinoma Induced by Non-necrotizing Dose of Diethylnitrosamine in Rats. *Journal of West China University of Medical Sciences(In Chinese)* 2001; 32: 555.
- [10] Sun Wei, HE FC. Applications of proteomics in hepatic diseases research. *Science in China Series C-Life Sciences(In Chinese)* 2004; 47: 101-6.
- [11] Serag H, Mason A, Key C. Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in the United States. *Hepatology* 2001; 33: 62-5.
- [12] Kolch W, Mischak H, Pitt A R. The molecular make-up of a tumour: proteomics in cancer research. *Clin Sci(Lond.)* 2005; 108 :369-83.
- [13] Wulfkuhle J D, Liotta L A, Petricoin E F. Proteomic applications for the early detection of cancer. *Nat Rev Cancer* 2003; 3: 267-75.
- [14] Powell K. Proteomics delivers on promise of cancer biomarkers. *Nature Med* 2003; 9: 980.
- [15] Li J, Zhang Z, Rosenzweig J, Wang YY, Chan DW. L Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clin Chem* 2002; 48:1296-304.
- [16] Engwegen JY, Gast MC, Schellens JH, Beijnen JH. Clinical proteomics: searching for better tumour markers with SELDI-TOF mass spectrometry. *Trends Pharmacol Sci* 2006;27:251-9.
- [17] Volker S, Matthias PAE, Thomas B. Advances in clinical cancer proteomics: SELDI-TOF-mass spectrometry and biomarker discovery. *Briefings in Functional Genomics and Proteomics* 2005; 4:16-26.
- [18] Pan YZ, Xiao XY, Zhao D, Zhang L, Ji GY, Li Y, et al. Application of surface-enhanced laser desorption/ionization time-of-flight-based serum proteomic array technique for the early diagnosis of prostate cancer. *Asian J Androl* 2006; 8: 45-51.
- [19] Tolson J, Bogumil R, Brunst E, Beck H, Elsner R, Humeny A, et al. Serum protein profiling by SELDI mass spectrometry: detection of multiple variants of serum amyloid alpha in renal cancer patients. *Lab Invest* 2004; 84:845-56.

