

## RESEARCH ARTICLE

# Pituitary Adenoma Biomarkers Identified Using Proteomic Fingerprint Technology

Kai-Yu Zhou\*, Hang-Huang Jin, Zhi-Qiang Bai, Chi-Bo Liu

### Abstract

**Objective:** To determine whether pituitary adenomas can be diagnosed by identifying protein biomarkers in the serum. **Methods:** We compared serum proteins from 65 pituitary adenoma patients and 90 healthy donors using proteomic fingerprint technology combining magnetic beads with matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). **Results:** A total of 42 M/Z peaks were identified as related to pituitary adenoma ( $P < 0.01$ ). A diagnostic model established based on three biomarkers (3382.0, 4601.9, 9191.2) showed that the sensitivity of diagnosing pituitary adenoma was 90.0% and the specificity was 88.3%. The model was further tested by blind analysis showing that the sensitivity was 88.0% and the specificity was 83.3%. **Conclusions:** These results suggest that proteomic fingerprint technology can be used to identify pituitary adenoma biomarkers and the model based on three biomarkers (3382.0, 4601.9, 9191.2) provides a powerful and reliable method for diagnosing pituitary adenoma.

**Keywords:** Pituitary adenoma - proteomic fingerprint - diagnostic model

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### Introduction

Pituitary adenoma is a common intracranial tumor, accounting for about 10% ~ 15% of intracranial tumors, some cases treated with the more complicated treatment operation, difficult to total tumor removal, and postoperative recurrence (Aghakhani et al., 2011; Barber et al., 2011). In order to explore the pathogenesis of pituitary adenomas, improve the level of diagnosis and treatment, so it is necessary to search for new specific biomarkers for the early diagnosis of pituitary adenoma. Proteomics research in recent years has made considerable progress, Especially the surface enhanced laser desorption ionization time of flight mass spectrometry technology (SELDI-TOF-MS) has emerged as the world in search of a variety of disease markers has provided a new technology platform (Martiny et al., 2012; Wu et al., 2012). The present study using matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) combined with WCX nanometer magnetic beads technique (Zhang et al., 2011) for the determination of known pituitary adenoma and serum of healthy volunteers in the protein differential expression. The application of bioinformatics technology can be used for diagnosis of pituitary adenoma protein combination model, seek for pituitary adenoma clinical early diagnosis of specific biomarkers.

### Materials and Methods

#### Subjects

The study included 65 pituitary adenoma patients from

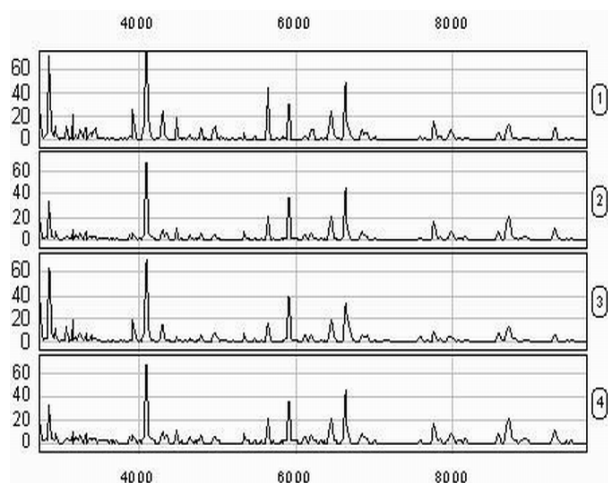
the Taizhou municipal hospital (January 2007 to March 2011). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Taizhou Municipal Hospital. Written informed consent was obtained from all participants. The control group consisted of 90 healthy volunteers who visited the hospital for general health exams. Written informed consent was obtained from each participant. The study was approved by the Review Board of Taizhou municipal hospital (TMH-2007-066). Serum specimens were collected from each participant.

#### Methods

Peripheral blood was collected and placed immediately in refrigerator at 4 °C for 1 ~ 2 h. The blood was then placed in 3000rpm centrifuge machine at 4 °C for 10 min to separate out the serum. Serum was transferred to the PCR tubes and then prepared with the WCX nanometer magnetic beads (Beijing Co. China) according to the manufacture's protocol.

Protein Biological System (PBS) IIC mass spectrometer (Ciphergen, USA) was used to read the chip information. ALL-IN-ONE standard protein NP20 was added for chip correction, so that the error of the molecular mass was < 0.1%. Chip reading instrument parameter settings were as follows: the laser intensity was 200, the detection sensitivity was 8, the range was optimized to 2000 ~ 20000, the highest molecular weight was 50000, and chip for each point on was the acquisition of 80 times. Data was analyzed using Ciphergen ProteinChip 3.2.1 software collection.

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**Figure 1. Type O Serum Protein Fingerprint Experiment**

**Table 1. Research Object Grouping**

Group	Sample type	Number of cases	Diagnosis of right cases	Detection rate (%)
Experimental	Pituitary tumor	40	36	90
Experimental	Healthy controls	60	53	88.3
Validation	Pituitary tumor	25	20	88
Validation	Healthy controls	30	25	83.3

#### Statistical analysis

Serum of 40 patients with pituitary adenoma and 60 age-matched controls were used to establish the model of the diagnosis of pituitary adenoma. The main processes of modeling included standardizing fingerprints, screening differences in the peaks using Biomarker Wizard software, and establishing the diagnostic model using Biomarker Pattern software. Blind analysis was conducted with the fingerprint data from 25 patients and 30 healthy controls.

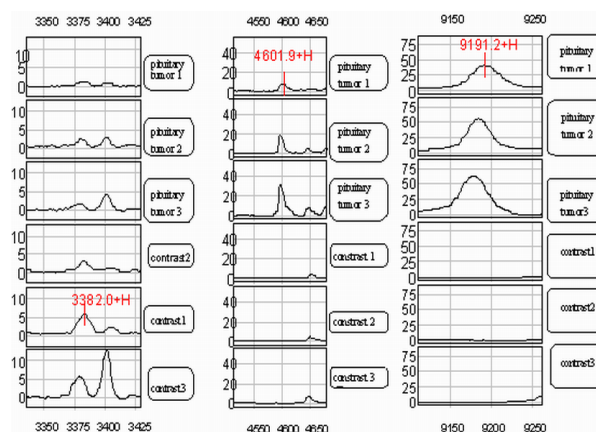
## Results

#### Optimization and evaluation

We randomly chose 4 healthy controls with O type blood (two male and two female) to test and optimize the experimental conditions. Analysis of serum peptides and low molecular weight proteins based on the peak intensity had coefficient variation (CV) within 10% under the optimized condition described in the methods section, which indicated that the experimental condition were suitable to test and compare proteins in different samples (Figure 1).

#### Data analysis and diagnostic model

Two hundred and fifty two protein peaks in the molecular mass range of 2000 ~ 50000 were detected from 40 patients with pituitary adenoma and 60 cases of healthy controls, in which 42 different proteins in pituitary adenomas group and the control group showed significant difference ( $P < 0.01$ ). A diagnostic model was established based on 3 protein peaks (M/Z: 3382.0, 4601.9, 9191.2) (Figure 2). Analyzing the 40 pituitary adenomas and 60 healthy donors samples according to this model showed the sensitivity of detecting pituitary adenomas was 90.00% (36/40) and specificity was 88.30% (53/60) (Table 1). These suggested that the combination of the 3 protein



**Figure 2. Establishment of a Pituitary Tumor Diagnostic Model Based on 3 Protein Peaks (M/Z 3382.0, 4601.9, 9191.2)**

marks could be used as a diagnostic model for pituitary adenoma.

#### Blind validation of diagnostic model

The validity of the model based on 3 protein peaks (M/Z: 3382.0, 4601.9, 9191.2) was tested by blind analysis using 25 pituitary adenoma patients and 30 healthy controls. The results showed that the diagnosis of pituitary adenoma had sensitivity of 88.00% (20/25) and specificity of 83.30% (25/30) (Table 1). These data further indicated that the combination of the 3 protein marks could be used as a diagnostic model for pituitary adenoma.

## Discussion

Pituitary adenoma is a common benign intracranial tumor. It presents diverse symptoms, some of which are destructive (Raappana et al., 2010). Nowadays, the research of pituitary adenomas are mainly concentrated on the following areas: 1) the hypothalamic pituitary secretion regulating system (Melmed, 2011); 2) cytokines and signal transduction proteins (Gurlek et al., 2007); 3) the expression of cell cycle regulatory proteins; 4) oncogenes and cancer related proteins (Yarman et al., 2010). A growing number of studies have confirmed that the pathogenesis of pituitary adenomas is attributed to the interaction between specific genes and proteins. Thus, fundamental study of pituitary adenoma can be based on comprehensive analysis of neuroendocrine proteins involved in metabolic processes (Asa et al., 2009; Beckers et al., 2009). This study investigated specific biomarkers of pituitary adenoma in serum. A diagnostic model was built according to the corresponding protein markers, providing potential methods for diagnosis and treatment of pituitary adenoma.

In order to study the pathogenesis of pituitary adenomas and improve its diagnosis and treatment, growing number of scholars focus on the feature of protein expression for pituitary adenoma (Cheng et al., 2005; Stilling et al., 2010; Tam et al., 2010; Azarpira et al., 2012; Garg, 2012). Ciphergen Biosystems has developed a protein chip technology, which may speed up the discovery of new biomarkers and overall enhances proteomics research (Cheng et al., 2005; Azarpira et al., 2012; Garg,

2012; Shi et al., 2012; Strathmann et al., 2012). When combined with the protein crystal structure analysis, this technology will be able to identify the different tumor biomarkers for early tumor diagnosis and more effective treatment (Guo et al., 2011; Liu et al., 2011; Zheng et al., 2011; Shanks et al., 2012). However, the research about screening invasive pituitary adenoma patients through detecting specific serum protein expression by MALDI-TOF-MS remains unclear. Our study used WCX nano magnetic microspheres combined with MALDI-TOF-MS to analyze the serum protein fingerprint in 40 patients with pituitary adenoma and 60 cases of healthy controls. The results showed that M/Z 3382.0, 4601.9, 9191.2 protein peaks were the best biomarkers for diagnosis of pituitary adenoma. The combination of the 3 proteins could be used as a model to screen and diagnose pituitary adenoma patients from healthy controls. Since protein expression could be slightly diverse in different human races, it could be interesting to conduct similar research in different populations.

This study has established a great base for our following investigations. We will further study the different subtypes of pituitary adenoma by analyzing the differences among protein fingerprints.

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