Detection and Prognostic Analysis of Serum Protein Expression in Esophageal Squamous Cell Cancer

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Abstract

Objective: To assess differences in serum proteins in esophageal squamous cell carcinoma patients. Methods: 144 esophageal squamous cell carcinoma patients and 50 healthy volunteers were included in this study, with surface-enhanced laser desorption-ionization time-of-flight mass spectrometry and weak cation exchange magnetic beads. Follow-up allowed the relations between serum proteins and prognosis to be analyzed. Results: A total of 93 protein peaks were detected (molecular weight range: 1500-30000), 10 demonstrating statistically significant differences. There were no differences in protein peaks between 92 patients with a survival more than 2 years and 52 patients with survival less than 2 years. There were two significantly different protein peaks between 45 stage II patients with a survival more than 2 years and 14 stage II patients with survival less than 2 years. There was one significantly different protein peak between 22 stage III patients with a survival more than 2 years and 29 stage III patients with survival less than 2 years. Conclusion: Differences of serum proteins in esophageal squamous cell carcinoma are related to prognosis of patients. The protein fingerprint can be helpful for clinical diagnosis and treatment.

Keywords: Esophageal cancer - prognosis - serum protein fingerprint

Introduction

Esophageal cancer is a common clinical malignancy. According to the Cancer Statistic Report from the International Agency for Research on Cancer (IARC), there were 462,000 new cases in 2002 and 386,000 deaths. Esophageal cancer is the 8th most common malignancy worldwide and one of the six most lethal diseases. The treatment of esophageal cancer is not effective enough, because the majority of patients are diagnosed too late. Specific markers will contribute to an early diagnosis and predict patients’ prognosis. Therefore, to seek efficient molecular markers is valuable for diagnosis and treatment of esophageal cancer.

Recently, the rapid development of proteomics promotes the studies on tumor markers. During the last decade, surface-enhanced laser desorption-ionization time-of-flight mass spectrometry (SELDI-TOF-MS) has been a widespread proteomics implement in oncology study. A diagnostic model for esophageal squamous cell carcinoma and cervical squamous cell carcinoma has been developed (Xia et al., 2008). According to the pattern of esophageal squamous cell carcinoma, serum protein changes are related to the occurrence of esophageal cancer. It still remains unclear if protein changes relate to prognosis. In the present study, SELDI-TOF-MS and weak cation exchange magnetic bead were used to examine the changes of serum protein expression in newly diagnosed patients with esophageal cancer to further identify esophageal cancer-related proteins. During the follow-up, the relationship between serum protein differences and prognosis was analyzed in esophageal cancer.

Materials and Methods

Patients

From August 2007 to June 2010, peripheral blood samples from 144 newly diagnosed cases with esophageal squamous cell carcinoma (age: 43-75, median age: 60) before surgery were collected. The control group included 50 sex- and age-matched healthy volunteers (age: 36-78, median age: 56). Pathological examination confirmed esophageal squamous cell carcinoma after gastroscopy. The exclusion criteria included hepatitis, acute infection, and concurrent tumors. All patients underwent surgical treatment. Surgical approach was upper abdomen-right chest or upper abdomen-right chest-left neck. Postoperative pathology examination was performed according to UICC 2009 version of the esophageal cancer staging (stage I: 28 cases, stage II: 59 cases, stage III: 51 cases, stage IV: 6 cases).
Follow-up
All patients completed telephone follow-up. The follow-up rate was 97.2% with 4 cases lost to follow-up.
years and 14 stage II patients with survival less than 2 years.

There was one significantly different protein peak between 22 stage III patients with a survival more than 2 years and 29 stage III patients with survival less than 2 years.

There was one significantly different protein peak between 22 stage III patients with a survival more than 2 years and 20 stage III patients with survival 1-2 years.

Discussion

SELDI-TOF-MS was first proposed in 1993. In recent years, this technology has made considerable progress. According to the conclusion drawn from a stage III multicenter clinical trial by U.S. National Cancer Institute (NCI) affiliated early disease detection network, SELDI-TOF-MS is the most promising cancer early detection methods after serum and instrument standardization quality control (Grizzle et al., 2004). Its principle is to use high-energy laser beam to make the analyte in the chip resolved to form ions. On the basis of different mass-to-charge ratio (M/Z), the time of flight of ion in the instrument field is various. As a result, a protein spectrum can be drawn. After computer analysis, data regarding protein molecular weights and concentrations can be recorded.

Its main advantages are listed below: 1) Samples are simple and convenient to access and even directly crude samples. The changes of protein (Panicker et al., 2009; Calvano et al., 2010) were detected in the cervix mucus protein of patients with cervical cancer and urine of prostate cancer patients. 2) Small sample volume and prompt detection. 3) High sensitivity and specificity (Guo et al., 2011). 4) Protein molecular weight range is wide. Low-molecular weight, low-abundance proteins can be found. 5) Widespread in clinical use: tumor etiology, diagnosis, efficacy monitoring of various treatment methods and prognostic evaluation of patients (Kohli et al., 2006; Ren et al., 2009).

But it still has several disadvantages in protein markers and early diagnosis of disease. First, most experiments focus on primary description of differential protein peak and molecular weight. Protein sequence, conformation, purification and features need more attention. Thereby some specific proteins can not be identified from much further way. In addition, the reproducibility of SELDI-TOF-MS needs to be established because of controversial conclusions regarding the same research subject.

Recently, many scholars have succeeded in finding new tumor markers in tumor such as cervical cancer (Piyathilake et al., 2007; Xia et al., 2008), ovarian cancer (Zhang et al., 2006), colorectal cancer (Xu et al., 2006), prostate cancer (Malik et al., 2007; McLerran et al., 2008), breast cancer (Ricolleau et al., 2006), bladder cancer (Langbein et al., 2006) by SELDI-TOF-MS, and established relevant diagnosis model of tumor protein fingerprint whose sensitivity and specificity are higher than existent tumor markers. Even more promisingly, the high-resolution mass spectrometer in combination with statistical advances had a higher sensitivity and specificity (Yu et al., 2005; Cadron et al., 2009).

At the same time, many researchers studied the changes of serum proteins in the occurrence and development of esophageal cancer and reported various proteins were related to the occurrence and metastasis of esophageal cancer (Breton et al., 2008; Liu et al., 2010). We have established a diagnosis model of esophageal cancer and cervical squamous cell carcinoma among patients of Zhejiang Province. Through the comparison between patients of esophageal cancer or cervical cancer and healthy volunteers, several significant differential protein peaks was identified. However, domestic and international researches on relevance of serum protein changes and prognosis in patients with esophageal cancer are less reported.

In this study, there were no significantly different protein peaks between 92 patients with survival more than 2 years and with 52 patients with survival less than 2 years. This was probably because of different pathological stages ant related interference factors; Therefore, patients of stage II and patients of stage III were compared, respectively. And it was concluded that differences of serum protein spectrum in esophageal squamous cell carcinoma were related with the prognosis.

Two statistically different protein peaks were also found after analysis of serum albumin in patients with lymph node metastasis or not. There were 6 statistically different protein peaks between patients without lymph node metastasis and those with distant metastasis in this study. It implied that there were significantly different gradients of relative content of serum proteins between early and middle-advanced esophageal cancer patients. The lack of expression of certain proteins might lead to tumor metastasis indicating that these proteins probably could be related markers of tumor metastasis. There was no statistically different protein peaks between patients with lymph node metastasis and those with distant metastasis in the present study. The underlying cause might originate from unknown distant micrometastasis in middle-advanced esophageal cancer patients with lymph node metastasis. One liver metastasis and 1 lung metastases were confirmed in this study which had no metastasis evidence preoperatively. The small cases of distant metastasis (n=2) in this study must be taken into account which might introduce a statistical error.

Nowadays, some scholars have also established

| Table 1. The Area Under the ROC Curve of 10 Differential Peaks in 144 Esophageal Squamous Cell Carcinoma Patients and 50 Healthy Subjects |
|---------------------------------|---------------- |
| Protein (m/z)                  | ROC Area  |
| 2768                           | 0.926     |
| 2745                           | 0.944     |
| 3403                           | 0.97      |
| 6638                           | 0.897     |
| 5972                           | 0.812     |
| 6440                           | 0.872     |
| 4797                           | 0.689     |
| 3269                           | 0.678     |
| 3321                           | 0.846     |
| 3978                           | 0.82      |

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diagnosis model of esophageal cancer protein fingerprint in different races, including esophageal squamous cell carcinoma and adenocarcinoma (Hammoud et al., 2007; Xu et al., 2009; Zhang et al., 2011). According to our research, there were 10 different protein expressions between esophageal cancer patients with a family history and patients without a family history. And these differential proteins were over expressed in esophageal cancer patients with a family history. Whether there are differences of protein expression between patients inform esophageal cancer epidemic areas – Tiantai and Xianju and other places will require further studies in the future.

References


