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Identification of differentially expressed serum proteins in gastric adenocarcinoma☆

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ABSTRACT

Gastric adenocarcinoma is an aggressive cancer with poor prognosis. Blood based biomarkers of gastric cancer have the potential to improve diagnosis and monitoring of these tumors. Proteins that show altered levels in the circulation of gastric cancer patients could prove useful as putative biomarkers. We used an iTRAQ-based quantitative proteomic approach to identify proteins that show altered levels in the sera of patients with gastric cancer. Our study resulted in identification of 643 proteins, of which 48 proteins showed increased levels and 11 proteins showed decreased levels in serum from gastric cancer patients compared to age and sex matched healthy controls. Proteins that showed increased expression in gastric cancer included inter-alpha-trypsin inhibitor heavy chain

Abbreviations: iTRAQ, Isobaric tags for relative and absolute quantitation; LC-MS/MS analysis, Liquid chromatography- tandem mass spectrometry; MRM, Multiple reaction monitoring; MMTS, Methyl methanethiosulfonate; TCEP, Tris-(2-carboxyethyl) phosphine

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H4 (ITIH4), Mannose-binding protein C (MBL2), sex hormone-binding globulin (SHBG), insulin-like growth factor-binding protein 2 (IGFBP2), serum amyloid A protein (SAA1), Orosomucoid 1 (ORM1) and extracellular superoxide dismutase [Cu–Zn] (SOD3). We used multiple reaction monitoring assays and validated elevated levels of ITIH4 and SAA1 proteins in serum from gastric cancer patients.

Biological significance
Gastric cancer is a highly aggressive cancer associated with high mortality. Serum-based biomarkers are of considerable interest in diagnosis and monitoring of various diseases including cancers. Gastric cancer is often diagnosed at advanced stages resulting in poor prognosis and high mortality. Pathological diagnosis using biopsy specimens remains the gold standard for diagnosis of gastric cancer. Serum-based biomarkers are of considerable importance as they are minimally invasive. In this study, we carried out quantitative proteomic profiling of serum from gastric cancer patients to identify proteins that show altered levels in gastric cancer patients. We identified more than 50 proteins that showed altered levels in gastric cancer patient sera. Validation in a large cohort of well classified patient samples would prove useful in identifying novel blood based biomarkers for gastric cancers.
This article is part of a Special Issue entitled: Proteomics in India.

1. Introduction

Gastric cancer is a highly aggressive cancer associated with high mortality. High incidence of gastric cancer has been observed throughout the world [1]. High mortality is often due to delayed diagnosis [2] due to non-specific symptoms observed in gastric cancer [3]. Serum-based biomarkers are of considerable importance in early diagnosis of various diseases including cancer. Proteins secreted from tumor tissues have a greater likelihood of reaching systemic circulation and therefore, secreted proteins could serve as potential biomarkers for early detection [4]. However, serum is a complex protein mixture consisting of proteins that exhibit a wide dynamic range of expression [5]. Ten most abundant proteins in serum constitute about 95% of the total protein content. Therefore, identifying candidate biomarkers for diseases from serum has been an immense challenge. To overcome this challenge, various depletion strategies have been developed to reduce sample complexity in serum [6].

Plasma-based tumor markers including CA 19-9, CA 125, and CEA (carcinoembryonic antigen) have been in clinical use for monitoring gastric cancer [7]. These markers have been shown to be useful to detect disease recurrence after curative surgery [8]. However, they display low sensitivity, lack specificity and often are not reproducible [9,10]. Several groups have employed quantitative proteomic approaches to identify novel secreted biomarkers in gastric cancer by analyzing secretome [11–13], and plasma [14].

In this study, we used isobaric tags for relative and absolute quantitation (iTRAQ) based quantitative proteomic strategy to identify proteins that show altered levels in serum of gastric adenocarcinoma patients. We identified 643 proteins of which 48 showed increased levels and 11 showed decreased levels in serum from gastric cancer patients. Large scale validation of these proteins might prove useful in identification of blood-based biomarkers of gastric cancer.

2. Materials and methods

2.1. Patient samples

Patient and control blood samples were collected after obtaining approval from the institutional review board at the Kidwai Memorial Institute of Oncology, Bangalore, India. Ten blood samples each were collected from patients who underwent curative surgery for the removal of tumor and had histologically confirmed gastric adenocarcinoma (details in Supplementary Table 1). None of the patients had previously undergone radio or chemotherapy. Corresponding age and sex matched control serum samples were obtained from individuals with no prior health conditions such as diabetes and cardiovascular disease. Blood was collected from patients and control individuals after obtaining informed consent. Serum was separated from control and test samples using standard centrifugation techniques. The samples were stored immediately at −80 °C.

2.2. Protein estimation and depletion of abundant proteins

The total protein amount was measured for each serum sample using Bicinchoninic acid (BCA) assay [15] (Pierce®. Cat#: 23225). Protein amounts were normalized and equivalent amounts of protein from each of the patient and control serum samples were pooled separately. 10 mg protein each from both patient and control samples were depleted of the top fourteen abundant serum proteins using a Human-14 Multiple Affinity Removal Column (Agilent Technologies, Santa Clara, CA. Cat# 5188-6557; 4.6 × 50 mm). Post-depletion, 600 μg protein from each of the samples was recovered.