JOURNAL OF PROTEOMICS 94 (2013) 473–485

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot

Up-regulation of type I collagen during tumorigenesis of colorectal cancer revealed by quantitative proteomic analysis

Xia Zou[a,1], *Bo Feng*[b,c,1], *Taotao Dong*[b,c], *Guoquan Yan*[d], *Binbin Tan*[a], *Hao Shen*[a], *Ao Huang*[b,c], *Xiu Zhang*[a], *Menghui Zhang*[e], *Pengyuan Yang*[d], *Minhua Zheng*[b,c], *Yan Zhang*[a,*]

[a]*Ministry of Education, Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai, China*
[b]*Department of Surgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China*
[c]*Shanghai Institute of Digestive Surgery, Shanghai, China*
[d]*Department of Chemistry and Institutes of Biomedical Sciences, Fudan University, Shanghai, China*
[e]*State Key Laboratory of Microbial Metabolism, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai, China*

ARTICLE INFO

Article history:
Received 7 May 2013
Accepted 15 October 2013
Available online 24 October 2013

Keywords:
Colorectal cancer
Collagen I
CTx
CRC tumorigenesis
APEX

ABSTRACT

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide. The discovery of non-invasive biomarker candidates for diagnosis and prognosis is important for the management of CRC. In this study, we performed proteomic profiling of serum from patients with different stages of CRC using a 2D-LC–MS/MS based approach combined with the APEX quantitative method. 917 proteins were identified and 93 were differentially expressed in normal and three patient groups (stages I, II and III). These proteins were predominantly involved in cell adhesion, immune response, coagulation process and metabolism. Importantly, we found collagen I dynamically changed from stages I to IV, with maximum expression in stage II, as detected in serum by MS analysis. Expression of collagen I was also validated in tumor tissues from the same group of CRC patients by real-time PCR and western blotting. Furthermore, we demonstrate that serum levels of collagen I degradation telopeptide (CTx) are correlated with staging and poor disease-free survival of CRC patients by ELISA analysis. These results suggest (1) serum proteomics may reflect biological changes in colorectal tumor tissues and (2) altered collagen I expression may be an early event in CRC tumorigenesis and CTx may provide additional information for prognosis of CRC.

Biological significance
In this work, we performed a systematic characterization of serum proteomic alterations in colorectal cancer (CRC) with different stages using a LC–MS based approach combined with the

Abbreviations: CRC, colorectal cancer; UICC, Union Internationale Contre Le Cancer; 2D-LC–MS/MS, two-dimensional liquid chromatography with tandem mass spectrometry; APEX, absolute protein expression; ECM, extracellular matrix; COL1A1, α1 chain of type I collagen; COL1A2, α2 chain of type I collagen; PICP, carboxyterminal propeptide of type I collagen; CTx, C-terminal telopeptide of type I collagen; MMPs, matrix metalloproteinases; CEA, carcinoembryonic antigen; ROC, receiver operating characteristic; AUC, area under the curve; EMT, epithelial-mesenchymal transition; Collagen I, equal to type I collagen in this study.

* *Corresponding author at:* Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, 800 Dong Chuan Road, Minhang, Shanghai 200240, China. Tel./fax: +86 21 34206778.
E-mail address: yanzhang2006@sjtu.edu.cn (Y. Zhang).
[1] These authors contributed equally to this study.

1874-3919/$ – see front matter. Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jprot.2013.10.020

APEX quantitative method, attempting to gain overview of relevant proteins in tumorigenesis and discover non-invasive CRC-derived markers. We found a significant up-regulation of collagen I based on the proteomics data, and confirmed its expression in tissue and serum of the same group of patients. In addition, we also demonstrated that serum levels of collagen I degradation telopeptide (CTx) are correlated with the staging and poor survival rate of CRC. Those findings imply that alternation of collagen I might be an early event during tumorigenesis of CRC, and might contribute to the metastasis of CRC under the degradation regulated by some specific proteases. This work provides evidence for the clinical application of serum proteomics, and would aid the understanding of the role of the ECM in the clinical progression of CRC.
Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.

1. Introduction

Colorectal cancer (CRC) is a prevalent and deadly malignant tumor worldwide. In developed countries like the United States, it is the third most commonly diagnosed cancer and accounts for almost 10% of overall cancer mortality [1]. In China, CRC is now the fourth leading cause of cancer death, and its incidence has rapidly increased during recent decades, especially in major cities where significant lifestyle alterations have occurred [2,3]. Although most patients with earlier stages of the disease can be cured by surgical excision, CRC continues to be a malignant disease with high mortality rates. Indeed, the disease outcome, prognosis and treatment regimens are highly dependent on pathologic tumor stage based on the size of the tumor, lymph node and metastasis. Because of the lack of obvious clinical symptoms at early stages of the disease, approximately 61% of CRC cases are diagnosed at an advanced stage with a poor five-year survival rate [4]. Therefore, the development of tools for early detection and for prognosis and prediction of treatment response is of great importance for the proper management of CRC.

Currently, various screening methods for the clinical detection of CRC are available, including colonoscopy, flexible sigmoidoscopy and fecal occult blood tests (FOBTs) [4,5]. Among these tests, colonoscopy represents the most sensitive for detection of adenomas and early-stage carcinomas; however it can be inconvenient, costly and bears potential risks. Conversely, as the most commonly used non-invasive screening option, FOBT is limited by its poor sensitivity [4–6]. In addition to the development of stool markers, non-invasive tests based on blood samples are also studied. Several serum molecules have shown potential clinical value, including carcinoembryonic antigen (CEA), CA19-9, CA242 and TIMP-1[7,8]. However, none of these are currently recommended for routine screening because of both low sensitivity and specificity. Novel non-invasive markers for diagnosis and prognosis of CRC are therefore necessary.

Proteomic analysis has emerged as a powerful tool for global protein profiling during the past few decades [9]. The characterization of proteins involved in carcinogenesis and CRC progression is considered a promising approach for the discovery of diagnostic and prognostic signatures in clinical samples. At the beginning of this century, the discovery of CRC biomarkers was predominantly focused on the analysis of serum peptides profiling generated by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) combined with bioinformatics [10–12]. The pattern diagnosis was considered useful if sample collection was standardized to a high level, however in most cases, differential peaks were not identified [13]. To solve this problem, several studies performed serum protein profiling using 2D gel- or LC–MS/MS-based proteomics strategies, and directly identified several serum proteins with diagnostic potential [14,15]. However, because of the complexity and large dynamic range of protein abundance in serum, few of these proteins were regarded as tumor secreted, thus limiting their clinical utility.

In this study, we attempted to identify non-invasive markers of CRC directly in serum. First, we removed 14 high-abundance proteins in serum to decrease the masking effect, and then performed systematic comparative proteomics of CRC patients with different stages using a 2D LC–MS/MS based approach combined with label-free quantitation. Using this approach, we identified 93 differentially expressed proteins with functions predominantly related to cell adhesion, immune response, the coagulation process and metabolism. To investigate whether these serum identified proteins were released from colorectal tumor tissues, we focused on collagen I and verified its expression in tumor tissues from the same group of patients. Finally, the association between collagen I expression and clinicopathological parameters of CRC were investigated.

2. Materials and methods

2.1. *Sample collection*

From May 2009 to May 2010, a total of 91 CRC cases (pathological stages I (n = 21), II (n = 41), III (n = 22) and IV (n = 7)) treated with laparoscopic surgery and 33 healthy individuals without gastrointestinal disorders or inflammatory diseases were recruited from Ruijin Hospital (Shanghai, China) in accordance with guidelines set by the Scientific and Ethical Committee at Shanghai Jiao Tong University. All participants gave written, informed consent and no participants had received any medication prior to sample collection. Diagnosis of CRC was confirmed by a final histopathology report and staging of these patients was performed according to the Union Internationale Contre Le Cancer (UICC). The inclusion criteria included patients 1) who suffered pathologically confirmed carcinoma of colon and rectum; 2) who did not have any severe comorbidities that need medications; 3) who received elective surgery. The exclusion criteria included patients who 1) suffered malignant lymphoma and benign tumor for colon and rectum; 2) received medications

Download English Version:

https://daneshyari.com/en/article/7637106

Download Persian Version:

https://daneshyari.com/article/7637106

Daneshyari.com