BIOCHEMICAL PHARMACOLOGY 73 (2007) 1422–1433

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Biomarker discovery for inflammatory bowel disease, using proteomic serum profiling

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ARTICLE INFO

Article history:
Received 14 November 2006
Accepted 15 December 2006

Keywords:
Proteomics
IBD
Biomarkers
SELDI-TOF-MS
Inflammation
Serum profiling

ABSTRACT

Crohn's disease and ulcerative colitis known as inflammatory bowel diseases (IBD) are chronic immuno-inflammatory pathologies of the gastrointestinal tract. These diseases are multifactorial, polygenic and of unknown etiology. Clinical presentation is non-specific and diagnosis is based on clinical, endoscopic, radiological and histological criteria. Novel markers are needed to improve early diagnosis and classification of these pathologies. We performed a study with 120 serum samples collected from patients classified in 4 groups (30 Crohn, 30 ulcerative colitis, 30 inflammatory controls and 30 healthy controls) according to accredited criteria. We compared protein sera profiles obtained with a Surface Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometer (SELDI-TOF-MS). Data analysis with univariate process and a multivariate statistical method based on multiple decision trees algorithms allowed us to select some potential biomarkers. Four of them were identified by mass spectrometry and antibody based methods. Multivariate analysis generated models that could classify samples with good sensitivity and specificity (minimum 80%) discriminating groups of patients. This analysis was used as a tool to classify peaks according to differences in level on spectra through the four categories of patients. Four biomarkers showing important diagnostic value were purified, identified (PF4, MRP8, FIBA and Hpα2) and two of these: PF4 and Hpα2 were detected in sera by classical methods. SELDI-TOF-MS technology and use of the multiple decision trees method led to protein biomarker patterns analysis and allowed the selection of potential individual biomarkers. Their downstream identification may reveal to be helpful for IBD classification and etiology understanding.

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2006.12.019

1. Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders affecting the gastrointestinal tract. The two main forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC). Their etiopathogenesis has not been fully elucidated but involves a complex interplay among genetic and environmental factors [1–3]. Recent studies on experimental animal models of IBD as well as the discovery of gene variants or loci, selectively associated with specific forms of IBD have highlighted the heterogeneity of mechanisms leading to IBD [4,5]. Despite this heterogeneity, clinical manifestations of these diseases appear quite stereotyped including diarrhea, abdominal pain, fever and degradation of the general physical condition.

There is currently no easy diagnostic tool for these pathologies. Biological markers potentially useful in IBD include proteins of inflammation such as C-reactive protein (CRP), fecal calprotectin and several antibodies [6,7]. However, these biomarkers have many limitations. Acute inflammatory markers, such as CRP or fecal calprotectin cannot differentiate between infectious colitis and flare of IBD [8–12]. Anti-*saccharomyces* antibodies (ASCA) and perinuclear anti-neutrophil cytoplasmic antibody (pANCA) are the only available commercial tests that can be helpful for CD and UC discrimination. Although, they show a quite good specificity, their sensitivity is rather low and they are therefore not recommended for broad clinical practice [13–16]. Finally, the recent identification of genetic factors predisposing to IBD did not lead to novel and relevant diagnostic tools since such factors are also present in a significant proportion of the general population [17,18]. Therefore, initial diagnosis still relies on the combination of several biological and morphological tests, including gastrointestinal endoscopies and histology, and is based on standardized validated diagnostic criteria [19,20]. However, even using these invasive methods, differential diagnosis between IBD and self-limited colitis as well as between the two main forms of IBD is still difficult. This is a relevant clinical question since optimal management and treatments may differ among these entities [21,22]. Beyond this initial diagnosis, there are various degrees of severity among IBD and responses to standard therapies vary between patients due to disease heterogeneity. Again, powerful predictive factors do not exist and clinicians often manage patients empirically and secondarily adapt the therapeutic strategy according to clinical evolution [23,24].

Analysis of the proteome signature of each patient, although technically difficult, could be a more pragmatic and realistic approach to these questions, in particular for inflammatory processes [25–27]. Indeed the proteome represents the net result of interactions between genetic background and environmental factors and may be considered as the signature of a disease, involving small circulating proteins or peptides from degraded molecules as often encountered in those types of inflammatory and dysimmune pathologies. Surface Enhanced Laser Desorption Ionization-Time of Flight-Mass Spectrometer (SELDI-TOF-MS) technology is a rapid and sensitive technique, which offers the possibility to analyze many samples in a very short time period [28,29]. The sensitivity of mass spectrometer allows detection of peptides at the femto molar range. This means that low abundant serum proteins can be detectable in appropriate conditions [30–33]. Many teams including ours have already undertaken studies of protein profiling with SELDI-TOF-MS, in order to discover new specific biomarkers for various pathologies at different stages and on different sample origins [34,35].

In this paper, we present a pilot study based on serum profiling and robust statistical approach which attempts to answer questions regarding the potential interest of proteomics in IBD study and classification. We compared profiles from IBD versus non-IBD inflammatory pathologies and healthy controls. The same samples were tested with non-invasive markers for IBD, namely ASCA and ANCA, for comparison. We further selected and identified several potential biomarkers among the highly discriminating ones, which might be interesting to better understand IBD pathophysiology.

2. Materials and methods

2.1. Patients

Experimental protocol was approved by the ethic committee of our academic hospital and patients enrolled gave their informed consent for the study. A total of 120 serum samples from patients affected by various pathologies and healthy controls were prospectively collected in 10 cm^3 serum separator vacutainer tube. Clotting was allowed to occur within a minimum of 30 min and a maximum of 4 h before centrifugation at 3000 rpm for 10 min. All sera were aliquoted and immediately frozen at −80 °C, until thawed for SELDI-TOF-MS analysis (Ciphergen Biosystems Fremont, CA, USA).

Samples were classified in four categories according to the considered pathologies: Crohn's disease (CD), ulcerative colitis (UC), healthy controls (HC) and inflammatory controls (IC). IC grouped patients presenting inflammatory pathologies affecting the bowel other than IBD, such as diverticulitis or pathogen caused enterocolitis, as well as two other chronic inflammatory diseases: asthma and rheumatoid arthritis. Diagnoses of IBD patients were realized by gastroenterologists specialized in IBD, according to widely accepted criteria [36]. CD was considered as clinically active or inactive according to Harvey–Bradshow index (HBI) [37]. UC was considered active when clinical symptoms were confirmed by the presence of significant lesions at rectosigmoidoscopy including erosions and spontaneous or contact bleeding. Vienna classification was used to describe the localization and behavior of CD population at the time of sampling [38,39]. The HC group was composed of 30 healthy controls showing CRP level <6 mg/l (CRPXL Tina-quant® ROCHE Diagnostics, GmbH). Diverticulitis clinical diagnoses were confirmed by abdomen CT scanner. Pathogen caused enterocolitis were defined by acute onset of intestinal symptoms associated with stool culture positive for a specific pathogen. Rheumatoid arthritis patients were fulfilling the 1987 ACR criteria [40] and asthmatics the 1987 ATS criteria [41]. ASCA tests (Euroimmun, Germany) (Inova diagnostis, USA) and ANCA (The Binding Site, UK) test were realized on every sample according to manufacturers' recommendations, in order to correlate our results to existing