Original Study

Peptide Patterns as Discriminating Biomarkers in Plasma of Patients With Familial Adenomatous Polyposis

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Abstract

We use the mass spectrometry technique for the detection of circulating peptides in patients with familial adenomatous polyposis (FAP). We describe a FAP-specific fingerprint that allows early identification of the disease and neoplastic transformation. Our results are relevant because they could postpone preventive surgery as long as possible, improving the quality of life of patients with FAP.

Background: Familial adenomatous polyposis (FAP) is one of the most important clinical forms of inherited susceptibility to colorectal cancer. So far, no accepted prognostic markers are present to monitor patients with FAP. Consequently, the major problem in managing patients with FAP is the difficulty to predict when the switch between adenoma and malignant carcinoma occurs, leading to the necessity of preventive surgery. Proteomics is one of the most suitable approaches to identify biomarkers, and it is widely used in cancer research. In this investigation, we studied the circulating plasma peptides in samples collected from patients with FAP and compared the obtained results with adenoma, colorectal cancer, and control samples to discover peptides able to distinguish different phenotypes. **Materials and Methods:** The peptide fingerprint was obtained by matrix-assisted laser desorption/ionization coupled to time-of-flight mass spectrometry. After statistical analysis, a subset of 45 ionic species was found differently expressed in the 4 groups considered, 12 of them peculiar to patients with FAP. Moreover, 4 ionic species were found significantly changed in the switch between adenoma and malignant carcinoma. **Results:** Potentially prognostic peptides identified by this study derive mainly from circulating proteins, some of which are involved in the inflammatory response, such as complement C3 and C4 subjected to an exoprotease activity that seemed pathology related. **Conclusions:** In this study, we defined for the first time a specific panel of peptides for monitoring patients with FAP that could be profitably used to monitor and predict the pathologic evolution in adenocarcinoma malignancy.

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Introduction

Colorectal cancer (CRC) is one of the most frequent malignancies in Europe. Approximately 85% of CRCs are considered sporadic, whereas only 15% are familial and less than 1% are familial adenomatous polyposis (FAP) (Orphanet). Characteristic features of FAP include the development of hundreds to thousands of adenomatous polyps beginning in early adolescence, with the development of CRC in the absence of treatment. Approximately 7% of patients develop CRC by age 21 years, and approximately 95% of patients develop CRC by age 50 years. In all patients with

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FAP, colon cancer is inexorable in the absence of colectomy. Although the classic course of FAP results in CRC, it also can become complicated by noncolonic expressions, in particular gastric and duodenal polyps, and is associated with elevated risk for duodenal, stomach, pancreatic, thyroid, liver, and central nervous system cancer.[1] Classic FAP disease presents a germline mutation in 1 *APC* allele (chr. 5q21-22) that, together with a further somatic mutation in the normal *APC* allele, seems to be sufficient to initiate the tumorigenesis, with the consequent development of a huge number of microadenomas in the colorectum and the small intestine.[2] More than 300 different mutation types of *APC* gene have been identified so far, and most of them are nonsense variations, leading to the formation of a truncated protein. In addition to the classic form, a mild form of FAP called "attenuated FAP" has been identified. Attenuated FAP is characterized by the presence of less than 100 adenomatous colorectal polyps, and a subgroup of these patients present germline mutations and biallelic variations of *MutYH* gene (chr. 1p34.1) that cause an autosomal recessive form called "*MutYH*-associated polyposis" (MAP).[3]

The most relevant problem in FAP disease is the difficulty in understanding the temporal window in which the switch between adenoma and malignant carcinoma occurs. In patients with FAP, the average age when colorectal polyps are detected is between puberty and 20 years.[4] Management of FAP includes surveillance colonoscopy every 1 to 2 years, starting at approximately 10 years of age. After polyp development is observed, annual colonoscopy is recommended. Colectomy is considered when more than 20 adenomatous polyps develop, when adenomas greater than 1 cm are noted, or when concerning histology appears, and is recommended when polyp burden precludes safe colonoscopic surveillance.[5] After colectomy, surveillance continues.[1] This causes an important loss of quality of life for these people because of the invasiveness of this clinical practice. The identification of prognostic biomarkers in subjects affected by this disease could represent a valuable possibility to replace colonoscopy in young subjects and to delay the surgery, improving the quality of life in patients with FAP.

Proteomic approaches are suitable for this purpose because the analysis of biological fluids, such as the plasma, is more accepted than colonoscopy. Quaresima et al[6] have identified different levels of specific serum proteins in patients with FAP compared with healthy subjects by liquid chromatography-mass spectrometry. In particular, they found that alpha-2-HS-glycoprotein and apolipoprotein D were increased in patients with FAP, whereas alpha 2-antiplasmin was decreased. In this study, the authors did not include patients with CRC in the group analysis lacking the prognostic impact of proteomic profile.

In addition to the proteome studies, the interest for the dynamic nature of sub-proteomes present in blood has increased recently.[7] The permeability between tissues and cell membranes is higher for small peptides than in their corresponding full-length proteins, and tumor circulating peptidome seems to be a possible source of disease-specific biomarkers.[8] Moreover, the proteolytic cascades within the tumoral tissues can generate fragments that diffuse into the circulatory system.[9] Several studies showed that the multipeptide signature represents a reliable approach to diagnose different types of cancer. Villanueva et al[9] analyzed the serum samples derived from healthy donors and patients affected by breast, bladder, or prostate cancer, highlighting the presence of a specific cancer signature. Pietrowska et al[10] compared the serum proteome profiles of healthy donors and patients with head and neck squamous cell cancer, colorectal adenocarcinoma, and non–small cell lung cancer. They characterized 2 different peptides (3766 and 5867 Da) highly expressed in all cancer samples. They also found 2 other peptides (11511 and 11667 Da, fragments of serum amyloid A) specifically associated with advanced stages of these cancers. They concluded that certain components of serum peptide signature are common in different cancer types, and they may reflect the general response of the organism to the disease, whereas other components may correspond to the clinical stage of the malignancy.

In this work, the peptidome of patients with FAP has been investigated for the first time; the direct comparison of peptides of patients with FAP with those of control, adenoma, and CRC subjects has been performed to identify pathology-specific signatures. The ionic species (m/z), corresponding to plasma peptides detected by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), have been subjected to statistical analysis, and those presenting different intensity in patients with FAP have been used to correctly discriminate between control subjects and adenoma/CRC subjects. Moreover, a subset of ionic species (m/z) showed statistically significant changes in the direct comparison of FAP, adenoma, and CRC subjects, thus providing the evidence of a possible prognostic biomarker. This work presents for the first time a simple and noninvasive analysis that could be helpful from a clinical point of view, not only in providing helpful information on the malignancy progression but also in postponing surgery as long as possible.

Materials and Methods

Patient Selection and Plasma Preparation

Plasma samples were collected from 13 patients with FAP (APC or MutYH mutated) (Supplemental Tables 1 and 2), 26 patients with adenoma, 58 patients with sporadic CRC, and 38 control subjects, and were negative at colonoscopy. The reason for the unbalanced groups is that FAP is a rare disease. A complete clinical history and written informed consent were obtained from each patient (Ethical Committee approval number 448).

Blood samples were collected in DB Vacutainer Blood Collection Tubes (Becton Dickinson and Company, Franklin Lake, NJ) containing K_3EDTA. Plasma was obtained after centrifugation for 10 minutes at 3000 rpm, and the aliquots were stored at −80°C until further analysis.

Sample Preparation

Plasma samples (200 μL) were diluted 1:4 in deionized water, and 500 μL of the solution was centrifuged for 20 minutes at 3000 *g* in Amicon Ultra-4 Centrifugal Filter Devices (Millipore, Darmstadt, Germany) with 30 KDa molecular weight cutoff. Before MALDI-TOF analysis, the concentrate was discarded from the filter unit sample to eliminate high molecular weight proteins, and the eluate was desalted and purified by ZipTip C18 pipette tips (Millipore, Merck KGaA, Darmstadt, Germany) following the procedure described in the user's guide.