Metabolite profiling and metabonomic approaches of fecal extracts from patients with chronic ulcerative colitis and colorectal cancer based on 9.4T NMR spectroscopy and pattern recognition

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Introduction The incidence of colorectal cancer (CRC) in China has increased dramatically in recent years and becomes a substantial cancer burden. The cumulative probability of developing CRC is significantly higher in chronic ulcerative colitis (UC) than in the general population [1]. Accurate diagnosis at an early stage of UC (precancerosis of CRC) and CRC is of great importance for optimum treatment and prognosis. Compare with the clinical radiological, endoscopic, and histopathological evidence all of which are invasive techniques, NMR-based metabolomics has been shown to be particularly useful for identifying disease biomarkers in the fecal extracts, and could potentially be used for disease surveillance [2-4]. This present study is a NMR-based metabolomics approaches coupled with pattern recognition methods to evaluate the ability to characterize the metabolic "fingerprint" of fecal extracts from patients with UC and CRC

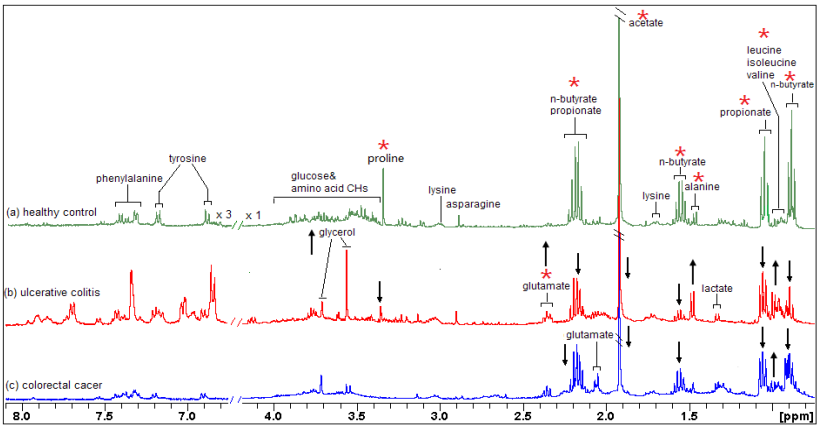

Fig.1 Typical 400 MHz mean ^{1}H NMR spectra of fecal extracts obtained from healthy controls (a), patients with UC (b) and with CRC (c), referenced to TSP (δ 0.00 ppm). The spectra in the aromatic region (6.6–8.2ppm) were magnified three times as compared to the aliphatic region (0.6–4.4ppm).

Materials and Methods Stool specimens were collected from healthy subjects (n=12, 5M, 7F, age 50 ± 12), UC patients (n=12, 6M, 6F, age 50 ± 15) and CRC patients (n=12, 5M, 7F, age 54 ± 13), prior to surgery for colonic resection. Samples were extracted with PBS/$D_2$0 buffer. A stock solution of TSP/$D_2$0 was added to supernatant prior to analysis by ^{1}H NMR spectroscopy. Spectra of the fecal extracts were recorded with a 400MHz Bruker Advance system at 300K using a standard presaturation pulse sequence for water suppression: TD=16k, SW=5555Hz, NS=16, RD=1.5s, AQ=1.47s. All spectra were processed using Bruker Topspin 2.1 and bucketed with Amix 3.9.1. The region of δ 4.4~ 6.6 was discarded to eliminate the effects of imperfect water suppression. Each bucket was normalized to the total integral of the spectrum prior to partial least squares discriminant analysis (PLS-DA).

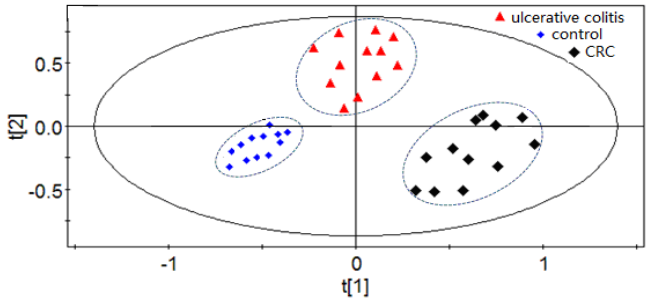

Fig 2. PLS-DA scores plot of fecal extracts from healthy controls (green boxes), and patients with UC (red triangles) and CRC (black

Results There were clear metabolic differences of fecal extracts between healthy controls, and patients with UC and CRC (Fig.1). The fecal extracts in both UC and CRC patients were characterized by reduced levels of short-chain fatty acids (SCFA, including acetate, propionate and butyrate) and proline in comparison with the healthy controls. The levels of isoleucine, leucine, valine, alanine and glutamate were found to be higher in both UC and CRC than in control. Glycerol resonances were a dominant feature of fecal spectra from patients with UC but were present in much lower intensity in CRC and controls. PLS-DA scores plot generated from ^{1}H NMR spectra of the fecal extracts showed good separation between each two groups, although the data points are located dispersedly due to individual differences and random noises (Fig.2).

Conclusions The most significant metabolites for classification include SCFA, alanine, isoleucine, leucine, valine, glutamate, glycerol and the spectra in the aromatic region (6.6–8.2ppm), suggesting changes in the gut microbial community or malabsorption [3] This work shows the potential of MR-based metabolomics of fecal extracts in providing useful non-invasive diagnostic information for UC and CRC diseases and may further our understanding of disease mechanisms.

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