Metabolic signatures of colorectal cancer in biofluids: NMR-based metabolomics of fecal extracts

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Introduction Colorectal cancer (CRC) is one of the most prevalent types of cancer death worldwide. Identifying reliable biomarkers would facilitate the development of novel technologies for CRC screening and early diagnosis, as well as improving therapeutic intervention and prognosis of this disease. Global proton nuclear magnetic resonance (^{1}H NMR)-based metabolomics on stool specimens opens up new possibilities for screening new, efficient and high-throughput CRC screening biomarkers[1-4]. Given that our previous study was exploratory and laboratory due to the limited number of samples [4], the purpose of this present study was to validate the ability of NMR-based metabolomics approaches coupled with orthogonal projection to least squares discriminant analysis (OPLS-DA) to characterize the metabolic "fingerprint" of fecal extracts for CRC routine screening on larger patient cohorts.

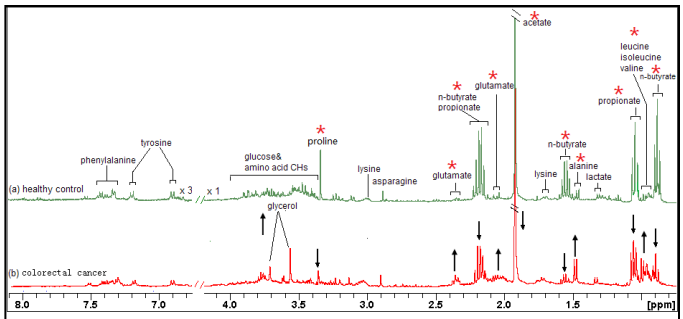

Fig.1 Typical 400 MHz mean ^{1}H NMR spectra of fecal extracts obtained from healthy controls (a) and patients with CRC, referenced to TSP (δ 0.00 ppm)

Materials and Methods Stool samples were collected from healthy individuals (n=36, 21M, 15F, age 50±12) and CRC patients (n=58, 31M, 27F, age 54±13). Samples were extracted with PBS/D_20 buffer. A stock solution of TSP/D_20 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 1D NOESY pulse sequence. Water suppression was achieved by irradiation of the water peak during the recycle delay (RD) and mixing time (t_m), with the following acquisition parameters: RD=1.5s; t_1=3μs; t_m =100ms; 90° pulse width=7.3us; NS=64; TD=16380; SW=5000Hz; AQ=1.47s. All spectra were preprocessed and then bucketed with the equal width of 0.02ppm. The region of δ 4.4~ 5.6 was discarded to eliminate the effects of imperfect water suppression. Each bucket was normalized to the total integral of the spectrum prior to OPLS-DA using the SIMCA-P+ package (V.13, Umetrics AB, Sweden).

Results There were clear metabolic differences of fecal extracts between healthy controls and CRC patients (Fig.1). Good discrimination between cancer and control groups was achieved by OPLS-DA scores plot generated from ^{1}H NMR spectra of fecal extracts (Fig.2(a)). Validation of this regression model produces R^2=0.842, Q^2=0.646, which suggests the model possessed a satisfactory fit with good predictive power. The main metabolites contributing to this discrimination, were short-chain fatty acids (including acetate, propionate and butyrate), praline, leucine, valine, glutamate, glycerol (Fig,2(b)). A random permutation test with 200 permutations was performed with OPLS-DA to further evaluate the robustness of this method and validate the biomarkers. As shown in Fig. 2(c), the validation plots assured that our original OPLS-DA models were not random and over fitting because both the permuted R^2 and Q^2 values to the left were significantly lower than the original points to the right and Q^2 regression lines have a negative intercepts.

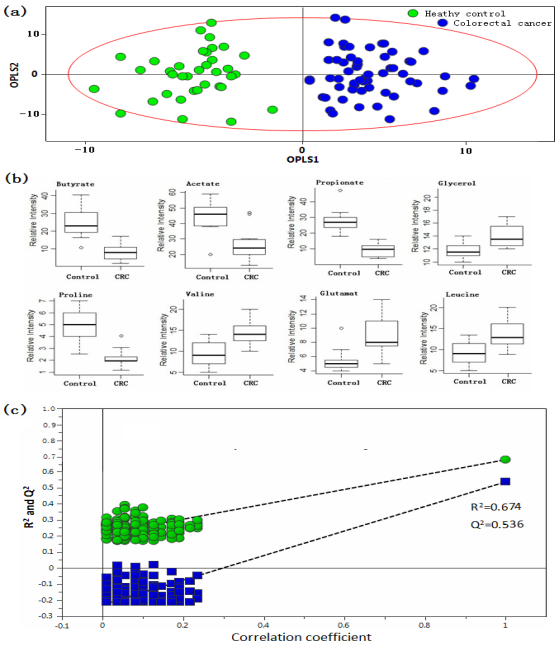

Fig 2. (a) OPLS-DA scores plot of fecal extracts based on 36 healthy controls (green dots) versus 58 CRC group (blue dots). (b) Box-and-whisker plots illustrating discrimination among the groups. (c)

Conclusion The most significant metabolites for classification include SCFA (butyrate, acetate, propionate), Proline, Glycerol, Valine and Glutamate, suggesting changes in the gut microbial community and malabsorption of nutrients [5]. These present results show the valuable potential of NMR-based metabonomics of fecal extracts to characterize the systemic metabolic disturbances underlying CRC and to identify possible early biomarkers for clinical prognosis.

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References [1] T. Bezabeh, et al, NMR Biomed. 2009. [2] D Monleon, et al. NMR Biomed. 2009. [3] Y Lin, et al. ISMRM.21 (2013). [4] Y Lin, et al. ISMRM.22 (2014). [5] Julian R. et al, J Proteome Res 2007