NMR-based metabolite profiling of fecal extracts from colorectal cancer in China: an initial study

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Introduction The incidence of colorectal cancer (CRC) in China has increased in recent years and becomes a substantial cancer burden, particularly in the developed areas. Therapeutic intervention for the CRC depends largely on the stage of the disease at the time of diagnosis [1]. However, due to a lack of an effective, expensiveness and preferably non-invasive way of detecting CRC at an early stage, the disease has often advanced significantly when its clinical manifestation becomes apparent. Proton Magnetic resonance Spectroscopy (^{1}H MRS) has been shown to be particularly useful for identifying metabolic profiles of fecal specimens, and disturbances in these profiles compared with the basal metabolic state may reveal potential biomarkers of CRC [2-4]. This present pilot study is a NMR-based metabolite profiling and metabolomics approaches aimed to evaluate the ability to characterize the metabolic "fingerprint" of fecal extracts, for routine screening of CRC in China.

Materials and Methods Stool specimens were collected from 33 CRC patients (21 M, 12 F, age 22~86) and 17 healthy subjects (8 M, 9 F, age 26~72) and 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 4.6 ~ 5.8 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). The quality of models was evaluated with the R^2 and Q^2 values, reflecting the explained variables and the model predictability.

Results Fig.1 shows the PLS-DA scores plot of the fecal extracts from healthy subjects and CRC patients. Validation of this regression model produces R^2=0.7912, Q^2=0.7829, which suggests the model to be robust for biological interpretation. There were clear metabolic differences of fecal extracts between the healthy and CRC by visual comparison (Fig.2.). Lower levels of short-chain fatty acids (SCFA) such as acetate(1.92ppm), propionate(1.06ppm, 2.19ppm) and butyrate (0.9ppm) can be seen in CRC group, compared to healthy controls, whereas lactate (1.33ppm), formate (8.46ppm), amino acids & glucose (3.6-4.2ppm) were higher in CRC than in the healthy controls.

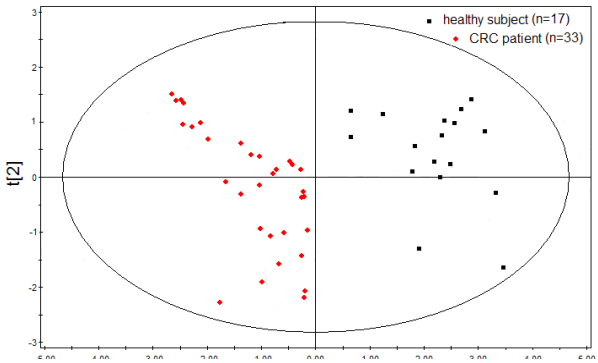

Fig.1. PLS-DA scores plot of the stool samples from healthy subjects and CRC patients （R2=0.7912 , Q2=0.7829）

Discussions and Conclusions This preliminary results based on ^{1}H NMR spectroscopy and PLS-DA demonstrated that metabolites differences of fecal extracts between CRC patients and healthy controls can be identified clearly. The observed low levels of SCFA (acetate, butyrate and propionate) are consistent with previous reports[3,4], which has been associated with the development of colorectal cancer, seems to be the most effective marker of fecal extracts for differentiating CRC patients from controls. The identity and significance of the other selected peaks (e.g., lactate, formate, amino acids & glucose) remain to be determined. A more important number of samples are needed to validate these results and allow improve in the discrimination between the two populations.

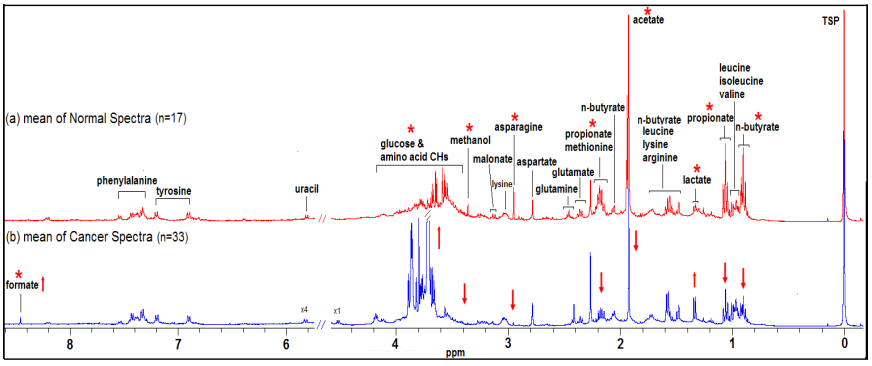

Fig.2. Mean value of the 1D typical ^{1}H MR spectra (400MHz) for (a) the 17 control samples and (b) 33 CRC samples, referenced to TSP (δ0.00 ppm)

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