Characterization and evaluation of metabolic biomarkers for human colon adenocarcinomas by ^{1}H HR MAS spectroscopy

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Introduction

Colon cancer is one of the most frequent types of cancer worldwide counting 677,000 deaths each year[1]. Together with rectal cancer, colon cancer is the third most commonly diagnosed type of cancer in the US and accounts for 10% of estimated new cases among leading cancer types[2]. Biochemical mechanisms causing development of colon cancer are still unknown, and new methods and research are therefore needed. High resolution proton magic angle spinning (^{1}H HR MAS) is an ex vivo method providing detailed information on metabolic composition of intact tissue, and new metabolic biomarkers for colon cancer can be important for clinical prognostic and diagnostic evaluation. The purpose of this study was to characterise the metabolic profile of human colon tissue using ^{1}H HR MAS and evaluate possible biomarkers for colon adenocarcinoma.

Methods

Sixty-two colon tissue samples were obtained from 31 different patients (mean age: 73 years (range: 48-93), 17 women and 15 men). Two samples were collected from each patient where one was excised from areas consisting healthy tissue (mucosa) and the other from areas with clinically proven colon adenocarcinoma representing various stages of disease (T1-4, N0-3, M0-1). Four of the patients were diagnosed with adenomas. After surgery, tissue samples were immediately frozen and stored at -80°C until HR MAS analysis. ^{1}H HR MAS was performed on a 14.1 T Bruker Avance DRX spectrometer equipped with a 4-mm ^{1}H/^{13}C MAS probe. Spectra were acquired at 4°C with a spin rate at 5 kHz. In addition to a single-pulse sequence, a Carr-Purcell-Meiboom-Gill (CPMG) spin echo sequence was acquired to suppress signals from overlapping lipids and macromolecules (128 transients, effective echo time 272ms). After HR MAS analysis, the tissue was embedded in formalin, sectioned and stained (H&E) for pathology reading. Spectra (1.4-4.8 ppm) were statistically analysed by principal component analysis (PCA, The Unscrambler 7.01, Camo, Norway).

Results

PCA, explaining 50% of the variance, showed a marked separation between the metabolic profiles of cancer and healthy spectra by the second principal component (PC2) (Fig. 1a). The four patients diagnosed with adenomas were separated from the healthy samples by PC1, but additionally from the adenocarcinomas by PC2 (Fig. 1a, black circles). The loading plot for PC2 (Fig. 1b, 19% of the variance) shows the metabolites responsible for separation between cancer and healthy/adenomas. The metabolic profiles of colon cancer tissue were characterized by having a higher level of taurine and lactate and a lower level of inositols (myo-inositol, scyllo-inositol), β-glucose, glycine and phosphocholine (PC) and glycerophosphocholine (GPC). The adenomas had higher levels of choline-containing compounds (choline, PC and GPC) and taurine than the healthy samples. Representative spectra (CPMG) from healthy and cancer tissue from the same patient are presented in Figure 1c (2.8-4.3 ppm), showing differences in metabolic composition.

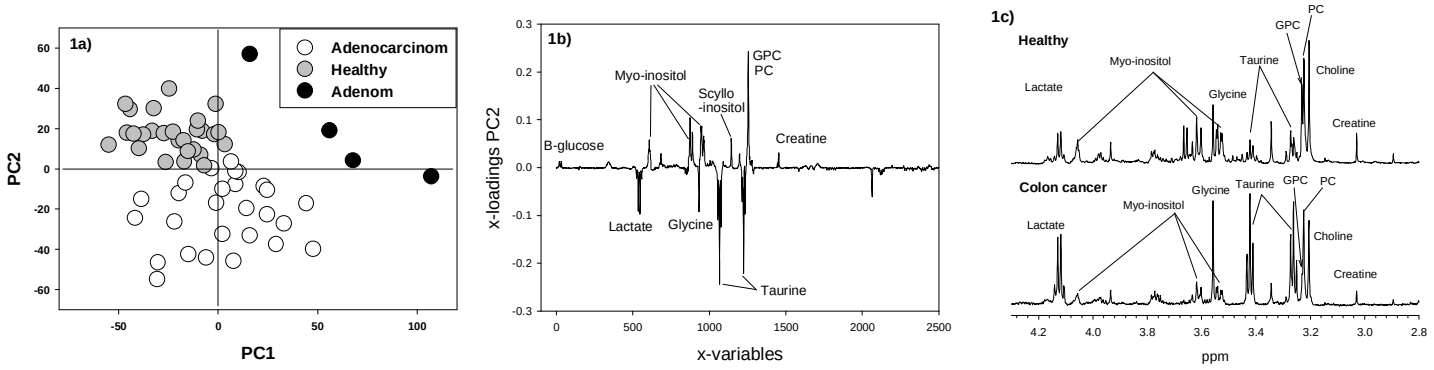

Figure 1. a) PCA scoreplot showing PC1 and PC2 b) Loading plot for PC2 c) Representative ^{1}H HR MAS spectra from healthy and cancer tissue from the same patient.

Discussion

PCA on human colon ^{1}H HR MAS spectra, revealed marked differences in metabolic profiles of adenocarcinomas and healthy tissue, and additionally adenomas and healthy tissue. From a clinical point of view, it is important that there was minimal overlap between the individual metabolic profiles of cancer and healthy tissue, and a very clear separation of adenomas from both healthy and cancer tissue. Increased levels of taurine and lactate in cancer is in agreement with previous studies[3,4], indicating possible mechanisms such as membrane stabilisation[3] and increased glycolytic flux[4], respectively. Decreased levels of inositols may indicate an unbalance in osmolyte function in cancer cells[6]. Surprisingly, GPC and PC decreased in cancer tissue compared to healthy tissue, which is opposite to what is reported in many other cancers. However, it is stated that the healthy human gastrointestinal system have relatively high levels of phospholipid intermediates[6], and cancer may disturb the normal highly efficient cell proliferation in colon mucosa. These metabolites are possible biomarkers for human colon cancer and could be used to improve clinical diagnosis and characterisation of colon cancer.

References

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