Metabolomic Characterization of Human Rectal Adeno-Carcinoma with Intact Tissue Magnetic Resonance Spectroscopy

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Introduction

The diagnosis of rectal cancer is currently dependant on biopsies taken from the suspicious lesion during colonoscopic or sigmoidoscopic examinations. While histopathology will remain the gold standard for the diagnosis of rectal cancer for years to come, it is possible that new technologies, such as *in vivo* MRS, may not only complement but even replace histopathology for the diagnosis and grading of rectal cancer tumors. To facilitate developments in this direction, we have investigated the utility of *ex vivo* intact tissue metabolomic profiles in differentiating biopsies from rectal tumors containing malignant cells from benign samples by spectroscopic results measured with HRMAS ^{1}HMRS.

Methods

Multiple biopsies of untreated rectal tumors that appeared macroscopically malignant, along with one to two biopsies from macroscopically benign rectal mucosa of the same patient, were obtained, immediately put into cryo vials, labeled and put on dry ice. Spectral analyses were performed on a 14.1 T spectrometer operating at 600 MHz, and data were processed with Nuts software. Samples were then formalin fixed, embedded in paraffin, cut into 5 mm sections, and stained with Hematoxylin and Eosin. Percentages of the pathological components were determined by the pathologist and correlated with spectral metabolite profiles by principle component analysis (PCA).

Results and Discussions

In this study a total of 23 samples were collected and analyzed from 5 patients. PCA revealed 3 principle components could significantly differentiate samples containing cancerous tissue from those without tumor tissue present: PC 2 ($P = 0.0041$), PC 4 ($P = 0.0238$), and PC 6 ($P = 0.0075$). This differentiating capacity was binary, and linear regression analysis determined that PCs 2 and 6 correlated with the % by volume of cancerous and benign epithelium (PC 4 had no significant correlation to % vol malignant cells, $P = 0.074$). PC 2 reflected 16.1% variance among the samples and correlated with % vol malignant tissue ($P = 0.0065$, $R^2 = 0.303$) and % vol normal epithelium ($P = 0.0051$, $R^2 = 0.318$). PC 2 reflects changes in several metabolites with known connection to oncological developments, including choline and phosphocholine. PC 6 reflected 5.50% variance and correlated with % vol malignant tissue ($P = 0.0200$, $R^2 = 0.232$) and % vol normal epithelium ($P = 0.0255$, $R^2 = 0.216$). PC 4, accounting for 11.0% variance, also correlated with the % vol normal epithelium ($P = 0.033$, $R^2 = 0.200$), and with % vol stromal tissue ($P = 0.0311$, $R^2 = 0.203$), but not with % vol cancer. PC 7, representing 4.43% variance, correlated with % vol inflammation ($P = 0.0026$, $R^2 = 0.360$).

The current study was undertaken as a pilot project to determine if metabolomic profiles could be constructed for human rectal adeno-carcinoma biopsies using HRMAS ^{1}HMRS, in the same manner that profiles have been created for various other tissues. Each of the four pathological features examined (malignant cells, benign epithelium, stromal cells, and inflammation) manifested different biochemical changes that could be significantly delineated by PCA. The most noteworthy being the PCs that correlate with % volume tumor or benign mucosa. Interestingly PCs that correlate with the same process often indicated metabolite regions that contributed in the same manner, but with differing magnitudes to the processes taking place. The study has important implications for future research. Given the strong preliminary data it is logical to assume that additional features, such as stage, grade, or tumor aggressiveness, may manifest in measurable metabolic differences. Further examinations correlating clinical features with metabolic profiles could increase the clinical utility of HRMAS ^{1}HMRS for rectal adeno-carcinoma, and provide information that current *in vivo* radiological means are unable to produce.

Conclusion

As an exploratory study designed to establish if the HRMAS ^{1}HMRS method can metabolomically characterize human rectal adeno-carcinomas, the current results are significant, both for further *ex vivo* and future *in vivo* investigations. The results indicate the ability to biochemically classify tissue samples as malignant or benign. Further studies may reveal if HRMAS ^{1}HMRS can accurately stage rectal tumors or indicate additional prognostic factors, such as a subset of more aggressive tumors that should be monitored post-surgery for recurrence. All these observations may present directive guidance for the future targeted developments of *in vivo* diagnostic methodologies.

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