Comprehensive Insight into Colorectal Cancer Metabolites and Lipids for Human Serum: A Proof-of-Concept Study

GC×GC-LR/HR-TOFMS for Colorectal Cancer Metabolomics and Lipidomics

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Colorectal cancer (CRC) is the third most frequently diagnosed cancer and the second leading cause of cancer-related deaths. The current endoscopic-based or stool-based diagnostic techniques are either highly invasive or lack sufficient sensitivity. Thus, there is a need for less invasive and more sensitive screening approaches. We conducted a study on 64 human serum samples from different groups (adenocarcinoma, adenoma, and control) using cutting-edge GC×GC–LR/HR-TOFMS techniques. We analyzed samples with two different sample preparation approaches for lipidomics (fatty acids) (25 µL serum) and metabolomics (50 µL serum). Samples were randomized with a QC sample (pooled human plasma) and NIST SRM 1950 for QAQC requirements.

The feature selection was based on three statistical criteria for multi-group analysis: false discovery rate (FDR) from one-way analysis of variance (ANOVA); variable importance projection score (VIP score) from partial least squares - discriminant analysis (PLS-DA); Mean Decrease Accuracy (MDA score) from the random forest (RF) algorithm.

For the Lipidomics study, 40 analytes were isolated, including saturated, monounsaturated, and polyunsaturated fatty acids, and cholestadiene isomers. Amongst them, 8 features (MSI confidence levels of 1 or 2) were identified as significant (VIP score >1, MDA cut-off >0,008). It revealed that specific PUFA (ω-3) molecules were inversely associated with increased odds of CRC, while some PUFA (ω-6) analytes shown a positive correlation.

For the metabolomics study, 105 metabolites were identified. The metabolite's nature ranged from amino acids, carboxylic acids, carbohydrates, fatty acids, nucleoside, purine, and vitamins. Some proteogenic amino acids (Ala, Glu, Met, Thp, Tyr, Val), Myo-inositol, and 3-hydroxybutyric acid were found to be dysregulated in CRC.

This study provides a more comprehensive insight into molecular-level changes associated with CRC and allows for a comparison of the efficiency of two different analytical approaches for CRC screening using the same serum samples and a single instrumentation.

Keywords: colorectal cancer; metabolomics; lipidomics; chemometrics, comprehensive gas chromatography, mass spectrometry, GC×GC–TOFMS, separation science