Colorectal cancer study with GC×GC-(HR)TOFMS

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1. Introduction

Colorectal cancer globally affects more than one million new persons each year, and kills more than 700,000. Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one of the approaches that can be used to help solve these issues.

2. Approach

An optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS method we developed and already applied successfully in Crohn disease (article under review) was used. It also included an in-house QC system based on all study samples aliquots, control charts to guarantee high-quality data, and data processing based on multiple statistical techniques.

Practically, we analyzed serum samples from patients affected by colorectal cancer (CRC, n = 18), by colorectal cancer in remission (R-CRC, n = 17), and samples from healthy patients matched for as many biases as possible (HC, n = 19 and R-HC, n = 17). The aim was to highlight candidate biomarkers able to discriminate between matched HC and CRC or R-CRC.

After the selection process, the discrimination potential of the candidates was assessed using supervised and unsupervised models (PLS & OPLS / PCA and HCA), discriminant analysis and ROC curves, with overfitting of the experimental data avoided by re-sampling and test validation testing.

The significant metabolites were identified using full mass spectrum, linear retention indices and accurate mass provided by state-of-the-art high-resolution (HR) time-of-flight mass spectrometry. Finally, confident identifications were used to study the main metabolic pathways altered in the disease, whether in active or in remission state.

3. Results

From 620 unique features automatically detected in the cumulative chromatogram of all samples, that covered more than 5 orders of signal magnitude, 589 were kept for their chromatographic quality after manual control. Data were scaled and corrected for the analytical variation measured in the QC samples, before statistical research lead to respectively 11 and 15 candidates for CRC and R-CRC, from which 6 and 11 could be annotated with level 2 confidence¹.

These most altered, i.e. significant, metabolites performed quite well, albeit not perfectly, in discriminating the disease from healthy samples. Indeed, if OPLS-DA Q² was limited to 0.35 and 0.47 respectively for CRC and R-CRC (permutation Q² : 0.38 and 0.41), AUC was > 0.8 and 0.9 and discriminant analysis p-values was < 0.00001 for both. Unexpectedly, R-CRC samples were easier to discriminate from matched healthy patients than CRC. The annotated candidates were associated to alterations of several pathways including pentose phosphate pathway, glycolysis or gluconeogenesis, synthesis and degradation of ketone bodies, citrate cycle and several amino acid and sugar metabolisms.

4. Discussion

This study not only found novel candidate biomarkers for colorectal cancer, in active and remission states, it also produced potentially original pathological insights through the metabolic pathways altered. Moreover, it confirmed the capability of GC×GC-(HR)TOFMS and the global analytical method developed to perform well in untargeted analysis.

References