GC-MS Orbitrap and GC×GC-(HR)TOFMS in colorectal cancer metabolomics

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Globally affecting more than one million new persons each year, and killing more than 700.000, colorectal cancer is the second leading cause of cancer-related deaths in women and the third in men. Nevertheless, diagnosis is still largely based on invasive tissue sampling, while gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics, by providing a global picture of the outcome of the disease, is one way to address these issues.

To do so, two techniques and their corresponding methodologies were applied in parallel. First, comprehensive GC×GC-(HR)TOFMS, using a completely optimized and validated (NIST SRM 1950) method, including data processing. Second, newly developed GC-MS Orbitrap with dedicated data preprocessing and high resolution accurate mass metabolomics (HRAM) library. Both were submitted to an in-house QC system based on all study samples aliquots and control charts to guarantee high-quality data.

Practically, replicates of 48 serum samples were analyzed on both platforms : samples from patients affected by colorectal cancer (CRC, n = 12), by colorectal cancer in remission (R-CRC, n = 12) and samples from healthy patients matched for gender and age at sampling (HC and R-HC, both n = 12), along with QC samples (n = 19 and n = 9 for GC×GC-(HR)TOFMS and GC-MS Orbitrap respectively). The aim was to highlight candidate biomarkers able to discriminate between pathological and healthy states through the selection of metabolites with significantly different distribution between matched HC and CRC or R-CRC.

Each technique was investigated in terms of peak capacity, data stability –with a consideration for the effect of the QC system- and identification power. The results were analyzed through their respective specificities, especially the trade between mass resolution power –mass accuracy- and chromatographic separation capacity.

The potential biomarkers obtained with both instruments were cross-confirmed and their discrimination potential 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 procedures.

They were then identified using full mass spectrum, linear retention index and exact mass. Since this step is a bottleneck in metabolomics, the influence of mass resolution was assessed for both instruments. Finally, confident identifications were used to determine the main metabolic pathways altered in the disease, whether in active or in remission state.