Exploring Gut Microbiota Metabolism – New Chemical Biology Tools for Metabolomics Analysis

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Abstract

One of the most exciting scientific developments in the past decade has been the realization that gut microbiota profoundly impact human physiology. The complex consortium of trillions of microbes possesses a wide range of metabolic activity. Due to the link to disease development from metagenomic analysis the targeted investigation of their metabolism represents a tremendous potential for the discovery of biomarkers and bioactive metabolites. Mass spectrometry-based metabolomics analysis is the method of choice for the analysis of known and discovery of unknown metabolites. Advanced Chemical Biology tools are still limited in metabolomics compared to other 'omics research fields. We have developed new state-of-the-art Chemical Biology methodologies for an enhanced metabolomics analysis using liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS).[1-7] These unique tools are aimed at overcoming limitations in mass spectrometry-based metabolomics analysis and are selective for microbiome metabolism. We are applying these methods for the analysis of human samples collected from pancreatic cancer patients.

We have designed and synthesized a unique chemoselective probe immobilized to magnetic beads that allows for facile extraction of metabolites and led to increased mass spectrometric sensitivity by a factor of up to one million.[1-5] An incorporated bioorthogonal cleavage site, which we have adapted from a protecting group that is labile under mild, palladium-catalyzed conditions facilitates efficient release of captured metabolite without altering their chemical structure. This method was utilized on human fecal and isolated microbiome samples. Our analysis of carbonyls, thiols, amines, and short-chain fatty acids (SCFAs) revealed previously unknown metabolites and due to conjugation of the mass-spectrometric tag and separation from the sample background the detection limit was at high attomole quantities.

We also utilized selective enzymatic treatment of metabolites in human samples for simplified identification of converted sulfated and glucuronidated metabolites to elucidate their chemical structure using mass spectrometry.[6,7] Analysis of pretreated samples using ultra high-performance liquid chromatography-mass spectrometric techniques led to the identification of 206 sulfated metabolites, exceeding the number of sulfated metabolites in metabolomics databases by a factor of three to four. Many of these identified phase II clearance products are linked to gut microbiota-human host co-metabolism. An optimized enzymatic method was applied for a dietary intervention study to investigate the dietary sulfatome.[5] Several previously unknown and undetected metabolites are derived from specific microbiota metabolism.

References

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