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

Mário S. P. Correia, Louis P. Conway, Caroline Ballet, Weifeng Lin, Abhishek Jain, Theodosia Vallianatou, and Daniel Globisch ; Uppsala University, Uppsala/Sweden

One of the most exciting scientific developments in the past decade has been the understanding that gut microbiota profoundly impact human physiology. The complex consortium of trillions of microbes possesses a wide range of metabolic activity. This metabolic interspecies communication represents a tremendous opportunity for biomarker discovery as only limited information on this co-metabolism has been elucidated on a molecular level.

The detailed investigation of metabolites in any type of human samples has been termed metabolomics and holds a great potential for the discovery of unknown biomarkers and bioactive metabolites. Mass spectrometric analysis is the method of choice for metabolic investigation due to its sensitivity to metabolites over several orders of magnitude. Global metabolomics is the newest 'omics-research field, for which advanced chemical tools are limited compared to other 'omics research fields such as transcriptomics and proteomics. Especially, the detailed and selective analysis of microbial metabolism remains a major challenge that requires more advanced techniques due to low metabolite concentrations.

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).[1-5] These unique metabolite-analyzing 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 discovery of unknown metabolites in human samples collected from pancreatic cancer patients to evaluate their potential as biomarkers.

Chemoselective probes for increased mass spectrometric sensitivity

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 six orders of magnitude.[1-3] 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 urine samples, the sample type most directly influenced by metabolically active microbial communities. Analysis revealed previously unknown metabolites and due to conjugation of the mass-spectrometric tag and separation from the sample background the detection limit for most metabolites was increased by up to a factor of one million.

Selective investigation of human host and microbiota co-metabolism

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.[4,5] 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 microbiota-human host co-metabolism.

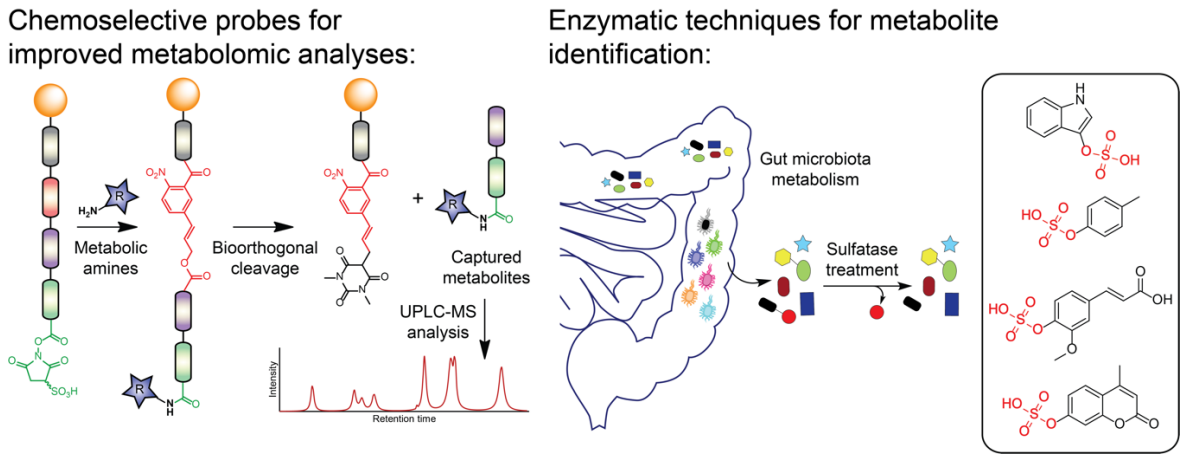

Figure 1. New Chemical Biology methods for advanced analysis of human samples

References

1. N. Garg, L.P. Conway, …, D. Globisch, *Angew. Chem. Int. Ed.* **2018**, *57*, 13805–13809.

2. L.P. Conway, …, D. Globisch, *Chem. Comm.* **2019**, *55*, 9080-9083. (Selected as Cover Art)

3. W. Lin, … , D. Globisch, *under review*.

4. C. Ballet, M.S.P. Correia, …, D. Globisch, *Chem. Sci.* **2018**, *9*, 6233–6239. (Hot Paper)

5. M.S.P. Correia, … D. Globisch, *ChemBioChem* **2019**, *20*, 1678–1683. (ChemBioTalents)