"Untargeted metabolomics uncovers N-lactoyl-amino acids and glutamate conjugates as substrates of ABCC5"

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Abstract:
Our group studies ATP-binding cassette (ABC) efflux transporters using metabolomics-based approaches. ABC transporters are integral membrane proteins and use the energy provided by the hydrolysis of ATP to transport specific substrates across membranes. Despite the fact that ABC transporters have been extensively studied, their physiological function is incompletely understood. This is mainly due to a lack of knowledge about their in vivo substrate spectrum. ABCC5, for instance, is present at high levels in the blood-brain barrier, neurons and glia, but its preferred substrates are unknown. In my talk I will show how we used untargeted metabolomics to shed more light on the in vivo substrate spectrum of this ubiquitous efflux transporter. Using HEK293-ABCC5 cells we, for instance, found that N-lactoyl-amino acids are transported by ABCC5. N-lactoyl-amino acids are ubiquitous pseudo-dipeptides of lactic acid and amino acids and were not known to exist in mammals. Intriguingly, we found that N-lactoyl-amino acids are formed by CNDP2-mediated reverse proteolysis a process previously considered negligible in vivo. The function of N-lactoyl-amino acids, if any, is unknown.

We also applied our metabolomics screens to tissue extracts of Abcc5−/− and wild-type mice and uncovered ABCC5 as a general carrier for compounds containing a glutamate-moiety. Glutamate conjugates are of physiological relevance because they can affect the function of glutamate, the principal excitatory neurotransmitter in the brain. Interestingly, ABCC5 also transports exogenous glutamate analogs, like the classic excitotoxic neurotoxins kainic acid, domoic acid and N-methyl-D-aspartate (NMDA), and the therapeutic glutamate analog ZJ43.

In conclusion, in my presentation I will demonstrate the power of metabolomics to identify new substrates of (orphan) transporters. The presented methodology is not limited to ABC transporters but can be applied to any transporter of interest.