How Lipidomics Unravels Sphingolipid Species and Related Pathway Transcripts as Biomarkers

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Sphingolipids play essential roles as structural components of cell membranes, in cell signalling, and in the regulation of apoptosis and survival. The disruption of their metabolism is associated with several diseases ranging from vascular- and metabolic disease to cancer. Searching for novel targets for vascular- and metabolic disease in the EUROSPAN study (n=4500), a genome-wide association between clinical and laboratory phenotypes, lipid species and single-nucleotide polymorphisms (SNPs) has been performed. Nine polymorphic gene regions involved in glycerophospholipid metabolism and five polymorphic candidate genes related to ceramide metabolism and corresponding glycerophospholipid and ceramide species levels in plasma have been identified, which correlated directly with disease phenotypes. The experimental proof and the molecular mechanism behind these associations came from in vitro experiments exposing human macrophages to the two prototypic atherogenic lipoprotein modifications, enzymatically modified LDL (E-LDL) and oxidised LDL (Ox-LDL), followed by lipidomic, transcriptomic and proteomic analysis of the incubated macrophages. Cholesterylester and total cholesterol increased during E-LDL loading while ceramide and lysophosphatidylcholine (LPC) were found higher during Ox-LDL loading. The ceramide species with a significant induction upon Ox-LDL loading include ceramide C16:0, C18:0, C22:0, C24:1 and C24:0, and the LPC species include LPC 16:0, LPC 18:1 and LPC 18:0. The in vitro responses to E-LDL and Ox-LDL were strongly similar to the lipid species abnormalities and the genes found in the EUROSPAN cohort and in case/control studies related to lipid metabolism, apoptosis/survival, and radical generation elimination. Specific LPC and ceramide species were identified as lipidomic “find me” and “eat me” signals differentially regulated by non- or pro-inflammatory clearance of either E-LDL or Ox-LDL and the corresponding lipid species identified in the EUROSPAN cohort are promising biomarkers for patient risk assessment.