Glycomics and Lipidopics

Extended Abstract

High-Throughput Screening of Lipidomic Adaptations in Cultured Cells

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Abstract:

High-throughput screening of biologically active substances in cell cultures remains challenging despite great progress in contemporary lipidomic techniques. These experiments generate large amounts of data that are translated into lipid fingerprints. The subsequent visualization of lipidomic changes is key to meaningful interpretation of experimental results. As a demonstration of a rapid and versatile pipeline for lipidomic analysis, we cultured HeLa cells in 96-well format for four days in the presence or absence of various inhibitors of lipid metabolic pathways. Visualization of the data by principle component analysis revealed a high reproducibility of the method, as well as drug specific changes to the lipidome. Construction of heatmaps and networks revealed the similarities and differences between the effects of different drugs at the lipid species level. Clusters of related lipid species that might represent distinct membrane domains emerged after correlation analysis of the complete dataset. Taken together, we present a lipidomic platform for high-throughput lipidomic analysis of cultured cell lines. High-throughput lipidomics has great potential when investigating the role of lipids in cellular metabolism. A large number of drugs is available to interfere with normal lipid metabolic pathways and contemporary lipidomic techniques can monitor the levels of hundreds of lipids simultaneously. However, true high-throughput lipidomics still involves the subsequent overcoming of several challenges. First, the clean-up of samples so their lipid composition may be measured. Established protocols for extraction of lipids involve liquid-liquid extractions after an initial one-phase system. In this one phase system, lipids and hydrophilic metabolites remain dissolved, whereas proteins precipitate and are removed by centrifugation. The subsequent induction of a two-phase system by the addition of water and/or organic solvent, separates hydrophilic- and hydrophobic metabolites. Together with additional washing steps to increase lipid recovery, the two-phase extraction process is laborious, time-consuming, and cumbersome to automate. Next, a choice has to be made between shotgun lipidomics or a liquid chromatography-mass spectrometry ($LC\text{-}MS^n$) approach. Shotgun lipidomics, i.e., the direct infusion of the lipid extract, relies solely on mass spectrometry techniques for lipid fingerprinting . The $LC\text{-}MS^n$ approaches based on either reversed phase- (RP), normal phase- (NP), or hydrophilic interaction liquid chromatography (HILIC) have all proven their value as they add retention time as an additional feature that can aid in the identification of lipid speci. In particular HILIC based separation of lipid classes allows for LCMS based lipidomic analysis in time-spans normally only achievable by shotgun lipidomics. Here, we used such a rapid HILIC-LCMS technique to obtain maximum sensitivity and specificity, while avoiding the ion suppression commonly associated with shotgun lipidomics. For the translation of LC-MS data to a (semi-)quantitative and annotated peak list, several strategies have been successfully demonstrated for high- as well as low resolution instruments. Typically, a lipidomics experiment results in the identification of several hundreds of lipid species. The interpretation of the obtained results is more challenging than in other -omics fields. For instance, in proteomics and genomics experiments, the changing proteins may be directly linked to enzymatic or signaling pathways, thus giving clear clues to altered cellular functions. Also in (hydrophilic) metabolomics, most metabolites are the product of one or very few enzymes, and are a substrate for very few other enzymes. Therefore, also in metabolomics, altered levels of metabolites can be directly mapped to changes in metabolic pathways. In lipidomics, this mapping is more challenging, but bioinformatics have become an integral part of the lipidomic pipeline. Lipidomic data contain an additional layer of detail. Whereas the two-dimensional (2D) metabolic maps typically present a lipid class such as phosphatidylcholine (PC) or phosphatidylethanolamine (PE) as a single metabolite, the lipid subclass and acyl composition add a third dimension to the map. Lipid functions can be dependent on this additional layer, for instance phospholipases may act only on a subset of lipid species within a lipid class and the occurrence of lipid microdomains is also regulated at the lipid species level. Interpretation of lipidomic data, therefore, is particularly challenging. Likewise, interference with lipid metabolism, be it drug induced or resulting from pathology, is likely to affect many lipid species. Discriminating between key primary effects and less relevant side-effects is impossible without considering the entire (phospho-)lipidome and understanding existing interactions between lipid species. Therefore, information-rich visualizations of the changes in

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the complex lipidome are important. Once this is achieved, multiple drugs or cellular conditions may be compared. Here, we compare the effects of nine different drugs on the cellular lipidome of the HeLa cell line. We chose drugs that are widely used in literature, are readily available and target a variety of cellular processes. These experiments were performed using a high-throughput approach, which enables a scientist to complete the entire lipidomic analysis from lipid extraction and LC-MS to visualizations within a single day. In this way, we could demonstrate a high reproducibility of the lipidomic changes in our experiments. Furthermore, we visualize similarities and differences between drugs and their effects on lipid species. Our use of inhibitors that interfere at different points in lipid metabolism, enabled us to investigate which lipid species strongly correlate to each other, either positively or negatively. Taken together, we demonstrate how lipidomics can be implemented in high content-omics screens

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