Metabolite Profiling with Isotopically Encoded Chemical Derivatization

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Overview

- A chemical modification platform for biomarker discovery through metabolite profiling is described.
- Quantitative analysis of peak intensity through isotope labeling chemical derivatization is demonstrated.
- Allows for increased throughput through pooling, split pooling, and improved quantitation.

Introduction

Human plasma contains a complex mixture of metabolites that reflects global changes from system-wide catabolism and anabolism, providing a viable resource for biomarker discovery. Recent developments in our laboratory have made it possible to mine large sets of data obtained from LCMS analysis of human plasma metabolites including the development of isotope labeling chemical derivatization, a novel approach to metabolite profiling. This approach applies the same principles as isotope labeling in proteomics to metabolomics, expanding the range of detectable metabolites by the thousands.

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Overview

Quantitative Metabolite Profiling Through Isotope Labeling

Chemical Derivatization enhances ionization, allowing the detection of a greater number of metabolites, some of which are otherwise undetectable. For example, ions of phenylalanine and cholesterol are absent in underivatized samples, whereas the corresponding butyl ester or aminated forms are readily detectable. For example, ions of phenylalanine phenylalanine, butyl ester

TIC of human serum (methanol extracted)

MS/MS

Ametabolite has been chemically derivatized underivatized

Amination

NH2

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Biomarker Discovery -- Results from Data Analysis of 500 Analyses

Data analysis begins with XCMS and the resulting features were further analyzed to give normalized plots of area sum for each feature detected. Three independent statistical tests: CART, PAM, and RF were used to evaluate the same data set for possible biomarkers for disease diagnosis. The resulting accuracy were plotted side by side showing an overall 76% chance of correct prediction both in the original dataset (Training) as well as new data (Validation). Over 10,000 features were detected by XCMS, however, only a fraction of these were selected to be useful for disease diagnosis. The correlation heat map and histogram illustrates the interrelationship of the top 50 biomarkers chosen. The correlation linkage may represent metabolites produced by the same or similar biochemical pathway.

Conclusion

This study presents an approach for metabolite profiling and biomarker discovery with advantages in enhanced ionization, improved quantification and increased throughput.