[sPHT-2] [11/15/2007 (Thr) 11:20 - 11:50 / 2nd FL]

Application of metabolic profiling for biomarker discovery

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Abstract

An important potential of metabolomics-based approach is the possibility to develop fingerprints of diseases or cellular responses to classes of compounds with known common biological effect. Such fingerprints have the potential to allow classification of disease states or compounds, to provide mechanistic information on cellular perturbations and pathways and to identify biomarkers specific for disease severity and drug efficacy. Metabolic profiles of biological fluids contain a vast array of endogenous metabolites. Changes in those profiles resulting from perturbations of the system can be observed using analytical techniques, such as NMR and MS.

^{1}H NMR was used to generate a molecular fingerprint of serum or urinary sample, and then pattern recognition technique was applied to identity molecular signatures associated with the specific diseases or drug efficiency. Several metabolites that differentiate disease samples from the control were thoroughly characterized by NMR spectroscopy. We investigated the metabolic changes in human normal and clinical samples using ^{1}H NMR. Spectral data were applied to targeted profiling

and spectral binning method, and then multivariate statistical data analysis (MVDA) was used to examine in detail the modulation of small molecule candidate biomarkers. We show that targeted profiling produces robust models, generates accurate metabolite concentration data, and provides data that can be used to help understand metabolic differences between healthy and disease population. Such metabolic signatures could provide diagnostic markers for a disease state or biomarkers for drug response phenotypes.

Keyword: Metabolic Profiling, Biomarker, Endogenous Metabolite, NMR, MS, Multivariate statistical data analysis

Introduction

One of new technologies in addition to genomics and proteomics is the emerging field of metabolomics. Metabolomics studies complement genomic and proteomic investigations by providing a quantitative description of the low molecular endogenous metabolites present in a biological sample such as urine, plasma. The metabolic profile of biofluids shows changes of their composition in response to toxic or disease-induced stress due to the system's attempt to maintain homeostasis. Understanding the biochemical reason for such a shift in metabolic space leads to the identification of biomarkers of disease, toxicity, and drug efficiency.

In such studies analysis is usually performed using high field ^{1}H nuclear magnetic resonance (NMR) spectroscopy and Mass Spectroscopy (MS), which give a metabolite profiling. Both are powerful analytical tools when combined with multivariate statistical analyses. While MS can be used for measuring metabolite concentrations well below the micromolar range, the measurement of even 40 metabolite concentrations from a number urine samples is laborious, requiring multiple internal standards. The same measurement using ^{1}H NMR spectroscopy requires only one internal standard. For screening purposes, NMR spectroscopy can be applied to biological samples, such as urine with minimal preparation or purification of metabolites, and is useful for measuring concentrations in the micromolar range and up, making it an ideal tool for metabolomics. In all previous studies of human urine, blood, or tissue, the analysis method of choice has been a combination of ^{1}H NMR spectroscopy with some form of spectral binning. Recently, the new technique of targeted profiling, which identifies as many metabolites as possible in an NMR spectrum before multivariate analysis, has been introduced as a technique to reduce dimensionality, allowing for easier interpretation of data. In this study, we compare the method of spectral binning to the method of targeted profiling of serum and urine to assess differences associated with disease. These data will show how metabolic changes may be affected by toxicological insult or disease development.

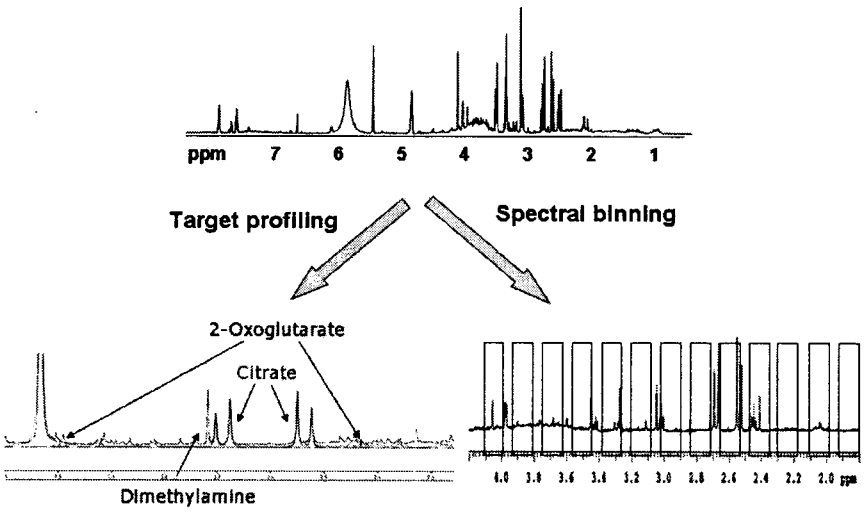

Figure 1. Two routes for metabolomic study by NMR

Experimental Method

^{1}H-NMR analysis

NMR spectral data were acquired on a Bruker AVANCE operating at 500 MHz and Varian NMR system operating at 600 MHz spectrometer for ^{1}H observation frequency For all samples, ^{1}H NMR spectra were measured with 64 scans into 76924 data points over a spectral width of 9615.4Hz, which resulted in an acquisition time of 4.00 s and a relaxation delay of 2.0 s. Solvent suppression of the residual water signals (a broad singlet at d1H 4.8) was achieved via the Noesypresat pulse sequence in which the residual water peak is irradiated during the relaxation delay (1.5 s) and during the mixing time (tm, 0.1 s).

Data reduction and Analysis.

Quantification for targeted profiling was achieved using the 600 MHz library from Chenomx NMR Suite 4.6 (Chenomx Inc., Edmomton, Canada), which uses the concentration of a Known reference signal (DSS, δ^1H 0.0) to determine the concentration of individual compounds. Integral bins were created in such a manner as to ensure that each resonance was in the same bin (0.04 ppm) throughout all spectra. All bins were normalized to the area of the DSS methyl peak. Due to variations in the effectiveness of the suppression of the water resonance, the region between δ 4.7 and 4.9 was excluded from the analysis. Partial Least Squares Discriminant Analysis (PLS-DA) was performed using standard procedures as implemented in SIMCA P+ (SIMCA-P+ 11, Umetrics AB, Umea, Sweden).

Result and Discussion

We investigated the serum metabolic changes in normal human, diabetes and nephropathy in order to study metabolic profiling and to discover the potential biomarkers. ^{1}H NMR and multivariate statistical data analysis (MVDA) was used to examine in detail the modulation of small molecule candidate biomarkers. As a result, it was possible not only to differentiate diabetes and nephropathy from the control but also to discover and identify the potential biomarkers. This approach using NMR and MVDA has the potential for discovery of novel or surrogate

biomarkers of disease, which may give mechanistic insight and act as a useful adjunct to existing measurement.

Figure 2 shows NMR spectral of human serum from human with control, diabetes, and nephropathy, with the major components identified. Close examination of ^{1}H NMR spectrum for all subjects reveals difference between the spectra obtained for sample classes. Figure 3A shows PLS-DA on metabolite concentrations derived from targeted profiling data. Figure 3B shows PLS-DA on serum spectra determined by spectral binning. This study indicates that analysis of metabolite concentrations in biofluids samples using spectral binning and targeted profiling methodology produces consistent and reliable results. Metabolite profiling may therefore be considered a robust technique for analysis of metabolomic data as it provides metabolite information in addition to accounting for the changes in NMR spectral peak position.

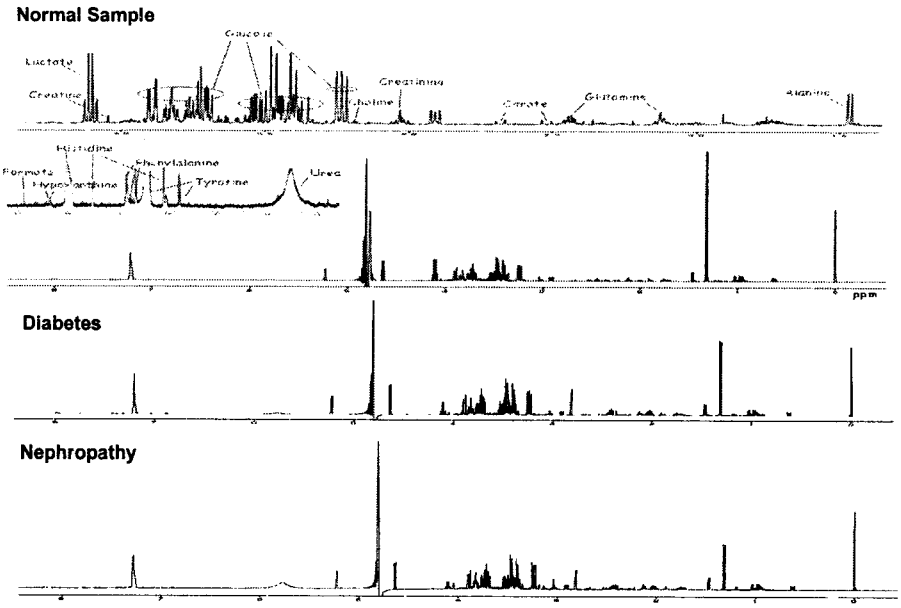

Figure 2. Comparison of representative NMR spectra of human serum samples from human with control, diabetes, and nephropathy.

(A) (B)

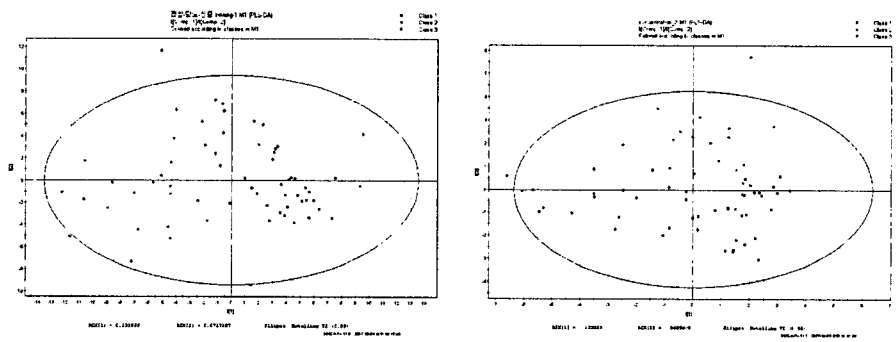

Figure 3. PLS-DA results of (A) targeted profiling and (B) spectral binning data obtained from ^{1}H NMR spectra from serum sample with normal human, diabetes, and nephropathy.

Results produced from metabolic profiling provide essential insights into metabolic pathways and processes. When used appropriately, metabolic profiling is a robust and informative technique for metabolomics research that could lead to effective biomarker identification.

Conclusion

A metabolomic method based on NMR and a multivariate statistical technique has been used to discover biomarkers for disease or drug response. These studies demonstrated that NMR based metabolomic approach is capable of identifying the metabolites that are important for the discrimination of classes of individuals of similar physiological conditions, and facilitating the search for metabolic markers under certain conditions. The field of metabonomics is gaining increasing interest across all disciplines, including functional genomics, integrative and systems biology, and surrogate biomarker discovery for drug discovery and therapy monitoring.

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