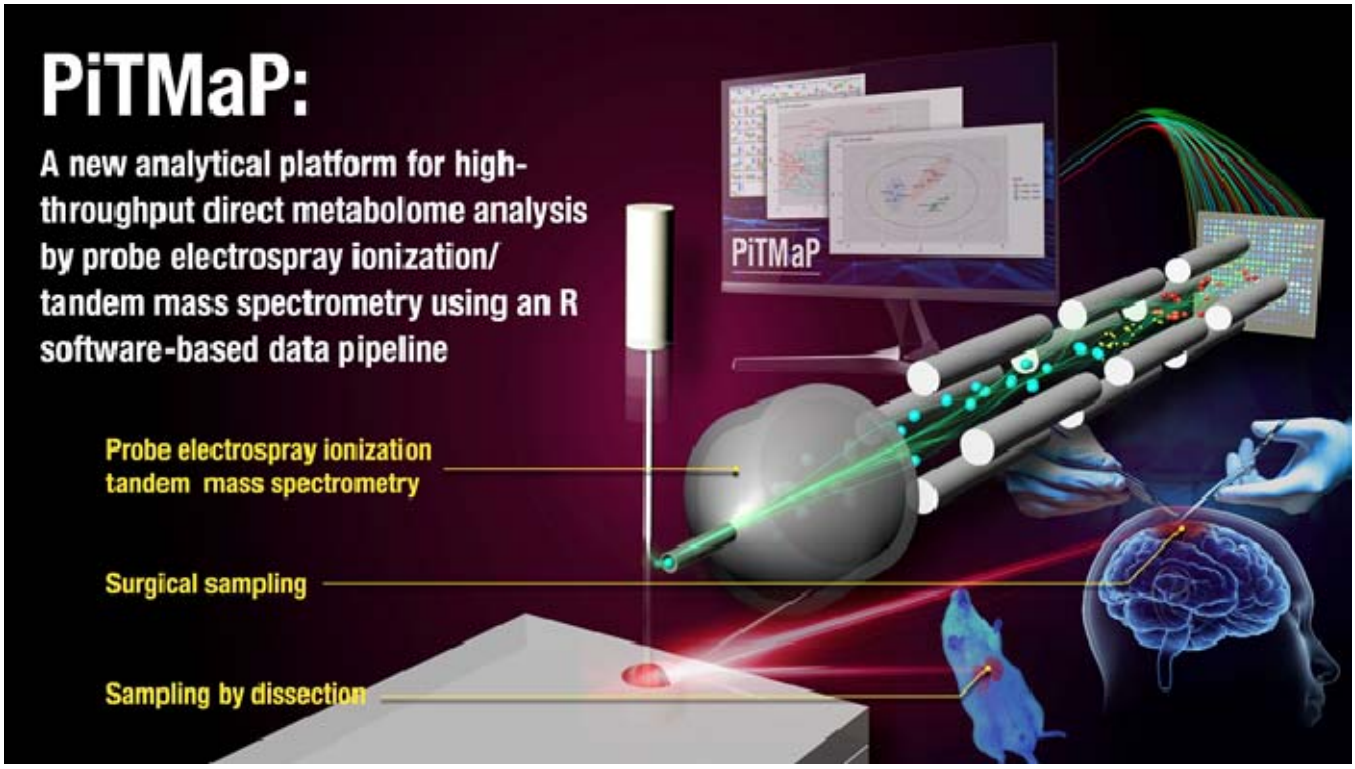
News Release



Title

PiTMaP: A new analytical platform for high-throughput direct metabolome analysis by probe electrospray ionization/tandem mass spectrometry using an R software-based data pipeline

Key Points

- ✓ A new analytical platform called PiTMaP was developed for high-throughput direct metabolome analysis by probe electrospray ionization/tandem mass spectrometry (PESI/MS/MS) using an R software-based data pipeline.
- ✓ PiTMaP was able to monitor 72 metabolites within 2.4 min per sample without tedious sample preparation, and the post-hoc data analysis was completed in ca. 1 min.
- ✓ PiTMaP was applied to APAP-induced liver injury model mice and human meningioma samples, demonstrating its convenience and feasibility. PiTMaP will thus become a next-generation universal platform to perform rapid metabolic profiling of biological samples.

Summary

The research group of Assoc. Prof. Kei Zaitsu (In Vivo Real-Time Omics Laboratory, Nagoya University), Assist. Prof. Seiichiro Eguchi (Tokyo Women's Medical University), and Senior Researcher Dr. Akira Iguchi (National Institute of Advanced Industrial Science and Technology (AIST)) have succeeded in developing a new analytical platform for high-throughput direct metabolome analysis by probe electrospray ionization/tandem mass spectrometry using an R software-based data pipeline, called PiTMaP.

PiTMaP was able to directly monitor 72 metabolites in tissue samples within 2.4 min per sample without tedious sample preparation, and it can i) automatically generate box-and-whisker plots for all metabolites, ii)

perform multivariate analyses such as principal component analysis (PCA) and projections to latent structures-discriminant analysis (PLS-DA), iii) generate score and loading plots of PCA and PLS-DA, iv) calculate variable importance of projection (VIP) values, v) determine a statistical family by VIP value criterion, vi) perform tests of significance with the false discovery rate (FDR) correction method, and vii) draw box-and-whisker plots only for significantly changed metabolites. These tasks could be completed within ca. 1 min. Finally, PiTMaP was applied to two cases: 1) an acetaminophen (APAP)-induced acute liver injury model and control mice and 2) human meningioma samples with different grades (G1–G3), demonstrating the feasibility of PiTMaP. PiTMaP will thus become a next-generation universal platform to perform rapid metabolic profiling of biological samples.

Our study has been published in *Analytical Chemistry* online on 25 May, 2020.

Research Background

Ambient ionization mass spectrometry (AIMS)-based techniques enable us to perform high-throughput and direct analysis of analytes not only in environmental samples but also in biological specimens. To date, our group has demonstrated that the combinational use of probe electrospray ionization (PESI), which was invented by Hiraoka et al., and tandem mass spectrometry (MS/MS) allows the direct profiling of endogenous metabolites in mouse liver and brain tissues without tedious sample preparation. However, only 26 metabolites can be monitored in our previous method, and thus, there is a need to increase target analytes to perform metabolome analysis by PESI/MS/MS. In addition, conducting a post-hoc data analysis such as multivariate analysis and tests of significance is time-consuming, even when the data acquisition is rapidly completed by PESI/MS/MS. Thus, the development of a high-throughput analytical platform for metabolomics requires a data pipeline.

Research Results

In this study, we developed a new platform for high-throughput direct metabolome analysis by PESI/MS/MS using an R software-based data pipeline, called PiTMaP. The number of targeted metabolites that can be simultaneously monitored was expanded using a scheduled-selected reaction monitoring (SRM) method, which is generally used in liquid chromatography tandem mass spectrometry. The method was able to directly profile 72 metabolites that are mainly related to central energy metabolic processes, such as glycolysis, tricarboxylic acid (TCA) cycle, pentose–phosphate pathway (PPP), β-oxidation pathway of fatty acids, and methionine pathway in tissue samples in 2.4 min per run. PiTMaP was also able to perform post-hoc data analysis, such as multivariate analyses, tests of significance considering the false discovery rate (FDR) correction. In addition, an algorithm that can select metabolites by the variable importance in projection (VIP) values was incorporated, allowing the objective determination of a statistical family. These tasks could be completed within ca. 1 min. Finally, we applied PiTMaP to an acetaminophen (APAP)-induced acute liver injury model and control mice or to human meningioma samples in order to confirm the feasibility of PiTMaP.

As shown in Figure 1, the APAP model and the control groups were clearly separated along with the first component axis in the PLS-DA score plots, suggesting that their metabolic profiles are apparently different. Also, PiTMaP demonstrated that 29 metabolites were significantly altered on the basis of the Welch’s t-test

with FDR correction as shown in Figure 2. In the significantly changed metabolites, reduced-form glutathione (GSH), taurine and some intermediates of the TCA cycle were significantly altered. It is well known that APAP is metabolized to an active metabolite, *N*-acetyl-4-benzoquinone imine (NAPQI), which is generally detoxicated by conjugation with GSH though large amounts of NAPQI, produced by an APAP overdose, cause acute liver injury. Also, the metabolites related to energy metabolism were significantly altered in the APAP-induced acute liver injury model mice, demonstrating that the energy metabolism was strongly disrupted in the APAP-induced acute injury. Consequently, PiTMaP was able to successfully capture the energy metabolism disruption via mitochondrial dysfunction and alteration of the antioxidative stress mechanism involving GSH.

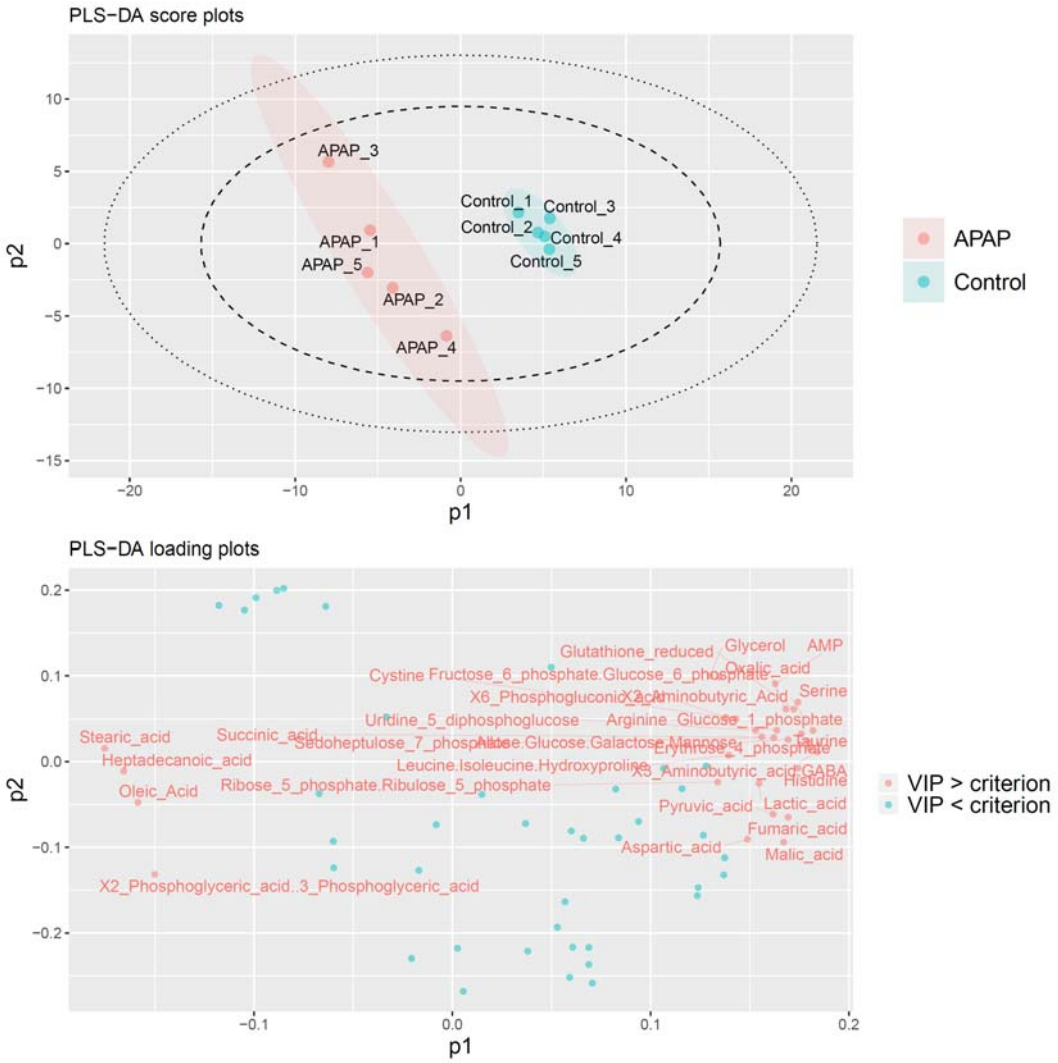


Figure 1 PLS-DA score and loading plots for the control and APAP-induced liver injury model mice with a VIP criterion of 1.0. Red: APAP model mice and blue green: control mice in PLS-DA score plots.

Figure 2 Box-and-whisker plots for significantly altered metabolites for control and APAP-induced liver injury model mice.

Next, we applied PiTMaP to human meningioma samples with different malignancy-grades (G1-G3). As shown in Figure 3, three groups were separated in PLS-DA score plots. In particular, the G1 and G2 groups were separated along with the first component axis, and the G3 group was separated from the G1 and G2 groups along with the second component axis, suggesting that the malignancy development of meningioma may not be metabolically continuous. As shown in Figure 4, PiTMaP successfully determined the significantly altered metabolites between G1 and G2, and between G1 and G3, though there was no significant difference between G2 and G3. Lactic acid was significantly increased in the cancerous G3 group mainly because of the upregulation of the anaerobic respiration. Also, stearic acid significantly decreased in the G2 and G3 groups compared to that in G1 though the reason why the stearic acid level was low in meningioma was not elucidated. Taurine was significantly increased in G3 in this study, probably because of the protection against tumor or cellular proliferation. PiTMaP could capture specific metabolic changes in human meningioma, demonstrating its feasibility.

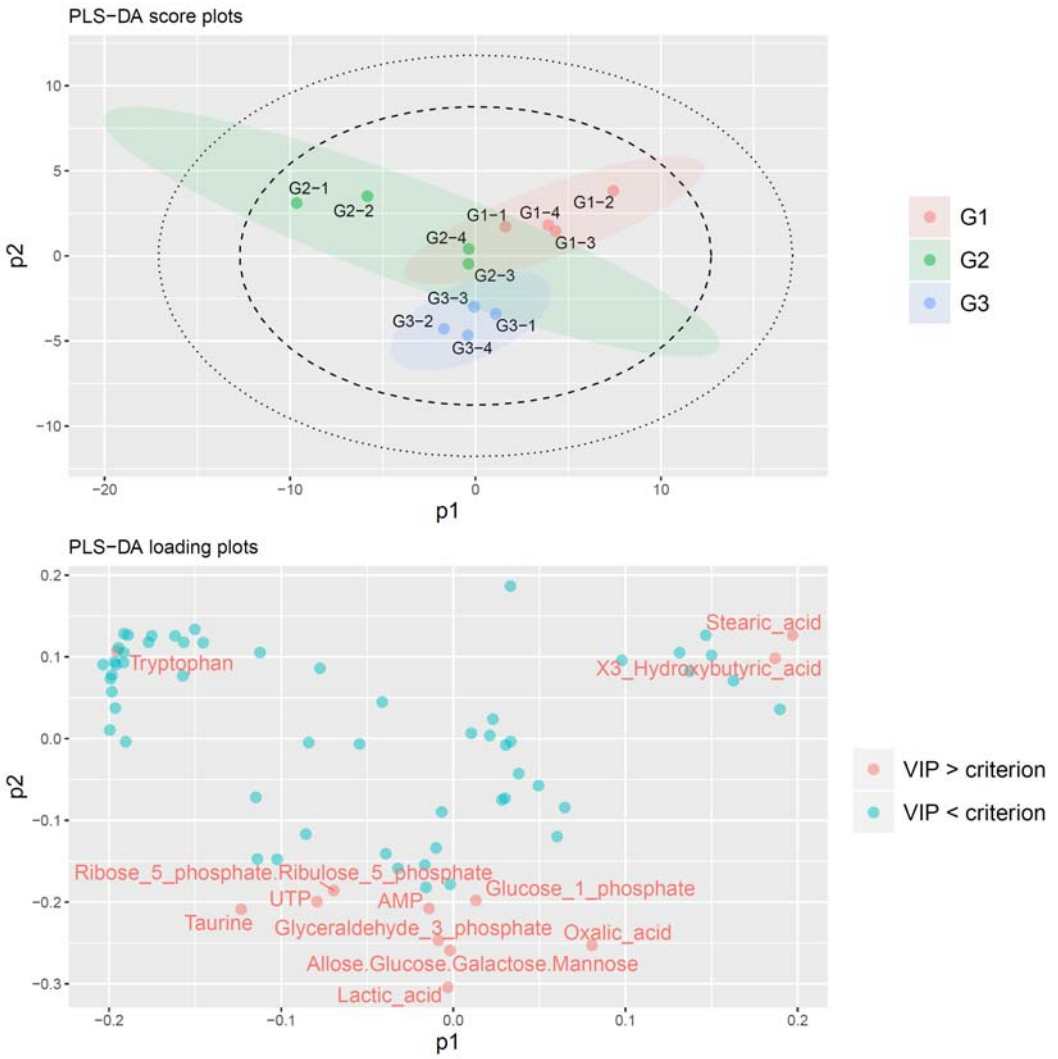


Figure 3 PLS-DA score and loading plots for human meningioma samples with a VIP criterion of 1.2. Red: G1, green: G2, and blue: G3 in PLS-DA score plots.

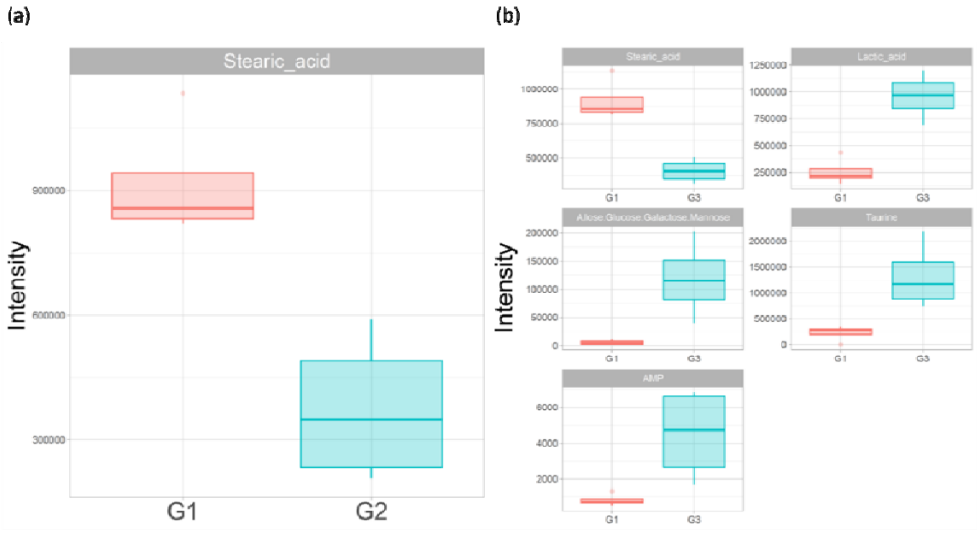


Figure 4 Box-and-whisker plots for significantly altered metabolites. (a) G1 vs G2 (red: G1 and blue green: G2), and (b) G1 vs G3 (red: G1 and blue green: G3).

Research Summary and Future Perspective

In this study, we developed a new analytical platform, PiTMaP, to perform direct metabolic profiling of tissue samples such as mouse liver and human brain samples. PiTMaP was able to monitor 72 metabolites within 2.4 min per sample without tedious sample preparation, and the post-hoc data analysis was completed in ca. 1 min. Finally, PiTMaP was applied to APAP-induced liver injury model mice and human meningioma samples, demonstrating its convenience and feasibility. PiTMaP will thus become a next-generation universal platform to perform rapid metabolic profiling of biological samples.

In future work, more target metabolites will be implemented to the method. In particular, secondary metabolites will be added to expand the method not only to plant metabolome but also to an exposome analysis. In addition, a graphical user interface (GUI) will be formed to manage the R software behind the GUI, which would facilitate the performance of metabolic profiling.

Publication

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