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Metabolic Changes in Lung Tissue of Tuberculosis-Infected Mice Using GC/Q-TOF

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Introduction

The global burden of tuberculosis (TB) is vast, with an estimated 9.6 million new TB cases and 1.5 million deaths due to the disease in 2014 alone [1]. Using metabolomics, new TB biomarkers can be identified to make progress in our understanding of the disease. In this study, we used a mouse model of Mtb infection to determine the metabolic profile of uninfected and infected lung tissues.

To identify new pathophysiological pathways involved in infection as well as biomarkers of TB, the untargeted metabolomics study was performed using uninfected and infected lung at 9 weeks following infection. After initial compound annotation, low-energy EI data were used to confirm the molecular ions and identify molecular formulas of putatively identified compounds and unknowns, respectively.

Experimental

Mice were infected with 5x104 CFU of Mycobacterium tuberculosis (Mtb) H37Rv via the intratracheal route. The dried extracts of lung tissue were derivatized by O-methoximation followed by trimethylsilylation. GC/MS analysis was performed using an Agilent 7890B GC system coupled to a novel high resolution 7250 GC/Q-TOF, equipped with EI source allowing low-energy ionization (Figure 1). In addition to the new low-energy EI source, the 7250 system is capable of higher resolving power (25,000 at m/z 272) and improved mass accuracy, as compared to 7200 GC/Q-TOF due to longer (1.5 m) flight tube and higher dynamic range while maintaining high resolving power. Instrument parameters are shown in Table 1.

Locked (RTL) Fiehn method was used to facilitate compound identification when using Fiehn.L RI library for initial compound identification. In addition, NIST.L as well as an accurate mass Metabolomics PCDL were also used to identify additional hits. Feature detection was performed using SureMass signal processing in Agilent MassHunter Unknowns Analysis B.08.00. Statistical analysis was performed in Mass Profiler Professional (MPP) version 13.0. Pathway Architect, an extension tool for MPP, was used to identify biochemical pathways associated with TB infection.

Experimental

Figure 1. Agilent 7250 GC/Q-TOF

GC and MS Conditions:	
Column	DB-5MS, 30 m, 0.25 mm, 0.25 µm, DuraGuard, 10m
Injection volume	1 µL
Split ratio	10:1
Split/Splitless inlet temperature	250 °C
Oven temperature program	60 °C for 1 min 10 °C/min to 325 °C, 9.5 min hold
Carrier gas	Helium at 1 mL/min constant flow
Transfer line temperature	290 °C
Ionization mode	Standard EI at 70 eV; low electron energy EI at 17 eV, 15 eV and 12 eV
Source temperature	200°C
Quadrupole temperature	150°C
Mass range	50 to 950 m/z
Spectral acquisition rate	5 Hz

Table 1. GC/Q-TOF conditions

Results and Discussion

Experimental setup and feature detection

To identify new pathophysiological pathways involved in infection as well as biomarkers of TB, an untargeted metabolomics study was performed using uninfected and infected lung tissue extracts at 9 weeks following infection.

Following feature detection and library search performed in the Unknowns Analysis (Figure 2), the results were exported as .CEF files for further processing in MPP.

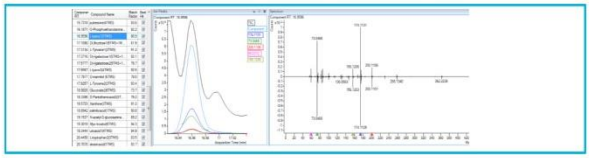


Figure 2. Feature detection and library search performed in Unknowns Analysis (using PCDL as an example)

Differential Analysis

In MPP, Principal Component Analysis (PCA) was utilized to evaluate clustering of the data. Distinct clusters, that represent clear separation between uninfected control and infected tissues were formed (Figure 3).

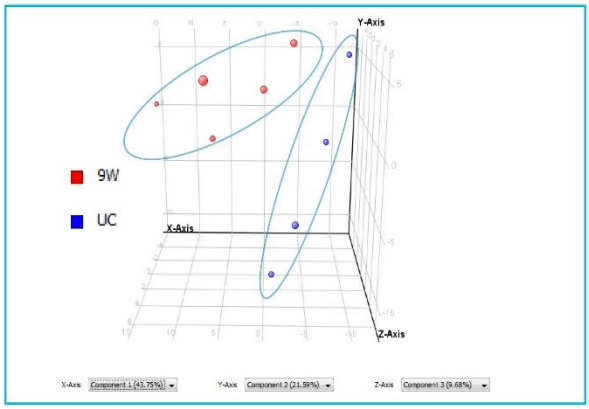


Figure 3. PCA plot. Distinct clusters from uninfected control (UC, blue circles) and 9 weeks following the infection (9W, red circles) lung tissues were observed.

Differential Analysis

Significant changes in the metabolome of lung tissue between infected and uninfected mice were further evaluated in MPP using Fold Change Analysis (Figure 4) as well as Heatmap (Figure 5). Alteration in profiles of many metabolites, in particular, amino acids and nucleobases have been observed. In addition, a change in the profiles of itaconic acid and kynurenine, have also been detected.

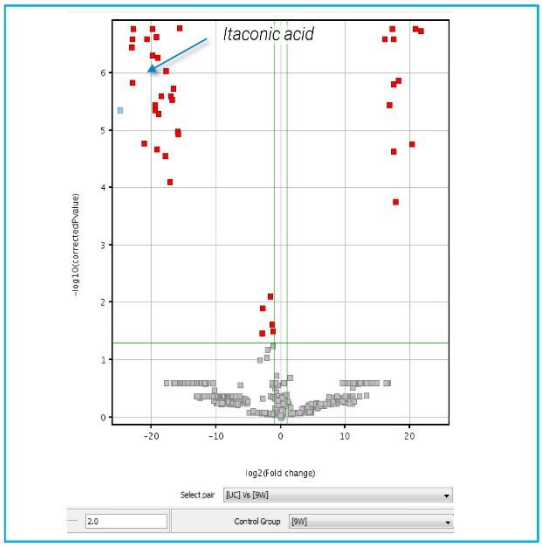


Figure 4. Volcano plot visualizing of log of fold change vs. log of p-Value for uninfected lung tissue vs. 9 weeks following TB infection.

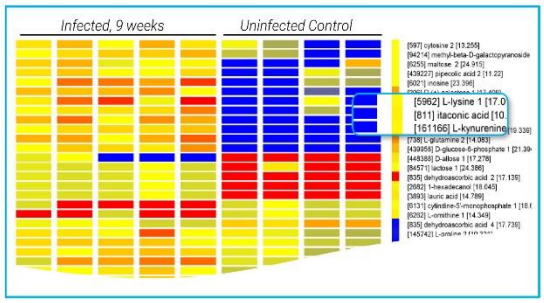


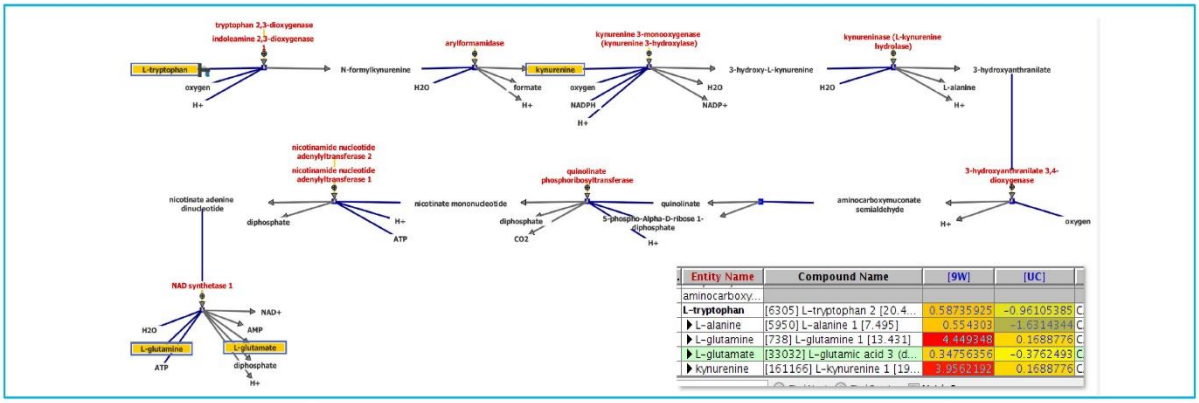
Figure 5. Heat map that highlights differentially regulated metabolites between uninfected and infected lung tissues

In addition, Pathway Architect was used to identify the biochemical pathways potentially associated with TB infection. Pathways of purine and pyrimidine metabolism as well as NAD biosynthesis II were among most significant. One of the examples is shown on Figure 6.

Unknowns identification and confirmation of tentative hits

After initial compound annotation and differential analysis in MPP, low-energy EI spectra were used to confirm the molecular ions and identify molecular formulas of putatively identified differential compounds and unknowns, respectively (Figure 7).

Results and Discussion



Entity Name	Compound Name	[9W]	[UC]
aminocarboxy...			
L-tryptophan	[6305] L-tryptophan 2 [20.4...	0.58735925	-0.96105385 C
L-alanine	[5950] L-alanine 1 [7.495]	0.554303	-1.6314344 C
L-glutamine	[738] L-glutamine 1 [13.431]	4.449348	0.1688776 C
L-glutamate	[33032] L-glutamic acid 3 (d...	0.34756356	-0.3762493 C
kynurenine	[161166] L-kynurenine 1 [19...	3.9562192	0.1688776 C

Figure 6. Example of Pathway Analysis Results: NAD Biosynthesis II

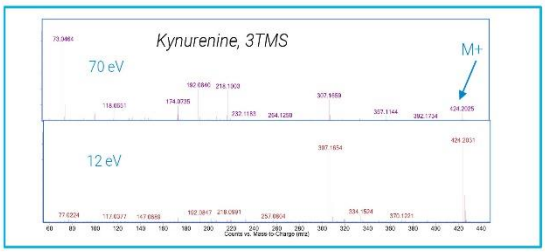


Figure 7. Confirmation of the molecular ion using low electron energy.

The first step in attempt to elucidate structure of unknowns was to use low electron energy to help identify molecular ion. MS/MS was further obtained at optimal electron energy to maximize the absolute abundance of M^+ use as a precursor. Unknown compound MS/MS spectra were then extracted using Find by Targeted MS/MS algorithm in Qual. The results were evaluated in Molecular Structure Correlator (MSC) (Figure 8).

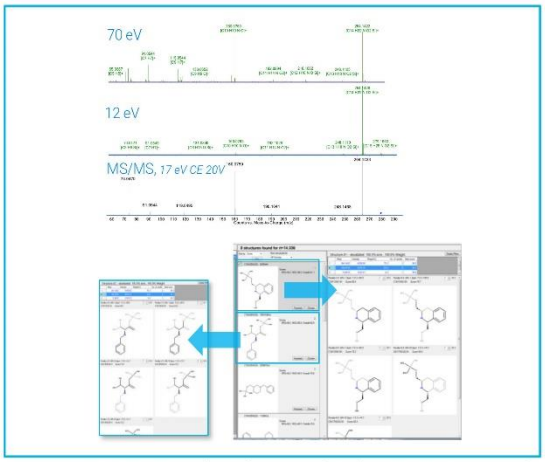


Figure 8. Structure elucidation of unknown compound using low electron energy and MS/MS with Molecular Structure Correlator (MSC). Shown are possible structures of an unknown compound.

Conclusions

The untargeted metabolomics study demonstrated an alteration in amino acids profile, as well as a change in kynurenine and itaconic acid profiles. Interestingly, itaconic acid is not generally classified as a mammalian metabolite, however, it has recently been shown to likely play a role in macrophage-based immune response [2].

Reference

[1] World Health Organization (http://www.who.int/mediacentre/factsheets/fs104/en/)

[2] Cheryl L. Strelko, Wenyun Lu, et al. J. Am. Chem. Soc., 2011, 133 (41), 16386–16389