Metabolomics and Lipidomics Approaches for Biomedical Research and Biomarker Discovery

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Waters Users Meeting ASMS 30 May, 2015
METABOLOMICS AND LIPIDOMICS

[APPLICATION NOTEBOOK]

DOWNLOAD THE APPLICATION NOTEBOOK

Waters

THE SCIENCE OF WHAT’S POSSIBLE.
**Metabolomics Approaches**

**Untargeted**
Scanning for differences using high-res instruments (semi-quantitative; statistics-based; hypothesis-generating)

**Structural Elucidation**
High-res instruments with fragmentation capabilities (chemical structure)

**Targeted**
Monitoring selected ions in high-res or nominal mass (quantitative; sensitive; requires internal standards)

**In situ; Imaging**
Scanning along x and y axes using high-res instruments (spatial information; molecular histology)
Metabolomics and Lipidomics

Separate (Chromatography; Ion Mobility)

Measure (Mass Spectrometry)

Process and Mine (Informatics and Statistics)
Typical Metabolomics Applications

WT/KO cells
- Add mixture of internal standards
- Bottom layer (organic phase)
- Lipidomics
- Database Search
- Pathway Analysis/Data interpretation

Gene function
- PNAS, 2015

Transgenic mice
- Prepare plasma and add internal standards
- Vortex
- Centrifuge
- Elute
- LC/TOF MS
- Global Metabolic Profiling
- Untargeted (screening)
- Targeted (monitoring)

Diet
- PLOS ONE, 2014

Treated mice
- Prepare plasma and add internal standards
- Vortex
- Centrifuge
- Elute
- LC/TOF MS
- Global Metabolic Profiling
- Untargeted (screening)
- Targeted (monitoring)

Environmental exposure
- J Proteome R, 2014

Human subjects
- Add mixture of internal standards
- Centrifuge
- Elute
- LC/TOF MS
- Global Metabolic Profiling
- Untargeted (screening)
- Targeted (monitoring)

Disease
- In Preparation

PNAS, 2015
PLOS ONE, 2014
J Proteome R, 2014
In Preparation
Metabolic Phenotyping Reveals a Lipid Mediator Response to Ionizing Radiation

Evagelia C. Laiakis,† Katrin Strassburg,‡,§ Ralf Bogumil,‖ Steven Lai,⊥ Rob J. Vreeken,‡,§ Thomas Hankemeier,‡,§ James Langridge,⊥ Robert S. Plumb,⊥# Albert J. Fornace, Jr.,† and Giuseppe Astarita‡,⊥,

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#Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London SW7 2AZ, United Kingdom

Poster 202 – Metabolomics: Untargeted Metabolite Profiling II
A mass-spectrometry-based metabolic phenotyping strategy to investigate the molecular response to ionizing radiation
Radiation Exposure

- Occupational exposure (medicine, manufacturing and construction)
- Medicine (research, diagnosis and therapy)
- Airline/Space Travel
Nuclear and Radioactive Accidents

Hiroshima    Nagasaki

Others:

Three Mile Island, US
Goiania, Brazil
Kozloduy, Bulgaria

Chernobyl

Fukushima Daiichi
Health Effects of Radiation Exposure

- Cancer
- Cardiovascular Disease
- Cognitive decline
Molecular Effects of Radiation Exposure

DNA

Proteins

Lipids/Metabolites
Aims of the study

1. Investigate the **biochemical mechanisms** underlying radiation exposure

2. Determine **biomarkers or biosignatures** associated with radiation exposure

Laiakis E. et al, J Proteome Res. 2014 Sep
Study Design

Sham Control + Irradiated

Blood collection

Serum preparation

LC/TOF MS
Global Metabolic Profiling

Biocrates kit
Targeted Metabolic Profiling

SPE clean up
Oxylipin Profiling

Liquid-liquid

LC/TOF MS
Global Metabolic Profiling

LC tandem MS
Targeted Metabolic Profiling

Endogenous metabolite

Internal standard

Cryostat sectioning

MS Imaging

Biocrates kit

Laiakis E. et al, J Proteome Res. 2014 Sep
Metabolomics Approaches

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Metabolomics: UPLC Separation

**Negative Ion**

Basic Conditions

1. Succinic Acid
2. Glyceraldehyde
3. FAD
4. Folic Acid
5. ADP-Glucose
6. UDP-Glucose
7. Fructose-6-Phosphate
8. Glucose-6-Phosphate
9. ATP
10. Sucrose
11. Lactose
12. Raffinose

**Positive Ion**

Acidic Conditions

1. 5-MTA
2. Hypoxanthine
3. Adenine
4. Adenosine
5. Xanthine
6. Phenylalanine
7. Inosine
8. Xanthosine
9. Taurine
10. Hydroxyproline
11. SAH
12. Cysteine

Lipidomics: UPLC Separation

Positive Ion

lysophospholipids

22:5 omega-3

13C 22:6 omega-3

22:5 omega-6

PC, SM, PG, PE

ChoE & TG

SM, DG, ChoE

Orthogonal Coordinates

PC, SM, PG, PE

lysophospholipids

ChoE & TG

Ion mobility Separation

**Poster 649 – Ion Mobility: Small Molecule and Metabolomics**

**The analysis of Bile Acids: Enhancement of specificity using an Ion Mobility-TOFMS based approach**
Data Processing and Mining

1. Individual ion maps

2. Data alignment and peak picking

3. ANOVA filtering and Multivariate Statistics

4. Filtering by ANOVA P value

5. Tentative Identifications

Data Processing and Mining

Laiakis E. et al, J Proteome Res. 2014 Sep
Database Search using Orthogonal Coordinates

Database Search using Orthogonal Coordinates

MetaScope search parameters

Define a set of MetaScope parameters that can be saved for later reuse. Learn more in the online reference.

Name:

Compounds database

Data format: Auto-detect

Search parameters

- Mass within: 5 ppm
- Retention time within: 1 minutes
- CCS within: 20 %

Additional compound properties source

- Read additional compound properties from this file

Fragment search method

- Do not use fragmentation data
- Perform theoretical fragmentation
- Relative mass error: 10 ppm
- Perform fragment database search

Save search parameters Cancel

Graph showing ppm m/z error vs. identifications for HMDB and in-house databases with and without RT.

Table showing possible identifications with their scores, mass errors, and fragmentation scores.
Database Search using Orthogonal Coordinates

MetaScope search parameters

- Name:
- Compound database: [Browse...]
- Data format: Auto-detect
- Search parameters:
  - Mass within: 5 ppm
  - Retention time within: 1 minutes
  - CCS within: 20 %
- Additional compound properties source:
  - Read additional compound properties from this file
  - [Check box]
- Fragment search method:
  - [Do not use fragmentation data]
  - Perform theoretical fragmentation: [Check box]
  - Relative mass error: 10 ppm
  - Perform fragment database search: [Check box]
- Save search parameters: [Button]
  - [Cancel]

HDMS<sup>E</sup>

Co-eluting ions
With ion mobility separation
Fragmentation
Fragments Transferred to TOF-MS

303.2324 (FA 20:4)

HDMS<sup>E</sup> Low Energy

303.2324 (FA 20:4)

HDMS<sup>E</sup> High Energy

<25 fragments
## Global Metabolic Profiling

<table>
<thead>
<tr>
<th>m/z</th>
<th>Tentative ID</th>
<th>ANOVA (p value)</th>
<th>Max Fold Change</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>818.6050</td>
<td>PC(P-18:0/22:6)</td>
<td>5.12E-06</td>
<td>8.0</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>764.5571</td>
<td>PC(P-16:0/20:5)</td>
<td>1.02E-05</td>
<td>11.3</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>804.5529</td>
<td>PC(P-16:0/20:4)</td>
<td>2.11E-05</td>
<td>9.8</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>794.6038</td>
<td>PC(P-18:0/20:4)</td>
<td>2.97E-05</td>
<td>5.7</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>792.5873</td>
<td>PC(P-18:0/20:5)</td>
<td>3.38E-05</td>
<td>8.1</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>790.5728</td>
<td>PC(P-16:0/22:6)</td>
<td>6.74E-05</td>
<td>5.9</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>703.5747</td>
<td>SM(d18:1/16:0)</td>
<td>2.09E-04</td>
<td>12.5</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>162.1117</td>
<td>Carnitine</td>
<td>1.50E-02</td>
<td>1.7</td>
<td>HILIC</td>
</tr>
<tr>
<td>166.0861</td>
<td>Phenylalanine</td>
<td>1.53E-02</td>
<td>1.2</td>
<td>HILIC</td>
</tr>
</tbody>
</table>
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Phosphotidylcholine (PC)

\[ \text{sn-1} \quad \text{sn-2} \]

\[ \text{sn-1-H}_2\text{O} \quad \text{sn-2-H}_2\text{O} \]
Targeted Metabolic Profiling: Metabolomics kits

<table>
<thead>
<tr>
<th>Metabolite group</th>
<th>No. of metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids and Biogenic amines</td>
<td>40</td>
</tr>
<tr>
<td>Acylcarnitines</td>
<td>40</td>
</tr>
<tr>
<td>Lyso-phosphatidylcholines</td>
<td>14</td>
</tr>
<tr>
<td>Phosphatidylcholines</td>
<td>74</td>
</tr>
<tr>
<td>Sphingomyelins</td>
<td>14</td>
</tr>
<tr>
<td>Hexose</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
</tr>
</tbody>
</table>

**Poster 401 – Metabolomics: Quantitative Analysis**
**Improved performance of targeted metabolome analysis with Waters Xevo® TQ-S and Xevo® TQ-S micro instruments**
Targeted Metabolic Profiling

Diacyl PCs

Ether PCs

Laiakis E. et al, J Proteome Res. 2014 Sep
Data Integration:
Molecular Effects of Radiation

Plasmalogens ➔ Oxidation ➔ Aldehydes

Diacyl PC ➔ PLA₂ ➔ Arachidonic acid ➔ ?

Metabolites
Eicosanoids: Bioactive Oxygenated PUFAs

Omega-6 metabolites: pro-inflammatory

Omega-3 metabolites: anti-inflammatory
Multiplexed Assay for Eicosanoids Profiling

1. Plasma sample
2. Add internal standard mix
3. Load
4. SPE clean up
5. Inject into UPLC/MS-MS

Selected Lipid

Internal Standard
Eicosanoids Profiling

Omega-6s

Omega-3s

Laiakis E. et al, J Proteome Res. 2014 Sep
Pathway Analysis

**Omega-6 metabolites:** pro-inflammatory

**Omega-3 metabolites:** anti-inflammatory
Data Integration: Molecular Effects of Radiation

Diacyl PC

\[ \downarrow \text{PLA}_2 \]

Arachidonic acid

\[ \downarrow ? \]

Metabolites

8-HETE
Data Fusion: Biosignature of Radiation Exposure

Top metabolites correlated with the irradiated phenotype
**In vivo responses to total body irradiation (TBI) in patients**

TBI received prior to hematopoietic stem cell transplant (n=15)

- Total Body Irradiation
- Serum Pre, 6hr, 24hr
- SPE clean up
- Xevo TQ-S
- Targeted Metabolomics

**Untargeted Metabolomics**

- Synapt G2 Si
- Untargeted Metabolomics
- Untargeted Lipidomics

**Targeted Metabolomics**

- TargetLynx for quantification
- Statistical Analysis and pathway assignment

**Statistical Analysis**

- Progenesis QI
- Pathway annotation
  - HMDB, KEGG, Lipidmaps

**Peak Picking**

- Validation through tandem MS or fragment matching through online databases

*Slide from Evagelia C. Laiakis*
Preliminary Results in Human Subjects

Slide from Evagelia C. Laiakis
Conclusions

Integration of multi-platform lipidomics data highlighted new biochemical pathways associated with radiation exposure.

The untargeted lipidomics approach uncovered a differential metabolism for diacyl phospholipids and plasmalogen.

The multiplexed assay identified marked alterations in a subset of pro-inflammatory lipid mediators.

Fusion of multi-platform data highlighted a metabolomics biosignature associated with exposure to radiation exposure.
Methodological References: Untargeted

A Facile Database Search Engine for Metabolite Identification and Biomarker Discovery in Metabolomics
Panagiotis Arapitsas,1 James Langridge,2 Fulvio Mattivi,1 Giuseppe Astarita2
1Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all’Adige, Italy
2Waters Corporation, Milford, MA, USA

Development of a Metabolomic Assay for the Analysis of Polar Metabolites Using HILIC UPLC/QTof MS
Giuseppe Paglia,1 James Langridge,2 and Giuseppe Astarita3
Center for Systems Biology, University of Iceland, Iceland; 2,3 Waters Corporation, Manchester, UK and Milford, MA, USA

Lipid Separation using UPLC with Charged Surface Hybrid Technology
Giorgis Isaac, Stephen McDonald, and Giuseppe Astarita
Waters Corporation, Milford, MA, USA
Methodological References: Targeted

Targeted Lipidomics of Oxylipins (Oxygenated Fatty Acids)
Katrin Strassburg,1,2 Billy Joe Molloy,5 Claude Mallet,3 André Duesterloh,4 Igor Bendik,4 Thomas Hankemeier,1,2 James Langridge,5 Rob J. Vreeken,1,2 Giuseppe Astarita3

Targeted Lipidomics Using the ionKey/MS System
Giuseppe Astarita,1 Angela Doneanu,1 Jay Johnson,1 Jim Murphy,1 James Langridge2
1 Waters Corporation, Milford, MA, USA

Targeted Metabolomics Using the UPLC/MS-based Absolute IDQ p180 Kit
Evagelia C. Laiakis,1 Ralf Bogumil,2 Cornelia Roehring,2 Michael Daxboeck,2 Steven Lai,3 Marc Breit,2 John Shockcor,3 Steven Cohen,3 James Langridge4 Albert J. Fornace, Jr.1 and Giuseppe Astarita1,3
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