Characterization of metabolic profiles and biomarker identification in biofluids, tissues and cells through NMR spectroscopy

Debora Paris - ICB days 22-23 October 2014
Metabolomic and biomarkers

**Metabolome**: the quantitative complement of all the low molecular weight molecules present in cells in a particular physiological or developmental state.

**Metabolomics**: “...the complete set of metabolites/low-molecular-weight intermediates, which are context dependent, varying according to the physiology, developmental or pathological state of the cell, tissue, organ or organism…” Oliver 2002

**Searching for biomarkers**: searching for a substance which could be potentially used as biological indicator; it must be characteristic, objectively measurable and statistically robust, to evaluate physiological process, pathological state, or to monitor drugs response and therapeutical interventions.
the “omic” place in the pyramid of life

- Genomics
- Proteomics
- Metabolomics

Environmental Influence
Physiological Influence

increasing number!!

30,000 Genes
3,000 Enzymes
1,400 Chemicals

Increasing number!!
NMR based metabolomic and MVA approach

- Minimal sample preparation;
- High throughput analysis;
- Inexpensive per-sample cost;
- Robust, semi-quantitative (fully?);
- Non-destructive analysis;
- Unbiased identification of $^1$H-containing metabolites;
- 2-D NMR methods for identification;
- Ideal for screening samples, followed by more sensitive MS analysis.

- Extracting information from data by using all the variables simultaneously;
- Convert large data set in graphical representation easier to interpret;
- Overview (Trends, QC, Patient monitoring);
- Classification (Diagnostic, Toxicity, disease progression);
- Discrimination (biomarkers candidates, comparing studies or instrumentation);
- Regression (Comparing blocks of omic data).

Enormous possibility for metabolic biomarker discovery and mechanistic action.
Application to airway diseases: Exhaled breath condensate analysis

EBC samples (3-4 ml)

Low metabolites concentration!
- water vapour
- volatile and non-volatile substances from lower airways

Main issue: to assess non-invasive and reproducible method
- Methodological aspects and standardization: limit detection, contaminants (saliva..), cleaning mediums..
- Characterize metabolic profiles of different airway diseases with common symptoms;
- Monitor treatment response and progression through EBC evolution and classification.

References:

  Sofia, M., Maniscalco, M., de Laurentiis, G., Paris, D., Melck, D., and Motta, A. NMR-based metabonomics of exhaled breath condensate as a potential tool to explore airway diseases.
NMR spectroscopy metabolomic profiling of exhaled breath condensate in patients with stable and unstable cystic fibrosis

- **Cystic fibrosis** (CF or mucoviscidosis) is a genetic disorder affecting most critically the lungs;
- It is characterized by abnormal transport of chloride and sodium across an epithelium, leading to thick, viscous secretions;
- Patient systematically undergo chronic bacterical infections in the altered mucus which form biofilm difficult to immune cells and anthropiotic to penetrate;
- Viscos secretions and persistent respiratory infections repeatedly damage the lung making the infections even more difficult to eradicate;

Does nuclear magnetic resonance (NMR) spectroscopy of exhaled breath condensate (EBC) discriminate between patients with unstable cystic fibrosis (CF), stable CF and healthy controls, and are selected metabolites responsible for between-group differences?

Unstable CF
- Higher systemic inflammation;
- Reduced FEV1 values;
- Higher white blood cells count;
Stable vs. Unstable Cystic Fibrosis

Control vs. Cystic Fibrosis

A

PLS-DA scores plot of filtered data

R2 = 0.84
Q2 = 0.79

B

PLS-DA 3D scores plot

R2 = 0.82
Q2 = 0.78

A

HS

Regression coefficients

B

sCF

Regression coefficients

uCF
Conclusions

• NMR spectroscopy of EBC is able to discriminate patients with CF from healthy subjects through PLS-DA on OSC filtered spectroscopic data (Y=HS);

• Acetate shows higher concentration in HS, while CF patients are characterized by high levels of ethanol and 2-propanol;

• Furthermore, PLS-DA on OSC filtered data Y=SCF discriminates patients with unstable CF from those with stable CF, and identifies the metabolites responsible for between-group differences;

• Stable CF shows high levels of ethanol and methanol, while unstable CF are mostly characterized by higher levels of acetate and 2-propanol with respect to stable CF;

• Probably elevated ethanol concentrations (enzyme-mediated product of reduction of acetone) may be related to reduced capacity of Pseudomonas aeruginosa to oxidase ethanol to acetate;

• Elevated EBC 2-propanol levels might be due to bacterical metabolism and/or increased lipolysis and lipid peroxidation;

Reference:

NMR Spectroscopy Metabolic Profiling of Exhaled Breath Condensate in Patients with Stable and Unstable Cystic Fibrosis.
**NMR-based Metabolomics Discriminates Primary Ciliary Dyskinesia from Cystic Fibrosis**

- **Primary ciliary dyskinesia (PCD)** is a genetic (inherited) disorder of the structure and/or function of cilia in ears, sinuses and some other structures.
- The sweeping, wave-like motion of cilia is important for keeping these areas clean and free from infection.
- Without properly functioning cilia, people with PCD are unable to protect their respiratory system. Frequent infections of the lungs, ears, throat, and sinuses are common and can lead to serious and permanent damage.

- The motion of cilia is also implicated in *situs inversus* or *situs ambiguous*.
- Fertility can be impaired for immotile sperm tails in males and ineffective ciliary activity in the fallopian tubes in females.


Does nuclear magnetic resonance (NMR) spectroscopy of exhaled breath condensate (EBC) discriminate between patients with primary ciliary dyskinesia (PCD) and cystic fibrosis (CF) and are selected metabolites responsible for differences?
PCD
• CF

1H NMR spectra of EBC

R2=0.88
Q2=0.79

R2=0.85
Q2=0.75

methanol
ethanol
acetoin

methanol
acetoin
SFAs
Conclusions

- In ciliary axonemes, **low ethanol concentration** stimulates cilia beating, which is hampered at high concentration. Moreover, low levels of NO are associated in PCD patients due to absent/reduced expression/activity of nitric oxide synthase (eNOS), suggesting that ethanol accumulation is due to a specific pathway dysfunction.

- **Short-chain SFAs** regulate several leukocyte functions, including their ability to migrate to the inflammation foci and to destroy pathogens. Because SFAs modulate neutrophil production of proinflammatory mediators, their decreased concentration might be associated with a lack of inflammation suppression in PCD and CF.

- **Increased methanol** in PCD and CF may be associated with airway inflammation, being a breakdown product of formaldehyde, which is observed to exacerbate airways inflammation.

- **Acetoin**, discriminant in our CF-PCD model, is also involved in inflammatory processes. Its reduction in PCD might be associated with a different airway inflammation pattern and eventually due to patients’ different pharmacological treatments.

Reference:

*Nuclear Magnetic Resonance–based Metabolomics Discriminates Primary Ciliary Dyskinesia from Cystic Fibrosis.*
**In-vivo NMR metabolic profiling of *Fabrea salina*** reveals sequential defense mechanisms against UV radiation

*Fabrea salina* is a hypersaline ciliate unicellular protozoo, known to be among the strongest UV-resistant microorganisms, but the molecular mechanisms of such resistance are almost unknown. This extremophile has an important role in the **stability of ocean ecosystem**, playing a prominent role in aquatic food webs.

<table>
<thead>
<tr>
<th>Spectral band</th>
<th>Regime 1 (UV-B+UV-A+Vis) irradiance values [W/m²]</th>
<th>Regime 2 (Vis) irradiance values [W/m²]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV-A</td>
<td>9.85</td>
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<tr>
<td>UV-B</td>
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</tr>
<tr>
<td>UV-B/UV-A</td>
<td>1.64</td>
<td>0.00</td>
</tr>
</tbody>
</table>

We investigate changes in the metabolic profile of living *F. salina* cells exposed to visible light and to a polychromatic UV-B+UV-A+Vis radiation, for several exposure times.
Specific metabolites variation according to temporal UV response

- Detox agent;
- C and N source;
- Enzymes denaturation protectant;
- Cytoplasmatic acidity regulator

- Detox agent;
- Cell membrane protectant;
- Oxygen complex stabilizer.

- Proper hydration maintenance;
- Proteins stabilizer.
Conclusions

• Unsupervised pattern-recognition analysis has been employed to compare NMR metabolic profiles, discovering some metabolites whose concentration changes specifically upon UV-exposure and in a dose-dependent manner;
• Different batch of F. salina cultures showed the same trend, but for intra batch differences;
• Such a variation was interpreted in terms of a two-phase cell reaction, involving at least two different pathways:
  • early response consisting in degradation processes with alteration of formate, acetate and saturated fatty-acids metabolites;
  • late response activating osmoprotection mechanisms which modifies the activity of betaine moieties and other functionally related metabolites.
• Within the latter, alanine, proline and sugars suggest a possible incipient protein synthesis as defense and/or degeneration mechanisms.
• NMR spectroscopy on in-vivo cells represents an optimal approach to investigate the effect of UV-induced stress on the whole metabolome of F. salina, by minimizing the invasiveness of the measurements.

References:


Monitoring progressive liver alterations during hepatic tumorigenesis

- Human hepatocellular carcinoma (HCC) is one of the most common tumors worldwide and its incidence is steadily increasing;
- Liver is the most frequent site of metastatic colonization, and moreover hepatic metastases are far more common than primary cancers in Western countries.
- Small hepatic lesions (≤ 1.5 cm in diameter) are frequently difficult to detect and characterize, and diagnostic inaccuracy may lead to incorrect patient treatment.
- Most frequent differential diagnosis is HCC against intrahepatic cholangiocarcinoma or metastatic adenocarcinoma, and serological markers (such as α-fetoprotein) are diagnostic when markedly elevated.


It is possible to discriminate metabolic profiles of primary HCC, chronic hepatitis-C virus related cirrhotic tissues, hepatic metastases from colorectal carcinomas, and non-cirrhotic normal liver tissues adjacent to metastases as controls?
Lactate-glucose conversion rate improves according to tissues malignant progression.

HCC appear to be auxotrophic for Arg.

Enhanced glycolysis in tumors
- Solid malignant tumors are characterized by pronounced tissue hypoxia and enhanced formation of lactate…;
- …but many tumors exhibit a strong generation of lactate even in the presence of oxygen;
- An elevated lactate concentration in primary lesions at first diagnosis was related to an increased risk of metastases in squamous cell carcinomas of the uterine cervix, of the head and neck, and in rectum adenocarcinomas;
- Upregulated glycolysis could develop growth advantages that promote unconstrained proliferation and invasion.

References:

  *Basic amino acids and dimethylarginines targeted metabolomics discriminates primary hepatocarcinoma from hepatic colorectal metastases.*

  *Monitoring progressive liver alterations during hepatic tumorigenesis by NMR profiling and pattern recognition.*
...and more applications and methodological aspects

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Metabolomic group pathway

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