QUANTIFYING METABOLIC FLUXES IN CANCER AND STEM CELLS

An integrative approach monitoring metabolic reprogramming

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Kempa Lab - Integrative Proteomics & Metabolomics
Genome-regulatory code

Heterochromatin
inactive

Euchromatin
active

Metabolite A

Metabolite B

transcription

translation

processing

degradation

DNA

mRNA

K27
Me

K4
Me

K4
Me

K27
Me

K4
Me

K27
Me

Ace

Me

Me

Me

K27
Me

K4
Me

K27
Me

K27
Me

K4
Me

K4
Me

S

T

Y
Genome-regulatory code

Genomics
three billion DNA base pairs

Transcriptomics
2 % of the genome

Proteomics
20’000 - 25’000 protein-coding genes

Metabolomics
~ 5 000
Reprogramming of metabolism

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Reprogramming of metabolism

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Reprogramming of metabolism

OXPHOS
Phenotype
finite life-span

DIFFERENTIATED
CELL

REPROGRAMMING

CANCER
CELL
Glycolytic
Phenotype
infinite life-span

PLURIPOTENT
CELL
Glycolytic
Phenotype
infinite life-span

DIFFERENTIATION

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oncogenic regulation

carbon re-routing

hypoxia
Warburg effect

mitochondrial activity

nutrient availability

regulation CCM enzymes
Reprogramming of metabolism

OXPHOS Phenotype finite life-span

DIFFERENTIATED CELL

REPROGRAMMING

HYPOXIA

METABOLIC PATHWAYS

ONCOGENIC REGULATION

CARBON RE-ROUTING

MITOCHONDRIAL ACTIVITY

NUTRIENT AVAILABILITY

REGULATION CCM ENZYMES

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Quantitative information turns functional
Quantitative information turns functional

Proteomics

LC-MS/MS
label-free, SILAC quantification
~ 3500 proteins
Quantitative information turns functional

**Proteomics**

LC-MS/MS  
label-free, SILAC quantification  
~ 3500 proteins

**pulsed stable isotope resolved metabolomics (pSIRM)**

GC-MS approach  
identification  
~ 100 metabolites  
absolute quantification  
~ 40 metabolites
Quantitative information turns functional

**Proteomics**

LC-MS/MS
label-free, SILAC quantification
~ 3500 proteins

**pulsed stable isotope resolved metabolomics (pSIRM)**

GC-MS approach
identification
~ 100 metabolites
absolute quantification
~ 40 metabolites

application of stable isotopes
—> time-resolved
—> position-specific
—> indirect measurement of flux

Pietzke & Zasada et al. Cancer & Metabolism 2014, 2:9
Non-stationary metabolic flux analysis

in cooperation with Katharina Nöh and Sebastian Niedenführ
Forschungszentrum Jülich GmbH

metabolic steady & isotopic non-stationary

Time courses for:
Glucose
Glutamine

Model parameter
168 labeling data point
5 measured rates
14 measured pool sizes

Pathways coverage
Glycolysis
TCA-Cycle
Pentose-Phosphate Pathway
Glutaminolysis
Amino acid synthesis
Biosynthesis

Niedenführ S, Nöh K, Wiechert W
Current Opinion in Biotechnology 2015, 34:82–90
GC-MS fragment mapping to molecule level

modified from S. Niedenführ
GC-MS fragment mapping to molecule level

I. Isotope standards

II. High-resolution mass spectrometry

III. In silico consistency check

modified from S. Niedenfűhr
GC-MS fragment mapping to molecule level

Glutamine DL (3 TMS)

Fragment identification

I. Isotope standards

\[ ^{13}\text{C}\text{-Glu} \]

\[ ^{13}\text{C}_{1,3,4,5}\text{-Glu} \]

II. High-resolution mass spectrometry

\[ \text{m/z} 220 \text{ to 260} \]

\[ 246.1 \text{ to 250.1} \]

\[ [\text{M-COOTMS}] \]

\[ [\text{M-117}] \]

III. in silico consistency check

\[ \text{Glu[1-4]} \text{ m+n, 1, 2, 3, 4} \]

Identified fragment

I. Chemical formula

\[ [\text{C}_{10}\text{H}_{24}\text{NO}_{2}\text{Si}_{2}]^{+} \]

II. Fragment and precursor mapping

Atoms found in fragment

modified from S. Niedenführ
GC-MS fragment mapping to molecule level

I. Isotope standards

II. High-resolution mass spectrometry

III. In silico consistency check

Identified fragment

I. Chemical formula

II. Fragment and precursor mapping

Correction for natural abundance

Atoms found in fragment

Fragment identification

Labels found in fragment
GC-MS fragment mapping to molecule level

Glutamine DL (3 TMS)

I. Isotope standards

\[^{13}\text{C}\text{-Glu}\]

\[^{13}\text{C}_{13,34,5}\text{-Glu}\]

II. High-resolution mass spectrometry

\[\frac{\text{M-117}}{\text{M-COOTMS}}\]

III. *in silico* consistency check

Correction for natural abundance

Measurement specification

\[\text{Glu}[1-4]\]

<table>
<thead>
<tr>
<th>m+0</th>
<th>m+1</th>
<th>m+2</th>
<th>m+3</th>
<th>m+4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>0.25</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Labeling enrichment

- Measured atom
- Not measured atom

modified from S. Niedenführ
Monitoring metabolic reprogramming - experimental setup

in cooperation with R. Bucowiecki and A. Prigione (MDC, Berlin, Germany)
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Re-arrangement of carbon fate
Re-arrangement of carbon fate
Re-arrangement of carbon fate
Routing of $^{13}\text{C}$-Pyruvic acid

- Lactic acid
- AcetylCoA
- Malic acid $m+2$
- Malic acid $m+3$
- Malic acid $m+4$
- Alanine 3 TMS
Summary

pluripotent vs. differentiated

pluripotent vs. tumorgenic

OXPHOS Phenotype finite life-span

DIFFERENTIATED CELL

CANCER CELL

Glycolytic Phenotype infinite life-span

Glycolytic Phenotype finite life-span
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Germany

… thank you for your attention