Workshop: Applications of Omics Technologies

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The current paradigm is still that cells are mere catalysts. Product quantity and quality are a result of information flow inside cells.

• **Transcriptomics** to QC hESC process: static-to-stirred culture system transition

- **Access pluripotency**

- **Screen culture conditions** to preserve stemeness phenotype

- **Final product characterization**

Transcriptomics to identify engineering targets in viral vector producer cells

Biological pathways recruited in retrovirus producer cells?

Which metabolic pathways?

6-fold to 30-fold increase on virus yields by medium design and gene manipulation

Rodrigues et al., 2013, *Metab Eng.* 20:131-45

Searching for novel molecules in the Receptome of human cardiac stem cells and their role in the cardiac regenerative process

More than 3000 proteins identified

- **40%** proteins with predicted TMDs  
  (e.g. ATPase, Ca++ transporting, cardiac muscle slow twitch-2,...)
- **20%** plasma membrane (e.g. Connexin-43,...)
- **5% Receptors** (e.g. EGFR, IGF2R, ACVR2A, CD70, TGFβR2,...)

**> 150 Receptors were identified!**
Stem cells characterization using Proteomics

Proteomics & MS-based methodologies constitute a powerful analytical toolbox enabling:

- Stem cell bioprocess optimization;
- Generation of comparability data that guarantee cell-based product’s quality and potency, overcoming some of the critical challenges in clinical development and approval of ATMPs;
- Identification of potential therapeutic targets by unveiling key proteins and pathways in the regenerative processes.

“You can miss the solution to the problem by using inappropriate methods of analysis”

The metabolism provides the **Building blocks** and **Energy** for

- Growth
- Recombinant protein production / virus replication

**Fluxome** (the *in vivo* fluxes through metabolic pathways) is a function of gene expression, translation, PTMs, protein-metabolite interactions.

**Fluxome**: integrative information
Fluxomics | 13C-metabolic flux analysis

- **13C MFA**: state-of-the-art tool for steady-state fluxome estimation

**Workflow:**
(1) 13C tracer experiments
(2) GC/LC-MS / NMR analysis of intracellular labelling patterns
(3) Analysis of uptake/secretion rates by exometabolome profiling (e.g. 1H-NMR)
(4) Data integration in computational metabolic models to determine fluxes

**Outcome:**
Altered pathways

**Fluxome comparison**
- Non-producer cells
- Uninfected cells
- Healthy cells
- Pluripotent cells
- Producer cells
- Infected cells
- Diseased cells
- Differentiated cells
**Fluxomics | Adenoviral vector production**

- Metabolic requirements for productive CAV2 replication in MDCK cells?

Parallel labelling cultures

- $[1,2^{13}C]Glc$ & $[U^{13}C]Gln$

- $13^C$ pattern analysis by GC/LC-MS
- Metabolite exchange rates (supernatant analysis)

Fluxome mapping using $13^C$-MFA

Cells possess a highly glycolytic and anaplerotic metabolism, upregulated by infection.

- **Fluxome modulations upon infection**

  - PPP flux increase 1.7-fold upon infection
  - Reductive carboxylation of glutamine-derived AKG for lipogenesis increase 2-fold upon infection

  - Upregulation/downregulation of **nucleic acid** or **fatty acid biosynthesis** improves/decreases CAV2 production?

1. Data from infection cultures

2. PLS model – Fluxes vs Productivity

3. Clustering of reactions

4. Implementation of suggested strategies

An up-regulated oxidative metabolism is beneficial for Bac replication

Pyruvate / αketoglutarate Supplementation: Productivity increase up to 10x

Carinhas et al. 2011. BMC Syst Biol 5:34
Metabolic reprogramming during neural stem cell (NSC) differentiation

- [1-\textsuperscript{13}C]Glucose fate in NSC and NSC-derived astrocytes

Fluxome mapping using \textsuperscript{13}C-MFA

- Significant metabolic differences between NSCs and astrocytes
- General downregulation of central carbon metabolism during astrocytic differentiation.

\begin{itemize}
  \item Significant metabolic differences between NSCs and astrocytes
  \item General downregulation of central carbon metabolism during astrocytic differentiation.
\end{itemize}

\textsuperscript{13}C MFA: uncovering metabolic determinants of stem cell fate.

Integrated Omics

Adapted from Nemutlu et al CMJ 2012; http://dx.doi.org/10.3325/cmj.2012.53.529
Thank you

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