Unveiling the metabolic plasticity underlying metastatic potential and drug resistance: a systems medicine approach to identify new drug targets

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http://www.bq.ub.es/bioqint/principaleng.html
Common biological capabilities sequentially acquired during cancer development which are essential to drive malignancy.

Metabolic reprogramming underlying metastatic potential or and drug resistance?

Cancer cells are perfect systems to invade other tissue: Could metabolism be used as therapeutic target against tumor progression and drug resistance?

Robust metabolic profile

FRAGILITY

Exploitable Target against tumor progression, metastasis and drug resistance?

- Tumor metabolism robustness counteracts single hits
- Multiple hit strategies can avoid bypass of single inhibitions
- Tumor metabolism response to multiple hits is unpredictable

Rational design of new therapeutical combinations is necessary
Understanding metabolic reprogramming in metastatic cancer or/and drug resistance will permit to discover new drug targets.

Metabolites are not only the “end point” also the “driving force”

**EXPERIMENTAL APPROACHES**

- Cytomics
- Genomics
- Proteomics
- Metabolomics

**“IN SILICO” SIMULATOR**

**Fluxomics** *(analyze the metabolic flux distribution in the cell metabolic network)*

**Highest capacity to predict phenotype:**
- Analyze metabolic adaptations supporting metastatic potential or/and drug resistance
- Design metabolic Interventions in drug development
Tracer based metabolic flux models and GSMM: two versatile, potent and informative tools in the study of the metabolic fingerprint of cancer cells
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**13C TRACER-BASED FLUXOMICS WORKFLOW**

**Incubation with tracer substrates**

For quantification of internal fluxes, the use of tracers is necessary:
- \([1,2^{13}C_2]\)-glucose
- \([U-^{13}C]\)-glutamine

**Study of isotopologue distribution**

- Glucose
- Lactate
- Glycogen
- Ribose
- Fatty Acids
- Krebs Cycle intermediates
- Amino acids

**Introduction of additional constraints**

- Enzymatic activities
- Metabolite uptake/release of:
  - Glucose
  - Glutamine
  - Lactate
  - Amino acids
  - Biogenic amines
- Targeted metabolomics (Biocrates kits etc...)

**Generation of fluxomic model**

**Qualitative:** from MIDA (Mass Isotopomer Distribution Analysis)

**Quantitative:** from software packages (i.e. ISODYN in-house and other in-house tools available through Fluxomics workflow Phenomenal: [https://public.phenomenal-h2020.eu/](https://public.phenomenal-h2020.eu/))

**Cell culture**

**LC/MS, GC/MS**

**LC/MS, Spectrophotometry**

**Computational**

Metabolization of 13C-labelled substrates

Incorporation of 13C in intracellular and excreted metabolites

**Fluxome:** Total set of fluxes in the metabolic network of a cell

Tracer based metabolic flux models and GSMM: two versatile, potent and informative tools in the study of the metabolic fingerprint of cancer cells
Genome-scale metabolic models (GSMM)

Mathematical representation of the metabolic reaction encoded by an organism’s genome.

GSMMs INCLUDE VARIED INFORMATION:

- List of reactions
- Reaction stoichiometry
- Reaction directionality
- Subcellular localization
- Transport reactions
- Gene – reaction associations

The latest version on 2014:

- Exchange reactions: Introduce/Release nutrients in/out the system
- Transport reactions: Metabolite exchange between cell and media
- Intra-cellular reactions: Produce intermediates of cellular processes
EXAMPLE: GSMMs AND FLUX BALANCE ANALYSIS (FBA) APLYED TO CHAracterlZE METAB>OLIC VULNERABILITIES IN METASTATIC AND NON METASTATIC CANCER CELL SUBPOPULATIONS

GSMMs of human metabolism flux map distribution: constraint based approach

- Stoichiometric constraints
- Thermodynamics constraints
- Transcriptomic profile
- Proteomic and Metabolic profile
- Calculate optimal steady-state flux distributions in metastatic and non-metastatic cancer cell subpopulations

Challenge: Different tumor subpopulations will have different vulnerabilities?
WE KNOW THAT tumor metastatic potential:

- Resides in a minority of malignant cells (tumor heterogeneity), known as tumor initiating cells (TICs) or cancer stem cells (CSCs)
- It uses at several critical steps the epithelial-mesenchymal transition (EMT)

WE AIM TO:

- Characterize TICs/CSCs and EMT metabolic patterns
- Unveil metabolic vulnerabilities of tumor invasion and metastasis
A dual cell model to study prostate cancer metastasis: the PC-3/M and PC-3/S cell model

Two related cell subpopulations isolated from the PC-3 prostate cancer cell line according to their metastatic and invasive potential

PC-3 cell line

Selection by limiting dilution: using the Matrigel invasiveness assay

PC-3/S cells
- Can be transformed into each other (factors, \(\downarrow\) or \(\uparrow\) genes, etc.)

PC-3/M cells

Cells isolated from nude mice liver metastases after intrasplenic injection of PC-3 cells.

↓ metastatic potential

↓ invasive

EMT

↑ metastatic potential

↓ invasive

TICs/CSCs (stem cell markers)

Metabolic characterization to find metabolic targets in order to:

- Kill these cells
- Promote the transformation of these distinct phenotypes

Here we propose an strategy to characterize the metabolism of these cells in order to find specific targets, combining and integrating experimental and computational methods:

- Detailed metabolic characterization of both PC-3/M and PC-3/S cell lines

- Novel genome scale metabolic network reconstruction analysis that integrates the transcriptomic and metabolic analyses on the two clonal cell lines
Here we propose an strategy to characterize the metabolism of these cells in order to find specific targets, combining and integrating experimental and computational methods:

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Aguilar, E Marín de Mas, I, Zodda E, Marin S., Morrish F; Selivanov V; Meca-Cortés O; Delowar H; Pons M; Izquierdo I., Celia-Terrassa T; de Atauri P; Centelles JJ; Hockenbey D; Thomson TM; Cascante M, Stem Cells, 2016
PC-3M have enhanced glycolytic metabolism and fuel TCA cycle using glutamine.

**Glucose consumption**
- PC-3M: 0.8 μmol/h · 10⁶ cells
- PC-3S: 0.2 μmol/h · 10⁶ cells

**Lactate production**
- PC-3M: 2.0 μmol/h · 10⁶ cells
- PC-3S: 3.0 μmol/h · 10⁶ cells

**Glutamine consumption**
- PC-3M: 0.15 μmol/h · 10⁶ cells
- PC-3S: 0.10 μmol/h · 10⁶ cells

**100% 1,2-¹³C₂-glucose**
- Glucose contributes to a greater extent to the labelling of several final glycolytic products in PC-3M.

**100% U-¹³C₅-glutamine**
- Glutamine contributes to a greater extent to the labeling of the TCA intermediates in PC-3M.
ISODYN MODEL PREDICTS:

- PC3-M (e-CSC) cells display enhanced glycolysis and fuel TCA cycle using glutamine
- Increased TCA cycle relative to glucose uptake and mitochondrial respiration fluxes in PC-3S cells
- Increased one-carbon metabolism in PC3-M cells

PC-3S (stable EMT and non-eCSC) have enhanced TCA cycle and mitochondrial respiration

Integration of $^{13}$C-based metabolomics data in a flux model generated using ISODYN software package to estimate quantitative flux maps in PC-3S and PC-3M cells:
Metastatic metabolic gene signature

- **Metastatic metabolic gene signature (MMGS):** (genes over-expressed in PC-3/M compared with PC-3/S)

<table>
<thead>
<tr>
<th>BCAT1</th>
<th>PDE3B</th>
<th>AGMAT</th>
<th>ASS1</th>
<th>GLDC</th>
<th>CA9</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS3ST3A1</td>
<td>ALDH1A2</td>
<td>GALNT14</td>
<td>GUCY1A3</td>
<td>DGKG</td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td>CBS</td>
<td>SLC27A2</td>
<td>ABCB11</td>
<td>SLC7A8</td>
<td></td>
</tr>
<tr>
<td>SLC22A3</td>
<td>ST6GAL1</td>
<td>ADCY7</td>
<td>SLC1A3</td>
<td>SLC03A1</td>
<td></td>
</tr>
</tbody>
</table>

- **MMGS is consistent with metabolic differences observed between PC-3/M and PC-3/S:**

<table>
<thead>
<tr>
<th>Glycine and Serine metabolism</th>
<th>GLDC, CBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched-chain amino acids</td>
<td>BCAT1</td>
</tr>
<tr>
<td>Glutamate and proline metabolism</td>
<td>ASS1, AGMAT</td>
</tr>
<tr>
<td>Synthesis of purines</td>
<td>GUCY1A3, PDE3B</td>
</tr>
</tbody>
</table>

- Higher metabolic flexibility in PC-3/M
- Higher activity of one carbon metabolism in PC-3/M

- Correlation between **MMGS** and prostate cancer progression by applying GSEA:

We found a **significant correlation** between MMGS and tumor progression in prostate cancer and in other 11 cancer types.
Here we propose an strategy to characterize the metabolism of these cells in order to find specific targets, combining and integrating experimental and computational methods:

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Computational approach based on CBM:

- Uses transcriptomic data of PC-3/M and PC-3/S to infer the activity state of their metabolic networks

Cancer-specific Metabolic model

Non-specific genome-scale metabolic network reconstruction

transcriptomic data of PC-3/M & PC-3/S

Metabolomics, measured metabolic fluxes and other omics data of PC-3/M & PC-3/S

Infer the activity state of metabolic networks
This approach uses an objective function that maximizes the similarity between gene expression and reaction activity state:

- Maximizes the number of active reactions associated to highly expressed genes
- Minimizes the number of active reactions associated to lowly expressed genes

This analysis was performed by using the software fasimu

Shlomi et al; Nat Biotechnol. 2008 Sep;26(9):1003-10
Large systems can be readily analyzed (1000s reactions):

- Explore metabolic capabilities (e.g. uptake rates, growth rate)
- Calculate optimal steady-state flux distributions (e.g. to maximize growth rate)
- Simulate different genotypes (perturbations to network structure, i.e. gene deletion) and nutrient environments

**WHAT CAN WE EXPLORED USING GSMMs AND FBA?**

**Tumoral Metabolism**

**Metabolic Reprogramming**

**Normal metabolism**

**Tumoral Metabolism**

**Identify Therapeutic Targets**

**KILL CANCER CELL**

(Challenge: Different tumor subpopulations PC-3/M & PC-3/S will have different vulnerabilities?)

**NOT KILL NORMAL CELLS**

**IDENTIFICATION OF NEW DRUG TARGETS AND NEW DRUG TARGET COMBINATIONS**
Prediction:
- Both, long chain fatty acid transport (LCFA) from cytosol into the mitochondria via CPT1 and LCFA β-oxidation to be more active in PC-3/M cells.

Validation:
- PC-3/M cells show higher sensitivity to CPT1 inhibition (*Etomoxir*).
- Levels of CPT1 protein higher in PC-3/M.
- Acylcarnitines (*readout of β-oxidation*) higher in PC-3/M cells.
- Higher LCFA (*DHA as a redout*) levels in PC-3/S as a consequence of low β-oxidation.

Prediction:
Eicosanoid metabolism is more active in PC3/S cells (*Arachidonic Acid is the precursor of this pathway*)

Validation:
Arachidonic and some other eicosanoids (*12-HETE*) higher in PC-3/S (12-HETE in prostate cancer tissues exceed by >9 normal prostate).

WHAT CAN WE EXPLORED USING GSMMs AND FBA?

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WHAT CAN WE EXPLORED USING GSMMs AND FBA?

Tumoral Metabolism

Metabolic Reprogramming

Normal metabolism

Identify Therapeutic Targets

KILL CANCER CELL: IDENTIFICATION OF NEW DRUG TARGETS AND NEW DRUG TARGET COMBINATIONS TO KILL BOTH PC-3/M & PC-3/S

NOT KILL NORMAL CELLS
CONCLUSIONS

• The complementary $^{13}$C-based fluxomics and genome scale flux models integrating “omics” (GSMMs) are useful tools to elucidate key players in metabolic reprogramming associated either to metastatic or to local invasive phenotypes.

• Combination of drugs that target specifically metabolic key players in metastatic cells with drugs that target bulk tumor cells can result in more efficient therapies.
WE KNOW THAT tumor cells frequently undergo metabolic reprogramming, BUT IT IS UNKNOWN how these metabolic changes relate to drug resistance.

WE AIM TO:

Unveil metabolic vulnerabilities of drug resistance
Proof of concept: Targeting metabolic reprogramming associated to CDK4/6 inhibition as a combined therapy in cancer treatment

- Cell cycle regulation is frequently altered in cancer
- Highly selective and potent small-molecule CDK4/6 inhibitors have been developed and approved for clinical development (e.g. PD0332991)
- Mechanisms of acquired resistance to CDK4/6 inhibitors are beginning to emerge

HCT116 cells

Human colorectal carcinoma

Identification of metabolic dependences and vulnerabilities associated with CDK4/6 inhibition

Metabolic reprogramming associated to CDK4/6 inhibition

- CDK4/6 depletion enhances glucose, glutamine, and amino acid metabolism

- CDK4/6 inhibition enhances mitochondrial metabolism and function

Metabolic reprogramming associated to CDK4/6 inhibition

- Metabolite consumption and production rates
- Mass isotopomer distributions
- Protein expression
- Enzyme activities
- Oxygen consumption
- Gene expression

- Flux Balance Analysis (FBA)
- Mass Isotopomer Distribution Analysis (MIDA)
- $^{13}$C Metabolic Flux Analysis ($^{13}$C-MFA)

Characterization of central carbon metabolism

Quantitative metabolic network model

• MYC, mTOR, and HIF-1α are key players in the adaptive cellular responses to CDK4/6 inhibition

• CDK4/6-Cyclin D complexes directly phosphorylate MYC and cause its degradation

• CDK4/6 inhibition leads to de novo addiction to MYC, and also to GLS1 and mTOR signaling, as well as to a compromised adaptation to hypoxia

CDK4/6 depletion or inhibition sensitizes cells to inhibition of MYC, glutaminase, mTOR or PI3K or to hypoxia

Combination Index (CI) equation of Chou and Talalay and CompuSyn software

<table>
<thead>
<tr>
<th>CI value</th>
<th>Agonistic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.10</td>
<td>Very strong synergism</td>
</tr>
<tr>
<td>0.10–0.30</td>
<td>Strong synergism</td>
</tr>
<tr>
<td>0.30–0.70</td>
<td>Synergism</td>
</tr>
<tr>
<td>0.70–0.90</td>
<td>Moderate to slight synergism</td>
</tr>
<tr>
<td>0.90–1.10</td>
<td>Nearly additive</td>
</tr>
<tr>
<td>1.10–1.45</td>
<td>Slight to moderate antagonism</td>
</tr>
<tr>
<td>1.45–3.30</td>
<td>Antagonism</td>
</tr>
<tr>
<td>&gt;3.30</td>
<td>Strong to very strong antagonism</td>
</tr>
</tbody>
</table>

These combination treatments present synergistic and selective antiproliferative effects to cancer cells

CONCLUSIONS

• The complementary $^{13}$C-based fluxomics and metabolic models integrating “omics” are useful tools to elucidate key players in metabolic reprogramming associated to drug resistance.

• CDK4/6 inhibition enhances glycolysis and mitochondrial metabolism and function through MYC stabilization, which upregulates GLS1 and mTOR and blunts hypoxic response. CDK4/6-Cyclin D1 complexes phosphorylate MYC triggering its degradation by the ubiquitin-proteasome pathway.

• As MYC is a key driver of cancer cell metabolic adaptations after CDK4/6 inhibition, combined inhibition of GLS1 and mTOR-dependent pathways synergistically and selectively inhibits cancer cell growth.
Making existing Metabolomics and Fluxomics tools available to serve Systems Medicine:

- **Use and development of e-infrastructures for analyzing metabolic phenotype data:**

  ![PhenoMeNaL](PhenoMeNaL) *(Phenome and Metabolome aNalysis)*

  Large-Scale Computing for Medical Metabolomics

- **PhenoMeNaL** will support the data processing and analysis pipelines for molecular phenotype data generated by metabolomics applications.

- **PhenoMeNaL** will provide services enabling computation and analysis to improve the understanding of the causes and mechanisms underlying health and diseases.

This project has received funding from the European Union’s Horizon 2020 research and Innovation programme under grant agreement No 65424132
Making existing Metabolomics and Fluxomics tools available to serve Systems Medicine:

Automatic workflows for $^{13}$C-fluxomics in the framework of PhenoMeNaI in development.
CONCLUSIONS

• The complementary $^{13}$C-based fluxomics and metabolic models integrating “omics” are useful tools to elucidate key players in metabolic reprogramming associated to metastasis and drug resistance

• $^{13}$C-based fluxomics tools and GSMMs in conjunction with simulation techniques such as FBA need to be integrated

• Automatic workflows for $^{13}$C-fluxomics can be developed to serve systems medicine (PhenoMeNal e-infrastructure in development)
Talks accessible in The Biomedical and Life Sciences Collection (TBLSC):


THANK YOU FOR YOUR ATTENTION

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