Synthetic lethality in cancer research via genetic Minimal Cut Sets

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Barcelona, November 12, 2018
Metabolism is a hot topic in cancer research.

Signals and tumor microenvironment define different metabolic programs for enhancing proliferation, dissemination and invasion.

Opportunity of identifying biomarkers and drug targets for cancer cells based on metabolic networks and -omics data.
Information included:

- Substrates and products for an enzyme; Stoichiometric coefficients; Reversibility; Compartments, input/output metabolites, Biomass equation

Gene-Protein-Reaction (GPR) rules:

<table>
<thead>
<tr>
<th>Example</th>
<th>Reaction EC Number</th>
<th>R</th>
<th>E</th>
<th>G</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2.1.2</td>
<td></td>
<td></td>
<td></td>
<td>fumA</td>
</tr>
<tr>
<td></td>
<td>5.3.3.8</td>
<td></td>
<td></td>
<td></td>
<td>fumB</td>
</tr>
<tr>
<td></td>
<td>4.2.1.17</td>
<td></td>
<td></td>
<td></td>
<td>fumC</td>
</tr>
<tr>
<td>2</td>
<td>1.1.1.35</td>
<td></td>
<td></td>
<td></td>
<td>fadB</td>
</tr>
<tr>
<td></td>
<td>5.1.2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.1.3.27</td>
<td></td>
<td></td>
<td></td>
<td>trpE</td>
</tr>
<tr>
<td></td>
<td>2.4.2.18</td>
<td></td>
<td></td>
<td></td>
<td>trpD</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Example</th>
<th>Reaction EC Number</th>
<th>R</th>
<th>E</th>
<th>G</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.3.99.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.1.1.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.3.1.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multienzyme complex
Max (MDH1B, MDH1)
SDHA and SDHB and SDHC and SDHD
Min(SDHA, SDHB, SDHC, SDHD)

- Genomics
- Transcriptomics
- Proteomics
- Lipidomics
- Metabolomics
- Fluxomics
- ....
Cancer-specific metabolic reconstructions

Contextualize the reference metabolic network of human cells based on available -omics data and, then, conduct gene knockout perturbations.
Cancer-specific metabolic reconstructions:

\[
\text{max} \quad \text{similarity with expression data}
\]

subject to

\[
S_v = 0
\]

\[
u_r \geq v_r \geq l_r
\]

\[
v_{\text{biomass}} \geq \varepsilon
\]
Cancer-specific metabolic reconstructions:

- Essential metabolites for cellular growth (biomass reaction)
  - Human biomass reaction (Folger et al, 2011)

<table>
<thead>
<tr>
<th>Coefficient Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.6508</td>
<td>h2o[c] H2O</td>
</tr>
<tr>
<td>-0.7045</td>
<td>atp[c] ATP(4-)</td>
</tr>
<tr>
<td>-0.3859</td>
<td>glu_L[c] L-glutamate(1-)</td>
</tr>
<tr>
<td>-0.3526</td>
<td>asp_L[c] L-aspartate(1-)</td>
</tr>
<tr>
<td>-0.0361</td>
<td>gtp[c] GTP</td>
</tr>
<tr>
<td>-0.2794</td>
<td>asn_L[c] L-asparagine</td>
</tr>
<tr>
<td>-0.5056</td>
<td>ala_L[c] L-alanine</td>
</tr>
<tr>
<td>-0.0466</td>
<td>cys_L[c] L-cysteine</td>
</tr>
<tr>
<td>-0.326</td>
<td>gln_L[c] L-glutamine</td>
</tr>
<tr>
<td>-0.5389</td>
<td>gly[c] Glycine</td>
</tr>
<tr>
<td>-0.3925</td>
<td>ser_L[c] L-serine</td>
</tr>
<tr>
<td>-0.3127</td>
<td>thr_L[c] L-threonine</td>
</tr>
<tr>
<td>-0.5921</td>
<td>lys_L[c] L-lysinium(1+)</td>
</tr>
<tr>
<td>-0.3593</td>
<td>arg_L[c] L-argininium(1+)</td>
</tr>
<tr>
<td>-0.153</td>
<td>met_L[c] L-methionine</td>
</tr>
<tr>
<td>-0.0233</td>
<td>pah_L[c] 1-phosphatidyl-1D-myoinosito(1-)</td>
</tr>
<tr>
<td>-0.039</td>
<td>ctp[c] CTP</td>
</tr>
<tr>
<td>-0.1545</td>
<td>phchol_hs[c] Phosphatidylcholine</td>
</tr>
<tr>
<td>-0.0554</td>
<td>pe_hs[c] phosphatidylethanolamine</td>
</tr>
</tbody>
</table>
Gene essentiality and drug targets:

- One of these metabolites is disrupted upon gene knockout

<table>
<thead>
<tr>
<th>Delete Gene 1</th>
<th>Delete Gene 2</th>
<th>Delete Gene 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reaction is affected</td>
<td>Reaction $v_5$ inactive</td>
<td>Reactions $v_6$ and $v_3$ inactive</td>
</tr>
<tr>
<td>Non-essential</td>
<td>Non-Essential</td>
<td>Essential</td>
</tr>
</tbody>
</table>

Diagram:

1. Delete Gene 1: No reaction is affected
2. Delete Gene 2: Reaction $v_5$ inactive
3. Delete Gene 3: Reactions $v_6$ and $v_3$ inactive
Gene essentiality and drug targets:

- Polyamines in cancer

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Enzyme(s)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>262 (AMD1)</td>
<td>adenosylmethionine decarboxylase</td>
<td>Essential</td>
</tr>
<tr>
<td>4507 (MTAP)</td>
<td>5′-methylthioadenosine phosphorylase</td>
<td>Essential</td>
</tr>
<tr>
<td>4953 (ODC1)</td>
<td>Ornithine Decarboxylase</td>
<td>Essential</td>
</tr>
<tr>
<td>6723 (SRM)</td>
<td>spermidine synthase</td>
<td>Essential</td>
</tr>
<tr>
<td>4143 (MAT1A) &amp; 27430 (MAT2B)</td>
<td>methionine adenosyltransferase</td>
<td>Synthetic</td>
</tr>
<tr>
<td>4143 (MAT1A) &amp; 4144 (MAT2A)</td>
<td>methionine adenosyltransferase</td>
<td>Synthetic</td>
</tr>
<tr>
<td>353 (APRT) &amp; 4860 (PNP)</td>
<td>purine-nucleoside phosphorylase adenine phosphoribosyltransferase</td>
<td>Synthetic</td>
</tr>
<tr>
<td>383 (ARG1) &amp; 4942 (OAT)</td>
<td>ornithine transaminase reversible arginase</td>
<td>Synthetic</td>
</tr>
</tbody>
</table>

Error in the database led to APRT as an essential gene in leukemic cells

Pey et al, 2017, Scientific Reports,
Large-scale validation of predicted essential genes:

- **Project Achilles data**: large-scale gene silencing (knocking out) experiments in order to identify and catalogue genetic vulnerabilities in cancer.

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**Lack of accuracy**

![Graph showing KS test results](image)

QUESTION

Why and when a metabolic gene is essential for a particular molecular context using our modeling perspective?
Minimal Cut Sets – MCSs (Steffen Klamt’s group)

Identification of groups of metabolic reactions, that, when simultaneously inhibited, render cellular proliferation impossible.
MCSs- Introduction

Based on:
- Optimization Theory
- Duality Theory
- Linear Algebra

Inputs:
- Template Metabolic Network
- Target metabolic task

Minimal Cut Sets:
- $r_1, r_4$
- $r_1, r_6$
- $r_2, r_6$
- $r_5, r_6$
- $r_3, r_4, r_5$
- $r_2, r_3, r_4$

L. Tobalina et al, 2016, Bioinformatics
MCSs – Results
MCSs – Limitations

PROBLEM

Due to complex GPR rules, minimal reaction knockout strategies may not be minimal at the gene level.

P. Jensen et al, 2011, BMC systems biology
MCSs – Limitations

GPR rules

<table>
<thead>
<tr>
<th>Minimal Cut Set</th>
<th>Gene knockout</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r_1, r_4$</td>
<td>$g_1, g_4$</td>
</tr>
<tr>
<td>$r_1, r_6$</td>
<td>$g_{1,3}$</td>
</tr>
<tr>
<td>$r_2, r_6$</td>
<td>$g_{2,3}$</td>
</tr>
<tr>
<td>$r_5, r_6$</td>
<td>$g_5$</td>
</tr>
<tr>
<td>$r_2, r_3, r_4$</td>
<td>$g_{2,3,4}$</td>
</tr>
<tr>
<td>$r_3, r_4, r_5$</td>
<td>$g_{2,3,4,5}$</td>
</tr>
</tbody>
</table>
genetic Minimal Cut Sets – gMCSs

Identification of groups of metabolic genes, that, when simultaneously inhibited, render cellular proliferation impossible.
gMCSs – Our Approach

Based on:
• Optimization Theory
• Duality Theory
• Linear Algebra

Inputs:
• Template Metabolic Network
• Target metabolic task
• GPR rules

GPR rules

genetic Minimal Cut Sets:
• $g_5$
• $g_1, g_4$
• $g_2, g_3, g_4$

I. Apaolaza, 2017, Nature Communications
gMCSs – Our Approach

- A more efficient tool for the calculation of gMCSs was later implemented in the COBRA Toolbox, the gMCS function.
- Technical details can be found in I. Apaolaza et al, 2018, Bioinformatics.
- Some results (see poster 92 of Luis V. Valcarcel):
  - 20,000 gMCSs for Recon3D in less than 48 hours (4 cores at 2.70 GHz, 16GB RAM).
Returning to our fundamental question:

A particular gene is essential if it is the only expressed gene in at least one gMCS

I. Apaolaza, 2017, Nature Communications
Essentiality of RRM1 (Ribonucleotide Reductate Catalytic Subunit M1) in different cancer cell lines

I. Apaolaza, 2017, Nature Communications
gMCSs – Cancer

- Reconstruction process is avoided to identify cancer-specific essential genes.

- Possibility to calculate gMCSs involving a particular gene knockout.

- Possibility to calculate gMCSs among a selected subset of genes (e.g. lowly expressed genes).

- The expression of partner genes of a cancer-specific essential gene (e.g. RRM1) can be used as response biomarkers.
gene & drug Minimal Cut Sets – gdMCSs

Minimal subsets of metabolic inhibitors (drugs) and gene knockouts that render cellular proliferation impossible.
**gdMCSs – Our Approach**

**Based on:**
- Optimization Theory
- Duality Theory
- Linear Algebra

**Inputs:**
- Template Metabolic Network
- Target metabolic task
- GPR rules
- Drug – Target Relationships

**Example:**
Will $d_1$ be effective for a given patient?

**Translation:**
Is there a gdMCS which contains $d_1$ and lowly expressed genes for the patient under study?

**Solution:**
\{d_1, g_4\} is a gdMCS. If $g_4$ is not expressed, the patient will benefit from a therapy with $d_1$.

In addition, $g_4$ is a biomarker for the effectiveness of therapy with $d_1$.  

**Drug Target Relationships:**
- $d_1$: $g_1$
Methotrexate

- Targets Dihydrofolate Reductase, DHFR.

- DHFR is a metabolic gene which converts dihydrofolate into tetrahydrofolate.

- Methotrexate is an interesting drug for our analysis since it mainly interacts with metabolic targets.
gdMCSs – Methotrexate

**HYPOTHESIS**

The expression level of TK1 will explain the effectiveness of Methotrexate.

<table>
<thead>
<tr>
<th>gdMCS_1</th>
<th>TK1</th>
<th>TK2</th>
<th>Methotrexate</th>
</tr>
</thead>
</table>

- TK2 commonly not expressed.
IC50 values of Methotrexate for 533 cell lines from Genomics of Drug Sensitivity (GDSC) and gene expression data from the Cancer Cell Line Encyclopedia.

We expect a higher expression of the partner genes (TK1 and TK2) in those cell lines with a higher IC50 value of Methotrexate.
gdMCSs – Genomics of Drug Sensitivity in Cancer (GDSC)

<table>
<thead>
<tr>
<th>gdMCS_1</th>
<th>SENSITIVE</th>
<th>RESISTANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK1</td>
<td>KE-37</td>
<td>A498</td>
</tr>
<tr>
<td>TK2</td>
<td>JVM2</td>
<td>LOUNH91</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>PF-382</td>
<td>U87 MG</td>
</tr>
<tr>
<td></td>
<td>P12-ICHIKAWA</td>
<td>BT549</td>
</tr>
</tbody>
</table>

N = 533

rho: 0.17977 - pval: 1.4918e-05

Maximum Expression of Partner Genes

log2(IC50)
Future Directions

• Integration of RNA-seq data from MM patients and healthy cells (Poster 92).

• Application of our approach to target tamoxifen-resistance breast cancer tumors.

• In-vitro validation of synergy of TK1 knockout and methotrexate.

• Minimal strategies involving nutrient restrictions and gene knockouts.

• Integration of tracer-based metabolomics data.

• Accounting for cellular adaptation to our intervention.

• Extend our approach to signalling and regulatory networks.
Acknowledgments

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- Jon Pey
- Alberto Rezola
- Angel Rubio

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- Ines Thiele
- Laurent Heirendt

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- Xabier Aguirre
- Edurne San Jose
- Estibaliz Miranda
- Leire Garate
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