Mathematical Models on Cancer Progression

In collaboration with:
C. Fornari, F. Cordero, G. Balbo and R. Calogero
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CSCs theory: a new point of view

• The evolution of many cancers is driven by Cancer Stem Cells (CSCs).

• Main CSC properties: (i) self-renewal; (ii) high proliferative potential; (iii) responsible of tumor heterogeneity.

• Tumor heterogeneity: CSCs, Progenitor Cells (PCs) and Terminal Cells (TCs).

• CSC-tumors have a hierarchical structure.

• CSCs influence tumors in: (i) carcinogenesis; (ii) tumorigenesis; (iii) tumor resistance.
CSC AS A TARGET OF THERAPIES

CSCs express drug resistance proteins, making traditional therapies unable to kill them.

Anti-CSC therapies can be:

- direct, as CSC ablation, increment of CSC differentiation,...
- indirect, as reduction of CSC angiogenic/vasculogenic functionality,...
MODELING CANCER

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Our Contributes
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Bio Data
Model Definition
Qualitative Analysis
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Discussions
**Why math?**

A model:

- should make predictions matching experimental values;
- should provide results helping to answer specific questions about the process under study.

Main issue

- Why could be difficult? -

Successes

- Provide a formal definition of the system.
- Validation of hypothesis;
- Suggestion of new hypothesis;

Issues

Not a completed knowledge of the system we do not know all the system dynamics.
Parameter estimation
low data coverage.
Which scale?
high-degree of precision vs. global description.
ErbB2+ breast cancer

- ErbB2 is a transmembrane protein, which belongs to the Epidermal Growth Factor Receptor family;
- 20% of breast cancers overexpresses ErbB2;
- ErbB2 overexpression disrupts normal cell control, promoting cell division;
- ErbB2 is an attractive target for therapies.
ErbB2\(^+\) breast cancer & ErbB2 vax

Data describe tumor progression in BALB/c mice after an injection of \(10^5\) TUBO cells.

- **TUBO** are cancer cells generated from a mammary carcinoma arising in a BALB/c neuT mouse.
- **BALB/c neuT mouse** is a BALB/c female mouse transgenic for the rat ErbB2 oncogene. It develops mammary tumor few weeks after its birth.
An attempt to make order in the tumor chaos:

- modeling the multiscale aspects of cancer, by means of a multilevel model;

- formalizing a problem solving approach, by means of a workflow definition.
A SYSTEMATIC APPROACH

Model definition:

- the biological system is encoded on a multilevel model (our *complete* model);
- as many sublevels as subphenomena;
- a different formalism can be adopted for each sublevel;
- sublevel number in accordance with the phenomenon under investigation.

Model Definition

- Molecular regulatory network
- Cell population model

Multilevel Interactions

- Choice of interaction variables
- Definition of interactions

Qualitative Model Analysis

- Check of model consistency and correctness
- Structure validation and steady state analysis

Quantitative Model Analysis

- Submodel temporal behavior evaluation
- Global temporal behavior analysis
Multilevel interactions:

- interaction points are selected within each sublevels;
- reassembling process.
A SYSTEMATIC APPROACH

Qualitative model analysis:
- analysis of model qualitative properties;
- check of model consistency with respect to data and literature;
- several methodologies can be adopted, in accordance with different sublevel formalisms;
- sublevel reduction and simplification, if necessary.
A SYSTEMATIC APPROACH

Quantitative model analysis:

- investigation of the model temporal behavior;
- validation of hypothesis through in-silico experiments;
- suggestion of new theories.
THE complete (OR MULTILEVEL) MODEL

2-level model on the ErbB2+ breast cancer progression

1. **molecular level**: assuming the ErbB2 overexpression, it describes regulation aspects of cell proliferation;

2. **cell population level**: assuming the CSC theory, it describes how cell subpopulations interact during tumor progression;

+ **therapies**: perturbations are performed at molecular level, and their effects can be observed also on population dynamics.
The complete (or multilevel) model

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Mathematical Models on Cancer Progression

Marco Beccuti

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Cordero et al., BMC Bioinfo, 2013

Cell Population level

Differentiation degree

Portion A

ErbB receptors family cascade and RAS activation

Portion B

Pip3 production and Akt activation

Portion C

Cyclin D1 - activation
BAD - phosphorilation
NF-kB - activation

Portion D

mTORC regulation

Portion E

TLR2 cascade

encoded in an ODE system

encoded in a Petri Net

Portion A

Portion B

Portion C

Portion D

Portion E

CSC

PCn

TC

Proliferation potential

Molecular Regulatory Network level

Cell Population level

ModeL Definition

ErbB receptors family cascade and RAS activation

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ODE system

Cell Population level

\[
\frac{dN_{CSC}}{dt} = p_3 \omega_{CSC} N_{CSC} - \eta_1 N_{CSC} - \delta_1 N_{CSC} + \gamma_{PC} \sum_{j=1}^{3} N_{PC_j}
\]

\[
\frac{dN_{PC_1}}{dt} = P_{ar3} \omega_{CSC} N_{CSC} - \gamma_{PC} N_{PC_1} - \eta_1 N_{CSC} - \eta_2 N_{PC_4} - \delta_2 N_{PC_3} - \omega_{PC} N_{PC_1}
\]

\[
\frac{dN_{PC_2}}{dt} = 2\omega_{PC} N_{PC_1} - \omega_{PC} N_{PC_2} + \eta_2 N_{PC_4} - \eta_2 N_{PC_1} - \eta_2 N_{PC_2} - \delta_2 N_{PC_3} - \gamma_{PC} N_{PC_2}
\]

\[
\frac{dN_{PC_3}}{dt} = 2\omega_{PC} N_{PC_1} - \omega_{PC} N_{PC_3} + \eta_2 N_{PC_4} - \eta_2 N_{PC_2} - \eta_2 N_{PC_3} - \delta_2 N_{PC_4}
\]

\[
\frac{dN_{TC}}{dt} = \eta_3 N_{PC_7} - \delta_3 N_{TC}
\]

Molecular Regulatory Network level

Petri Net

Cordero et al., BMC Bioinf, 2013

9 cell pop
10 parameters

111 compounds
124 reactions
235 parameters
Molecular Regulatory Network Definition

- The net represents the signaling cascade controlled by the ErbB receptor family;
- it describes proliferation in CSCs and PCs;
- all reaction rates and initial concentrations are based on the paper of Birtwistle et al., *Molecular Systems Biology*, 2007.
**Cell population model**

\[
\frac{dN_{CSC}}{dt} = Psy\omega_{CSC}N_{CSC} - \eta_1N_{CSC} - \delta_1N_{CSC} + \gamma_{PC} \sum_{j=1}^{3} N_{PC_j}
\]

\[
\frac{dN_{PC_1}}{dt} = Pasy\omega_{CSC}N_{CSC} - \gamma_{PC}N_{PC_1} - \eta_1N_{CSC} - \eta_2N_{PC_1} - \delta_2N_{PC_1} - \omega_{PC}N_{PC_1}
\]

\[
\frac{dN_{PC_j}}{dt} = 2\omega_{PC}N_{PC_{j-1}} - \omega_{PC}N_{PC_j} + \eta_2N_{PC_{j-1}} - \eta_2N_{PC_j} - \delta_2N_{PC_j} - \gamma_{PC}N_{PC_j} \quad j = 2...3
\]

\[
\frac{dN_{PC_i}}{dt} = 2\omega_{PC}N_{PC_{i-1}} - \omega_{PC}N_{PC_i} + \eta_2N_{PC_{i-1}} - \eta_2N_{PC_i} - \delta_2N_{PC_i} \quad i = 4...6
\]

\[
\frac{dN_{PC_7}}{dt} = 2\omega_{PC}N_{PC_6} + \eta_2N_{PC_6} - \eta_3N_{PC_7} - \delta_2N_{PC_7}
\]

\[
\frac{dN_{TC}}{dt} = \eta_3N_{PC_7} - \delta_3N_{TC}, \quad (1)
\]

- It takes into account the **main properties** of CSCs: 
  (i) tumorigenic capacity, (ii) self-renewal and (iii) differentiation into non-stem cells.

- It guarantees the **hierarchical organization** of the tumor:  
  (i) CSCs give rise to PCs characterized by rapid proliferation rate; 
  (ii) PCs are able to completely differentiate into TCs.
From the regulatory level a set of key proteins (cycD, NF-kB and BAD) is selected.

Cell proliferation parameters are deduced from these target proteins.

\[
\begin{align*}
\frac{dN_{CSC}}{dt} &= P_{asgCSC}N_{CSC} + \gamma_{PC} \sum_{j=1}^{3} N_{PC_j} - \eta_1 N_{CSC} - d_1 N_{CSC} \\
\frac{dN_{PC_1}}{dt} &= P_{asgCSC}N_{CSC} - \omega_{PC}N_{PC_1} - \gamma_{PC}N_{PC_1} - \eta_1 N_{CSC} - \eta_2 N_{PC_1} - d_2 N_{PC_1} \\
\frac{dN_{PC_j}}{dt} &= 2\omega_{PC}N_{PC_{j-1}} - \omega_{PC}N_{PC_j} - \gamma_{PC}N_{PC_j} + \eta_2 N_{PC_{j-1}} - \eta_2 N_{PC_j} - d_2 N_{PC_j} & j = 2...3 \\
\frac{dN_{PC_i}}{dt} &= 2\omega_{PC}N_{PC_{i-1}} - \omega_{PC}N_{PC_i} + \eta_3 N_{PC_{i-1}} - \eta_3 N_{PC_i} - d_2 N_{PC_i} & i = 4...6 \\
\frac{dN_{PC_7}}{dt} &= 2\omega_{PC}N_{PC_6} - \eta_3 N_{PC_6} - \eta_3 N_{PC_7} - d_2 N_{PC_7} \\
\frac{dN_{TC}}{dt} &= \eta_3 N_{PC_7} - d_3 N_{TC}
\end{align*}
\]
**Effects of ErbB2 Vaccination**

A. “Normal” breast cancer growth: massive production of TCs, whose increment becomes exponential after time 1000.

B. Effects of ErbB2 vax: in-silico vaccinations are performed at molecular level, but their effects are reflected on the population one.

Cordero et al., BMC Bioinformatics, 2013
**TLR2 and CSC Proliferation**

**TLR2 is linked to the NF-κB oncogenic activity.**

**Hyp:** do perturbations on TLR2 affect CSC proliferation?

---

**A. ErbB2 vax:** control scenario.

**B. ErbB2 & TLR2 joint vax:** the TC number decreases, but the growth speed does not change.

Maybe CSC differentiation?
Towards the *essential* model

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<th>Solutions</th>
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The essential model

A BASIC, but NON SIMPLICISTIC mathematical framework, that is a mathematical-biological joint effort to investigate the CSC role in tumor progression.
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### The essential model

A BASIC, but NON SIMPLICISTIC mathematical framework, that is a mathematical-biological joint effort to investigate the CSC role in tumor progression.
CSC TUMORIGENIC POTENTIAL

**HYP: the tumorigenic potential of a cellular population is larger if it is enriched for CSCs.**

**IN VITRO EXPERIMENT**

Mammosphere assay \[ TUBO \rightarrow P1 \rightarrow P2 \rightarrow P3 \]

From TUBO cells several passages of CSC enriched mammospheres are generated.

The number of spheres increases progressively from TUBO to P3, suggesting the presence of an increased number of CSCs.
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IN VIVO AND IN-SILICO VALIDATION

Transplantation assay and data fitting.

3 experimental conditions: \(10^5\) TUBO, \(10^3\) TUBO and \(10^3\) P3 cells are implanted subcutaneously in syngeneic BALB/c mice.

In-silico growth rates are evaluated through data fitting, with Malthus model.

\[ V'(t) = \beta_i V(t) \]

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Fornari et al., submitted
THE CELL POPULATION MODEL

Ordinary Differential Equation (ODE) system:

\[
\frac{dN_{\text{CSC}}}{dt} = P_{sy} \omega_{\text{CSC}} N_{\text{CSC}} + \gamma_{\text{PC}} N_{\text{PC}1} - \eta_{1} N_{\text{CSC}} - \delta_{1} N_{\text{CSC}}
\]

\[
\frac{dN_{\text{PC}1}}{dt} = (1 - P_{sy}) \omega_{\text{CSC}} N_{\text{CSC}} - \omega_{\text{PC}} N_{\text{PC}1} - \gamma_{\text{PC}} N_{\text{PC}1} + \eta_{1} N_{\text{CSC}} - \eta_{2} N_{\text{PC}1} - \delta_{2} N_{\text{PC}1}
\]

\[
\frac{dN_{\text{PC}2}}{dt} = 2 \omega_{\text{PC}} N_{\text{PC}1} + \eta_{2} N_{\text{PC}1} - \eta_{3} N_{\text{PC}2} - \delta_{2} N_{\text{PC}2}
\]

\[
\frac{dN_{\text{TC}}}{dt} = \eta_{3} N_{\text{PC}2} - \delta_{3} N_{\text{TC}}
\]
**Kinetic Parameter Grouping**

**NEW MODEL PARAMETERS**

\[-\eta_1 + P_{sy} \omega_{CSC} - \delta_1 = a \iff \text{CSC variation, without de-diff};\]
\[\eta_1 + \omega_{CSC}(1 - P_{sy}) = b \iff \text{increasing rate of } PC_1\text{s};\]
\[\delta_2 + \eta_2 + \gamma_{PC} + \omega_{PC} = c \iff \text{decreasing rate of } PC_1\text{s};\]
\[\eta_2 + 2\omega_{PC} = d \iff \text{increasing rate of } PC_2\text{s};\]
\[\delta_2 + \eta_3 = e \iff \text{decreasing rate of } PC_2\text{s}.\]

**ADVANTAGES:**

- downsize of system complexity;
- principal dynamics among subpopulations are highlighted;
- aggregated parameters are easier to be measured experimentally, with respect to original parameters.
Possible scenarios:

\[
\begin{align*}
(i) & \quad \text{if } a < -\frac{b}{c} \gamma_{PC}, \quad \rightarrow \quad \text{tumor extinction, A;} \\
(ii) & \quad \text{if } a = -\frac{b}{c} \gamma_{PC}, \quad \rightarrow \quad \text{tumor stabilization, B;} \\
(iii) & \quad \text{if } a > -\frac{b}{c} \gamma_{PC} \quad \rightarrow \quad \text{tumor exponential growth, C.}
\end{align*}
\]

CSC and PC\textsubscript{1} variations determine the three possible system evolution scenarios, in accordance with the CSC theory.
Parameter Space is Enclosed by Biological Constraints

Biological knowledge and experimental data are used to define parameter properties. This allow us to:

- reduce parameter space dimension;
- better characterize the model.

Specifically, we impose conditions on:

1. **tumor growth rate**, by means of Malthus fitting;
2. **PC₁ de-differentiation**, being the PC₁ plasticity a rare event;
3. **subpopulation proportions**, as evinced from FACS analysis + biological knowledge.
4. **Reduced Parameter space** is explored with standard Minimum Least Square (MLS) technique to produce the best fit of breast cancer data.
**SUBPOPULATION PROPORTIONS, BY FACS ANALYSIS**

Sca-1$^+$ or CD44$^+$/CD24$^-$ cells are counted to estimate the CSC enrichment of different mammosphere passages.

Proportions between other cell subpopulations are derived from the literature.
The *essential* model reproduces cancer progression in terms of:

- tumor volume, panel A;
- cell subpopulation dynamics, panel B;

It also reflects the estimated subpopulation proportions, panel C.
Model results suggest new experiments:

Evaluate how subpopulation proportions behave over time *in vitro* and *in vivo*.
**DISCOVERING HIDDEN RELATIONSHIPS AMONG PARAMETERS**

There is a balance between actions that remove and generate cells within the same cell compartment:

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<td>Sca-1$^+$ cells</td>
<td>$b - c$ (PC$^1$)<em>; $e - d$ (PC$^2$)</em></td>
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<tr>
<td>CD44$^+$/CD24$^-$ cells</td>
<td>$b - a$; $c - \gamma$ (CSC)*</td>
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* $b$, $c$ increasing/decreasing rate of PC$^1$s; $e$, $d$ increasing/decreasing rate of PC$^2$s; $a$ CSC variation; $\gamma$ PC$^1$ de-differentiation.

- correlations are independent of the three injection-scenarios;
- correlations are influenced by the marker considered.
- by literature CD44$^+$/CD24$^-$ is considered a good marker for CSCs.

**Hypothesis:** May Sca-1$^+$ be a PC marker?
DISCOVERING HIDDEN RELATIONSHIPS AMONG PARAMETERS

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* b, c increasing/decreasing rate of PC_1 s; e, d increasing/decreasing rate of PC_2 s; a CSC variation; γ PC_1 de-differentiation.

- correlations are independent of the three injection-scenarios;
- correlations are influenced by the marker considered.
- by literature CD44^+ / CD24^- is considered a good marker for CSCs.

Hypothesis: May Sca-1^+ be a PC marker?
**May Sca-1\(^+\) be a PC marker?**

- The amount of CD44\(^+\)/CD24\(^-\) cells increases in the mammosphere passages.
- The amount of Sca-1\(^+\) cells increases too, and it is larger than those with CD44\(^+\)/CD24\(^-\) phenotype.
- There is a constant number of Sca-1\(^+\) cells that is also CD44\(^+\)/CD24\(^-\).
SOME CONSIDERATIONS

The complete model:

- is a multilevel model;
- is able to provide a qualitative description of the tumor growth assuming different drug therapies;
- too parameters to be estimated: in-silico and in vivo time scales are uncoupled.

The essential model:

- is able to reproduce a real tumor growth: in-silico and in vivo time scales are coupled;
- can be used to gain further knowledge from data:
  1. inferring properties which might require many biological experiments;
  2. suggesting hidden properties as subpopulation dynamics and relations among cellular events.
Acknowledgments

Modeling and Simulation Group

- Gianfranco Balbo
- Francesca Cordero
- Chiara Fornari

Molecular Biotechnology Center

- Raffaele Calogero
- Federica Cavallo
- Stefania Lanzardo
- Laura Conti
Thank you for your attention

Questions?
Bibliography

- Grange et al. Sca-1 identifies the tumor-initiating cells in mammary tumors of balb-neuT transgenic mice, Neoplasia, 10, 2008.
- Tang, Understanding cancer stem cell heterogeneity and plasticity, Cell research, 2012.
PNs are bipartite graphs with two types of nodes - places and transitions - and connected by directed arcs.

The state of the system is given by the distribution of tokens over places, while model dynamics are captured by state changes, i.e. by movements of tokens over the net.

When applied in systems biology:

- places represent biological entities (enzymes, compounds, etc.),
- transitions correspond to their interactions,
- tokens stand for the quantities of the entities.
**Petri Net - Solution**

**Biochemical Reaction**

\[
Pip_3 + Pten \xrightleftharpoons[k_{53}]{k_{54}} Pip_3 : Pten
\]

**Petri Net (PN)**

**Ordinary Differential Equation (ODE)**

Before k53 fires

\[
\frac{dX_{Pip_3}(t)}{dt} = -k_{53}X_{Pip_3}(t)X_{Pten}(t) + k_{54}X_{Pip_3:Pten}(t),
\]

\[
\frac{dX_{Pten}(t)}{dt} = -k_{53}X_{Pip_3}(t)X_{Pten}(t) + k_{54}X_{Pip_3:Pten}(t),
\]

\[
\frac{dX_{Pip_3:Pten}(t)}{dt} = k_{53}X_{Pip_3}(t)X_{Pten}(t) - k_{54}X_{Pip_3:Pten}(t)
\]

After k53 fires
Why PNs?

1. PNs provide an useful graphical representation of biological pathways;

2. they allow to analyze qualitative and quantitative properties of the system;

3. they allow to stochastically and deterministically evaluate system behavior.
Molecular Regulatory Network - PN -

Marco Beccuti

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Molecular Regulatory Network Definition

Cordero et al., Bioinformatics 2013
VACCINATION EFFECTS AT MOLECULAR LEVEL

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Background
CSC theory: a new point of view
Modeling Cancer

Our Contributions
The “complete” model
ErbB2+ breast cancer
Model interactions
Some considerations
The “essential” model
Bio Data
Model Definition
Qualitative Analysis
Quantitative Analysis
Discussions
CSC AS A TARGET OF THERAPIES

CSC express drug resistance proteins, making traditional therapies unable to kill them.

Anti-CSC therapies can be:

- direct, as CSC ablation, increment of CSC differentiation,...
- indirect, as reduction of CSC angiogenic/vasculogenic functionality,...
ErbB2 vaccination (DNA electroporation)

Mice were vaccinated with a plasmide encoding for the extracellular and transmembrane domain of the rat ErbB2.

TLR2 and CSC invasivness

- **siRNA of TLR2:**
  - *in vivo*: reduction of the tumor take in 9/14 mice (reduction of cell proliferation and increase of apoptosis),
  - *in vitro*: reduction of the mammosphere formation.

- **TLR2** was stimulated with HMGB1, that activates the TLR2 pathway:
  - *in vivo*: TLR2 is overexpressed in tumor cells,
  - *in vitro*: stimulation of the mammosphere formation.
LINEAR REGRESSION ON PARAMETER VALUES - SCA-1\textsuperscript{+} CELLS -
Linear regression on parameter values - CD44⁺/CD44⁻ cells -
MAMMOSPHERE PASSAGES

- after 2 days of culture, floating spherical mammospheres (P1) were developed,
- after 10 days they formed *golf-ball* like structures, that became hollow inside;
- after 3 weeks these structure did not grow or expand further;
- P1 mammospheres were dissociated after 7 days and propagated into secondary (P1) and tertiary (P3) sphere passages.

Long-term P1 culture showing mammosphere development, scale bar 100 µm, 20X.

Cavallo et al., 2013
**De-differentiation**

- The de-differentiation is a process in which a specialized cell takes on a more primitive state.
- The de-differentiation rate is very low with respect to other cellular kinetics.
- The plasticity of non-CSCs occur more prevalently under "induced" conditions:
  - accompanying tumor progression (hypoxia, EMT, inflammation, microenvironment changes) *in vivo*;
  - after therapies *in vivo*;
  - after experimental manipulations *in vitro.*
**Petri Net - Formalism**

PNs are a bipartite graph defined by the tuple $(P, T, W, m_0)$, where

- $P$ is a finite set of **places**;
- $T$ is a finite set of **transitions**;
- $P$ and $T$ are such that $P \cap T = \emptyset$;
- $W : (P \times T) \cup (T \times P) \rightarrow N$ defines the arcs of the net and assigns to each of them a multiplicity;
- $m_0$ is the **initial** marking which associates with each place a number of **tokens**.

Combining the information provided by the flow relations and by the weight function, we obtain the Incidence Matrix