

Systems Biology approach and Mathematical Modeling for analyzing phase space switch during Epithelial-Mesenchymal Transition

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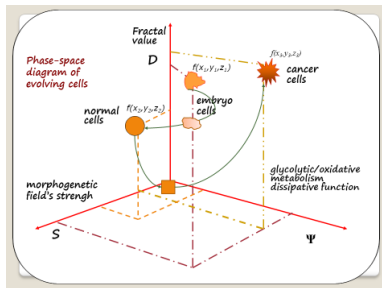
work in progress with

Mariano Bizzarri, Alessandra Cucina, Simona Dinicola,
Corrado Mascia, Pierfrancesco Moschetta

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epithelial-mesenchymal transition / metastable states / systems biology
mesoscopic description / mathematical modeling / multi-scale differential
equations / slow-fast dynamics / stability analysis

phenotypic transition = critical event during cell development and differentiation → onset of degenerative diseases



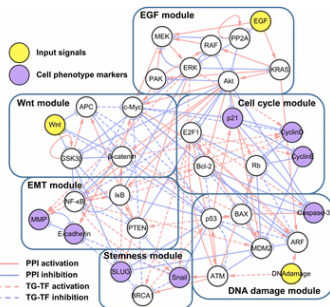
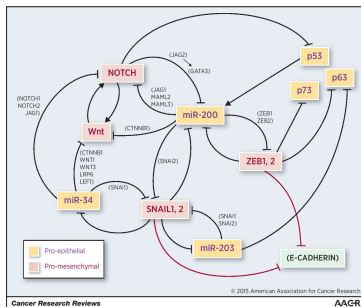
cell differentiation includes morphological, physiological and molecular changes, but the progressive process is still poorly understood...

in biological experiments, such transformation behaves like **first order phase transitions** also involving the overall system in coherent and global (phase) changes

→ efficiently addressed by adopting the formalism of phase space theory

- P.C. Davies, L. Demetrius, J.A. Tuszyński, Cancer as a dynamical phase transition, Theoretical Biology and Medical Modelling **8** (2011)

(primary) causative role of molecular dynamics = cell fate specification occurs as deterministic process with coordinated regulation of gene expression patterns in response to intrinsic/extrinsic chemical signals driving the cell population into specific differentiating pathway
 → the phase space is reconstructed by computing GRNs from data provided by high-throughput experiments



- C.P. Bracken, Y. Khew-Goodall, G.J. Goodall, Network-based approaches to understand the roles of miR-200 and other microRNAs in cancer, *Cancer Research* **75** (13) 2015

→ inherently stochastic with increasing fluctuations at critical transitions between (stable) differentiated phenotypes/states

- A. Paldi, What makes the cell differentiate?, Progress in Biophysics and Molecular Biology **110** (1) 2012

Systems Integrated versus Reductionist approach in Carcinogenesis

1) environmental factors efficiently constrain processes occurring at lower scales and 2) higher order levels of matter aggregation (tissues, organs, ...) exhibit emerging properties that cannot be anticipated from fundamental laws or by analyzing single components

- A. Giuliani, Collective motions and specific effectors: a statistical mechanics perspective on biological regulation, BMC Genomics **11** (Suppl.1) 2010
- B. Bravi, G. Longo, The unconventionality of Nature: biology, from noise to functional randomness, Lecture Notes in Computer Science 9252, Springer 2015

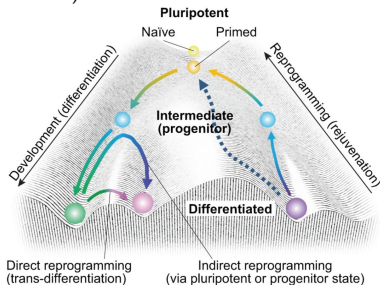
→ BIOPHYSICAL CONSTRAINTS and MULTISCALE NONLINEAR DYNAMICS at the root of CANCER PHENOTYPE

- M. Bizzarri, A. Cucina, F. Conti, F. D'Anselmi, Beyond the oncogene paradigm: understanding complexity in carcinogenesis, Acta biotheoretica **56** (3) 2008

phenotypic plasticity of complex living systems = resilience (robustness) to perturbations at molecular/microscale + extreme sensitivity to small fluctuations of environmental conditions

- W.L. Tam, R.A. Weinberg, The epigenetics of epithelial-mesenchymal plasticity in cancer, Nature Medicine **19** (11) 2013
- C.H. Waddington, The strategy of the genes. A discussion of some aspects of theoretical biology, 1957 (reprint edition Routledge Library Editions 2014)

cell phenotypes are stable attractors in the **Waddington landscape** for representing phase space transitions (semantic misunderstanding for metastable states with geometrical characterization as saddle points)



→ to identify control parameters that drive the systemic order parameters to instability when approaching their critical values

it is mostly from macroscopic changes that diseases, and especially cancer, are diagnosed... the **mesoscopic framework** entails both cells and tissues by integrating different levels of system organization

→ (not exhaustive/exclusive and independent) order parameters that display self-similarities at various scales

- M. Bizzarri, A. Giuliani, A. Cucina, F. D'Anselmi, A.M. Soto, C. Sonnenschein, Fractal analysis in a systems biology approach to cancer, *Seminars in Cancer Biology* **21** (3) 2011

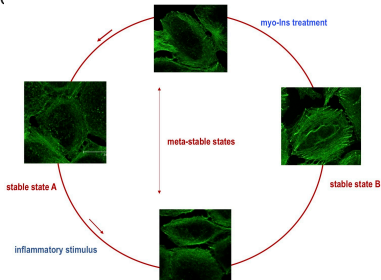
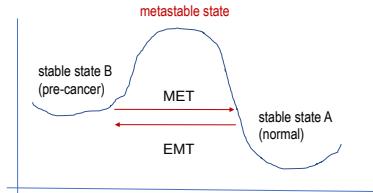
→ correlations between local processes and global structure (systems biology approach to the tyranny of scales problem)

- S. Green, R. Batterman, Biology meets Physics: reductionism and multi-scale modeling of morphogenesis, *History and Philosophy of Biological and Biomedical Sciences* **61** (2017)

→ collective behavior of microscale variables (enzymatic-genetic networks) also supports the emergence of coordinated/ordered macroscopic features (fractal morphology, cell population connectivity and motility, cytoskeleton rearrangement)

Epithelial-Mesenchymal Transition (EMT) is the biological process through which cells undergo multiple biochemical and/or structural changes to assume mesenchymal phenotype = enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, increased production of extracellular matrix components

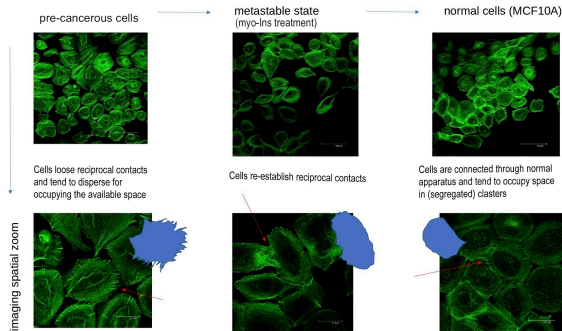
- J.P. Thiery, J.P. Sleeman, Complex networks orchestrate epithelial-mesenchymal transitions, Nature Reviews Molecular Cell Biology 7 (2) 2006



reversible process under specific environmental constraints

→ compatible with mathematical modeling exhibiting hysteresis loops

Mesenchymal-Epithelial Transition (MET) is required for transforming somatic cells into pluripotent stem cells, under the beneficial effects of external stimuli = promising studies for cancer cells reprogramming



- M.A. Esteban, X. Bao, Q. Zhuang, T. Zhou, B. Qin, D. Pei, The mesenchymal-to-epithelial transition in somatic cell reprogramming, *Current Opinion in Genetics & Development* **22** (5) 2012
- M. Moroni, L. Zanlorenzi, Complete regression following sorafenib in unresectable, locally advanced hepatocellular carcinoma, *Future Oncology* **9** (8) 2013

experimental setting as recorded by molecular and morphological analyses

→ normal MCF10A breast cells exposed to micromolar concentrations of **Transforming growth factor- β** (pro-inflammatory molecular effector) undergo conspicuous EMT within about 5 days

- J. Xu, S. Lamouille, R. Derynck, TGF-beta-induced epithelial to mesenchymal transition, Cell Research **19** (2) 2009

→ phase transition is switched backward within 24-48 hours by adding **myo-Inositol** treatments (with apparent effect only at metastable states, namely bifurcation/symmetry breaking points)

- S. Dinicola, G. Fabrizi, M.G. Masiello, S. Proietti, A. Palombo, M. Minini, A.H. Harrath, S.H. Alwasel, G. Ricci, A. Catizone, A. Cucina, M. Bizzarri, Inositol induces mesenchymal-epithelial reversion in breast cancer cells through cytoskeleton rearrangement, Experimental Cell Research **345** (1) 2016

→ low Fetal Bovine Serum concentration added to the culture medium implies negligible growth rate and constant density/space distribution

system process referred to (sufficiently dense) cell population samples subjected to control parameters

→ modifications in order parameters = from differentiated tissues to highly heterogeneous and motile aggregates

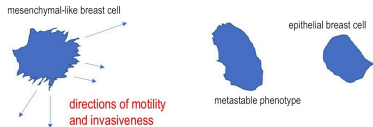
1) **E-cadherin downregulation** (reduced density values along membrane borders and impaired formation of cell-to-cell adhesion structures)

- M. Maeda, K.R. Johnson, M.J. Wheelock, Cadherin switching: essential for behavioral but not morphological changes during an epithelium-to-mesenchyme transition, *Journal of Cell Science* **118** (5) 2005

2) **cell shape** and **fractal dimension** (marker of cell malignancy, with substrate detachment and striving to occupy any available space)

- A. Pasqualato, A. Palombo, A. Cucina, M.A. Mariggiò, L. Galli, D. Passaro, S. Dinicola, S. Proietti, F. D'Anselmi, P. Coluccia, M. Bizzarri, Quantitative shape analysis of chemoresistant colon cancer cells: correlation between morphotype and phenotype, *Experimental Cell Research* **318** (7) 2012

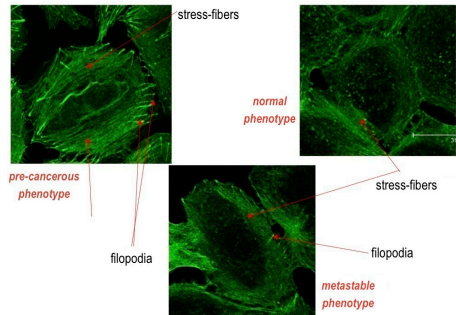
quantitative assessment of subtle morphological changes is predictive of further cell fate commitment



3) cytoskeleton architecture rearrangement and stress fibers (ultimate emergence of filopodia/pseudopodia for increased cellular contractility, migration and invasiveness)

- J. Weichsel, N. Herold, M.J. Lehmann, H.G. Krüsslich, U.S. Schwarz, A quantitative measure for alterations in the actin cytoskeleton investigated with automated high-throughput microscopy, Cytometry Part A **77** (1) 2010

transformations are appreciated through an integrated parameter that extracts the relative strength of structural edges compared to their surroundings and is also connected with relative E-cadherin density values along membrane borders with respect to its overall concentration
→ **coherency**

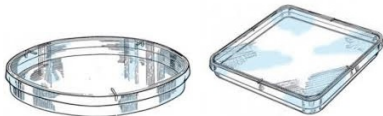


the (simplest but essential) **mathematical framework** consists of two coupled first order ordinary differential equations

$$\tau \frac{du}{dt} + u = w, \quad \frac{dw}{dt} + w = \lambda g(u),$$

with nonlinear structural function (multiple non-trivial equilibria) and external constraints given by constant control parameters $\tau, \lambda > 0$

Petri dish = two-dimensional support/reference domain



→ dynamical process with slow-fast dynamics

$$\Phi_1(u, w; \tau, \lambda) = \frac{1}{\tau} (w - u), \quad \Phi_2(u, w; \tau, \lambda) = \lambda g(u) - w,$$

assuming that u represents E-cadherin boundary distribution of cell populations and w stands for the coherency (order parameters)

→ multi-scale approach (macroscopic observables are interpreted as systemic averages of microscopic/molecular densities)

the stationary solutions $\begin{cases} \Phi_1(u, w; \tau, \lambda) = 0 \\ \Phi_2(u, w; \tau, \lambda) = 0 \end{cases}$ are located at the

intersection of the curves $w = u$ and $\lambda g(u) = w$ laying on the phase plane = **two-dimensional projection of the phase space**

→ for any fixed $\lambda > 0$, they are the zeros of $h(u; \lambda) := u - \lambda g(u)$ and stability properties are deduced from its first order derivative

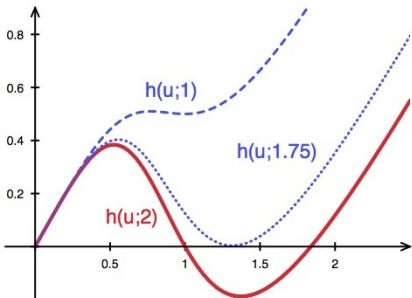
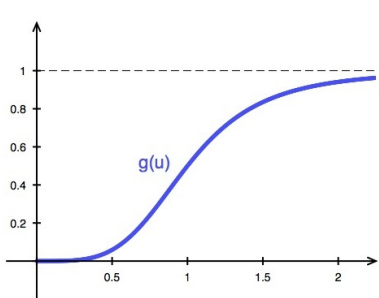
$$\frac{dh}{du}(u; \lambda) = 1 - \lambda \frac{dg}{du}(u)$$

(this approach is equivalent to the standard spectral analysis)

so that **the equilibrium is stable if $\frac{dh}{du}(u; \lambda)$ is positive**

otherwise, it is unstable/metastable

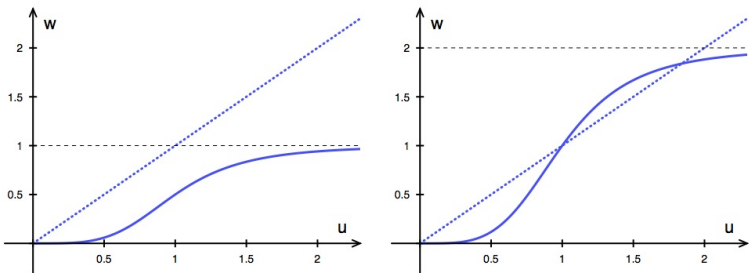
→ for $g(0) = \frac{dg}{du}(0) = 0$ the critical point $(u, w) = (0, 0)$ is the stable stationary state associated with the original (unperturbed) phase of the system (normal cells)



→ any subsequent intersection gives rise to additional (biologically relevant) equilibria = cell system phases during EMT and/or MET

→ **alternating stable and unstable/metastable states** in the case of simple zeros of h (under the generic assumption that $h(u; \lambda) = 0$ implies $\frac{dh}{du}(u; \lambda) \neq 0$, that is the so-called transversality condition)

→ two distinct ranges of values for the control parameter λ leading to quite different emerging scenarios



these two regimes are separated by (non-generic) critical value $\lambda = \lambda_c > 0$ which produces only two equilibria

→ minimal but reliable modeling supporting phase transitions exhibiting the typical destabilization mechanism of phenotypic cell differentiation

→ importance of identifying/quantifying the critical threshold for **adjusting control parameters to predictively regulate the system response**

alternative representation consisting of the one-field equation
= differential modeling for second-order phase transitions

$$\tau \frac{d^2 u}{dt^2} + (1 + \tau) \frac{du}{dt} + u - \lambda g(u) = 0$$

- R.E. O'Malley, Singular perturbation methods for ordinary differential equations, Applied Mathematical Sciences 89, Springer 1991

together with the **energy estimate** for time-decreasing potential functions

$$\underbrace{\tau \frac{d^2 u}{dt^2} \frac{du}{dt} + \left(u - \lambda g(u) \right) \frac{du}{dt}}_{\frac{d}{dt} \left\{ \frac{\tau}{2} \left(\frac{du}{dt} \right)^2 + \frac{1}{2} u^2 - \lambda G(u) \right\}} = - (1 + \tau) \left(\frac{du}{dt} \right)^2$$

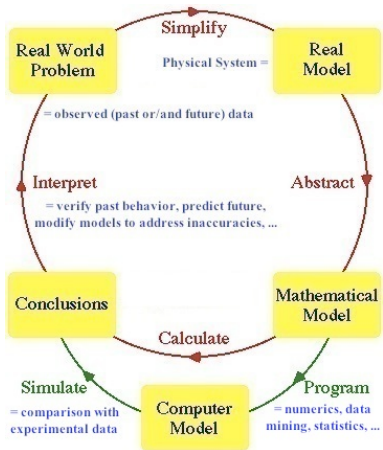
where G denotes a primitive of g (such that $\frac{dG}{du}(u) = g(u)$ for any u)

→ that shows the dissipative structure of the dynamical process

= **Lyapunov functional designated for being an INTRINSIC ENTROPY**

→ identifying markers of transformation for early diagnosis,
drug targets and therapeutically efficient interventions

methodological pathway for **adaptive design of *in silico* experiments**



→ measurement quantifications and data fitting = applied mathematics for determining the attractor manifolds of different equilibria by extrapolating information from experimental data

→ mathematical modeling and data mining = to capture the emergence of higher level dynamics and give evidence of precursive factors for phase space transitions (in order to induce medical actions before moving too far from stable/healthy equilibria)

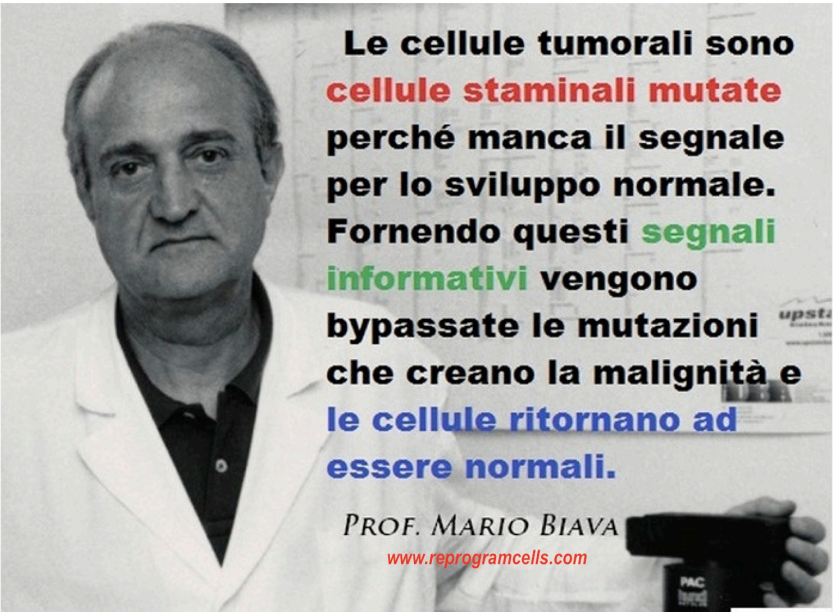
in grateful memory of Prof. Gilberto Flores Gallegos

glossary

high-throughput screening (HTS) is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry

genetic regulatory networks (GNRs) are collections of molecular regulators that interact with each other and with other substances in the cell to govern the gene expression levels of mRNA and proteins

- H.S. Khalil, H. Tummala, N. Zhelev, ATM in focus: a damage sensor and cancer target, BioDiscovery **5** (1) 2012 – www.biodiscoveryjournal.co.uk/Archive/A18.htm



Le cellule tumorali sono
cellule staminali mutate
perché manca il segnale
per lo sviluppo normale.
Fornendo questi segnali
informativi vengono
bypassate le mutazioni
che creano la malignità e
le cellule ritornano ad
essere normali.

PROF. MARIO BIAVA

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