The problem of tumor resistance / recurrence

Hallmarks of cancer
Hanahan & Weinberg, 2011

Why do tumors come back – often in a more malignant form?
How can non-specific perturbations (cytotoxic drugs, irradiation, toxins) invariably produce the highly sophisticated phenotype (= resistant, stem cell-like) of recurrent tumors?

Somatic Evolution of Drug Resistance: a new model

**STANDARD VIEW**

**NEO-DARWINISM**

- **Mutation**
- **Selection**
- **Resistant cells**

**DRUG**

- by chance or drug-induced
- frequent random or directed may be reversible

**Susceptible state**

**Resistant state**

**INSTRUCTION**

- selection

**NO MUTATIONS!**

**We proposed a new picture:**

- **DRUG**
  - rare random pre-existing
- **Resistant cells**

- **instruction** + (non-genetic) selection

- But what is this “landscape”? (→ mechanism behind the metaphor)
- Whence the “urge” (directionality) towards the resistant, stem –cell like state?
TALK OUTLINE

Theoretical concepts
- Gene networks and the Epigenetic Landscape,
- Cancer as attractors

Experiments: *to give a feel of state transitions*
- High-dimensional attractors,
- Non-genetic heterogeneity,
- State transitions

Application to Cancer
- Resistance development:
  Non-Darwinian dynamics

Cancer progression: *an unarticulated paradox*

DEVELOPMENT:
Gene expression program change = state transition (− same genome)

MULTI-STEP PROGRESSION:
"somatic evolution driven by mutations"

**DEVELOPMENT**

“Epigenetic” Phenotype Switch

**CANCER**

Genetic Mutation

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**Quasi-discrete Phenotype Transformations**

**CONCEPTS**

**Current molecular biology description**

From GENES to CELL PHENOTYPE SWITCH

- high-dimensional!
  
  (n genes change expression)

\[ S^A = [x_1^A, x_2^A, \ldots, x_n^A] \]

\[ S^B = [x_1^B, x_2^B, \ldots, x_n^B] \]

- ~ 3000 genes
  - Huang et al., PRL 2005

GEDI map:
- one transcriptome
- Pixel position = gene
- Color = mRNA level

Eichler et al., Bioinformatics 2003

**ISB**
The phenotype diversity *sans* mutation: The tree of development

CONCEPTS

- neuron
- liver
- muscle
- kidney
- prostate

The phenotype diversity *sans* mutation: The tree of development

CONCEPTS

- cancer ??
- neuron
- lung
- endoderm
- neural crest
- skin ectoderm
- neural ectoderm
- prostate
- liver
- muscle
- kidney
- placental meso
Dynamical systems view

- high-dimensional systems
- heterogeneous ensemble of systems

\[ S^A = [x^A_1, x^A_2, \ldots, x^A_N] \]
\[ S^B = [x^B_1, x^B_2, \ldots, x^B_N] \]

Local dynamics:
\[ \dot{x} = F(x) \]
- multistability
- but we need: relative stability between >2 attractor states!

Global dynamics

Dynamical systems framework
Local dynamics: multi-stability

Global dynamics

Dynamical systems framework

\[ \dot{x} = F(x) \]

- a network state \sim a cell state

- Relative stability between >2 attractor states!

CONCEPTS

From gene regulatory network to quasi-potential landscape

1. A fully specified gene regulatory network
   \[ \dot{x} = F(x) \]
   - There is no function \( U(x) \) that satisfies \( F(x) = -\nabla U(x) \)
   - Decompose vector field: \( F(x) = -\nabla U(x) + G(x) \)

2. Steady-state probability distribution (measurable) (J. Wang)
   \[ U = -\ln(P(x)) \]
   \[ P(A \rightarrow B) = e^{-2\Delta U_{AB} / \gamma^2} \]
   - Cond. probability for \( A \rightarrow B \)
   - Wentzell-Freidlin quasi-potential

\( \Delta U_{AB} = \Delta V_{AB} = \frac{1}{2} \min_{x \in \mathbb{R}^n} \left( \int_{t_i}^{t_f} ||\dot{x} - F(x)||^2 dt \right) \)

WITH E. AURELL (ZHOU J., ET AL., J. ROYAL SOC. INTERF., 2012)
Meaning of the Quasi-potential landscape

A fully specified gene regulatory network

\[ \dot{x} = F(x) \]

unique mapping

Cell population

REMEMBER FOR LATER

Change in specification of the network (incl. mutation, etc.) \( \Rightarrow \) change in shape (=topography) of the landscape

Transient perturbations of expression variables \( \Rightarrow \) attractor transition

Waddington’s “Epigenetic Landscape”

Similar ideas (“Biological cell state as attractor”)

- 1940 Waddington
  (‘valleys’ in landscape)
- 1949 Delbrück (bistability)
- 1961 Jacob & Monod (gene circuits)
- 1969 Kauffman (networks)

Modern quasi-potential landscape

based on notion of network dynamics
(but: often still equally metaphoric)

Mathematical equivalency
Molecular basis

We can roughly estimate
the landscape topography
Utility of quasi-potential: Directionality near Bifurcation

CONCEPTS

\[
\begin{align*}
\frac{dX}{dt} &= a_1 X - a_2 X^2 + b_1 Y^2 - b_2 X^2 Y \\
\frac{dY}{dt} &= a_3 X - a_4 X^2 + b_3 Y^2 - b_4 X^2 Y \\
\end{align*}
\]

\[\text{quasi-potential } U\]

\[\text{LOCAL STABILITY\nreversal of relative stability\nbifurcation point}\]

\[\text{parameter change} \quad (\text{decrease in auto-stimulation decreases})\]

Wang et al., Biophys. J. (2010).

IN SILICO EVOLUTION:
The Epigenetic Landscape grew downwards during evolution...
Simulation gene network evolution (Gene duplication + rewiring + selection) with Max Aldana (UNAM)

\[\text{with } 100,000 \text{ attractors}\]

\[\text{new attractors have to be accessible to existing attractors}\]

\[\text{many attractors are abandoned or never used}\]

\[\text{There is a global slope.}\]

\[\text{Attractors near the top of the landscape are phylogenetically older and ontogenetically more immature}\]

Qiu et al., in preparation
The Cancer Attractor Hypothesis

ASSEMBLING THE CONCEPTS TO DREAM MODEL:
The epigenetic landscape of the entire genome (CARTOON!)

lowest attractors = terminally differentiated cell types
- Stable • discrete • no fate option ("restricted")

OK, still qualitative – but: in principle inevitable.
→ allows specific qualitative predictions . . .
Unoccupied attractors represent cancer cells.

Huang, Ernberg & Kauffman, 2011

Evolution of chreods carved out a smooth path to assure safe descent to mature cell types → tumor suppressor genes

Properties of cancer attractors

Never evolved to serve tissue function → “asocial”, primordial cell functions, unstable
No access to normal development → when occupied: cells stuck in immature state

CONCEPTS

EXPANDING AN OLD HYPOTHESIS:

Kauffman, 1971

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  - State transitions

Application to Cancer
- Non-genetic dynamics
  - Drug screening: The Perturbation space
  - Resistance development: Non-Darwinian
SUMMARY: Manifestations of high-dimensional heterogeneity and dynamics on the attractor landscape


SUMMARY: OUTLIER (tail fraction) CELLS: are primed to transition into neighbor attractor

Theory predicts many non-intuitive properties for state transitions

Rugged landscape: fractionated response to perturbation

Bifurcation → “rebellious” cells

Heterogenization → Heterotypic cell-cell interactions
Differential growth rates → complex population dynamics
…

The canonical bi-potential framework

Don’t forget: Complex landscape – We have more than just two potential wells

Dispersal into many new states - some in “opposite direction”
EML progenitor, P

Sca1+  Sca1++

erythroid (E)  CD11b-

myeloid (M)  +IL3/GM-CSF

Sca1- CD11b+

Split into three intermediate populations (heterogeneization due to asynchronous transition)

Transient Rebellious cells!

EML progenitor, P

Sca1+  Sca1++

erythroid (E)  CD11b-

myeloid (M)  +IL3/GM-CSF

Sca1- CD11b+

GATA1 +  GATA1 -

PU.1 -  PU.1 +

FACS sort individual cells

GATA1  PU.1

undesired! rebellious cells

Biased destabilization

Single cell RT-PCR
TALK OUTLINE

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  - Cancer as attractors

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- High-dimensional attractors,
- Non-genetic heterogeneity,
- State transitions: Rebellious cells

Application to Cancer
- Non-genetic dynamics
  - Drug screening: The Perturbation space
  - Resistance development: Non-Darwinian

Mutation or chronic perturbation can allow cells to enter an the unoccupied attractors

MODEL
- unites genetic and non-genetic causes of cancer
- explains multiple discrete substates in clonal cancer populations
Cancer cells also partition into discrete types

Major Lung cancer histologic types
(> 90% of all cases)

- Squamous carcinoma: 33%
- Adenocarcinoma: 25%
- Small cell carcinoma: 25%
- Large cell carcinoma: 16%

Minna et al., 1985

Tumors partition into discrete, stable types:
Highly specific patterns that do not exist in healthy organisms

Breast cancer: natural types

- Luminal
  - MDA453
  - ZR75-1
  - MCF7
  - HCC1428
  - SUM52
  - MDA134

- Basal
  - MDA436
  - MDA157
  - HCC1954
  - BT20
  - MDA468
  - HCC70
  - HCC1187
  - HCC1143
  - HCC1937
  - HCC3153

No continuum (despite genomic chaos) but: characteristic, quasi-discrete distinct patterns → best explained by attractor states.

Data from Kao et al., PLoS One, 2009

(A) ETS pathway
(B) EMT
BRCA1 mutants

Partitioning into types of characteristic patterns

(breast cancer cell lines)
Dynamics of MDR1-low and MDR1-high subpopulations

Clonal HL60 (leukemic) cells

Reversibility: Dynamic and Non-genetic

MDR1-high expressing cells: The MDR1 gene confers multi-drug resistance (MDR)

Can one select for the MDR\textsuperscript{HIGH} state – or are they induced?

MDR1 (one state space dimension)

Re-equilibriates in 17d
REMEMBER:

NEO-DARWINISM

A new, more accurate picture:

by chance or drug-induced

frequent random or directed
may be reversible

Susceptible state

Resistant state

DRUG

Mutation

Resistant cells

selection

instruction or (non-genetic) selection

Why would chemotherapy - or any other cytotoxic cell stress – cause a stem-like resistant state?

Differential fitness of the two subpopulations

MDR1-LOW MDR1-HIGH

no drug + vincristine

Does this suffice for selection ???
SPECULATION - MORE COMPLEX MODEL

Can chemotherapy also trigger “rebellious cells”? → even more malignant

Inevitable consequence of change in parameters that promote transition into the benign attractor:

→ also access to more malignant (stem-cell like) state

Pisco et al., Nature Comm. 2014

Rapid appearance of Multi-drug-Resistance (MDR) + cells after vincristine

Drug efflux capability

Expression of MDR1 protein

Vincristine + anti-MDR1 mAb

vincristine

+ calceinAM (dye)

to visualize resistant cells

calcein AM efflux accumulation

vincristine

vincristine + anti-MDR1 mAb

Expression of MDR1 protein

vincristine

A

A’

C

B

destabilization

State space coordinate

differentiated/apoptotic

even more stem-like

2/14/2014
MDR1 expression after chemotherapy: instruction vs. selection?

Darwinian Selection
(fast selection of a non-genetic stable alternative state)

or

Lamarckian Instruction
(induction of “rebel” cells by therapy stress)

or both

A simple quantitative model

The drug modulates:
- transition rate constant $k$
- effective growth rate $g$

INSTRUCTION (“LAMARCKIAN” mechanism)

SELECTION (Non-genetic “DARWINIAN” mechanism)
Modeling: Growth and phenotype switch kinetics

- Unlimited exponential growth of both subpopulations
- \( \frac{x_L}{x_H} \) steady state \( \approx 50 \) is invariant & stable
- Presence of MDR+ cells after 24h: \( x_H \approx 40\% \)

**Effective growth rate:**

<table>
<thead>
<tr>
<th>Condition</th>
<th>( g_L )</th>
<th>( g_H )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.50 /d</td>
<td>( \sim ) 0.10 /d</td>
</tr>
<tr>
<td>Vincristin</td>
<td>0.25 /d*</td>
<td>0.37 /d*</td>
</tr>
</tbody>
</table>

*Initial growth rate first day

The only “proof” of cell-individual adaption (instruction):

- Single-cell longitudinal monitoring of phenotype change

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**ISB**

The only “proof” of cell-individual adaption (instruction):

- Single-cell longitudinal monitoring of phenotype change

- Instruction not selection

- + vincristine
EXPERIMENT: blocking fitness advantage ...

To really exclude "selection of the fittest" by vincristin

fitness phenotype = MDR1 pump function from MDR1 expression induction

VERAPAMIL vincristin

MDR1 expression

it's not selection

Transcriptome analysis of vincristine-induced genes

⇒ check other dimensions

Drug resistant, stem-cell like

This is induction and not selection

Genes induced:
- Stress-response genes
- Inflammatory genes
- Stemness genes
- Detoxification

Pisco et al., Nature Comm. 2014
**Functional role of the Wnt pathway in “induced” resistance**

Wnt-pathway targets up-regulated by vincristine:

- RSPO2
- LRP5/6
- FZD7
- DVL2
- DAAM
- β-catenin
- EETS
- PTGS2

= > 4-fold increase 48h after chemotherapy (vincristine)

**no drug**

**vincristine (60h)**

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**Wnt pathway required for survival in vincristine**

**Drug efflux**

- MDR1 expression

<table>
<thead>
<tr>
<th>Control</th>
<th>HU60 β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>10nM</td>
<td>50</td>
</tr>
</tbody>
</table>

Wnt-signaling is required for:

- induction of MDR1,
- efflux phenotype and
- prolonged survival of cells in vincristine.

**MDR1-expression**

- % survival

<table>
<thead>
<tr>
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<th>Control</th>
<th>HU60 β-catenin</th>
</tr>
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<td>Vincristine</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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“stemness” stress-response & resistance?
NON-DARWINIAN evolution of resistance

NEO-DARWINISM

DRUG

Mutation
(pre-existing !)

Resistant cells

INDUCTION

Drug
non-genetic
heterogeneity

+SELECTION

+ many new stem-like
“atavistic” survival functions

“Survival of the fittest”
(Darwin/Spencer)

“What doesn’t kill me makes me stronger”
(Nietzsche)


BROAD LESSONS

Why do tumors come back – often in a more malignant form?

Cancer is not (just) a “genetic disease”!

There is an inherent limitation to killing cancer cells.
– partial destruction is not partial success ...
  but can be worse in the long term.

It is not all “mutation + selection”!
– there is enormous non-genetic plasticity of phenotype
Further Reading