Towards Quantitative Endogenous Network Theory of Cancer Genesis and Progression: beyond cancer as disease of genome

Workshop on the Economy of a Cell:

Resource Allocation, Trade- Offs and Efficiency in Living Systems

Trieste, Italy; June 23-27, 2014

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Major and interconnected research projects

a. *Carcinogenesis: endogenous molecular-cellular network dynamics*
beyond "cancer as diseases of genome"

b. Develop whole organism platform on kinetic modeling of large metabolic networks
dealing with incomplete kinetic parameters

c. Development of mathematical and computational methodologies on stochastic processes

d. Towards new theoretical foundation of evolutionary biology

Kinetics of Global Metabolic Networks

- Simplified reaction reactions: generic rate equation

  Generic Enzymatic Rate Equation under Living Conditions,

  L.W. Lee, L. Yin, X.M. Zhu, and P. Ao, J. Biol. Syst. **15** : 495-514 (2007) .

  Generic Enzymatic Rate Equation.

  M.J. Xu, PH Lin, X.M. Zhu, P. Ao. Progress in Biochemistry and Biophysics . **38**: 759-767 (2011)

- Finding viable kinetic parameters by robustness principle

  Towards Kinetic Modeling of Metabolic Networks with Incomplete Parameters,

  W. Zheng, X.M. Zhu, Y.C. Chen, P.H.Lin, P. Ao, *Proceedings of the 2013 IEEE Conference on Systems Biology*. **2013**

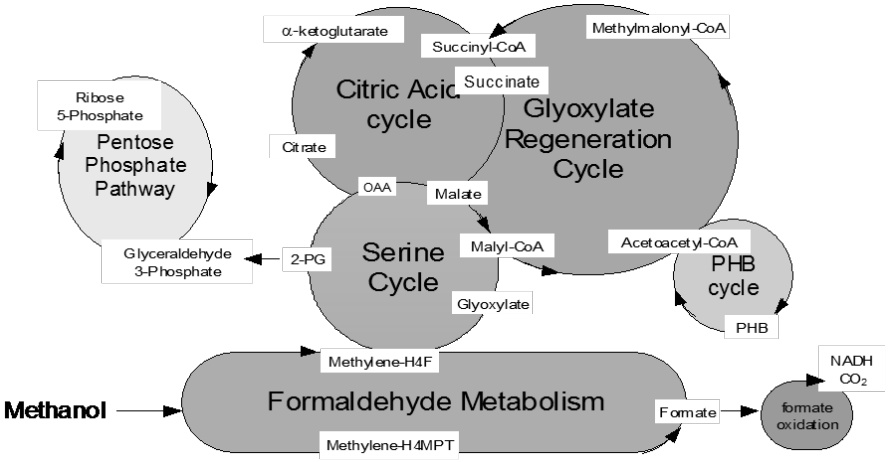
- Applications

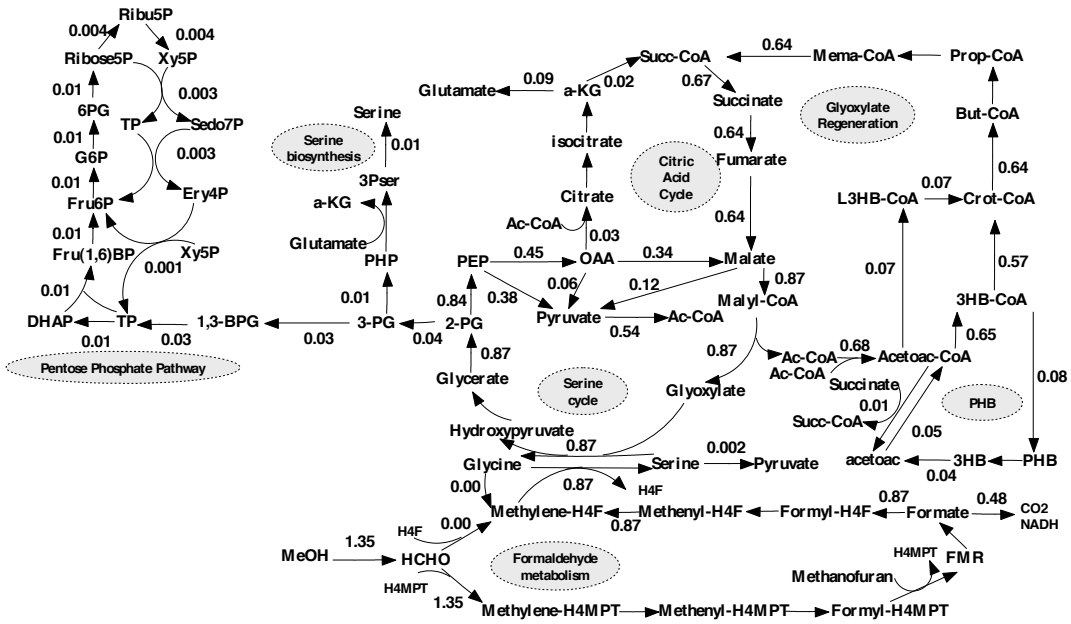
  Towards Kinetic Modeling of Global Metabolic Networks: *Methylobacterium extorquens* AM1 Growth as Validation,

  P Ao, LW Lee, ME. Lidstrom, Lan Yin, and XM Zhu, Chinese Journal of Biotechnology **24** (2008) 980 - 994.

Using bacterium AM1 to gain understanding on "combustion" and "parts manufacturing".

Metabolism and genetics





Systems Biology: endogenous molecular-cellular network cancer theory

Quantitative Implementation of Endogenous Molecular-Cellular Network Hypothesis in Hepatocellular carcinoma,
G.W. Wang, X.M. Zhu, J.R. Gu, P. Ao. Interface Focus 4 (**2014**) 20130064.

From Phage lambda to human cancer: endogenous molecular-cellular network hypothesis
G.W. Wang, X.-M. Zhu, L. Hood, P. Ao. Quantitative Biology 1 (**2013**) 32-49.

Towards Predictive Stochastic Dynamical Modeling of Cancer Genesis and Progression.
P. Ao, D. Galas, L. Hood, L. Yin, X.M. Zhu. Interdiscip Sci Comput Life Sci 2 (**2010**) 140–144

Global view of bionetwork dynamics: adaptive landscape.
P. Ao. J. Genet. Genomics 36 (**2009**) 63-73

Cancer as Robust Intrinsic State of Endogenous Molecular-Cellular Network Shaped by Evolution.
P. Ao, D. Galas, L. Hood, X.-M. Zhu, Medical Hypotheses 70 (**2008**) 678–684.

Orders of Magnitude Change in Phenotype Rate Caused by Mutations.
P. Ao, Cellular Oncology (**2007**) 29: 67-69.

This program aims to establish theoretical protocols and computational tools, which can provide a unified framework **to organize, to explain, and to predict** biological phenomena on cancer and related robust complex diseases.

"**If this even becomes possible, drug development will be more a matter of dry bioinformatics than wet biology at the laboratory bench.**" (RA Weinberg, 2007)

Milestones in Cancer Research

(Nature, Milestones of timeline, 2006)

1889 Seed and soil hypothesis
1890 Cancer as a genetic disease
1909 Immune surveillance
1910 Viruses and cancer
1915 Hormones and cancer
1937 Cancer stem cells
1939 Angiogenesis
1950 Smoking and cancer
1953 Two-hit hypothesis
1960 Chromosome translocations
1971 Tumour suppressor genes
1972 Apoptosis and cancer
1975 Tumour microenvironment
1976 Clonal evolution & multistep tumourigenesis
1976 Cellular homologues of viral oncogenes
1978 Oncogenes encode proteins that regulate cell growth
1979 First human oncogene
1983 Oncogene co-operation
Cancer epigenetics
1989 Cell cycle and DNA damage checkpoints
1990 Genetic basis for cancer predisposition
Mechanisms of genetic instability in cancer
1999 Cancer profiling
2001 Targeted cancer therapy

Cancer Complexity Slows Quest for Cure

EC Hayden, Nature, September 11, **455** (2008)148:

- Hopes that large studies of cancer genomics will justify their high cost by offering a fast track to cures have been dealt a blow by a series of papers.
- …
- "It is apparent from studies like ours that it is going to be even more difficult than expected to derive real cures," says Vogelstein.

Omic-related papers:

1923-2000:	~ 40,000
2000-2005:	~ 50,000
2005-now:	~100,000

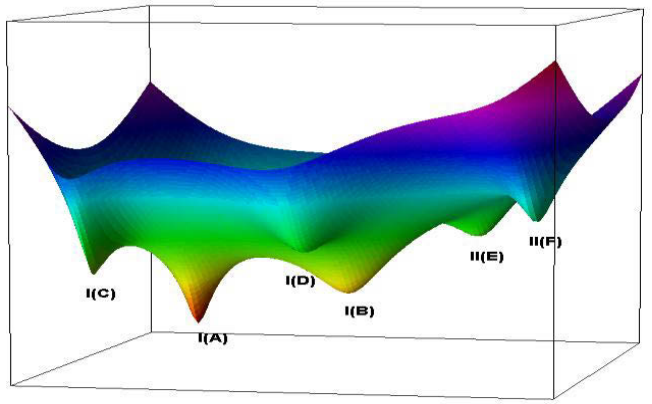
Focusing on genes and mutations may be misplaced.

Beneath cancer's daunting complexity may lie a simplicity that gives grounds for hope.

Network Dynamics and Diseases

Developing endogenous network theory for complex diseases, such as cancer, based on molecular-cellular processes and evolution.

Ao, Cell. Oncology, 2007; Biol. Theory 2007;
Ao, Hood, Galas, Zhu, Med. Hypotheses, 2008



- **Three basic considerations:**
  - Endogenous network shaped by evolution
  - Stochastic nonlinear dynamics: adaptive landscape
  - Enough experimental data to start with

- **Hypothesis:**

A complex disease an intrinsic robust state in the functional landscape of the endogenous network not optimized for the interest of whole organism.

P. Ao, D. Gala, L. Hood, X.-M. Zhu, Medical Hypotheses, **70** (2008) 678-684.

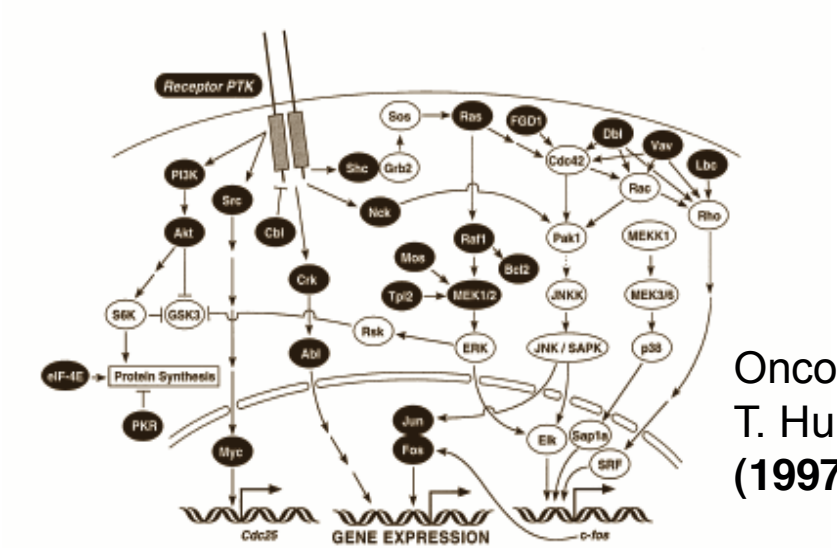


Figure 2. Membrane-to-Nucleus Signaling Pathways Involved in Cancer
Proteins that have been implicated as oncoproteins or tumor suppressor proteins are in white letters on black fill.

Oncoprotein networks, T. Hunter, Cell 88 **(1997)** 333-346.

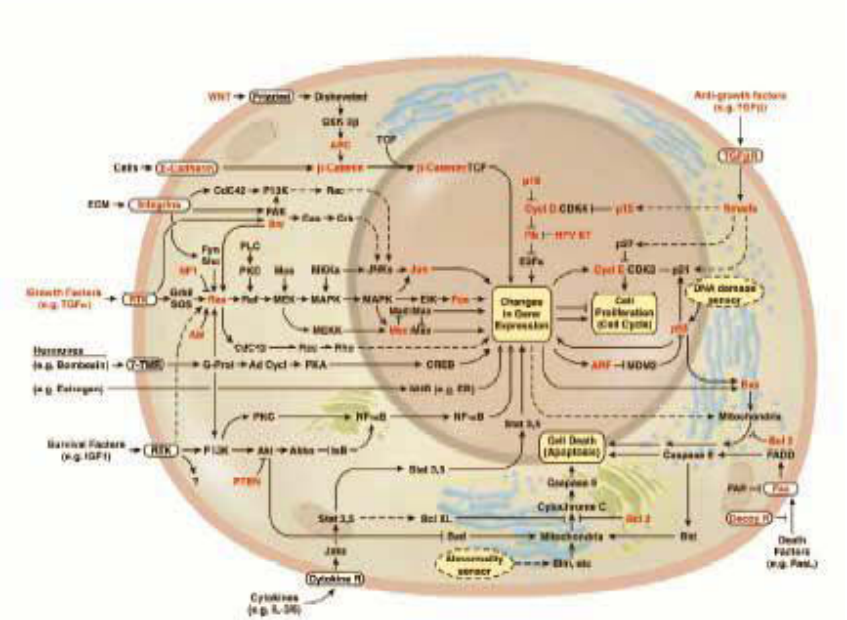


Figure 2. The Emergent Integrated Circuit of the Cell
Progress in dissecting signaling pathways has begun to lay out a circuitry that will likely mimic electronic integrated circuits in complexity and finesse, where transistors are replaced by proteins (e.g., kinases and phosphatases) and the electrons by phosphates and lipids, among others. In addition to the prototypical growth signaling circuit centered around Ras and coupled to a spectrum of extracellular cues, other component circuits transmit antigrowth and differentiation signals or mediate commands to live or die by apoptosis. As for the genetic reprogramming of this integrated circuit in cancer cells, some of the genes known to be functionally altered are highlighted in red.

The Hallmarks of Cancer, **D. Hanahan and R.A. Weinberg,** Cell 100 **(2000)** 57–70.

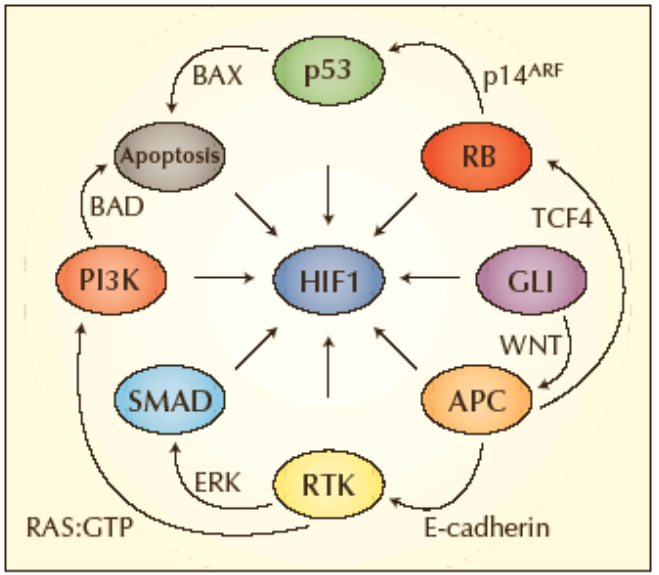


Figure 9 Overview of cancer gene pathways. The major pathways regulating cell birth and cell death are depicted as ovals color-coded to match Figs. 1–8. The schematics in Figs. 1–8 emphasize the genes that have been shown to be genetically altered in human tumors, though many other genes participate in these pathways. Additionally, some of the same genes appear in more than one pathway and there is substantial 'cross-talk' between pathways. Selected mediators of this cross-talk are indicated in the loops that connect the pathways. More detailed information about these pathways can be found in several comprehensive reviews (refs. 11,12,15,31,103–116).

Cancer genes and the pathways they control, B. Vogelstein and K.W. Kinzler, Nature Medicine 10 **(2004)** 789-799.

Wright's Adaptive Landscape

S. Wright, 1932

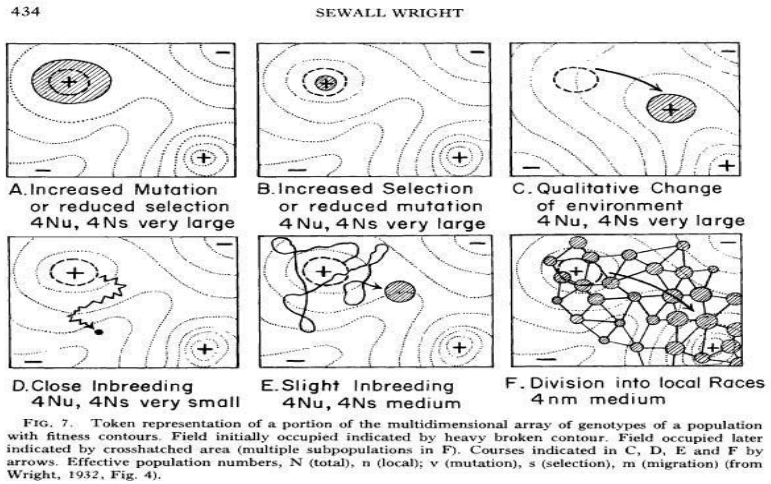
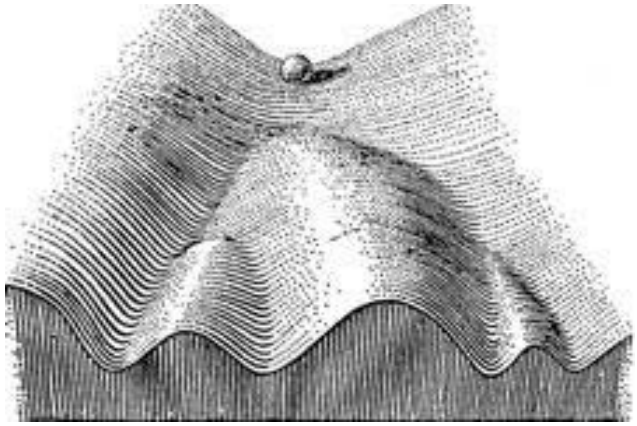
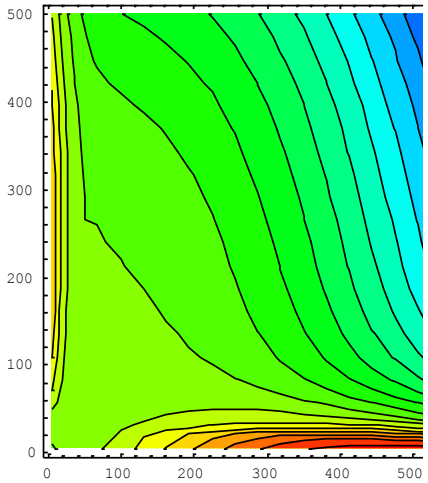


FIG. 7. Token representation of a portion of the multidimensional array of genotypes of a population with fitness contours. Field initially occupied indicated by heavy broken contour. Field occupied later indicated by crosshatched area (multiple subpopulations in F). Courses indicated in C, D, E and F by arrows. Effective population numbers, N (total), n (local); v (mutation), s (selection), m (migration) (from Wright, 1932, Fig. 4).

Waddington's Developmental Landscape

C. Waddington, 1940





Genetic Switch as multiple equilibria,
M. Delbruck, 1949;
Phage lambda genetic switch,
Zhu, Yin, Hood, Ao, 2004

Neural computing landscape,

J. Hopfield, 1982

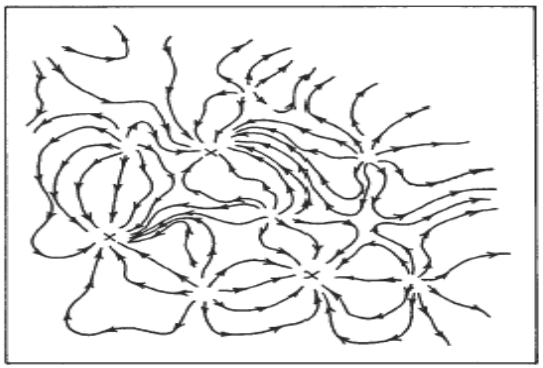
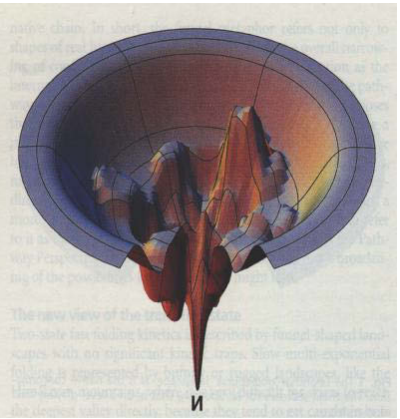
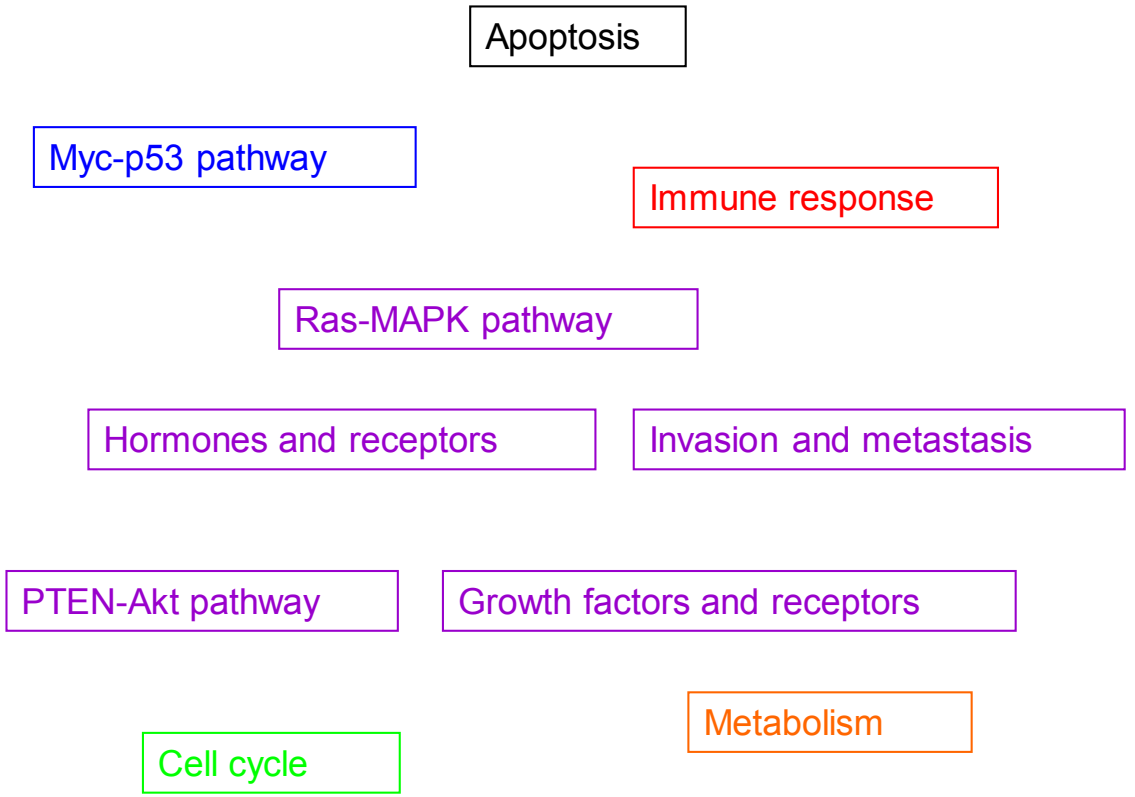


FIG. 1. The flow field of a simple analog computer. The stable points of the flow, marked by x's, are possible answers. To initiate the computation, the initial location in state space must be given. A complex analog computer would have such a flow field in a very large number of dimensions.



Protein folding funnel landscape

Endogenous Network:
major pathways



The minimum set of pathways and modules of the endogenous network.
Endogenous molecular and cellular agents first form pathways and modules.
Pathways and modules cross talk to each other to form the endogenous network.

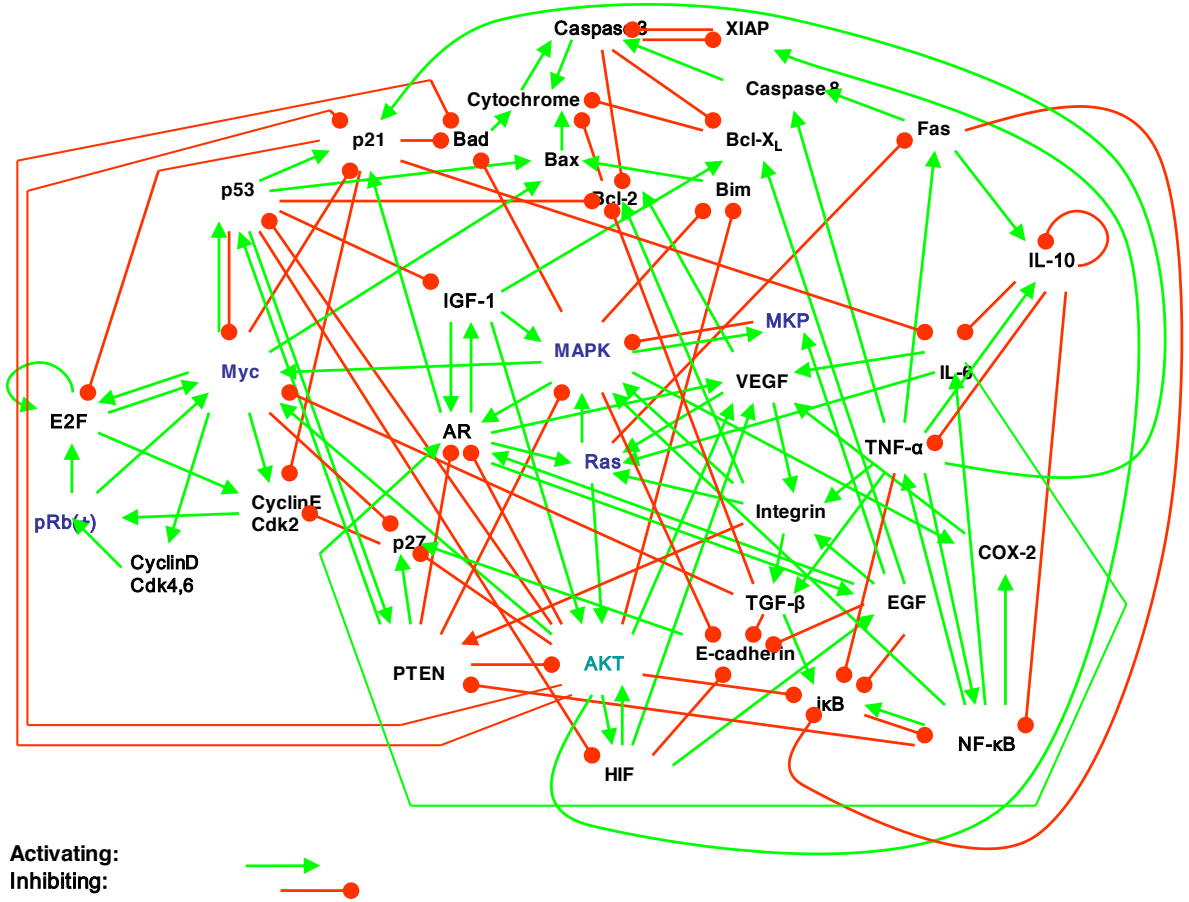
Table I. Molecular interactions from experiments to establish the mathematical model.

From Zhu, Hood, Galas, Ao (2008) (in press)

	Activated(up-regulated, transcribed) by	Inhibited (down-regulated, cleaved) by	Reference
pRb (+)(phosphorylated)	**Cyclin D/Cdk4,6, Cyclin E/Cdk2**		35,36
Cyclin D/Cdk4,6	**Myc**		36,38,43
Cyclin E/Cdk2	**Myc, E2F**	**p21, p27**	35,37-39
Myc	**pRb (+),E2F, Akt, MAPK**	**P53, TGF-β**	42,43
E2F	**E2F, Myc**	**pRb(-), p21**	43
p21	**p53, TNF-α, Androgen R**	**Myc, Akt**	39,40,43
p27	**PTEN, E-cadherin**	**Myc, Akt**	65,105
p53	**Myc, PTEN**	**Akt**	40,43
Caspase3	**Cytochrome c, Caspase8**	**XIAP**	45,46,48,50,51
Cytochrome c	**Caspase3, Bad, Bax**	**Bcl-2, Bcl-xL**	45-49,54,56,57
Caspase8	**Fas, TNF-α**		45,46,50,51
XIAP	**Akt,**	**Caspase3**	45-50
Bcl-2	**VEGF, Integrin**	**Caspase3, p53, TGF-β**	45,46,53
Bcl-xL	**EGF, IGF-1R**	**Caspase3**	45,46,53,61
Bim		**Akt, MAPK**	45,46,53
Bad		**p21, Akt, MAPK**	45,46,53
Bax	**Myc, p53, Bim**		42,45,46,53,55,58
Ras	**VEGF, IL-6, Integrin, Androgen R**		64,83

Endogenous Network:

interacting agents



Endogenous molecular and cellular network including the important regulations responsible for prostate cancer. The network was constructed from experimental data in literature, most of them of normal biological functions (From Zhu, Galas, Hood, Ao (in preparation)).

Cancer Is Fundamentally Stochastic

Effective stochastic differential equations:

$$d\mathbf{X}/dt = \mathbf{f}(\mathbf{X},t) - \mathbf{x}/\tau_0 + \zeta(\mathbf{X},t) \quad (S)$$

$$\mathbf{X} = (X_1, X_2, \ldots, X_n)^{\tau}, \quad \tau\text{: transpose}$$

$f_A(x) = a\,x^m / (1 + a\,x^m)$, sigmoidal (step-like) function

$f_I(x) = 1 - f_A(x) = 1 / (1 + a\,x^m)$

for example, $m = 3$, $a = 10$; $f_A(0) = 0$, $f_A(1) \sim 1$

$N_i = 0$: i^{th} gene has no activity (no corresponding protein);

$N_i = 1$: i^{th} gene has full activity (largest number of protein allowed); $\tau_0 = 1$

Scaled (normalized) dynamical variables to minimize the demand on input.

Gaussian and white noise:

$$\langle \zeta \rangle = 0, \quad \langle \zeta(\mathbf{X},t)\, \zeta^{\tau}(\mathbf{X},t') \rangle = 2\, D(\mathbf{X})\, \delta(t-t')$$

Following the indications from experimental data.

Robustness assumption: Major network properties are **not** fine tuned.

First 10 deterministic part of sde's

$$dx(1)/dt = (10(x^3(3) + x^3(11) + x^3(12))/(1 + 10(x^3(3) + x^3(11) + x^3(12))))\times (1/(1 + 10(x^3(13) + x^3(31)))) - x(1);$$

$$dx(2)/dt = (10(x^3(15) + 0.1)/(1 + 10(x^3(15) + 0.1))) \times (1/(1 + 10(x^3(3)))) - x(2);$$

$$dx(3)/dt = (10(x^3(1) + x^3(14))/(1 + 10(x^3(1) + x^3(14)))) \times (1/(1 + 10(x^3(2)))) - x(3);$$

$$dx(4)/dt = (10(x^3(6) + x^3(8))/(1 + 10(x^3(6) + x^3(8)))) - x(4);$$

$$dx(5)/dt = (10(x^3(5) + x^3(7))/(1 + 10(x^3(5) + x^3(7)))) \times (1/(1 + 10((1-x(4))^3 + x^3(10)))) - x(5);$$

$$dx(6)/dt = (10x^3(7)/(1 + 10(x^3(7)))) - x(6);$$

$$dx(7)/dt = (10(x^3(4) + x^3(5) + x^3(15) + x^3(23))/(1 + 10(x^3(4) + x^3(5) + x^3(15) + x^3(23)))) \times (1/(1 + 10(0.5x^3(9) + 0.2x^3(36)))) - x(7);$$

$$dx(8)/dt = (10(x^3(5) + x^3(7))/(1 + 10(x^3(5) + x^3(7)))) \times (1/(1 + 10(x^3(10)) + x^3(20)))) - x(8);$$

$$dx(9)/dt = 0.31((x^3(7) + x^3(16))/(1 + (x^3(7) + x^3(16)))) \times (1/(1 + 10(x^3(15)))) - x(9);$$

$$dx(10)/dt = (10(x^3(9) + x^3(22) + 0.3x^3(33))/(1 + 10(x^3(9) + x^3(22)) + 0.3x^3(33)))) \times (1/(1 + 10(x^3(7) + x^3(15)))) - x(10);$$

…

x(1) = cxtochrome c

x(2) = XIAP

x(3) = caspase-3

x(4) = pRb(+),
pRb(-)= 1-x(4)

x(5) = E2F (free of pRb-)

x(6) = CyclinD/Cdk4,6

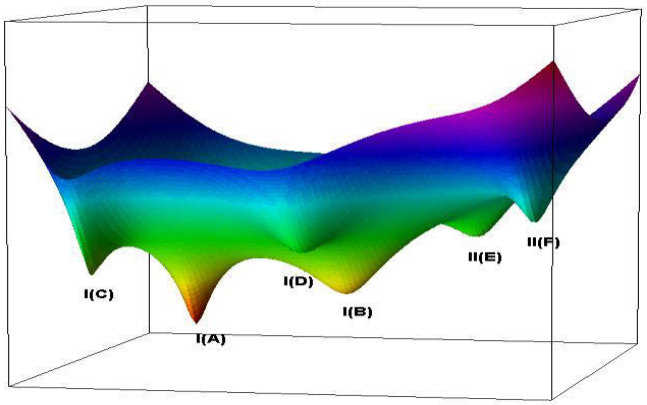
x(7) = Myc

x(8) = CyclinE/Cdk2

x(9) = p53

x(10) = p21

Functional Landscape



Schematic endogenous functional landscape for prostate with 6 main stable functions.
37 dimensions

Main robust states of the network: stable minima in the functional landscape space

The protein profile suggests that II(E) and II(F) are very likely correlated with cancer.

Stable state	Function	Molecular signature
I(A)	Arresting	Cell cycle off. Apoptosis off. Immune off
I(B)	Proliferating	Cell cycle on. Apoptosis off. Immune off.
I(C)	Apoptosis	Cell cycle off. Apoptosis on. Immune off.
I(D)	Apoptosis	Cell cycle on. Apoptosis on. Immune off.
II(E)	Growth with high metabolism	Cell cycle on. Apoptosis off. Immune on.
II(F)	Apoptotic with high metabolism	Cell cycle on. Apoptosis on. Immune on.

Table II. Positions of possible stable states in functional landscape

From Zhu, Hood, Galas, Ao (2009) (in preparation)

Rescaled activity (or expression level) reduces the demand on the parameters

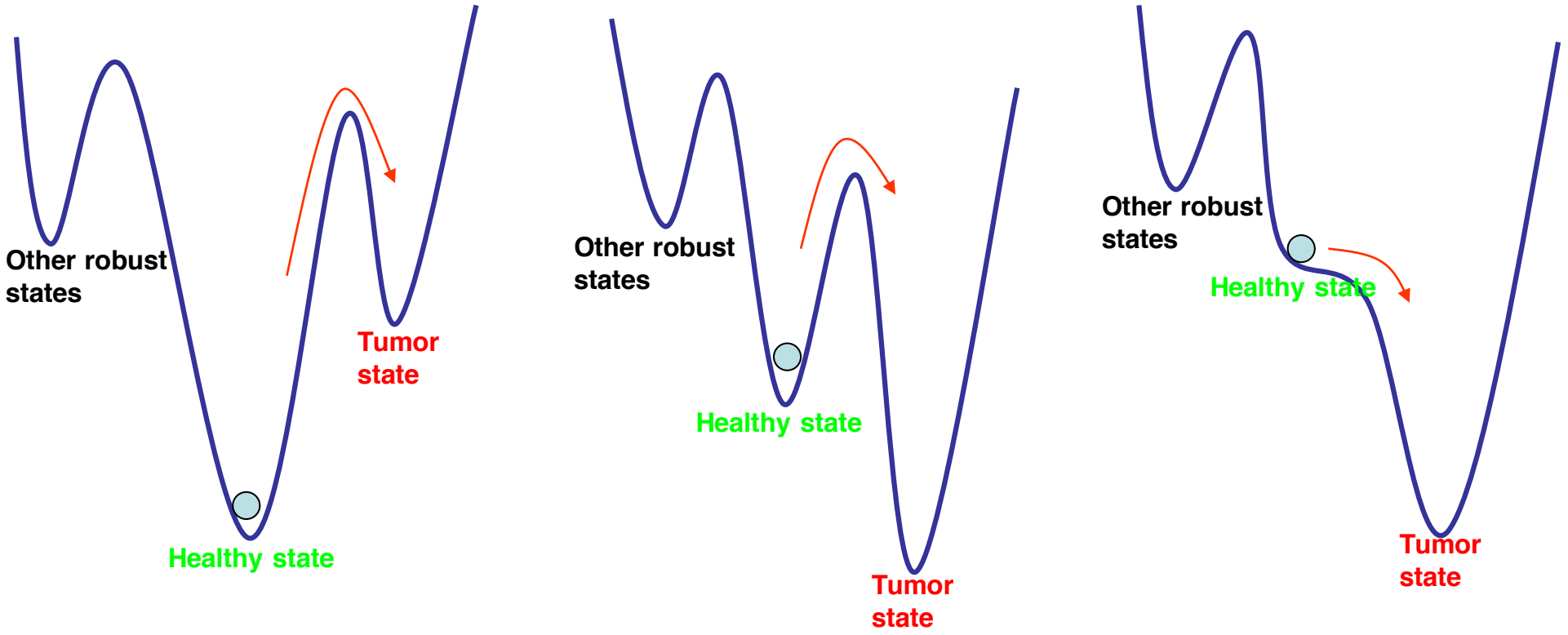
Maximum activity: 1
Minimum activity: 0

	I(A)	I(B)	I(C)	I(D)	II(E)	II(F)
Cytochrome c	0.04	0.08	0.86	0.86	0.00	0.85
XIAP	0.50	0.50	0.07	0.07	0.77	0.10
Caspase 3	0.00	0.00	0.86	0.86	0.09	0.87
pRb(+)	0.00	0.93	0.00	0.93	0.89	0.89
E2F	0.00	0.93	0.00	0.93	0.86	0.86
CyclinD/CDK4,6	0.02	0.86	0.02	0.86	0.59	0.59
Myc	0.13	0.84	0.13	0.84	0.53	0.53
CyclinE/CDK2	0.00	0.92	0.00	0.92	0.84	0.84
p53	0.09	0.16	0.09	0.16	0.01	0.01
p21	0.05	0.01	0.05	0.01	0.11	0.11
Bad	0.26	0.26	0.26	0.26	0.06	0.06
Bax	0.08	0.29	0.08	0.29	0.07	0.07
Bcl-2	0.48	0.47	0.09	0.09	0.19	0.08
Caspase 8	0.04	0.04	0.04	0.04	0.48	0.48
Akt	0.02	0.02	0.02	0.02	0.63	0.63
PTEN	0.74	0.74	0.74	0.74	0.32	0.32
NF-κB	0.26	0.26	0.26	0.26	0.55	0.55
HIF	0.00	0.00	0.00	0.00	0.72	0.72
EGF	0.50	0.50	0.50	0.50	0.79	0.79
p27	0.83	0.12	0.83	0.12	0.16	0.16
iκB	0.60	0.60	0.60	0.60	0.25	0.25
TNF-α	0.16	0.16	0.16	0.16	0.44	0.44
MAPK	0.26	0.26	0.26	0.26	0.51	0.51
Ras	0.18	0.18	0.18	0.18	0.81	0.81

Functional Landscape

(determined by nonlinear interactions within the endogenous network and its interactions to other levels)

of Endogenous Dynamical Network with healthy, tumor, and other robust biological states: three typical situations



I: preventable/curable

II: curable/treatable

III: incurable/untreatable

The vertical scale illustrates the relative stability of robust states, healthy, tumor and others, in the multiple dimensional state space.

The landscape concept follows what reviewed by Zhu, Yin, Hood, Galas, Ao on modeling of lambda genetic switch. in ***Introduction to Systems Biology***, 2000.

A brief discussion of history of landscape concept and its usage in biology can be found in P. Ao, *Laws in Darwinian evolutionary theory*, Physics of Life Reviews **2** (2005) 117-156.

Initial Experimental Validations

- Prostate Cancer

  Consistency among experimental data implies that there may indeed be a core network.

  Consistency with theoretical predictions implies that such mathematical model may indeed be correct.

- Leukemia: CML

- Hepatocellular carcinoma (HCC)

Chronic Myeloid Leukemia

proteins, hormnes, etc	I(A): (noraml)	gene	log(ratio), CP	log(ratio), AP	log(ratio), BC	I(B): (chronic phase)	I(B)-II(E) (accelerated phase)	II(A): (blast crisis)	r: right; w: wrong
Cytochrome c	0. 04	Cytochrome c	0.007500298	-0.173573275	-0.2749898	0. 08	0. 01	0	r r r
XIAP	0. 5					0. 5	0. 6	0. 77	
Caspase 3	0					0	0. 09	0. 09	
pRb(+)	0	RB1	-0.02340131	0.359629581	0.444844091	0. 93	0. 89	0. 89	w r r
E2F	0	E2F5	-0.00806339	0.652361166	0.831233078	0. 93	0. 88	0. 86	w r r
CyclinD/CDK4,6	0. 02		0.003611066	0.303090059	0.559535536	0. 86	0. 68	0. 59	r r r
Myc	0. 13	MYCL1	-0.02968787	-0.391017737	-0.64657657	0. 84	0. 6	0. 53	www
CyclinE/CDK2	0	CDK2AP1	0.000311783	0.27653861	0.313775662	0. 92	0. 8	0. 84	r r r
p53	0. 09	TP53I11	-0.00029215	-0.201807578	-0.65126646	0. 16	0. 05	0. 01	w r r
p21	0. 05	PAK4	0.007022029	-0.223846794	-0.1879491	0. 01	0. 11	0. 11	www
Bad	0. 26					0. 26	0. 11	0. 06	
Bax	0. 08					0. 29	0. 1	0. 07	
Bcl-2	0. 48	BCL2	0.017421845	0.399760103	0.609259831	0. 47	0. 24	0. 19	r ww
Caspase 8	0. 04					0. 04	0. 34	0. 48	
Akt	0. 02	FLJ10111	0.014376216	-0.11855937	-0.19535138	0. 02	0. 37	0. 63	r ww
PTEN	0. 74	PINK1	-0.02884286	-0.170412126	-0.49640671	0. 74	0. 46	0. 32	r r r
NF-κB	0. 26					0. 26	0. 43	0. 55	
HIF	0					0	0. 34	0. 72	
EGF	0. 5	EGFR-RS	0.010144436	0.330470246	0.612509998	0. 5	0. 59	0. 79	r r r
p27	0. 83					0. 12	0. 22	0. 16	
ikB	0. 6	IKBKG	-0.00940305	-0.33163751	-0.34250343	0. 6	0. 42	0. 25	r r r
TNF-α	0. 16	TNFSF4	-0.01035776	0.977510388	0.847869808	0. 16	0. 37	0. 44	r r r
MAPK	0. 26		0.009285026	0.270846089	0.433078557	0. 26	0. 49	0. 51	r r r
Ras	0. 18	NRAS	0.028317121	0.260572439	0.407931981	0. 18	0. 73	0. 81	r r r
COX-2	0. 26	COX11	0.020621697	0.55757579	0.583467024	0. 26	0. 66	0. 74	r r r
VEGF	0. 19	VEGFB	0.017184631	0.118985275	0.272996986	0. 19	0. 83	0. 93	r r r
IL-6	0. 16					0. 16	0. 37	0. 44	
IL-10	0. 04					0. 04	0. 28	0. 34	
Fas	0. 04	FAIM	0.008222487	0.350064521	0.740909031	0. 04	0. 15	0. 18	r r r
Bim	0. 26					0. 26	0. 11	0. 06	
Bcl-xL	0. 56					0. 56	0. 69	0. 87	
Integrin	0. 39					0. 39	0. 57	0. 65	
Androgen R	0. 13					0. 13	0. 36	0. 54	
IGF-1R	0. 02					0. 02	0. 31	0. 61	
MKP	0. 56	DUSP10	0.037154577	0.284152541	0.645319979	0. 56	0. 7	0. 74	r r r
TGF-β	0. 37	YR-29	0.019577459	0.366250242	0.62084987	0. 37	0. 65	0. 73	r r r
E-Cadherin	0. 36	PCDHGC4	0.000148711	-0.220939937	-0.27218245	0. 36	0. 15	0. 07	r r r

Note 1: For biology in our cancer modeling, Ao, Galas, Hood, and Zhu, 2008, Cancer as robust intrinsic state of endogenous molecular-cellular network shaped by evolution. Medical Hypotheses, **70**: 678-684.
Note 2: Predictions are normalized values for each endogenous agent respectively. Should comparing them horizontally. Full activity 1; no activity 0.
Note 3: Predictions were from results obtained by Zhu, Hood, Galas, Ao for prostate cancer (submitted to J. Biol. Syst., 2008)
Note 4: June, 2008. Ping's attempt to understand Jerry Radich's 2006 PNAS chronic myeloid leukemia paper (Radich et al, PNAS **103** (2006) 2794-2799). Here, Data were from Table 4.

Core endogenous network of liver tissue

minimal molecular-cellular agents, core signaling transduction and transcription pathways

Cell Cycle
cyclin D-CDK4/6, cyclin E-CDK2, Rb, E2F, p21

Cell Death
Bcl-2, tBid, XIAP, Bax, cytchrome c, caspase 9, caspase 8, caspase 3

Liver specific modules
FOXA2, HNF4a, C/EBP, HGF

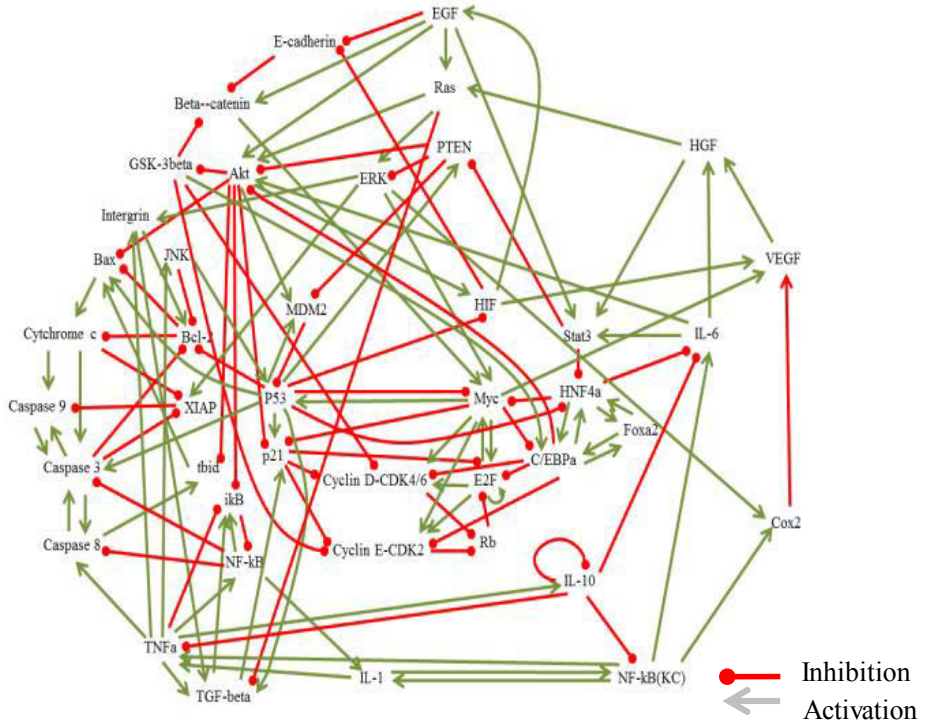
Immune response
NF-kB, TNFa, IL-1
IL-6, stat3, IL-10

Metabolism
HIF, Myc, Akt

Angiogenes
VEGF

Cell adhesion
TGF-bete, E-cadherin, Integrin

Modularization



Core endogenous molecular-cellular network for liver

Validation 1: hallmarks of each stable state

Stable states

	A	B	C	D	E
X1=cyclin D-CDK4	0.0000	0.8781	0.8709	0.0000	0.8781
X2=cyclin E-CDK2	0.0000	0.8781	0.8709	0.0000	0.8781
X3=Rb	1.0000	0.0688	0.0704	1.0000	0.0688
X4=E2F	0.0000	0.8923	0.8851	0.0000	0.8923
X5=P21	0.0000	0.0454	0.0533	0.0000	0.0454
X6=C/EBPa	0.9642	0.0076	0.0092	0.9642	0.0076
X7=Foxa2	0.9457	0.0000	0.0000	0.9457	0.0000
X8=HNF4a	0.9457	0.0000	0.0000	0.9457	0.0000
X9=Bcl-2	0.0000	0.4962	0.2049	0.0000	0.0878
X10=tBid	0.0000	0.0000	0.0076	0.8785	0.1095
X11=XIAP	0.0000	0.8669	0.8660	0.0000	0.0950
X12=Bax	0.0000	0.0464	0.0597	0.8715	0.0539
X13=Cytchrome c	0.0000	0.0004	0.0020	0.8687	0.0016
X14=Caspase 9	0.0000	0.0000	0.0000	0.9387	0.8828
X15=Caspase 8	0.0000	0.0000	0.1857	0.8975	0.8904
X16=Caspase 3	0.0000	0.0000	0.0181	0.9566	0.9331
X17=Ras	0.0000	0.8953	0.8902	0.0000	0.8953
X18=ERK	0.0000	0.8669	0.8648	0.0000	0.8669
X19=JNK	0.0000	0.0000	0.6173	0.0000	0.0000
X20=Akt	0.0000	0.8880	0.8857	0.0000	0.8880
X21=PTEN	0.0000	0.1075	0.1084	0.0000	0.1075
X22=HIF	0.0000	0.5008	0.4549	0.0000	0.5008
X23=Myc	0.0000	0.5331	0.4822	0.0000	0.5331
X24=P53	0.0000	0.4212	0.4517	0.0000	0.4212
X25=EGF	0.0000	0.5567	0.4850	0.0000	0.5567
X26=TGF-beta	0.0000	0.1075	0.1124	0.0000	0.1075
X27=E-cadherin	1.0000	0.2512	0.3244	1.0000	0.2512
X28=b-catenin	0.0000	0.5374	0.3914	0.0000	0.5374
X29=GSK3	1.0000	0.1250	0.1258	1.0000	0.1250
X30=Integrin	0.0000	0.8669	0.8899	0.0000	0.8669
X31=HGF	0.0000	0.8804	0.8866	0.0000	0.8804
X32=TNFa	0.0000	0.0000	0.5443	0.0000	0.0000
X33=NF-kB	0.0000	0.0000	0.6148	0.0000	0.0000
X34=NF-kB(KC)	0.0000	0.0000	0.5236	0.0000	0.0000
X35=IL-1	0.0000	0.0000	0.7899	0.0000	0.0000
X36=IL-6	0.0000	0.0000	0.3713	0.0000	0.0000
X37=IL-10	0.0000	0.0000	0.3888	0.0000	0.0000
X38=Stat3	0.0000	0.8953	0.8961	0.0000	0.8953
X39=Cox-2	0.0000	0.8669	0.8877	0.0000	0.8669
X40=VEGF	0.0000	0.0026	0.9006	0.0000	0.9028
X41=MDM2	0.8889	0.3504	0.9333	0.8889	0.8889
X42=ikB	0.8889	0.0015	0.0855	0.8889	0.8889

Model:

	Quiescence	Proliferation		Death	
	A	(B, C)		(D, E)	
Cell cycle	Off	On	On	Off	On
Glycolysis	Off	On	On	Off	On
Liver specific function	On	Off	Off	On	Off
Cell death	Off	Off	Off	On	On
Cell adhesion	On	Off	Off	On	Off
Immune response	Off	Off	On	Off	Off
Angiogenesis	Off	Off	On	Off	On

Physiological and clinical:

	Normal Liver	Cancer
Cell cycle	Off	On
Glycolysis	Off	On
Liver specific function	On	Off
Cell death	Off	Off
Cell adhesion	On	Off
Immune response	Off	On
Angiogenesis	Off	On

Hanahan, D. and Robert A. Weinberg (2011). "Hallmarks of Cancer: The Next Generation."

Perfect match: stable state A as normal liver; C as HCC

Validation 2: Molecular-cellular agents test

Stable states **(model)**

	Normal	HepG2	HCC
cyclin D-CDK4	0.0000	0.8781	0.8709
cyclin E-CDK2	0.0000	0.8781	0.8709
Rb	1.0000	0.0688	0.0704
E2F	0.0000	0.8923	0.8851
P21	0.0000	0.0454	0.0533
C/EBPa	0.9642	0.0076	0.0092
Foxa2	0.9457	0.0000	0.0000
HNF4a	0.9457	0.0000	0.0000
Bcl-2	0.0000	0.4962	0.2049
Bid	0.0000	0.0000	0.0076
XIAP	0.0000	0.8669	0.8660
Bax	0.0000	0.0464	0.0597
Cytchrome c	0.0000	0.0004	0.0020
Caspase9	0.0000	0.0000	0.0000
Caspase8	0.0000	0.0000	0.1857
Caspase3	0.0000	0.0000	0.0181
Ras	0.0000	0.8953	0.8902
ERK	0.0000	0.8669	0.8648
JNK	0.0000	0.0000	0.6173
Akt	0.0000	0.8880	0.8857
PTEN	0.0000	0.1075	0.1084
HIF	0.0000	0.5008	0.4549
Myc	0.0000	0.5331	0.4822
P53	0.0000	0.4212	0.4517
EGF	0.0000	0.5567	0.4850
TGF-beta	0.0000	0.1075	0.1124
E-cadherin	1.0000	0.2512	0.3244
b-catenin	0.0000	0.5374	0.3914
GSK3	1.0000	0.1250	0.1258
Integrin	0.0000	0.8669	0.8899
HGF	0.0000	0.8804	0.8866
TNFa	0.0000	0.0000	0.5443
NF-kB	0.0000	0.0000	0.6148
NF-kB(KC)	0.0000	0.0000	0.5236
IL-1	0.0000	0.0000	0.7899
IL-6	0.0000	0.0000	0.3713
IL-10	0.0000	0.0000	0.3888
Stat3	0.0000	0.8953	0.8961
Cox-2	0.0000	0.8669	0.8877
VEGF	0.0000	0.9028	0.9006
MDM2	0.8889	0.3504	0.9333
ikB	0.8889	0.0015	0.0855

Normal liver and HCC

	Gene symbol	Model	Experiments	HCC1	HCC2	HCC3
Cyclin D-CDK4/6	CCND1	Up	Up	Up	Up	Up
Cyclin E-CDK2	CCNE1	Up	Up	Up	Up	Up
Rb	Rb1	Down	Down	Up	Down	Down
E2F	E2F4	Up	Up	Up	Up	Up
P21	CDKN1A	Unchange	Down	Up	Up	Up
C/EBPa	CEBPA	Down	Down	Up	Down	Down
Foxa2	FOXA2	Down	Down	Up	Down	Down
HNF4a	HNF4A	Down	Down	Down	Down	Down
Bcl-2	BCL2	Up	Up	Down	Un	Up
tBid	BID	Unchange	Down	Down	Down	Down
XIAP	XIAP	Up	Up	Up	Up	Up
Bax	BAX	Unchange	Down	Up	Down	Down
Cytchrome c	CYCS	Unchange	Unchange	Up	Un	Un
Caspase 9	CASP9	Unchange	Unchange	Up	Up	Un
Caspase 8	CASP8	Up	Unchange	Up	Up	Up
Caspase 3	CASP3	Unchange	Unchange	Down	Up	Down
Ras	KRAS	Up	Up	Up	Up	Down
ERK	MAPK1	Up	Up	Up	Up	Down
JNK	MAPK8	Unchange	Up	Down	Down	Up
Akt	AKT3	Up	Up	Up	Up	Up
PTEN	PTEN	Down	Down	Down	Down	Un
HIF	HIF1A	Up	Up	Up	Up	Un
Myc	MYC	Up	Up	Up	Up	Up
P53	TP53	Up	Down	Down	Up	Down
EGF	EGFR	Up	Up	Up	Up	Up
TGF-β	TGFB2	Up	Up	Up	Up	Up
E-cadherin	CDH1	Down	Down	Down	Down	Down
β-catenin	CTNNB1	Up	Up	Up	Un	Up
GSK-3beta	GSK3B	Down	Down	Down	Down	Down
Integrin	ITGB2	Up	Up	Up	Up	Up
HGF	HGF, MET	Up	Up	Up	Up	Up
TNFa	TNF	Up	Up	Up	Down	Up
NF-kB	NFKB1,RELA	Up	Up	Down	Up	Up
NF-kB(KC)	NFKB1,RELA	Up	Up	Down	Up	Up
IL-1	IL1B,IL1BR1	Up	Up	Up	Up	Up
IL-6	IL6, IL6R	Up	Up	Up	Up	Up
IL-10	IL10, IL10R	Up	Up	Up	Up	Up
Stat3	STAT3	Up	Up	Up	Up	Up
Cox-2	COX2	Up	Up	Up	Up	Up
VEGF	KDR	Up	Up	Un	Up	Down
MDM2	MDM2	Up	Up	Up	Up	Up
ikB	NFKBIA	Down	Down	Un	Up	Un

86% 72% 76% 71%

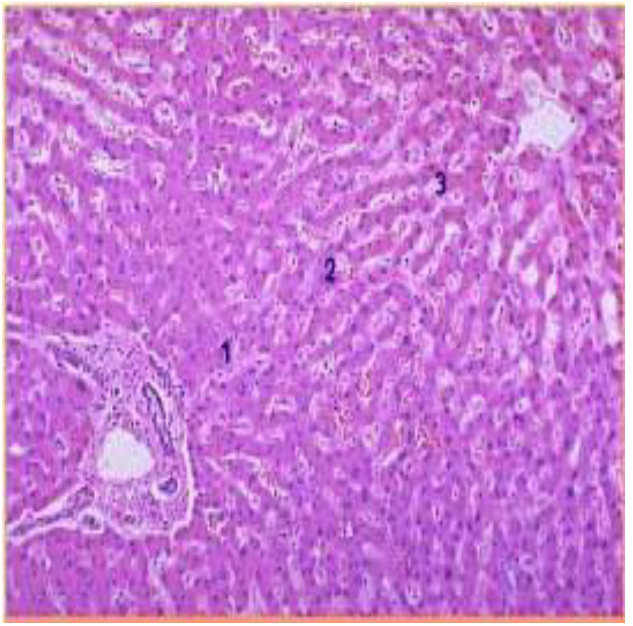
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33006,2012; 3-paired HCC tissue samples and adjacent normal liver tissues

Hepatocellular carcinoma and normal state

HCC state

Oncogenes (Ras, Myc)

Regression probability? Defined factors?

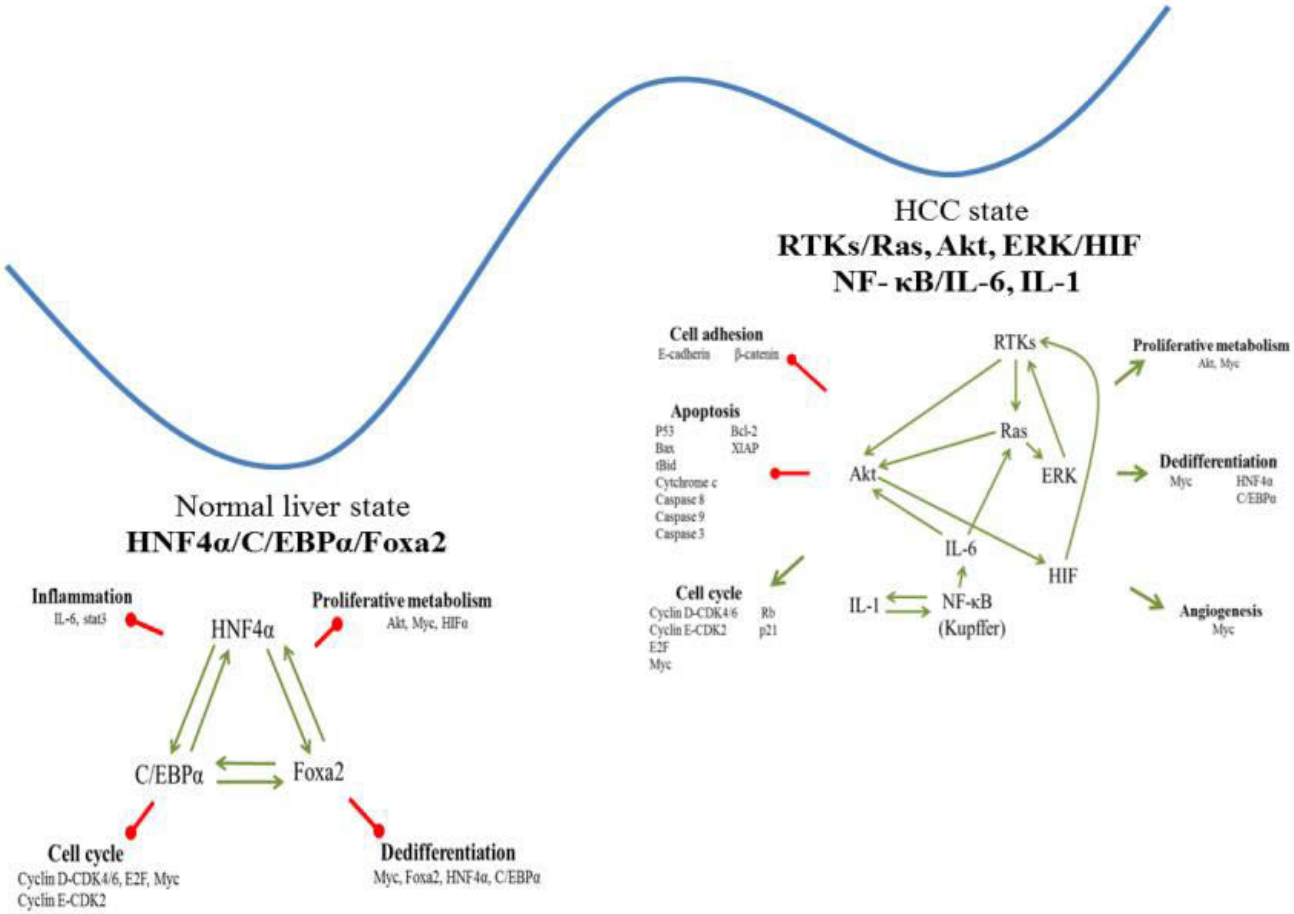


Normal liver state

Spontaneous regression of hepatocellular carcinoma and review of literature.
Journal of Gastroenterology and Hepatology, 2000 15:1079-1086

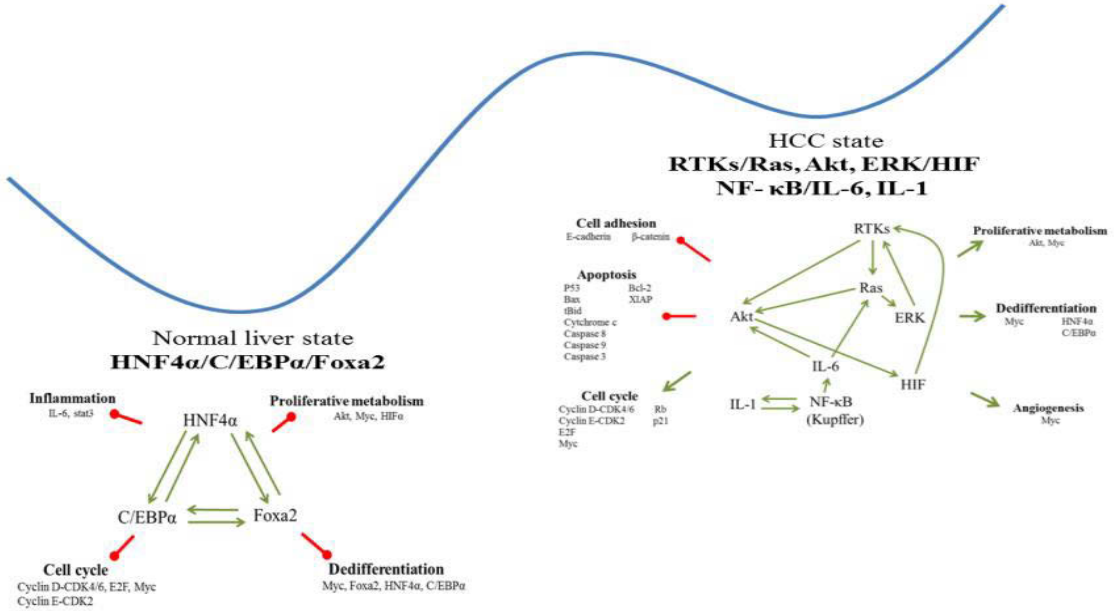
Can we induce HCC state to normal-like liver state by defined factors? How?

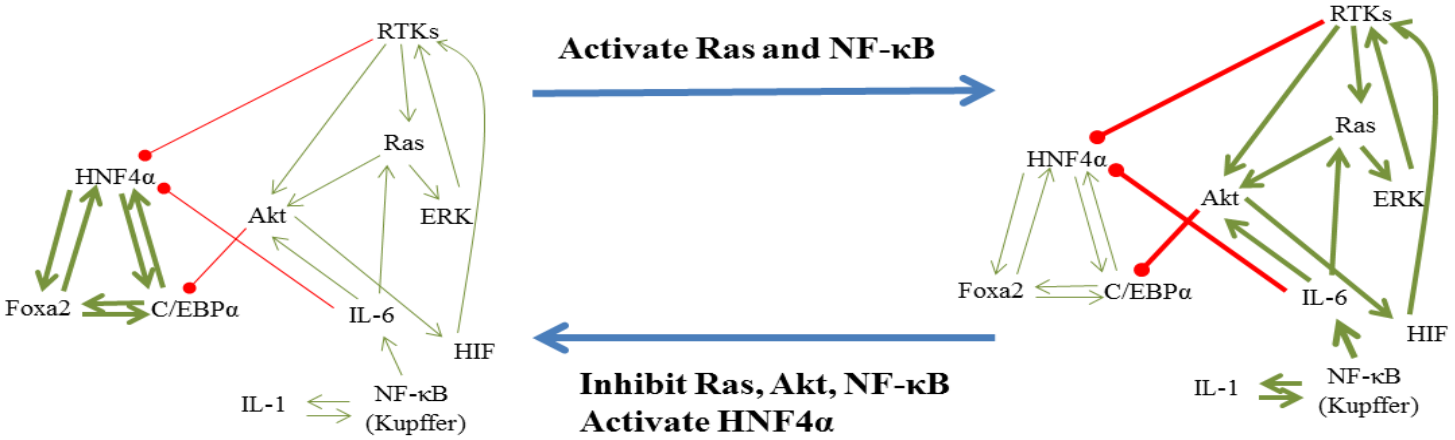
Predications: maintenance of stable states at molecular level



Positive feedback loops provide a general strategy for the establishment and maintenance of stable states

Induce HCC to normal liver by defined factors



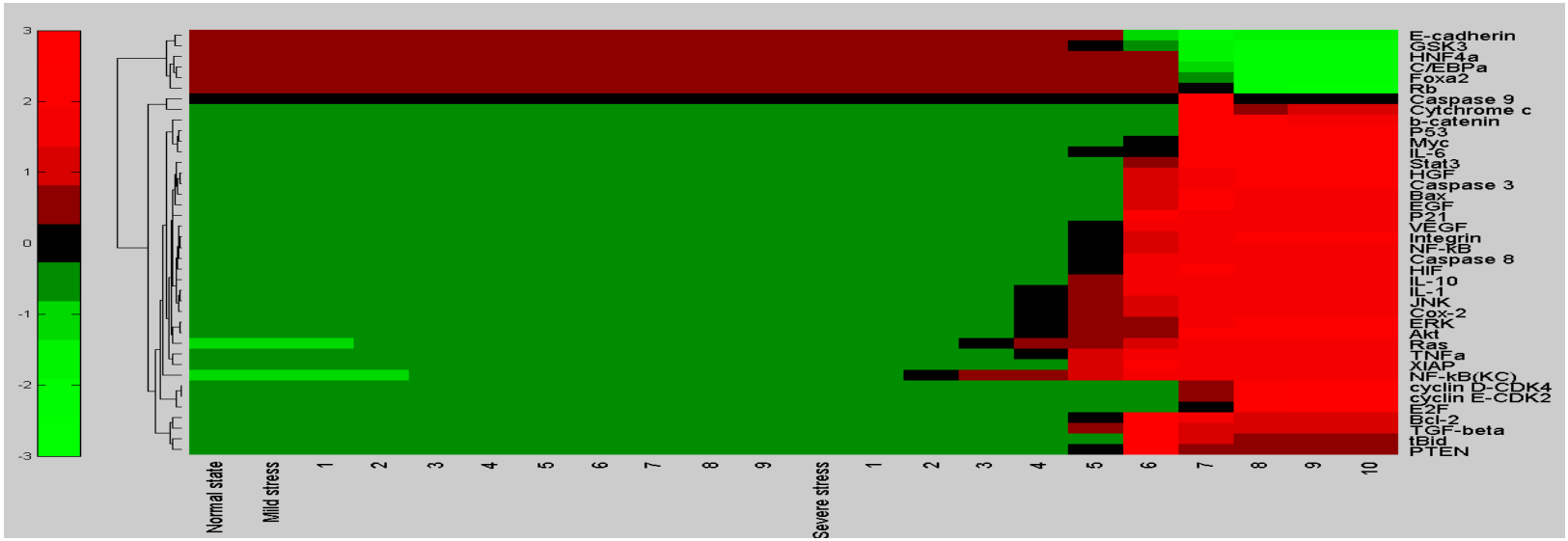


Induce HCC to normal by inhibiting **Ras, Akt, NF-κB**, and activating **HNF4α**

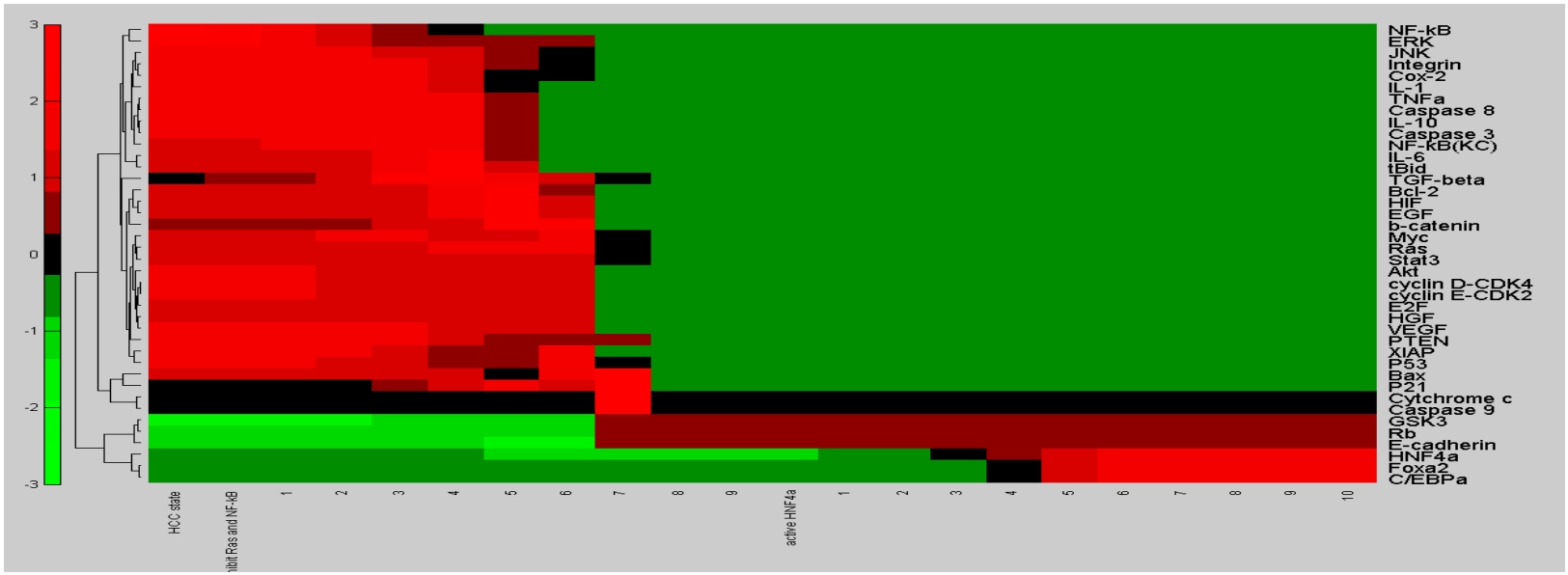
Inhibit cell proliferation and inflammation, induce differentiation

Dynamics of HCC progression and regression

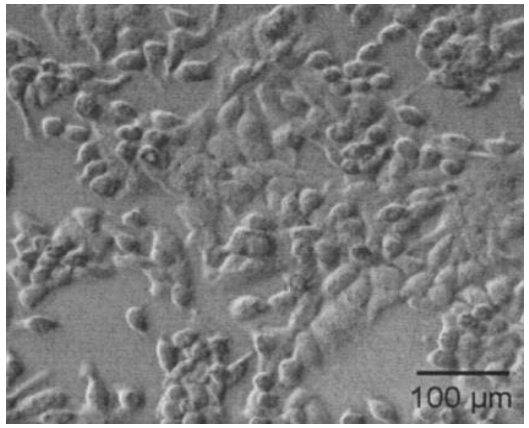
Progression



Regression



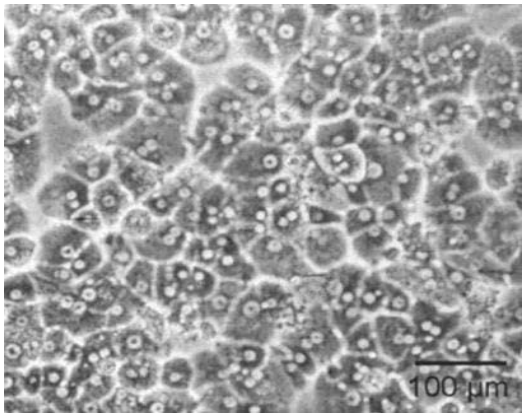
Summary for a set of testable predictions



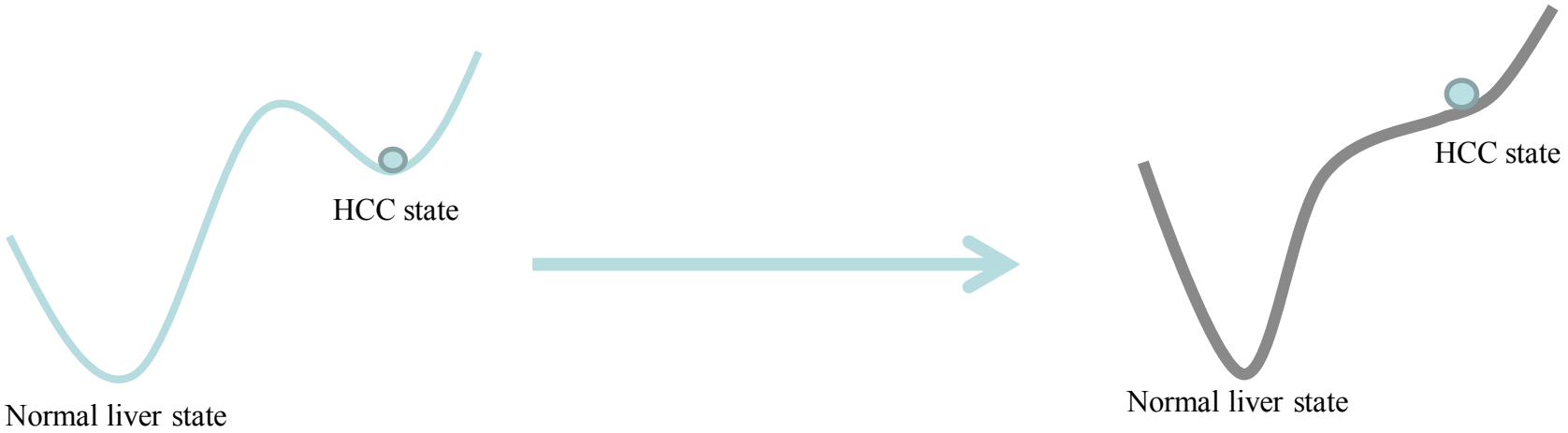
HCC state

Ras, NF-κB

Ras, Akt, NF-κB, HNF4α



Normal liver state



HCC progression and regression are asymmetric
HCC may be induced to normal-liker liver by defined factors

More cancer problems addressable

- **What are the essential features that a mathematical model for cancer must possess?**
  (Hanahan and Weinberg, 2000; Varmus, 2006; Feinberg, Ohlsson, Henikoff, 2006; Sporn, 2006;)
- **Does the basic mathematical model already contain them?**
- **How are they explained?**
  (dormancy; "double" hit *vs* multi-steps; androgen withdraw; double edge/context dependence/multiple roles; as unhealed wound; as aberrant developmental process; as survival of stressed unicellular organisms;)
- **Are there any obvious contradictions/difficulties to explain well established observations?**
- **What are the major controversial features in current cancer theories?**
  (single cell progenitor vs stem cell; more benign tumors at advanced age; spontaneous appearing and disappearing of lesions; drug resistance; exercise (simulating annealing?); sleeping/ circadian (elevated temperature?);)
- **How can those issues be addressed within the basic mathematical model?**
- **What are the most pronounced predictions which can be tested experimentally to demonstrate its distinct character and/or predictable power?**
  Very plausible prediction with the existence of functional landscape: Cancer-like cells (or neoplasia?) with NO genetic defect can arise. The only difference may be in protein and/or epigenetic profile.
  An entry point:
- **What are the most wanted features from a medical point of view to prove its utility?**
- **Are there any insights/suggestions on prevention, diagnostics, and cure/care?**
- **Is the basic mathematical model scalable/extendable?**
- **Are there requirements for interrogating technologies?**
  (high throughput and high sensitivity; DNA, protein, function/physiology; real time; single cell; bioinformatics;)

Mathematical Question:
existence of potential function

- **Biology:**
  " … the idea that there is such a quantity (adaptive landscape—P.A.) remains one of the most widely held popular misconceptions about evolution".
  S.H. Rice, in *Evolutionary Theory: mathematical and conceptual foundations* (2004)
- **Chemistry**:
  "The search for a generalized thermodynamic potential in the nonlinear range has attracted a great deal of attention, but these efforts finally failed."
  G. Nicolis in *New Physics*, pp332 (1989)
- **Physics**:
  "Statistical physicists have tried to find such a variational formulation for many years because, if it existed in a useful form, it might be a powerful tool for the solution of many kinds of problems. My guess … is that no such general principle exists."
  J. Langer in *Critical Problems in Physics*, pp26 (1997)
  and, check recent issues of Physics Today, Physical Review Letters, …
- **Mathematics**:
  gradient *vs* vector systems, unsolved (Holmes, 2006)
  dissipative, $\nabla \bullet \mathbf{f} \neq 0$ **; asymmetric,** $\nabla \times \mathbf{f} \neq 0$ **(absence of detailed balance) ; nonlinear ;**
  stochastic with multiplicative noise
- **Economy (econophysics), finance, engineering, …**

- **We have found a general solution (similar results have been obtained by several other groups):**
  C. Kwon, P. Ao, and D.J. Thouless. Proc. Nat'l Acad. Sci. (USA) **102** (2005) 13029-13033.
  R.S. Yuan, and P. Ao. J. Stat. Mech. (2012) P07010.

Biological Question:
Quantitative Evidence

- **Answer from stability puzzle of phage lambda genetic switch**

  X.-M. Zhu, L. Yin, L. Hood, and P. Ao,

  Journal of Bioinformatics and Computational Biology 2: 885-817 (2004).

- We obtained two major predictions: cooperation energy and extrinsic noise
- Genetic and environmental factors are quantitatively described.

  (There are lots of recent and related experimental works to support them.)

Phage	Lysogenization frequency	Relative CI level in lysogenic state	Relative Cro level in anti-immune state	Fraction switched to lytic state
	Theoretical (experimental)	Theoretical (experimental)	Theoretical	Theoretical (experimental)
λ^+	**90% (63%)**	**100% (100%)**	**100%**	**5×10^{-7} (4×10^{-7})**
$\lambda O_R 121$	**70% (57%)**	20% (25-30%)	100%	**3×10^{-6} (3×10^{-6})**
$\lambda O_R 323$	10% (33%)	70% (60-75%)	70%	2×10^{-5} (2×10^{-5})
$\lambda O_R 3'23'$	80% (60%)	50% (50-60%)	140%	5×10^{-7} (5×10^{-7})

Acknowledgements

Co-workers:

- Xiaomei Zhu, GenMath, Seattle, USA
- Lan Yin, Beijing Univ., Beijing, P.R. China

- Prof. Chen Zhu's lab, leukemia
- Prof. Gu Jianren's lab, liver cancer

- Lee Hood, Inst. Systems Biology, Seattle
- David Galas, Inst. Systems Biology, Seattle

- David Thouless, Physics, Univ. Washington
- Hong Qian, Applied Math, Univ. Washington
- Chulan Kwon, Myongji Univ., S. Korea

Funding:

- Institute for Systems Biology
- USA **NIH**
- China: 985, 973

Thank You!