Cancer Evolution Games

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KITP
Cooperation and the Evolution of Multicellularity
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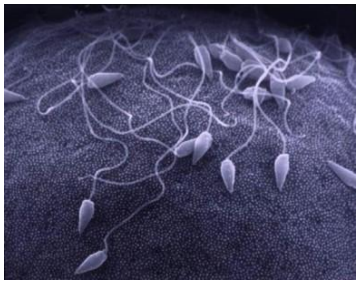
Key Biological Questions

Development: How does Fertilized Egg Self-Organize into an Organism **without** a road map or plan?



→

http://www.stanford.edu/group/Urchin/LP/
[Lauren Palumbi]

http://www.kvarkadabra.net/images/articles/Regeneracija-organov_1_original.jpg

Homeostasis: How does an Organism Maintain itself without an absolute standard of reference?

→

Key Biological Questions

Developmental Diseases: How does Failure of Homeostasis Lead to Redeployment of Developmental Mechanisms in Pathological Ways?

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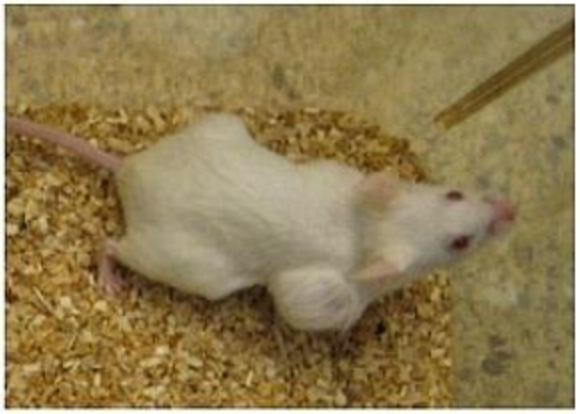
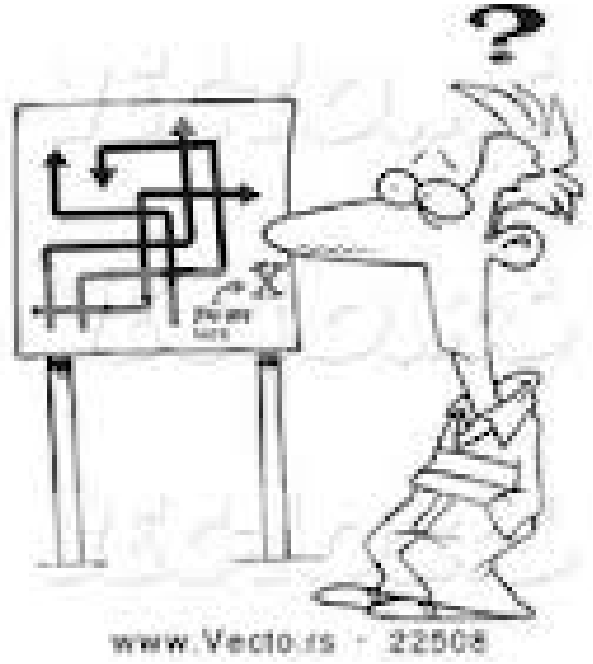


e.g., liver cirrhosis, cancer, diabetic retinopathy, polycystic kidney disease, osteoporosis,..

Effect on Organism of Up or Down-Regulating a Gene in a Cell



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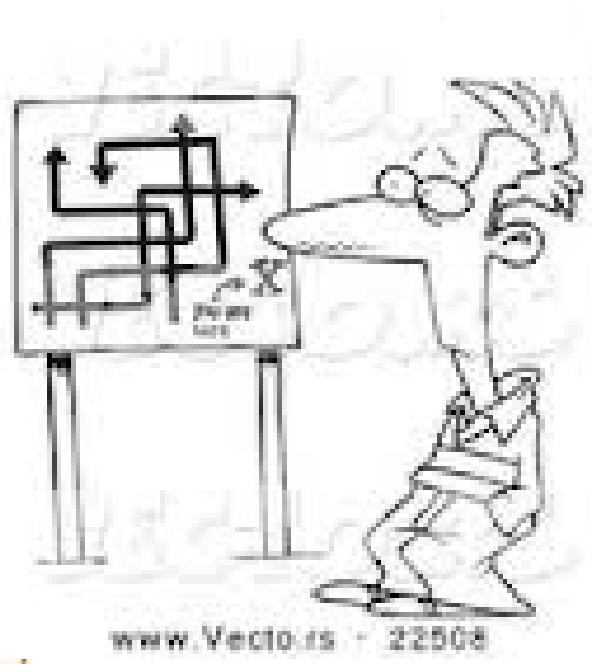

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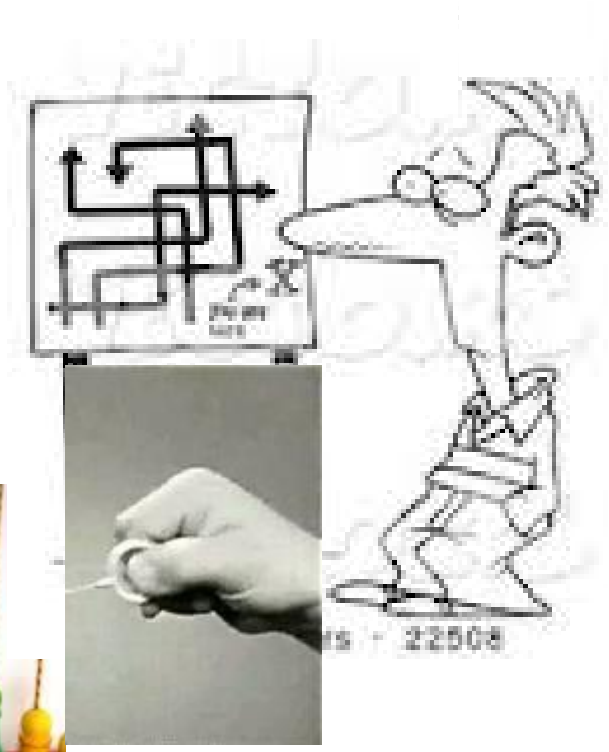


Effect on Organism of Up or Down-Regulating a Gene in a Cell

Effect on Organism of Up or Down-Regulating a Gene in a Cell

“Nature has a Criminal Mind”

Prof. Marcelo Magnasco, Rockefeller University

Motivation for Study of Cancer

- Cancers are a very complex spectrum of diseases
- Many therapies make cancers more aggressive and may lead to reduced survival
- Therapies are often highly toxic to non-cancer cells (side effects)
- Therapies are often ultimately ineffective
- Cancer is currently treated without sufficient fundamental biological understanding
- Molecular and genetic approaches to developing novel cancer therapies have had limited success
- Cancer and therapies involve many systems and components interacting in complex patterns of feedback

- **Will restrict to solid tumors in this talk**

Where Do We Start?

Can’t model everything in detail

Key Motivation for Multicell Modeling

Cells: Know Their Internal State
Respond to Local Environment
Remodel their Environments
Change their Own Behaviors

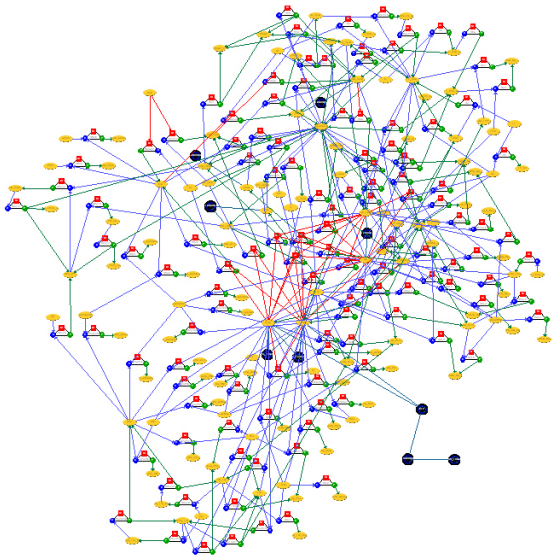
Cells have no Roadmap
Cells don't know they are in an organism

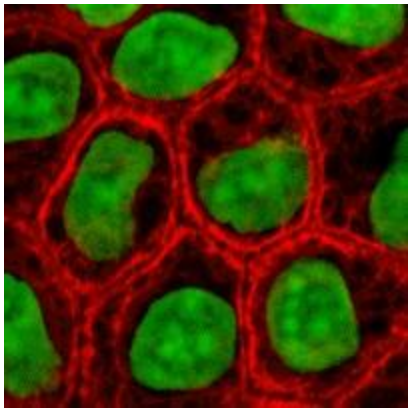
http://reslife.tamu.edu/images/maps/map3.gif

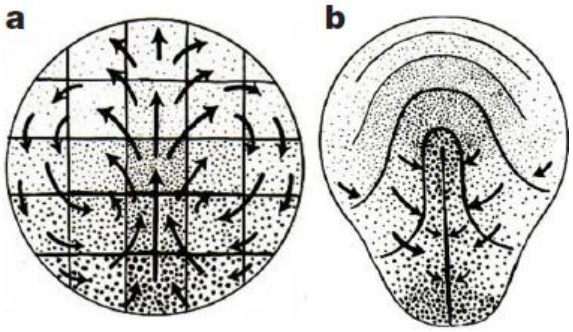
Unlike an Airplane, Procedural Control (Programs) are Rare—Almost All Structures and Behaviors are Emergent (Self Organized)!

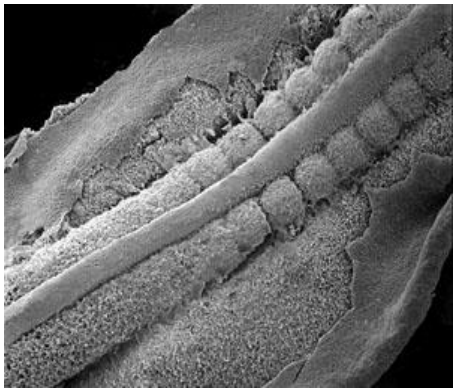
Multicell Modeling

- Separate Analysis into Three Components
  - From molecular event to molecular network behavior
  - From network behavior to cell behaviors
  - From cell behavior to tissue behaviors

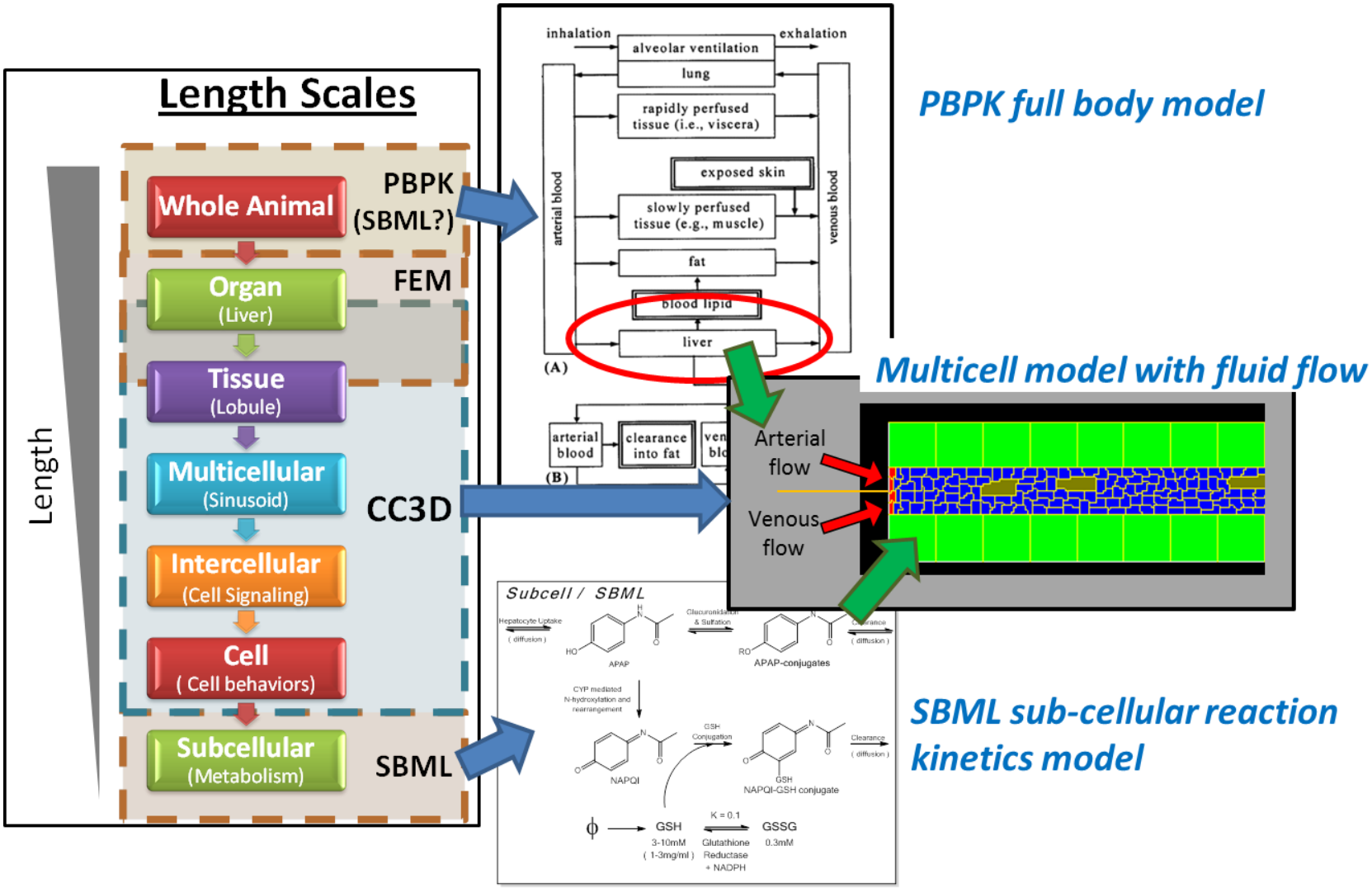








Multiscale, Multicell Virtual Tissue

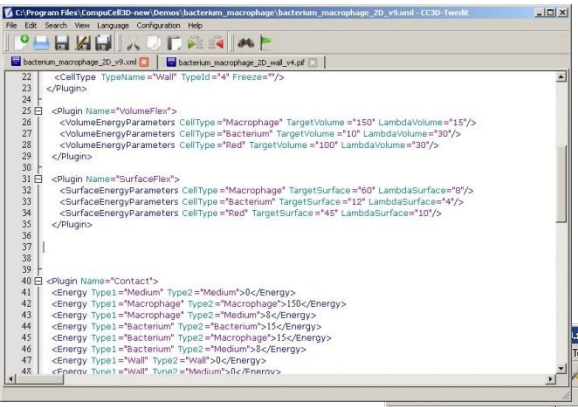


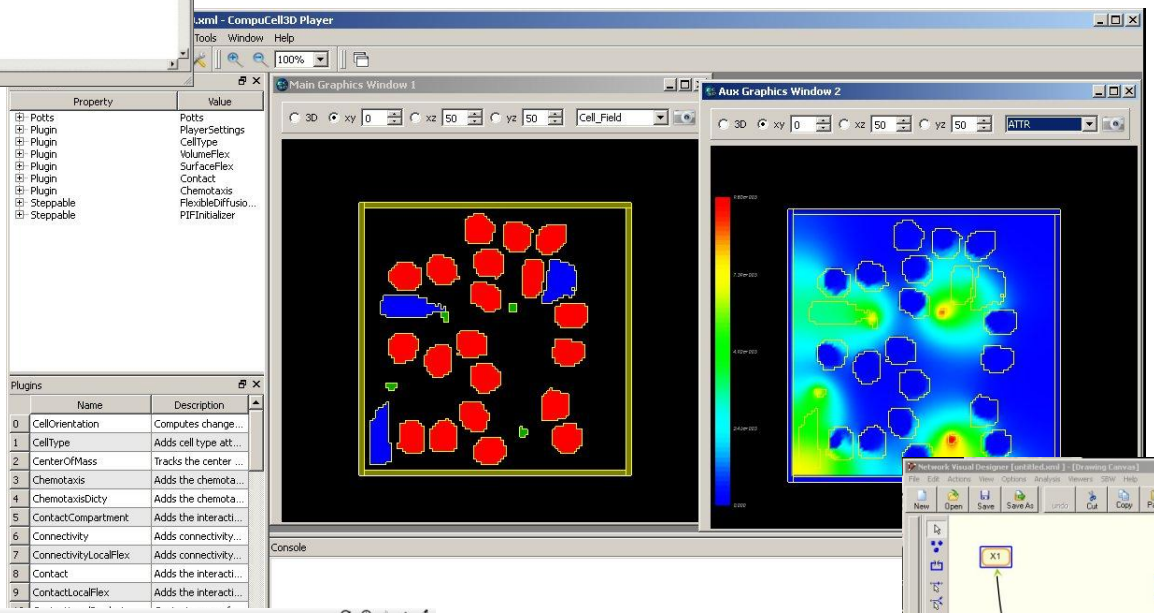
CompuCell3D - Simulation Environment for Multi-Cell, Multi-Scale Models

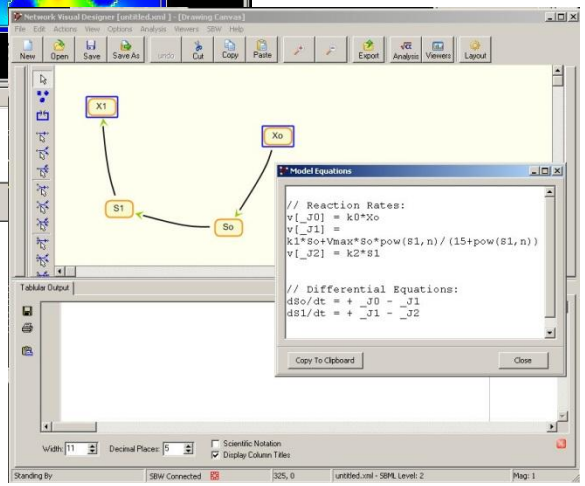
Python and XML model scripting

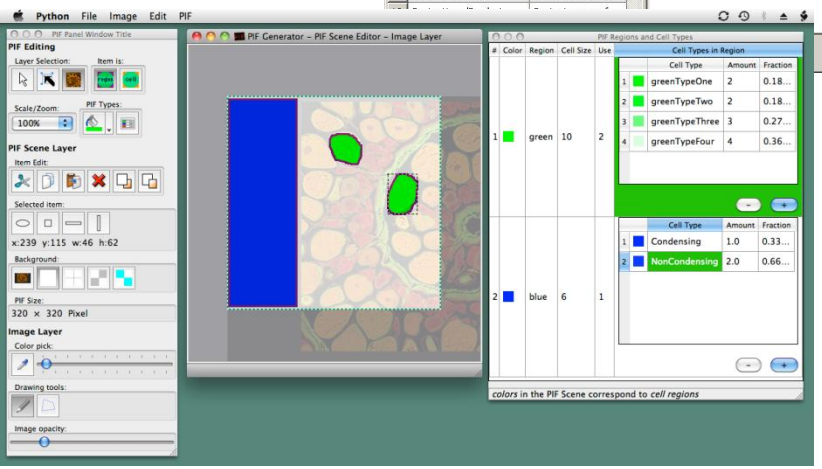
Graphical specification of initial conditions

SBML models (e.g. defined using SBW)









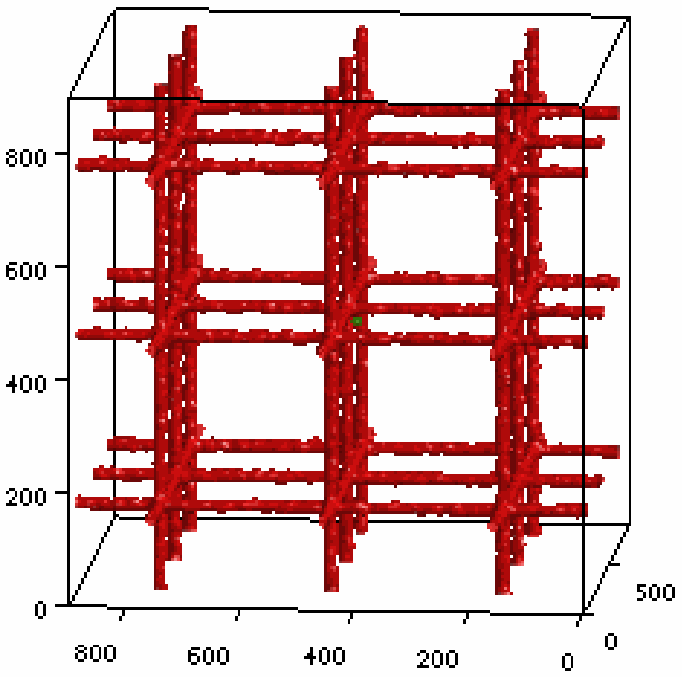
www.compucell3d.org

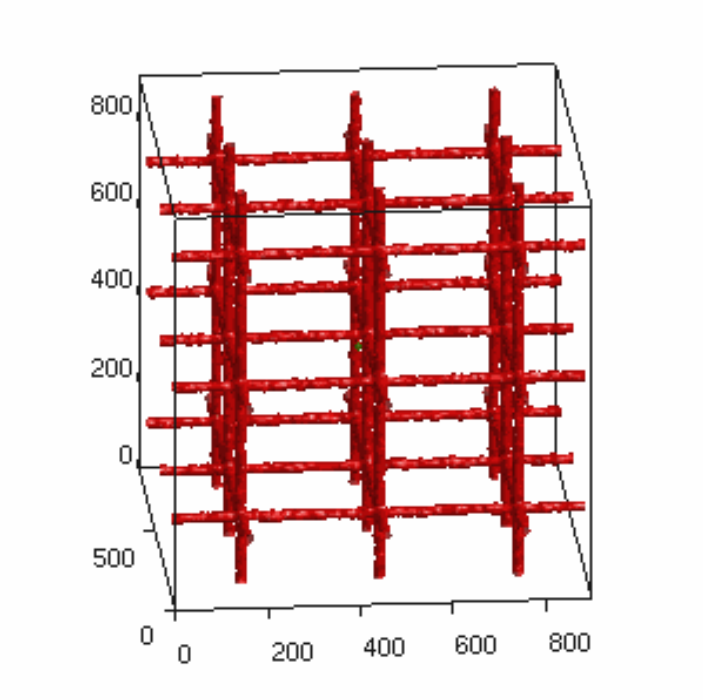
Available Mechanisms in CompuCell3D

- Dynamics of Intracellular and Intercellular Biochemical Signaling, Metabolic and Regulatory Networks (RK)
- Whole Organism, Physiologically-based Pharmacokinetic Modeling (PBPK)
- Reaction, Decay and Diffusion of Soluble Components (PDEs)
- Elastic, Viscoelastic and Plastic Materials (FE)
- Fluid Flows (GGH)
- Cell Behaviors (GGH)
  - Cell Adhesion
  - Membrane Areas
  - Mitosis
  - Apoptosis
  - Chemotaxis
  - Haptotaxis
  - Viscosity
  - Filopodia (FE)
  - Inertial/Persistent Motion
  - Explicit External Forces
  - Gravity
  - Compartmental Cell Models
  - Cell Polarity
  - Complex Cell Shapes
  - Cell Differentiation
  - Cell Compartments
  - Filopodia
- Secretion and Absorption
- …..

Simulated Neoangiogenesis Effects on 3D Vascular Tumor Growth (75 days)

Axes are in μm





- Proliferative
- Hypoxic
- Necrotic
- Preexisting Capillaries
- Tumor-Induced Capillaries
- White—Stromal Tissue

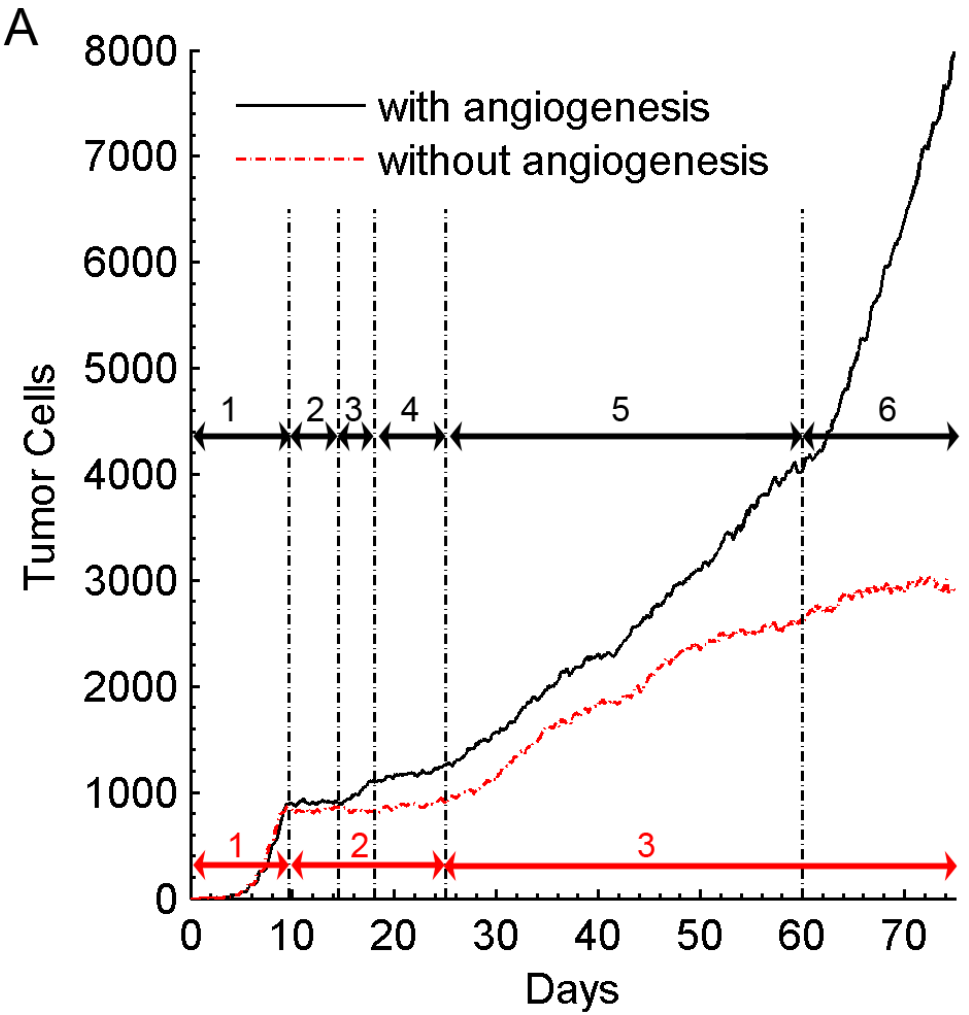
Simulated Neoangiogenesis Effects on 3D Vascular Tumor Growth

With Angiogenesis

1. exponential growth phase
2. no growth
3. linear-spherical phase
4. slow growth
5. linear-cylindrical phase
6. linear-sheet phase

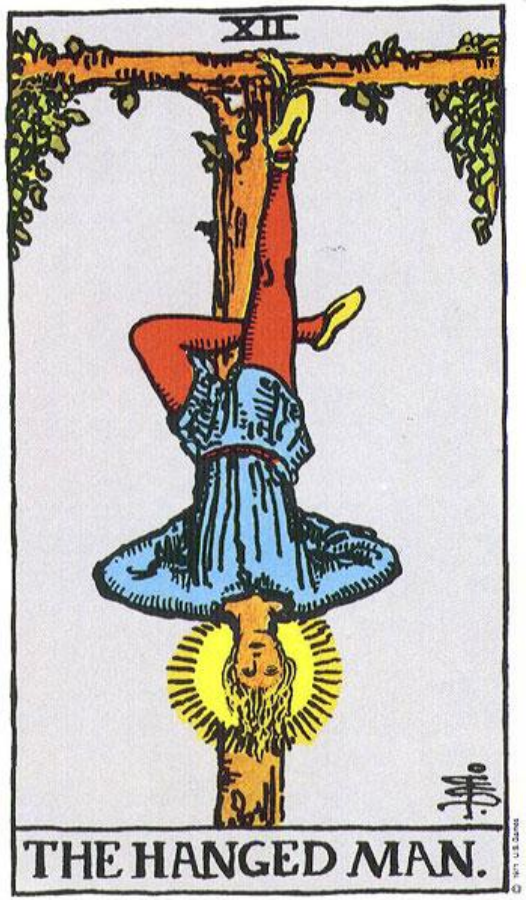
Without Angiogenesis

1. exponential growth phase
2. slow growth
3. cylindrical phase

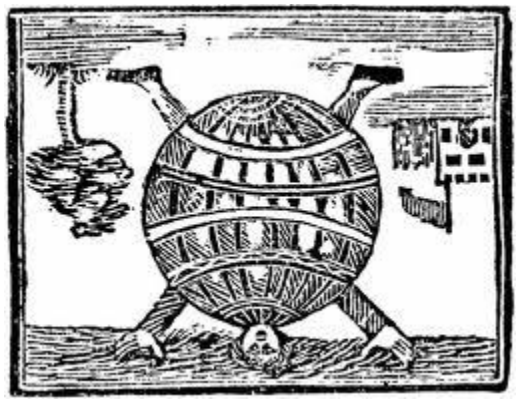


Abbas Shirinifard, J. Gens , Benjamin Zaitlen , Nikodem Poplawski , Maciej Swat , James Glazier, Sep 7, PLoSOne

What Story Would We Tell if We Were Thinking About Cancer for the First Time?



I don't think we'd tell the story exactly the way we find it in the textbooks



Where would we start?

Development is a self-organized process, *i.e. cells build the environment that they themselves respond* to—leads to emergence and tight coupling

Homeostasis in tissues is often taken for granted, but is poorly understood

Maintenance of tissues requires continuous activity of developmental mechanisms

Lack of absolute references make drift during tissue turnover inevitable

Tumor initiation is a failure of homeostasis

Where would we start?

Understanding somatic evolution is essential to understanding homeostasis, solid tumor initiation and progression

Why?

- **Rapid Replication**
- **Behavioral Variation (of many types)**
- **Variation is Heritable (persistence depends on mechanism)**
- **Extremely Strong Selection (99%+ of a cell's offspring die)**

Need to understand from the point of view of the tumor cells, rather than from that of the host (*e.g.* don't talk about deregulation or misregulation, but reregulation)

Musings on Solid Tumors I

Primary Solid Tumors are Diseases of Cell Behaviors (because selection acts on cells—but post-metastasis may be different, see below)

Genetics is important when a particular behavior or behaviors correlate strongly with specific genetic changes

As long as transmissible variation in behaviors occurs, the evolution of tumors will depend primarily on selection. However, most research focuses on generation of variation, rather than on selection.

Paradox I: Lander, Lowengrub, Enderling

After Partial resection (chemotherapeutic killing, irradiative killing), regrown tumor is often more aggressive

Percentage of stem-like cells in tumor increases in time.

Partial resection, radiation and chemotherapy increase percentage of stem-like cells.

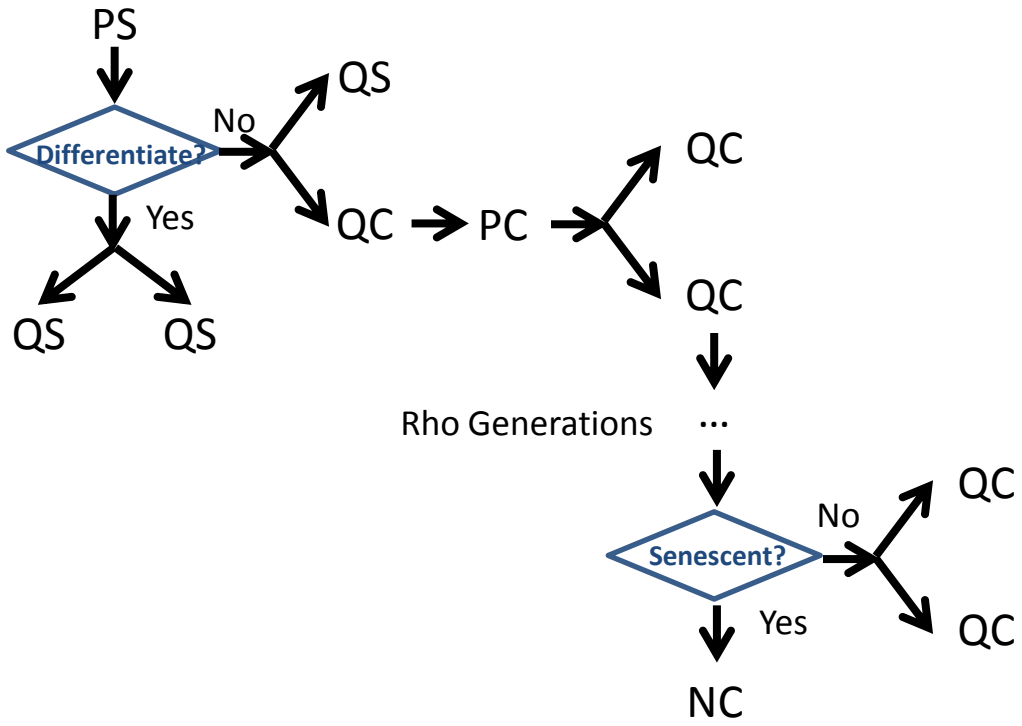
Simulations in Collaboration with Heiko Enderling

First Game

- Objects:
  - Cells
    - “Stem-like”
    - “Somatic Cancer”
    - Stroma and surrounding ECM represented by Medium
- Behaviors and Dynamics
  - Constant Cell Growth
  - Cell Division
  - Cell Random Motility
  - Cell Death due to:
    - Compression
    - Senescence
  - Cell Differentiation
  - Generation Counter for Cell Cycles
  - Cell Mutation
- Interactions
  - Cell-Cell Adhesion (via “Cadherins”)
  - Cell Competition for Space

Cells Types and States

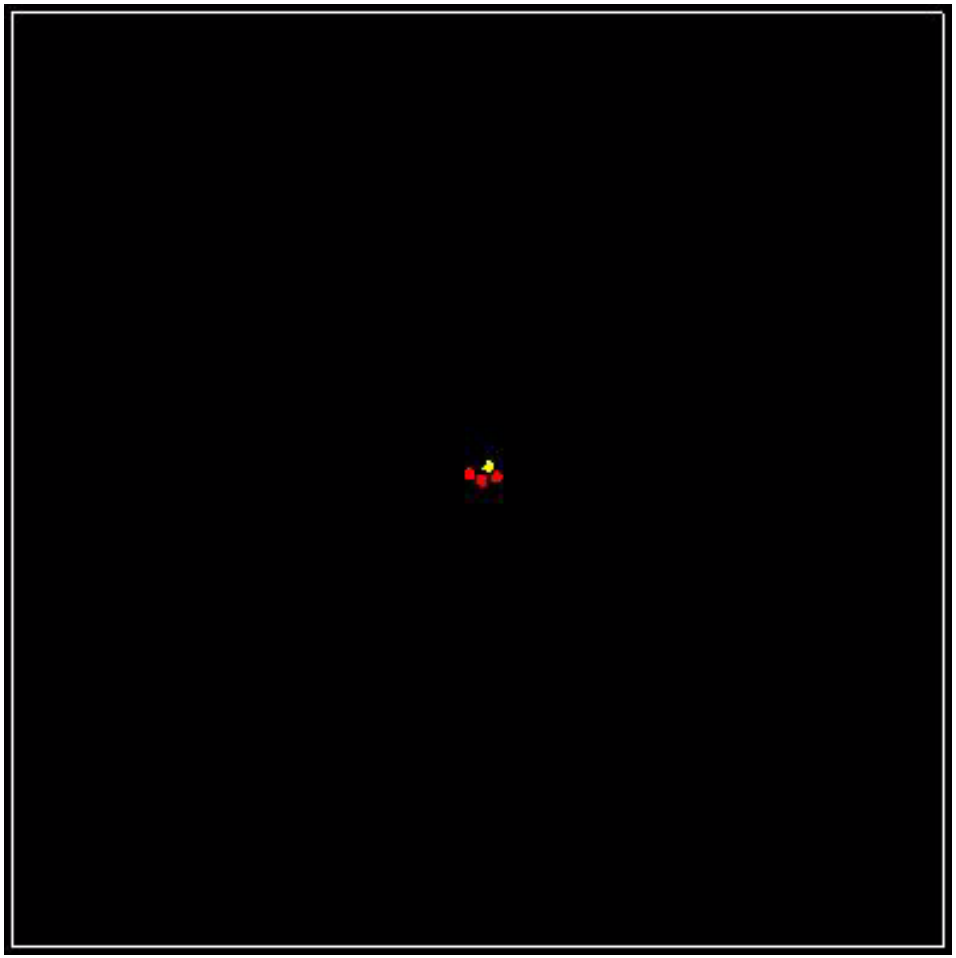
- Cells: (S) Stem-like, (C) “Somatic” Cancer
- States: (P)roliferating, (N)ecrotic

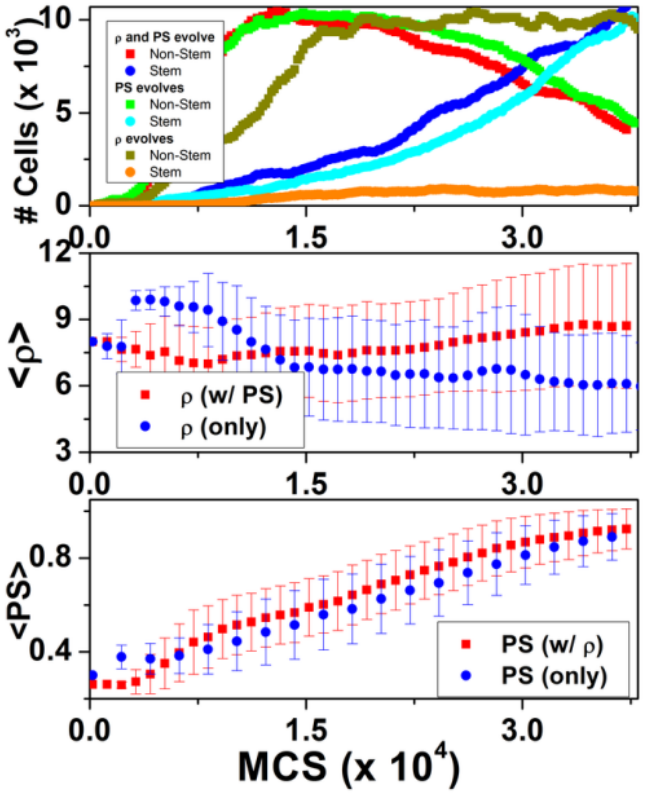


Heritable Mutations May Occur at any Division

Paradox I: Lander, Lowengrub, Enderling

After Partial resection (chemotherapeutic killing, irradiative killing), regrown tumor is often more aggressive





Heiko Enderling

Stem-Like Cell Evolutionary Effects

- Percentage of stem-like cells in tumor increases in time as long as stem cells proliferate
- Partial resection, radiation and chemotherapy increase percentage of stem-like cells

Musings on Solid Tumors II

- Metaphor: War on cancer
- Paradox (I): Attacking things makes them more aggressive

- Suggested Resolution:
  - The niche opened by the lifting of nutrient/space limitation favors more aggressive phenotypes

- Suggested Action:
  - Take tumor normalization seriously—no more “targets”
    - Stop looking for ways to kill cancer cells
    - Look for ways to work with somatic evolution to coax cancers back to non-threatening behaviors
    - To do so need to understand how to keep the cells in a tumor “happy”

Paradox II

- **Paradox: If epithelial to mesenchymal transition is a common feature of cancer progression, why are the majority of cancers epithelial rather than mesenchymal in origin?**
- **Possible Answer:**
  - **Many epithelial tissues have a relatively large number of fast replicating stem cells**
  - **Variation between stem cells will lead to phenotypic drift in the population**
  - **Beyond the range at which the epithelial structure can compensate for this drift, develop tissue disorganization**
  - **At this point other pro-progression mechanisms kick in**
- **Surprising correlates:**
  1) **Cancers can develop with NO mutations (may take a very long time)**
  2) **All stem cells "are" cancer cells**

Paradox III

- **Paradox: Progression Doesn't Make Obvious Sense**
  - **Mutation is undirected and acts on all cell behaviors simultaneously.**
  - **Why is there the appearance of directional quasi-deterministic progression leading to hallmarks appearing in a standard sequence?**
- **Suggested Answer:**
  - **The emergent environment of the tumor leads sequentially to selection favoring mutations of different types and in specific directions**

Second Game: Hypotheses to Test

Solid Tumor Progression Results from:

- Resource limitation.
- Spatial Heterogeneity which favors reduced cell-cell adhesion.
- Red Queen Paradox—as cell behaviors evolve, environment, competition and selection pressures evolve (hence apparent progression).

- Key Questions—
  - What types of heterogeneity drive progression?
  - How can we bias environment externally to favor less invasive types?]
- Focus—
  - Evolution of Cell Adhesion and Invasiveness

Adhesion

- Cell-Cell Adhesion: Homotypic Cadherin
- Cell-ECM Interaction: Heterotypic Integrin-Fibonectin
- **Cadherin and Integrin expression evolve independently**

Toy Model Concepts

- Objects:
  - Cells
    - “Stem-like”
    - “Somatic Cancer”
    - Stroma and surrounding ECM represented by Medium
  - Fields
    - Limiting Diffusing Nutrient Field
    - [Survival Factor]
- Behaviors and Dynamics
  - Cell Growth and Division
  - Cell Random Motility
  - Cell Death due to:
    - Starvation/Hypoxia/Pressure
    - Senescence
    - Immune Killing
  - Cell Differentiation
  - Cell States (Proliferating, Quiescent, Necrotic)
  - Generation Counter for Cell Cycles
  - Cell Mutation
  - Field Diffusion
  - Field Decay
  - Field Production
- Interactions
  - Cell Absorption of Nutrient
  - Cell Secretion and Absorption of Survival Factor
  - Cell-Cell Adhesion (via “Cadherins”)
  - Cell-ECM Adhesion (via “Integrins”)
  - “Immune” Killing of Cells

Limiting Diffusing Nutrient ("Glucose")

- A uniform source representing stromal background concentration of 5mM (the typical concentration in culture medium)
- Consumption:
  - Michaelis-Menten function of the local glucose concentration
- Diffusion
- Parameters set to make gradients much steeper than in reality to allow faster, smaller simulations

Q → P Transition

- Cells have metabolic resting consumption;
- If consumed nutrient is

> metabolic resting need

→ Q cell accumulates "**health**";

→ **Q → P**

Q/P ⟶ N Transition

- Cells have metabolic resting consumption;
- If consumed nutrient is

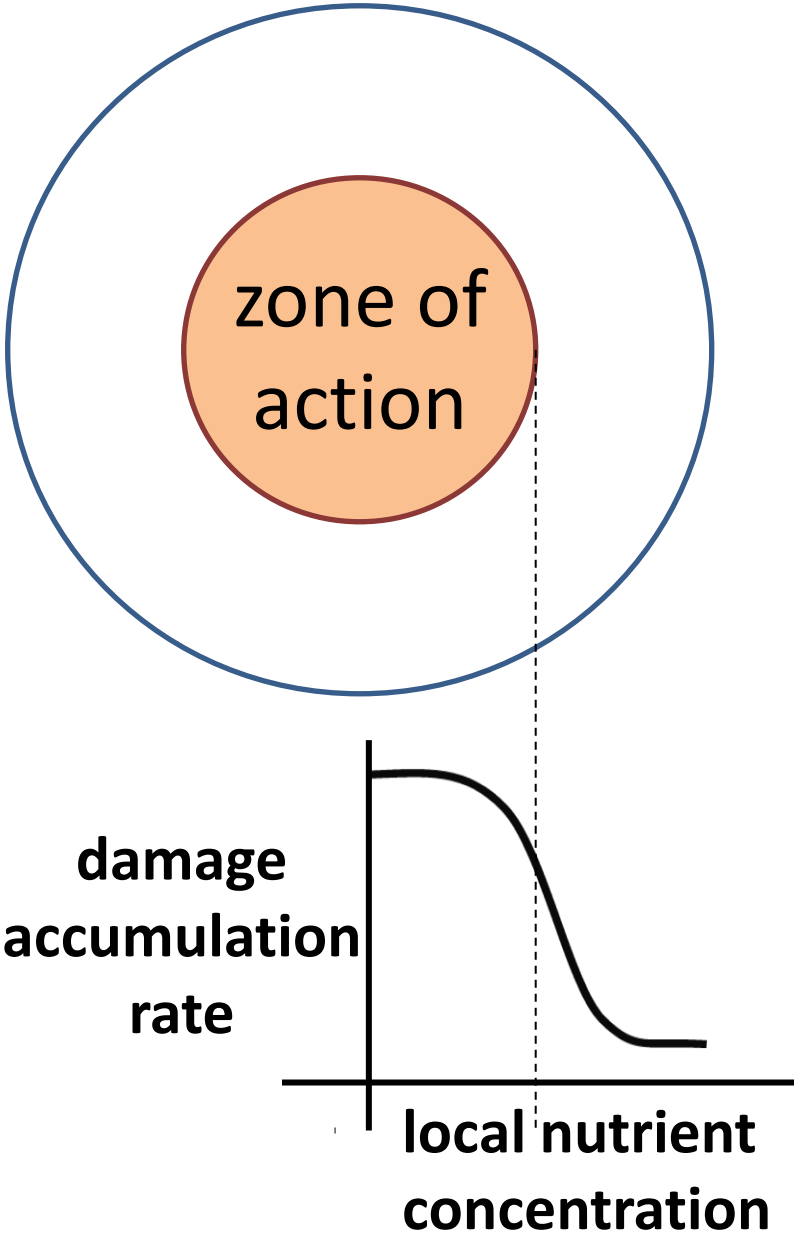
< metabolic resting need (or under pressure)

→ Q/N cell accumulates "**damage**";

→ **Q/N ⟶ P**

Killing Mechanisms: **Starvation**

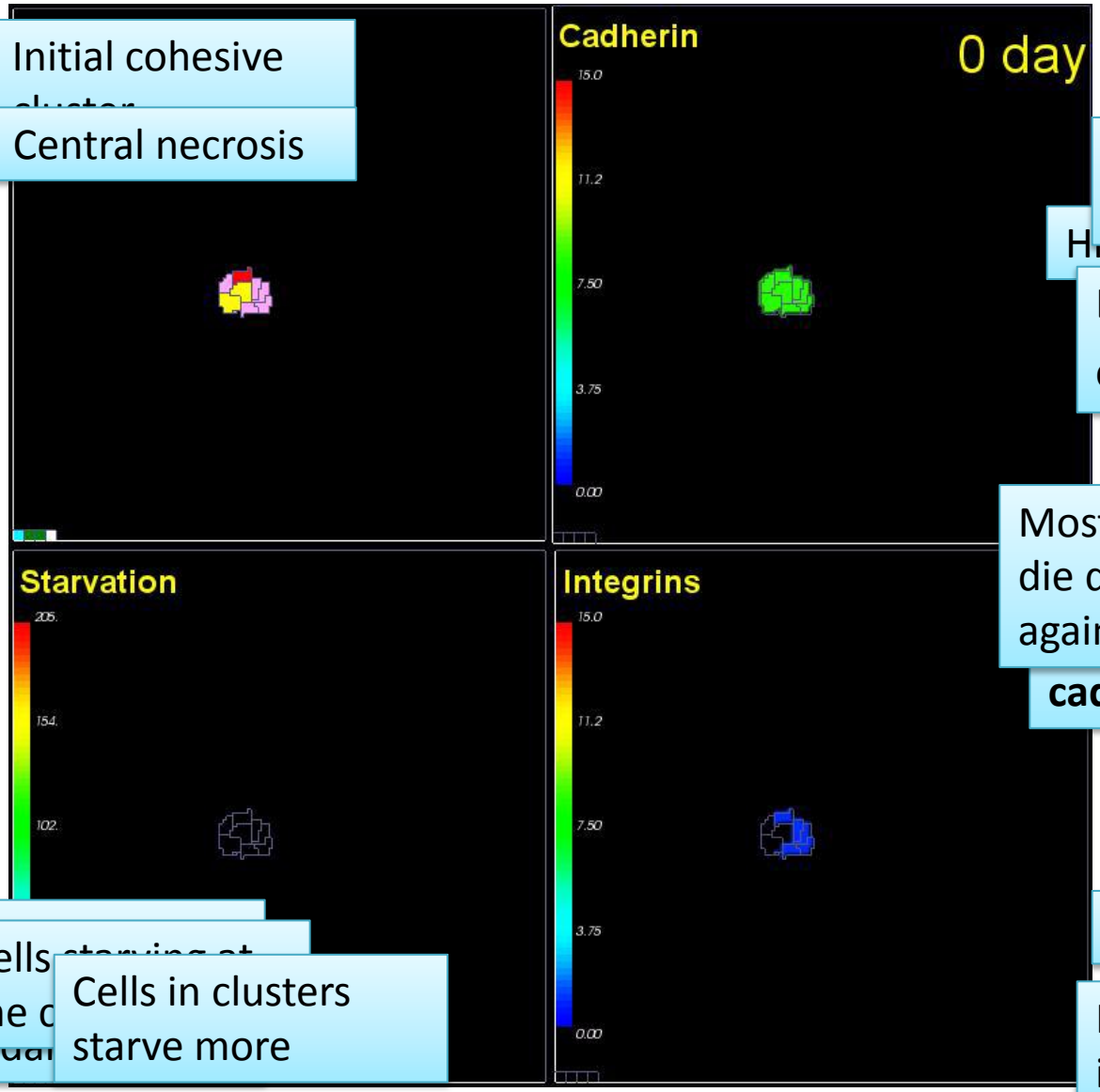
- Cells have metabolic resting consumption
- Cell accumulates "**damage**" if local concentration of Glucose is less than 1/10 of normal concentration (5mM/10 ~ 0.5mM)
- Damage accumulation rate is a Hill-function of local concentration of Glucose
- Cells experiencing less than 0.5 Glucose for more than 24 die to accumulated damage.
- Quiescent cells have higher thresholds for accumulated damage



Scenarios: Nutrient-Gradient-Induced Evolution

- Cadherin and integrin evolve independently
- Cell cycle is about one day initially and later when glucose levels are low
- Tumor cells consume 3X of typical experimental value (10e-17 mol/cell/sec)
- Maximum compact diameter ~ 200 µm
- Healthy Periphery ~ two cell diameter

Nutrient-Gradient-Induced Evolution



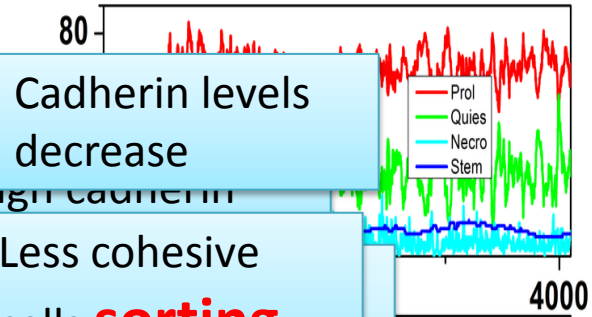
Initial cohesive cluster

Central necrosis

Cadherin levels decrease

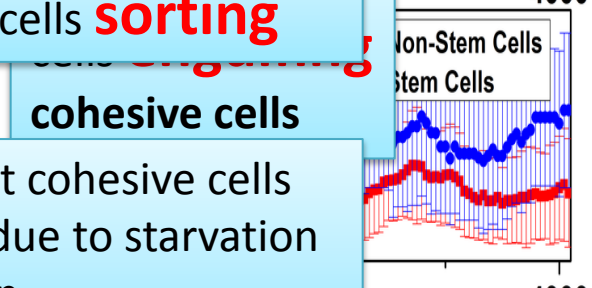
High cadherin

Less cohesive cells **sorting**

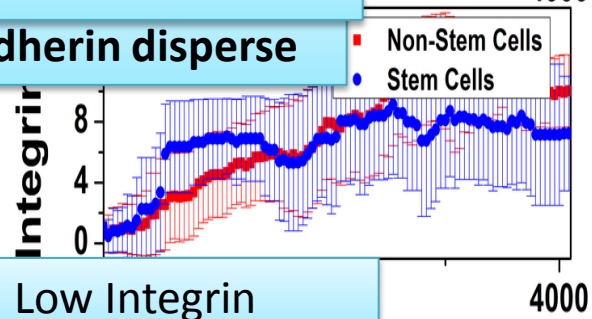


cohesive cells

Most cohesive cells die due to starvation again



cadherin disperse



Low Integrin

Integrin levels increase

Cells in clusters starve more

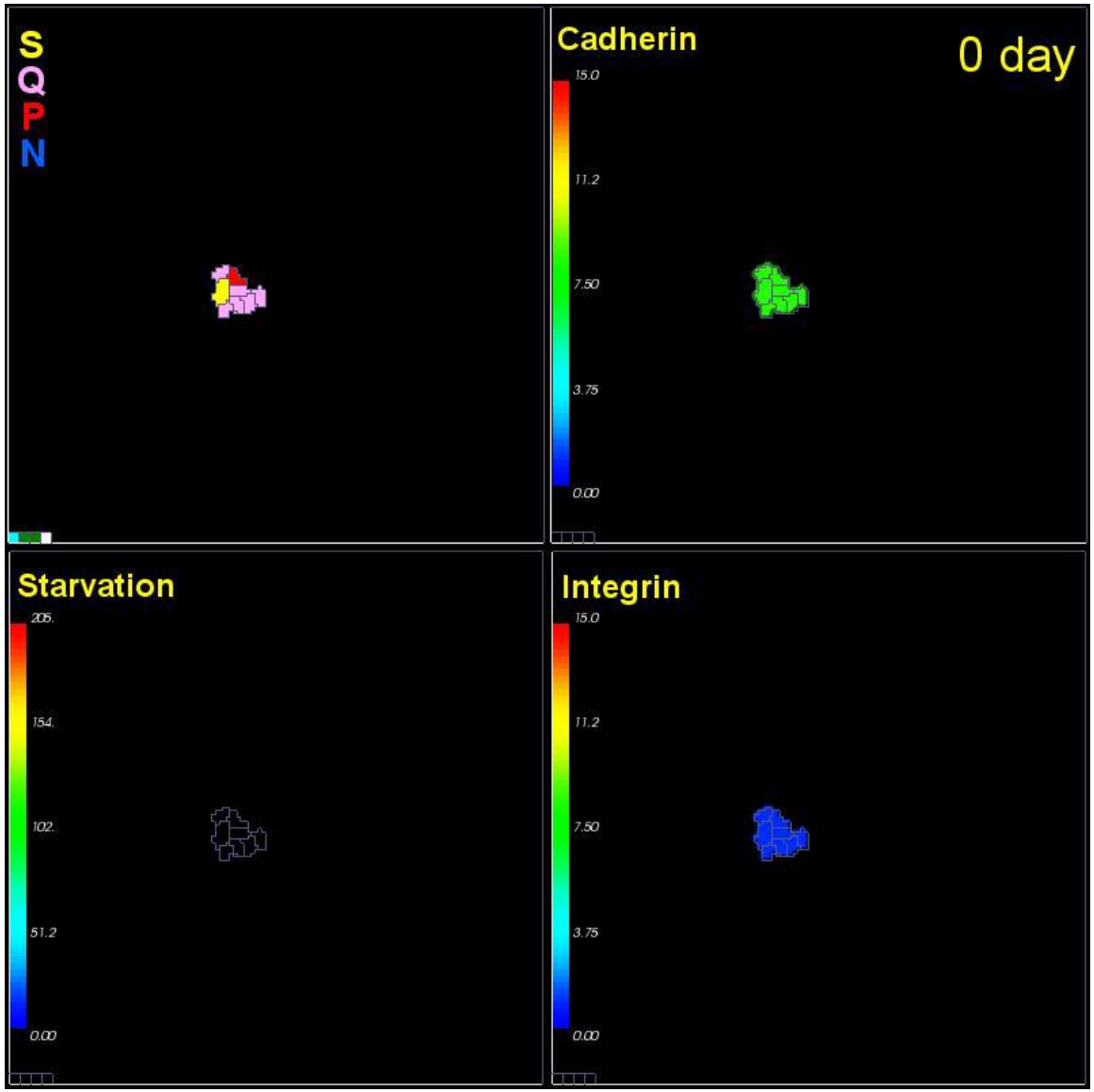
Cells starving at the c

Le ce less dar

Nutrient-Gradient-Induced Evolution

- "Nutrient" field + gradient →
  - cohesion goes down (Cadherin down)
  - Invasiveness goes up (Intergrin up)
  - Morphology: dispersed cells
  - High risk of tumor spreading
- But,…

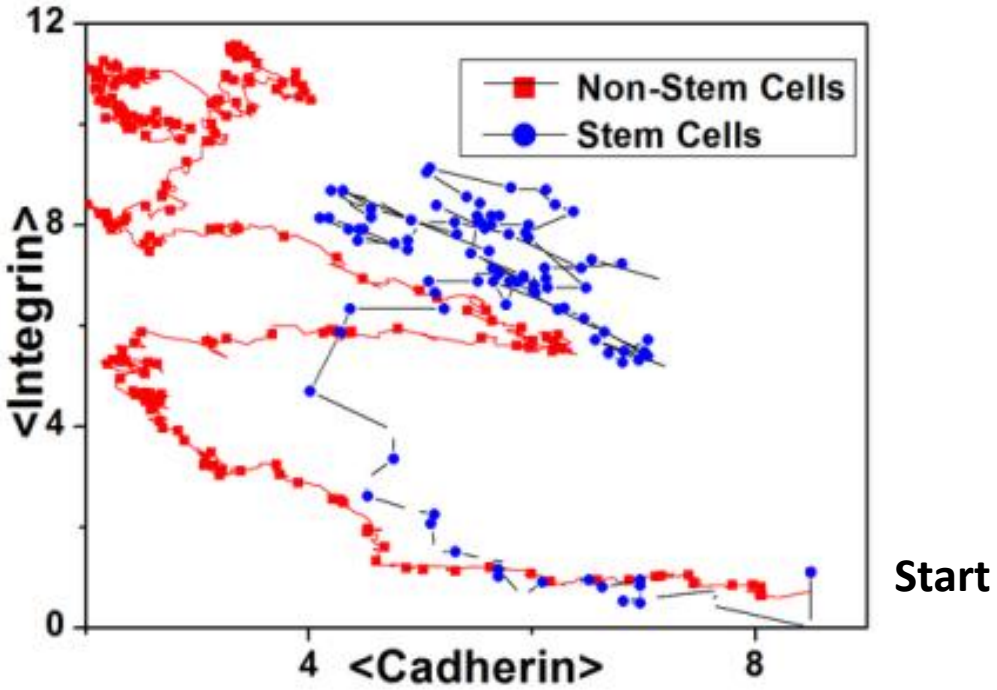
Remission due to Stem-Like Cell Starvation



Nutrient-Gradient-Induced Evolution

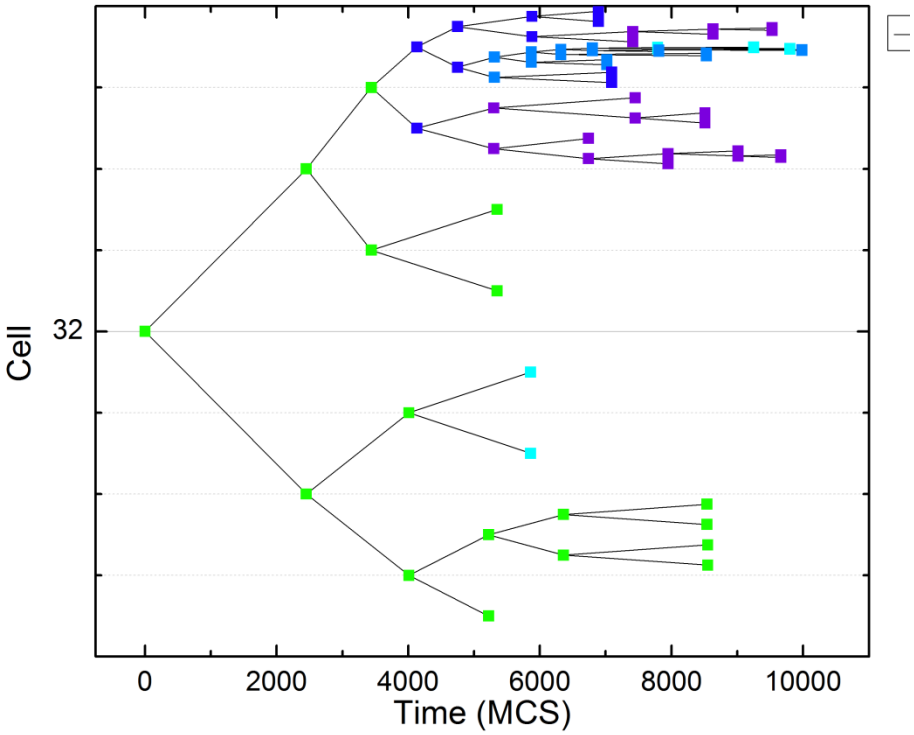
- Nutrient" field + gradient →
  - cohesion goes down (Cadherin down)
  - Invasiveness goes up (Intergrin up)
  - Morphology: dispersed cells
  - High risk of tumor spreading
  - Small chance of stochastic remission

Canalization



Cadherin Levels Decrease **before** Integrin levels Increase

Development of Cell Types



Cells Specialize into high adhesion and low adhesion lineages.

Paradox III

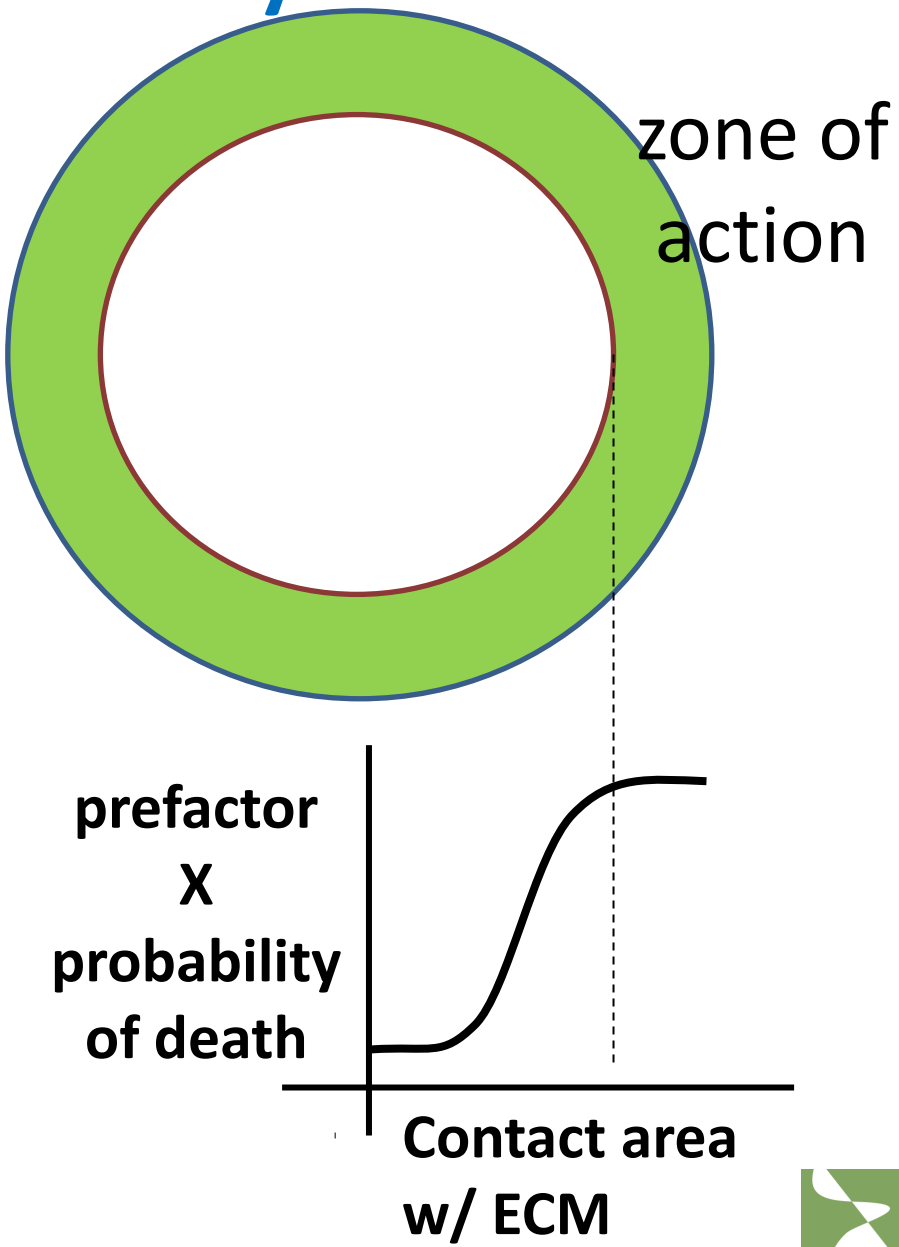
- **If nutrient limitation leads to invasiveness, why don't all replicating stem cells become cancers rapidly?**

- **Observation: Immune deficiency (after transplant or due to disease) can lead to rapid cancer progression.**

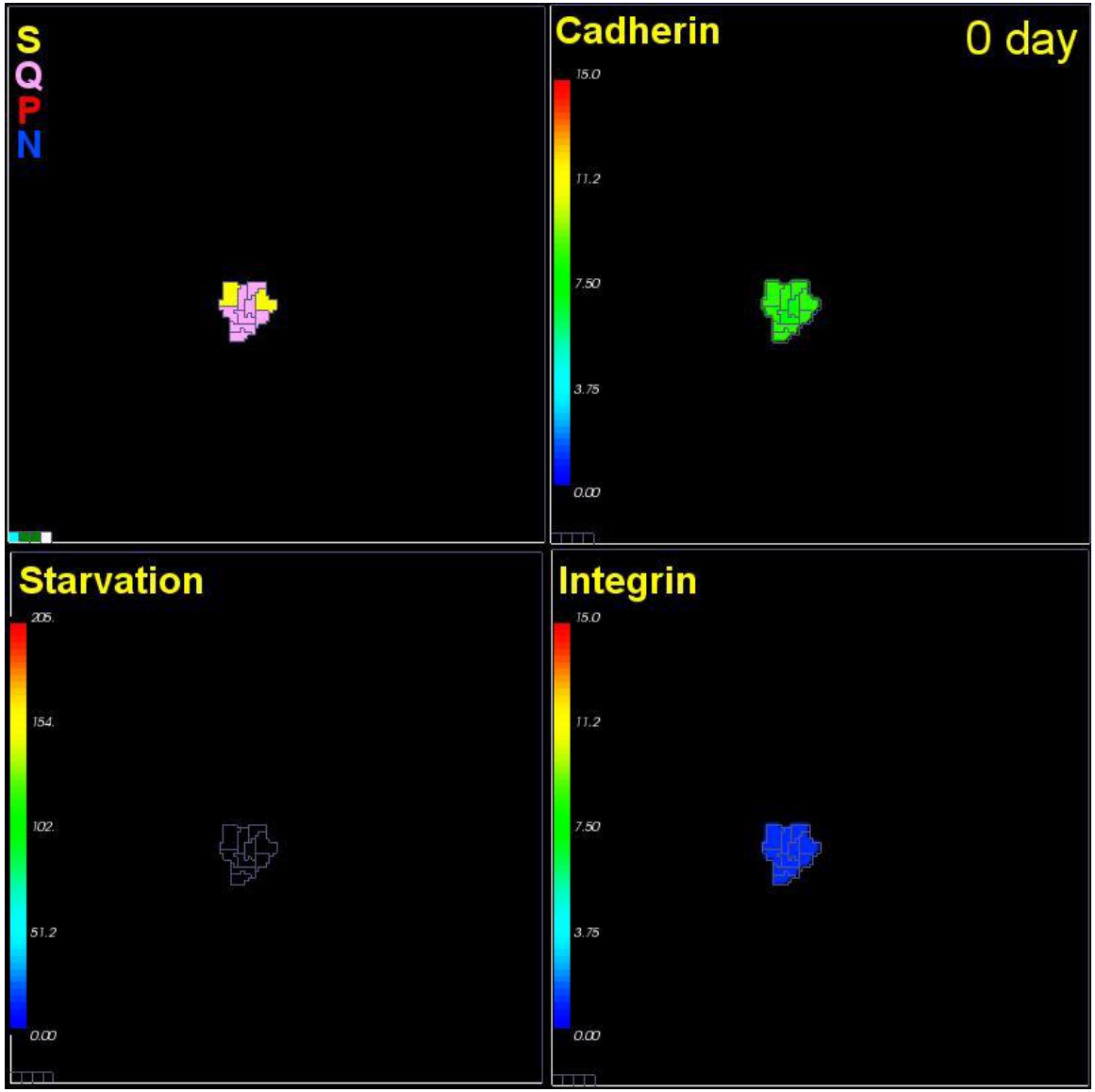
- **Hypothetical Answer to Test:**
  - **A functional immune system prevents progression**

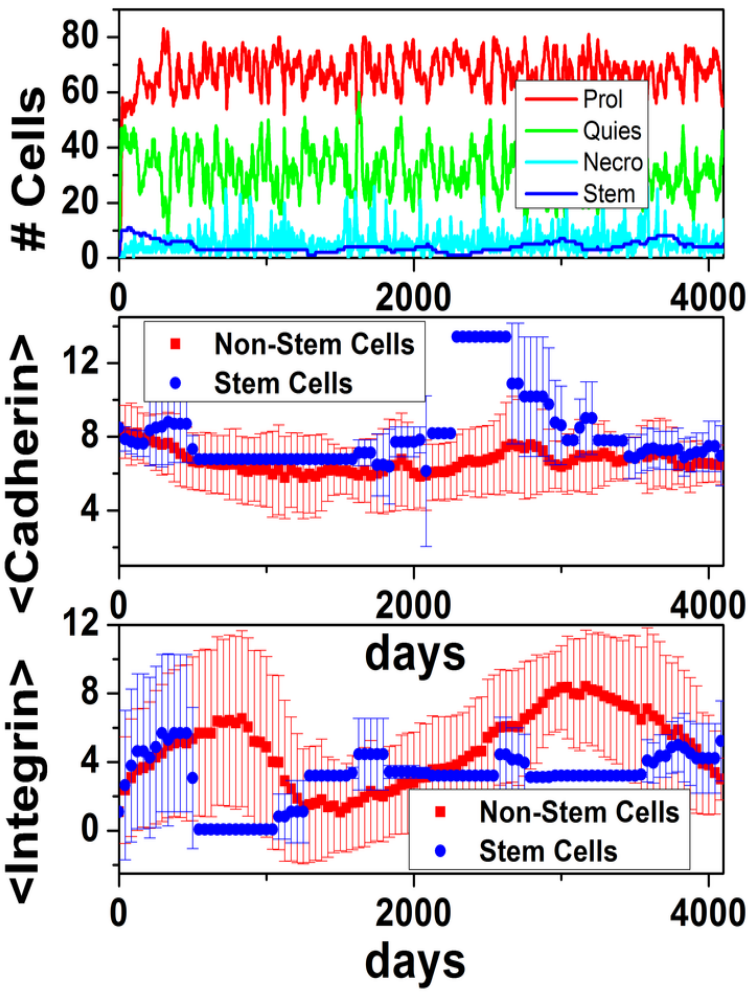
Killing mechanisms: **Immune system**

- Defined by the cell neighborhood (a Hill function of contact area between neighbors)
- The more exposed the cell is (less neighbors), the bigger the chance to die
- Prefactor determines MAX death probability when a tumor cell entirely detaches from the cluster
- Shape of Hill-function determines the depth of affected layer (Green Zone)



Nutrient Gradient and Strong Immune

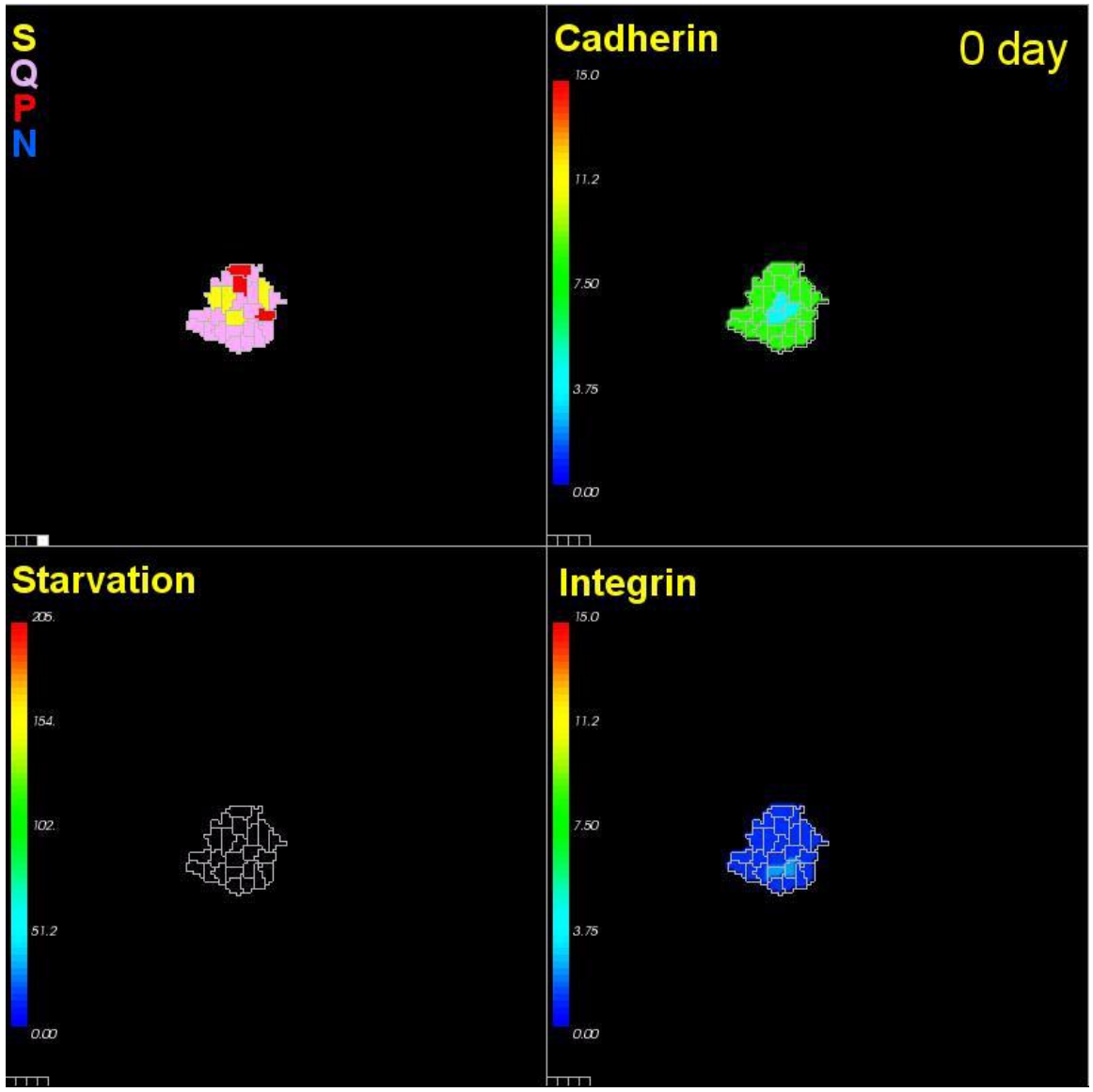


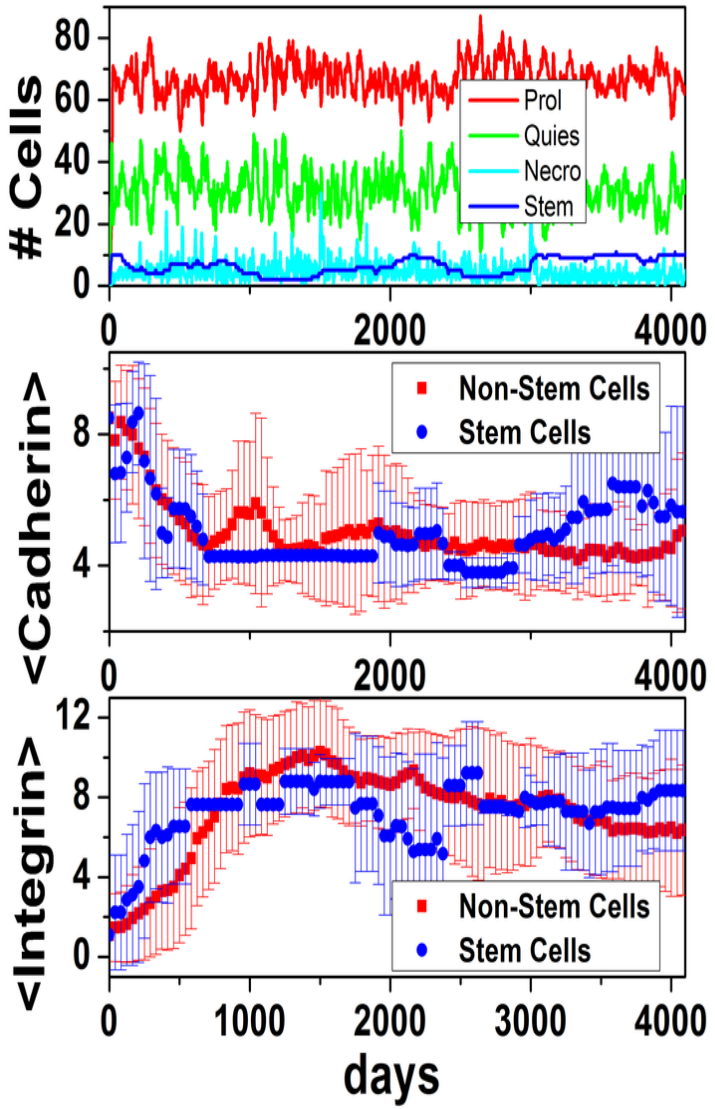


Nutrient Gradient and Strong Immune

- Cohesion remains high (Cadherin up);
- Morphology: single compact cluster
- S cells do not usually survive (finite chance of remission)
- Low risk of tumor spreading (benign tumor)

Nutrient Gradient and Weak Immune





Nutrient Gradient and Weak Immune

- Cohesion decreases (Cadherin down)
- Invasiveness goes up slightly (Integrins up)
- Morphology: single compact cluster, with single or few-cell spread
- Moderate risk of tumor spreading
  Low chance of remission

Nutrient Gradients and Immune Effects ⇒ Adhesion Changes ⇒ Metastasis

	Cohesiveness	Integrins	Morphology	Remission	Spread	Classification
Strong Immune	++	+	Compact	+	-	Benign
Weak Immune	--	+	Compact + Metastases	-	+	Metastatic

Musings on Solid Tumors III

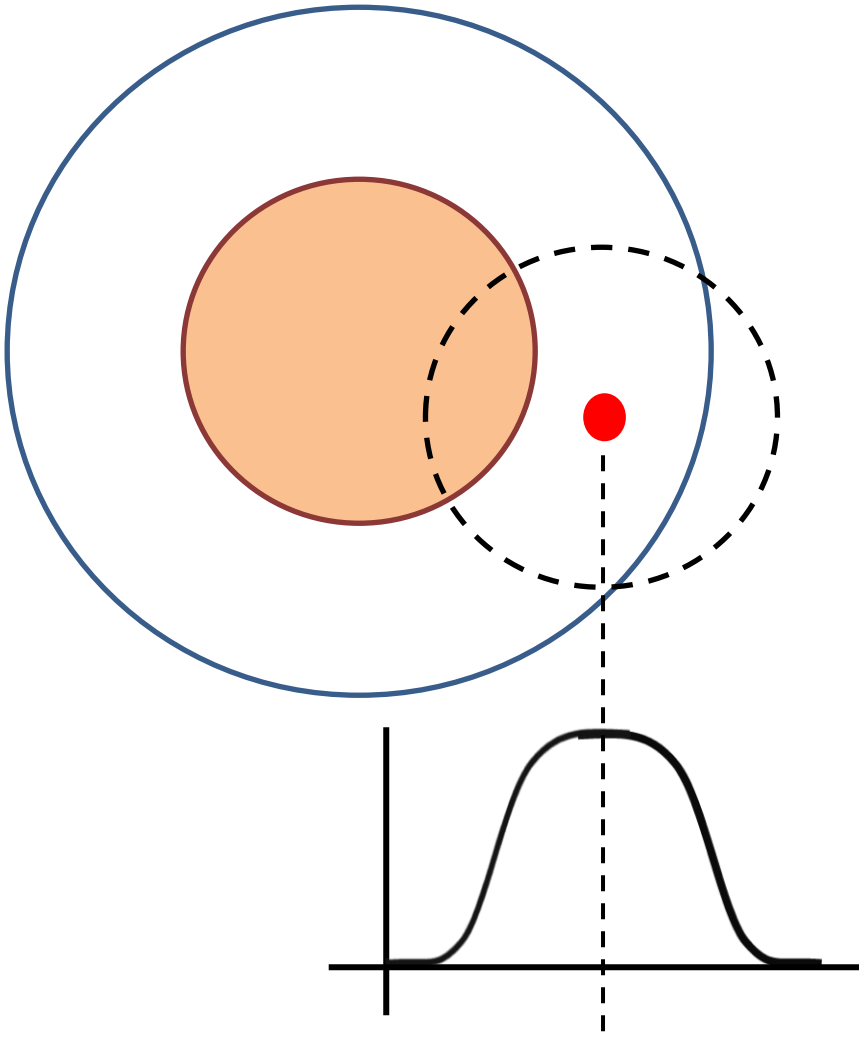
- Even simple models in which tumors can evolve only their cell-cell and cell-ECM adhesion show nontrivial evolution:
  - In the absence of other effects, nutrient gradients lead to:
    - decreasing cell-cell adhesion
    - Increasing cell-ECM adhesion
    - Increasing invasiveness
    - Invasion primarily by single cells
    - In small simulations, starvation can lead to remission
    - Evolution is canalized (cell-cell adhesion changes before cell-ECM adhesion)
  - A toy model of the immune system produces an interesting result:
    - Strong immune effects lead to benign tumors
    - Strong immune effects can lead to tumor remission by an immune-killing mechanism

Paradox IV

- **Paradox: If tumors evolve towards higher "stemness," why have somatic cells at all?**
  - **Somatic cells compete for resources with stem-like cells**
- **Possible Answers:**
  - **Somatic cells may perform functions necessary to stem-like cell survival (especially shared goods)**

Survival factor (SF)

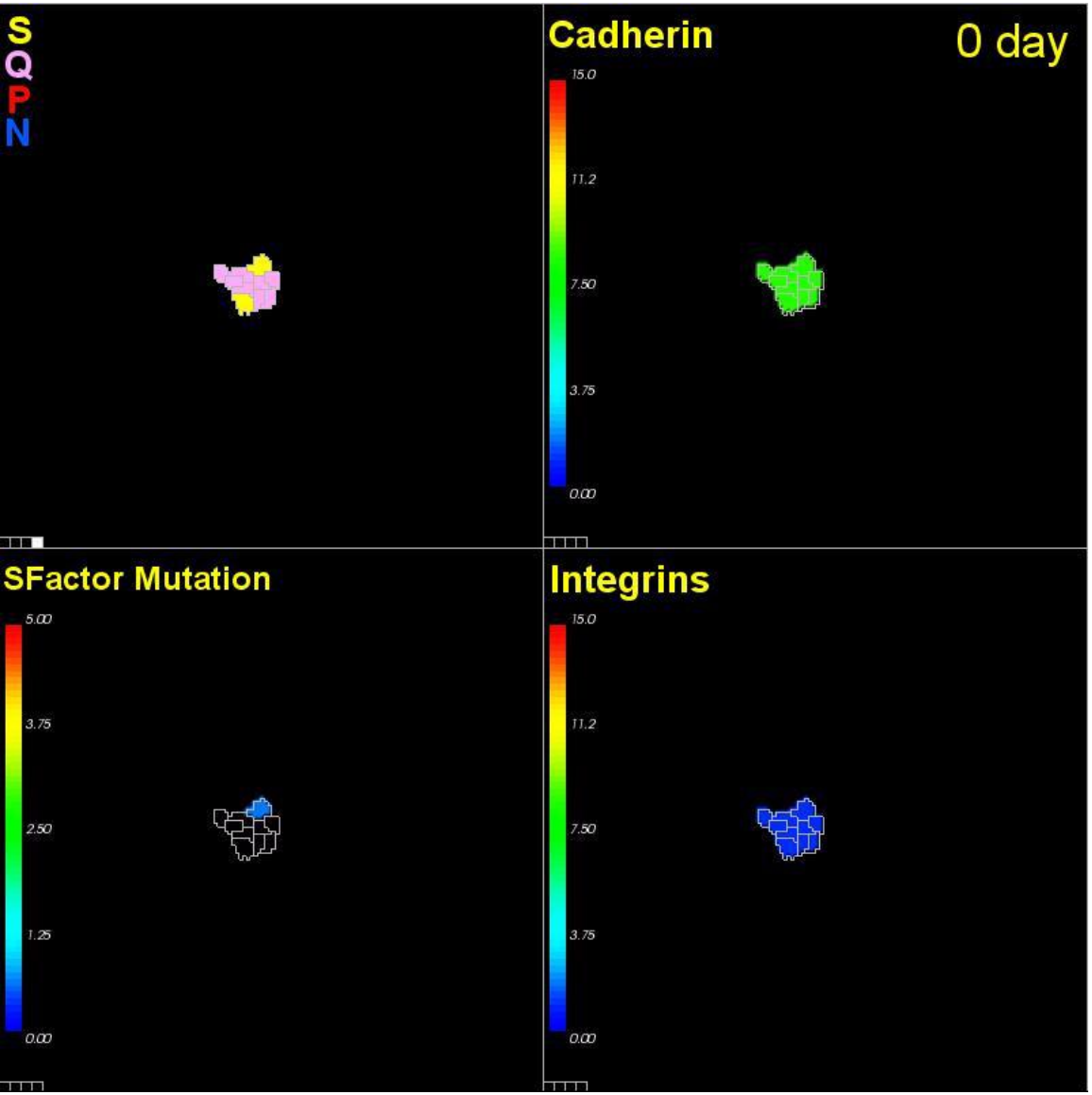
- SF diffuses and decays
- All cells may acquire the ability to produce
- P (non-stem) cells only produced when local Glucose concentration is greater than 0.5mM
- Ability is inherited and may increase during new divisions;
- P cells producing SF grow slower (rate is calculated using a Hill-funtion)
- S Cells consume SF
- SF slows starvation damage accumulation (Hill function)
- Range of effectiveness is determined by the of diffusion length and shape of the Hill-function used to calculate reduced accumulation rate

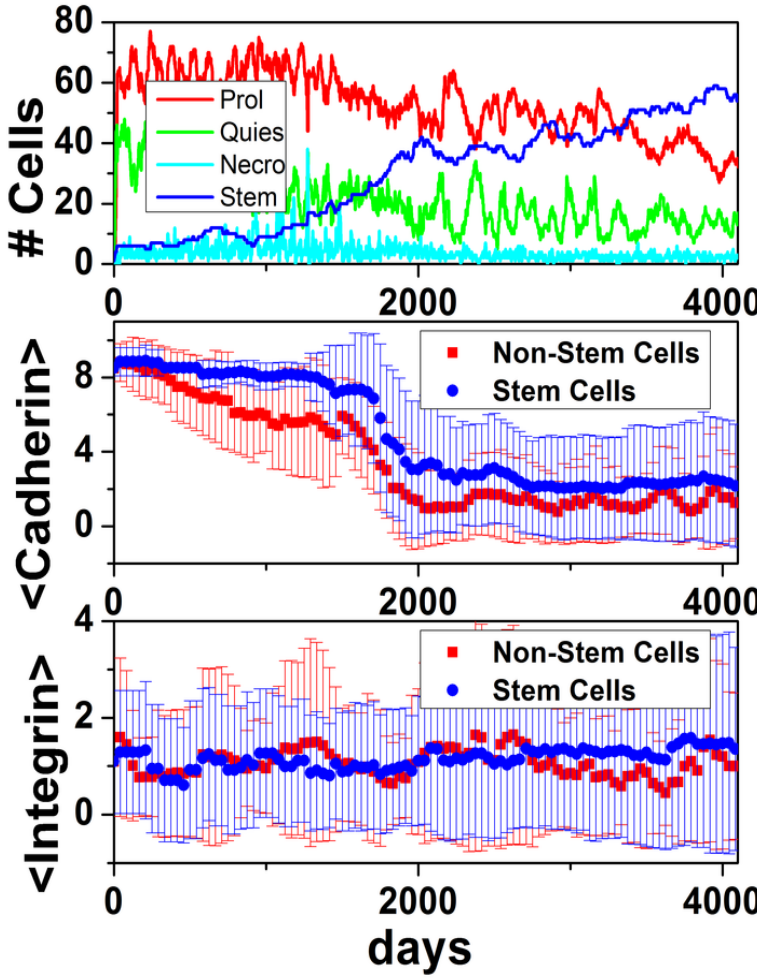


Survival Factor Evolutionary Effects

- A cell that secretes SF can slow the damage accumulation rate 2 times at 3 cell diameters
- Cadherin and integrin both evolve independently
- SF secretion potential (a multiplicative factor that scales the secretion rate) evolves

Survival Factor Evolutionary Effects





Musings on Solid Tumors IV

- Cohesion goes down slightly
- Single-cell Invasiveness is minimal, but
- High risk of tumor spreading via formation of nodules
- Nodules recapitulate distributions of primary tumor (quasi-organisms)

Musings on Solid Tumors V

Specific metabolic defects could be causative of certain cancers.

Could we treat tumor with therapeutic agents that kill only cells with these specific metabolic defects?

Problem: Since the cancer cells are highly heterogeneous, some cells in the tumor will not be killed and, since their metabolic networks change rapidly via somatic evolution, they will rapidly evolve resistance to the therapy).

Happy Accident: If the surviving tumor cells become resistant to the chemotherapy by restoring the function of the missing metabolic pathway and recover normalized metabolism, they should no longer be cancerous. *I.e.*, the treatment will have cured the cells by working with, rather than against, somatic evolution.

Paradox V

Metastasis seems evolutionarily unfavorable (most metastatic cells die)

Possible Resolution:

Metastasis as Epiphenomenon (an accidental result of selection for something else)

Why is metastasis often associated with vascularization?

Vascularization increases the temporal variability of resources

Spatio-temporal gradients select for motility

Spatiotemporal scales determine rate and type of selection

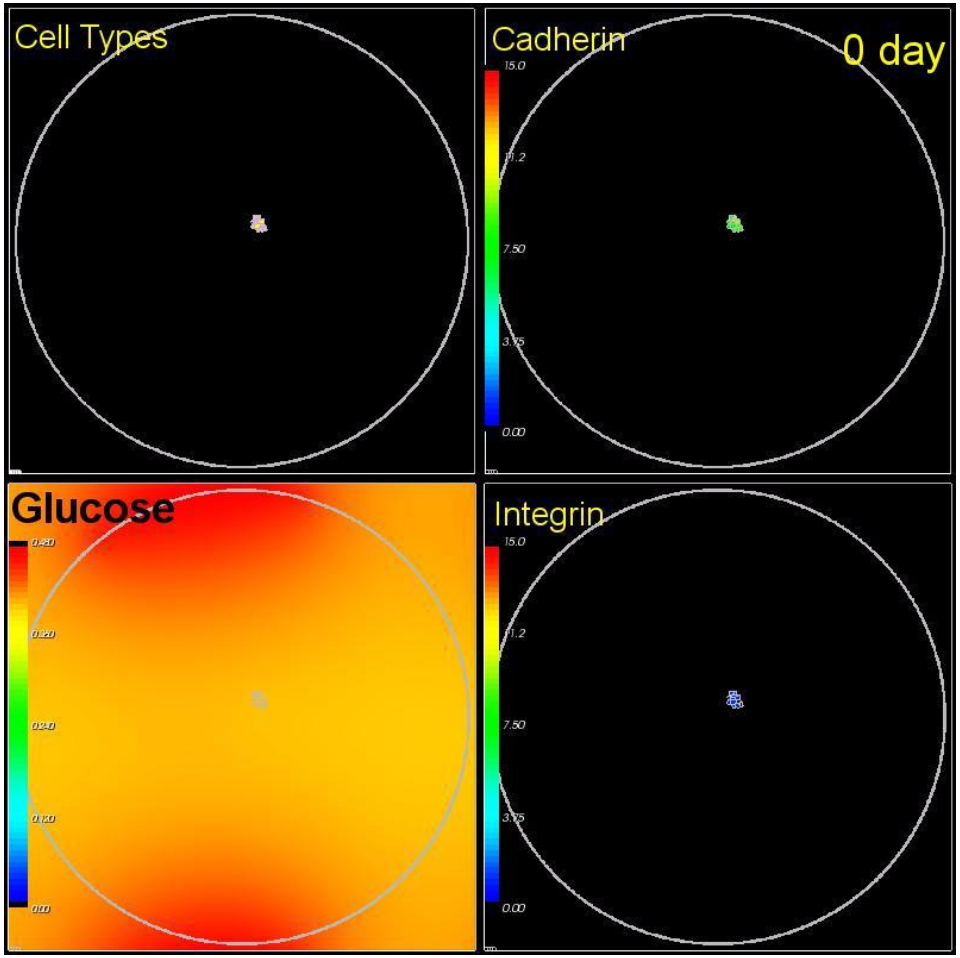
Multicell Metastasis

Quasiorganisms

Time Varying Nutrient Gradient

- Cadherin and integrin evolve independently
- No immune system, no S Factor
- Glucose source rotates every ~ 10 days
- Tumor cells consume 10e-17 mol/cell/sec
- Maximum compact diameter ~ 1 mm
- Cells one cell diameter
- Cell cycle is about one day initially and later up to two weeks when glucose levels are low
- Healthy Periphery ~ 10 cell diameter ~ 150 µm

Time Varying Nutrient Gradient



Time Varying Nutrient Gradient

- A variation time on the order of the cell-death accumulation time drives decrease of cell-cell adhesion and increase of cell invasiveness much more rapidly than a static gradient.

Provisional Conclusions

- **Genomic and proteomics on single cells may never be sufficient to 'understand' cancer**
- Even simple models in which tumors can evolve only their cell-cell and cell-ECM adhesion show nontrivial evolution:
  - In the absence of other effects, nutrient gradients lead to:
    - decreasing cell-cell adhesion
    - Increasing cell-ECM adhesion
    - Increasing invasiveness
    - Invasion primarily by single cells
    - In our small simulations, starvation can lead to remission
    - Evolution is canalized (cell-cell cadhesion hanges before cell-ECM adhesion)
  - Adding in a simple model of the immune system produces an interesting result:
    - Strong immune effects lead to benign tumors
    - Strong immune effects can lead to tumor remission by an immune-killing mechanism
  - Adding in a diffusible Survival Factor produces:
    - Multicell Metastasis
    - Quasiorganisms
- We can understand key aspects of cancer progression and invasiveness by considering tumors from an evolutionary/ecosystem perspective

Suggestions?