Multiscale Models of Solid Tumor Growth and Angiogenesis:
The effect of the microenvironment

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Motivation

• Provide biophysically justified *in silico* virtual system to study
• Help experimental investigations; design new experiments
• Therapy protocols
Outline

• Introduction to tumor growth
  Multiscale complex soft matter problem

• Models and analysis of invasion

• Numerical methods and results

• Models of angiogenesis

• Nonlinear coupling of angiogenesis and invasion
The Six Basic Capabilities of Cancer
(Hanahan and Weinberg, 2000)

• Genetic-Level (Nanoscopic)
  – Self-sufficiency in Growth Signals
  – Insensitivity to Growth-inhibitory Signals
  – Evasion of Programmed Cell Death
  – Limitless Replicative Potential

• Tissue-Level (Microscopic)
  – Tissue Invasion and Metastasis
  – Sustained Angiogenesis
Cartoon of solid tumor growth

- Genetic mutations
- Avascular growth: Diffusion dominated
- Angiogenesis
- Vascular growth: invasive metastasis malignancy

**Goal:** Model all Phases of growth
Cancer: Multiscale Problem

**Subcell Scale**
- Gene expression
- Protein synthesis
- Biochemical reactions
- Size: nanometers

**Cell Scale**
- Cell proliferation, quiescence, apoptosis, and necrosis
- Cell-cell and cell-matrix adhesion
- Cell size: ~10 microns

**Tissue Scale**
- Tumor growth and spread
- Invasion
- Biomechanical stress
- Billions to trillions of cells
- Size: 1 to 10 centimeters

- Complex, soft matter microstructure
- Processes at multiple scales
- All scales coupled


Modeling

- Continuum approximation: super-cell macro scale (Collective motion)

- Role of cell adhesion and motility on tissue invasion and metastasis
  Idealized mechanical response of tissues

- Coupling between growth and angiogenesis (neo-vascularization): necessary for maintaining uncontrolled cell proliferation

- Genetic mutations: random changes in microphysical parameters cell apoptosis and adhesion
Key variables

Minimal set.

- the mass fraction of the viable tumor cells $\rho_V$,
- the mass fraction of the dead (e.g. necrotic) tumor cells $\rho_D$,
- the mass fraction of both viable and dead tumor cells $\rho_T$,
- the mass fraction of the host (healthy) cells $\rho_H$,
- the mass fraction of the water $\rho_W$,
- the cellular, necrotic, host and water velocities $u_V$, $u_D$, $u_H$ and $u_W$.

Tumor fraction: $\rho_T = \rho_V + \rho_D$.

Will discuss refinements later.
Equations governing tumor growth and tissue invasion


\[
\begin{align*}
\frac{\partial \rho_V}{\partial t} + \nabla \cdot (u_V \rho_V) &= -\nabla \cdot J_V + S_V, \\
\frac{\partial \rho_D}{\partial t} + \nabla \cdot (u_D \rho_D) &= -\nabla \cdot J_D + S_D, \\
\frac{\partial \rho_H}{\partial t} + \nabla \cdot (u_H \rho_H) &= -\nabla \cdot J_H + S_H, \\
\frac{\partial \rho_W}{\partial t} + \nabla \cdot (u_W \rho_W) &= S_W,
\end{align*}
\]

\textbf{J} -- Adhesion fluxes \quad \textbf{S} -- Net sources/sinks of mass

Adhesion

Fundamental biophysical mechanism.

Cell-cell binding through cell-surface proteins (CAMs, cadherins)

- Cell-sorting due to cell-cell adhesion

Chick embryo
Armstrong (1971)

5 hours 19 hours 2 days

- Cells of like kind prefer to stay together.

Cell-ECM binding through other cell-surface proteins (integrins)
Adhesion Energy

• Assume tumor cells prefer to be together. Different phenotypes may have different adhesivity (can extend the model)

\[
E = \int_{\Omega} \left( f(\rho_T) + \frac{\varepsilon^2}{2} |\nabla \rho_T|^2 \right) d^3x,
\]

Double-well potential Gradient energy (allows intermixing)

• Thermodynamic consistency:

\[
\mathbf{J}_V = -M \rho_V \nabla \frac{\delta E}{\delta \rho_V}, \quad \mathbf{J}_D = -M \rho_D \nabla \frac{\delta E}{\delta \rho_D}, \quad \mathbf{J}_H = - (\mathbf{J}_V + \mathbf{J}_D)
\]

where \[
\frac{\delta E}{\delta \rho_V} = \frac{\delta E}{\delta \rho_D} = f'(\rho_T) - \varepsilon^2 \nabla^2 \rho_T.
\]

Generalized Cahn-Hilliard equation

Other approaches: Nonlocal energy (Katsulakis et al.), Armstrong et al. (2006)
Constitutive Assumptions

Simplest assumptions. Can be generalized. (X.Li, L., Cristini, Wise)

- Water density is constant: \( \rho_W(x, t) = \bar{\rho}_1 \).
- Close-packing: \( \rho_T + \rho_H = \bar{\rho}_0 \).
- Cell-velocities are matched using Darcy’s law:

\[
\mathbf{u}_V = \mathbf{u}_D = \mathbf{u}_H = -\mu \left( \nabla \rho - \frac{\delta E}{\delta \rho_T} \nabla \rho_T \right)
\]

Water decouples

Excess adhesion

Cell mobility: reflects strength of cell-cell and cell-matrix adhesion

Oncotic (hydrostatic) solid pressure

(arises from thermodynamic considerations)
Constitutive Assumptions Contd.

Cell proliferation:

$$S_V = \tilde{\lambda}_M n / \bar{n}_\infty \rho_V - \tilde{\lambda}_A \rho_V - \tilde{\lambda}_N \mathcal{H}(\bar{n}_N - n) \rho_V,$$

mitosis \hspace{1cm} apoptosis \hspace{1cm} necrosis

Viability level of nutrient 

Nutrient (oxygen)

Heaviside function

Necrotic cells:

$$S_D = \tilde{\lambda}_A \rho_V + \tilde{\lambda}_N \mathcal{H}(\bar{n}_N - n) \rho_V - \tilde{\lambda}_L \rho_D,$$

lysing (enzymatic degradation)

Host domain:

$$S_H = 0,$$

Water:

$$S_W = -(S_V + S_D + S_H) = -\tilde{\lambda}_M n / n_\infty \rho_V + \tilde{\lambda}_L \rho_D$$
Evolution of nutrient

Oxygen:

\[ 0 = \nabla \cdot \left( D\left( \rho_T \right) \nabla n \right) + T_C \left( n_c, n, p, \delta_c \right) - \nu_U n \rho_V \]

= 0 (quasi-steady assumption). Tumor growth time scale (~1 day) large compared to typical diffusion time (~1 min)

Source due to capillaries (angiogenesis)

uptake by viable cells
Interpretation

In $\Omega_H$,

- $D$ is an indirect measure of perfusion
  \[ i.e., D \text{ large} \rightarrow \text{nutrient rich} \]

- $\mu$ is a measure of mechanical/adhesive properties of extra-tumor tissue
  \[ i.e., \mu \text{ small} \rightarrow \text{tissue hard to penetrate} \]
  \[ \text{ (less mobile)} \]

- Although a very simplified model of these effects, this does provide insight on how the microenvironment influences tumor growth.
The equations (nondimensionalized)

\[ \mathcal{L} = \left( \frac{D_T}{\bar{\nu}_U} \right)^{\frac{1}{2}} \text{ and } T = \bar{\lambda}^{-1}_M, \]

\[ \frac{\partial \rho_T}{\partial t} = M \nabla (\rho_V \nabla \mu) + S_T - \nabla \cdot (u \rho_T), \]

\[ \mu = f'(\rho_T) - \varepsilon^2 \nabla^2 \rho_T, \]

\[ \frac{\partial \rho_D}{\partial t} = M \nabla \cdot (\rho_D \nabla \mu) + S_D - \nabla \cdot (u \rho_D), \]

\[ \nabla \cdot u = S_T, \]

\[ S_T = S_V + S_D \]

• Only one Cahn-Hilliard Equation to be solved for \( \rho_T \)

• Generalizes to multiple species easily.
Nondimensional parameters

\[ \lambda_H = \lambda_B = \lambda_A = 0 \]

Microenvironmental:

- Diffusion ratio: \( \kappa_D = D_H / D_V \)
- Mobility (adhesion) ratio: \( \kappa_\mu = \mu_H / \mu_V \)

Cell-based:

- Adhesion: \( G = \frac{\lambda_M}{\lambda_R} \)
- Intermixing: \( \varepsilon \)
- Necrosis: \( G_N = \lambda_L / \lambda_M, \quad \overline{G}_N = \lambda_N / \lambda_M \)
- Viability: \( N = \frac{n_N}{n_{\infty}} \)
Spherical Solutions

- Balance between proliferation/necrosis/lysing.
- Viable tumor cells move to center. (water moves outward)
- Necrotic boundary is diffuse

Growth to a steady spheroid
Convergence to sharp interface

\[ \varepsilon = 0.10 \quad \varepsilon = 0.05 \quad \varepsilon = 0.025 \]

• Method of matched asymptotic expansions can be used to suggest convergence to classical sharp interface models as \( \varepsilon \to 0 \) provided \( M \) is bounded
Tumor Spheroids: Validation *in vitro*

In vitro growth: No vascularization (diffusion-dominated)

Dormant (steady) states

One micron section of tumor spheroid showing outer living shell of growing cells and inner core of necrosis.

3-D video holography through biological tissue
P. Yu, G. Mustata, and D. D. Nolte, Dept. of Physics, Purdue University
Tumor Modeling: The basic model

Model validation:

- Agreement w/ observed growth
- Determine microphysical parameters

In vitro data: Karim & Carlsson Cancer Res.
Microphysical parameters

- $A=0$, $G_N = \begin{cases} 4.0 & u_{118} \\ 0.31 & u_{251} \end{cases}$, $N \approx 10^{-2}$

- $\lambda_M \approx 0.3 \text{ day}^{-1}$
- $\lambda_C \approx 2 \text{ s}^{-1}$
- $D \approx 3 \times 10^{-3} \text{ mm}^2 / \text{s}$
- $L \approx 4 \times 10^{-2} \text{ mm}$

$G$ is not determined:
- Experiments
- Stability analysis

(approximately 7 cells)
Morphological stability

**Perturbation**
\[ r_\Sigma = R(t) + \delta(t) \begin{cases} \cos(l\theta) & \text{in } 2D \\ Y_{im}(\theta, \phi) & \text{in } 3D \end{cases} \]

**Underlying Growth**
\[ G^{-1} \frac{dR}{dt} = -\frac{AR}{d} + \begin{cases} I_1(R) / I_0(R) & \text{in } 2D \\ \coth(R) - 1 / R & \text{in } 3D \end{cases} + F(N, G_N, R) \]

\[ G_N = G_{N}^{\text{steady}} (R, N, A) \text{ such that } dR / dt = 0 \]
(balance between proliferation, necrosis and apoptosis)

If \( N=0 \), then reduces to \( A = A^{\text{steady}} (R) \)

**Shape evolution**
\[ \left( \frac{\delta}{R} \right)^{-1} \frac{d}{dt} \left( \frac{\delta}{R} \right) = H_{\text{growth}} (l, R, A, G, G_N, N) - H_{\text{decay}} (l, R, A, G, G_N, N) \]

**Self-similar evolution**
\[ G = G_{\text{crit}} (l, R, G_N, N, A) \text{ such that } d(\delta / R) / dt = 0 \]

If \( N=0 \), then can also get \( A = A^{\text{crit}} (l, R, G) \)
Diffusional Instability--Avascular

3D: Li, Cristini, Nie and Lowengrub, DCDS-B, In review

2D: Avascular (tumor spheroid) (low cell-to-cell adhesion)

3D:

\[ G > G_{\text{critical}} \]

- Growth-by-bumps
  - ejection of cells from bulk
- topology change

Highly vascularized

Boundary integral method
Diffusional Instability

- Perturbed tumor spheroids/Complex Morphology

Frieboes, et al.

Glioblastoma

Velocity field (simulation)

Swirling ejection from bulk

- Theory:
  Possible mechanism for invasion into soft tissue

Nonlinear Simulations
Numerical Scheme

• Implicit time discretization (Gradient Stable)
  fully implicit treatment of system

• Second order accurate, centered difference scheme.
  Conservative form. Adaptive spatial discretization.
  Wise, Kim, Lowengrub J. Comp. Phys., in review

• Nonlinear, Multilevel,
  multigrid method
Advantages of Multigrid

• Complexity is $O(N)$
  Optimal convergence rate

• Handles large inhomogeneity/ nonlinearity seamlessly (no additional cost)
  • Smoothing is performed by, for example, the nonlinear Gauss-Seidel method.
  • Local linearization. No global linearization, for example via Newton’s Method, is needed.

• Flexible implementation of b.c.’s (compare with pseudo-spectral, spectral methods)

• Seamlessly made adaptive

• Hard to analyze: quantify smoothing properties of the nonlinear relaxation scheme
Well-perfused host domain

$\chi_D$ large

$\chi_\mu = 1$

- Small nutrient gradients in host
• Tumor develops folds to increase access to nutrient
Large nutrient gradients

\[ \chi_D = \chi_\mu = 1 \]

- Large nutrient gradients in host

- Tumor breaks up in its search for nutrient
Morphology diagram

Effect of microenvironment

- 3 distinct regimes:
  - Fragmented (nutrient-poor).
  - Fingered (high tissue resistance)
  - Hollowed (low tissue resistance, nutrient-rich)

\( A=0, \ G=20, \ G_N = 1 \)
\( N=0.35 \)

Macklin, Lowengrub JTB, in press
Hypoxia leads to cluster invasion, \textit{i.e.,} inhomogeneous nutrient distribution, imperfect vasculature.

Strong metastatic potential.

Implications for antiangiogenic therapy.

Combine with anti-invasive therapy.

G55 human glioblastoma tumors in vivo becoming invasive after antiangiogenic therapy.

Effect of Cell-based Parameters

- Increasing $G$ or $G_N$ enhances instability
- Increasing $G_N$ decreases necrotic core

$\chi_D = 1$, $\chi_\mu = 1$

Necrosis (degradation)

Behavior qualitatively similar
**Invasive fingering**

\[ \chi_D = 50, \quad \chi_\mu = 1 \]

- Growth into lower mobility regions results in larger invasive tumors
- Implication for therapy (decrease adhesion)

**Area ratios**

- Proliferating
- Necrotic
- Captured

**Shape parameter**

**Length scale**

Thick: \( \chi_\mu = 1 \)
Thin: \( \chi_\mu = 0.25 \)
Dependence on cell-based parameters

- Increasing $G$ or $G_N$ enhances instability
- Increasing $G_N$ decreases necrotic core
- May cause transition from fingering to compact, hollow (1D-like)

In vitro tumor spheroid


\[ \chi_D = 50, \quad \chi_\mu = 1 \]

Necrosis (degradation)
Hollow/Necrotic Growth

\[ \chi_D = 100, \quad \chi_\mu = 50 \]

- Repeated capture and coalescence leads to hollow/necrotic structure

Area ratios

shape parameter

length scale
Dependence on cell-based parameters

\[ \chi_D = 50, \quad \chi_\mu = \infty \]

- Strong effect on morphology—compact, 1D-like, hollow
- Increasing \( G \) or \( G_N \) enhances instability
- Increasing \( G_N \) decreases necrotic core
- Strong effect on morphology—compact, 1D-like, hollow

*In vitro* tumor spheroid


Necrosis (degradation)
Invasion Summary

• Microenvironment is a primary determinant for tumor growth and morphology
  (fragmented, invasive fingering, hollow/necrotic)

• Internal structure (e.g. size of necrotic, proliferating regions) determined by cell-based parameters

• Implications for therapy

• Experimental evidence for this behavior?
Comparison with experiment

Frieboes et al., Cancer Res. (2006).

- Model is qualitatively consistent with experimental results
Angiogenesis

Angiogenic factors:
- VEGF (Vascular Endothelial cell Growth Factor)
- FGF (Fibroblast Growth Factor)
- Angiogenin
- TGF (Transforming Growth Factor),....
Mathematical model

Anderson, Chaplain, McDougall, Levine, Sleeman, Zheng, Wise, Cristini,

Tumor Angiogenic Factor: $c$

Tumor angiogenic factor (e.g., VEGF-A): potent mitogen, drives motion

\[ 0 = D_c \nabla^2 c - \beta_D c - \beta_U c e + S_c (\rho_T, \rho_D) c \]

Uptake by the endothelial cells

Decay

Endothelial Cell (localized) density

production

Cell receptor ligand $f$ (e.g., Fibronectin) in the ECM.
Regulates cell adhesion and motion

\[ \frac{\partial f}{\partial t} = \eta_P e - \eta_U f e - \eta_N \chi_{\Omega_N} f, \]

production degradation

Matrix degradation by vascular endothelial cells

Decomposition of the tumor vasculature.
Gradient-based, biased circular random walk

Othmer, Stevens; Planck-Sleeman

Idea: track the capillary tip. Use the trace to describe the vessel. Not lattice-based.

• Endothelial cell travels with speed $s$ with direction given by the polar and azimuthal angles

• Endothelial cells tend to move up the gradients of $c$ and $f$ (chemotaxis, haptotaxis)

• Reinforced random walk for angles. Master equation:

$$ p(\theta, t + \Delta t) - p(\theta, t) = \hat{\tau}^+(\theta - \delta, t) \cdot p(\theta - \delta, t) + \hat{\tau}^-(\theta + \delta, t) \cdot p(\theta + \delta, t) $$

$$ - (\hat{\tau}^+(\theta, t) + \hat{\tau}^-(\theta, t)) \cdot p(\theta, t). $$

Prob. Density function Transition rate (gradient approach from Othmer-Stevens)
Model contd.

• Branching: Tip is allowed to split with a certain probability. (always takes 60 degree angle, from Exps).

• Anastomosis: If vessels are close, they may merge with a certain probability. If merged vessels are from different roots (i.e. pressure drop across) then may release nutrient (simple model of blood flow)

Nonlinear coupling with tumor:
  • Release of TAF by tumor cells affects EC motion
  • Source of nutrient from neovasculature affects tumor evolution via mitosis

(in reality is much more complicated but this is a start)
Simulation of Tumor-Induced Angiogenesis

Parameters appropriate for glioblastoma

Frieboes, Wise, Zheng, Lowengrub, Cristini, Neuroimage (in prep)
Vascular cooption

- Initial capillaries present
- Growing tumor surrounds vessels
- Uses up available vasculature
- Secondary angiogenesis
- Observe bursts of growth as the nutrient supply increases (like a fire)

Histology Slices

- Note nutrient supply localized near red (nutrient-releasing) vessels
- Observe corresponding (tumor) near vessels cell growth
• Regions of hypoxia separate cell clusters
Implications for therapy


Anti-invasive therapy

increase adhesion

Anti-angiogenic therapy

vessel disruption

Vascular normalization

2D: Cristini, et al., Cancer Res. (2006)
Next Steps

• More complex/realistic biophysics

• Improved invasion models

• Improved Angiogenesis models

• Integrative models—match parameters with experiments. Collaboration with Bullitt (Angiogenesis) Gatenby (Invasion and Morphologic instability)

• Hybrid continuous/discrete models

• Finite, complex domains

• More realistic mechanical response

• Even biophysically simplified modeling can provide insight though