Impact of metabolic heterogeneity on tumor growth, invasion, and treatment outcomes

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***Abstract*— A hybrid multiscale mathematical model of tumor growth is used to investigate how tumoral and microenvironmental heterogeneity affect treatment outcomes. A key component of this model is normal and tumor metabolism and its interaction with microenvironmental factors. In early stages of growth, tumors are stratified, with the most aggressive cells developing within the interior of the tumor. Simulations suggest that in some cases chemotherapy may increase the metabolic aggressiveness of a tumor due to drug-mediated selection.**

I. Heterogeneity: an important determinant of tumor progression

Tumor heterogeneity at the genetic scale has been known for decades and until recently was largely viewed as a whole tumor metric. Historically, molecular techniques average genomic signals from large numbers of cells from single biopsies, thus smoothing and potentially hiding underlying variations. However, a potential issue with this approach was recently highlighted by Swanton and colleagues [1], who showed that multiple biopsies from the same tumor display distinct genetic profiles and yet are phenotypically similar. This genotypic divergence and phenotypic convergence has previously been hinted at theoretically [2] and may be a predictable evolutionary consequence of the tumor ecosystem [3, 4]. The intricate dialogue between tumor cells and environment selects for clones that are best adapted phenotypically to survive, regardless of specific mutations that may facilitate tumor progression. Furthermore, this environment is temporally and spatially heterogeneous largely due to variations in blood flow, resulting in local fluctuations of nutrients, growth factors and other cellular populations (e.g. normal cells, stromal cells and immune cells). These dynamics occurring within the cancer ecosystem are almost impossible to dissect via experimentation alone.

A serious effort is being put into quantifying this heterogeneity and understanding how it evolves as the tumor progresses and how it relates to overall outcome [5-7]. However, we are far from understanding how the microenvironment modulates this heterogeneity and drives the overall phenotypic behavior of the tumor cell population. Furthermore, the impact of heterogeneity on treatment outcomes remains poorly understood.

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II. Therapy rapidly alters the selection pressures

Using a two-dimensional hybrid cellular automaton model, we have grown tumors in a vascularized tissue and applied therapy to them [8]. The heterogeneity was investigated on two metabolic axes: glycolytic capacity and resistance to extracellular acidosis. Absent treatment, a subset of tumor cells evolve under environmental selection pressure to become glycolytic and acid resistant. These properties cause increased tumor invasiveness under the right environmental conditions. Figure 1 shows the results of applying a chemotherapeutic agent in the model; in panels (a-f) the simulated tumor is shown, where the left column is the untreated case, the central column is an early application, and the right column is a late application. Panel (g) shows the growth curves for the three cases. The color of the tumor cells corresponds to the phenotype, and in all cases a heterogeneous mix is observed. Green tumor cells are metabolically normal, while purple cells are more aggressively glycolytic and acid-resistant. A segregated spatial structure is seen in the simulations; the aggressive cells develop in the center of the tumor, where hypoxia is more likely to occur due to dysfunctional vasculature.

This spatial heterogeneity has a profound impact on how the tumor responds to therapies given only months apart. The early application, in the central column, has the effect of delaying growth on the order of a year or two, compared to controls. On the other hand, applying the same therapy regimen just a few months later results in an *acceleration* of tumor growth, meaning that it would have been better to not treat at all. This occurs because the heterogeneity of the tumor at the time of the later treatment is poised for invasion, but not yet invasive. The therapy selects for these invasive cells, both spatially and temporally, and thus allows them to become actively invasive. This is compared to the early treatment case, where the invasive cells were not fully developed; the early treatment regimen has the effect of selecting for cells which are more resistant to invasion, due to their acid-resistant but low-glycolytic phenotype. In our publication [8], we have examined a number of therapies, showing how tumor heterogeneity has an impact on how therapies either succeed or fail.

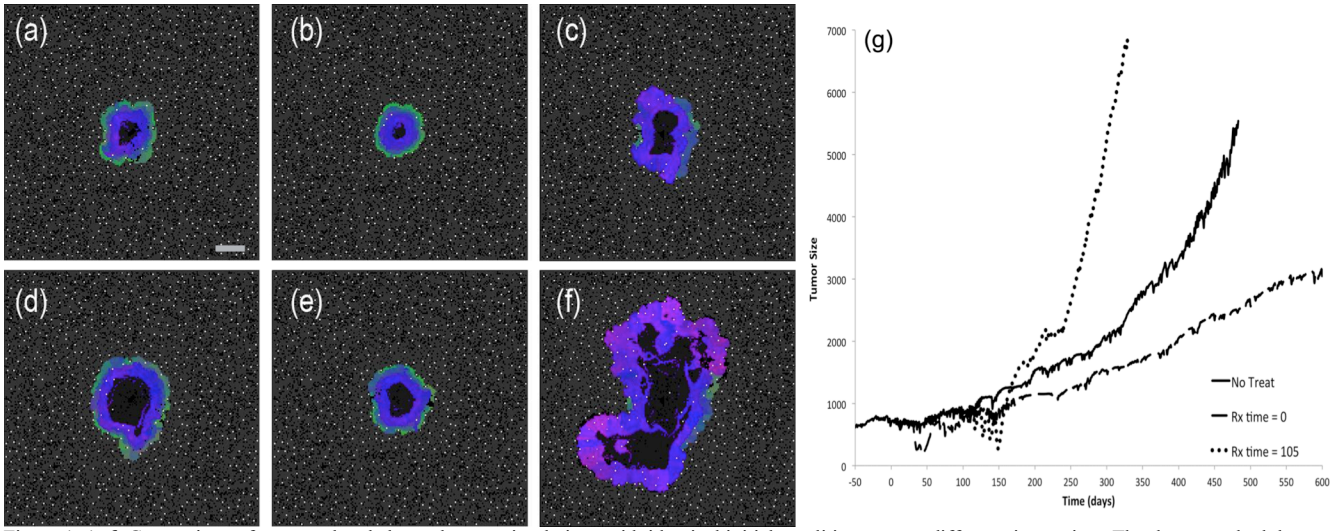

Figure 1. (a-f) Comparison of untreated and chemotherapy simulations with identical initial conditions at two different time points. The therapy schedule was 5 pulses, 2 weeks apart. The left column is an untreated simulation; the central column was pulsed with cytotoxic therapy starting at t_{rel}=0; the right column starts the identical treatment at t_{rel}=105. The top panels (a-c) show the state of the three simulations at t_{rel}=264, i.e. shortly after the tumor in the right column has finished the therapy. The bottom set of panels (d - f) shows the state of the tumors at t_{rel}=380. Scale bar is 400 microns. (g) Growth curves for untreated (solid), early (dashed), and late (dotted) chemotherapy from (a-f). Tumor size on the vertical axis is the diameter in microns.

III. Quick Guide to the Methods

A. Equations: The concentration of a molecule (C(x)) across a tissue is described by

$$\frac{\partial C}{\partial t} = D\nabla^2 C + f(\mathbf{C}, \mathbf{p}), \quad (1)$$

with diffusion constant D, and f describing the production and consumption of the molecule depending on the concentrations of extracellular molecules ($\mathbf{C}(x)$) and cellular parameters ($\mathbf{p}(x)$) at position x. Cells primarily produce ATP from glucose (G), using either an efficient aerobic pathway that requires oxygen (O), or using glycolysis, an inefficient anaerobic pathway that produces protons (H). The model assumes that cells meet a target level of ATP demand by preferentially using the aerobic pathway, and making up the difference by increasing flux through the glycolytic pathway in hypoxic regions. Oxygen consumption (f_O) and glucose consumption (f_G) are determined by the need to meet normal ATP demand (A_0), given by

$$f_O = -V_O \frac{O}{O+k_O}, \; f_G = -\left(\frac{p_G A_o}{2} + \frac{27 f_O}{10}\right)\frac{G}{G+k_G} \quad (2)$$

For tumor cells, if the coefficient p_G>1, the tumor will consume more glucose than needed to meet normal ATP demand, representing constitutively activated glucose consumption seen in many tumors. The actual ATP production rate for the cell (f_A) is determined from nutrient consumption rates, given by

$$f_A = -\left(2f_G + \frac{27 f_O}{5}\right). \quad (4)$$

Proton production (f_H) is linked to the amount of glycolysis that does not feed the aerobic pathway, given by

$$f_H = k_H \frac{p_G V_o + f_O}{5}, \quad (5)$$

where parameter k_H accounts for proton buffering.

This metabolic program is implemented into each cell of a hybrid cellular automaton (HCA) model. One cell type is permitted per grid point, either a normal cell, tumor cell, necrotic cell, or blood vessel. For each time step dt, Eq. (1) is solved over the domain of the HCA for the steady state. Then, cells in the grid are put through a decision process based on the metabolic state of each cell. Cells with enough ATP to meet the threshold of proliferation will advance their cell cycle. Cells that have completed the cell cycle will proliferate if there is adjacent space. The cycle is not advanced if the cell is quiescent due to lowered ATP production. Cells with production less than a death threshold are removed.

Tumor cells have two heritable traits: excess glucose consumption, p_G from Eq. 2; and resistance to extracellular acidosis. These traits are passed from a parent cell to its daughters modified by a small equally-weighted variation, variation chosen at random. The model is agnostic with respect to specific biological mechanisms that underlie this drift, which could include gradual accumulation of mutations, regulation of gene transcription by epigenetics or aneuploidy, or changes in the number or structure of organelles.

A point-source vasculature is used to simulate blood vessels that spatiotemporally deliver nutrients and remove waste products. The field of vessels is seeded using a circle-packing algorithm based on vessel densities *in vivo*. This initial distribution can be altered by the creation of new vessels through angiogenesis, or by vessel degradation. For angiogenesis, new vessels are added to regions of hypoxia until there is enough oxygen delivery to remove the hypoxic state. Vessels are degraded over time due to surrounding tumor growth until they are lost from the tissue. These two opposing vascular forces impact the gradients of diffusible molecules.

Chemotherapy is pulsed through the vasculature, diffusing through the tissue subject to Eq. (1). Cell death depends on the concentration of the drug at the cell position.

B. Type of settings in which these methods are useful

HCA models have been used extensively to model cancer [9], including applications to angiogenesis [10-13], cell motility and invasion [14-18], tumor evolution and microenvironment [19-35], tumor-immune interactions [36], and metastasis [37, 38].

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