Mathematical Oncology

Prediction of Drug Response in Breast Cancer Using Integrative Experimental/Computational Modeling

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Abstract

Nearly 30% of women with early-stage breast cancer develop recurrent disease attributed to resistance to systemic therapy. Prevailing models of chemotherapy failure describe three resistant phenotypes: cells with alterations in transmembrane drug transport, increased detoxification and repair pathways, and alterations leading to failure of apoptosis. Proliferative activity correlates with tumor sensitivity. Cell-cycle status, controlling proliferation, depends on local concentration of oxygen and nutrients. Although physiologic resistance due to diffusion gradients of these substances and drugs is a recognized phenomenon, it has been difficult to quantify its role with any accuracy that can be exploited clinically. We implement a mathematical model of tumor drug response that hypothesizes specific functional relationships linking tumor growth and regression to the underlying phenotype. The model incorporates the effects of local drug, oxygen, and nutrient concentrations within the three-dimensional tumor volume, and includes the experimentally observed resistant phenotypes of individual cells. We conclude that this integrateivc method, tightly coupling computational modeling with biological data, enhances the value of knowledge gained from current pharmacokinetic measurements, and, further, that such an approach could predict resistance based on specific tumor properties and thus improve treatment outcome. [Cancer Res 2009;69(10):4484–92]
Quick Guide to Equations and Assumptions

Equation 1: \[ \frac{\partial \Phi_V}{\partial t} + \nabla \cdot (u_V \Phi_V) = M \nabla \cdot \left( \Phi_V \left( \frac{\partial E}{\partial \Phi_V} \right) \right) + S_V \]
\[ \frac{\partial \Phi_D}{\partial t} + \nabla \cdot (u_D \Phi_D) = M \nabla \cdot \left( \Phi_D \left( \frac{\partial E}{\partial \Phi_D} \right) \right) + S_D \]

The three-dimensional in vitro environment is modeled as a mixture of viable tumor tissue (volume fraction \( \Phi_V \)), dead tumor tissue (\( \Phi_D \)), and interstitial fluid (\( \Phi_{IS} \)). These partial differential equations are derived from the conservation of mass of these quantities (22, 23). From left to right in each equation, the terms represent change of volume fraction with respect to time; tissue advection (bulk transport) by the overall mixture as it moves with local velocity \( u \); net tissue diffusion due to the balance of mechanical forces, including cell-cell adhesion (related to mobility constant \( M \); refs. 22, 23) and cell-cell adhesion and repulsion (osmotic pressure), modeled using the variational derivative of the adhesion energy \( E \) (for specific forms of this energy, see ref. 22); and net source of tissue due to the balance of mitosis, apoptosis, and necrosis.

In Words: The temporal rate of change in viable and dead tumor tissue at any location within the tumor equals the amount of mass that is pushed, transported, and pulled due to cell motion, adhesion, and tissue pressure, plus the net result of production and destruction of mass due to cell proliferation and death.

**Major Assumptions of the Model**

The tumor is a mixture of cells, interstitial fluid, and ECM, and we treat the motion of interstitial fluid and cells through the ECM as fluid flow in a porous medium. Therefore, no distinction between interstitial fluid hydrostatic pressure and mechanical pressure due to cell-cell interactions is made. Cell velocity is a function of cell mobility and tissue onscotic (solid) pressure (Darcy’s law); cell-cell adhesion is modeled using an energy approach from continuum thermodynamics (22).

Equation 2:
\[ S_V = (\lambda_M(\sigma, d) - \lambda_A(\sigma, d) - \lambda_N[H(\sigma_N - \sigma)]) \Phi_V \]
\[ S_D = (\lambda_A(\sigma, d) + \lambda_N[H(\sigma_N - \sigma)]) \Phi_V - \lambda_D \Phi_D \]

These equations specify net sources \( S_V \) and \( S_D \) of mass for viable and dead tumor tissue, respectively. This directly links the conservation of mass equations, Eq. 1, to the cell phenotype through hypothesized phenomenological functional relationships that include diffusion of cell substrates and drug (of local concentration \( \sigma \) and \( d \), respectively) through tumor interstitium (see ref. 22 and references therein). For the viable tissue (first equation), the terms on the right-hand side represent, from left to right, volume fraction gained from cell mitosis (rate \( \lambda_M \)) and lost to cell apoptosis (\( \lambda_A \)) and necrosis (\( \lambda_N \)). For the dead tissue (second equation), the terms on the right-hand side represent, from left to right, increase in volume fraction from cell apoptosis and necrosis and decrease in volume fraction from cell disintegration by lysis (\( \lambda_D \)). All rates are inverse time.

In Words: Mass of viable tumor tissue increases through cell proliferation and decreases through cell apoptosis and necrosis. Mass of dead tumor tissue increases through cell apoptosis and necrosis and decreases through cell lysising. These equations provide a means of incorporating the biology of the problem into the physical conservation laws in Eq. 1.

**Major Assumptions of the Model**

Cells are composed entirely of water, which is a reasonable first approximation in terms of volume fraction (see discussion in ref. 22). Cell mitosis is proportional to substrates present (47). As mitosis occurs, an appropriate amount of water from the interstitial fluid is converted into cell mass. Substrate depletion below a level \( \sigma_N \) leads to necrosis (26, 7). Cell lysis represents a loss of mass as cellular membranes are degraded and the mass is converted into water that is absorbed into the interstitial fluid. The simulated interface between viable and necrotic tissue is forced to be of biologically realistic thickness (10–100 \( \mu m \)) through the Heaviside function \( H \) (see refs. 22, 23). Mitosis and apoptosis rates are functions of drug (\( d \)) and substrate (\( \sigma \)) concentrations. Note that the assumption that parameters measured for drug sensitivity in monolayer, modulated by diffusion gradients, can adequately represent response in vitro may not be correct for real tumors (e.g., it disregards drug resistance from growing with an ECM).

Equation 3:
\[ \eta^{-1} \frac{\partial \sigma}{\partial t} = L^2 \nabla^2 \sigma - \sigma \]

This is a partial differential equation that describes cell substrate concentration \( \sigma \) across the tumor viable region. The first term on the right-hand side models diffusion of the substrates into the tumor spheroid with penetration length \( L \). The second term models substrate uptake by the cells (with rate \( \eta \)). An analogous equation is posed for drug concentration \( d \).
In Words: The temporal rate of change of cell substrates or drug across the tumor viable region equals the net amount that diffuses into the region minus the amount taken up by the tumor cells.

**Major Assumptions of the Model**

Diffusion of cell substrates and drug into the tumor, combined with uptake in the tumor interior, creates and maintains a gradient of these substances through the three-dimensional tumor tissue, which is assumed to be fairly compact (noninvasive/infiltrative).

Although *in vitro* spheroids are a gross simplification of the complex *in vivo* condition, they allow capturing the effect of three-dimensional tissue architecture in generating diffusion gradients of drug and cell substrates under controlled conditions without the complicating effects of blood flow (1). Spheroids develop a layer of viable cells surrounding a necrotic core (26). The thickness of this layer (~100 μm), maintained by substrate diffusion gradients, “mimics” the viable tissue thickness supported by diffusion from surrounding blood vessels *in vivo* (26). This provides a more controllable experimental model than *in vivo*, which is necessary to test and calibrate the computational model parameters. In turn, computer simulations of the model enable formulating and testing the functional relationships that quantify the dependence of drug resistance on diffusion gradients and particular tissue characteristics (e.g., tissue compactness as a function of cell packing density; ref. 27). We conclude that computational modeling tightly integrated with tumor biology extends the information that can be learned from pharmacokinetic experiments (28–30), offering a promising possibility of ultimately quantifying and predicting treatment response from individual tumor characteristics.

**Materials and Methods**

Doxorubicin was used as the cytotoxic drug in the experiments. Experiments used MCF-7 wild-type (WT) breast cancer cell lines to represent drug-sensitive tumor cells and MCF-7 40F breast cancer cell lines to represent drug-resistant tumor cells. Details of experimental methods are given below.

**Observation of cell substrate gradients.** Confluent MCF-7 WT (breast cancer) cell monolayers were incubated with 0.25% (w/v) trypsin (Sigma) and 0.02% (w/v) EDTA (Sigma) for 4 min at 37°C. Complete medium was added to this mixture to inhibit enzyme activity, and the cell suspension was passed through a 24-gauge needle six times to ensure a single-cell population. A 10-ml High Aspect Ratio Vessel (HARV; Synthecon) was seeded at 2×10^6 cells/ml and rotated at 15 rpm at 37°C in a humidified atmosphere of 5% CO₂ in air. Aggregates formed within 6 h and were maintained in RCCS culture for a total of 30 d. During this time, medium in the HARVs was replenished 50:50 every other day. Spheroids were fixed in formalin, embedded in paraffin, and 5-μm sections cut for immunohistochemistry.

To observe hypoxia, unfixed spheroids were incubated in 200 μmol/L pimonidazole (Chemicon) in complete medium for 2 h at 37°C. Cells for monolayer culture were seeded at 20,000 per well in 24-well Costar 3527 cell culture plates (Corning). Cells for spheroids were seeded at 50,000 per well in 24-well Costar 3473 cytophobic plates and shaken at 100 rpm for 10 min on day 1. Both the cells in monolayer and spheroids were incubated for 3 d and then exposed to doxorubicin (Bristol-Myers Squibb) concentrations ranging from 0 to 16,384 nmol/L in 4× nanomolar increments (0, 4, 16, 64, etc.) for 96 h, representing at 256 nmol/L a typical area under the curve (AUC) in *in vivo* (29). Negative controls were seeded and incubated under the same conditions without drug. Three end points were concurrently measured as fraction of negative control proliferation using tritiated thymidine (Amer sham) incorporation assay (31), viability using trypan blue exclusion counts, and metabolic activity using XTT (sodium 3-[1-(phenylamino)-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate) assay (32).

All experiments were done in triplicate. Photographs were taken with a digital camera through a Zeiss microscope (>100 magnification).

**Mathematical model of tumor growth and drug response.** Briefly, the equations (22, 23) are formulations of mathematical models used in engineering to describe phase separation of two partially miscible components (viable and necrotic tumor tissue), diffusion of small molecules (cell substrates and drug), and conservation of mass (Quick Guide, Supplementary Fig. S1; refs. 33–36). Mass conservation equations describe growth (proliferation as a function of total number of cycling cells) and death from the cytotoxic effects of the drug (apoptosis as a function of a rate constant dependent on the unique cell sensitivity and as a function of cycling cells). These are combined with diffusion of small molecules to a reaction-diffusion equation. Rate constants for proliferation and apoptosis are modified by functions that represent their dependence on cell nutrients and oxygen (proliferation) and drug concentration (death), along with a dependence on spatial diffusion of these substances. By dimensional analysis of the reaction-diffusion equation, the diffusion penetration length is \( L = (D/\eta)^{1/2} \), where \( D \) is the corresponding diffusion constant and \( \eta \) is a characteristic cellular uptake rate. Large differences in concentration will occur if \( L \) is much smaller than the tumor radius (linear dimension) in the absence of blood vessels, creating a steep gradient. By using \( L \) and knowing the spheroid size, one can determine the steepness of these gradients by solving the reaction-diffusion equation. We note that gradients are affected by cell packing density, intracellular uptake, and duration of drug exposure, as well as conditions specific to particular experiments.

**Estimation of mathematical model parameters.** In our model, cell substrates are represented by glucose and oxygen, with \( L \) estimated from independent measurements taken from the literature. For glucose, \( D = 1 \times 10^{-10} \ cm^2/s \) (37) and uptake rate may be as large as \( 1 \times 10^{-3} \ s^{-1} \) (38), whereas for oxygen, the corresponding values are \( 1 \times 10^{-5} \ cm^2/s \) (39) and \( 1 \times 10^{-7} \ s^{-1} \) (40). Diffusion penetration lengths thus calculated (\( L \approx 100 \mu m \)) are consistent with our experimental observations (immunohistochemistry) and with previous (murine mammary tumor EMT6/Ro spheroid) observations showing glucose concentration decreasing by 65% (41) and oxygen decreasing by 90% (42) across the tumor viable region.

Although gradients of drug into tumor tissue have been observed with a number of different cell types, many of these observations have been 12 In pharmacokinetics, the AUC is a calculation to evaluate the total exposure of the body to a given drug. In a graph plotting plasma drug concentration versus time, the area under the curve is the AUC.
qualitative. Thus, published values for diffusion constants and uptake rates can vary considerably. We estimate doxorubicin diffusion length by considering that the distance at which the penetrating concentration will be 50% of the source is ln 2 times the diffusion length—equivalent $L$, here is $-90 \mu m$ for a 2/3 drop across the spheroid viable region ($\approx 100 \mu m$). This is consistent with in vitro doxorubicin penetration into Chinese hamster lung cell spheroids at 24 h (43) reporting an average 2/3 drop of external concentration across the viable region. Other studies have shown doxorubicin penetration in vivo (mouse model) decreasing by half within 40 to 50 $\mu m$ of blood vessels by 3 h (4), as well as penetration in humans (biopsies) decreasing by half within 50 $\mu m$ of blood vessels after 2 h of a bolus and within 60 to 80 $\mu m$ after 24 h in breast cancer tissue (2). By fitting the solution of the unsteady Eq 3 to these data, we could indirectly estimate average cellular uptake rates $\eta$ of doxorubicin by breast cancer cells in three-dimensional tissue of $-1 \text{ day}^{-1}$, consistent with the independent penetration length estimate $L = (D/\eta)^{1/2}$ from the steady-state profiles.

The rate $\lambda_{N}$ (inverse time) measures the change in cell number in a population due to mitosis, normalized by total cell number (control = cells with no drug), and was thus calibrated by matching proliferation data from our experiments, using as initial guess for $\lambda_{M}/\lambda_{NC}$, the in vitro cell proliferation as fraction of control divided by in vitro cell viability (total number of cells $N$ in monolayer) as fraction of control, yielding an estimate of cell proliferation $-1 \text{ day}^{-1}$.

Apoptosis rate $\lambda_{A}$ (inverse time) over the period $0 \leq t \leq T$ (total experiment time) was estimated as a function of local concentrations of substrate $\sigma$ and drug $d$ as $\lambda_{A}(\sigma, d) = \lambda_{A}(\sigma_{\infty}, d) + \lambda_{A}(\sigma)$, where $\lambda_{A} = -T^{-1}\log(N(\sigma)/N(\sigma_{\infty}))$ and $\lambda_{A}(\sigma) = -T^{-1}\log(1 + \sigma(1 - \sigma/\sigma_{\infty}))$. Death rate $\lambda_{A}$ was calculated from measurements of cell viability $N$ as fraction of negative control $N_{c}$ over time in monolayer cell cultures at various drug concentrations. Physiologic resistance based on cell cycle status was modeled with $\lambda_{A}$. Decreased substrate concentration in three dimensions results in cell populations that cycle less and therefore have reduced sensitivity to doxorubicin. In the above formula, enhanced survival was assumed to be linearly dependent on substrate concentration with a fitting constant calibrated from previous observations (12) showing an average MCF-7 viability increase 2.2 to 4 times for glucose deprivation of up to 50% at drug concentrations similar to our study (i.e., $2.2 \leq 1 + \sigma/2 \leq 4.0$). In our experiments, glucose concentration in the medium was $\sigma_{\infty} = 2.0 \text{ g/L}$. Note that for $\sigma = \sigma_{\infty}$ the above formula gives $\lambda_{A} = 0$ and thus $\lambda_{A} = \lambda_{A}^{*}$. Necrosis parameters for diffusion-limited growth $\lambda_{N}$ (rate of necrosis) and $\sigma_{\infty}$ (substrate limit for cell viability) were calibrated by matching simulation results to in vitro spheroid growth curves and histologic data under conditions with no drug, as described previously (45), so that the simulated spheroid radius and viable region thickness matched in vitro observations. These parameters are directly responsible for the steady spheroid size (and extent of necrosis) after a growth period because they regulate the balance of mass growth due to cell proliferation in the viable region and mass loss due to cell disintegration in the necrotic center (45).

Based on our in vitro measurements of spheroid and necrotic volumes, we found a stationary average spheroid radius of $\approx 0.8 \text{ mm}$, with viable region of thickness $\approx 0.1 \text{ mm}$ (see also ref. 46). The latter is consistent with the estimated cell substrate penetration length $L$ calculated above. The model calibration based on these quantities consistently yielded $\lambda_{A}/\lambda_{N} = 0.7$ and $\sigma_{\infty}/\sigma_{\infty} = 0.5$.

Higher cell densities were observed for resistant spheroids, which had a more compact morphology (27). Based on these observations, higher cell adhesion parameter values (22) were used in the model equations to simulate resistant spheroids than for sensitive ones. We used a recently presented calibration procedure (45) to determine the relative values for sensitive and resistant cell cultures. This observation and the resulting different parameters for the two cell phenotypes underscore the need to explicitly model cell adhesion forces when constructing models of cancer growth.

**Results**

The hypothesized functional relationships of the computational model link growth and regression of the tumor mass to the underlying phenotype as described in Eqs. 1–3 (Quick Guide). The model is fully constrained (Materials and Methods) through experimentally based parameters (Fig. 1). To quantify physiologic resistance in three-dimensional tumor tissue, diffusion gradients

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15 Cytotoxicity of MCF-7 cells to doxorubicin may be affected more by glucose deprivation than by hypoxia (6, 44). The glucose-regulated stress response (10) has been correlated with resistance to topoisomerase II–directed chemotherapeutic drugs such as doxorubicin (10, 11) through a decreased expression of topoisomerase II in MCF-7 cells (12).
are incorporated in the functional relationships and the model is calibrated to compute these gradients. Accordingly, immunohistochemical measurements were done on drug-sensitive tumor spheroids cultured in vitro (Fig. 2) to confirm that in our experiments (a) cell substrate concentration decreases across the radius of the spheroid, (b) apoptosis correlates with hypoxia, and (c) proliferation correlates with substrate availability (43, 47). Increasing positivity for pimonidazole protein adducts across the viable rim (~100 μm) in the direction of the spheroid center indicates a gradient of oxygen. Across the viable region, up-regulation of Na+/H+ transporters detected by increasing positivity for NHE-1 is observed, showing a gradient of pH. Na+/H+ transporters are up-regulated in response to acidosis. Up-regulation of GLUT-1 transporters (adaptation to hypoxic/hypoglycemic stress) is shown in the increasing positivity for GLUT-1 (48) in perinecrotic regions, indicating gradients of glucose and oxygen. The frequency of apoptotic cells increases toward the center as shown by increasing positivity of terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining. End-stage apoptotic bodies are concentrated at the perinecrotic necrotic regions, with most cells in the center being apoptotic or necrotic. Cell proliferation is limited to the viable rim, as shown by Ki-67 nuclear staining.

According to the computational model, the solution of the reaction-diffusion, Eq. 3, yields substrate gradients (Fig. 3A) developing across a viable region of width ~0.1 mm. The computed width of this region is consistent with previous observations by other investigators (e.g., ref. 26). Both glucose and oxygen substrate concentrations were calculated in the model to drop by at least 50% across the spheroid viable region, with an average concentration (σ/σ∞) ≈ 0.7 in the living tumor tissue. This is in agreement with measurements of glucose (41) and oxygen (e.g., ref. 42) in spheroid viable regions from previously reported in vitro measurements (Materials and Methods).

Similarly, the model computes the drug gradients that develop within the viable tumor spheroid tissue. The calculated drug concentration profile reaches steady state in ~24 hours and decays by 2/3 across the viable region to yield an average drug concentration of 50% that of outside the spheroid (Fig. 3B). Note that the drug penetration length is comparable to the penetration length of substrates (Fig. 2). Because doxorubicin is a cell cycle–specific drug and the proportion of cells that are cycling correlates with the substrate availability, the drug would be less effective as the proportion of cycling cells is diminished. Not only is its concentration decreased by the diffusion barrier but also its efficacy is impaired as a result of the substrate gradients. The physiologic resistance predicted by the model based on the hypothesized functional relationships would, in principle, apply to any cell cycle–specific drug.

Cell proliferation (ratio of proliferation-to-viability counts) in vitro under conditions with diffusion gradients (i.e., in spheroids) versus monolayers was lower by at least 50% for both cell lines at drug concentrations d > 64 nmol/L representing clinically relevant dosages14 (Fig. 4A). Further, increased viability for drug-sensitive cells in three dimensions corresponded with ~50% lower cell metabolic activity (ratio of metabolic-to-viability counts) in vitro (data not shown). These data indicate that cell quiescence was significant at drug dosages similar to in vivo conditions. The cell apoptosis parameter of the computational model calculated from the monolayer cell viability data increases with drug concentration (Fig. 4B).

The functional relationships of the computational model (Eqs. 1–3) linking growth and regression of the tumor mass to the underlying phenotype are validated by quantifying the physiologic resistance introduced by the diffusion gradients. Cell viabilities predicted by the model agree well with the ones observed experimentally in three-dimensional spheroids (Fig. 5). In contrast, monolayer cell viability data are a poor prognosticator of drug resistance in three-dimensional tumors. An average survival increase (as fraction of control) of 250% over monolayer was predicted for drug-sensitive (MCF-7 WT) spheroids under various drug concentrations (Fig. 5A), whereas for drug-resistant (MCF-7 40F) spheroids, the corresponding increase was 280% (Fig. 5B). The median dose15 for MCF-7 40F was ~20% higher in spheroid versus monolayer than for MCF-WT, implying not only that the
three-dimensional morphology promoted the net survival for both cell types but also that the resistant phenotype was further favored by the three-dimensional configuration. We noted that drug-resistant spheroids maintained more compact, nearly spherical shapes (Fig. 6A) that simply decreased in size as cells were killed, whereas drug-sensitive spheroids formed irregular, looser shapes (Fig. 6B) that fragmented, particularly at higher drug concentrations. The tumor morphology may depend on the competition between heterogeneous cell proliferation caused by diffusion gradients of cell substrates, driving shape instability, and stabilizing mechanical forces such as cell-cell and cell-matrix adhesion, as we quantitatively showed in previous work (45). Here, we observe a similar instability for drug-sensitive cells, indicating that their adhesion is below the threshold necessary to maintain tumor shape stability. This is confirmed through the computational model, where variation in the parameter values for cell adhesion (22) reproduces these morphologies (Fig. 6C and D).

Discussion

We have used an integrative method that tightly couples computational modeling with biological data to quantify physiologic resistance in three-dimensional tumor tissue in vitro. The model hypothesizes functional relationships that mechanistically link the growth and regression of a tumor mass to the underlying phenotype. These relationships incorporate the complex interplay between tumor growth, cell phenotype, and diffusion gradients.

![Figure 3](image3.png)

**Figure 3.** Computational model computes the gradients in three-dimensional (3-D) tissue. Cross section of a tumor spheroid in the computational model (A; dotted line, tumor boundary; dashed line, perinecrotic area) showing calculated diffusion gradients (arrow) of cell substrate concentration $r/r_{\text{c}}$ (as fraction of external concentration in the medium) using diffusion penetration length parameter $L \approx 100 \mu m$. Necrosis becomes significant at substrate level $r/r_{\text{c}} < 0.5$. B, doxorubicin concentration $d/d_{\text{c}}$ in tumor tissue (normalized with concentration in the medium) versus distance from the tumor/medium interface, estimated for the model to be consistent with data in refs. 2, 43 and others (Materials and Methods). By solving the unsteady Eq. 3, the drug gradient across the viable region can be calculated at various times: bottom curve, 2 h; middle curve, 24 h; topmost curve, steady-state, showing $-2/3$ drop from the tumor edge to the perinecrotic region.

![Figure 4](image4.png)

**Figure 4.** Cell proliferation and apoptosis. A, cell proliferation (ratio of proliferation-to-viability counts; $n = 3$) in vitro was lower by at least 50% under conditions with diffusion gradients (spheroids) versus monolayers for both cell lines at drug concentrations $d > 64 \text{ nmol/L}$ representing clinically relevant dosages. B, drug-induced cell apoptosis death rate parameter $k_{\Lambda}(r, d)$ (inverse time) versus doxorubicin concentrations $d$ calculated (Materials and Methods) from measured in vitro monolayer viability counts ($n = 3$); glucose concentration $r_{\text{c}} = 2.0 \text{ g/L}$.
caused by heterogeneous delivery of oxygen and cell substrates and removal of metabolites, and are thus able to calculate tumor drug response as a predictable process dependent on biophysical laws. The results underscore the importance of a quantitative approach in evaluating chemotherapeutic agents that takes into consideration diffusion in addition to such chemoprotective effects as cell phenotypic properties and cell-cell and cell-extracellular matrix (ECM) adhesion (49). Although the model was calibrated with in vitro data for breast cancer, this approach may also apply to drug response of other tissues.

By modeling transport of drug and cell substrates, this work creates a quantitative tool that could in the future be used to predict resistance in patients based on their tumor cell properties, thus improving treatment outcome. In particular, the proposed functional relationships help to quantify resistance in human breast cancer due to local cell substrate depletion. Our integrative experimental/computational approach provides insight into the physical dynamics of solid tumors and validates the hypothesized functional relationships as adequate to describe the observed phenomena. Although more complex functional forms could conceivably also provide a reasonable match between model and experimental results, quantifying the diffusion effect with a minimal mathematical description is the preferred approach, unless it is known to be wrong, as it facilitates insight into the system and provides for more economical simulations. We explicitly chose not to use complex relationships that would contain more parameters than the number of independent measurements available for calibration, thus resulting in an underdetermined problem.

Cell packing density affects the magnitude of diffusion gradients and may pose a barrier to effective drug penetration (27). This density can vary between cell lines and tumors and reflect variations in drug resistance (compare Fig. 6). How this relates to cell adhesion molecule expression (e.g., integrin and E-cadherin) is unclear. Mechanisms of cell packing may include stronger cell adhesion forces due to higher E-cadherin expression; this should also limit proliferation, which does not seem to be the case here for the drug-resistant cells at lower drug concentrations. Additionally, cellular stress affects the quantity and strength of cell adhesion molecules. We have previously investigated the effect of the tumor microenvironment on tissue morphology (45, 50), suggesting that marginally stable environmental conditions could directly affect morphogenesis and present an additional challenge to therapy (45, 48) in vivo by increasing tumor cell invasiveness and leading to complex infiltrative morphologies, depending on the magnitude of cell adhesion forces that tend to maintain compact, noninvasive tumors (50). Diffusion gradients combined with higher cell packing density augment drug resistance synergistically, as observed in our experiments with the higher median dose for the more compact, drug-resistant spheroids, yet it may be difficult to deterministically gauge their combined effect (5). Synergism may be due to increased

Figure 5. Validation of the hypothesized functional relationships. This is done by the computational model quantifying the physiologic resistance introduced by the diffusion gradients. Cell viabilities as fraction of control \( N/N_c \) versus doxorubicin concentrations \( d \); glucose concentration \( r_1 = 2.0 \text{ g/L} \); time \( T = 96 \text{ h} \) of drug exposure; \( n = 3 \). A, MCF-7 WT drug-sensitive cells. B, MCF-7 40F drug-resistant cells. White columns, three-dimensional in vitro tumor spheroids (dark gray columns, in vitro monolayer data reported for comparison); light gray columns, predictions of the computational model (error bars reflect variation in the apoptosis rate parameter—see Materials and Methods).

Figure 6. Correlation of tumor morphologic stability with drug resistance. A, compact, nearly spherical morphology of a drug-resistant MCF-7 40F tumor spheroid in vitro is contrasted with the looser, irregular morphology of a drug-sensitive MCF-7 WT spheroid (B). In the model, stable (C) and unstable (D) morphologies depend on variation in the parameter values for cell adhesion forces (45). Bar, 100 \(\mu\text{m}\).
drug binding to ECM in tumor areas proximal to the drug source, whereas substrate and drug penetration to distal areas is significantly reduced due to higher cell packing, thus exacerbating the resistance effect of the diffusion barrier.

Note that the presumption that the necrotic region is not a risk factor for progression may not be necessarily true because selection pressures for resistance and induction of mutation are strongest in hypoxic, perinecrotic areas. Thus, inducing a large necrotic fraction during treatment may paradoxically further select for drug resistance.

The class of prediction modeling presented in this article, based on an assimilation of complex processes with a fully three-dimensional physical environment, offers the capability of complementing current pharmacokinetic measurements. A more expansive integration of theoretical model parameters with biological data could help to move toward prediction of treatment response based on in vitro and in vivo tumor information, and determine the correspondence between in vitro measurements and the in vivo condition to refine and validate the assumption that this approach can describe in vivo tumors. Incorporation of patientspecific tumor phenotypic and microenvironmental parameters into the model could enhance clinical strategies and prognosis evaluation. We further envision that these methods will enhance current pharmacokinetic models used in designing and interpreting phase II clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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