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Mathematical Modeling of the Interactions between Cellular Programs in Response to Oncogene Inactivation

Incorporation of both Cell Intrinsic and Cell Extrinsic (Immune Mediated) Effects

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Abstract—**Some tumors' addiction to the over-expression of a single oncogene provides a weakness for a molecularly targeted therapy to exploit. A number of targeted therapy drugs (e.g., imatinib, erlotinib, etc.) produce dramatic tumor regression but are often eventually followed by tumor relapse. Understanding the complex interaction of mechanisms behind the tumor's overall response to oncogene inactivation is key to preventing tumor relapse. It is becoming clear that there is a general sequence of cellular responses and that the latter immune-mediated steps are key to eliminating residual disease. What is still unclear is exactly how these mechanisms work together to produce the overall tumor regression response.**

We have built a novel integrative computational model that includes the various cellular response mechanisms of tumors to oncogene inactivation. This consists of cell intrinsic programs (e.g., apoptosis, proliferation, differentiation, dormancy) and immune-mediated cell extrinsic programs (e.g., senescence, inhibition of angiogenesis). Our model unifies these different response programs in order to allow for predictions of the time-course of events following oncogene inactivation and their impact on tumor burden.

Eventually, we will use this model to make and validate predictions of the effect of various therapeutic strategies and interventions (e.g., adjunct chemotherapy, immune system modulation). These predictions will be validated *in vivo* using conditional expression transgenic mouse models of MYC-induced lymphoma, osteosarcoma, and hepatocellular carcinoma.

Keywords-mathematical model, oncogene addiction, oncogene inactivation, cellular programs, immune system, bioluminescence imaging, cancer

I. INTRODUCTION

First proposed by Bernard Weinstein in 1997, oncogene addiction is the phenomenon whereby the inactivation of a single oncogene, even if brief, may lead to sustained tumor regression, providing a weakness for a molecularly targeted therapy to exploit [1]. For example, imatinib causes dramatic tumor regression in gastrointestinal stromal tumors (GIST), chronic myelogenous leukemia (CML), and other tumors by inhibiting the *Bcr-Abl* oncogene; erlotinib causes dramatic tumor regression in non-small cell lung cancer (NSCLC) and pancreatic cancer, and other tumors by inhibiting EGFR; and numerous other examples of targeted therapies exist. These drugs induce dramatic tumor regression without the side effect profile of non-specific chemotherapies.

Inactivation of the oncogene, whether through a targeted drug or modeling a targeted drug using the conditional expression of a transgene in a mouse model, produces a complex array of responses at the cellular level including apoptosis, cell cycle arrest, differentiation, senescence, inhibition of angiogenesis, etc. Some of these programs are cell intrinsic (cell autonomous) while others are cell extrinsic, involving complex host interactions with the immune system. While these different response mechanisms have been studied and modeled individually, there has been far less investigation into integrating the overall sequence and interactions of tumor responses into a unified model that can inform the design and optimization of therapeutic strategies. Understanding how and why some tumors relapse while others do not as well as how and why the specific cellular program responses depend on the tissue-specific and host immune background is of crucial importance for designing the most effective therapies.

Previously, we have built and validated a model of tumor growth and regression kinetics in response to oncogene inactivation [2]. This model explicitly incorporated apoptosis and proliferation but no other cellular programs. Here, we have created a mathematical model that captures the tumor growth kinetics as a function of all of the aforementioned cellular programs. We are building on this to develop and calibrate a novel integrative computational model of the tumor responses to oncogene inactivation (cell intrinsic and cell extrinsic) that we will use to predict, optimize, and validate various therapeutic strategies.

We will use the model in order to study the major cellular processes involved in *MYC*-induced lymphoma, osteosarcoma, and hepatocellular carcinoma and test different therapeutic strategies.

Much work has been done in characterizing tumor growth kinetics *in vivo* and mathematically modeling the cell intrinsic mechanisms involved in the response to oncogene inactivation. *In vivo* observations about cell extrinsic mechanisms in response to oncogene inactivation have been published recently, but little if any mathematical or computational modeling has been done to complement these theories. This work is among the first of its kind in modeling the more complex immune-mediated responses that are critical in determining what factors are involved in tumor relapse and how to prevent it.

II. Biological Model

We utilize the tetracycline (Tet) system to conditionally control the expression of the *MYC* oncogene in mouse models [3, 4]. This models the effect of targeted therapeutics that would shut down the over-expression of *MYC* as a treatment for the tumor. In the Tet-Off system, doxycycline (dox) is added to the drinking water to inhibit binding of the tet-transactivating promoter (tTA) to the Tet-O promoter, and thus inactivates transcription of *MYC*. In the Tet-On system, dox allows binding of reverse tTA (rtTA) and thus activates transcription of *MYC* [5]. MYC can even be titrated with a threshold on tumor regression occurring at $\leq$0.05 μg/mL of dox (in $\leq$0.2 ng/mL plasma concentration) in a Tet-On system [6]. We (and others) have collected data from various conditional mouse models of *MYC* and will concentrate on the tumor type specific responses to MYC inactivation seen in various tumor types including lymphoma (apoptosis, proliferative arrest, differentiation, senescence, anti-angiogenesis, tumor relapse) [7-9], osteosarcoma (proliferative arrest, differentiation, senescence) [10, 11], and hepatocellular carcinoma (apoptosis, differentiation, senescence, dormancy) [12].

In tumor dormancy, cells restore their neoplastic property upon MYC reactivation. In order to improve therapy, it is important to distinguish when MYC inactivation leads to complete tumor regression characterized by permanent loss of malignant phenotype and when it simply results in a reversible state of tumor dormancy [13, 14].

MYC inactivation in MYC-induced lymphoma leads to differentiation, apoptosis and complete tumor regression. Therefore there is permanent loss of a neoplastic phenotype upon MYC inactivation. In osteogenic sarcoma, MYC inactivation induces differentiation, proliferative arrest but does not induce significant apoptosis. MYC reactivation in these apparently differentiated cells either has no consequence or leads to apoptosis. Only in very rare cells is there restoration of neoplastic properties. In hepatocellular carcinoma, MYC inactivation leads to differentiation and then eventually gradual apoptosis of most of the tumor cells. Upon reactivation of MYC, these differentiated cells quickly become tumorigenic [15].

Senescence is the process by which normal cells are restrained from malignant transformation. Cellular senescence resulting from MYC inactivation has recently been shown to depend on the host immune system. Oncogene inactivation induced senescence is the irreversible cell cycle arrest of normal cells in the event of inactivating an oncogene. Tumor regression upon inactivation of MYC oncogene is associated with cellular senescence. Cellular senescence is important in order to have sustained tumor regression upon MYC inactivation [16].

p53 has been shown to suppress tumor angiogenesis and regulate thrombospondin-1 (TSP-1), an antiangiogenic protein, expression [17]. The loss of p53 upon MYC inactivation leads to a deficit of TSP-1 and this inhibits angiogenesis thus impeding tumor regression. Restoration of p53 leads to sustained tumor regression upon MYC inactivation. Therefore either p53 or TSP-1 is required upon MYC inactivation to shut down angiogenesis and induce sustained tumor regression [18].

Tumors undergo regression initially regardless of the status of the host immune system. But in hosts that are immune compromised, there is incomplete tumor elimination. An intact immune system is required for oncogene inactivation induced senescence, inhibition of angiogenesis and chemokine expression resulting in sustained tumor regression. CD4+ (but not CD8+) T cell deficiency was enough to impede sustained tumor regression. The secretion of TSP-1 a potent antiangiogenic protein is markedly decreased in immune compromised versus wild-type hosts. TSP-1 expression requires host immune cells particularly CD4+ T cells. Reconstitution of immune compromised mouse with CD8+ T cells still showed significant minimum residual disease whereas reconstitution with CD4+ T cells showed no minimum residual disease like in wild-type hosts upon MYC inactivation. Hence restoration of CD4+ T cells only was sufficient to eliminate minimum residual disease and lead to sustained tumor regression. CD4+ T cells are the crucial host effector population necessary for tumor regression upon MYC inactivation. TSP-1 is important in immune effectors for sustained regression upon MYC inactivation and overexpression of TSP-1 in immune compromised hosts is sufficient to increase the duration of sustained tumor regression upon MYC inactivation [19, 20].

III. Computational Model

A. Cellular Programs

We created a new mathematical model (Fig. 1) of tumor growth/regression kinetics incorporating cell intrinsic mechanisms (apoptosis, proliferative arrest, differentiation/dormancy) and immune-mediated cell extrinsic mechanisms (senescence and anti-angiogenesis). The stochastic model consists of 10 cellular states (MYC on, MYC off, undergoing apoptosis, proliferating, differentiated, senescent, etc.) with probabilistic transitions and the ability to control the expression of transgenic MYC using the tetracycline system. The core of the model is in the 'MYC on' and 'MYC off' states, controlled in the conditional transgenic mouse model through doxycycline (dox) in the water. 'MYC off' tumor cells have been shown to develop mechanisms to turn MYC back on without doxycycline through tTA, Notch, MAPK or Wnt pathways and are represented by the 'Escaped' node. Tumor cells may undergo proliferation or apoptosis and 'MYC off' tumor cells may

alternatively undergo differentiation or oncogene inactivation induced senescence.

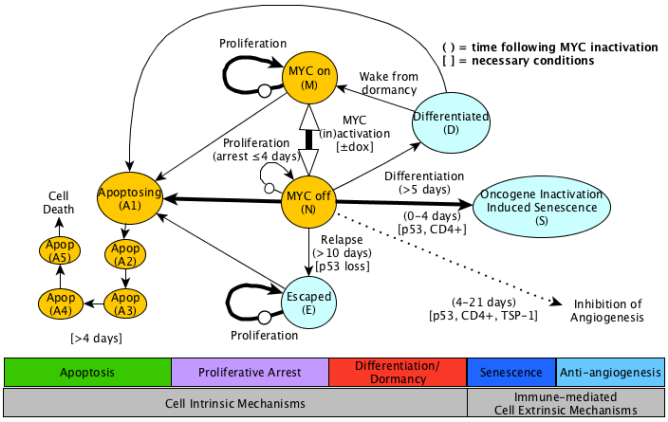

Figure 1. Computational model of cellular states. Current model shown in gold. Proposed additions shown in blue. Note that transitions can theoretically happen between almost any two states, Only the more probable transitions are drawn explicitly in this diagram. The white arrowheads corresponding to ±dox indicate that this is an independent variable controlled experimentatly. The dotted arrow to shutdown of angiogenesis represents an influence rather than a state transition.

The structure of our model is based on *in vivo* observations. We add an explicit transition in the model from 'MYC off' to 'Differentiated' to represent differentiation. We tested parameters over a number of values and chose values that matched experimental data the closest. Additionally, we add a transition from 'Differentiated' back to 'MYC on' to represent that some tumor cells (e.g., hepatocellular carcinoma) that have differentiated to an apparently normal state may indeed be dormant with the ability to regain neoplastic properties [15, 21]. The transition from 'MYC off' to 'Oncogene Inactivation Induced Senescence' is dependent on both p53 as well as $CD4^+$ T-cells.

Tumor cells can exist in one of ten separate states where the number of cells in each state are as follows. M is the number of cells where MYC is 'on', N are cells in the MYC 'off' state (and still under the control of the tetracycline system), A_i are cells which have irreversibly committed to apoptosis (and the fives sub-states represent the time delay from commitment to actual cell death), D are cells that have differentiated back into a quasi-normal state (although in some tumor types they retain their neoplastic capability if MYC is re-activated), S are cells that have irreversibly committed to oncogene-inactivation induced senescence (OIIS), and E are cells that have escaped their addiction to MYC (e.g., through mutations in the tetracycline control elements or by activating expression of genes downstream of MYC).

Fig. 2 shows the initial deterministic model with ordinary differential equations for each of the 10 states. We ran simulations using a time step of 0.02 days per iteration, which we represent as *delta_t*. Model parameters, are explained in TABLE I.

B. Governing Equations

$$\frac{dM}{dt} = B\left(M, K_{M_{prolif}} * dt\right) + B(D, K_{wake} * dt) - B\left(M, K_{M_{apop}} * dt\right)$$

$$\frac{dN}{dt} = B\left(N, K_{N_{prolif}} * dt\right) - B(N, K_{diff} * dt) - B\left(N, K_{N_{apop}} * dt\right) - B(N, K_{senesc} * dt) - B(N, K_{relapse} * dt)$$

$$\frac{dA_1}{dt} = B\left(M, K_{M_{apop}} * dt\right) + B\left(N, K_{N_{apop}} * dt\right) + B\left(E, K_{E_{apop}} * dt\right) + B\left(D, K_{D_{apop}} * dt\right) - B\left(A_1, K_{A_1} * dt\right)$$

$$\frac{dA_2}{dt} = B\left(A_1, K_{A_1} * dt\right) - B\left(A_2, K_{A_2} * dt\right)$$

$$\frac{dA_3}{dt} = B\left(A_2, K_{A_2} * dt\right) - B\left(A_3, K_{A_3} * dt\right)$$

$$\frac{dA_4}{dt} = B\left(A_3, K_{A_3} * dt\right) - B\left(A_4, K_{A_4} * dt\right)$$

$$\frac{dA_5}{dt} = B\left(A_4, K_{A_4} * dt\right) - B\left(A_5, K_{death} * dt\right)$$

$$\frac{dE}{dt} = B(N, K_{relapse} * dt) + B\left(E, K_{E_{prolif}} * dt\right) - B\left(E, K_{E_{apop}} * dt\right)$$

$$\frac{dD}{dt} = B(N, K_{diff} * dt) - B(D, K_{wake} * dt)$$

$$\frac{dS}{dt} = B(N, K_{senesc} * dt)$$

Figure 2. Set of differential equations governing the cell intrinsic and cell extrinsic programs in response to oncogene inactivation in oncogene addicted tumors.

The data shows that there is variability in relapse kinetics, which a deterministic model cannot capture. Hence, stochasticity was added to the model. Through the use of random sampling from a binomial distribution to represent a stochasticity in the number of cells transitioning from one state to another, we are able to recapitulate the variability in tumor relapse (Fig. 3).

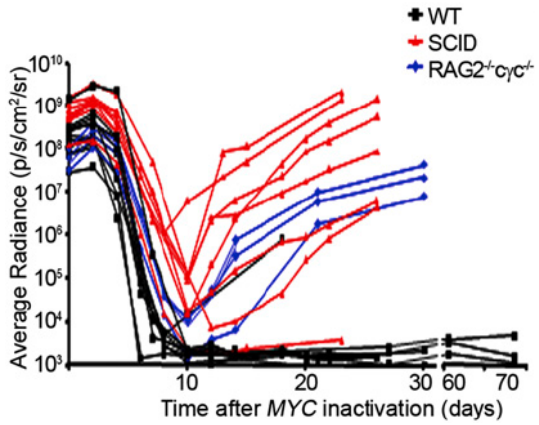

Figure 3. Tumor regression and relapse kinetics as measured by bioluminescence imaging. Excerpted from [19].

C. Model Parameters

TABLE I. MODEL PARAMETERS AND VALUES GOVERNING THE RESPONSE TO ONCOGENE INACTIVATION.

Parameters	Estimated Value (day^{-1})	Description
K_{N_prolif}	0.1-0.5	Transition coefficient from the MYC off state to proliferating
K_{diff}	0.02-0.5	Transition coefficient between MYC off state and differentiation state
K_{N_apop}	0.4-2	Transition coefficient between MYC off and the first apoptosing states
K_{senesc}	0.01-0.1	Transition coefficient between MYC off state and Oncogene Inactivated Induced Senescence state
$K_{relapse}$	0-0.1	Transition coefficient between MYC off state and Escaped state
K_{wake}	0.1	Transition coefficient between Differentiated state and MYC on state
K_{E_prolif}	0-0.5	Transition coefficient from the Escaped state to proliferating
K_{M_apop}	0-0.05	Transition coefficient between MYC on and the first apoptosing state
K_{M_prolif}	0-0.5	Transition coefficient from the MYC on state to proliferating
K_{E_apop}	0-0.2	Transition coefficient between Escaped state and the first apoptosing state
K_{death}	1	Transition from the last apoptosing state to cell death
K_{D_apop}	0-0.1	Transition coefficient between Differentiated state and the first apoptosing state.
K_{Ai}	1	Transition between the apoptosing states and then leading to cell death

IV. RESULTS

After running simulations, our model recapitulates features such as the different rates and delays in the tumor kinetics measured from *in vivo* experimental data from mouse models. Several emergent behaviors of the model have come to light. Empirically, proliferative arrest immediately follows oncogene inactivation but there is a 4-5 day delay in apoptosis. We model this with a chain of 5 sub-states from irreversible commitment to apoptosis to actual cell death, which we call apoptosing states. We varied the number of sub-states and transition rates to match the empirically observed delay.

Furthermore, the rate of mutations leading to tumor relapse (which is captured in the term $K_{relapse}$) had almost no bearing on the kinetics of the relapse. Instead tumor relapse kinetics are dominated by K_{E_prolif}. We performed a basic simulation of regressing tumors followed by increasing the rate of tumor cells from 'MYC Off' to 'Escaped' (increasing the term $K_{relapse}$) that would have otherwise gone to 'Oncogene Inactivation Induced Senescence'. The rate governing mutations leading to the transition from 'MYC off' to 'Escaped' had little effect on deterministic simulations but a significant effect on our stochastic model. The relapse of tumors due to absence of immune-mediated senescence and shutdown of angiogenesis is demonstrated in Fig. 5. This indicates that the immune system has a huge role to play in sustained tumor regression. From Fig. 4, we see that in a WT mouse (which has an intact immune system), there is sustained tumor regression.

Empirically, the regression kinetics had little variability (in Fig. 3, the curves all overlap) while the relapse growth kinetics showed greater variability (timing of the curves varies). Only adding stochasticity to our deterministic model can capture this variability in relapse in the different mouse models. The result from a run of 20 simulations is displayed in Fig. 6.

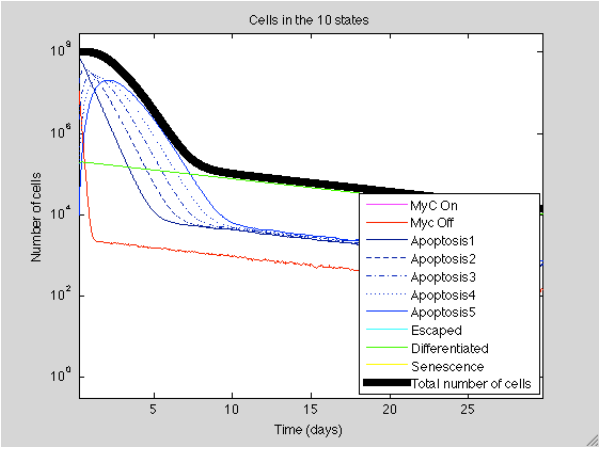

Figure 4. Graph depict simulation of a tumor with no escape of tumor cells from the conditional control.

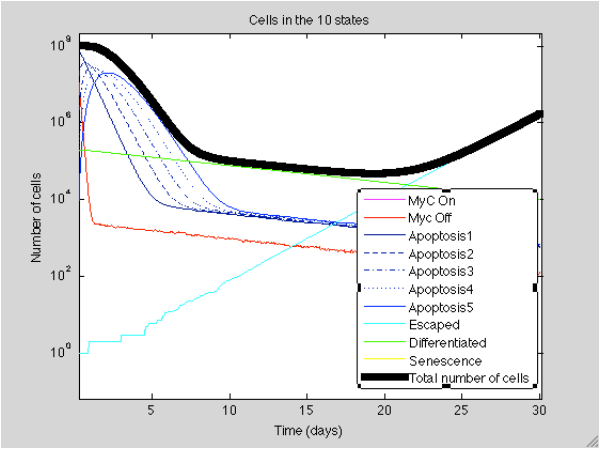

Figure 5. Graph shows a tumor with actually a very low rate of cells escaping from Tet control (or activating endogenous Myc).

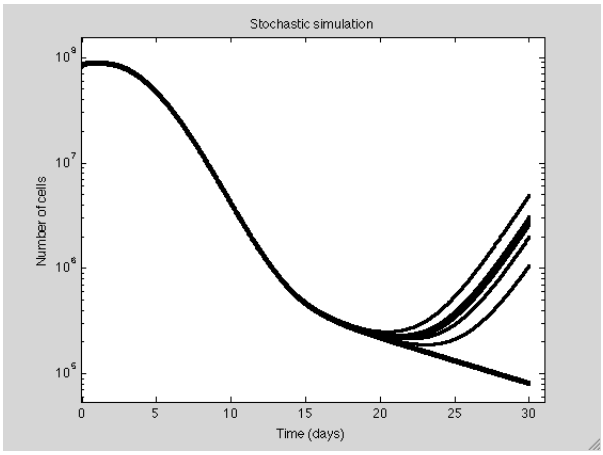

Figure 6. Shows variability in relapse kinetics as captured using stochastic simulations.

V. DISCUSSION

The initial results from our new model are helping to uncover the sequence of and interaction between cellular mechanisms involved in tumor response to oncogene inactivation such as might be encountered using targeted therapeutics, which will eventually help to optimize treatment. Avoiding immune destruction is a crucial hallmark because it has been shown that immune cells, $CD4^+$ T-cells in particular, are a required component for senescence, shutdown of angiogenesis and chemokine expression that result in sustained tumor regression [19]. This is captured in our model. If residual disease reaches a low enough level, this can prevent relapse. Large tumors have a great number of cells that can transition through an extremely low-likelihood event to the 'Escaped' state. If the tumor is small enough, not enough cells remain to make it likely that any one will achieve the extremely low-likelihood 'Escaped' state. Tumor burden is also an important factor in tumor regression mediated through the immune system. The immune system might not be able to attack and eliminate tumor fast enough if tumor burden is too high. Currently our model does not account for this but we are adding more sophisticated immune system components to the model in order to show this. Our model is simple, consisting of only time-homogeneous transitions of cells that are statistically independent, and yet is able to recapitulate the complex response of tumors to oncogene inactivation.

Our model offers more fidelity than models in the literature that just capture tumor cells in a single variable in that we are able to capture the various states that the cells are in which we are not able to quantitatively capture over time *in vivo* as yet. We are working on imaging methods to be able to quantitatively image distinct cellular states such as apoptosis, senescence, proliferation, etc., which will eventually allow us to further validate our simulation results. Future work will include validating novel predictions from our model *in vivo*.

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