Mathematical models of heterogeneity in cancer cell growth: a review

Priscilla S. Macansantos

University of the Philippines Baguio, Baguio City 2600, Philippines

psmacansantos@up.edu.ph

Abstract. Cancer is characterized by unregulated growth of certain cells in the body, often leading to a rapid growth of tumors in vital organs. Various treatments have been proposed and given to cancer patients, including combinations of radiation, chemotherapy, and immunotherapy, with varying rates of success. Characterization of the disease (and the search for a cure) is made more challenging by the observed heterogeneous behavior and variability of growth rates of cells, particularly cells forming tumors in various stages of development. Heterogeneity refers to apparently dissimilar traits and behavior of individual cells or cell subpopulations, despite originating from a common tumor or parental line. In the last several decades, developments in mathematical biology, together with increasing availability of sophisticated laboratory equipment (aided by powerful computers) has provided a framework for the quantification and study of cell traits, including variability. We review some recent work on heterogeneity and growth variability in the context of mathematical models proposed. In the models presented, variance in cell proliferation rate distribution signals heterogeneity, so that mechanisms tuning variance are considerations for treatment strategies. We look into previous work and studies on sources of variability and stochasticity, and some numerical approaches are discussed, in order to deal with huge gene networks implicated in the complex process of cell division, proliferation and heterogeneity.

Keywords: cancer heterogeneity, growth variability, mathematical modeling

1. Introduction

Cancer is a family of diseases afflicting a considerable portion of the world's population. Cancer is characterized by unregulated growth of certain cells in the body, often leading to a rapid growth of tumors in vital organs. Various treatments have been proposed and given to patients, including combinations of radiation, chemotherapy, and immunotherapy, with varying outcomes. Characterization of the disease (and the search for effective therapies) is made more challenging by the observed heterogeneous behavior and variability of growth rates of cells, particularly cells forming tumors in various stages of development. Here, heterogeneity refers to apparently dissimilar traits and behavior of individual cells or cell subpopulations, despite originating from a common tumor or parental line. In Quaranta (2009), the authors note that *"Mapping quantitative cell traits (QCT) to underlying molecular defects is a central challenge in cancer research because heterogeneity at all biological scales, from genes to cells to populations, is recognized as the main driver of cancer progression and treatment resistance*." Heterogeneity, long observed in laboratory experiments on cancer cells, poses difficulties as well as opportunities for deeper understanding of the disease. In Cleary (2014), evidence is provided "that interclonal cooperation can be essential for tumor maintenance," thus supporting the contention that heterogeneity can and does have a function on tumor progression and persistence.

Quantitative analysis and modeling of heterogeneity and growth variability of cell populations has generated much interest and has given rise to a variety of mathematical models and approaches. In the last several decades, developments in mathematical biology, together with increasing availability of sophisticated laboratory equipment and powerful computers has provided a framework for the quantification and study of cell traits, including variability (see e.g., Quaranta (2008)). In this paper, we review recent studies modeling heterogeneity, using quantitative tools to improve understanding of this extremely challenging feature of cancer progression. Section 2 includes a discussion of growth models proposed, in which variance plays a key role in the process of fitting the model to experimental data. Section 3 discusses protein control of cellular growth as likely a major source of variability, with transcription and translation rates of inhibitory proteins accounting for variability. Section 4 includes a brief discussion on recent discrete stochastic computational tools introduced to study gene regulatory responses, such as the Finite State Projection (FSP). Finally, Section 5 discusses *in silico* experiments on intratumor heterogeneity models, with a confounding mathematical insight regarding the extraordinary evolvability of cancer.

2. Heterogeneous growth models and the key role of variance

In recent work in the Quaranta Laboratory, Frick (2014) introduced the novel clonal Fractional Proliferation (cFP) methodology/assay to investigate the growth of EGFR-addicted PC9 lung cancer cell lines treated with erlotinib, an EGFR small molecule inhibitor. Following earlier work of Tyson *et al* (2012) he used the notion of clonal drug-induced proliferation (DIP) rate as a measure of long-term clonal fitness in a cell population. In brief, drug induced proliferation (DIP) rate is defined to be proliferation rate of a single-cell derived clone, at steady state (after approximately 72 hours in drug), hence DIP rate is slope of $\mathbf{log_2}$ growth curve at 3-10 days. DIP rate quantifies steady state cell response to drug, by way of clonal proliferation rate, and this approach allows quantification of heterogeneous response of tumor cells to drug. Further, an HG (Heterogeneous Growth) model of exponential growth incorporating both mean $\boldsymbol{\mu}$ and variance $\boldsymbol{\sigma^2}$ of drug-induced proliferation (DIP) rate distributions is considered, leading to a mathematically-informed prediction of time to rebound (TTR, time required for the population to regain its initial size y_0 in continuous drug treatment). The HG model is given by the equation

$$y = y_0 \exp(\mu t+\sigma^2 t^2/2) \qquad (1)$$

where y is population size. By including variance of the clonal DIP rate distribution in the HG exponential model, population rebound (after an initial period of decrease) is accounted for, and this is also in better fit with data from experiments.

Variability of growth rates has been observed, both in drug-treated and untreated tumors and cancer cell lines. In Greene et al (2015), two mathematical models are proposed to predict the growth of a single ovarian cell line, OVCAR-8: a stochastic (individual) agent-based model (ABM), and a corresponding integro-differential equation model. In these models, a dynamic transition between proliferative and quiescent states characterizes the growth of cancer cells. In the agent-based model, cellular decision-making to transition from one state to the other is a function of the global dynamic cell density, together with intrinsic variations of the cell cycle and death process lengths. The ABM is then approximated by a system of integro-differential equations (IDEs) that approximate the expected values of the cellular compartment sizes over time. Apart from observations on remarkably good fit between the two models for sufficiently large sample size (and the parameter estimation process using experimental data and simulations), a significant finding from the study is that "the most important parameter which dictates the qualitative structure of the population growth dynamics is the standard deviation $\boldsymbol{\sigma}$ of the cell cycle." The authors then hypothesize that "variations in both the cell-cycle and apoptotic lengths are central contributing factors to the overall dynamics." Mathematically, a connection to growth/proliferation rate variance is clear.

In a separate study (Hamon *et al*, 2014), researchers present evidence of variability of growth rates for clones inside the same cell lines. A log-quadratic model (incorporating variance), as opposed to a log-linear model, is suggested to explain growth data from the group's experiments. With the proportion and mitotic rate of CSC (or CSC-like cells) being highly variable in cancer tumors, relative growth rates of fast-growing clones are predicted by the classical exponential model to increase exponentially with time, regardless of initial rates . The group grew two different non-interacting human cancer cell lines, separately and together in cell cultures with unlimited nutrient supply. Experiments utilized two well known laboratory strains: RL (non-Hodgkin's lymphoma B cell line: ATCC CRL-22618) and THP-1 (cell line derived from an acute monocytic leukemia patient: TCC TIB-2029). Both strains were grown together in the same solution, with different initial proportions of the faster-growing RL (0.5%, 1%, 5%). The exponential model predicts that the relative proportion of RL *vs.* THP-1 would increase exponentially in time, at a rate which is independent from the initial proportion. Observed growth rates did not confirm this prediction (see paper for graphs illustrating growth rates from data). A mathematical formulation that takes into account variable growth rates yields

$$log(N(t)) = a + log\ (E(\ e^{Bt})) \quad (2)$$

where $N(t)$ is cell population size at time t, E indicates expectation, and B refers to some distribution of growth rates. If μ is the expectation of the distribution B, σ its standard deviation, and γ its skewness, then the Taylor expansion of $log\ (E(\ e^{Bt}))$ is

$$log\ (E(\ e^{Bt})) = \mu t + \sigma^2 t^2/2 + \sigma^3\ \gamma t^3\ /6 + o(t^3\ ). \quad (3)$$

If B has the normal distribution, then

$$log\ (E(\ e^{Bt})) = \mu t + \sigma^2 t^2/2 \quad (4)$$

yielding finally the growth model

$$log\ N(t) = a + bt + ct^2. \quad (5)$$

In equation (5), $b = \mu$ and $c = \sigma^2/2.$ Hence, the log-quadratic model explains the phenomenon observed in the experiments, which is that (apparent) ratio growth rate depends on initial proportion. More significantly, evidence for the intrinsic variability of growth rates is again provided, and given a mathematical formulation, with variance playing a key role in the refined mathematical growth model.

3. Sources o variability

Of particular interest to cancer research are sources of variability and heterogeneity, as these clearly impinge on treatment strategies such as molecular-targeted therapy, to control the disease. In many instances, drug interventions are only initially effective, but become ineffective after some time. Similar to microbial populations, drug-tolerant persistent subpopulations have been observed in tumor populations, and tumor re-growth has been attributed to this phenotype.

Rocco *et al* (2012) make use of the concepts of epigenetic landscape (see Waddington) and ergodicity breaking to study so-called intrinsic noise (related, for instance to the bursting activity of gene expression or to repartition of protein molecules in daughter cells at cell division) – which contributes to phenotypic heterogeneity. The study, though dealing with bacterial populations and the presence of persistent subpopulations (to antibiotic treatment), is relevant to cancer cell growth rates in response to perturbation, as it relates gene expression to cell cycle length, and growth rate variability. The authors note that the term 'persister' was first used by Bigger in 1944 to "describe the ability of a small fraction of isogenic cells of *Staphylococcus aureus* to survive prolonged exposure to bactericidal concentrations of penicillin." In Balaban (2004), the authors demonstrate that persister cells were either slow-growing or non-growing at the time of antibiotic administration. Balaban et al (2004) developed a persistence model of two pre-existing subpopulations (normal and persistent cells) with a constant rate of stochastic switching between the two cell types. Rocco *et al* (2012) instead introduce an ergodicity-breaking model for persistence, proposing a mechanism that slows down the intrinsic fluctuations associated with gene expression and protein repartitioning

during cell division. The model gives a mathematical argument for why this mechanism is sufficient to account for the emergence of phenotypic heterogeneity in clonal populations.

The epigenetic landscape is a hyper-surface in multidimensional configuration space, (whose axes indicate expression of each gene in the cell), which plots the inverse probability that a cell is in a given state. The familiar picture of hills and valleys, giving a pictorial depiction of network dynamics, is one that derives from the notion of the energy landscape for Hamiltonian systems. Valleys correspond to metastable states, and their basins of attraction are seen as belonging to the same phenotype. The phenomenon of multiple phenotypes then corresponds to observable/realizable basins of attraction of multiple metastable states. At equilibrium, cells move around because of stochastic fluctuations, whose magnitude determine whether the cell moves from one basin of attraction to another. Two phases are defined for the system – the ergodic phase and the weakly non-ergodic phase – the distinction between the two phases determined by what is referred to as "permanence or sojourn time," τ_p, the average time a cell has to wait for it to be exposed to a large enough fluctuation enabling it to move to an adjacent metastable state. If τ_p is much smaller relative to some pre-determined observation time, cells "hop" around rapidly among the available basins of attraction, the observed time-averaged behavior is the same for any observed cell, and the time average of a relevant variable (such as a specific protein concentration) is the same as the population ensemble average. In this instance, there is only one average phenotype observed or present in the population. This is called the ergodic phase. Otherwise, when τ_p is large, population ensemble average is no longer predictive of cell behavior since each cell "maintains its individuality over the period of observation," the time-averaged variable of interest varies from cell to cell, hence the possibility of realizing multiple phenotypes; this phase is referred to as non-ergodic. Ergodicity breaking occurs in the transition from the ergodic to the non-ergodic phase.

The idea in the ergodicity-breaking analysis is that distinct phenotypes arise from mechanisms slowing down dynamics. In the Rocco (2012) study, protein control of cellular growth rate is shown to exhibit ergodicity breaking. Assuming that growth is inhibited by a (growth-inhibiting) protein, distinct growth phenotypes are seen to emerge due to a slow-down of protein fluctuations and the emergence of weakly ergodic components accounts for bacterial persistence. With protein accumulation in the cell resulting in increasingly longer cellular division times, the effectiveness of cellular division is decreased as a randomization process responsible for protein levels mixing.

In setting up the mathematical model, the equation for protein-dependent cellular growth rate is given by a Hill function $g(p(t))=g_0/(1+kp(t))$ with protein concentration $p(t)$ quantified as cell number per unit volume, and cellular division is assumed to occur upon doubling of volume. A differential equation (extending the exponential growth model) is introduced for cellular volume growth in terms of protein concentration, namely,

$$dV/dt=\{ g_0/(1+kp(t))\} \; V(t) \qquad (6)$$

In the above expression, $g_0 = ln2/T_0$ is the maximal cellular growth rate, with T_0 being zero protein division time, and k is a parameter quantifying growth-inhibitory strength of the growth inhibiting protein. To model production of the inhibitory protein, a model for gene expression is adopted from Friedman (2006), incorporating transcription and translation rates, as well as degradation rates. Division time is approximated based on the differential equation for volume, as well as protein concentration $p(t)$, a stochastic variable. Depending on whether fluctuations of $p(t)$ are very fast or very slow with respect to the cell cycle, the ergodic regime (or the weakly non-ergodic regime) are shown to arise. Bursting activity and protein degradation are among the stochastic processes accounting for protein fluctuations within generations. In the case of fast gene expression fluctuations, analysis of the master equation (a partial differential equation for protein distribution $w(p,t)$ over time, see Rocco (2012)) leads to the conclusion of ergodicity if the mean number of transcriptional bursts per cycle, a, is greater than 1, accompanied by the assumption that τ_p is smaller

than cell cycle length. In the case of slow gene expression fluctuations (from the assumption of small transcription and slow protein degradation), weak ergodicity breaking arises if observational time is less than that of cell division (equivalently, $a<1$). When the above conditions are accompanied by fast translation, "phase space islands" appear, due to cells undergoing rare transcriptional events, with, however, efficient translation. Hence, cells will be in one of two states – one with little protein content because of rare transcription, or the other with large amount of protein due to efficient translation. The authors note that "The two portions of phase space respectively characterized by negligible and very large protein contents appear to be weakly connected phase space islands, with negligible transition probabilities between them over large but finite observational times. " The translation rate k_2 can then be seen as a parameter that controls the landscape morphology, by inducing a transition from a single well to a double-well in the growth rates landscape, corresponding to the emergence of a bimodal probability distribution for growth rates due to ergodicity breaking.

Simulations of the growth model lead to several conclusions: the models are consistent with the established link between increased level of persistence and slow-growing (and starved) cells; the model provides an explanation to the observation that overproduction of any gene which slows growth appears to increase persistence. Finally, the authors conclude : *"Our view is that the population of persisters, pre-existing to antibiotic exposure, is anyway present because of stochastic fluctuations of any growth-inhibiting protein, and is not related to the specific regulated tuning of the expression of any specific gene. In this respect, subpopulations of normal and persister cells emerge naturally as a consequence of the growth phenotypic heterogeneity resulting from the mechanism of ergodicity breaking."*

Though the context of the above analysis is bacterial populations and persistence of a small subpopulation in antibiotic treatment, an application of this analysis to cancer cell populations is not implausible, see e.g. Carja and Plotkin (2015). Nonetheless, tremendous complexity in the genetic circuits and networks implicated in the development of cancer poses questions as to which combination of proteins (and which mechanisms in the process of gene expression) are to be monitored as one looks into possibly targeting these to control or manage growth and fluctuations.

4. Sources of stochasticity

In Sherman (2015), investigation of global (extrinsic) mechanisms is made to more fully characterize stochastic gene expression, which is a multi-step process, including transcription and translation. Towards this end, Sherman *et al* (2015) study sources of stochasticity that influence the expression of a yeast heat shock gene SSA1. With processes underlying gene expression producing cell-to-cell heterogeneity of RNA and protein counts between genetically identical cells, the authors note that the theoretical basis for intrinsic noise - the ON-OFF model- has become the consensus model. However, extrinsic stochasticity arising from such factors as differences in cell volume, cell cycle position, mitochondrial content, cotranscriptional regulation have not been studied as extensively, and Sherman *et al* (2015) introduce a hybrid intrinsic model with extrinsically varying transcription rate which appears in better fit with their experimental data than the ON-OFF model, and which leads the authors to conjecture that factors that influence the global transcription rate (are) the underlying cause of correlated fluctuations in stochastic gene expression.

With advances in experimental methods and technology, investigation and modeling of gene dynamics has drawn much interest. Among recent discrete stochastic computational tools introduced to study gene regulatory responses is Finite State Projection (FSP), (Munsky et al 2015). FSP uses "fluctuation fingerprints" to test mechanisms (and parameter combinations) in a variety of stochastic models with full probability distributions, with the end in view of identifying those models that reproduce and predict results of single molecule fluorescent in situ hybridization (smRNA-FISH) experiments, a method to image individual molecules of RNA. Since gene expression is controlled by

the presence and abundance of transcription factors, varying gene states give rise to different rates of transcript production. A framework to model gene regulatory processes is the chemical master equation, which is the collection of equations characterizing transitions from one gene state to another, taking the lattice of possible gene states and RNA counts as a Markov chain. Because there is a conceivably huge number of states and data, the chemical master equation is "simplified" and subsequently analyzed using finite state projection, which involves the selection of a finite subset of states that retains most of the probability for a specified time interval. To test the FSP method, experiments on bacteria, yeast and human cells were undertaken, and researchers observe that experimental data on RNA allows for a more systematic and incisive analysis of gene dynamics.

Even as large-scale dynamical networks in cancer genes are being uncovered by researchers, there is an emerging view that despite tremendous complexity, it is possible and necessary to uncover and formulate some manner of organization of species – whether these are genes, proteins, transcription factors – that leads to an understanding of the mechanisms of the disease on a manageable number of scales and dimensions. One sees the method of identifying "stable motifs" to control gene regulatory networks (see Zanudo and Albert (2015)) as one such approach. Recently, Jiang (2014) looked into a number of statistical metrics based on the pairwise similarity of transcriptional and genomic profiles and through simulations, evaluated these metrics and selected mean dispersion distance as the best measure of transcriptional diversity. Using this measure, it was observed that among basal-like breast cancers, those that were chemotherapy resistant were significantly more diverse than chemotherapy sensitive cancers.

5. In silico experiments on intratumor heterogeneity models

With high intratumor heterogeneity presumably leading to therapy resistance, Niida A. et al (2015) introduce a cellular automaton model for tumor evolution to identify possible principles underlying intratumor heterogeneity. Noting that somatic mutations and evolutionary selection for growth advantage characterize cancer, and observing that no model has so far reproduced the recently emerging view of highly branched cancer evolution, the authors build a BEP (Branching Evolutionary Process) model to reproduce branching cancer evolution *in silico*. Running simulations with a large number of parameter settings on a supercomputer to identify conditions leading to high intratumor heterogeneity, the authors study the following statistics (among others) to measure intratumor heterogeneity: population entropy $\boldsymbol{\varepsilon}$ (from a distance matrix among genotypes of randomly sampled cells), number of founder mutations $\boldsymbol{\rho}$ to characterize mutation profiles, and a measure of self-similarity, $\boldsymbol{\Theta}$.

In running simulations to test parameter dependence of the statistics, they then provide the following observations: (1) assuming that differences in the number $\boldsymbol{d}$ and strength $\boldsymbol{f}$ of driver genes contribute to differing degrees of intratumor heterogeneity, hence focusing on driver gene size and strength, simulations show that a multiple number of driver genes of moderate strength lead to high intratumor heterogeneity; (2) when cells harbor any strong driver gene, intratumor heterogeneity is low for any number of driver genes. Two different modes of selective sweep are seen, namely, in the latter case, the driver mutation rapidly dominates the population, while, in the case of multiple driver mutations of moderate strength, cells gradually increase their growth rates, providing cells with chances to acquire combinations of driver and passenger mutations. This results in what is termed selective sweep-derived heterogeneity. The authors qualify that these results are based on low mutation rate $\boldsymbol{r}$ (.0001); parameter dependence of $\boldsymbol{\varepsilon}$ on $\boldsymbol{d}$ and $\boldsymbol{f}$ changes on increasing mutation rates, with population entropy $\boldsymbol{\varepsilon}$ relatively high, with low $\boldsymbol{d}$ or low $\boldsymbol{f}$. Moreover, cluster heat maps of mutation profile matrices demonstrated cell-wise dendograms with fractal-like patterns harboring self-similarity with increasing $\boldsymbol{r}$, leading authors to suggest use of the term fractal heterogeneity. Calculating $\boldsymbol{\Theta}$, the rate of such cell-wise dendograms exhibiting self-similarity for each parameter setting resulted in $\boldsymbol{\Theta}$ being close to 1 when $\mathbf{r}=0.01$. Inspection of mutation profile matrix heat maps

leads Niida et al to suggest that the “fractal heterogeneity is caused by neutral evolution; that is, numerous neutral mutations that do not affect growth rate were produced by hypermutation and diverse subclones harboring different neutral mutations underwent genetic drift.” Citing recent multiregional sequencing (e.g., of colorectal cancer) where highly branched patterns without clear subclonal driver mutations are observed, the authors hypothesize that branched cancer evolution is mainly driven by neutral evolution and intratumor heterogeneity is essentially fractal. This, they say, seems consistent with emerging evidence that resistance to some targeted therapies may be due to the outgrowth of preexisting low frequency cancer cell populations, as different subclones with varying neutral mutations would expand into potentially resistant subpopulations.

Extending the BEP model to include a stem cell hierarchy, the rate s of symmetric division of stem cells is brought into the simulations, and a remarkable simulation outcome has to do with apparent transitions between different phases of cancer evolutionary dynamics. For instance, a ***d-f*** heat map of ε with $s = 0.01$ shows a clear boundary between two distinct phases, bringing to mind the phenomenon characterized by a change in dynamics of cellular automata (on changing values of a system parameter across a critical threshold), from highly ordered to highly disordered, with the boundary referred to famously by Kauffman and others as the “edge of chaos.” Referring to Kauffman’s theory on evolution being maximized at the edge of chaos, the authors invoke their BEP model and simulation outcomes to hypothesize that *“cancer is also evolving at the edge of chaos, which underlies its extraordinary evolvability.“*

6. Conclusion

Clearly, mathematical concepts and tools are increasingly brought to bear on the modeling and analysis of mechanisms of cancer development and evolution. In the mathematical models studied in this paper, variance in growth rate distribution accurately characterizes heterogeneity in cancer. A factor that has been studied to account for variability and the observed persistence of a small population of cells – resistant to drug - is the growth dynamics of growth inhibiting proteins as well as rates of gene transcription and translation. Stochasticity in gene expression is a significant issue, and given the complexity of gene networks, several approaches have been suggested to study these on a manageable number of states, using finite state projection and the concept of stable motifs. The challenge brought on by tremendous complexity of cell growth mechanisms on various scales is in part addressed by computational tools that make possible handling massive data and a huge number of parameters .

In conclusion, we see that mathematical concepts and tools have become indispensable in the study of the confounding phenomenon of heterogeneity in various contexts. Admittedly, simplifying assumptions are made in all modeling processes, yet despite these, the mathematical analyses are borne out by experimental data. Though more work has to be done to better understand the progression of this highly complex disease, it is clear that mathematical approaches and concepts facilitate the efforts towards dealing with heterogeneity in cancer.

We conclude with a statement from Beerenwinkel et al (2015):

Intra-tumor genetic heterogeneity is often portrayed as a major challenge for successful targeted treatment. However, evolutionary analysis of the process leading to the observed heterogeneity could turn this perceived weakness into a strength by tailoring treatment specifically to the unique evolutionary scenario within each patient.

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