DRUG EFFECT STUDY ON PROLIFERATION AND SURVIVAL PATHWAYS ON CELL LINE-BASED PLATFORM: A STOCHASTIC HYBRID SYSTEMS APPROACH

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Abstract—In this paper, a model that combining cell population and genetic regulation within a single cell by using stochastic hybrid systems is proposed. The objective is to study the response of a population of cancer cells to various drugs that targeting the proliferation and survival pathways. The proposed model captures both the dynamics of the cell population and the dynamics of gene regulations within each individual cell. We use drug Lapatinib applied to colon cancer cell line HCT-116 as an example to validate the proposed model. Simulation results demonstrate the phenomena that observed in TGen experiments.

Index Terms—drug effect, pathway, hybrid system

I. INTRODUCTION

The current drug development process, especially in oncology, is expensive, prolonged and unsustainable. With the revolution in molecular biology and high-throughput technologies in recent years, systems biology is fundamentally changing the practice of cancer biology and directly address pressing challenges in the development of new anti-cancer therapies, particularly the lack of efficacy or toxicity due to poor understanding of the biological system they attempt to affect. Increasingly, cancer therapy such as molecularly targeted agents is being designed to interrupt key components of critical pathways within this complex system.

However, how to analyze the therapeutic effects of such molecularly targeted agents within the context of biological networks poses many challenges. For example, most biological processes correlate with cancer involves many different but interconnected phenomena, which occur at different spatial and temporal scales. From the modeling viewpoint, how to construct multi-scale quantitative predictive models based on biological evidence and underpinned and parameterized by biological data will no doubt be very useful. Equally important, the dynamical characteristics within the biological processes at different scale is being recognized very critical and too important to be neglected. Advanced experimental technologies and computational methods need to be applied together in mutually complementary fashion to address the challenges mentioned. This paper strives to provide an integrative preclinical model combining experimental methods and theoretical analysis to study the dynamics of pathways that are critical to cancer under drug perturbation, in order to assessing the effect of therapeutics.

II. BACKGROUND

When it comes to drug therapeutics and disease modeling, the major goal is to understand how the systems changes when perturbed and how to modify the system to achieve a desired outcome. Understanding cells’ responses to a drug requires experimental designs that ask very specific questions about what is happening in the cell in the absence of a drug and how the cell activities change when the drug is present. The objectives of the experimental protocol is to efficiently capture cell process dynamics in response to drugs and thereby obtain a deeper understanding of the genetic regulatory mechanisms, the point being to make preclinical research more predictive.

A. Experimental Setup

A series of experiments have been conducted on cancer cell lines by applying various drugs at Translational Genomics Research Institute (TGen) [1]. The ability to follow specific cellular activities during the response to a drug is based on the ability to measure the abundance and position in a cell of fluorescent molecules [2]. With fluorescent molecules in the very small volume of a cell, one can make quantitative and positional measurements using only light to probe the cells. This assay is not destructive of the cells, so repeated serial assays of the same cell population to be sampled many times allows the dynamics of the responses to be followed over a time course.

In the TGen experiments, first we infect colorectal cancer cell lines HCT-116 with the desired packaged reporter. Then plate cells/reporter pair in a media containing a live-cell nuclear stain. Allow the cells to attach to the plate and grow overnight. Add drug Lapatinib to the appropriate wells (we have 6 wells (biological duplicates). In order to remove
environmental effects, such as growth factor depletion, there are 6 control wells (no drug added). These measurements are carried out with an automated epifluorescent microscope. A single assay is carried out by epifluorescent imaging of a portion of the bottom of a well, producing an image of the cells in that region (200-500) bearing fluorescent reporters. Fluorescent intensity data can be extracted from these images using specialized image analysis tools developed at TGen [1]. The dynamic evolution of the drug/cell interactions is captured along time, and specifically the dynamical change is observed by the desired packaged reporters to show the difference in proliferation signaling and survival regulation.

**B. Stochastic Hybrid Systems**

In general, hybrid system models combine continuous dynamics and discrete logic. These models typically include dynamic equations of the continuous state, a set of rules governing the transitions between discrete modes, and reset maps determining jumps of the state at transition times. Stochastic Hybrid Systems (SHSs) introduce randomness into hybrid system modeling. Depending on how the randomness is introduced, several different format of SHS models have been proposed, such as in [3], [4], [5]. In this study, we follow the model setup in [4].

Hybrid systems provide a rigorous foundation for modeling biological systems at desired levels of abstraction, approximation, and simplification [6]. For example, systems that exhibit multi-scale dynamics can be simplified by replacing certain slowly changing variables by their piecewise constant approximations. Additionally, sigmoidal non-linearities are commonly observed in biology and the corresponding models often use sigmoid functions. These can be approximated by discrete transitions between piecewise-linear regions. In some instances, symbolic upper and lower bounds are more useful than deterministic approximations because they capture all critical behavior of the system [6], [7], [8]. In addition, gene expression is a sequence of biochemical reactions which are inherent stochasticity. Those biochemical reactions depend on the molecular events and the difference in the internal states of cells like random births, deaths, and collisions of molecules [9]. Furthermore, the environment is complicated and the subtle environment difference may result in fluctuations in gene expression as well. Thus, incorporating stochasticity in the model is necessary, and this makes stochastic hybrid system an attractive candidate for our study on drug effect modeling.

**III. Drug Effect Modeling**

The measure of the repression of the proliferation pathway at the cell population level is the percentage change of non-proliferating cells during the TGen experiment on HCT-116 cell line. In order to link this measure to the drug effect on the proliferation pathway at the molecular level, an integrated cell population and individual cell gene regulation model is proposed based on the experimental setup and the stochastic hybrid system theory.

![Fig. 1. The proliferation pathway and the survival pathway with inputs of several drugs [1].](image)

A. Population Model

Denote \( \rho \) the ratio of non-proliferating cells, the dynamics of \( \rho \) is given by

\[
d\rho \over dt = \gamma (1 - \rho) - \beta \rho
\]

where \( \gamma \) is the drug effect coefficient, \( \beta \) is a balancing factor modeling various other factors such as the logistic constraints. Since the focus of this study is on the effect of Lapatinib on cell proliferation, the drug effect coefficient is assumed to be inversely proportional to the average concentration level of [ERK] in cells. In other words, the intended effect of Lapatinib is to suppress the Raf/Ras pathway (i.e., reduced concentration level of [ERK]) and as a result, prevent cancer cells from proliferating. Thus the drug effect coefficient is related to the average concentration level of [ERK] as follows,

\[
\gamma = \gamma_0 / E([\text{ERK}])
\]

The expectation \( E \) is taken over the cell population. The above equation bridges the population model with the gene regulation model in individual cells, which will be discussed in the next section.

B. Gene Regulation Model in Individual Cell

One form of gene regulation in a pathway with a switch that follows a Markov Chain can be modeled as follows.

\[
dx_i \over dt = \begin{cases} 
\alpha_j x_j - \alpha_i x_i, & s_1 \text{ is off} \\
\alpha_j x_j \eta_{\text{drug}} - \alpha_i x_i, & s_1 \text{ is on} 
\end{cases}
\]

where \( x_i \) and \( x_j \) are gene expression (protein) levels, \( \alpha_j > 0 \) and \( \alpha_i > 0 \) are synthesis and degradation factors, respectively. \( \eta_{\text{drug}} \) is the drug effect factor. \( s_1 \) is a switch determined by the drug effect. It is assumed that the evolution of \( s_1 \) follows a Markov chain with state transition matrix defined by

\[
M_1 = \begin{pmatrix}
1 - m_0 & m_0 \\
m_1 & 1 - m_1
\end{pmatrix}
\]
where \( m_0 \) (\( m_1 \)) is the probability that \( s_1 \) switches from off to on (on to off), respectively. \( m_0 \) (\( m_1 \)) is affected by the drug effect as follows,

\[
m_0 = \frac{\gamma}{1 + \gamma} \quad (5)
\]

\[
m_1 = \frac{1}{1 + \gamma} \quad (6)
\]

Applying the above proposed model to Fig 1, as currently understood by biologists, the proliferation and survival pathway model can be obtained.

**IV. SIMULATION RESULTS AND ANALYSIS**

In this simulation study, we validate the proposed model by firstly performing a baseline run of the pathway model, then comparing the output of the combined model with the experimental results from TGen [1].

An outline of the simulation procedures is given in Algorithm 1.

**Algorithm 1:** Procedures for simulating the combined model

- **Input:** Parameters of the pathways and the drug
- **Output:** Percentage of non-proliferating cells
- for each observation interval (an hour) do
  - for each proliferating cell do
    - determine the switch’s state by sampling a Markov chain;
    - solve the pathway equations;
    - if \([FOS] < a threshold\) then
      - declare this cell non-proliferating;
    - update the drug effect coefficient;

A baseline run of the pathway model with HCT-116 cancer cell line when applied drug Lapatinib is performed and the results of the gene expression levels are given in Fig 2. It is observed that Lapatinib suppressed the proliferation of the cancer cells, as indicated by the [ERK] level. It is interesting to notice that the survival pathway is not suppressed. At the beginning of applying the drug, the concentration level of [PI3K] drops. However, it quickly recovers due to the crosstalk and feedback loops in the pathways.

In order to further validate the proposed model, we perform simulations using the proposed combined cell population and individual cell pathway regulation model to capture an important measure of drug effectiveness, the percentage change of non-proliferating cells along time when Lapatinib is applied to HCT-116 cancer cell line. The experimental result and the simulation result are shown in Fig 3 and Fig 4, respectively. Similar trend is observed in both figures. The percentage of non-proliferating cells remains flat during the initial 10-15 hours and this commitment time is also observed in the TGen experiment.

**V. CONCLUSION**

This is our first attempt to explore a hierarchical model that combining cell population dynamics and gene regulation in individual cells. The goal is to study the response of a population of cancer cells to various drugs that targeting the proliferation and survival pathways. We use drug Lapatinib applied to colon cancer cell line HCT-116 as an example to validate the proposed model. Simulation results demonstrate similar phenomena that observed in TGen experiments. The next step would be identification of the parameters and validate the model across different drugs and cancer cell lines.
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