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Stochastic modeling of cellular colonies with quiescence: An application to drug resistance in cancer

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Abstract

Several cancers are thought to be driven by cells with stem cell like properties. An important characteristic of stem cells, which also applies to primitive tumor cells, is the ability to undergo quiescence, where cells can temporarily stop the cell cycle. Cellular quiescence can affect the kinetics of tumor growth, and the susceptibility of the cells to therapy. To study how quiescence affects treatment, we formulate a stochastic birth–death process with quiescence, on a combinatorial cellular mutation network, and consider the pre-treatment (growth) and treatment (decay) regimes. We find that, in the absence of mutations, treatment (if sufficiently strong) will proceed as a biphasic decline with the first (faster) phase driven by the elimination of the cycling cells and the second (slower) phase limited by the process of cell awakening. Other regimes are possible for weaker treatments. We also describe how the process of mutant generation is influenced by quiescence. Interestingly, for single-drug treatments, the probability to have resistance at start of treatment is independent of quiescence. For two or more drugs, the probability to have generated resistant mutants before treatment grows with quiescence. Finally, we study the influence of quiescence on the treatment phase. Starting from a given composition of mutants, the chances of treatment success are not influenced by the presence of quiescence.

Keywords: Stochastic modeling; Chronic myeloid leukemia (CML); Cancer therapy; Cell cycle; Imatinib; Evolution

1. Introduction

Tissues and organs are maintained at some defined homeostatic level by so-called tissue stem cells which can divide and differentiate throughout the life span of the organism. Many regulatory mechanisms are involved in this process. One of them is called cellular quiescence (Cheshier et al., 1999; Weissman, 2000; Fuchs et al., 2004; Moore and Lemischka, 2006; Pelayo et al., 2006; Arai and Suda, 2007). When stem cells enter quiescence, they temporarily stop to progress through the cell cycle (i.e. they do not divide) until they become activated again. This occurs for example if there is currently no need for the production of additional tissue cells.

Several cancers are thought to originate from mutations that occurred in tissue stem cells, and the tumor can contain primitive cancer cells with stem cell like properties (Reya et al., 2001; Burkert et al., 2006; Jordan et al., 2006; Houghton et al., 2007). An example is chronic myeloid leukemia (CML) (Sawyers, 1999; Shet et al., 2002; Melo et al., 2003; Calabretta and Perrotti, 2004; Yoshida and Melo, 2004). Such primitive cancer cells have been observed to undergo quiescence (Holyoake et al., 1999, 2001). In particular, with CML, among the primitive dividing leukemic cells it has been reported that there is a small subset of quiescent cells which have been found to be locked in the G0 phase of the cell cycle (Holyoake et al., 1999, 2001). This is in contrast to the healthy stem cell compartment, where a majority of stem cells can be quiescent in G0 at any one time (Cheshier et al., 1999). Entry of leukemic cells into the quiescent state is highest among the most primitive leukemic cell populations, associated with down-regulation of IL-3 and G-CSF gene

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expression (Holyoake et al., 1999, 2001). Quiescent cells have the ability to spontaneously exit G0 and enter a continuously cycling state.

Quiescence in tumor cells can have important implications for the kinetics of tumor growth and also for the response to specific therapies (Al-Hajj et al., 2004; Jones et al., 2004; Barnes and Melo, 2006). Anti-cancer drugs have even been observed to promote a state of quiescence in primitive tumor cells (Graham et al., 2002). An example is CML (Graham et al., 2002), which is treated by the targeted small molecule inhibitor imatinib (or Gleevec) and has shown the best levels of success in cancer therapy so far (Gorre et al., 2001; O'Dwyer et al., 2002; Daley, 2003; Deininger and Druker, 2003; John et al., 2004; Shah et al., 2004; Tauchi and Ohyashiki, 2004; Yoshida and Melo, 2004). An important problem with the treatment of cancers is the evolution of mutant tumor cells that have become resistant to the drugs in use. For example, while CML can be treated with imatinib during the earlier stages of the disease, drug resistant mutants pose an important obstacle during the later stages of the disease (Gorre et al., 2001; O'Dwyer et al., 2002; Shannon, 2002; Deininger and Druker, 2003; Druker, 2003, 2004; Gambacorti-Passerini et al., 2003; Yee and Keating, 2003; Daub et al., 2004; Nardi et al., 2004; Shah et al., 2004; Tauchi and Ohyashiki, 2004). Because the generation of drug resistant mutants depends on the particular kinetics of tumor cell growth, this process is also likely to be influenced by the occurrence of cellular quiescence in the tumor cell population. This paper aims to examine the effect of cellular quiescence on the response of the tumor to drug therapy, and on the potential of the tumor to escape therapy as a result of drug resistant mutants.

We present mathematical methods to study the emergence of drug resistance in the setting of multiple-drug treatment, in colonies of cells with quiescence. Our stochastic model is a generalization of our previous work (Komarova and Wodarz, 2005; Komarova, 2006) and includes quiescence of cells. The method is reminiscent of that developed by Moolgavkar et al. (1988), and it continues the tradition of Goldie and Coldman (1998) of stochastic modeling of drug resistance in cancer (see also Goldie and Coldman, 1983a,b; Goldie and Coldman, 1985; Coldman and Goldie, 1986). Our paper examines (1) how quiescence influences a colony's growth before treatment and its decay during treatment, in the absence of mutations and (2) how it effects the generation of resistant mutants before and after the start of treatment. Here are our main findings regarding question (1):

- If the death rate is larger than the sum of the division rate and the quiescence rates, then treatment (in the absence of resistant mutants) proceeds in two exponential phases (a biphasic decline). The first phase is governed by the death of cycling cells, and the second (slower) phase is characterized by awakening and elimination of quiescent cells. The characteristic time of colony eradication, as well as the switching time, are calculated analytically.
- For smaller values of the death rate, we could have a one-stage decline or, if the initial number of quiescent cells is large, a reversed biphasic decline, where the first (slower) phase is characterized by cell awakening, which is followed by their elimination.
- In the absence of treatment, the colony will grow exponentially. If the rate of quiescence is smaller than the net growth rate of cycling cells, then the colony will be dominated by cycling cells. Otherwise, the colony is dominated by quiescent cells, and the growth is limited by their awakening.

In our investigation of question (2) above, that is, the generation of resistance in colonies with quiescence, we have found the following:

- Consider the regime of treatment. Starting with a given number of cells and a given composition of resistant and susceptible mutants, the probability of treatment success does *not* depend on the quiescence parameters. In other words, the presence of quiescence in the colony does not make the treatment success less (or more) likely. It of course influences the time it takes to treat, see above.
- Consider the pre-treatment phase and suppose a colony grows to a given size, N. The probability of having mutants resistant to one drug (one-hit mutants) at size N does *not* depend on quiescence parameters. Neither does it depend on the death rate in the colony. However, the expected number of one-hit mutants grows with quiescence and with the death rate of the colony.
- In a similar setting, and given that the growth is not quiescence dominated (that is, the division rate of cells is larger than their death rate plus the quiescence rate), the probability to have mutants resistant to two or more drugs depends on the presence of quiescence. It grows with the rate of quiescence and decays with the rate of awakening. These dependencies become stronger for larger numbers of drugs.

The rest of the paper is organized as follows. In Section 2 we exclude the possibility of mutations, and concentrate on the stochastic processes of birth, death and quiescence. We present methods to calculate the time of colony extinction (duration of treatment), in the absence of resistant mutants. We find the duration of treatment and the time of the biphasic switch as functions of all the parameters. In Section 3, we study generation of drug resistance by mutations in a population of cells with quiescence. We start by analyzing the phase of treatment and find that, given a colony of size N (with a defined composition of resistant and susceptible cells), the probability of treatment success does not depend on the quiescence parameters. Also, we investigate the microevolution of the cell population before the start of treatment, and calculate the probability of having resistance in a colony of size N (pre-existence of