

Effect of aspirin on tumor cell colony formation and evolution

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Supporting information

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1 The probability of single mutant generation

To describe the stochastic dynamics of cellular growth, and in particular, to calculate the chances of generating mutants, we formulate a fully stochastic model of cellular expansion, see also [1].

We consider a stochastic continuous time birth-death process, which represents the growth of a colony of tumor cells. The following parameters are used to describe the kinetics of wild-type cells: R_w stands for the division rate, and D_w for the death rate, with $D_w < R_w$. Wild type cells can mutate with probability u per cell division. Mutant cells are characterized by the division rate, R_m and the death rate, D_m . We assume that initial, there are N_0 wild-type cells and 0 mutant cells in the colony. The size of the colony at the time when the number of mutants is assessed is denoted by N . Please note that in the main text, we referred to the wild type division and death rates as R and D respectively, such that

$$R \equiv R_w, \quad D \equiv D_w.$$

In the supplement, since equations contain more types, the notation R_w and D_w is used for the wild type cells.

To describe the birth-death process with mutations, we can use the standard method of probability generating function, see e.g. [1]. Denoting by $\varphi_{i_w, i_m}(t)$ the probability to have i_w wild type cells and i_m mutant cells at time t , the Kolmogorov forward equation for this quantity can be written as:

$$\begin{aligned} \dot{\varphi}_{i_w, i_m} &= R_w(1-u)\varphi_{i_w-1, i_m}(i_w-1) + R_m\varphi_{i_w, i_m-1}(i_m-1) \\ &+ R_w u \varphi_{i_w, i_m-1} i_w \\ &+ D_w \varphi_{i_w+1, i_m}(i_w+1) + D_m \varphi_{i_w, i_m+1}(i_m+1) \\ &- \varphi_{i_w, i_m}(R_w + R_m + D_w + D_m), \end{aligned} \tag{1}$$

see Chapter 4.2, “The Basic Model of Cancer Growth and Generation of Mutations” for details. We define the probability generating function as

$$\Psi(\xi_w, \xi_m; t) = \sum_{i_w, i_m} \varphi_{i_w, i_m}(t) \xi_w^{i_w} \xi_m^{i_m}, \quad (2)$$

and obtain the following first order partial differential equation:

$$\begin{aligned} \frac{\partial \Psi}{\partial t} &= \frac{\partial \Psi}{\partial \xi_w} (R_w(1-u)\xi_w^2 + D_w - \xi_w(R_w + D_w - R_w u \xi_m)) \\ &+ \frac{\partial \Psi}{\partial \xi_m} (R_m \xi_m^2 + D_m - \xi_m(R_m + D_m)). \end{aligned} \quad (3)$$

PDE (3) can be solved by the standard method of characteristics. The initial value problem satisfied by the functions $\xi_w(t)$ and $\xi_m(t)$ is given by:

$$\dot{\xi}_w = R_w(1-u)\xi_w^2 + D_w - \xi_w(R_w + D_w - R_w u \xi_m), \quad (4)$$

$$\dot{\xi}_m = R_m \xi_m^2 + D_m - \xi_m(R_m + D_m), \quad (5)$$

$$\xi_w(0) = 1, \quad \xi_m(0) = 0. \quad (6)$$

The quantity $\xi_w(t)^{N_0}$ defines the probability of not having any mutants at time t . The time when the colony reaches size N is given by T : $N_0 e^{(R_w - D_w)T} = N$. The quantity of interest is the probability $P_{\text{res}}(T)$ to generate mutants by time T given that the colony has survived. To obtain this quantity, we can rewrite $\xi_w(t)^{N_0}$ as

$$\xi_w(t)^{N_0} = \text{Prob}(\text{Extinct}) + (1 - \text{Prob}(\text{Extinct})) P_{\text{no mut}}^1,$$

where the probability of colony extinction is well approximated by $\text{Prob}(\text{Extinct}) = (D_w/R_w)^{N_0}$, and $P_{\text{no mut}}^1$ is the probability of no one-hit mutants conditional on the colony non-extinction (the superscript 1 refers to the fact that one-hit mutants are considered; this will be generalized to more hits later). Therefore, solving for $P_{\text{no mut}}^1$, and using $P_{\text{mut}}^1 = 1 - P_{\text{no mut}}^1$, we obtain

$$P_{\text{mut}}^1(t) = \frac{1 - \xi_w(t)^{N_0}}{1 - (D_w/R_w)^{N_0}}.$$

Below we are using $N_0 = 1$. Therefore, we have

$$P_{\text{mut}}^1(T) = \frac{1 - \xi_w(T)}{1 - D_w/R_w}. \quad (7)$$

2 Multiple mutant generation

The probability of double-hit (or in general, m -hit) mutant generation in a colony of size N has been studied in [2, 1], in the context of resistance to drugs in cancer treatments. Each cell can acquire mutations of m types, in general

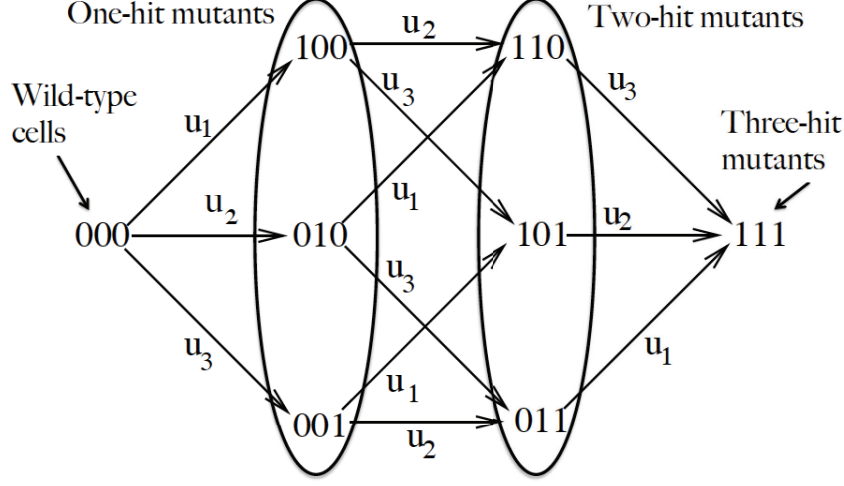


Figure 1: Mutation diagram corresponding to three possible mutations. The binary number, s , for each node describes the mutant types: 0 stands for “wild-type” and 1 for “mutant” with respect to a particular allele.

with rates u_1, \dots, u_m . Using simple combinatorics, we obtain that there can be up to $n = 2^m - 1$ different mutant cell types. Figure 1 illustrates the mutation network for $m = 3$. We label each phenotype by a binary number of length m , where “1” indicates mutation and “0” wild-type with respect to a particular allele. Each type s is characterized by the division rate R_s and the death rate D_s . In particular, the wild type has the division rate $R_0 = R_w$ and the death rate $D_0 = D_w$. To describe stochastic evolution of a cellular colony with mutations, we introduce the function

$$\varphi_{i_0, \dots, i_n}(t),$$

the probability to have i_s cells of type s at time t , where $0 \leq s \leq n = 2^m$ are binary numbers. We can write down the Kolmogorov forward equation,

$$\dot{\varphi}_{i_0, \dots, i_n} = \sum_{s=0}^n Q^{(s)},$$

where $Q^{(s)}$ is the contribution obtained from considering probabilities of reproduction and death of cell-type s ,

$$\begin{aligned} Q^{(s)} = & \varphi_{\dots, i_s-1, \dots}(i_s-1)R_s \left(1 - \sum_j u_j^{s, out}\right) + i_s R_s \sum_j \varphi_{\dots, i_s, \dots, i_j-1, \dots} u_j^{s, out} \\ & + \varphi_{\dots, i_s+1, \dots}(i_s+1)D_s - \varphi_{\dots} i_s (R_s + D_s). \end{aligned}$$

In this equation we used the following short-hand notation: φ_{\dots} stands for $\varphi_{i_0, \dots, i_n}$, and the only explicit subscripts indicate the indices which are different

from (i_0, \dots, i_n) . We further denoted by $u_j^{s,out}$ the mutation rates corresponding to the arrows originating at type s and pointing to type j in a mutation diagram, see e.g. figure 1 for the case $m = 3$. In analogy with equation (2), we define the probability generating function, $\Psi(\xi_0, \dots, \xi_n; t)$,

$$\Psi(\xi_0, \dots, \xi_n; t) = \sum_{i_0, \dots, i_n} \varphi_{i_0, \dots, i_n} \prod_{s=0}^n \xi_s^{i_s}. \quad (8)$$

This function satisfies the following first order partial differential equation (a generalization of (3)):

$$\frac{\partial \Psi}{\partial t} = \sum_s \frac{\partial \Psi}{\partial \xi_s} \left[\xi_s^2 R_s (1 - u^{s,out}) + D_s + \xi_s R_s \sum_j \xi_j u_j^{s,out} - (R_s + D_s) \xi_s \right], \quad (9)$$

where we used the notation

$$u^{s,out} = \sum_j u_j^{s,out}.$$

(details of the derivation of equation (9) can be found in [1]). As before, this equation can be solved by the standard method of characteristics. The equations for characteristics are given by:

$$\dot{\xi}_s = R_s (1 - u^{s,out}) \xi_s^2 + \left[R_s \sum_j u_j^{s,out} \xi_j - (R_s + D_s) \right] \xi_s + D_s, \quad 0 \leq s \leq n. \quad (10)$$

Although the methodology described here is very general, we proceed by assuming the existence of certain symmetries in the coefficients. We assume that:

- All the mutation rates are equal, that is, $u_j = u$.
- All the types with 1, 2, etc mutations, have equal kinetic parameters.

To simplify equation (10), we note that all the mutant types can be separated into classes such that in each class k , cells contain k mutations. For each k , the class consists of all variables ξ_s such that the binary numbers s contain exactly k nonzero entries. Therefore, we can denote by ξ_k , with $0 \leq k \leq m$, the class of variables describing k -hit mutants (with the total possible number of mutants m). We further make the assumption that

- Within each class, the birth and death rates are equal.

In this case, it does not matter which k out of m mutations a cell contains. The total number of distinct equations in this case is not $n = 2^m + 1$, but $m + 1$.

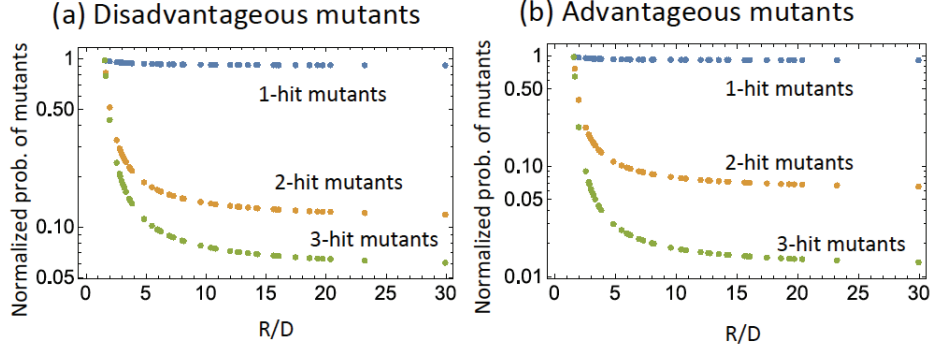


Figure 2: The probability of having one-, two-, and three-hit mutants in a colony of $N = 10^{10}$ cells, under mutation rate $u = 10^{-9}$. The values of division and death rates of wild-type cells were taken from the set of measured values for the cell lines under different aspirin doses. (a) Mutations are disadvantageous, such that each additional mutation gives a 2% reduction in the division rate. (b) Mutations are advantageous, such that each additional mutation gives a 2% increase in the division rate. Only the relative probabilities are shown, where the calculated probabilities are normalized to be one for the lowest $R/D \equiv R_w/D_w$ value.

Then, simplifying equation (10), we obtain the following initial value problem:

$$\begin{aligned} \dot{\xi}_k^{(m)} &= R_k(1 - (m - k)u)(\xi_k^{(m)})^2 + D_k \\ &\quad - \xi_k^{(m)}(R_k + D_k - (m - k)R_k u \xi_{k+1}^{(m)}), \quad 0 \leq k \leq m, \end{aligned} \quad (11)$$

$$\xi_k^{(m)}(0) = 1 \text{ for } 0 \leq k \leq m - 1, \quad \xi_m^{(m)}(0) = 0, \quad (12)$$

where we introduced superscript (m) which refers to the number of mutations, m , in the system. This is a multi-mutation generalization of system (4-6). The probability to have m -hit mutants at time T is given by equation similar to equation (7):

$$P_{\text{mut}}^m(T) = \frac{1 - \xi_0^{(m)}(T)}{1 - D_0/R_0}, \quad (13)$$

where $\xi_0^{(m)}(t)$ is the zeroth component of the solution of system (11-12). These calculations were used to investigate the probability of having one-, two-, and three-hit mutants, see figure 2.

Further, in figures 3 and 4 we plot the relative probabilities of mutant existence for one-, two-, and three-hit mutants as functions of the aspirin dose; these figures present the same information as figure 2, except they show the connection with the aspirin dose. These two figures are similar to figure 3 of the main text, where the case of neutral mutants is showcased.

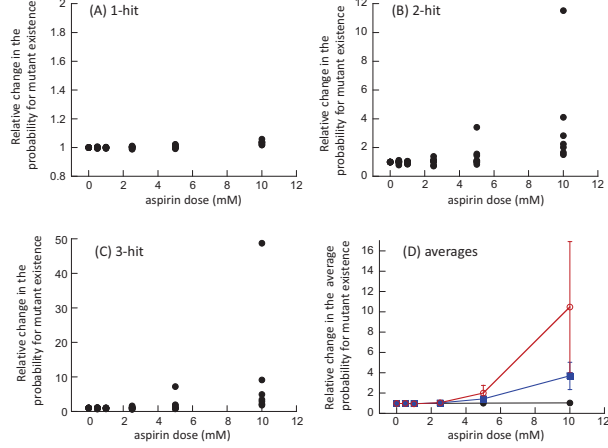


Figure 3: Effect of aspirin on the probability for a cell to exist that is characterized by (A) 1, (B) 2, and (C) 3 independent advantageous mutations by the time a cell colony has grown from 1 to 10^{10} cells (assuming a mutation rate $u = 10^{-9}$). A 2% fitness advantage was assumed, expressed by an increase in the division rate of cells. The relative change in the probabilities is shown, dividing the probability for a mutant to exist in the presence of aspirin by the probability in the absence of the drug. The dots represent the different cell lines. (D) This graph plots the average over all cell lines for each dose, along with error bars that represent the standard error.

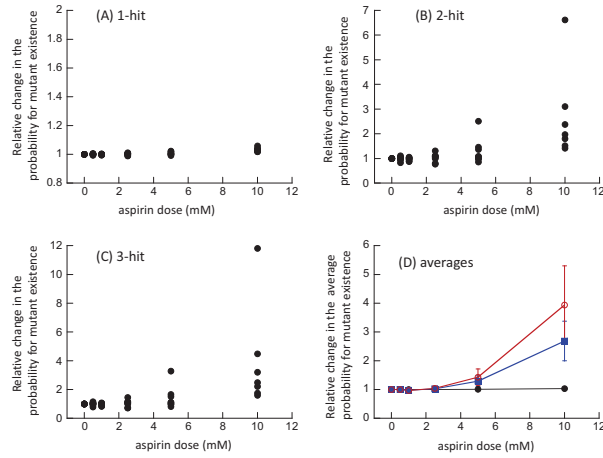


Figure 4: Same as in figure 3, but for the case where the mutants have a 2% disadvantage expressed as a reduction in the division rate.

3 The mean number of mutants

The mean number of mutants at colony of size N was found in [3], see eq. (1) in that paper. In our notations, we have

$$M_0 = \frac{Nu(1 - 1/N^{1-\alpha})}{(1 - D_w/R_w)(1 - \alpha)P}, \quad (14)$$

where

$$\alpha = \frac{R_m - D_m}{R_w - D_w}, \quad P = 1 - \exp\left(-\frac{NuF}{1 - D_w/R_w}\right), \quad F = \int_0^1 \frac{1 - D_m/R_m}{1 - y^\alpha D_m/R_m} dy,$$

α denoting the relative fitness of mutants and P the probability of non-extinction of mutated cells. For example, for neutral mutants and in the case of large $Nu > 1$, we have $R_m = R_w$, $D_m = D_w$, and $P \approx 1$. Then the expected number of mutants is simply given by

$$M_0 = \frac{Nu \ln N}{1 - D_w/R_w}, \quad (15)$$

where the numerator is exactly the expected number of mutants in the absence of cell death, calculated first by [4]. The denominator corrects for the actual expected number of divisions needed to grow to size N in the presence of cell death. The same expression as (15) can be obtained by solving the initial value problem describing the expected number of cells,

$$\dot{x}_w = (R_w(1 - u) - D_w)x_w, \quad (16)$$

$$\dot{x}_m = R_w u x_w + (R_m - D_m)x_m, \quad (17)$$

$$x_w(0) = 1, \quad x_m(0) = 0. \quad (18)$$

Setting $R_m = R_w$ and $D_m = D_w$ and evaluating the solution for x_m at $t_* = \ln N / (R_w - D_w)$, we obtain exactly the expression in (15).

In fact, system (16-18) can be solved numerically for advantageous or disadvantageous mutant cases. After calculating t_* such that $x_w(t_*) + x_m(t_*) = N$, we obtain the expected number of mutants at size N as

$$M_0 = x_m(t_*). \quad (19)$$

In the limit of large Nu , these solutions are very close to the predictions of formula (14).

References

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