

Predicting Tumor Cell Repopulation After Response: Mathematical Modeling of Cancer Cell Growth

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Abstract. *The kinetics of regrowth of tumor cells after treatment may offer a new end-point for clinical trials. Based on our testing, it is best described in this manner: $y(t) = a \cdot \exp(-b \cdot t) + c \cdot \exp(d \cdot t)$. Human malignant glioma cells U87 MG were treated with etoposide and allowed to regrow after treatment. The cell number versus time data were fitted mathematically to the two-term exponential model. Parameters b and d were independent of the drug concentration, while a increased c decreased as the drug dose increased. The concentration independence of b and d indicated that both cell proliferation and cell death kinetics were independent of the drug treatment, which suggests constant times for cell cycle and apoptosis. The concentration dependence of c suggests that the time until the cells started regrowing depended on the treatment, repair mechanisms taking longer after heavy damage. The two-term exponential model predicted tumor repopulation in this *in vitro* system. These results indicated that the velocities of the logarithm of cell growth and cell death were independent of drug treatment, while the recovery time of the tumor repopulation was dependent on the drug dose. The two-term exponential model can be used to predict tumor repopulation in an *in vitro* system and this model will be further tested using clinical data.*

Tumor repopulation is a phenomenon which has been extensively studied in many types of cancer (1, 2). After low-dose chemotherapy or radiotherapy, or with prolonged delays between treatments, tumor tissue will regrow and repopulate previously cleared locations. Rapidly growing tumor tissue can repopulate tumor sites within 2 weeks from treatment. To more accurately describe this phenomenon,

the tumor size must be assessed at multiple time-points. Clinical and radiographic data measuring the growth of recurrent tumors might serve this purpose, but there are also many possible mathematical models to describe tumor repopulation. To find the most appropriate model would require numerous additional patient studies. Preclinical models may be more appropriate to generate mathematical models that can be tested later in the clinical setting.

A typical cell culture growth curve consists of a lag-phase, a log-phase, a stationary-phase and a decline-phase. When cells are exposed to cytotoxic treatment, including chemotherapy or radiation, the process of apoptosis can be initiated. The apoptotic process requires approximately 24 hours to complete, with several hours passing before nuclear fragmentation can be detected (3). *In vitro*, a population of tumor cells exposed to cytotoxic therapy will either grow at a slow pace or remain stable, followed by reduced cell numbers as a result of apoptosis. The velocity of this decrease in cell number has not previously been described, but will depend, in part, upon the cell type and the dose intensity of the treatment. Non-lethal treatment will allow cell regrowth following a similarly shaped growth curve as untreated cells, possibly with a different velocity.

In order to quantify the growth and apoptotic rates of malignant cells following cytotoxic treatment, and to develop a mathematical model predicting the rate of cellular regrowth, human glioma cells were treated *in vitro* with etoposide, a cytotoxic chemotherapy agent, at varying doses for different incubation times, and mathematical modeling was used to describe tumor cell repopulation.

Materials and Methods

Cell lines and reagents. The human glioblastoma cell line (U87 MG) was a gift from the laboratory of Dr. Joya Chandra in the Division of Pediatrics at the M.D. Anderson Cancer Center, TX, U.S.A.

The U87 cell line was grown and maintained in DMEM/F12 containing 1% sodium pyruvate, 1% non-essential amino acids, 1% sodium bicarbonate, 2% glutamine, 1% penicillin/streptomycin and 10% fetal bovine serum. The cells were cultured in a standard

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incubator at 37°C with 5% CO₂. The cell culture reagents were purchased from the Invitrogen Corporation (Carlsbad, CA, USA). The chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), while etoposide was purchased from the M.D. Anderson Pharmacy (Houston, TX, USA).

Cell growth and viability assays (MTT assays). Tumor cells were grown and harvested in the log-phase, and 3,000 cells per well were plated in 96-well tissue culture plates (B.D. Bioscience Falcon, Bedford, MA, USA). The cells were allowed to adhere and grow for 24 h before etoposide was added at appropriate doses. The etoposide was serially diluted in complete medium and added to the wells at doses ranging from 0.0044 µM to 70.75 µM. Twenty-four h after the addition of etoposide, the medium was removed and the cells were rinsed with phosphate-buffered saline (PBS). Three hundred µl of fresh medium with fetal calf serum (FCS) was added to each well and the cells were returned to the incubator. The cells were harvested at 24-h intervals and MTT assays were performed as previously described (4). Briefly, the medium was aspirated from each well, 100 µl per well of MTT reagent (1 mg/ml) was added and the cells were incubated for 1 h at 37°C. One hundred µl of buffer (20% SDS, 30% PBS, 50% dimethylformamide) was added to each well and assayed in an ELISA plate reader at 570 nm. All the experiments were performed in quadruplicate. The data was transferred to Microsoft Excel files and used to generate all the further calculations and graphs.

Mathematical modeling. We hypothesized that the tumor cells, following cytotoxic therapy, could be classified into two groups. The first group consists of cells that undergo apoptosis, while the second group consists of cells that continue to grow after recovery.

Accordingly, the tumor cell repopulation was modeled using the following formula:

$$(1) \quad y(t) = a * \exp(-b*t) + c * \exp(d*t)$$

where $y(t)$ denotes the tumor cell number at time t after the drug is removed. The parameters a , b , c and d have positive values.

Specifically, parameter a represents the number of cells in the first group and parameter c represents the number of cells in the second group at the specified time-point. According to this model, the cell number in the first group should decrease exponentially, i.e., the first term in model (1) has a negative exponent $-b*t$; the cell number in the second group should increase exponentially, i.e., the second term in model (1) has a positive exponent $d*t$.

Direct fitting of the data to the model (1) has been shown to be unstable (5). The parameters a , b , c and d would have very different values if different starting points were used in the fitting procedure. In order to account for this instability, model (1) assumes that the probability of each cell being alive at time t is $\exp(-b*t)$. That is, parameter b is the same for all cells in the first group. Similarly, for the second group, model (1) assumes that all cells grow as if there were no drug treatment. That is, parameter d is the same for all cells in the second group. With the assumption that parameters b and d remain fixed, fitting the cell number results in model (1) becomes stable.

For high drug doses, it was assumed that most tumor cells would undergo apoptosis. The surviving cells will slowly regrow, however, in the first few days; the remaining measurable cells come from the first group. Therefore, the value of c is small and the second term

$c * \exp(d*t)$ in model (1) can be effectively ignored. Therefore, the cell number resulting from high drug doses can be fitted to the single-term exponential model:

$$(2) \quad y(t) = a * \exp(-b*t)$$

On the other hand, for low drug doses, it is assumed that most tumor cells will survive the drug treatment. That is, the number of tumor cells that undergo apoptosis is very small and the contribution to the final cell number is negligible. Therefore, the cell number measured after low drug doses can also be fitted to the single-term exponential model:

$$(3) \quad y(t) = c * \exp(d*t)$$

Thus, very high and very low doses of cytotoxic therapy were used to determine parameters b and d separately. The resulting b and d values were held constant and were used to fit the cell number results from intermediate drug doses to model (1) where only parameters a and c were unknown.

The fitting procedure for each of the formula (1), (2) and (3) was done using the "fit" function provided by the software MATLAB (Mathworks, Natick, MA, USA). Since all the parameters were positive, the lower bounds were specified as 0 (rather than the default negative infinity) while using the "fit" function.

Results

U87 human glioma cells were treated *in vitro* with varying doses of etoposide, and MTT assays were performed on the remaining cells to determine the cell numbers. There was a dose- and time-dependent drug effect. Figure 1 shows the cell growth in response to varying drug doses. When the etoposide doses were high (e.g., >1.13 mM), the cell numbers had not recovered by 120 h. When the doses were low (e.g., ≤ 0.28 mM), the cell numbers increased exponentially with time.

Determination parameters b and d . In order to calculate the value of parameter b , the data from the two highest etoposide doses was used and fitted to model (2). The calculated value was $b = -5.386 \times 10^{-2}$. In order to calculate the value of parameter d , the data from the two lowest etoposide doses was used and fitted to model (3). The calculated value was $d = 2.289 \times 10^{-2}$.

Determining parameters a and c . Using the values obtained for parameters b and d , the cell number data for all drug doses was fitted to model (1). The calculated values for parameters a and c are shown in Table I and Figure 2.

Determination of the relationship between parameter c and dose. The value of parameter c were fitted to the chemotherapy doses (Figure 2) using the following sigmoid model:

$$(4) \quad c(x) = p_1 / [1 + p_2 * \exp(p_3 * \log(x))] \text{ or } c(x) = p_1 / (1 + p_2 * (x^{p_3})),$$

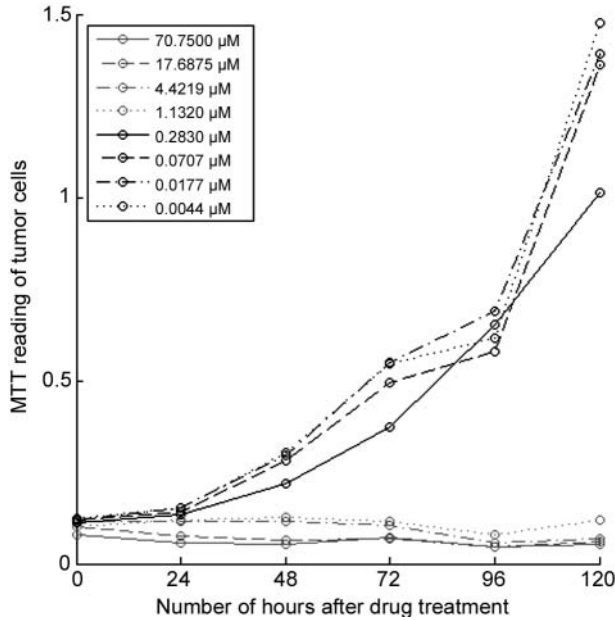


Figure 1. Graph of cell numbers over time with varying etoposide doses. The vertical axis shows the optical density readings in MTT tests representing tumor cell numbers, while the horizontal axis shows the time in hours after removal of the drug.

where x denotes the dose. The coefficients are $p_1=0.086$, $p_2=14.74$ and $p_3=3.033$, respectively. Therefore, the tumor cell number $y(t)$ can be modeled as a function of t .

$$(5) \quad y(t) = c * \exp(0.0289 * t) \text{ and } c = 0.086 / (1 + 14.74 * \text{dose}^{3.033}).$$

Discussion

Tumor cell repopulation after exposure to cytotoxic therapy has been studied in a variety of systems (6-12). In an attempt to more accurately describe this phenomenon, an *in vitro* model system was explored and a mathematical model was generated for use in clinical practice. Our data fit well with the exponential model:

$$y(t) = a * \exp(-b * t) + c * \exp(d * t).$$

The etoposide doses correlated with parameters a and c in the model, but not b or d , which suggests that the velocity of cell death or growth after treatment was independent of the drug dose.

One of the goals of this study was to determine the relationship between the drug doses and the values for parameters a and c in model (1). Model (1) implies that increasing etoposide doses are associated with increased values of a and decreased values of c . However, the values

Table I. Calculated values for parameters a and c fitting the exponential model for different drug doses.

Doses	a	c
70.7500 μM	7.287E-2	9.790E-4
17.6875 μM	9.073E-2	4.903E-4
4.4219 μM	1.283E-1	1.851E-6
1.1320 μM	1.212E-1	3.312E-3
0.2830 μM	4.304E-2	6.497E-2
0.0707 μM	1.715E-2	8.192E-2
0.0177 μM	3.253E-2	8.592E-2
0.0044 μM	1.707E-2	8.877E-2

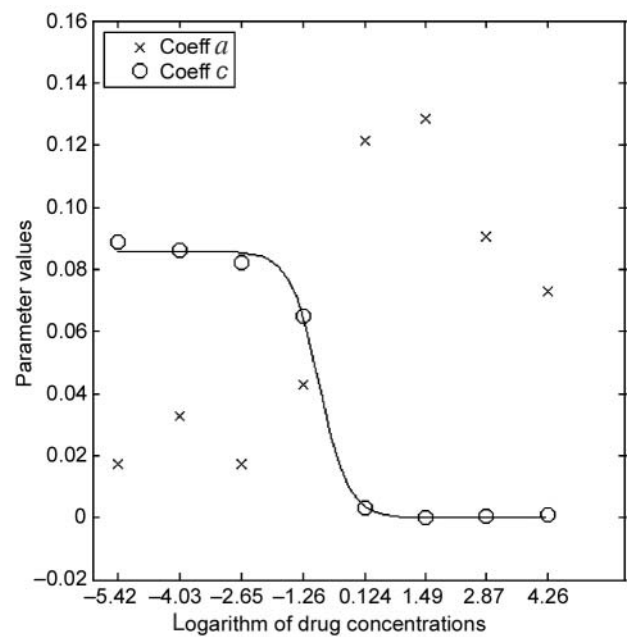


Figure 2. Graph of parameters a and c in model (1) against drug doses. The vertical axis shows the values of a and c , and the horizontal axis shows the (natural) logarithm of the drug doses. The values of parameter c and the drug doses were fitted to model (4).

of parameter a obtained from the fitting procedure may not fully agree with this implication. The number of cells in the first group, $a * \exp(-b * t)$, was generally small. Therefore, small errors in the MTT readings may have affected the accuracy of the value of a . However, since $a * \exp(-b * t)$ decreased exponentially as t increased, the accuracy of the value for parameter a is unimportant for describing tumor cell repopulation, which is reflected by exponential growth. Thus, no attempt was made to pursue precise values for a or to find the correlation between a and etoposide doses.

The value of parameter c , on the other hand, was directly related to drug concentration. At low drug doses, c could be

determined with high precision. However, at high drug doses, the value of parameter c was small and the accuracy of the MTT readings were reduced. Since the c values for these high doses were small, true differences could not be detected in the first few days using the MTT readings. To better estimate the c value, one could either predict the value using model (4) or record more MTT readings at later time-points.

The MTT readings were notably high at 72 h, particularly for the two highest and the two lowest drug doses. In order to examine the relevance of this finding, mathematical analysis was repeated excluding the 72-h data. The results were only minimally different (data not shown), and therefore model (1) appears to be robust with respect to experimental noise.

The values of parameter c were fitted to the drug doses using model (4). Model (4) can be used to predict tumor cell repopulation with values determined for the parameters p_1 , p_2 and p_3 . Parameter p_1 in model (4) corresponds to parameter c from the corresponding control setting, that is, when the tumor cells were untreated. Thus, $c=p_1$ when $dose=0$.

The sum of parameters a and c may not necessarily be a constant for different drug doses, since the amount of dead cells with different drug doses varied considerably and this variable was not included in model (1).

Interestingly, model (3) was equivalent to model (6):

$$(6) \quad y(t) = \exp(d^*(t-t_0)).$$

The rationale for model (6) is that different drug doses would result in different lag-phase durations (*i.e.*, t_0) during tumor cell repopulation. If model (6) were rewritten as:

$$(7) \quad y(t) = \exp(-d^*t_0) * \exp(d^*t),$$

and regarding $\exp(-d^*t_0)$ as parameter c , then model (3) is equivalent to model (7) and, hence, equivalent to model (6).

In conclusion, the tumor cell numbers in response to cytotoxic chemotherapy fitted well to the two-term exponential model (1) describing tumor cell repopulation. The model was robust to experimental error in the MTT readings, as long as the error did not change the trend of the readings as a time series. However, errors at early time-points might affect the accuracy of parameter c and the determination of the cell repopulation time. These errors can be addressed either by predicting the value of parameter c with model (4) or by obtaining data at later time-points. These results indicate that the velocities of cell

growth and cell death are independent of drug treatment, while the velocity of tumor repopulation is dependent in this respect. The results will be further tested in the future, using other cell lines and *in vivo* model systems, to determine whether mathematical modeling of an *in vitro* system is an appropriate tool to use for calculating tumor cell repopulation in clinical practice.

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