Mathematical model for Kinetic Heterogeneity of an Experimental Tumour Revealed by BrdUrd Incorporation

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Abstract

The mean and variance of time between biological organ system due to some factors related disease or dysfunctions are obtained by generalized. This model suggests that a person unable to balance the body conditions due to the environmental factors which are the effects of human health conditions. The cell population takes into account the cell–cell heterogeneity of the progression rate across cell cycle phase within the tumour, and also allows for a non constant DNA synthesis and parabolic DNA synthesis rate across S phase. Sequential DNA–BrdUrd distributions were obtained in vivo from a human ovarian carcinoma transplanted in mice.

In this paper the mean and variance of time between failures of biological organ System. We use the mathematical model by defining the space $(\boldsymbol{\Omega}, \mathfrak{F}, \boldsymbol{P})$ *a complete probability spaces and* $(\boldsymbol{E}, \boldsymbol{\xi})$ *be a measurable space. For any set* $\boldsymbol{X\epsilon\,\xi}$, $M(A)$ *has an exponential distribution with parameter* $\mu(A)$ *and it is used the suitable assumption and the corresponding results are obtained. If the tumour cell levels exceed a threshold level, the organ system reaches threat state or dysfunction state.*

Keywords: *Exponential distribution, branching process, BrdUrd-DNA, flow cytometry, relative movement.*

Subject classification: 60J85

1. Introduction

Growing cell populations are characterized by the variability of the proliferative pattern. In particular, the cell cycle duration and the residence times in the different cycle phases can vary even within a genetically homogeneous population that grows in a homogeneous environment, such as an in vitro culture. Direct evidence of this variability was provided by time-lapse cinematography [18] or, indirectly, by its effects on the progression of a labelled subpopulation [19, 10]. The technique based on cell labelling by a radioactive DNA precursor and subsequent autoradiography evidenced a wider variability occurring in the in vivo growing populations, such as in the experimental and spontaneous tumours [19] for a comprehensive review of these results. This is not surprising since the concentration of oxygen and nutrients in tumours can be quite heterogeneous [15] and because of the possible presence of different phenotypes with different kinetic characteristics [6]. The current method for assessing the kinetics of proliferating cell populations is based on the incorporation of the label BrdUrd followed by flow cytometry [13, 11, 12]. By means of this technique, that has replaced the 3H-thymidine incorporation method and the autoradiography, it is possible to follow the time course of the BrdUrd-labelled (and of the unlabelled) subpopulations as related to their DNA content. Some expressions for observable quantities related to the evolution of DNA–BrdUrd histograms have been derived by assuming that there is no cell–cell variability of the transit times in S and G2M phases [24, 26, 7]. The in vivo cell kinetics of experimental tumours has been studied under this assumption [9, 20, 25], but a more refined approach is advisable in view of the proliferative heterogeneity of experimental tumours. Mathematical and simulation models that predict the evolution of DNA–BrdUrd distributions assuming independently distributed phase transit times have been developed by [27, 23 21, 4, 5].

Human are highly adapt in nature. Most of the people, perceived threats to their well being often are psychological. A failed relationship between people triggers distress affecting smooth functioning of an organ system. A major challenge in biology is to determine the kinetics of proliferating cell populations is based on the incorporation of the label BrdUrd followed by flow cytometry. The most reliable method of labelling is through genetic modification leading to the expression of a reporter gene in a random sample of cells. Recently it has become possible to activate genetic labeling at a defined time in transgenic mice, enabling the kinetics of labelled cells to be studied with single cell resolution in vivo.

In this model the damage from a life distribution of a pure jump process using BrdUrd labelling and flow cytometry. This method, that exploits more completely the information contained in the data, is applied to the study of a human ovarian tumour implanted in nude mice. This damage is accumulated and when exceeding some given level results in a failure of a system, mission etc. Experimental data (HOC18) of the relative movement together with the constant DNA synthesis rate and parabolic DNA synthesis rate. If the tumour cells levels exceed a threshold level, the organ system reaches threat state or dysfunction state.

2. EXPERIMENTAL PROCEDURE

2.1. Experimental protocol.

The human ovarian carcinoma xenograft HOC18, was derived from a primary ovarian tumour from a 62-year-old patient. It is maintained and grows subcutaneously in nude mice as previously described [16]. The volumetric doubling time was 9.8 ± 2.5 days. In the experiments reported here, BrdUrd (50 mg kg−1) was injected into the peritoneal cavity of the mice 39 days following tumour implant, with tumours weighing 300–500 mg. BrdUrd reaches the tumour and labels the DNA-synthesizing cells [12]. The cells in S phase at the time of the BrdUrd exposure became therefore BrdUrd-labelled (BrdUrd+). At the subsequent times, these cells or their descendants remained BrdUrd+ and were definitely distinguishable from cells in G2, M and G1 phases at the initial time (BrdUrd-unlabeled cell, BrdUrd−). Three mice were killed at t = 0.5, 2, 4, 8, 14, 20 h after BrdUrd injection, the tumour was minced and the cells were suspended and fixed in 70% ethanol. For the in vitro study, cells from a human ovarian carcinoma line (IgroV-1) were cultured as previously described [8]. Cells in the exponential phase of growth were pulse labelled with BrdUrd (20 min labelling with 20 mM BrdUrd). At t = 0, 3, 6, 9, 15 h post labelling, the cells were detached, counted and fixed in 70% ethanol.

2.2. Flow cytometry.

For the flow cytometric analysis according to the DNA– BrdUrd method, the fixed cells were stained with propidium iodide (PI) and anti- BrdUrd fluoresceinated antibody, as described before [22]. Briefly, each sample of ethanol-fixed cell suspension was centrifuged and incubated with 3N HCl for 20 min, to obtain partially denatured DNA. After neutralizing with 0.1M $Na_2B_4O_7$, the cell pellet was resuspended with 50 µl Tween 20, 0.5% in PBS. After that, 50 µl of bovine serum albumin 0.5% in PBS and 20 µl of anti-BrdUrd mouse monoclonal antibodies were added and the mixture was incubated for 60 min at room temperature. After washing with PBS, cells were incubated for 1 h with fluorescein (FITC)-conjugated F(ab')2 fragments of goat antimouse IgG (Jackson,West Grove, PA) diluted 1:50 in PBS with 0.5% Tween 20 and 1% NGS. After incubation with antibody, cells were centrifuged, resuspended in 2 µg ml−1 PI in PBS plus RNAse, incubated overnight and analyzed by flow cytometry with laser excitation tuned at 488 nm, using a FACSCalibur (Becton Dickinson) flow cytometer. The green fluorescence of fluorescein was detected in the 515–555 nm wavelength band and the red PI fluorescence above 630 nm. The fluorescence intensities of each cell were accumulated to form a biparametric histogram [3].

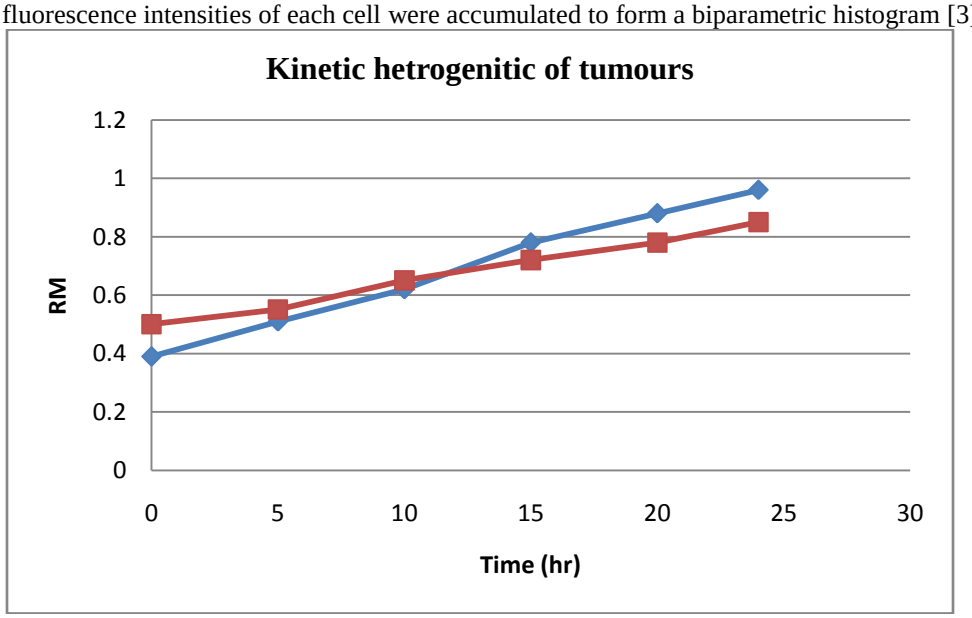

Fig1. Experimental data (HOC18) of the relative movement together with constant DNA synthesis rate (Red) and parabolic DNA synthesis rate (Blue).

3. Mathematical Model

Suppose that the cells are subject to affected (damage), the number of affected cells overtime is assumed to be an increasing pure jump process [17].Denote such a process by $\boldsymbol{X = x_i}$. Let $(\boldsymbol{\Omega, \mathfrak{F}, P})$ be the probability space in which $\boldsymbol{X}$ is defined .The life distribution properties of the application part under Suitable condition on the parameters of the damage process is obtained. In the application part the different disjoint sets are defined in the following manner [1, 2].

Let $(\boldsymbol{\Omega, \mathfrak{F}, P})$ a complete probability space $(\boldsymbol{E, \xi})$ be a measurable space.

$\boldsymbol{\xi}$ - is the collection of all subsets of $\boldsymbol{E}$,

$\boldsymbol{\Omega}$ - is the measure space,

$\boldsymbol{\mathfrak{F}}$-is the measurable spaces – all subsets of $\boldsymbol{\Omega}$,

$\boldsymbol{P}$ -is the probability measures

4. Results

A mapping $\boldsymbol{M}: \boldsymbol{\Omega \times \xi \to \overline{R}_+}$ is said to be random measures $M(\boldsymbol{E, \xi})$ provided that:

i) $\boldsymbol{\omega \to M(\omega}$, A) is $\boldsymbol{\mathfrak{F}}$ measurable for each **A** in $\boldsymbol{\xi}$.

ii) $\boldsymbol{A \to M(\omega}$, A) is a measurable on $(\boldsymbol{E, \xi})$ for each $\boldsymbol{\omega}$ in $\boldsymbol{\Omega}$.

A random measure $\boldsymbol{M}$ on $(\boldsymbol{E, \xi})$ is said to be an exponential random measures with mean measures $\frac{\boldsymbol{1}}{\boldsymbol{\mu}}$, and variance measure $\frac{\boldsymbol{1}}{\boldsymbol{\mu^2}}$ Provided:

i) For any $n \geq 2$ and disjoint sets $\boldsymbol{A_1 A_2 \ldots\ldots\ldots A_n}$ in, $\boldsymbol{M(A_1), M(A_2) \ldots\ldots\ldots\ldots\ldots M(A_n)}$ are independent random variables.

ii) For any $\boldsymbol{A}$ in $\boldsymbol{\xi}$, $\boldsymbol{M(A)}$ has an exponential distribution with parameter $\mu(A)$, that is

$\boldsymbol{P[M(A) = k] = \mu(A) \exp(-\mu(A)k)}$, $k \in N$ [14].

A survival probability of $\overline{F}$, R_+ is said to be :

(i) Increasing failure rate (IFR) if $\overline{F}$ convex with respect to an exponential function on R_+

(ii) Decreasing failure rate (DFR) if $\overline{F}$ concave with respect to an exponential function on R_+

(iii) Decreasing failure rate average (DFRA) $\overline{F}$ if is antistar-shaped with respect to an exponential function on R_+

(iv) Increasing failure rate average (IFRA) $\overline{F}$ if is star-shaped with respect to an exponential function on R_+

(v) New better than used (NBU) if $R \equiv -\text{In F}$ is a superadditive function on R_+

(vi) New worse than used (NWU) if $R \equiv -\text{In F}$ is a subadditive function on R_+

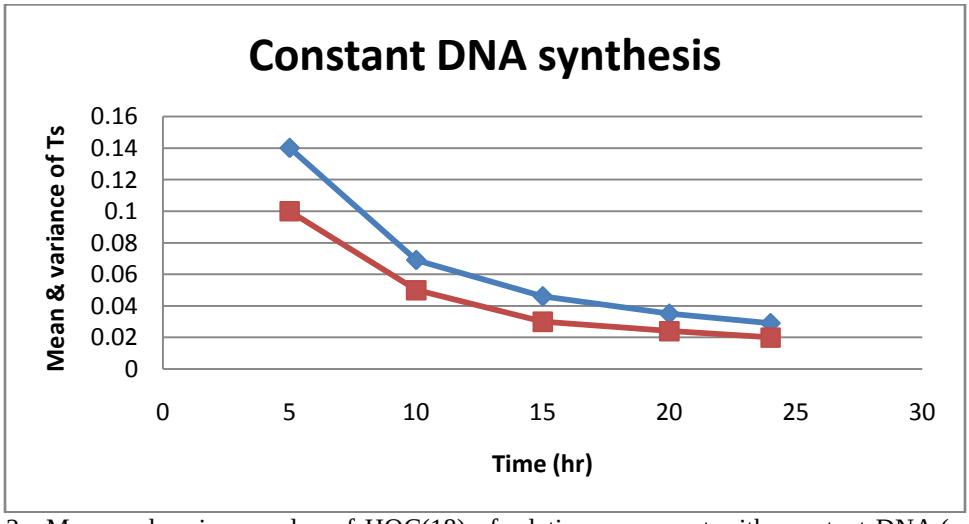

Fig 2. Mean and variance value of HOC(18) of relative movement with constant DNA.($\mu = 1.449$ mean – blue, var-Red)

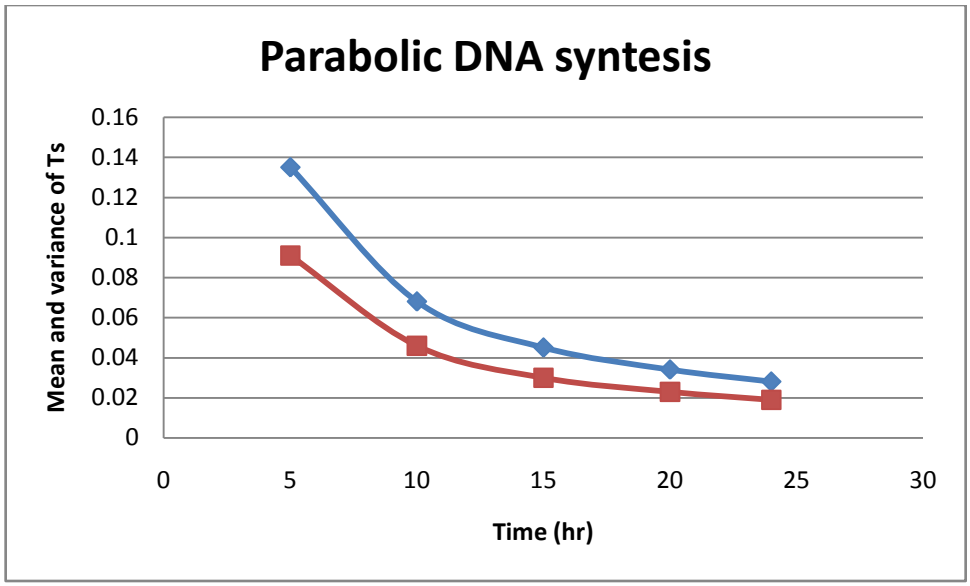

Fig3. Mean and variance value of HOC (18) of relative movement with parabolic DNA.($\mu = 1.481$ mean – blue, var-Red)

5. Application

An approximation of statistical measure DNA synthesis, we can use them to characterize the kinetics of heterogeneity of tumour cell population. Flow cytometry method, and additional data on the relative movement, show that the overall cell population of HOC(18) cells grow exponentially, also providing the independent to estimate the population statistical measure. In order to estimate $E(T_s) = \frac{1}{\mu}$ and $Var(T_s) = \frac{1}{\mu^2}$. Either BrdUrd labelled or unlabellled cells and fit the statistical measure with any one of the cell cycle phases. We just focused here on the outline of the statistical measure of S phase cells within the population of BrdUrd labelled cells. The curve of Fig. 2 & 3 show that the result of pure jump damage process with those statistical measure. Usually it is not possible to define the individual age of cells within a growing population ($\mu = 1.449\ and\ 1.481$ are constant and parabolic DNA synthesis respectively). After substituting the numerical value for the constant as well as the parabolic DNA synthesis cell line, we can conclude that its cell cycle lasts approximately 30 hr. BrdUrd lebelling is applied extensively for the quantification of DNA synthesis in high throughput assays of cell proliferation (proliferating cells in vivo and in vitro) are based on the detection of incorporation of the thymidine analog 5'-bromo-2'-deoxyuridine (BrdUrd), often combined with DNA content measurements. They may be either single time-point measurements or use the time-lapse strategy. The incorporated BrdUrd is detected either cytochemically, based on the use of the DNA dyes, or immunocytochemically, using fluoresceinated BrdUrd-antibodies. The time-lapse measurement of the cohort of BrdUrd-labelled cells allows one to estimate their rate of progression through different points of the cell cycle.

Conclusion

In this paper to obtained the value of mean and variance of DNA synthesis of HOC(18) from relative movement of DNA synthesis very closed and also estimated parameters are similar. We aim at assessing the speed of kinetic heterogeneity of (tumour) a cell population reached by considering the mean cell cycle duration and its variance as the main significant of parameters. A cancer cell line growing in vitro under optimal conditions can be view as a heterogeneity cell population with simple dynamics. Since the time is important factors of a successive affected of a cell in a particular time interval is considered and find the mean and variance of the cells at different levels.

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