Biogerontology **3:** 175–182, 2002.

Research article

A model of accelerated aging induced by 5-bromodeoxyuridine

Alexander A. Butov[1], Maxim A. Volkov[1], Vladimir N. Anisimov[2], Mary E. Sehl[3] & Anatoli I. Yashin[4,*]

[1]*Department of Applied Mathematics, Faculty of Mechanics and Mathematics, Ulyanovsk State University, Leo Tolstoi 42, 432700 Ulyanovsk, Russian Federation;* [2]*Laboratory of Carcinogenesis and Aging, N.N. Petrov Research Institute of Oncology, Pesochny-2, St. Petersburg 197758, Russia;* [3]*Division of Biology and Medicine, Brown University School of Medicine, Box G-8122, Brown University, Providence, RI, 02912, USA;* [4]*Laboratory of Advanced Statistical Methods, Max Planck Institute of Demographic Research, Doberaner Str. 114, 18057 Rostock, Germany; *Author for correspondence (e-mail: yashin@demogr.mpg.de; fax: +49-381-2081-169)*

Received 17 September 2001; accepted in revised form 6 November 2001

Key words: accelerated aging, aging, carcinogenesis, genetics of aging, mutagenesis, processes, random, semimartingale methods, simulation, 5-bromodeoxyuridine

Abstract

We consider a scheme of aging with two possible mechanisms of senescence processes: aging with apoptosis and necrosis for differentiated cells and a multistage process of malignant transformation. Our model describes the multistage phenomena of aging and carcinogenesis by a set of stochastic equations using multivariate and diffusion processes. This model fits experimental data on the acceleration of aging processes from postnatal exposure to 5-bromodeoxyuridine (BrdU), and the induction of genome instability. The methods of stochastic modeling and computer simulation of the processes of aging and carcinogenesis have been used for the verification of the biological suggestions.

Introduction. The importance of experiments with 5-bromodeoxyuridine

This work is devoted to investigation of accelerated aging and perspectives of theoretical and experimental control of aging processes by means of treatment with 5-bromodeoxyuridine (BrdU) (Anisimov and Osipova 1992, 1993; Anisimov 1994, 1995, 1997) and the extension of this work using proper computer simulation modeling.

A great number of various aging theories are known, and nearly all of them are in some sense justified: anything alive is subjected to destructive exposures and influences, uses certain mechanisms to counteract the damage of these exposures, and finally exhausts the individual ontogenetic program and resources in this struggle. These destructive processes are so numerous and intensive that the question about causes of aging is less interesting compared to the question: why does it occur so slowly? We can designate (very conventionally) the following as indicators (and perhaps as causes) of cell aging:

(I) damage to lipid structures (primarily membranes and mainly by free radicals),

(II) damage of protein structures and accumulation of such proteins in an organism,

(III) damage to DNA.

Item (III) can have a relatively large significance for tissues with proliferation activity when compared to its contribution in postmitotic tissues. Therefore, mutagenesis with possible subsequent malignant transformation of cells can be considered an important parameter of cell aging in tissues with proliferation activity. In postmitotic tissues, the effect of mutagenesis can result in disturbances of protein synthesis (II) and, as a consequence, an increase in phenomenon (I). The intensity of natural damages to DNA (III) is very high [in a human cell, spon-

taneous depurinization takes place at a rate of up to 10,000 acts per day and spontaneous deamination of adenine and cytosine occurs at a rate of hundreds of events per day (Singer and Berg 1991)]. As a result, permanently working mechanisms of DNA repair have evolved. It turns out that in both of the most intensive natural mutational processes (depurinization and mentioned deamination), thymine is not present [mutations related to it are significantly more rare (Singer and Berg 1991)], and therefore the reparation schemes for thymine may have evolved less intensively. Hence, if a researcher wants to induce uniformly distributed point mutations (and simultaneously to minimize damages in other structures) in laboratory animals then it is meaningful to use analogues of thymine as a mutagen. One of such analogues is 5-bromodeoxyuridine, which incorporates into cellular DNA in the place of thymidine. In addition, BrdU can be used because, unlike purine-analogues, it is not involved in energy production (as it is the case with ATP, ADP, AMP and GTP, GDP, GMP) or in cell-signaling and interactions (as in the case with GTP, etc.) and hence can reach required levels of mutation, while being less toxic than in the case of 'spoiled' analogues of purines. This ability to induce uniformly distributed point mutations with a chosen intensity using BrdU is based on the fact that the number of mutations is linearly dependent on the concentration of BrdU (Morris 1991; Anisimov 1994, 1995, 1997).

Point mutations in cells of postmitotic tissues (at small levels of exposure) also result in a decrease in the level of protein synthesis that is proportional to the number of point mutations. BrdU has been shown to induce a senescence-like phenomenon in mammalian cells (Michishita et al. 1999), induce the expression of senescence marker genes in human embryonic lung fibroblast cells and cervical tumor HeLa cells (Suzuki 2001), affect the level of diverse chemicals involved in immune function [e.g., induction of production of interferon in B cells of the Namalwa line (Shuttleworth 1982)], and increase the parameters of the rate of aging in mortality curves for rats (Anisimov 1997).

Formulating hypotheses about the mechanisms of acceleration of aging caused by damage to DNA (III) also provides new opportunities both in the laboratory and in simulation of controlled acceleration of aging processes. Correct control of such acceleration poses an important and very complex problem. Hence, it is possible to investigate the role of component (III) in the processes of aging and carcinogenesis. Recently, the study of cellular senescence and immortalization has become increasingly important in both aging and cancer research. It has been found that some common genetic changes (e.g., activated telomere maintenance) known to occur in carcinogenesis have a key role in the immortalization process (Aragona et al. 2000; Krupp et al. 2000; Reddel 2000).

Multistep models of cellular aging and immortalization have been developed in an attempt to explain delayed genomic instability, in which initiation of carcinogenesis is linked not only to a direct increase in chromosomal aberrations and mutation rate of oncogenes and tumor suppressor genes, but also to enhanced levels of aberration and mutation in distant progeny and a predisposition to immortalization (Simons 2000). In addition there have been multilevel models of carcinogenesis (examining the heterogeneous decrease in growth rate and accumulation of damages with age at the chromosome, cell, tissue and organism level) in which there was observed a convergence of findings related to cancer in culture and the organism (Rubin 1999).

Investigations of chromosomal mechanisms of immortalization are needed to understand the multistep process of carcinogenesis in human cells (Namba et al. 1996; Lee and Wei 1997). Studies of chromosomes that induce senescence in given cell line in cell culture reveal that multiple pathways to senescence and carcinogenesis may exist (Sasaki et al. 1994). Studies have also been done concerning the progress of secondary genetic events during multistage carcinogenesis and their dependence on the condition of the initiated target cell and adaptive functions of cells of various ages (Simons 1999). Pointed multistep processes of mutagenesis and malignant transformation are considered in the presented work as the first step (to some extent – simplification) of quantitative modeling of simultaneous processes of carcinogenesis and mutational factors of aging. Sure in general the description of the carcinogenesis phenomenon must include varying pathways of malignant transformation, and the processes of aging are overwhelming. But presented below first-step quantitative model of accelerated aging can be considered as an attempt to construct a tool for possible comprehension of mutational factors of aging.

All the processes controlled by the level of BrdU exposure in the experiment are investigated in the dynamic stochastic processes describing the changes in the numbers of cells in tissues with proliferation activity at various phases of their evolution and mutual

transformations: during interphase, in the mitosis phase, in preinitiated, initiated and transformed conditions, processes of apoptosis and necrosis and immune elimination of the transformed and tumoral cells. The levels of proliferation factors and protein synthesis, levels of promoters and initiators in the model are hypothesized to affect the processes of birth and death of cells, immune function, the intensity of the transitions of cells from one condition to another etc. We hypothesize in the model that intensities of such transitions are linearly dependent on some obvious factors and allow the presence of stochastic disturbances in the cases when dependencies are not obvious. This approach helped to formulate the stochastic mathematical model suitable for the simple procedure of simulation computer modeling. Ontogenetic processes, the times and causes of death of laboratory animals, observed in experiment, admits the comparison of results of simulation modeling with real biological experimental data.

Here we try to consider the processes of aging as a complex phenomenon including changes in functional and proliferating ability of cells, immune response and cellular immortalization events. All these destructions (induced by free radical damages, mutagens etc.) result in the changes of functional ability of tissue, carcinogenesis and immune defense of the modeled organism. The model permits to consider simultaneous behavior of all the characteristics of organisms, simulated mortality curves, changes in numbers of tumor occurrence (induced not only by mutagens but accelerated aging in immune function and functional ability of tissue). So we hypothesize here that the processes of aging in cells and tissues are cumulative destructions induced by mentioned above reasons I, II and III (the frameworks of the model are restricted by the reason III and to some extent – by the reason II in order to debug the modeling of mutational factors of aging). An attempt to simulate quite sophisticated phenomena of accelerated aging (and carcinogenesis simultaneously!) caused by additional mutagenic factors permits us to allow the meaning of mutational factors in aging not only verbally, but in quantitative speculations.

One of the additional reasons for the investigation of BrdU to be considered is the importance of azidothymidine (a thymine-analogue similar to BrdU in mutagenic action), which is widely used in human treatment.

The description of the model

We consider the mathematical model of processes of aging and carcinogenesis in tissues with proliferation activity in terms of random processes. This work is based on the experimental data published earlier (Napalkov et al. 1989; Anisimov and Osipova 1992, 1993; Anisimov 1994, 1995, 1997). Rats aged 1, 3, 7, and 21 days were given BrdU subcutaneously at a single dose 3.2 mg/animal.

For the mathematical model, a scheme of cellular aging with two possible ways of progression of these processes is designed (see Figure 1): aging with apoptosis and necrosis for differentiated cells and a multistage process of malignant transformation (Anisimov 1998).

This is the scheme of cell transitions from one state to another. The process N_t represents the number of cells in the interphase state at time $t \geq 0$ with some 'ideal' number of cells N in the adult organism; N_t^S is the number of cells in the mitotic phase state; A_t is the counting process of transition events from the mitotic state with N_t^S to the interphase state with N_t; and A_t^S is the counting process of the backward transitions. Process B_t is the number of events of cell death during aging (apoptosis and necrosis). The number of cell replications during development (or resulting from damages of tissue) is designated A_t^D and the process of elimination during mitosis is B_t^S. The number of cells that are damaged (initiation events), A_t^P, is under usual conditions in dynamic balance with the process of reparation A_t^R. In reality different tissues demonstrate dramatically different behavior in numbers of proliferating cells. Thus numbers of stem cells seems to be close to the initial value during the whole life, while the hepatocytes demonstrates substantial increase of N_t^S with time, and muscle cells – substantial decrease of proliferating cells after some period of development. So this behavior of N_t^S reflects the averaged levels of normalized tissues with proliferation activity. The effective cumulative volume of tissues (with the averaged reduced proliferation level) is taken to be $1.5 \cdot 10^{10}$ cells (which corresponds to 15 grams of a living proliferating tissue in adult rat – i.e., the cumulative number of cells with a common proliferation level being equivalent to the sum of varied amounts of tissue proliferating with various rates). Appropriate preinitiated (reversibly initiated) cells with number N_t^P, in the case of an additional initiation are transformed to a subpopulation of initiated cells (i.e., irreversibly initiated)

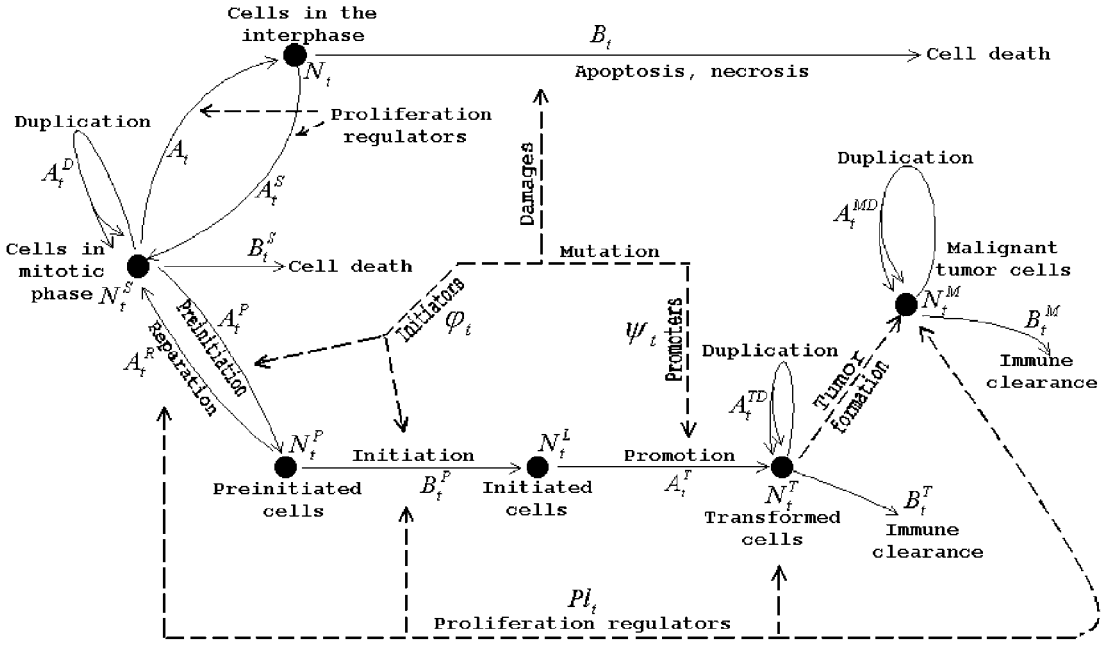

Figure 1. The scheme of phase states and transitions of cells in a tissue.

with N_t^L at time $t \geq 0$. The appropriate process of transitions B_t^P is a fixation of initiation. Exposure of initiated cells to a mutagen-promoter causes a stream of transitions A_t^T, with formation of the subpopulation of transformed (neoplastic) cells with number N_t^T. We suppose that the transformed cells can provide tumor growth A_t^{TD}, accompanied by the immune checking with the number of eliminated cells B_t^T. After tumor formation we consider the subpopulation of malignant tumor cells with number N_t^M. They provide tumor growth A_t^{MD} and immune clearance with number of eliminated cells B_t^M. We hypothesize that the intensities of the processes A_t^{MD} and B_t^M are greater than appropriate intensities of A_t^{TD} and B_t^T, because the number of tumor cells becomes greater and they proliferate and simultaneously are subjected to the immune clearance more intensively. Levels of mutagens and growth factors define the intensities of the appropriate processes of transitions, being variable proportionality coefficients.

In the model, the following phase states of cells are considered: interphase of a healthy cell, mitosis phase, preinitiated, initiated and transformed states of the cell, and the phase of tumor growth. Apoptosis, necrosis and immune clearance are the phenomena of cell elimination. Promoters and initiators are considered to act separately. A system level of proliferation regulation and the average protein and carbohydrate 'nutrition' of cells are taken into account. We suppose that the most of the mutations (both under influence of tumor promoters and initiators) fall to the mitotic phase of the cellular cycle. The level of damage caused by free radicals (and the appropriate processes of aging), under unstressed conditions is assumed to exceed a cumulative level of DNA damages by natural mutagens and damages resulting from transcription not less than 5 times. The level of damage resulting from natural basal mutagens is hypothesized to be equal to that resulting from transcriptions. The level of DNA repair is assumed to provide restoration of damages to the basal state of equilibrium in development and adult life. When exposed to BrdU, the contribution of the mutational factor to aging is hypothesized to increase substantially and become the prevailing factor (it is assumed that immediately after BrdU treatment, the number of promotion and initiation events exceeds 200, and thereafter the concentration of BrdU in tissues and the corresponding frequency of mutations decreases exponentially).

Simulation results and discussion

Modeling is carried out on the basis of the recurrent algorithms constructed on the stochastic equa-

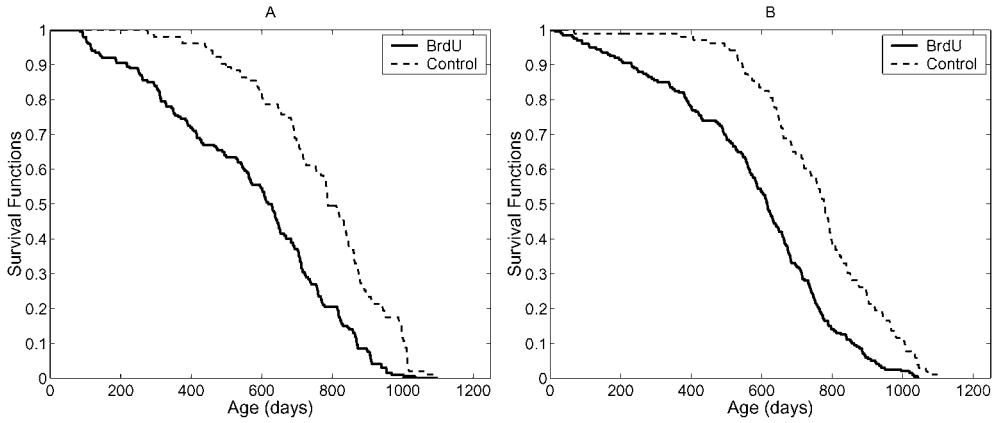

Figure 2. Empirical (A) and modeled (B) survival functions for the rats subjected to BrdU exposure (BrdU) and for control group (Control); 200 rats in the BrdU treated group, 103 rats in the control group.

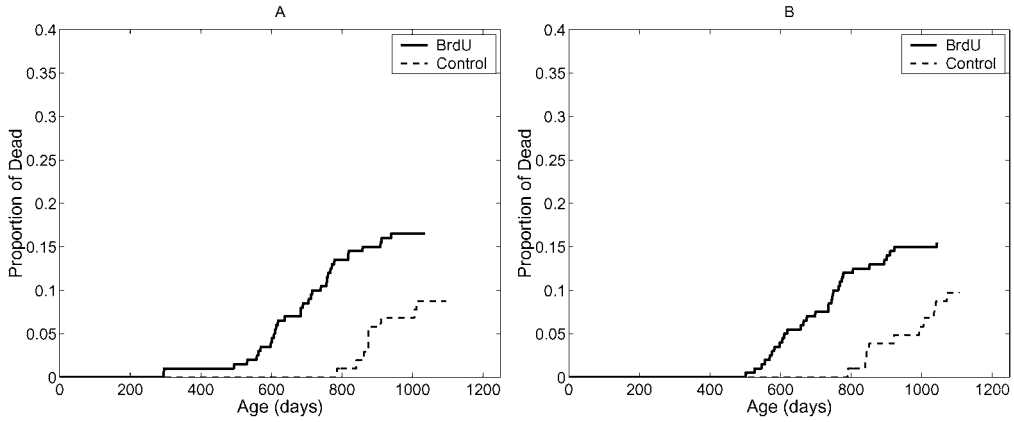

Figure 3. The proportion of rats died from cancer subjected to BrdU exposure (BrdU) and for control group (Control) in the experiment (A) and in the model (B); 200 rats in the BrdU treated group, 103 rats in the control group.

tions in terms of semimartingale characteristics of the processes (Liptser and Shiryaev 1986) and the phenomena mentioned above. Empirical survival functions are presented in Figure 2A. The proportion of rats died from cancer induced by 4-times BrdU exposure in the experiment is shown in Figure 3A constructed for experimental rats that died because of the critical size of a tumor. In the model we consider development of rats from birth till death. The modeled rat develops according with the equations given in Appendix. Death of modeled rat occurs in the following cases: the functional insufficiency of tissue, the critical size of a tumor or the accumulation of the intercellular damages. In such a way the set of rat lives is simulated, and the proper times of death are fixed. We use these times of death for constructing the modeled survival functions (Figure 2B). Figure 3B shows the proportion of deaths in the case of tumors induced by 4-times BrdU exposure in model. Sample sizes are the same in the experiment and in the model (103 rats for control group and 200 rats for BrdU treated group).

We compare Figure 2A and Figure 3A with appropriate Figure 2B and Figure 3B and observe the similarity between modeled and empirical curves both for control groups and at BrdU influence. Our results confirm the conclusion that under BrdU treatment there is an accelerated aging in tissues with proliferating cells and an increment of deaths from tumor growth. These results can serve as an indirect validation of the hypothesis about the influence of levels of tissue damages during mutagenesis and oxidative stress both on the rates of aging and on the rate of carcinogenesis. It is worthy to note that this conclu-

sion was also confirmed in observations *in vitro*. It is shown that BrdU addition in cell cultures of humans and animals accelerates their aging (Michishita et al. 1999).

Considered structure of mutagenesis in the model is to some extent simplification (in cumulative proliferating tissue sure must encounter pathways with different numbers of stages of carcinogenesis). Nevertheless the described two-stage process of tumor formation (with preliminary described processes of mutations, reparations, fixation of mutations, etc.) fits to experimental data and is quite stable while simulation.

Simulation modeling provides an opportunity to observe the dynamics on average for a population, of the development of ontogenetic phenomena, survival functions and changes in death caused by tumors after BrdU treatment on the basis of simulation of individual physiological processes.

Acknowledgements

This research was partly supported by NIH/NIA grant PO1 AG08761-01 and by RFBR grant 01-01-00735.

Many thanks are due to Professor J.W. Vaupel for the opportunity to complete this work in the Max Planck Institute for Demographic Research, Rostock, Germany.

Appendix

In accordance with the scheme shown in Figure 1, we use the following balance equations:

$$N_t = N_0 + A_t - B_t - A_t^S, \tag{1}$$

$$N_t^S = N_0^S + A_t^D + A_t^S + A_t^R - A_t^P - A_t - B_t^S, \tag{2}$$

$$N_t^P = N_0^P + A_t^P - A_t^R - B_t^P, \tag{3}$$

$$N_t^L = N_0^L + B_t^P - A_t^T. \tag{4}$$

While the number of transformed cells is below the level V we suppose that they change according to the equation:

$$N_t^T = N_0^T + A_t^T - B_t^T + A_t^{TD}. \tag{5}$$

After exceeding the level V the number of tumor cells is described as

$$N_t^M = N_0^M - B_t^M + A_t^{MD}. \tag{6}$$

All these processes have the proper intensities of transitions. The value $a_1 L_t N_t^S \cdot Pl_t$ is the intensity for the process A_t, $b_1 N_t H_t$ – for the process B_t, $a_2 L_t N_t \cdot Pl_t$ – for A_t^S, $a_3 L_t N_t^S \cdot Pl_t$ – for A_t^D, $a_4 N_t^P \cdot Pl_t$ – for A_t^R, $a_5 N_t^S \cdot Pl_t \cdot \varphi_t$ – for A_t^P, $b_2 N_t^S H_t$ – for B_t^S, $b_3 N_t^P \cdot Pl_t \cdot \varphi_t$ – for B_t^P, $a_6 N_t^L \psi_t$ – for A_t^T, $b_4 N_t^T \cdot Pl_t \cdot J_t$ – for B_t^T, $a_9 N_t^T \cdot Pl_t$ – for A_t^{TD}, $b_5 N_t^M \cdot Pl_t \cdot J_t$ – for B_t^M, $a_7 N_t^M \cdot Pl_t$ – for A_t^{MD}. Here, for example, in the equation for A_t^R the expression $a_4 N_t^P \cdot Pl_t$ means that at time t, for small time interval Δ, the number of cells passing from the state with N^P into the state with N^S is proportional to the number of preinitiated cells N^P and to the proliferation factor Pl with coefficient of reparation a_4, and similarly in all other equations, where H is the level of accumulated damage, φ is the level of mutagen-initiators, ψ is the level of mutagen-promoters, J is the process of immune clearance ability.

The specified processes are regulated by ontogenetic, tissue, proliferating and synthetic factors according to the following expressions: the programmed (ontogenetic) level of development is described as $\Gamma_t = \Gamma_\infty - (\Gamma_\infty - \Gamma_0) \cdot \exp\{-rt\}$, the level of tissue 'completeness' is defined by the expression $n_t = N_t/N$, the level of tissue 'loss' is equal to $L_t = 1 - (N_t + N_t^S + N_s^P)/(N \cdot \Gamma_t)$ (where $N_t + N_t^S + N_s^P$ is the total number of cells participating in normal functioning of tissue at time t; $N \cdot \Gamma_t$ is the ontogenetically proposed number of cells at time t in accordions with the hypothetical programmed level of development Γ_t). The parameters of function Γ_t are chosen in order to fit the changes of real weight of cumulative proliferating tissue in developing rat (from birth up to death). The 'loss' of tissue is related both to apoptotic or necrotic processes, immune clearance and to insufficiency of proliferation activity (that can occur e.g., during BrdU treatment). The functional ability of the tissue is supposed to be proportional to the level of proliferation factors Pl_t that corresponds to the ontogenetic level: $\Phi_t = n_t \cdot Pl_t / \Gamma_t$.

The external exposure and internal damaging factors in the equations take into account the main conditions of the experiment. The mutagen-initiators are defined as $\varphi_t = \varphi^b + \varphi^{\exp} \cdot I(t \in \Re_\varphi^{\exp})$, where φ^b is the basal level, $\varphi^{\exp}$ is the additional intensity of mutation induced by the experiment during the time of BrdU administration $\Re_\varphi^{\exp}$ – 1st, 3rd, 7th and 21st days (where $I(\cdot)$ is the indicator function: $I(true) = 1$, $I(false) = 0$). The level of mutagen-promoters is equal to $\psi_t = \psi^b + \psi^{\exp} \cdot I(t \in \Re_\psi^{\exp})$, where ψ^b is the basal level and $\psi^{\exp}$ is the level of additional intensity of mutation induced by the experiment during the time of BrdU exposure $\Re_\psi^{\exp}$ – 1st, 3rd, 7th and 21st days. The cumulative concentration of mutagens (promoters and initiators) $\mu_t = \varphi_t + \psi_t$ influences the change of general system parameters – both the immunity level and damages of type (II). The level of free radicals is responsible primarily for damages of type (I), and it is defined as $R_t = R^b + X_t$ where R^b corresponds to the basal level and the process $X = (X_t)_{t \geq 0}$ evolves as an Ornstein-Ulenbeck process with $dX_t = -\lambda \cdot X_t dt + \Theta dW_t$, $\lambda > 0$, W_t – a standard Wiener process. The frequency of anabolic regulated mutations d^b is assumed to be constant. An intensity of general destruction is defined as $D_t = R_t + \mu_t + d^b$, with proportions $R^b = 0.8 \cdot D^b$, $\mu^b = 0.1 \cdot D^b$, $d^b = 0.1 \cdot D^b$, where $D^b = R^b + \mu^b + d^b$, and $\mu^b = \varphi^b + \psi^b$. The level of general full damage is defined as $H_t = H_0 + \int_0^t D_s ds$ with initial value $H_0 > 0$.

The level of proliferation factors Pl_t is defined by the expression $dPl_t = -\kappa \cdot Pl_t dH_t = -\kappa \cdot Pl_t D_t dt$, $Pl_0 > 0$. The level of immunity J_t (considered as intensity of immune clearance) is hypothesized to decrease according to the formula $dJ_t = -J_t dH_t = -J_t \cdot D_t dt$ (the rate of J_t decrease is proportional to the level of intensity of destruction). This equation was considered as the first linear approach due to simplification.

We hypothesize that the death of an organism occurs for several reasons: in the case of tissue functional ability exhaustion, the time of death is $\tau_\Phi = \inf\{t : t > 0, \Phi_t \leq \Delta_\Phi\}$; in the case of an immunity exhaustion, the time of death is $\tau_J = \inf\{t : t > 0, J_t \leq \Delta_J\} = \inf\{t : t > 0, H_t \geq H^{\max}\}$; in the case where the tumor

Table 1. Coefficients used in the model. a_1 is the intensity coefficient for the process A_t; a_2 is the intensity coefficient for the process A_t^S; a_3 is the intensity coefficient for the process A_t^D; a_4 is the intensity coefficient for the process A_t^R; a_5 is the intensity coefficient for the process A_t^P; a_6 is the intensity coefficient for the process A_t^T; a_7 is the intensity coefficient for the process A_t^{MD}; a_8, b_6 and T^{EXP} are the parameters for exponentially distributed random variable τ^{EXP}; a_9 is the intensity coefficient for the process A_t^{TD}; b_1 is the intensity coefficient for the process B_t; b_2 is the intensity coefficient for the process B_t^S; b_3 is the intensity coefficient for the process B_t^P; b_4 is the intensity coefficient for the process B_t^T; b_5 is the intensity coefficient for the process B_t^M; σ_1 is the variance coefficients for the process n_t; σ_2 is the variance coefficients for the process n_t^M; N_0 is the initial number of cells in the interphase; N_0^S is the initial number of cells in a mitotic phase; N_0^P is the initial number of preinitiated cells; N_0^L is the initial number of initiated cells; N_0^T is the initial number of transformed (neoplastic) cells; N is the number of cells corresponding to 15 grams of alive proliferating tissue; κ is the regulating coefficient for the process Pl_t; r is the regulating coefficient for the process Γ_t; Γ_0 is the initial value and Γ_∞ is the maximum level of the process Γ_t; the threshold $H^{\max}$ is for the process H_t; φ^b is the basal level of mutagen-initiators and $\varphi^{\exp}$ is the additional intensity of mutagen-initiators induced by the BrdU administration; ψ^b is the basal level of mutagen-promoters and $\psi^{\exp}$ is the level of additional intensity of mutagen-promoters induced by the BrdU exposure; R^b is the basal level of free radicals; d^b is the level of anabolic regulated mutations; λ and Θ are the variance coefficients for the process X_t; Δ_Φ is the threshold for the process Φ_t; $N \cdot \Delta_N$ is the thresholds for the process N_t^M; V is the threshold for the process N_t^T.

Parameter	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9
Value	12	0.18	6	0.6	4000	0.1	0.1	20	0.016
Parameter	b_1	b_2	b_3	b_4	b_5	b_6	σ_1	σ_2	V
Value	0.01	0.016	0.3	0.05	0.15	632.9	0.005	0.01	1000
Parameter	N	N_0	N_0^S	N_0^P	N_0^L	N_0^T	Γ_∞	Γ_0	κ
Value	$1.5 \cdot 10^{10}$	$3.7 \cdot 10^7$	$0.9 \cdot 10^7$	$0.9 \cdot 10^7$	$1.0 \cdot 10^6$	10	1.4	0.00375	0.3
Parameter	Δ_N	T^{EXP}	r	φ^b	φ^{exp}	ψ^b	ψ^{exp}	R^b	d^b
Value	0.1	2100	0.0025	0.00015	0.03015	0.00015	0.03015	0.0024	0.0003
Parameter	λ	Θ	Δ_Φ	$H^{\max}$					
Value	0.1	0.0018	0.08	3.0					

exceeds some critical volume the time of death is $\tau_M = \inf\{t : t > 0, N_t^M \geq N \cdot \Delta_N\}$. The sharp increase of sensibility of rats to the pressure of environment after BrdU administration is described with exponentially distributed time of death τ_{EXP} with parameter $1/\{T^{EXP}(a_8 - b_6\varphi^{\exp})\}$. Thus, the death of an organism, τ, is determined as $\tau = \min\{\tau_\Phi, \tau_J, \tau_M, \tau_{EXP}\}$.

In the case of a large number of cells in groups (1) – (6), the procedure of simulation includes normalization and an appropriate diffusion approximation with conversion to the diffusion type equations. For example, Equations (1) and (6) are replaced by $dn_t = L_t \cdot \{a_1 n_t^S \frac{N_0^S}{N_0} - a_2 n_t\} \cdot Pl_t dt - b_1 H_t n_t dt + \sigma_1 (n_t)^{1/2} dW_t^1$ and $dn_t^M = n_t^M \cdot (a_7 - J_t b_5) \cdot Pl_t dt + \sigma_2 (n_t^M)^{1/2} dW_t^2$ respectively, and analogous diffusion approximation takes place for those processes whose values are too large for imitation in the form of point processes.

The choice of coefficients and parameters for the model (see Table 1) is defined by the values of appropriate biological characteristics and experimental conditions. Coefficient $a_1 = 12$ is the intensity of transitions from the mitotic phase to the interphase and it means that divided cell transits to the interphase approximately in 2 hours. The intensity of backward transition is the coefficient $a_2 = 0.18$. It means that the differentiated cell can transit to the mitotic phase approximately once in 5–6 days. Coefficient $a_3 = 6$ is the intensity of duplication of cells in mitotic phase and it means that cell coming in the mitotic phase can divide approximately in 4 hours. Coefficient $b_1 = 0.01$ is the intensity of cell death in the interphase and it means that cell in the interphase can die approximately in 100 days. Coefficient $b_2 = 0.016$ is the intensity of cell death in the mitotic phase and it means that one of 60 cells in the mitotic phase can die approximately in a day. Coefficient $a_4 = 0.6$ is the intensity of reparation of the preinitiated cells and it means that the preinitiated cell can be repaired approximately ones in a day or two. Coefficient $a_5 = \varphi^b \cdot a_4$, because the processes of damage and reparation are in equilibrium under usual conditions (so $a_5 = 4000$). Coefficient $b_3 = 0.3$ is the intensity of irreversible transitions of the preinitiated cells to the initiated state and it means that the preinitiated cell can become initiated approximately in 3 days. Coefficient $a_6 = 0.1$ is the intensity of transitions of the initiated cells to the transformed state and it means that the initiated cell can become transformed approximately in 10 days. Coefficient $a_9 = 0.016$ is the intensity of duplication of transformed cells and it means that the doubling of tumor occurs approximately in 60 days. Coefficient $b_4 = 0.05$ is the intensity of immune clearance of transformed cells and it means that the immunity can kill the transformed cell approximately ones in 20 days. Coefficient $a_7 = 0.1$ is the intensity of duplication of malignant cells and it means that the doubling of malignant tumor occurs approximately in 10 days. Coefficient $b_5 = 0.15$ is the intensity of immune clearance of malignant cells and it means that the immunity can kill the malignant cell approximately ones in 6–7 days. The numbers $\sigma_1 = 0.005$ and $\sigma_2 = 0.01$ are the variance coefficients for the processes n_t and n_t^M. Parameter $N = 1.5 \cdot 10^{10}$ is the number of cells corresponding to 15 grams of living proliferating tissue. Parameter $N_0 = 3.7 \cdot 10^7$ is the initial number of

cells in the interphase, and it is equal to 1/400 part of 15 grams of the living tissue (the weight of a rat at birth is approximately 1 gram and approximately 400 grams of adult one). The proportion $N_0/N_0^S = 1/4$ (N_0^S is the initial number of cells in a mitotic phase) takes place, because the cell is approximately 80% of time in the interphase and approximately 20% of time in the mitotic phase at the beginning of development. Parameter $N_0^S = N_0^P = 0.9 \cdot 10^7$ (N_0^P is the initial number of preinitiated cells), because the processes of damage and reparation are in equilibrium under usual conditions. Parameter $N_0^L = 10^6$ is the initial number of initiated cells and it is the between value for N_0^P and N_0^T. Parameter $N_0^T = 10$ is the initial number of transformed (neoplastic) cells, and it means that there are approximately 10 transformed cells in healthy tissue. Parameter $V = 1000$ is the number of transformed cells, and this amount of cells is the tumor formation. Parameter $\Delta_N = 0.1$ means 10% of the whole cell mass (the organism die when number of malignant cells exceeds 10% of the tissue). We define the coefficients $r = 0.0025$, $\Gamma_0 = 0.00375$ and $\Gamma_\infty = 1.4$ in order to accord to the average weight changing of rat during development. Coefficient $\kappa = 0.3$ under such value of parameter $H^{\max} = 3$ (the process H_t is the level of general full damage) provides the decrease of anabolic potential of proliferation in 2 times while aging. Parameters $a_8 = 20$, $b_6 = 632.9$ and $T^{EXP} = 2100$ are used for the generation of exponentially distributed random variable τ^{EXP}. Parameters $\varphi^b = 0.00015$ (the basal level of mutagen-initiators), $\varphi^{exp} = 0.03015$ (the additional intensity of mutagen-initiators induced by the BrdU administration), $\psi^b = 0.00015$ (the basal level of mutagen-promoters), $\psi^{exp} = 0.03015$ (the level of additional intensity of mutagen-promoters induced by the BrdU exposure), $R^b = 0.0024$ (the basal level of free radicals), $d^b = 0.0003$ (the level of anabolic regulated mutations), $\lambda = 0.1$ and $\Theta = 0.0018$ provides the level of mutagenes. Parameter $\Delta_\Phi = 0.08$ is the threshold for the functional ability of the tissue. So the majority of parameters are determined by rat ontogenesis and real parameters of mutagenesis, reparation, proliferation of 'healthy' and immortalized cells. Adjustment of the model is carried out (by least squares method) simultaneously for four patterns – survival curves and curves of the proportion of rats died from cancer for control and BrdU-treated groups. The parameters of the model adjustment are the changes in the levels of mutagen-initiators and mutagen-promoters, the intensity of immune clearance and the threshold of admissible levels of damages.

References

Anisimov VN (1994) 5-Bromo-2′-deoxyuridine-induced sole perturbation of DNA is sufficient for initiation of both aging and cancer *in vivo*. J Exp Clin Cancer Res 13: 13–38

Anisimov VN (1995) Carcinogenesis induced by neonatal exposure to various doses of 5-bromo-2′-deoxyuridine in rats. Cancer Lett 91: 63–71

Anisimov VN (1997) The role of 5-Bromodeoxyuridine-induced genome instability in mechanisms of accelerated aging and carcinogenesis. Adv Gerontol 1: 50–56

Anisimov VN (1998) Age as a risk factor in multistage carcinogenesis In: Balducci L, Lyman GH and Ershler WB (eds) Comprehensive Geriatric Oncology, pp 157–178. Harwood Academic Publishers, Amsterdam

Anisimov VN and Osipova GY (1992) Effect of 5-bromo-2′-deoxyuridine on life span, estrous function and tumor incidence in rats – an argument in favor of mutation theory of aging? Mutat Res 275: 97–110

Anisimov VN and Osipova GY (1993) Life span reduction and carcinogenesis in the progeny of rats exposed neonatally to 5-bromo-2′-deoxyuridine. Mutat Res 295: 113–123

Aragona M, Maisano R, Panetta S, Giudice A, Morelli M, La Torre I and La Torre F (2000) Telomere length maintenance in aging and carcinogenesis. Int J Oncol 17: 981–989

Krupp G, Bonatz G and Parwaresch R (2000) Telomerase, immortality and cancer. Biotechnol Annu Rev 6: 103–140

Lee SW and Wei JY (1997) Molecular interactions of aging and cancer. Clin Geriatr Med 13: 69–77

Liptser RS and Shiryaev AN (1986) Theory of martingales. Nauka, Moscow

Michishita E, Nakabayashi K, Susuki T, Kaul SC, Ogino H, Fujii M, Mitsui Y and Ayusawa D (1999) 5-Bromodeoxyuridine induces senescence-like phenomena in mammalian cells regardless of cell type or species. J Biochem 126: 1052–1059

Morris SM (1991) The genetic toxicology of 5-bromodeoxyuridine in mammalian cell. Mutat Res 258: 161–188

Namba M, Mihara K and Fushimi K (1996) Immortalization of human cells and its mechanisms. Crit Rev Oncog 7: 19–31

Napalkov NP, Anisimov VN, Likhachev AJ and Tomatis L (1989) 5-Bromodeoxy-uridine-induced carcinogenesis and its modification by persistent estrus syndrome, unilateral nephrectomy, and X-irradiation in rats. Cancer Res 49: 318–323

Reddel RR (2000) The role of senescence and immortalization in carcinogenesis. Carcinogenesis 21: 477–484

Rubin H (1999) Cell damage, aging and transformation: a multilevel analysis of carcinogenesis. Anticancer Res 19: 4877–4886

Sasaki M, Honda T, Yamada H, Wake N, Barrett JC and Oshimura M (1994) Evidence for multiple pathways to cellular senescence. Cancer Res 54: 6090–6093

Simons JW (1999) Genetic, epigenetic, dysgenetic and non-genetic mechanisms in tumorigenesis. II. Further delineation of the rate limiting step. Anticancer Res 19: 4781–4789

Simons JW (2000) Coming of age: 'dysgenetics' – a theory connecting induction of persistent delayed genomic instability with disturbed cellular ageing. Int J Radiat Biol 76: 1533–1543

Singer M and Berg P (1991) Genes and Genomes: A Changing Perspective. University Science Books, Mill Valley, California

Shuttleworth J, Morser J and Burke DC (1982) Control of interferon mRNA levels and interferon yields in butyrate and 5′-bromodeoxyuridine-treated Namalwa cells. J Gen Virol 1: 25–35

Suzuki T, Minagawa S, Michishita E, Ogino H, Fujii M, Mitsui Y and Ayusawa D (2001) Induction of senescence-associated genes by 5-bromodeoxyuridine in HeLa cells. Exp Gerontol 36: 465–474