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STEM CELL DIVISION STRATEGIES AGAINST CANCEROGENESIS IN COLON CRYPTS. A 2D CELLULAR AUTOMATON MODEL

Marzena Dołbniak

Department of Automatic Control, Electronic and Computer Science
Silesian University of Technology,
ul. Akademicka 16, 44-100 Gliwice,
marzena.dolbniak@polsl.pl

ABSTRACT

This paper is concerned with two types of stem cell division and their impact on cancerogeneis. We analyse symmetric, asymmetric and mixed type divisions and their relation to time needed to develop double hit mutants in a population, an event which is considered to be an initiation of cancer. We propose a 2-dimensional cellular automaton system, representing a colon crypt in which stem cells can divide and differentiate. Our results suggest, that a combination of both division types delay the process of cancerogenesis even more.

INTRODUCTION

Cell differentiation is an important process that takes place in complex organisms, e.g. during organ development, haematopoiesis or cell renewal in various tissues. It is initiated by stem cells, which either stem cells or differentiated cells during divisions. If two identical daughter cells are produced (two SCs or two differentiated cells), then the process is called a symmetrical divisions. Otherwise, if one progeny is a differentiated cell and the other a stem cell, we call it an asymmetrical division (Fig 1).

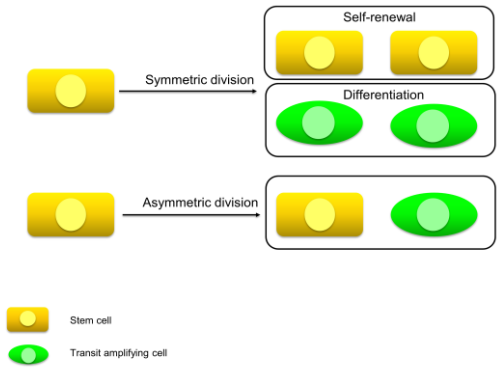

Figure 1. Symmetric/asymmetric stem cell divisions.

During differentiation cells become more specialized acquiring specific functions. The asymmetric division is considered to be a strategy to maintain normal homeostasis [2]. Symmetric division is often observed during development and after injury. The question about the role of division type in carcinogenesis remains unanswered [3, 7].

Experimental research focused on stem cells is very difficult [8]. Therefore, mathematical models prove to be very useful in this area, helping to analyse hypotheses about divisions and their influence on carcinogenesis. Theoretical modelling studies analyse for example, what impact size and dynamics of stem cells have on cancer risk [5].

Our goal is to analyse which, if any, type of division enhances the chances of cell population for entering the pathway leading to cancer. More precisely, we would like to know what is the impact of the division strategy on time in which cancerous mutations are produced.

This problem already has been analysed using numerical simulations based on the Morgan model and analytical methods in [9]. However, authors of this work have not included the structure of tissue and spatial properties of cellular population.

We decided to model colon crypts because of its cylindrical structure and properties being well known. Inside the crypt cellular proliferation and migration are tightly regulated [5].

MATHEMATICAL MODEL

We proposed a cellular automaton (CA) system in which each cell corresponds to a biological cell. Six different cell types are considered: stem cells (SC), SC with one mutation, SC with two mutations, transit amplifying cells (TA), TA cell with one mutation, TA cell with two mutations. TA cells arise from the stem cells and divide a finite number of times until they become differentiated.

Initially, in the populations there exist only wild-type stem cells located together in one area and wild-type TA cells. It is assumed that the SCs constitute 20% of the population, with the remaining subpopulation being the TA cells. Possible states and transitions rules are presented in Fig 2. SC and TA cell with one mutation can divide using the same division decision pattern. We assumed that cells with two mutations possess a knockout tumor suppressor gene, so the growth rate of such a cell can increase dramatically and cells gain cancerous properties. This assumption is based on the "two-hit hypothesis", which implies that both alleles of a tumor-suppressor gene must be affected before an effect is manifested. This is because if only one allele for the gene is damaged, the second can still produce the correct protein. In other words, mutant tumor suppressors alleles are usually recessive whereas mutant oncogene alleles are typically dominant [6]. So, when a double mutated cell appears in the population, the simulation is stopped and the iteration number is recorded. This number is subsequently used as an index indicating the time of cancer initiation.

The problem of early growth of cancer subpopulation following the appearance of the first double mutated cell was not analysed in this project.

The mutation probability u_1 was estimated to observe stochastic tunnelling [4]. This phenomenon is observed when cells having two particular mutations may arise in a fixed-size population even in the absence of an intermediate state in which cells having only one mutation take over the population. The extreme case is represented by $u_1 << 1/N^2$, where all cells in a population must mutate before a single cell with double mutation appears. Mutation rates leading to the acquisition of first and second hits were the same.

The parameter r, defines the impact of mutations on cell proliferations properties. A mutation can be neutral ($r = 1$), increase ($r > 1$) or decrease ($r < 1$) proliferation abilities of the cell.

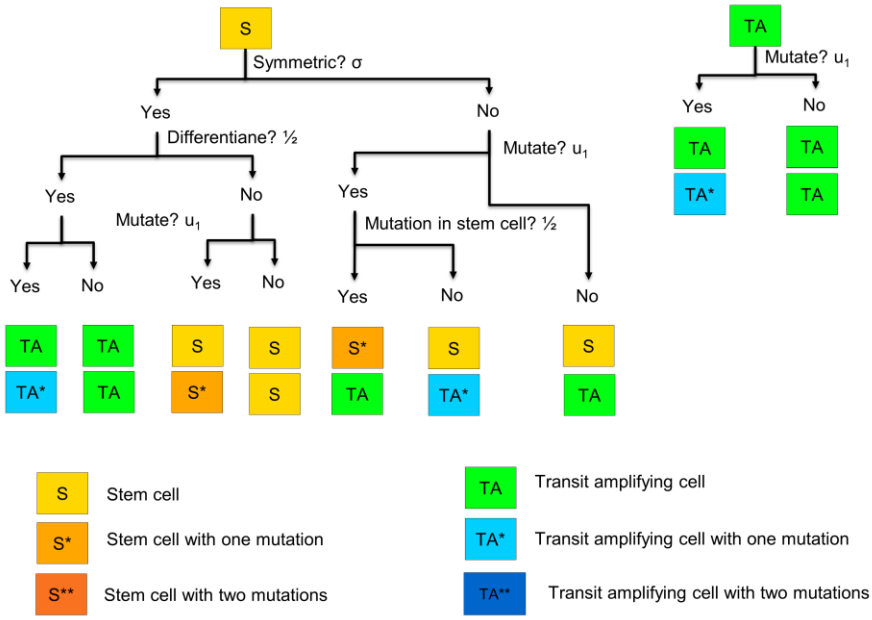

Figure 2. Division tree of wild-type stem cell and wild-type TA cell.

We have considered different values of probability of a symmetric cell division σ. In homeostatic systems, the fraction of self-renewal and differentiate symmetric cell divisions has to be balanced, to achieve this, we assumed that the probability of differentiation during symmetric division is equal to 0.5. This assumption was not sufficient to balance two populations of cells. Both cell types may persist in the population only if an additional rule was introduced. We proposed two CA systems. The first model assumed that after symmetric division with differentiation the next symmetric division is self-renewal. This helped to keep the stem cell population constant, nevertheless SCs migrated from one place to another. This was not consistent with our knowledge about colon crypt structure. SCs usually stay at the bottom of the crypt. The second model assumed that cells located in first and second row can only divide symmetrically and cells which are located in third and next rows can differentiate. All parameters are explained in Table 1.

Table 1. Description of parameters used in model

Parameter	Description
σ	Probability of a symmetric cell division
r	Impact of mutation on cell proliferations properties
u_1	Mutation rates leading to the acquisition of first and second hits
N	Total population size

To imitate the cylinder structure of the colon crypt we defined periodic left and right boundaries. Cells can not migrate from top to bottom and otherwise.

All simulations were run with initial conditions presented on Fig 3A. During every interaction one randomly chosen TA cell died (Fig. 3B) creating an empty spot. One randomly chosen cell from the neighbourhood was chosen to divide on empty spot (Fig. 3C). Stem cells are considered to be immortal. We analysed two types of neighbourhood: Moore (four neighbours) and von Neuman (eight neighbours) [1].

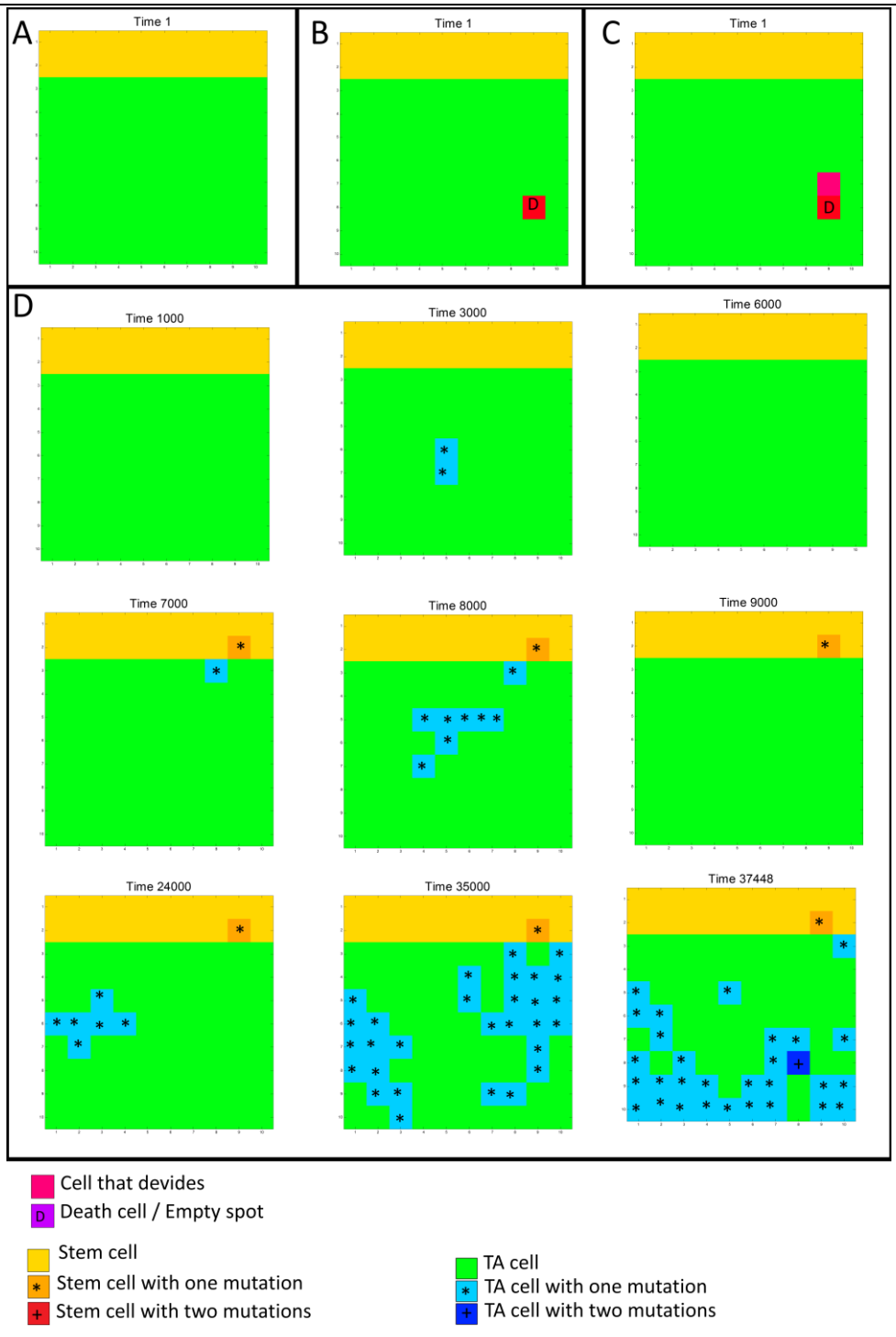

Figure 3. (A) Initial condition of cellular automaton. (B-C). Mechanism of dead cell replacement. (D). Example of simulation results for asymmetric division.

RESULTS AND DISCUSSION

We implemented two models, in the first one SCs migration was possible, in the second cells stayed in their compartments. Results obtained from both models were similar. We also analysed two possible neighbourhoods types, but they do not influence the model dynamics. The results shown in this paper were obtained for the compartmental model with von Neuman neighbourhood.

Firstly, we analysed differences in population dynamics when only one division type for cells was possible ($\sigma = 0$ or $\sigma = 1$). An example of simulation for asymmetric case is presented on Fig. 3D.

The most important observation is that under the assumption of asymmetric division mutations in stem cells persist in whole population. Once a mutation appears in SC cell, it will not disappeared from the SC subpopulation. On the other hand, during symmetric division, stem cells with mutation can differentiate and both mortal progenies can die before they mutate again. In both cases some subpopulations of mutated TA cells appeared and disappeared, but they never dominate the whole system.

Secondly, we calculated the probability of occurrence of double-hit mutant occurred in a population. Our results are consistent with the non-spatial model [9]. They suggest that symmetric division might have a cancer-delaying effect, especially, when mutations result in slower proliferation. Nevertheless, for mutations which cause higher proliferations abilities (r >1.3) for both method of division we have obtained the same probability (Fig. 4A).

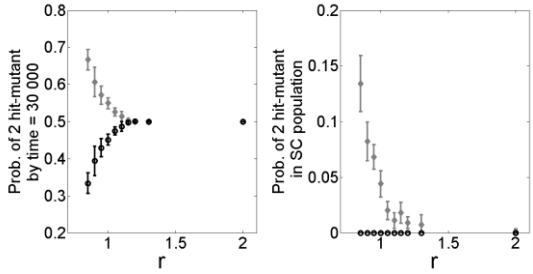

Figure 4 (A) Probability of double-hit mutant occurred in SC or TA cells populations. (B) Percentage of double-hit mutant in SC population.
Black circles – symmetric division, grey diamonds – asymmetric division.

We would like to analyse our results in the context of stem cancer cells (SCC). A controversial SCC hypothesis says that cancer is maintained by small fraction of cells with stem-like properties. The theory does not explain the source of these specific cells, one of the possibilities is that double-hit mutation occurred in a normal stem cell modifies it to become cancerogenous, but the "stemness" properties remain. The stem cell-like cancer cells localised in colon crypt were discovered and analysed. They expressed intestinal stem cells markers and display multipotency, as well as the ability to self-renew [10].

Results from our model suggest that double-hit mutants in SCs occur only when cell divides asymmetrically and the fraction of mutated SCs depends on mutation properties. If we assume that only this mutant can lead to further cancerogenic transformation this would suggest that first mutation of tumor suppressor gene deregulate cells by slowing down the proliferation (Fig 4B).

During the next step of our research we focused on neutral mutations ($r = 0$). We ran 15 000 simulations to find what division strategy results in the longest time before double-hit mutant appears in a population. Surprisingly we discovered that it is a combination of symmetric and asymmetric divisions (Fig. 5). These results suggest that both strategies might be necessary to delay cancerogenesis. This could explain why both strategies are observed in the tissue

also supported by the finding that individual stem cell have ability to switch between both division strategies [6].

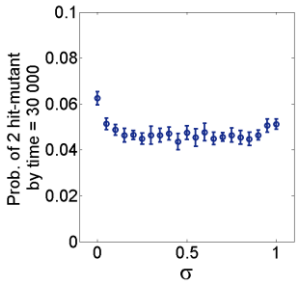

Fig 5. The probability of 2-hit mutant generation as a function of σ, the probability of symmetric stem cell divisions.

CONCLUSIONS

The results obtained after including spatial effects in the model differ from Moran model analysed in [9]. There the conclusion was that if cell divide symmetrically then it takes more time to develop cancer. Our results suggest, that a combination of both division types delay the process of cancerogenesis even more.

Our simple model includes only important behaviour of stem and TA cells. In future work we would like to look at non-constant populations, division rates at various heights in epithelial tissues, migration of cells, replenishing after catastrophic cell death, other fitness rates, and more types of mutations.

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