Study of Cancer Hallmarks Relevance Using a Cellular Automaton Tumor Growth Model

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Abstract. We studied the relative importance of the different cancer hallmarks in tumor growth in a multicellular system. Tumor growth was modeled with a cellular automaton which determines cell mitotic and apoptotic behaviors. These behaviors depend on the cancer hallmarks acquired in each cell as consequence of mutations. Additionally, these hallmarks are associated with a series of parameters, and depending on their values and the activation of the hallmarks in each of the cells, the system can evolve to different dynamics. Here we focus on the relevance of each hallmark in the progression of the first avascular phase of tumor growth and in representative situations.

1 Introduction and Previous Work

Cancer is a disease which arises from mutations in single somatic cells. These mutations alter the proliferation control of the cells which leads to uncontrolled cell division, forming a neoplastic lesion that may be invasive (carcinoma) or benign (adenoma). These two properties are in turn driven by what mutations the cells have acquired. In the invasive case the tumor grows in an uncontrolled manner up to a size of approximately 10^6 cells [4]. At this size the diffusion driven nutrient supply of the tumor becomes insufficient and the tumor must initiate new capillary growth (angiogenesis). When the tumor has been vascularized the tumor can grow further and at this stage metastases are often observed.

Although there are more than 200 different types of cancer that can affect every organ in the body, they share certain features. Thus, Hanahan and Weinberg described the phenotypic differences between healthy and cancer cells in a landmark article entitled "The Hallmarks of Cancer" [7]. The six essential alterations in cell physiology that collectively dictate malignant growth are: self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. In a recent update [8] the authors included two more hallmarks: reprogramming of energy metabolism and evasion of immune destruction, that emerged as critical capabilities of cancer cells. Moreover, the authors described two enabling characteristics or properties of neoplastic cells that facilitate acquisition of hallmark capabilities: genome instability and tumor-promoting inflammation (mediated by immune system cells recruited to the tumor site).

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In Artificial Life terms [10], tumor growth in multicellular systems is an example of emergent behavior, which is present in systems whose elements interact locally, providing global behavior which is not possible to explain from the behavior of a single element, but rather from the "emergent" consequence among the interactions of the group. In this case, it is an emergent consequence of the local interactions between the cells and their environment. Emergent behavior was studied in Artificial Life using models like Cellular Automata (CA) and Lindenmayer Systems [9][10]. As indicated by Ilachinski [9], CAs have been the focus of attention because of their ability to generate a rich spectrum of complex behavior patterns out of sets of relatively simple underlying rules and they appeared to capture many essential features of complex self-organizing cooperative behavior observed in real systems.

One of the traditional approaches to model cancer growth was the use of differential equations to describe avascular, and indeed vascular, tumor growth. CA approaches make easy the modeling at cellular level, where the state of each cell is described by its local environment. Thus, different works have appeared which used the CA capabilities for different purposes in tumor growth modeling [11]. For example, Bankhead and Heckendorn [2] used a CA which incorporated a simplified genetic regulatory network simulation to control cell behavior and predict cancer etiology. Ribba et al. [12] used a hybrid CA which combined discrete and continuous fields, as it incorporated nutrient and drug spatial distribution together with a simple simulation of the vascular system in a 2D lattice model, and with the aim of assessing chemotherapy treatment for non-Hodgkin's lymphoma. In the CA model of Gerlee and Anderson [4] each cell was equipped with a micro-environment response network (modeled with a neural network), that determined the behavior of the cell based on the local environment. Their focus was on the analysis of tumor morphologies under different conditions like oxygen concentration. Gevertz et al. [5] used a CA model to study the impact that organ-imposed physical confinement and heterogeneity have on tumor growth, that is, to incorporate the effects of tissue shape and structure.

Previous works have used CA models based on the presence of the hallmarks. For example, Abbott et al. [1] investigated the dynamics and interactions of the hallmarks in a CA model in which the main interest of the authors was to describe the likely sequences of precancerous mutations or pathways that end in cancer. They were interested in the relative frequency of different mutational pathways (what sequences of mutations are most likely), how long the different pathways take, and the dependence of pathways on various parameters associated with the hallmarks. In the work of Basanta et al. [3], a 2D cellular automaton modeled key cancer cell capabilities based on the Hanahan and Weinberg hallmarks. The authors focused their work on analyzing the effect of different environmental conditions on the sequence of acquisition of phenotypic traits and tumor expansion. Their results indicated that microenvironmental factors such as the local concentration of oxygen or nutrients and cell overcrowding may determine the expansion of the tumor colony.

We also used a CA model which determines the behavior of cells based on the Hanahan and Weinberg hallmarks. Nevertheless, our aim is different, as our simulation tries to determine the dependence of the cellular system behavior, at cellular level, on the presence of the different cancer cell hallmarks and their key defining parameters. We focused here on the dependence of the emergent tumor growth behavior on each individual hallmark, studying their relative importance in tumor development in the first avascular phase. These dependences are difficult to foresee without a model and associated simulating tool.

As indicated recently by Hanahan and Weinberg [8], in addition to providing a solid basis for cancer research, the hallmarks have served to identify certain cell functions that have become therapeutic targets. However, the utility of such attempts has been limited because tumor cells have demonstrated an ability to develop resistance to drugs that disrupt a single pathway. This adaptability of cancer cells suggests to Hanahan and Weinberg that simultaneous targeting of two or more hallmark pathways may be a more effective approach to therapy. So, our study can help to discern what are such most relevant hallmarks which can be targeted and in each multicellular system situation.

2 Methods for the Cellular System Modeling

2.1 Cancer Hallmarks

In the simulation each cell resides in a site in a cubic lattice and has a "genome" associated with different cancer hallmarks. The essential alterations in cell physiology that collectively dictate malignant growth are [6][7]:

SG. Self-Growth: Growth even in the absence of normal "go" signals. Most normal cells wait for an external message (growth signals from other cells) before dividing. Cancer cells often counterfeit their own pro-growth messages.

IGI. Ignore Growth Inhibit: As the tumor expands, it squeezes adjacent tissue, which sends out chemical messages that would normally bring cell division to a halt. Malignant cells ignore the commands, proliferating despite anti-growth signals issued by neighboring cells.

EA. Evasion of apoptosis: In healthy cells, genetic damage above a critical level usually activates a suicide program (programmed cell death or apoptosis). Cancer cells bypass this mechanism.

AG. Ability to stimulate blood vessel construction: Tumors need oxygen and nutrients to survive. They obtain them by co-opting nearby blood vessels to form new branches that run throughout the growing mass (angiogenesis).

EI. Effective immortality: Healthy cells can divide no more than several times (< 100). The limited replicative potential arises because, with the duplication, there is a loss of base pairs in the telomeres (chromosomes ends which protect the bases), so when the DNA is unprotected, the cell dies. Malignant cells overproduce the telomerase enzyme, avoiding the telomere shorthening, so such cells overcome the reproductive limit.

Table 1. Definition of the parameters associated with the hallmarks

Parameter name	*Default value*	*Description*
Telomere length (tl)	100	Initial telomere length in each cell. Every time a cell divides, the lenght is shortened by one unit. When it reaches 0, the cell dies, unless the "Effective immortality" hallmark (EI) is ON.
Evade apoptosis (e)	10	A cell with n hallmarks mutated has an extra n/e likelihood of dying each cell cycle, unless the "Evade apoptosis" hallmark (EA) is ON.
Base mutation rate (m)	100000	Each gene (hallmark) is mutated (when the cell divides) with a $1/m$ chance of mutation.
Genetic instability (i)	100	There is an increase of the base mutation rate by a factor of i for cells with this mutation (GI).
Ignore growth inhibit (g)	10	As in [1], cells with the hallmark "Ignore growth inhibit" (IGI) activated have a probability $1/g$ of killing off a neighbor to make room for mitosis.
Random cell death (a)	1000	In each cell cycle every cell has a $1/a$ chance of death from several causes.

MT. Power to invade other tissues and spread to other organs: Cancers usually become life-threatening only after they somehow disable the cellular circuitry that confines them to a specific part of the organ in which they arose. New growths appear and eventually interfere with vital systems.

GI. Genetic instability: It accounts for the high incidence of mutations in cancer cells, allowing rapid accumulation of genetic damage. It is an enabling characteristic of cancer [8] since, while not necessary in the progression from neoplasm to cancer, makes such progression much more likely [3]. The simulation implies that the cells with this factor will increase their mutation rate.

2.2 Event Model

In our modeling, each cell genome indicates if any hallmark is activated as consequence of mutations. Metastasis and angiogenesis are not considered, as we are interested in this work in the first avascular phases of tumorigenesis. So, every cell has its genome which consists in five hallmarks plus some parameters particular to each cell. All the parameters are commented in Table 1. The parameters *telomere length* and *base mutation rate* can change their values in a particular cell over time, as explained in the table. The cell's genome is inherited by the daughter cells when a mitotic division occurs. The default values indicated in Table 1 are the same as those used in [1]. Also, Basanta et al. [3] worked with parameters, such as base mutation rate (10^{-5}) and mutation rate increase for cells with acquired genetic instability ($i = 100$), with the same default values.

In the simulation of the cell life cycle, most elements do not change observably each time step. The only observable changes to cells are apoptosis and mitosis. In a tissue, only a fraction of all cells are undergoing such transitions at any

given time. We used an event model, similar to that used by Abbott et al. [1], summarized in Algorithm 2.1 and which takes into account the main aspects of the cell cycle from the application point of view. A mitosis is scheduled several times in the future, being a random variable distributed uniformly between 5 and 10 time steps, simulating the variable duration of the cell life cycle (between 15 and 24 hours). Finally, a grid with 10^6 sites represents approximately 0.1 mm^3 of tissue.

Algorithm 2.1. EVENT MODEL FOR CANCER SIMULATION()

```
t ← 0    // Simulation time. Initial cell at the center of the grid.
SCHEDULE A MITOTIC EVENT(5, 10) // Schedule a mitotic event with a random time
        //  (ts) between 5 and 10 time instants in the future (t+ts). The events
        // are stored in an event queue. The events are ordered on event time.
while event in the event queue
do { POP EVENT( ) // Pop event with the highest priority (the nearest in time).
     t ← t of popped event
     RANDOM CELL DEATH TEST( ) // The cell can die with a given probability.
     GENETIC DAMAGE TEST( ) // The larger the number of hallmark mutations,
                            // the greater the probability of cell death. If
                          // "Evade apoptosis" (EA) is ON, death is not applied.
     MITOSIS TESTS( ) :
       GROWTH FACTOR CHECKING( ) // cells can perform divisions only
         // if they are within a predefined spatial boundary which sufficient
         // growth factor; beyond this area cells cannot perform mitosis,
         // unless the hallmark "Self-growth" (SG) is ON.
       IGNORE GROWTH INHIBIT CHECKING( ) // If there are not empty cells in
         // the neighborhood, the cell cannot perform a mitotic division. If the
         // "Ignore growth inhibit" hallmark (IGI) is ON, then the cell competes
         // for survival with a neighbor cell and with a likelihood of success.
       LIMITLESS REPLICATIVE POTENTIAL CHECKING( )// If the telomere length
         // is 0, the cell dies, unless the hallmark "Effective immortality"
         // (Limitless replicative potential, EI) is mutated (ON).
     if the three tests indicate possibility of mitosis
       then
       PERFORM MITOSIS( ) :
         // Increase the base mutation rate if genetic instability (GI) is ON.
         // Add mutations to the new cells according to base mutation rate(1/m).
         // Decrease telomere length in both cells.
       PUSH EVENTS( )
         // Schedule mitotic events (push in event queue) for both cells:
         // Mother and daughter, with the random times in the future.
       else PUSH EVENT( )
         // Schedule a mitotic event (in queue) for mother cell.
   }
```

The simulation begins by initializing all elements of the grid to represent empty space. Then, the element at the center of the grid is changed to represent a single normal cell (no mutations). Mitosis is scheduled for this initial cell. After the new daughter cells are created, mitosis is scheduled for each of them, and so on. Each mitotic division is carried out by copying the genetic information

(the hallmark status and associated parameters) of the cell to an unoccupied adjacent space in the grid. Random errors occur in this copying process, so some hallmarks can be activated, taking into account that once a hallmark is activated in a cell, it will be never repaired by another mutation [1].

Frequently, cells are unable to replicate because of some limitation, such as contact inhibition or insufficient growth signal. Cells overcome these limitations through mutations in the hallmarks. Regarding hallmark *self-growth* (SG), as in [1] and [3], cells can perform divisions only if they are within a predefined spatial boundary, which represents a threshold in the concentration of growth factor; beyond this area (95% of the inner space in each dimension, which represents 85.7% of the 3D grid inner space) growth signals are too faint to prompt mitosis (unless hallmark SG is ON). Moreover, cells undergo random cell death with low probability ($1/a$ chance of death, where a is a tunable parameter).

So, our model corresponds to an "on-lattice model" as called by Rejniak et al. [11], where the model is constrained by a cubic lattice structure that defines the locations of cells and cell-cell interaction neighborhods, although there are other models that describe the spatial and morphological features of cancer development in a more biologically plausible way like the Cellular Potts or the Voronoi diagram-based off-lattice models [11].

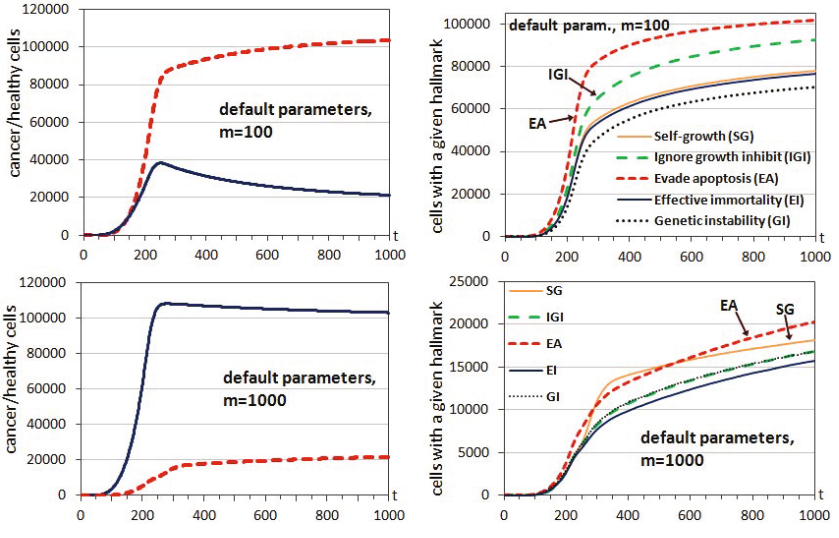

Fig. 1. Left: Evolution through time iterations of the number of healthy cells (continuous lines) and cancer cells (dashed lines) for different base mutation rates ($1/m$) and default parameters. Right: Evolution of the number of cells with a hallmark acquired.

3 Results

3.1 Simulations with Different Hallmark Parameters

First, we run several simulations with representative hallmark parameters. Figure 1 shows the evolution over time of the number of healthy and cancer cells for two

different values of the parameter m, which defines the base mutation rate, maintaining the rest of the parameters in their default values and using the same grid size (125000) employed in [1]. The number of time iterations was 1000 in the different runs. Given the stochastic nature of the problem, the graphs are always an average of 5 different runs. A cell was considered as cancerous if any of the hallmarks was present. As expected, with increasing base mutation rate ($1/m$), the increase in cancer cells becomes faster. For lower values of the base mutation rate it is difficult to obtain rapid cancer progression, so we selected those two high values.

The right part of Figure 1 shows the time evolution of the cells with a given hallmark and such standard parameters. Despite the rapid and initial cancer cell progression, with $m = 100$, two hallmarks present an advantage for cancer cell proliferation: *evade apoptosis* (EA) and *ignore growth inhibit* (IGI). The first one dominates in the cancer cell population because, as there are many mutations in the cells, the apoptosis mechanism eliminates many of the mutated cells, except those that have the hallmark EA acquired, which escape such control so they proliferate in the cell population. The second hallmark is necessary when the space is full, because in this situation there are no vacant sites for cell proliferation, except for those with hallmark IGI acquired (the free space limitation can be ignored by such cells). Using a lower base mutation rate ($m = 1000$), the hallmark *self-growth* SG is relatively more predominant than IGI, as cells with SG acquired proliferate rapidly when the cells have reached the limits of the area filled with growth factor. Remember that these hallmarks, that allow the cells to escape those limits, are acquired by the offspring, so the daughters can continue proliferating.

In Figure 2 we repeated the simulations but using a parameter set that facilitates the appearance of cancer cells. We selected values as the ones used by Abbott et al. [1] ($m = 100000$, $tl = 35$, $e = 20$, $i = 100$, $g = 4$, $a = 400$ and a grid size of 125000) for the determination of possible mutational pathways, that is, the sequence of appearance of hallmarks that end in a tumor growth. For example, the lower value of tl implies fewer mitoses in healthy cells, and the lower value of a facilitates that more vacant sites are available for cancer cells to propagate, in connection with the higher probability of replacing neighbors when making room for mitosis (lower value of g).

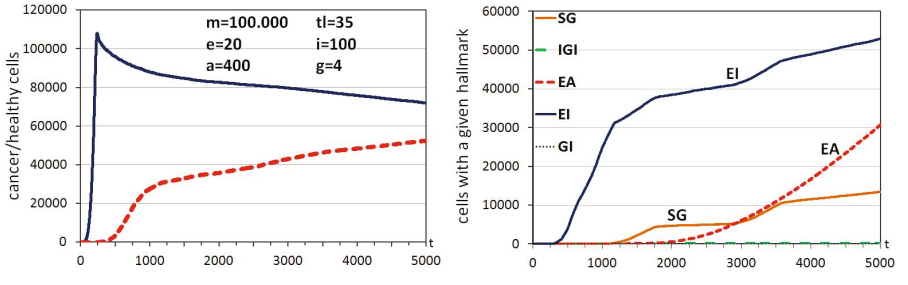

Fig. 2. Left: Time evolution of the number of healthy cells (continuous line) and cancer cells (dashed line) with a parameter set which facilitates cancer growth. Right: Time evolution of the number of cells with a hallmark acquired. All the graphs are an average of 5 independent runs.

The right part of Figure 2 shows the time evolution of the cells with a given hallmark and such parameter set. The dominant hallmark in the tumor growth is now *effective immortality* (EI), allowing the progression of the cells with such mutation even when the telomere length reaches its limit. Such cells have a clear advantage with respect to the other cells, which die after the maximum number of 35 divisions. This explains the rapid proliferation of the hallmark EI, before iteration 1000, when the healthy cells have performed their maximum number of mitotic divisions. Figure 3 shows snapshots at different time states of the multicellular system in a run with such parameters. In this case, we used a grid size of 10^6, for a better visualization of the tumor progression. These snapshots show again how EI is the dominant hallmark in such conditions (green color cells in Figure 3 have hallmark EI acquired).

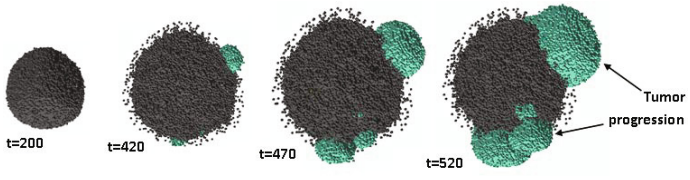

Fig. 3. Snapshots at different time steps using the parameters of Figure 2

3.2 Relevance of Hallmarks

Our aim is to inspect the relative importance of each hallmark in the emergent behavior of tumor growth. To answer this, we can analyze the growth behavior when the individual hallmarks are not present or do not imply any effect on the cellular behavior. This is the same as considering that mutations do not activate a particular hallmark. We selected two of the previous representative cases to study the effect of not considering the individual hallmarks, that is, to inspect the relative importance of each hallmark in the cancer growth behavior. First, Figure 4 (Left part) shows the evolution across time iterations of the number of cancer cells (grid size=125000), using the default parameters with $m = 100$, when all the hallmarks are considered (previously shown in Fig. 1), and when a particular hallmark is not taken into account in the rules of apoptotic and mitotic behaviors. As seen in Figure 4, the most important hallmark regarding the growth of cancer cells is *evade apoptosis* (EA), since its elimination implies a high decrease in the number of cancer cells. This is because, without the consideration of EA, all the cancer cells have a probability of death by apoptosis, so cancer cell proliferation is highly decreased.

The next most important hallmark is *ignore growth inhibit* (IGI), since its elimination implies also an important decrease in the number of cancer cells. This is because when the grid is almost full of healthy or cancer cells, after time iteration 200, the main limit for the mitotic divisions is the available free space. In this situation, the cancer cells with the hallmark IGI activated have an advantage, as they can replace (with a given probability) a neighbor cell to replicate. So, if this advantage does not exist when hallmark IGI is not considered, the cancer cells tend

to remain stable in number, even with this very high base mutation rate $(1/m)$. A hallmark with similar relevance is *genetic instability* (GI), as without its consideration there are fewer mutations or acquisition of hallmarks. The previous effects are not present with the elimination in the simulation of the other hallmarks, as it implies a smaller decrease in the number of cancer cells.

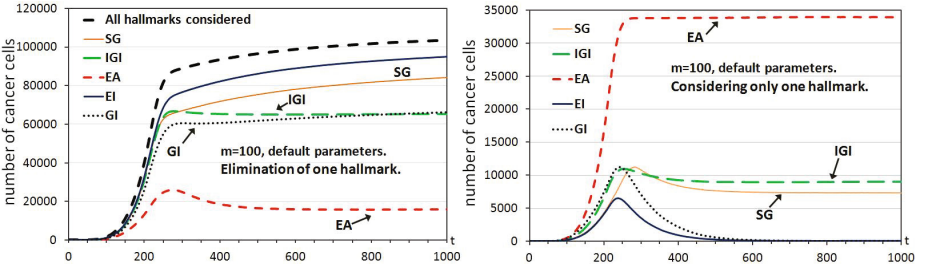

Fig. 4. Left: Effect of elimination of an individual hallmark. Right: Number of cancer cells when only one hallmark is considered. Simulations with parameter default values and $m = 100$, averaged with 5 independent runs.

The right part of Figure 4 shows the same evolution when only one particular hallmark is considered. As the Figure denotes, hallmarks EA and IGI are again the most relevant, and because the same reasons exposed. Note that now, when only *genetic instability* (GI) is considered, the number of cancer cells with only such a mutation cannot growth across time iterations. This is because GI only increments the mutations in such cells for the acquisition of the other hallmarks that have a possible effect on the proliferation of cancer cells. Note also the difference between the hallmark relevance and the number of cells with a given hallmark (Fig. 1), since the relative relevance between EA and other hallmarks is not reflected in Figure 1.

In Figure 5 we repeated the same analysis with the parameter set previously used in Fig. 2, which facilitates the appearance of cancer cells. As the Figure shows,

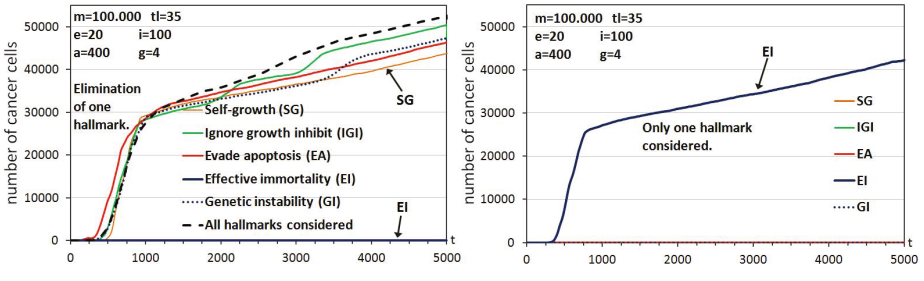

Fig. 5. Number of cancer cells when an individual hallmark is not considered (Left) and when only one hallmark is considered (Right). Simulations with parameter values of Figure 2, averaged with 5 independent runs.

when we do not consider the hallmark *effective immortality* (EI) in the simulation, the number of cancer cells is maintained to a minimum (close to 0, dark blue line). This is because, in this case, the great advantage of the limitless replicative potential is never present, so all cells have the same limit of replications imposed by the initial telomere length. The other hallmarks do not have relevance except the low relevance of *self-growth* (SG), as not considering it eliminates the final possible progression of cancer cells in the area without growth factor.

4 Conclusions

We used a cellular automaton model to simulate tumor growth at cellular level, based on the cancer hallmarks acquired in each cell. We focused here on the relevance or relative importance of the different hallmarks in the avascular tumor progression. The experimentation performed showed that the effect of elimination of hallmarks is different depending on the main advantage of cancer cells to propagate. With high mutation rates, the most relevant hallmark is *evade apoptosis*. If the space is full of cells, a relevant hallmark is *ignore growth inhibit*, as it allows cancer cell proliferation when there is no available free space. When the cells have reached the proliferation limit imposed by the telomeres, then the most important hallmark for cancer proliferation is *effective immortality*, given its advantage with respect to cells without it in such stage. So, the simulations can help to analyze what are the most relevant hallmarks which can be targeted and in each multicellular system situation.

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