HHS Public Access

Author manuscript

Wiley Interdiscip Rev Syst Biol Med. Author manuscript; available in PMC 2021 September 01.

Published in final edited form as:

Wiley Interdiscip Rev Syst Biol Med. 2020 September ; 12(5): e1487. doi:10.1002/wsbm.1487.

Aspirin and the chemoprevention of cancers: a mathematical and evolutionary dynamics perspective

Natalia L. Komarova,
Department of Mathematics, University of California Irvine, Irvine, CA 92697

C. Richard Boland,
Department of Medicine, UCSD School of Medicine, San Diego, CA

Ajay Goel,
Department of Molecular Diagnostics and Experimental Therapeutics, Beckman Research Institute, City of Hope Comprehensive Cancer Center 1218 S. Fifth Avenue, Suite 2226 Biomedical Research Center Monrovia, CA 91016.

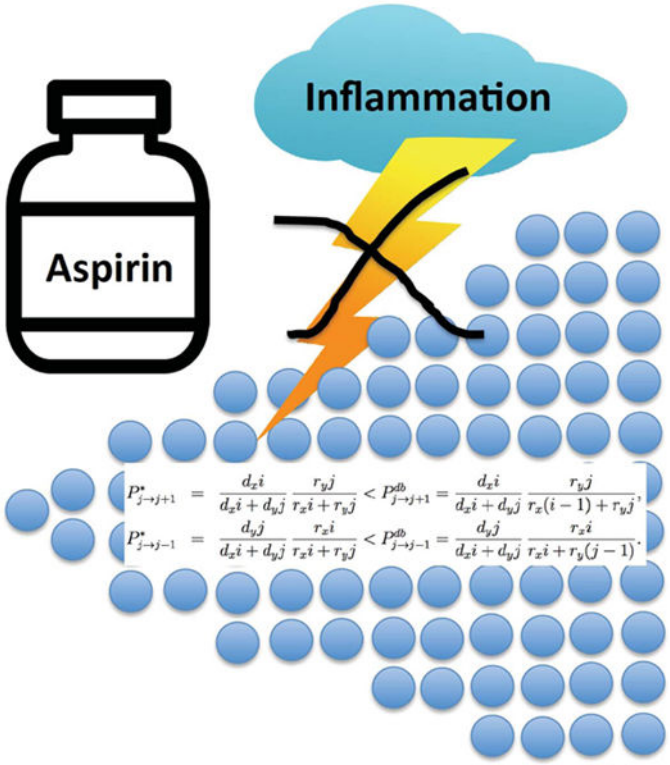
Dominik Wodarz
Department of Population Health and Disease Prevention, Program in Public Health, Susan and Henry Samueli College of Health Sciences, University of California, Irvine, CA 92697.

Abstract

Epidemiological data indicate that long-term low dose aspirin administration has a protective effect against the occurrence of colorectal cancer, both in sporadic and in hereditary forms of the disease. The mechanisms underlying this protective effect, however, are incompletely understood. The molecular events that lead to protection have been partly defined, but remain to be fully characterized. So far, however, approaches based on evolutionary dynamics have not been discussed much, but can potentially offer important insights. The aim of this review is to highlight this line of investigation and the results that have been obtained. A core observation in this respect is that aspirin has a direct negative impact on the growth dynamics of the cells, by influencing the kinetics of tumor cell division and death. We discuss the application of mathematical models to experimental data to quantify these parameter changes. We then describe further mathematical models that have been used to explore how these aspirin-mediated changes in kinetic parameters influence the probability of successful colony growth vs extinction, and how they affect the evolution of the tumor during aspirin administration. Finally, we discuss mathematical models that have been used to investigate the selective forces that can lead to the rise of mismatch-repair deficient cells in an inflammatory environment, and how this selection can be potentially altered through aspirin-mediated interventions.

Graphical Abstract

* komarova@uci.edu.



1. INTRODUCTION

The treatment of select cancers has seen transformational changes in recent years, resulting in significant improvement of patient survival. Prominent examples are the development of targeted therapies against leukemias using tyrosine kinase inhibitors [1], or the immunotherapeutic approaches against melanoma and other cancers [2]. Nevertheless, in most cases, the disease eventually relapses and progresses towards a fatal outcome. Therefore, cancer prevention approaches remain one of the most impactful measures at reducing mortality [3]. The effect of nutrition, the environment, and exposure to carcinogenic substances on cancer incidence has been much discussed in the literature [4]. Another approach to reduce the chances of carcinogenesis is chemoprevention, and recently, the daily long-term administration of low-dose aspirin has received attention [5]. The incidence of different cancers has been shown to be reduced by long-term preventative treatment with aspirin [5–9], and colorectal cancer (CRC) has been a particular focus in this respect [10–16]. Aspirin has been shown to impact not only the incidence of sporadic CRC cases, but also that of Lynch Syndrome, which is characterized by a genetic predisposition to CRC due to mutations in mismatch repair genes. Despite the documented contribution of aspirin to reducing CRC incidence, the epidemiological patterns are complex.

In epidemiological data from the CAPP2 trial [12], the outcome of aspirin chemoprevention depended on the details of the clinical protocol, and on the nature of the patient cohort. In patients with average risk of sporadic CRC development, a sizable reduction in CRC incidence has ben documented, with the effect depending on the dose and duration of

treatment [14,15]. In patients who took aspirin for loner than 10 years, CRC incidence was reduced by 21–23%. For the highest aspirin dose (≥14 325mg aspirin tablets per week), however, incidence was reduced by up to 70% [14,15]. In patients with Lynch Syndrome who took 600mg of aspirin every day for a minimum duration of two years, the incidence of CRC was reduced by 63% [12]. To observe this result, however, patients had to be on aspirin on average for a duration of 29 months, and had to be followed up for 55 months. In the absence of such a relatively long follow-up, a significant reduction in tumor incidence (mostly adenomas) was not seen [17].

Besides studies that demonstrate a protective effect of aspirin on colorectal cancer incidence, however, there are also recent data that cast doubt on this effect [18,19]. The results from the CAPP2 trial outlined above, however, make it clear that the observed protective effect might require a certain minimum duration of treatment, and also a relatively long follow-up period. These insights not only make it complicated to interpret data from clinical trials, but also indicate a high level of complexity in the mechanisms that lead to cancer protection by aspirin.

The array of mechanisms underlying aspirin-mediated cancer prevention is not well understood [20,21]. Aspirin is known to reduce the degree of inflammation in vivo, and inflammation is a documented promoter of carcinogenesis [22], also in the case of CRC [23]. Reduction of inflammation might certainly be a key reason for the reduction in cancer incidence as a result of aspirin treatment. In addition, however, experiments have elucidated a direct impact of aspirin on cancer cells themselves, which can alter the dynamics of cellular growth and hence the time it takes for pathology to occur [20]. Cox-independent and dependent mechanisms have been implicated, but a more detailed picture of the molecular events that contribute to the observed effects of aspirin is still not available [20,21].

Molecular biology approaches have been instrumental in trying to decode the mechanisms in which aspirin reduces disease incidence. A different and complementary approach, however, has received much less attention: the evolutionary dynamics perspective. Carcinogenesis is a somatic evolutionary process in which cells obtain a variety of genetic as well as epigenetic changes, and these can render the affected cells advantageous, leading to their selection and rise. Therefore, processes which can interfere with clonal evolution can contribute to cancer prevention, and it is likely that to some extent, aspirin acts in this way. A core tool in the analysis of evolutionary processes are mathematical models and computer simulations [24]. Hence, there is great potential for additional insights that can arise from studying mathematical evolutionary models of cell populations in the presence of aspirin. This review summarizes some recent work on this topic, which is just beginning to be explored. We thereby concentrate on two focus areas: (i) the direct effect of aspirin on the cell growth and death kinetics, which can have an important impact on the probability to progress to measurable disease; (ii) the effect of inflammatory processes on the evolution of cancer cells, with special focus on the evolution of mutator phenotypes [25] that can drive carcinogenesis.

2. Effect of aspirin on basic cell growth kinetics

One of the basic effects of a chemoprevention method is its effect on cell growth kinetics, i.e. on the division and death rate of cells. This effect could have far-reaching consequences, due to its implications for the probability of colony extinction. The role of aspirin administration and aspirin dose for the cell division and death kinetics of colorectal cancer cell lines has been investigated in an in vitro setting [26]. Experiments were performed with eight CRC cell lines (HCT116, HCT116+Chr3/5, RKO, SW480, HCT15, Caco2, HT29, and SW48) with known mutational backgrounds [27–29]. They were allowed to grow exponentially in vitro over 108 hours. Temporal measurements were taken of the number of living and dead cells, and the cell cycle distribution of the cells over time was also determined. These experiments were performed with and without aspirin treatment, and different doses of aspirin were used (0.5, 1.0, 2.5, 5.0, and 10 mM). Kinetic parameters were measured for the different cell lines and conditions, by fitting a mathematical model to the data [26].

A minimally parameterized model was used, which was appropriate for the estimation of kinetic parameters in an exponentially growing population. The model is expressed by ordinary differential equations that describe the development of live and dead cells over time [26]:

$$\begin{aligned} \dot{x} &= (r-d)x, \\ \dot{y} &= dx - \alpha y, \end{aligned} \tag{1}$$

where x stands for the number of live cells, y for the number of dead cells, and the parameters are: r, the division rate of cells; d, the death rate of cells; and α, rate of dead cell removal. This model was fit to data, and the model parameters were determined. A significant negative correlation between the cellular growth rate and aspirin dose was found (Figure 1A). The majority of this negative correlation was accounted for by variation in the rate of cell division, r, which also displays a significant negative correlation with aspirin dose (Figure 1B). Altogether, cell death rates showed a significant positive correlation with aspirin dose, but the magnitude of this effect was less pronounced (Figure 1C). In addition, it turned out that the effect of aspirin on cell growth depends on the growth and division rates of the cell line in the absence of aspirin treatment (Figure 2). The faster the cells divide, the more pronounced the negative effect of aspirin treatment. Interestingly, this also correlates with the genetic makeup of the cell lines. In particular, *PIK3CA*-mutants were more sensitive to aspirin-mediated inhibition than wild-type cells (Figure 2). PI3Ks are important RAS-induced cell mediators of survival and proliferation [30]. The two effects were found to be related because *PIK3CA* mutational status correlated with the growth rate of the cells in the absence of aspirin. Cells with the *PIK3CA* mutation grew faster in the absence of aspirin than wild-type cells (Figure 2). Hence, the question arises whether the mutational status of cells directly influences the response to aspirin, or whether the rate of cell growth primarily drives treatment responses, with the PIK3CA mutation acting indirectly to promote the treatment response though elevated rates of cell division.

Correlations with other genetic markers were also investigated but none were found [26]. Importantly, the response of cells to aspirin was independent of the DNA mismatch repair (MMR) status of the cells. The cell lines that were exposed to aspirin included both MMR-proficient (CACO2, SW480, HT29 and HCT116+3+5) and MMR-deficient (SW48, HCT116, RKO and HCT15) cells lines.

3. Effect of aspirin on the probability of successful colony formation

Division and death rates are key parameters that determine the probability that one cell successfully gives rise to clonal growth, or conversely, that the colony goes extinct during its attempt to expand. The above-described parameter estimates are valuable because they give us a sense of the magnitude of the aspirin-induced change in parameter values. This information was used in the context of a stochastic birth-death process to examine to what extent the observed magnitude of parameter changes can impact the probability of successful tumor cell colony formation [31]. This in turn can be related to the magnitude of the protective effect provided by aspirin: the higher the probability that the colony will go extinct, the smaller the fraction of mutated or initiated cells that will give rise to disease.

When examining the probability that a single tumor cell yields an expanding colony of cells for different doses of aspirin, it is crucial to consider the ratio of the cellular division to death rate, R/D. This parameter influences both the growth and the evolutionary dynamics of the cell population. From the parameter estimates discussed above, it follows that this ratio significantly declines with aspirin dose (Figure 3A). From the model, we can calculate the probability that a single tumor cell successfully grows (rather than going extinct). This is given by 1-D/R [31]. This probability was found to decrease with aspirin dose [31], especially for higher aspirin doses (5mM and 10mM, Figure 3B). A 30% reduction in the probability to grow successfully (rather than going extinct) is observed for the highest aspirin dose, and the degree of reduction was found to be lower for smaller aspirin doses (Figure 3B), [31].

For cases where tumors do grow successfully, aspirin can impact the rate at which they increase, i.e. the time it takes the cells to reach a certain size. The average time it takes one cell to grow up to a size of 10^{10} cells (a detectable tumor) is given by $\ln(10^{10})/(R-D)$ [31]. As can be see in Figure 3C, this time is increased on average 4-fold for 10mM aspirin, while lower aspirin doses results in less pronounced delays (Figure 3C).

These results suggest that the impact of aspirin on the kinetic parameters of tumor growth alone can have a substantial impact on tumor incidence, either leading to a reduction of the fraction of spontaneous tumors that successfully grow and cause disease, or resulting in a delay of tumor growth towards a detectable disease burden. This assumes that there is a direct link between the generation (and non-extinction) of the first malignant tumor cell clone and the observed cancer incidence. In some cases this is likely to be true (e.g. in Lynch Syndrome), while in other cases, it is important to keep in mind that this relationship might be more complex.

4. Aspirin and the evolutionary potential of tumors

The following outlines a stochastic birth-death process that can be used to study evolutionary dynamics in the setting of exponential growth [31]. Wild type cells are assumed to divide with a rate R, and death occurs with a rate D. During a division event, cells can acquire a point mutation given by the probability u. This gives rise to a one-hit mutant with a defined fitness. The mutant can either be neutral, i.e. characterized by identical parameters compared to the wild-type, or advantageous/disadvantageous, i.e. characterized by higher or lower division rates relative to the wild-type. The same processes govern the dynamics of the one-hit mutant and they can in turn give rise to 2-hit mutants etc. The probability that one- , two-, or three-hit mutants exist once the tumor has reached a defined size can be obtained from a probability-generating function [31]. Another evolutionary measure that can be considered is the mean number of mutants.

First, we ask how many mutant cells exist on average by the time a tumor has grown to the threshold of detection, e.g. 10^{10} cells. This can be predicted by the stochastic birth-death process model outlined above, parameterized by the estimates of the division and death rates discussed in previous sections. According to the model, the number of mutants at the threshold size is determined by the number of cell divisions that occur during this growth process [32]. The higher the number of cell divisions, the larger the probability that a mutant is generated. According to Figure 4a, the number of cell divisions that are needed to reach 10^{10} cells is larger for higher aspirin doses. Therefore, aspirin chemoprevention leads to a larger mutagenic potential of tumors. While for neutral mutants, the relative increase in the number of mutant cells is the same as the relative increase in the number of cell divisions that occur until the size threshold has been reached (Figure 4A), the relative increase in mutant numbers is less extensive for disadvantageous mutants (Figure 4B), and more extensive for advantageous mutants (Figure 4C). Thus, overall, aspirin-mediated chemoprevention is predicted to increase the number of mutants observed at a given tumor population size, and this increase is predicted to be stronger for a larger relative mutant fitness

Next, we consider a different evolutionary measure, i.e. the probability that a mutant with one, two, three etc point mutations exist at the time when the tumor reaches a certain size [31]. This can be an important determinant of tumor responses to drug therapy. Particularly in the context of small molecule inhibitors, drug resistance can be induced by one point mutation [33]. Often, drug resistant mutants are thought to pre-exist before therapy is initiated. In this case, therapy results in the selection of these pre-existing mutants [34–36]. If only mutants resistant against 1 drug (e.g. 1 point mutation) pre-exist, treatment failure can be avoided by a combination of two drugs (this assumes absence of cross-resistance, see [37] for the expansion of this theory to the case of cross-resistant mutations). According to similar principles, cases where mutants pre-exist that are resistant to two drugs (as a result of two independent mutational events) can be successfully treated with a combination of three drugs, as long as triple resistant mutants are not present. As seen in Figure 5A, the chances that a one-hit mutant exists at a seize threshold is not significantly impacted by aspirin. In contrast, the chances that 2- or 3-hit mutants are present is significantly influenced by

aspirin (Figure 5B, C), with the impact being stronger for 3-hit than for 2-hit mutants (compare Figure 5B and C).

This set of results gave rise to a surprising insight [31]: Although mathematical modelling predicts that aspirin lowers the probability of successful colony formation, and hence reduces or delays cancer incidence, a tumor that does manage to arise during long-term aspirin administration has a higher evolutionary potential. Therefore, when comparing tumors of the same size, one that arose during aspirin administration and the other not, then the tumor that arose during aspirin administration is predicted to contain a larger mutational burden, and hence has a higher degree of virulence and more pre-existing therapy resistance.

According to this notion, a tradeoff is indicated between the administration of aspirin and the potential of the tumor to evolve [31]. If this prediction is epidemiologically verified, chemoprevention approaches should be guided by these results. If the onset of malignant cancer is delayed long enough by aspirin to avoid the emergence of a highly evolved tumor, the tradeoff would not present a problem. In any case, however, detailed monitoring of patients that are subject to chemoprevention would be advisable, such that when a tumor does arise, it is detected at a relatively small size, thus limiting the number of accumulated mutations upon detection. If the tumor is only detected later, at a larger size, treatment might be significantly more problematic due to the high predicted mutational burden. We emphasize that these theoretical notions still need to be investigated and confirmed epidemiologically.

5. In vivo dynamics

The above-described insights were based on experiments in which colorectal cancer cell lines have been grown in vitro. While this is a good system for the purpose of model parameterization, it is also the biologically least realistic setting. Similar experiments were performed in an in vivo setting, using colorectal cancer cell lines to grow mouse xenografts in the absence of aspirin, and in the presence of varying aspirin doses. To estimate model parameters, the 3D spatial nature of the growing xenografts had to be taken into account in the computational modelling. This can be achieved by a 3D agent-based model, but fitting such a model to data is associated with a number of challenges. An important characteristic of spatially restricted growth is that cells around the surface of the expanding mass contribute most to cell colony expansion (in contrast to cells that are located more centrally within the mass). Ordinary differential equations can be constructed to approximate this scenario. The volume of the tumor, V, is assumed to grow through its surface, S, at a rate given by the expected change in the cell number, calculated per cell, per division. We assume that n outermost layers of the tumor contribute to the expansion. Hence, the growth of the tumor over time is given by

$$\frac{dV}{dt} = (L - D)Sn$$

The quantities Sxn, as well as V, are measured in terms of individual cell volumes. S is related to V according to $S = 6^{2/3}\pi^{1/3}V^{2/3}$. Hence, we can re-write the above ODE as

$$\frac{dV}{dt} = n(L - D)6^{2/3}\pi^{1/3}V^{2/3}$$

This model was confirmed to be an accurate representation of the average growth dynamics of the 3D agent-based model, and fit the xenograft growth data well. The resulting parameter estimates and their dependence on aspirin dose are shown in Figure 6A. The results confirm the patterns that were observed *in vitro*. Thus, aspirin induced a dose-dependent reduction in the rate of cell division, and a dose-dependent increase it the death rate of cells. It was again investigated how the aspirin-induced parameter changes influenced the probability for a single tumor cell to successfully establish a growing colony (rather than going extinct). This analysis was performed in a more general way that went beyond the particular parameters that were measured for the xenografts. Hence, a number of different virtual cell lines were generated in the computer, characterized by different turnover rates, i.e. by different ratios of death rate / division rate, in the absence of aspirin. These parameters were then changed by the same amount as determined in the experiments using the highest aspirin dose, and the impact on the probability of successful colony formation was computationally determined. Consistent with the above-discussed models, aspirin treatment was predicted to reduce the probability of successful colony formation (Figure 6B). The extent of this reduction, however, depended on the turnover of the virtual cell population in the absence of aspirin. For low-turnover cases, the reduction was minimal, and the extent of the reduction increased significantly with higher turnover rates (Figure 6B). This could help us understand heterogeneity in protective effects exerted by aspirin in different tumor types. According to the model, significant protective effects are expected for higher turnover tumors, while aspirin is predicted to not have pronounced protective effects against low-turnover tumors.

6. Exploring potential indirect effects of aspirin

Aspirin is a drug that reduces the extent of inflammation in tissues. Inflammation is considered a key driver of carcinogenic processes, altering the cellular microenvironment such that cellular stress and DNA damage occurs more frequently. This can directly cause mutations that might contribute to carcinogenesis, and it might also result in altered selection pressures that favor cell clones with increased carcinogenic potential. One example of cells with increased carcinogenic potential might be clones characterized by genetic instability [38,39]. There can be large scale genetic instability [40], such as chromosomal instability, where cells lose whole chromosomes or chromosome fragments. Alternatively, smaller scale instabilities can be observed such as microsatellite instability, where point mutations in microsatellite and other sequences frequently occur due to defects in mismatch repair (MMR) mechanisms [41]. The emergence of MMR-deficient cells has been associated with inflammatory conditions [42]. For example, inflamed but non-neoplastic sections of the colon in patients with the inflammatory disease ulcerative colitis have a prevalence of MMR-deficient cells, which may predispose the tissue to the development of cancer [23]. The inflammatory conditions in the colon likely generate an environment in which MMR-deficient cells enjoy a selective advantage over MMR-competent cells, setting the scene for their expansion and the predisposition for malignant transformation. Therefore, there seems to be a strong connection between MMR deficiency (such as developed by Lynch syndrome

patients) on the one hand, and inflammation (and aspirin's anti-inflammatory properties) on the other, see e.g. the data from the CAPP2 trial [12], where aspirin was shown to play a role in cancer prevention in Lynch syndrome patients.

The mechanisms underlying the selection for and the rise of MMR-deficient cells in inflamed tissues are not fully understood, and theories have been suggested. The rate of growth is an important component of the fitness of cells. How fast the cell population grows is determined in part by how fast cells progress through the cell cycle. The cellular microenvironment can play an important role in modulating the rate of cell cycle progression. In particular, the acquisition of genomic damage or alterations can play a major role in this respect [43]. Damage of a cell can trigger cell cycle checkpoints (such as mismatch repair or MMR mechanisms), and this can lead to temporary cell cycle arrest. The arrest halts the cell cycle and gives the cell a window of opportunity to repair the damage, but comes with the cost that cell cycle progression and hence reproduction is delayed [44,45]. In this situation, mutants with a defect in such cell cycle checkpoints would avoid this delay in reproduction, and hence would enjoy a selective advantage [46]. It has been experimentally demonstrated that an intact MMR system mediates the G2/M cell cycle checkpoint, which is missing in cells with microsatellite instability and can be reversed by restoring the defective MMR gene [47,48]. Based on these observations, it has been suggested that the presence of frequent DNA damage can select for MMR-deficient cells, leading to their emergence [49]. The phrase "don't stop for repairs in a war zone" has been coined to describe this scenario [50–53]: In this analogy, cells are compared to race cars in an adverse environment in which they are shot at. One strategy for the race car is to stop every time it gets hit. The alternative strategy is to ignore the damage and keep driving. If the rate at which the cars are hit by bullets is high, the latter strategy was considered advantageous. While the car accumulates damage, it keeps driving, even if slowly. This car would still make more progress than a competitor who constantly stopped for repair. This is illustrated in Fig. 7A.

This theory makes intuitive sense, and in order to test it, a set of mathematical models has been designed to describe the selection of MMR-deficient cells in microenvironments that induce genetic damage [54]. Interestingly, the models revealed that these dynamics are more complex than initially thought. Consider two cell populations [54]: One cell population has the ability to enter cell cycle arrest upon damage, which we refer to as the "arresting cell population". The other cell population has lost this ability, and we refer to this as the "non-arresting cell population", see Fig. 7B. The model depicted in Fig. 7B simplified the cell cycle process in the sense that only two cell cycle stages are taken into account: Division occurs in stage 2. Cells that belong to this stage divide with a defined probability. Following division, the daughter cells enter stage 1, which encompasses all the cell cycle stages during which no division occurs. Cells in stage 1 have a chance to die, and a chance to progress to stage 2. For the non-arresting cell population, these are all the processes that occur. For the arresting cell population, however, cells enter an arresting cell population following cell division, which can be called "stage 0". Death does not occur during stage 0, because cells are assumed to undergo repair while in this stage. It is important to note that all cells either do or do not arrest following division, depending on whether they are classified as "arresting" or "non-arresting". Further, the model does not track the consequence mutations

that have been accumulating in “non-arresting” cells. These are obviously simplifications and represents an extreme scenario, but this model captures the essence of the “don’t stop for repair in a war zone” argument. A stochastic agent-based model was used to capture these assumptions, keeping track of individual cells [54]. N “spots” are considered, capturing the carrying capacity of the cells, in other words the maximal number of cells that the system can contain. A spot can have two states: empty of filled by a cell. During each update, a cell is chosen randomly from the whole system, and one of the processes outlined above will occur with defined probabilities. Upon division offspring cells are placed into a randomly chosen spot provided that it is empty. A time step in this model is defined by sampling as many times as there are cells in the system.

In this setting, the two cell populations compete with each other for space. Some interesting basic dynamics have been observed [54]. When the two cell populations are initially at low numbers, a phase of exponential growth is observed in which the growth of the the arresting cell population is slower than that of the non-arresting one (Figure 7(C)), which makes intuitive sense. When the populations fill up the space and converge towards equilibrium level, however, it turns out that they are competitively neutral, i.e. an infinite number of equilibria exist depending on initial conditions [54]. Hence, the lack of cell cycle arrest does not bestow a selective advantage on the non-arresting cell population, as has been argued previously [50–53].

A different evolutionary measure to determine whether a phenotype enjoys a selective advantage is its fixation probability. To study that, we place a single non-arresting cell into an arresting cell population at equilibrium. Interestingly, we observe [54] that the fixation probability of the non-arresting cell is lower than the fixation probability that is expected for a neutral mutant (Figure 8). The longer the duration of cell cycle arrest, the lower the fixation probability of the non-arresting cells becomes (Figure 8). Hence, when considering fixation probabilities, non-arresting cells that are placed into an arresting cell population have the properties of disadvantageous mutants: the longer the cell cycle arrest of the established population, the larger this disadvantage. This is a stark contrast to the “don’t stop for repair in a war zone” notion, i.e. that non-arresting cells are advantageous.

This leads us to ask the following question: What potential micro-environmental conditions can bestow a selective advantage to non-arresting cells? Mathematical models identified one possible mechanism that could render non-arresting cells advantageous [54]: the periodic disturbance of tissue homeostasis, i.e. a repeated reduction of the number of tissue cells below equilibrium levels. This can be brought about by the presence of inflammation, which, in addition to causing DNA alterations, can also result in cell death [55]. This can be captured by the same basic model described above, by adding the assumption that the total cell population size is periodically reduced to a certain extent (e.g. by 10%) see Figure 9A. Upon reduction of the cell population size, new growth can ensue in order to reconstitute the tissue. Due to the faster growth rate of non-arresting cells compared to arresting cells (Fig. 7C), each such disturbance enriches the relative abundance of the non-arresting cells. With many repeated disturbances, this can result in the dominance of the non-arresting cell population (Figure 9A).

Let us also examine these processes in the context of the fixation probability of the non-arresting cell population [54]. While we established above that the fixation probability of a non-arresting cell is lower than that expected for a neutral mutant, a different result is observed if the frequency of tissue disturbance is high enough: Now, the fixation probability for the non-arresting cell population grew to higher levels than the fixation probability for a neutral mutant (Figure 9B). That is, if tissue disturbance occurs frequently enough, the non-arresting cell population behaves like an advantageous mutant.

The above discussion assumed that a non-arresting and an arresting cell population competed with each other, and that the arresting population is constantly being hit by DNA-damaging agents that induce cell cycle arrest, i.e. it assumed a highly inflammatory environment. A more realistic model would assume that cell cycle arrest and repair do not always occur in a cell, but occur with a defined probability that is influenced by the amount of damage that the cell has been expose to. It has been shown that results remain robust under these more realistic assumptions [54].

In summary, this analysis has shown that the theoretical concept of a non-arresting mutant advantage (“don’t stop for repairs in a war zone hypothesis”) [50–53] does not work in the absence of additional assumptions. On the contrary, computational models suggest that non-arresting cells behave either selectively neutral or like a disadvantageous population, depending on the evolutionary measure under consideration. Additional mechanisms, such as periodic tissue disturbance, have to be invoked to explain the selection of MMR-deficient cells under inflammatory conditions. This theory can serve as a stepping-stone towards building more complex models of aspirin and its role in altering selection forces in the presence of MMR deficiencies. Future models may include more details of cells’ life cycle, spatial heterogeneity, and an explicit incorporation of immune responses.

7. Future Perspectives

This paper has provided an overview of insights about the mechanisms underlying aspirin-mediated chemoprevention of colorectal cancer, that have been obtained through the lens of evolutionary thinking and mathematical models. An important finding was that a direct effect of aspirin on the kinetics of cell division and cell death might be an important component that contributes to protection from disease. Aspirin reduces the overall tumor cell growth rate, driven mostly by a reduction in the rate of cell division, and to a lesser extent by an increase in the rate of cell death. These changes were found to have a significant effect on the probability for a single transformed cell to give rise to successful clonal expansion. Aspirin treatment could lead to an up to 30% reduction in the probability to grow successfully (rather than go extinct), which could certainly account for some of the protection observed in epidemiological data. If tumors do grow during long-term aspirin administration, however, the mathematical models further suggested that the expected mutational load in such tumors is larger than in tumors developed in the absence of aspirin. Hence, a tumor generated and grown during long-term aspirin usage might be more virulent and less treatable. This is an important model prediction that remains to be addressed experimentally.

A future research direction resulting from this work is a detailed exploration of how the changes in the tumor growth kinetics that are brought about by aspirin translate into a change in the age-incidence of colorectal cancer. Based on the documented magnitude of the aspirin-induced changes, in vivo models of colorectal carcinogenesis can be constructed and those models can be used to make predictions about age incidence. There is a rich literature on using stochastic models of carcinogenesis to make predictions about age-incidence curves [56–61]. An exploration of this kind will provide more quantitative insights into the extent to which the direct aspirin-induced changes in growth parameters can explain the epidemiological data.

More work also remains to be done to further investigate the role of inflammation and the microenvironment for the observed aspirin-mediated chemo-prevention. Mathematical models indicate that inflammation and the occurrence of elevated levels of DNA damage can influence the selection of MMR-deficient cells in complex ways, and these dynamics remain to be addressed experimentally. While molecular experimental approaches continue to develop the picture of how aspirin acts to prevent disease, a more explicit evolutionary approach would be highly beneficial [61,62]. Whether cancer cells grow uncontrolled or fail to rise above a certain level is determined by a complex interplay of factors that affect the fitness of different cell types. Understanding those factors, and the way in which aspirin alters this selective environment will be crucial to develop new treatment approaches, with the goal to guide the evolutionary trajectory of cells towards a non-disease state, a principles that has been discussed in the literature in different contexts [63].

Acknowledgments

Funding Information

This work was funded by NIH grant U01CA187956

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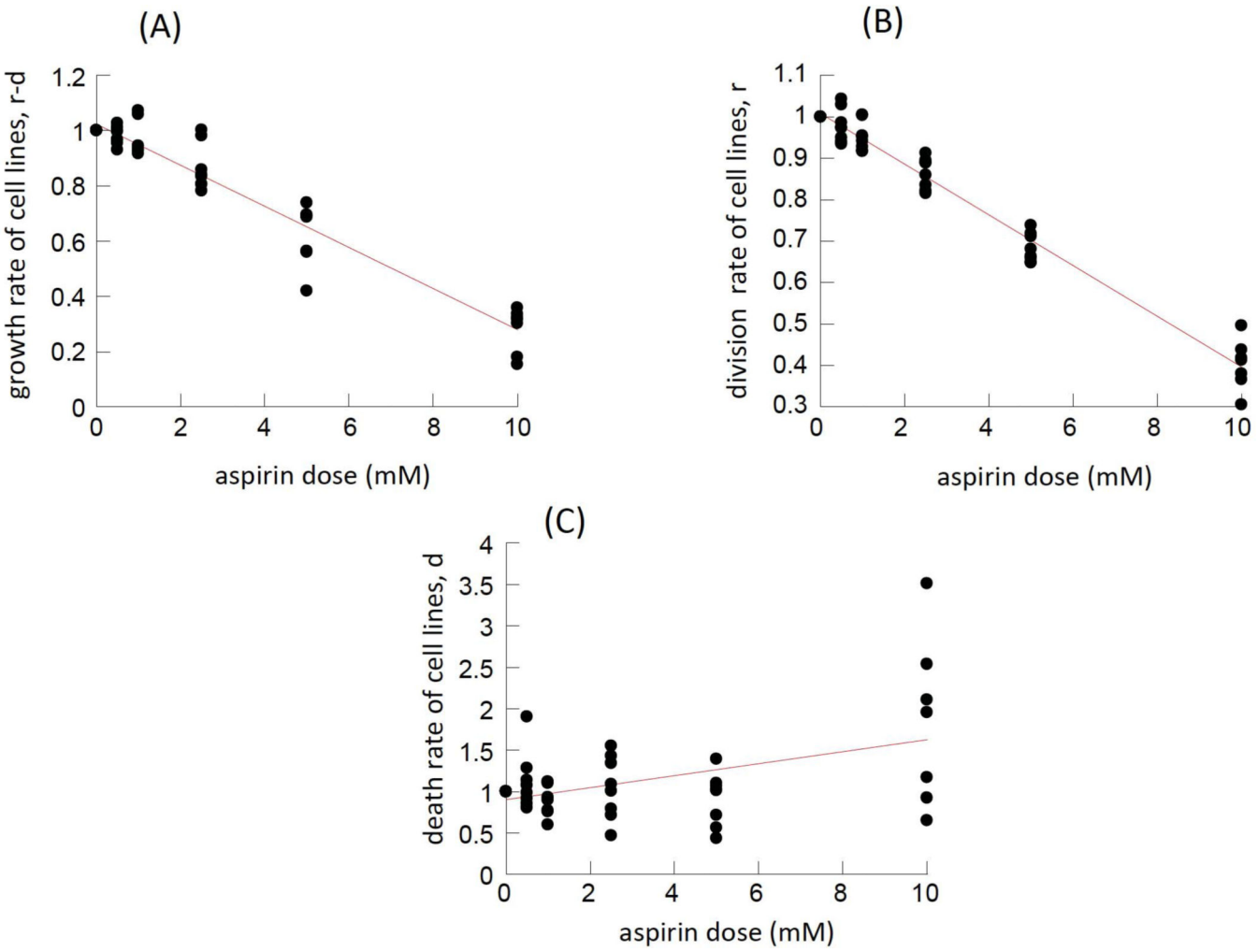

Figure 1.

Dependence of kinetic parameters on aspirin dose, as estimated from mathematical models [26]. (A) Overall growth rate versus dose shows a significant negative correlation ($p<10^{-4}$). (B) Rate of cell division versus dose shows a significant negative correlation ($p<10^{-4}$). (C) Death rate of cells versus dose shows a significant positive correlation ($p=0.0013$).

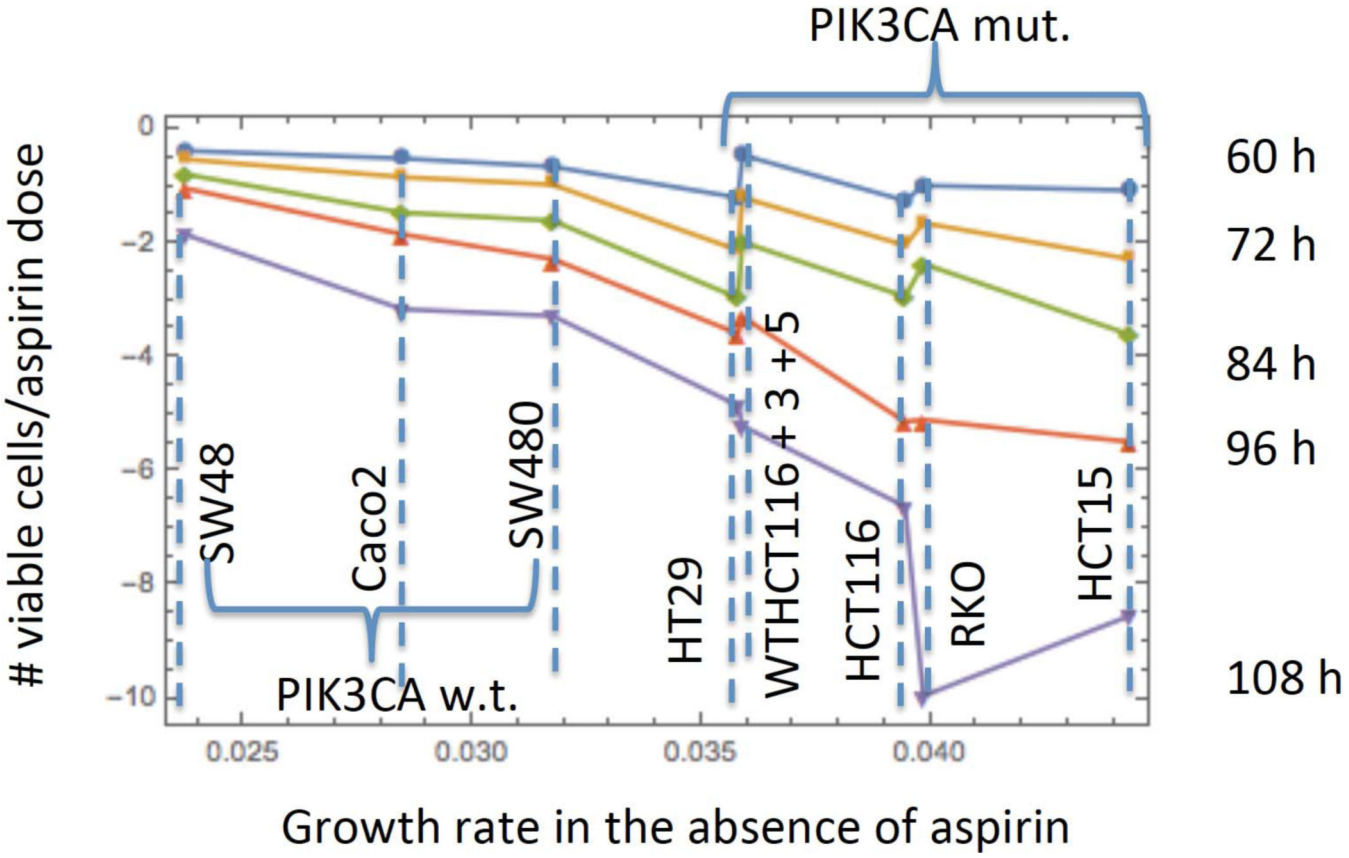

Figure 2.

The effect of aspirin on the number if viable cells, as a function of the growth rate in the absence of aspirin [26]. The different curves correspond to different time points. For all of these time-points, the negative correlation is statistically significant with p-value <0.05 (starting with t=72 h, p<0.01). The cell lines are marked next to their growth rates. The three PIK3CA w.t. lines correspond to the highest growth rates.

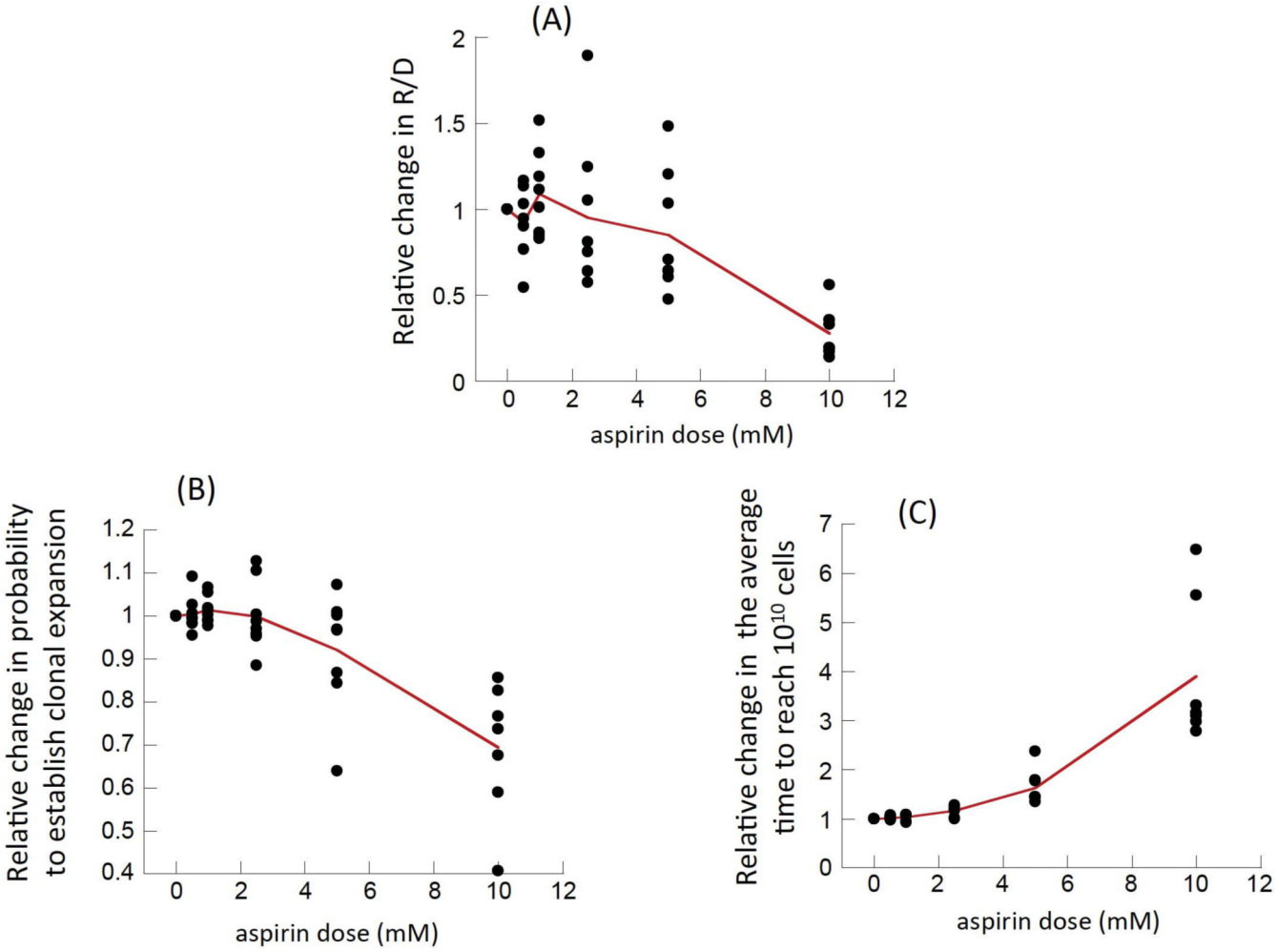

Figure 3.

Effect of aspirin on the basic parameters and the dynamics of tumor cell growth, estimated by the application of mathematical models to experimental data [31]. (A) Effect on the ratio of the rate of cell division to cell death, R/D. The value of R/D for each aspirin dose is divided by the value in the absence of the drug, yielding the relative fold-change in this measure brought about by aspirin treatment. (B) Effect on the probability for one cell to successfully establish clonal expansion rather than going extinct through stochastic effects. The graph shows the relative change in the probability to establish growth, brought about by aspirin. That is, the probability to establish growth in the presence of aspirin is divided by the probability in the absence of the drug. (C) Effect on the time it takes for one cell to expand to a population of 10^{10} cells. Again, the relative change is shown, dividing the time in the presence of aspirin (conditioned on non-extinction) by the time in the absence of aspirin (conditioned on non-extinction). For all graphs, the dots represent all the different cell lines that were used. The line shows the average over all cell lines for each aspirin dose.

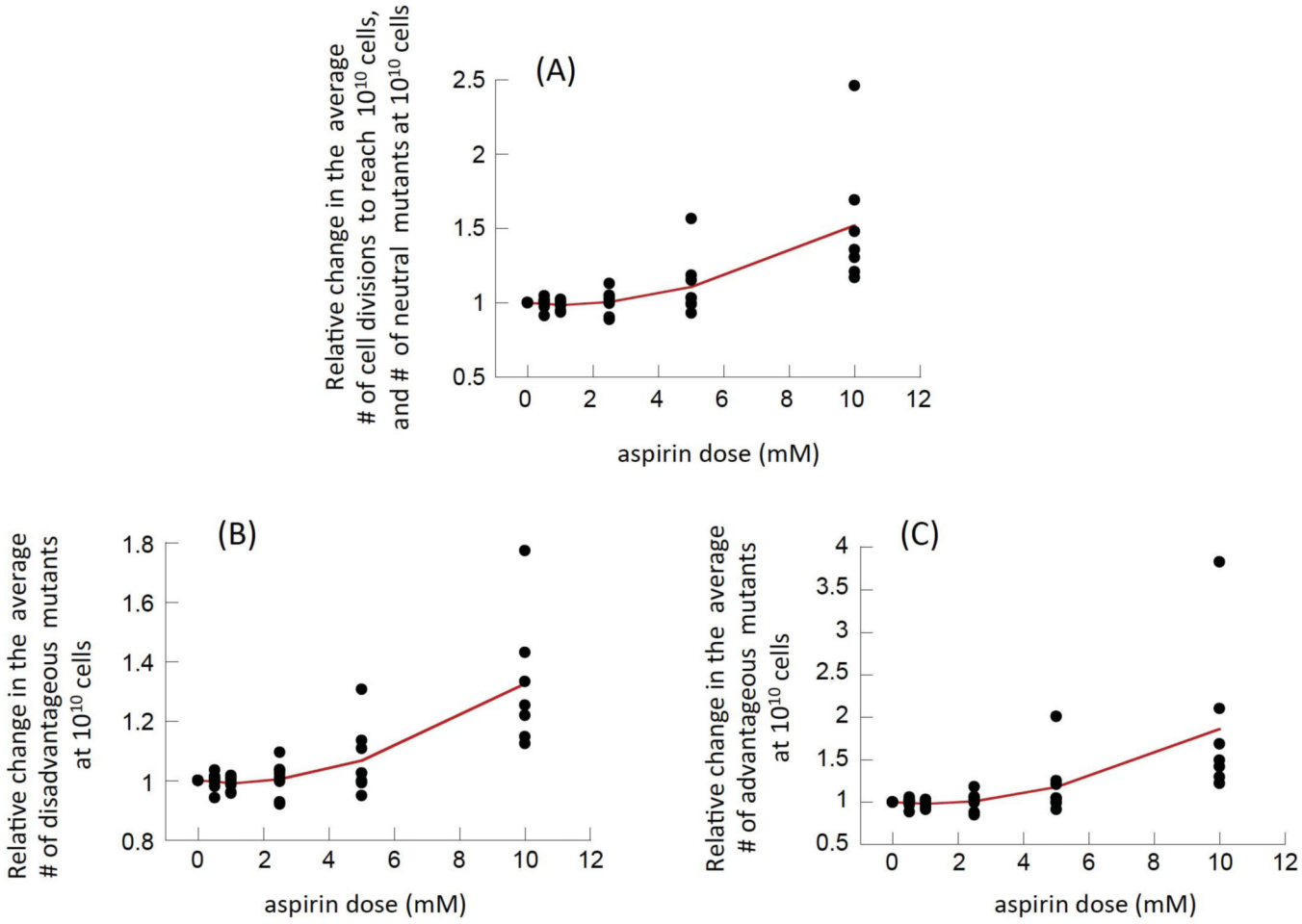

Figure 4.

Effect of aspirin on basic evolutionary dynamics [31]. (A) Relative change in the number of cell divisions required to expand from 1 to 10^{10} cells, brought about by aspirin. The number of cell divisions in the presence of aspirin was divided by the number in the absence of the drug. This measure shows the increase in the evolutionary potential of the cell population. The relative aspirin-induced change in the average number of neutral one-hit mutants when the cell colony has reached 10^{10} cells is identical and thus not plotted separately. (B) Relative change in the average number of disadvantageous mutants that are predicted to be present when the cell colony has grown from 1 to 10^{10} cells. (C) Relative change in the average number of advantageous mutants that are predicted to be present when the cell colony has grown from 1 to 10^{10} cells. The dots in the plots correspond to predictions for the different cell lines for each aspirin dose, and the line represents the average over all cell lines for each dose.

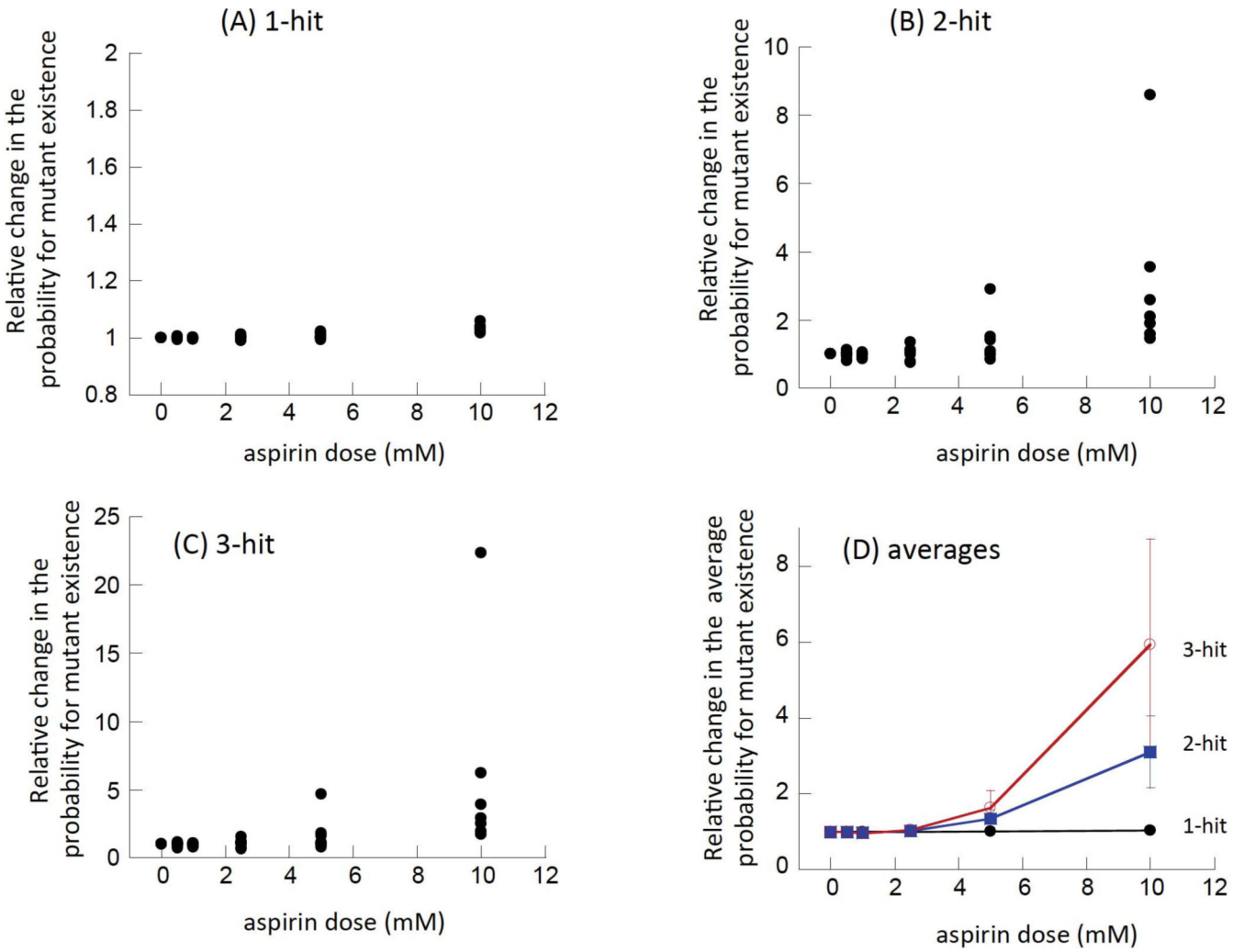

Figure 5.

Effect of aspirin on the probability for a cell to exist that is characterized by (A) 1, (B) 2, and (C) 3 independent neutral mutations by the time a cell colony has grown from 1 to 10^{10} cells [31]. Again, the relative change in the probabilities is shown, dividing the probability for a mutant to exist in the presence of aspirin by the probability in the absence of the drug. The dots represent the different cell lines. (D) This graph plots the average over all cell lines for each dose, along with error bars that represent the standard error.

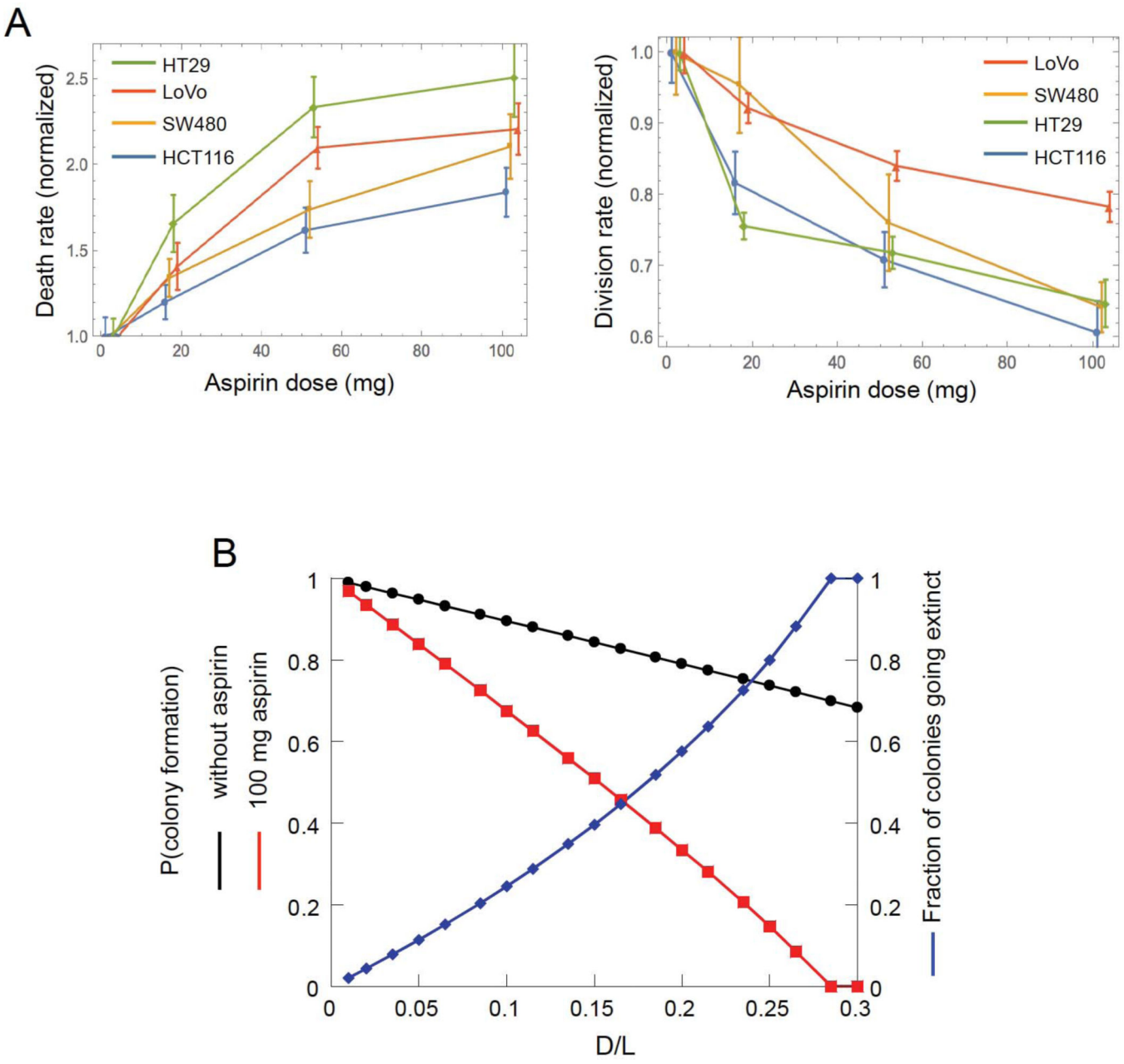

Figure 6.

(A) Effect of aspirin treatment on the division and death rates of cells in the mouse xenografts, as determined by model fitting to experimental data [64]. (B) Effect of aspirin on the probability to establish successful colony growth in a set of virtual cell lines that are characterized by different turnover rates, defined by the ratio of death rate (D) / division rate (L), in the absence of aspirin. Both parameters were changed by an amount that was given by the average change across all cell lines at maximal aspirin dosage. The black line shows the probability of successful colony formation in the absence of aspirin, while the red line shows the same in the presence of maximal aspirin dosage (starting from a singe cell). The

blue line shows the probability that a cell clone goes extinct. The probabilities were determined by simulating a 3D agent-based model of cell growth [64].

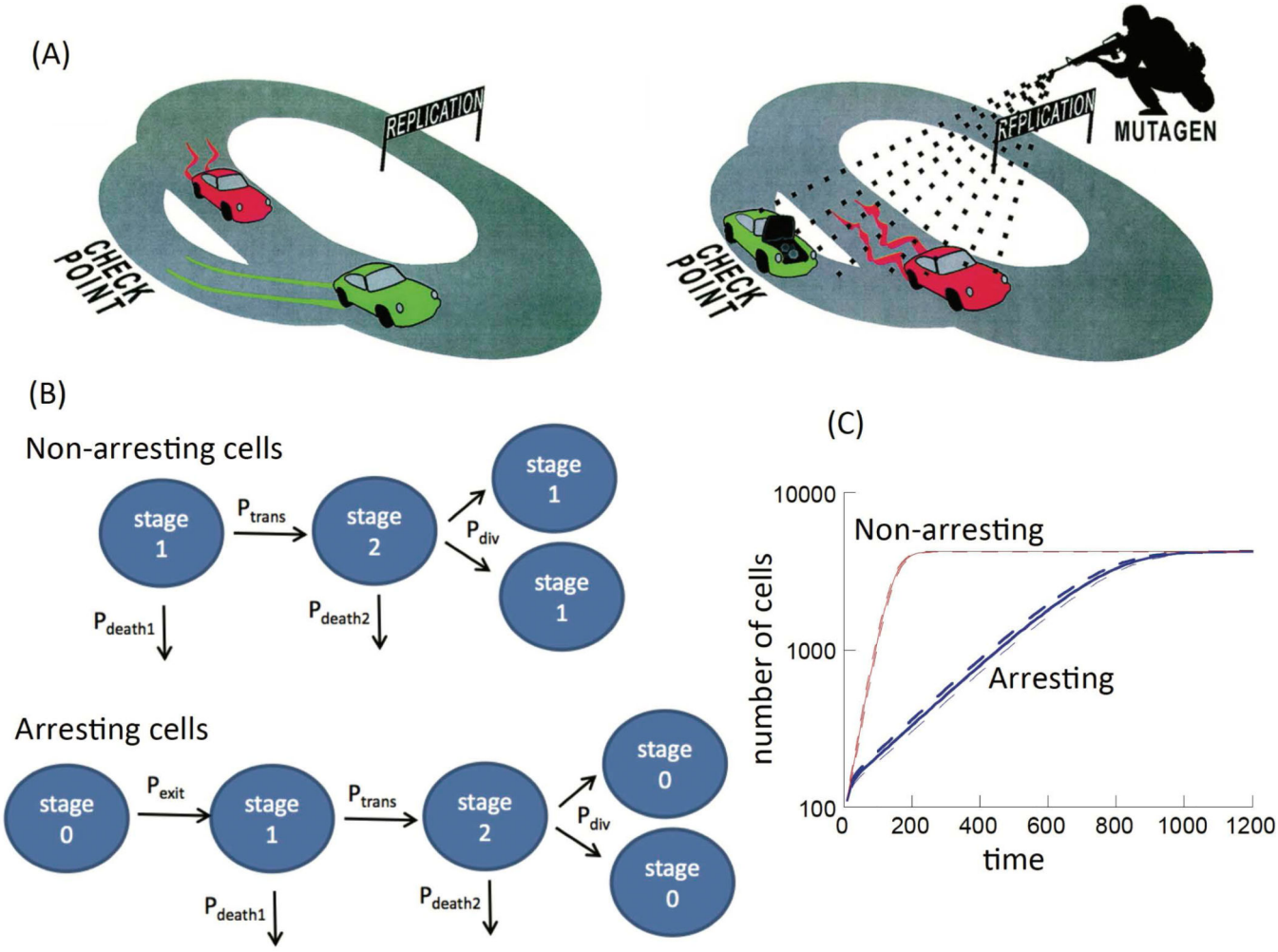

Figure 7.

Competition of arresting and non-arresting cells. (A) ``Don't stop for repair in a war zone" (from [53]). (B) A schematic showing the life-stage strategies of arresting and non-arresting cells [54]. (C) Computer simulations showing the growth of an arresting and non-arresting cell population in isolation, i.e. in the absence of competition [54]. An agent-based model was simulated and the average trajectories are shown (solid lines). The dashed lines are the average ± standard deviation.

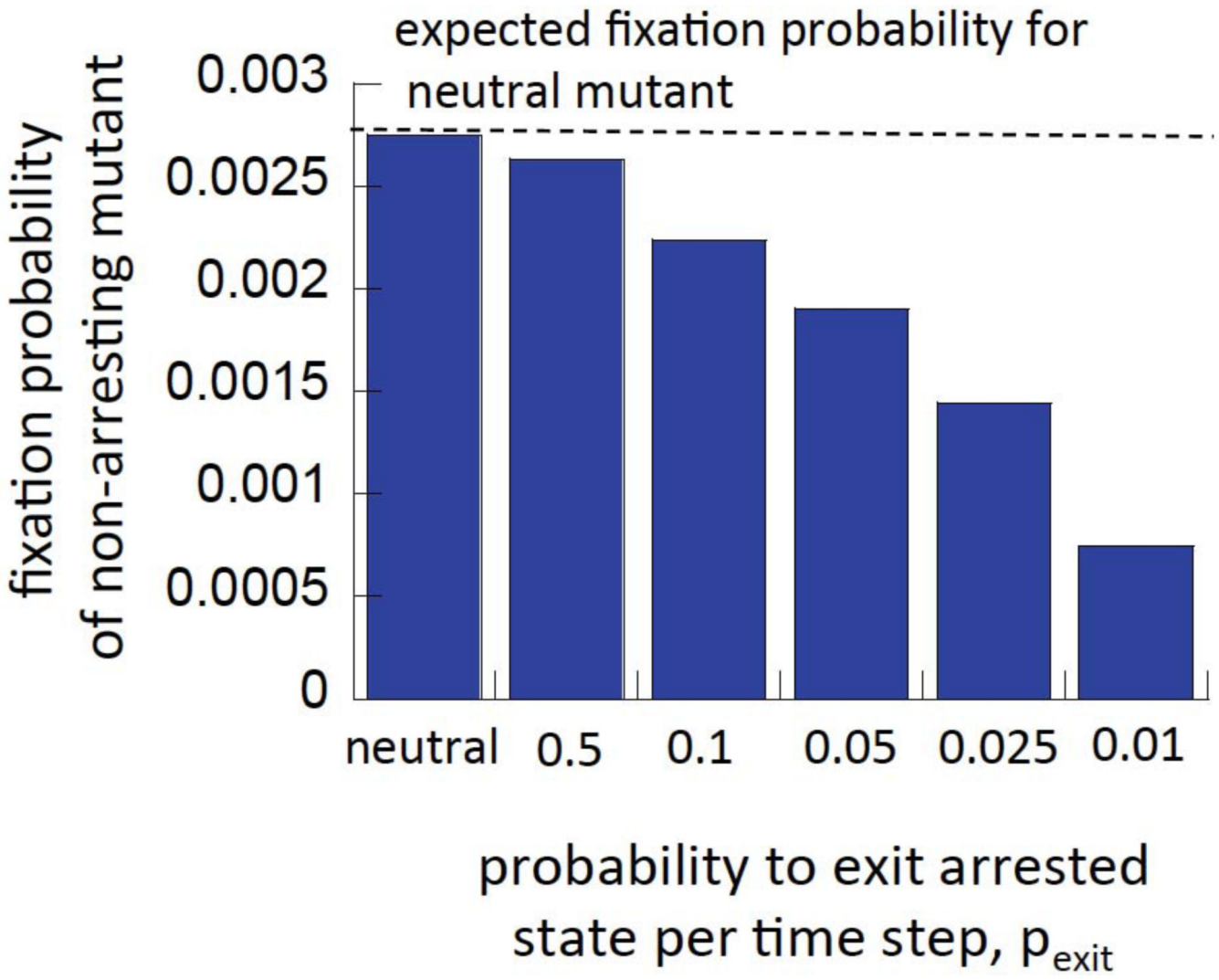

Figure 8.

Fixation probability of a single non-arresting cell placed into an established arresting cell population at equilibrium [54]. Simulations for each case were run repeatedly (>10^6 times), recording the fraction of fixation events. The "neutral" bar is the control simulation where the fixation probability was determined for a mutant that is equivalent to the established cell population (i.e. arresting, with same parameters). The horizontal line indicates the expected fixation probability for a neutral mutant, given by 1/M, where M is the average number of arresting cells in isolation around equilibrium. The remaining bars show the fixation probability of a single non-arresting cell placed into an established arresting cell population, characterized by different probabilities to exit the arresting state, P_{exit}. The lower the value of P_{exit}, the lower the fixation probability for the arresting cells.

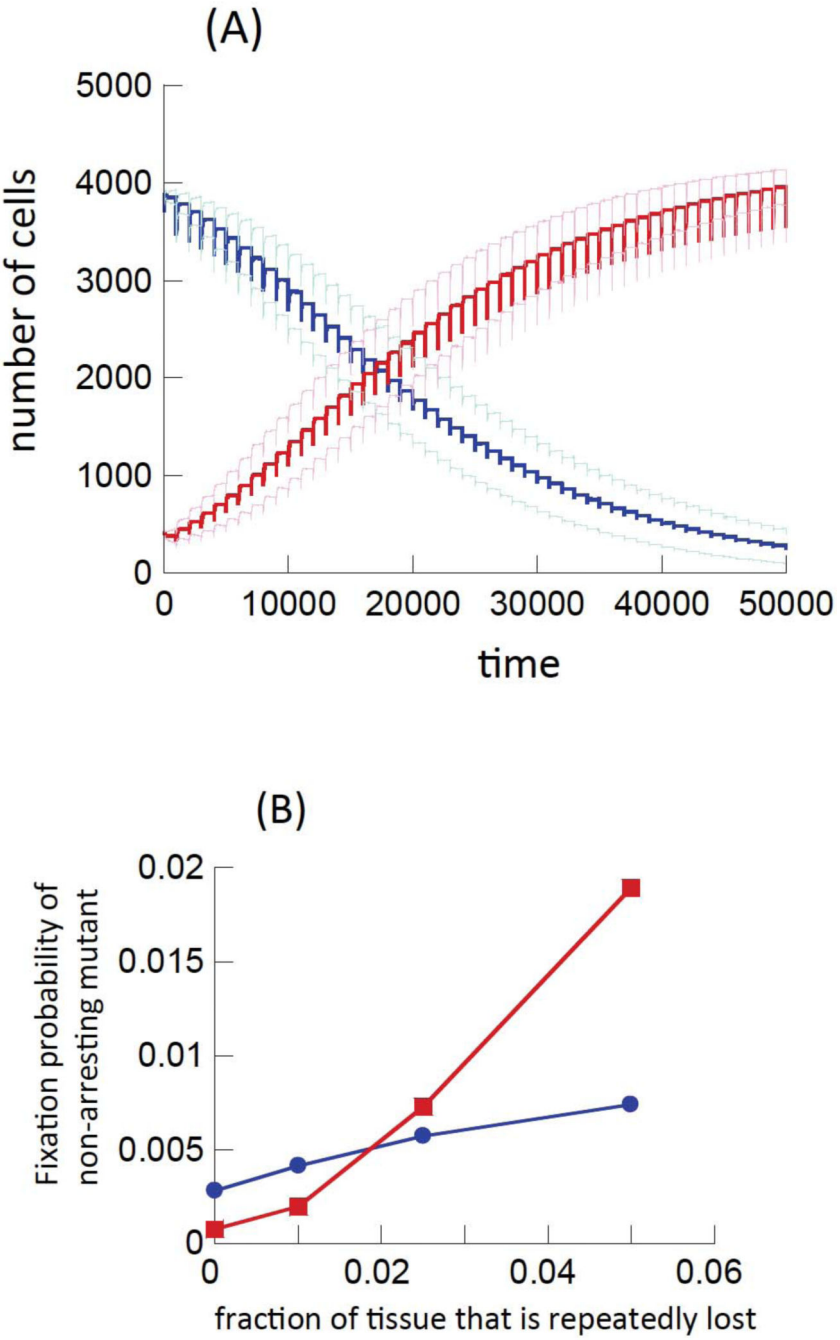

Figure 9.

Simulations of the agent-based model, assuming repeated disturbance of tissue homeostasis [54]. Every 1000 time steps, the overall population was reduced by 10%, leading to subsequent tissue regeneration (seen in the rugged shape of the lines). (A) The competition dynamics of non-arresting and arresting cell populations are shown. The blue and red lines are the average numbers of arresting and non-arresting cells, respectively. The light curves around the average lines represent average ± standard deviation. (B) Fixation probability of one non-arresting mutant placed into a population of arresting cells at equilibrium (red line),

running > 10^6 iterations of the simulation [54]. The fixation probability is plotted against the magnitude of tissue homeostasis disturbance, expressed as the fraction of cells that is removed every 300 time steps. The blue line indicates the neutral control, where the fixation probability of one arresting cell was determined when placed into an established arresting population with identical parameters.