p53 Gene Expression in Cancer Evolution: Equilibrium of Expression Rate Dependent on Mutation Rate

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Abstract

One known contributor to increased cancer risk is the level of expression of the p53 gene which produces proteins that assist in proofreading replicated DNA during the cell cycle and can terminate the cell if a mutation is detected. As important as the p53 gene is in stabilizing the body system from acquiring potentially harmful mutations, it requires a significant amount of activation energy. Our study aims to replicate this biological process to gain insight on the relationship between p53 gene expression and energy cost. We have built an adapted simulation from the Empirical Software that emulates this process by creating a world of organisms prone to mutations in the p53 gene. We implemented our simulation by adjusting the rates of malignant and benign mutations, symbolic of cancerous and silent mutations, and the rates of p53 gene expression. After running 3500 simulations with varying starting p53 gene expression and malign and benign mutation rates and recording average p53 gene expression rates in the simulation, we observed that the organisms reached a lower equilibrium of average p53 rate when the amount of mutation rates, malignant or benign, were higher- a novel relationship between the p53 gene in cell behavior. Moving forward, we hope to improve our emulation of cancerous and silent mutations in cell replication to spotlight the relationship more effectively between overall cell mutation rates and p53 gene expression equilibrium.

Introduction

Countless amounts of time and resources have been dedicated to learning more about all different aspects of cancer-- the source for over 600,000 deaths in 2020 in the United States (National Cancer Institute, 2015). Despite this seemingly unending fate of tragedy, scientists of the last century have made some incredible breakthroughs in the field of cancer research-- identifying high risk behaviors, developing novel treatments, determining mutated genes in cancerous cells (Flores, 2016). Despite these accomplishments, there remains many aspects of cancer that we know very little about.

In 1979, it was discovered that the p53 gene, the producer of the p53 protein, is commonly mutated in cancerous cells (Joerger & Fersht, 2008). In the result of this revelation, the scientific community has deemed this gene the “tumor suppressor gene” and is essential in detecting early stages of cancer (Joerger & Fersht, 2010; Nagao et al., 1994). The p53 gene operates by producing the p53 protein which effectively binds to the DNA and in turn stimulates another gene to produce a protein called p21 that interacts with a cell division-stimulating protein, cdk2 (Deepa et al., 2020). When p21 is complexed with cdk2 the cell cannot pass through to the next stage of cell division resulting in cell death (Deepa et al., 2020; Nagao et al., 1994). P53 only activates the p21 complex when it detects a change in nucleotides for the DNA it is bound to.

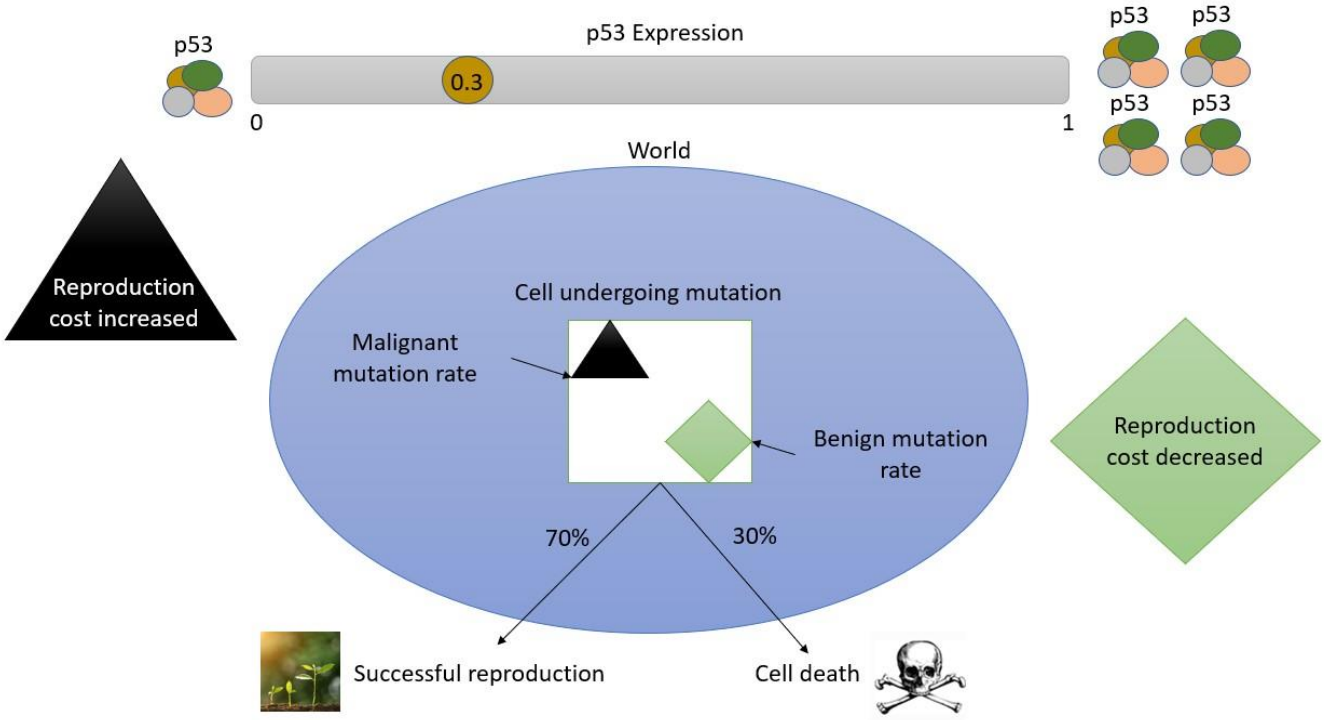
One tradeoff to the activation of the p53 gene and production of the p53 protein is the activation energy required-- in any biological process, the system pays a price in energy (Joerger & Fersht, 2010). Our simulation aims to emulate this relationship- is it always advantageous for cells to

spend energy to produce the p53 protein to protect themselves from harmful mutations, or are there cases where it is beneficial to bypass the extra screening of the replicated DNA and save resources? To answer this question, we will develop a simulation that mimics the trade-off between using energy to generate p53 proteins and saving energy for ancillary purposes such as reproduction by changing the necessary threshold of resources required to produce offspring. We implement this trade-off by enabling malignant and benign mutations which increase or decrease the cost of initiating reproduction. Malignant mutations increase the reproduction cost in effort to emulate cancerous mutations which hinder the process of stable cell reproduction, and benign mutations do the opposite by decreasing the reproduction cost in effort to symbolize mutations that are advantageous to the organism. We hypothesize that the average p53 gene expression rate will increase in organisms with predisposed high cancerous mutation rates, raising the rate of apoptosis, and it will decrease in organisms with low cancerous mutation rates, lowering the rate of apoptosis.

Methods

Evolutionary simulations are made by creating a simplified version of the environment and applying evolutionary pressures. A cell is added to this environment with the ability to mutate and reproduce, then this simulation is run, letting a large colony of cells to form. As the simulation progresses, we can extract information from each cell to get a general idea of how the evolutionary pressures affected the environment and the evolution of the cells.

To model this environment, we modified the existing system, Empirical, to fit our model (*Empirical*, 2021). The environment consists of a grid of cells with an infinite number of resources available, food. Each update, the cell receives 0.5 food. If a cell



High-level overview of our simulation. The top bar indicates the current p53 level in the cell, this p53 level is what dictates the 30% chance the cell dies. The black and green shapes represent malignant and benign mutation respectively. The malignant mutation causes an increase in reproduction cost as explained by the text in the triangle and the benign decreases the reproduction cost as shown in the diamond.

has 10 or more food, it begins the process of reproduction and loses all its food. In the case where reproduction occurs, the cell has a 40% chance of initiating mutation. During mutation, the cell first checks if there will be a benign mutation by generating a random number between 0 and 1 and checking if the random number is lesser than the value we have set as the benign mutation rate. If there is no benign mutation, the cell uses the same number and checks whether it is less than the benign mutation rate plus the malignant mutation rate. In the case where the mutation is neither benign nor malignant, the food threshold for reproduction remains the same-- this is symbolic of a missense mutation in biology where there is no consequence from the change in genetic code. If a benign mutation occurs, the cell reduces its cost of reproduction by generating a random number between 0 and 0.5. If the cell gets a malignant mutation, it increases its reproduction cost by a random number generated between 3 and 9. The random numbers are generated using a normal distribution with mean as 0.25 and 6 respectively, and standard deviation 0.0625 and 0.75, respectively. The cost of reproduction has no upper bound, but it cannot decrease below 1. If the mutation took place, another random number between 0 and 1 is generated, if the number is less than the current p53 expression rate of the cell, the cell is killed, and no offspring is created, otherwise the cell reproduces, and adds or subtracts a number with a mean of 0 and a standard deviation of 0.1 to its current p53 expression rate. This simulation was set to run for 2500 updates, with a maximum population size of 10000, if the max population size is achieved, and a cell reproduces, it will kill a cell nearby and take its place. The values changed between the runs are the benign and malignant mutation rate as well as the initial p53 value. We used malignant and benign mutation rates of; 0.0, 0.25, 0.5, 0.75, 0.9 and p53 values of; 0.0, 0.05, 0.06, 0.08, 0.13, 0.15, 0.2. We ran each combination of benign rate, malignant rate, and initial p53 on 20 seeds to check for variance.

Results

p53 Gene Expression

To examine the effects of p53 gene expression when we keep the starting p53 gene expression value constant, we ran simulations where we altered the

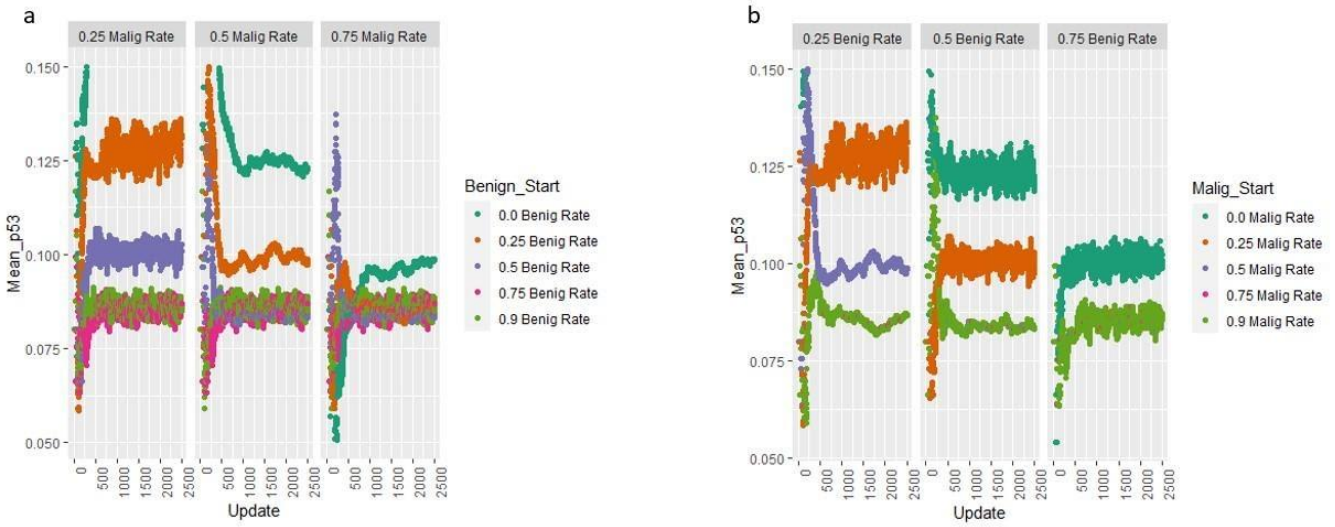


Figure 1. Effects of differentiating default benign and malign mutation rates on mean p53 gene expression when default p53 expression rate is held constant at 0.08. (a) and (b) depict how mean p53 rate of organisms in simulation balance as updates increase. (a) uses default benign mutation rate as explanatory variable and groups data on default mutation rate. (b) does same as (a) but uses default benign and malignant mutation rates inversely. Note that simulation does not allow for sum of malignant and benign mutation rates to exceed 1; in the case where they do exceed 1, the simulation gives priority to benign mutation rate and ignores excessive benign mutation rate (i.e., if the malignant mutation rate is 0.5 and the benign mutation rate is 0.75, the simulation honors 0.75 benign mutation rate and malignant mutation rate maxes out and is treated as 0.25).

starting values of the malignant and benign mutation rates and kept the starting p53 gene expression rate constant at 0.08.

We observed all possible combinations between the different starting values of benign and malignant mutation rates, which revealed the pattern of the mean p53 gene expression rate reaching lower equilibrium when the rate of mutation increased-- regardless of whether the mutation was benign or malignant as seen in Figure 1. Fig. 1(a) represents the data separated into three graphs grouped by distinct malignant mutation rates and the explanatory variables are the different starting values of benign mutation rate. In Fig. 1(a), we see that as the sum of the benign and malignant mutation rates increase shows this pattern where the p53 gene expression rate flattens out after around 500 updates at lower p53 values depending on how great the sum of the mutation rates is; once the sum of the benign and malignant mutation rates exceeds 1, the data remains the same as the mutation rate cannot be greater than 1. Fig. 1(b) corroborates this pattern; the p53 gene expression rates behave in the same manner, flattening out at decreasing values as the sum of the mutation rates increase when we separate the data into three graphs grouped by distinct benign mutation rates and use the different starting values of malignant mutation rates as the explanatory variables.

These data contradict our original hypothesis that the p53 gene expression rate would increase with higher malignant mutation rates and decrease with high benign mutation rates. One explanation for this is that the effect of the benign mutation rate overpowers the malignant mutation rate, where the decreased reproduction cost in response to a benign mutation populates the simulation far more frequently than organisms that have gained the malignant mutations, so the mean p53 gene expression rate in the simulation is saturated by organisms with low reproduction cost rates from gaining benign mutations. However, we view the same low p53 gene expression equilibrium point when the starting malignant mutation rate is high, and the starting benign mutation rate is low. Another explanation is that the organisms are more inclined to accept the mutation and run the risk of gaining a malignant mutation if it saves energy and avoids killing the cell. Another aspect of this data is that the mean p53 gene expression rate does not fall to 0, rather it finds an equilibrium value where the organisms in the simulation find the rate to be optimal; this reveals that the organisms do find a need to have present some p53 in the chance that an organism gains a malignant mutation, however, the organisms prefer to keep the p53 rate low when there is a higher chance of gaining mutation.

Biologically, these findings reveal a unique priority system in cells-- that it is advantageous to run the risk of gaining a potentially harmful mutation that could affect the ability to reproduce if there is a chance of gaining a beneficial mutation and saving from killing the cell. This relationship reveals that cells prioritize the ability to reproduce efficiently over the sanctity of preserving the original genetic DNA.

Benign and Malignant Mutation Rates

To investigate the relationship between the starting benign and malignant mutations rates, we held the default benign or malignant mutation rate constant and examined the mean p53 rate of the organisms in the simulation when we varied the default p53 gene expression rate and the malignant or benign mutation rate.

We observed all possible combinations between the different starting values of benign mutation rates and p53 gene expression rates when we held the starting malignant mutation rate constant at 0.5, and all possible combinations between the different starting values of malignant mutation rates and p53 gene expression rates when we held the starting benign mutation rate constant at 0.5 as seen in Figure 2. Fig. 2(a) shows the relationship between the mean p53 gene expression rate when the data are separated by three different malignant mutation rates and the explanatory variable being the distinct starting p53 gene expression rates when the benign mutation rate is defaulted to 0.5, and Fig. 2(b) is the same as Fig. 2(a), but the roles of

the starting benign and malignant mutation rates are reversed. Fig. 2(c) displays the data separated into three different starting p53 rates and the explanatory variables are the distinct starting benign mutation rates when the malignant mutation rate is defaulted to 0.5, and Fig. 2(d) is the same as Fig. 2(c), but the roles of the starting benign and malignant mutation rates are reversed.

Fig. 2(a-b) reveals that regardless of whether the starting benign or malignant mutation rates were set constant, the data with different starting p53 gene expression rates trended towards the same equilibrium after about 500 updates. When the non-constant starting mutation rates were at 0.25, the p53 gene expression rate equilibrium was higher, at around 0.10, and when the non-constant starting mutation rates were 0.5 or 0.75, the p53 gene expression rate equilibrium dropped to about 0.08.

This again shows that the equilibrium p53 rate decreases when the net starting mutation rate

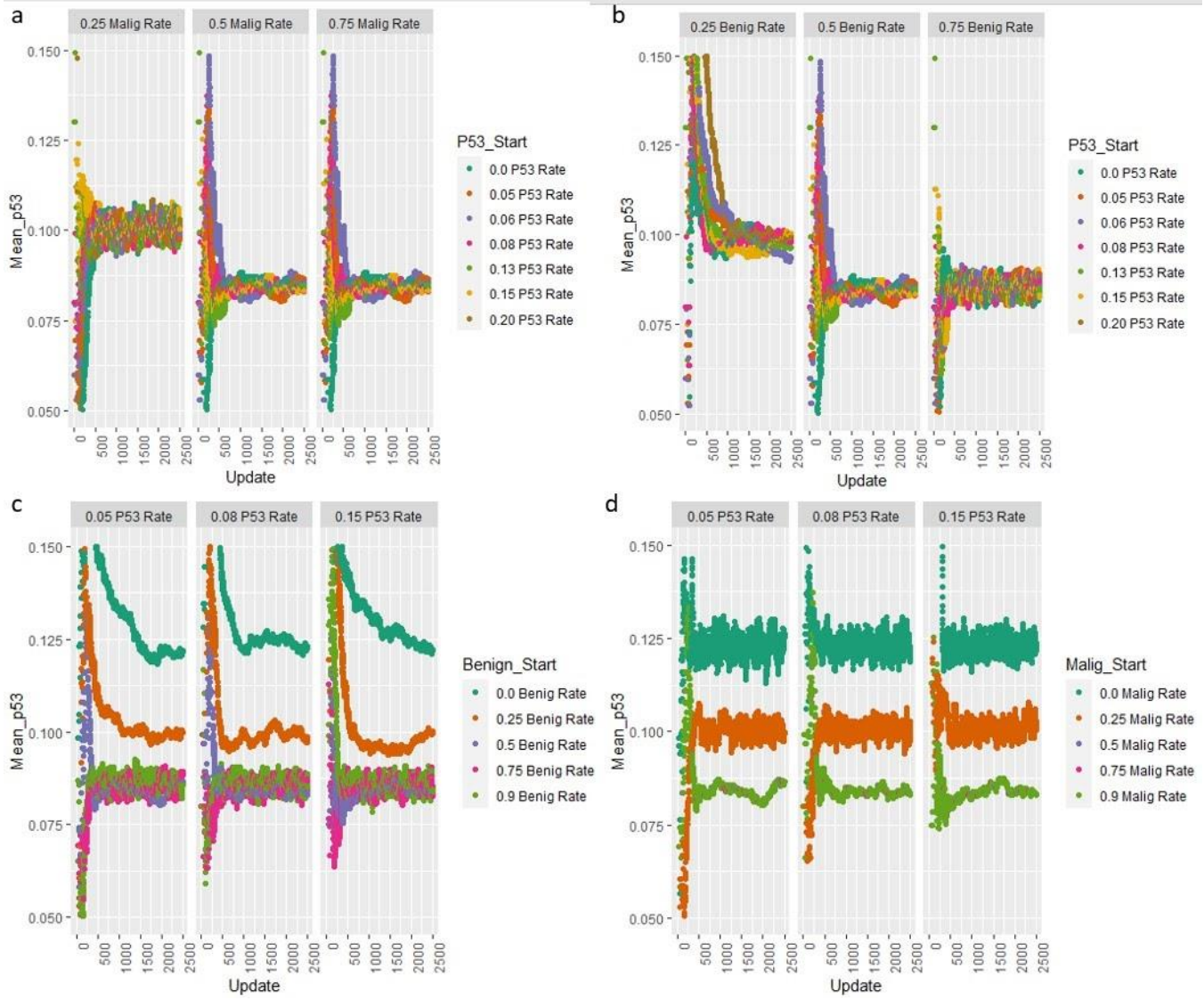


Figure 2. (a) shows the effect of differentiating default malignant mutation rates and p53 gene expression rates on average mean p53 gene expression rate when benign mutation rate is held constant at 0.5. (b) shows the effect of differentiating default benign mutation rates and p53 gene expression rates on average mean p53 gene expression rate when malignant mutation rate is held constant at 0.5. For (a) and (b), data were explained by p53 gene expression rate and grouped by either malignant or benign mutation rate. (c) and (d) display the same data but are explained by either malignant or benign mutation rate and grouped by p53 gene expression rate. Note that our simulation does not allow for the sum of malignant and benign mutation rates cannot exceed 1; in the case where they do exceed 1, the simulation gives priority to the malignant mutation rate and will ignore the excessive benign mutation rate (i.e. if the malignant mutation rate is 0.5 and the benign mutation rate is 0.75, the simulation would honor the 0.5 malignant mutation rate and the benign mutation rate would max out and be treated as 0.5).

increases. Despite the differences in effect of the mutations, the increased probability of gaining a benign or malignant mutation decreases the p53 gene expression rate.

Fig. 2(c-d) corroborates this trend where the mean p53 gene expression rate is not differentially influenced based on the altering mutation rates, however, there are a few interesting distinctions between Fig. 2(c) and Fig. 2(d). For one, there is more variability in Fig. 2(d) when the net mutation rate is higher, and lower variability when the net mutation rate reaches 1. Additionally, Fig. 2(c) does not show a clear equilibrium point for mean p53 gene expression rate when the starting benign mutation rates are less than 0.5.

One explanation for the difference in variability between Fig. 2(c) and Fig. 2(d) is the number of organisms present in the simulation when the benign mutation rate is low. Because there are few organisms, the data does not have opportunity to mutate the p53 gene expression often. This also explains the difficulty in Fig. 2(c) for the 0.0 and 0.75 benign mutation rate data to find equilibrium; because there are fewer opportunities for mutation, there are fewer opportunities for the p53 rate to find balance.

In the real world, these findings imply that cells prefer to risk gaining mutations if there exists an opportunity that the cell could gain an advantageous mutation that would benefit their efficiency during cell reproduction. One consequence of this behavior is that organisms that are more prone to mutation are in fact also more likely to possess lower expression rates of the p53 gene; this is potentially very problematic. In life, various activities such as smoking, exposure to radiation, and excessive amounts of sun exposure without protection have been found to increase the risk of developing cancer. As we know, the reason is that these behaviors put humans at risk for developing mutations-- not necessarily cancerous mutations, but mutations altogether. Now, our data suggests that with higher levels of mutation, the p53 expression rate decreases. So, for organisms that have higher exposure to at risk behavior like smoking, they are also more prone to having low p53 gene expression which is the driving force behind stifling mutations.

Conclusion

Our study aimed to better understand the relationship between the rate of p53 gene expression depending on the cost of reproducing cells. To do this, we implemented the Empirical software to develop a simulation where we looked at the average p53 gene expression rate in all organisms in the environment based on the rate of mutation which affects the energy cost of the organism performing reproduction. In our implementation, we enabled two types of mutations-- benign and malignant-- which are symbolic of advantageous and cancerous mutations. Benign mutations decrease the energy cost for the organism to reproduce, and malignant mutations increase the energy cost for the organisms to reproduce. In our simulation, we altered the rates of cells gaining benign and malignant mutations and observed how the p53 gene expression rates responded. We hypothesized that the average p53 gene expression rate would increase in organisms with high malignant mutation rates, raising the rate of apoptosis, and it will decrease in organisms with low cancerous mutation rates, lowering the rate of apoptosis. We observed that the p53 gene expression rate reaches an equilibrium value dependent upon the net rate of mutation rate, but there was no difference depending on the type of mutation.

In the system, p53 does not tend to reach an optimal rate, as seen by the p53 level decreasing when malignant mutation rate increases. This p53 rate is not optimal as there is a greater chance for malignant mutations, yet the only resistance to these mutations, the p53, is being removed. The p53 rate does not change based on whether the malignant or benign mutation rate is high, its change depends on mutation rate. Despite this, since the threat of malignant or benign mutation is constant in humans, p53 would have reached an equilibrium in our bodies.

These results are encouraging and raise further questions that are worth investigating. For one, we

would like to emulate the p53 gene more accurately by implementing the functionality where the gene halts the replication process and enables the DNA mutation to be repaired. Enabling DNA repair may increase the p53 gene expression considering the reward of fixing the mutation and avoiding cell death. We also plan to further develop our simulation to implement an environment where cells within spatial radius of one another would gain mutual effects from positive or negative mutations—this would give us a better idea of how cancerous cells affect their neighbor cells and how the p53 gene operates on the scale of an entire organ or organism rather than in individual cells. We believe that implementing a more radial effect of a malignant mutation would better mimic a real tumor mutation, but also involve kin selection. We could see if kin selection would be a driving force in increasing or decreasing the amount of p53 in a cell.

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