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Abstract

Cellular senescence, a mechanism employed by cells for thwarting proliferation, has shown to play an important role in protecting cells against cancer development in recent experimental observations, indicating that a deeper understanding of the cellular senescence pathway can help exploit its capacity for more effective cancer treatment. Furthermore, some experimental evidence points out that inhibition of CDK2 or Skp2 can be the critical trigger for cellular senescence. However, no mathematical model has been developed to highlight cellular senescence until now. In this study, we first implement a mathematical model of G1/S transition involving the DNA-damage pathway to highlight cellular senescence by lowering the critical trigger- CDK2. For this, we focus on the behaviour of two important proteins (E2F and CycE) for several reduced CDK2 levels under two DNA-damage conditions by calculating the probability (β) of DNA-damaged cells passing the G1/S. Our recently published results from the same model indicated that a large percentage of damaged cells pass G1/S under normal CDK2 levels, reaching β values of up to 65% under high level of DNA damage. The current study reveals that reduced CDK2 levels can significantly lower the percentage of damaged cells passing the G1/S; in particular, 50% reduction in CDK2 achieves 65% reduction in the passage of damaged cells. Furthermore, the model analyses the relationship between CDK2 and its CKIs in search of other effective ways to bring forward cellular senescence. Results show that the degradation rate of p21 and initial concentration of p27 can be effectively used to lower the senescence threshold. Specifically, p27 is the most effective, followed by

CDK2 and p21. However, the combined effect of CDK2 and CKIs is dramatic with CDK2/p27 combination almost totally arresting the passage of damaged cells. Biologists may wish to validate the efficacy of these targets for treating cancer.

CDK2 and CKI targeting can significantly lower the cellular senescence bar – reveals a mathematical model of G1/S checkpoint pathway

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1 Introduction

The work in this article was motivated by the article of Collado, & Serrano[1] on "Senescence in tumours: evidence from mice and humans" and the accompanying cancer related overview by Serrano[2] entitled "A lower bar senescence" in News and Views of the 18th of March 2010 issue of Nature. They highlight new findings on cellular senescence, a physiological mechanism employed by cells for thwarting the proliferation of cells through an irreversible state of cell cycle arrest whereby a normal cell loses the ability to divide and promote cell proliferation. A crucial fact that is emphasized is that the backdrop in which senescence is played out lies in the strategy used by cells to initially allow a considerable proportion of cells carrying oncogenes to pass through the cell cycle in consideration of the fact that, at a given time, there are probably millions of cells that are oncogenically primed and catching all these initially would be an exhausting task for a cell. This leads to the accumulation of these cells in the tissue. At the time of publication of the above results and comments, our recent work[3] on a mathematical model of the G1/S checkpoint pathway including the DNA damage pathway was revealing this phenomenon showing that under DNA damage stress, a significant proportion of

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damaged cells pass the G1/S checkpoint as healthy cells; for example, under low- and high- level of DNA damage stress, 64% and 66%, respectively, of damaged cells pass the checkpoint. The research in the current paper was inspired by the corroboration of this phenomenon by the mathematical model.

The second fact highlighted in Collado, & Serrano[1] and Serrano[2] is that cancer progresses in 3 stages beyond the healthy stage: pre-tumor, pre-malignant, and malignant, where transition from one stage to the next is generally accompanied by increased levels of senescence induced stresses. Most human and mouse tumour cells, however, stop proliferating and undergo senescence at the pre-malignant stage, where a non-invasive tumour is formed, indicating that it is at this level that senescence inducing stresses reach sufficient intensity to be effective.This suggests that once allowed through the cell cycle much of the cells carrying oncogenes are allowed to further proliferate in the pre-tumoral stage – the first stage of tumorigenesis- with little or no senescence. Moreover, mutations that disable senescence are instrumental in the transition from oncogene-harbouring cells to malignant stage, highlighting the importance of senescence in countering malignancy. Summarising the work of Lin et al.[4] and Campaner et al.[5] who elucidate the molecular mechanism of cancer associated senescence, Serrano[2] emphasises the clinical relevance of lowering the bar for senescence in curing cancer and raises hopes for and questions about possibilities for doing it so that oncogenically primed cells are targeted early on before reaching the pre-malignant stage as happens normally. Serrano[2] aptly points out that

"Encouraging cancer-prone cells to senesce [before reaching pre-tumoral or pre-malignant stages] might therefore be a way to nip this disease in the bud". Inspired by the ability of the model used in our study[3] to reveal normal biological process of initially allowing a large number of damaged cells pass the cell cycle, in this paper, we investigate if our model can further highlight the possibilities for lowering the senescence bar.

Cellular senescence was first described by Hayflick and Moorhead[6] more than four decades ago. They showed that normal cells entered an irreversible state of cell growth arrest in response to the uncontrolled proliferative capacity of normal cells. For the reasons highlighted previously, eliminating the cells that tend to become cancerous in the pre-malignant stage is an intelligence response adopted by cells to efficiently address the problem of abnormal cell proliferation. Considering the prevalence of cancer today, manipulating the threshold for senescence to encourage cancer cells to senesce early can lead to better protection against cancer. Thus, a deeper understanding of the pathway to cellular senescence can play an important role in exploiting this route for effective cancer treatment.

Both Lin et al.[4] and Campaner et al.[5] found that the inhibition of the activity of cyclin-dependent kinases (CDKs), such as CDK4, CDK6 and CDK2, plays a significant role in establishing cellular senescence in order to protect cells against cancer, particularly, inhibiting CDK2 activity as a critical factor to lower the bar for triggering senescence

in cancerous cells. All these CDKs are important proteins to mediate the initiation of G1 phase and control the G1/S transition in the cell cycle. Furthermore, senescence-inducing stressors inhibit the activity of CDKs by controlling their inhibitory proteins (called CDK inhibitory kinases or CKIs), which include p21, p27 and p16[7,8]. Furthermore, Skp2 inhibition might be critical to lower the bar for senescence in oncogenically primed cells based on recent studies[2,4,5]. Skp2 can mediate the degradation of some CKIs, such as p21 and p27, and Skp2 inhibition can increase the expression of CKIs in the cell cycle, which also results in inhibiting the activity of CDK2s.

Although there is some experimental evidence pointing out that the inhibition of CDK2 or Skp2 can be the critical trigger for senescence, currently, there aren't any mathematical models developed to highlight the cellular senescence under DNA damage conditions. Senescence in this respect leads to an exciting question: Can a mathematical model highlight the cellular senescence and formulate scenarios for adjusting the threshold for senescence to evaluate its efficacy and outcomes? The purpose of this paper is to elucidate such an approach based on a mathematical model of G1/S transition pathway incorporating DNA-damage signal transduction; more specifically, we address the possibility of lowering the probability of a DNA-damaged cell passing as a healthy cell in the G1/S phase transition in response to lowering the threshold for senescence. Meanwhile, we also evaluate the relationship between CDK2 and its CKIs under different DNA damage situations in search of alternative

approaches to lowering the senescence bar through these relationships in search of the most effective means of bringing senescence forward.

2 Model description

The latest mathematical model of the G1/S phase transition was published in 2008 by Iwamoto et al.[9], which simulates the G1/S transition incorporating the DNA damage signal transduction pathway. In this mathematical model, there are 28 ordinary differential equations with 75 kinetic parameters, which display interactions among the chemical species (for instance, E2F, CycE, CycA, CDK2, CDK4/6, p21, p27, p16, p53, Mdm2 and so on) in the G1/S transition integrating DNA damage signal (See Fig.1). Notably, all proteins crucial for triggering senescence are involved in the G1/S transition.

In our earlier work[3], we evaluate the probability (β) of a DNA-damaged cell passing as a healthy cell in the G1/S phase transition in the presence of various levels of perturbation in the key kinetic parameters associated with the model. In order to achieve this, three important steps were followed: 1) we first decided on the important biomarkers for the G1/S transitions and selected E2F and CycE (The accumulation of E2F and CycE activity could be a critical trigger for the cells to make a decision whether to enter S phase or not); then identified the significant kinetic parameters influencing these biomarkers under three different DNA-damage situations (No DNA damage, Low-level DNA damage and High-level DNA damage) based on Local

Sensitivity Analysis (LSA) where the effect of each parameter is assessed while other parameter being held at constant values (out of 75 parameters, we identified 8, 15 and 14 for E2F, and 10, 17 and 16 for CycE for the three DNA-damage conditions, respectively.); 2) we then calculated the probability density function (PDF) of the peak time (PT) of the two biomarkers in response to various levels of perturbations to the important kinetic parameters through a Global Sensitivity Analysis (GSA) where these parameters are varied simultaneously; 3) based on the PDF of PT from GSA, we computed Type II error or power (β) using statistical hypothesis testing, where the type II error in this case indicates the probability of a damaged cell wrongly passing as a healthy cell. The details of the procedure and results can be found in Ling et al.[3] and the relevant results extracted from the paper are shown in Table 1 that summarizes the probability of a damaged cell passing the checkpoint based on the PDF of PT of E2F and CycE under two DNA damage levels. The results show that the probability of a damaged cell passing the checkpoint became very large (more than 0.38) when the level of change of key parameters exceeded $\pm 30\%$. This can reach the level of 0.69 for $\pm 50\%$ perturbation to the parameters. Our results, in terms of percentage of damaged cells that pass G1/S checkpoint, agree with Collado et al.[7] finding and Serrano's[2] assertion: a large number of damaged cells undergo proliferation without being caught at DNA damage checkpoints in the pre-tumoral stage (initial proliferation of cells carrying oncogenes). In the next section, we investigate if the mathematical model supports the possibility of lowering the bar for

senescence in order to catch damaged cells early in the cell division without having to wait for premalignant stage as happens normally.

3 Results and Discussion

3.1 Effect of CDK2 on lowering the bar for cellular senescence

Table 2 shows the probability β of damaged cells passing G1/S checkpoint based on the PDF of PT of biomarker E2F under two different DNA-damage conditions for three reduced CDK2 levels: -10%, -30% and -50%, respectively. Table 3 displays the value of β for the three decreased CDK2 levels for CycE under the two DNA-damage conditions. According to the results from the behaviour of E2F and CycE under reduced and normal CDK2 levels, the probability of a damaged cell passing G1/S have decreased with inhibiting activity of CDK2; more importantly, there is a significant decrease in β based on both E2F and CycE when the CDK2 level is reduced to 50% of the normal level.

According to Table 1, under normal CDK2 level, the percentage of tumor cells passing G1/S increases with the level of DNA damage and the range of parameters for both biomarkers that indicate similar trends for β. For example, when the level of DNA damage is high, E2F and CycE indicate that 68.9% and 65.8%, respectively, of damage cells pass G1/S for $\pm 50\%$ perturbations to the key parameters. In contrast, Table 2 indicates that for the same high damaged conditions and parameter ranges, this percentage based on E2F can be reduced to 42.2% by decreasing the CDK2 levels

by 50%. This amounts to a reduction of 38.8%. For the low DNA damage, lowering CDK2 by 50% amounts to 42.7% reduction in damaged cells passing G1/S. The above observed trend is confirmed by the trends in PDF of PT of CycE in Table 3. Here, 50% reduction of CDK2 level under the high-level DNA damage situation results in 64.59% reduction in the percentage of damaged cells passing G1/S. For the low-level DNA damage, 50% reduction of CDK2 produces a 64.63% reduction. These results indicate that damaged-cells enter an irreversible state of cell cycle arrest, such as cellular senescence or apoptosis, in response to low CDK2 levels, which is consistent with Campaner and colleagues's[5] results that mice deficient in CDK2 became more sensitized to cellular senescence under the oncogenic stress caused by Myc oncogene or oncoprotein. Thus, our model supports the biological findings related to senescence; more importantly, it reveals the effect of reducing CDK2 levels in terms of the reduction of the percentage of damaged cells passing the G1/S checkpoint.

3.2 Effectiveness of CKIs in lowering the bar for senescence

Here, we investigate the behaviour of CKIs in response to reduced CDK2 levels, by reducing CDK2 in three different levels under two different DNA damage conditions, for example, CDK2-10%, CDK2-30% and CDK2-50%, respectively. Analytical results show that the change of CDK2 has littler or no effect on p16 and p27 at both low-level and high-level DNA damage. The reason is that most p27 molecules bind to CycD/CDK4/6 complex at the beginning of G1 phase and only few p27 molecules

bind to CycE/CDK2-P complex near the G1/S transition. However, the variation of CDK2 has a significant effect on p21 level in these two DNA damage situations. In the event of DNA damage, p21 is activated by p53 resulting in a dramatic effect on its concentration as required for promoting its binding to CycE/CDK2-P complex for arresting cell cycle. Fig.2 shows the behaviour of p21 for four levels of CDK2 under low- and high-level DNA damage as well as p21 behaviour for healthy cells. It shows that p21 level under damage conditions is much higher than that under no DNA damage (healthy cell) due to its increased production in response to DNA damage. Furthermore, p21 level in high-level DNA damage is lower than that in low-level DNA damage (20-25% difference between the maximum levels) because p53 is used to trigger the apoptosis pathway for high-level DNA damage. In both DNA damage conditions, the effect of lowering CDK2 is initially insignificant on the p21 level that kept constant until CycE level increases closer to the G1/S transition (at around 1500 time steps in Fig.2) for all four CDK2 levels. Then, the reduced CDK2 level comes into effect influencing the behaviour of p21. Increased CycE production in the cell cycle requires, more CDK2 for binding to CycE to form CycE/CDK2 complex, and this complex can further associate with p21 to form the triple complex p21/CycE/CDK2. This process results in a dramatic decrease in p21 level; however, p21 level increases at the end of G1 phase because CycE level decreases in the S phase thereby releasing more p21 in the cell cycle transition. Reducing CDK2 levels results in producing less CycE/CDK2 to associate with p21 thereby increasing the

concentration of p21. For this reason, p21 level in the cell cycle is the highest for 50% reduction of CDK2 levels compared to the two smaller reductions.

Now we turn to investigating the behaviour of CDK2 in response to increased levels of its CKIs. First we analyse the effect of degradation rate of CKIs on CDK2 concentration by decreasing their corresponding kinetic parameters in the mathematical model. According to the model results presented in Fig.3, there is an effect of adjusting the degradation rate of p21 on the variation of CDK2 for the two DNA damage situations. However, the variation of the degradation rate of p27 and p16 makes little or no effect on CDK2 levels under these two DNA damage conditions (not shown). According to Fig.3, the influence of the degradation rate of p21 on CDK2 is predominant during the G1/S transition. The reason is that the concentration of CDK2 is mainly controlled by CycE in G1/S transition. For example, at the beginning of G1 in the cell cycle of a healthy cell, the CycE level is kept at a low level which keeps CDK2 and CycE in balance. However, the concentration of CycE significantly increases during G1/S transition, which results in an imbalance between CycE and CDK2. Therefore, more CDK2 is required to associate with CycE. In the DNA damage situation, p21 is activated in the G1 phase and it plays an important role in reducing CDK2 levels. The reason is that increased p21 binds to CycE/CDK2 complex to inhibit E2F, which in turn inhibits CycE levels, leading to delayed and slower production of CycE under damage conditions compared to the healthy condition. This reduces the need for as high CDK2 levels as in the healthy

condition. Therefore, lower p21 degradation rates leads to higher CDK2 levels as indicated in Fig.3. Higher levels of free CDK2 indicate the presence of low CycE levels giving rise to further delays in the progression of these cells through G1/S transition. According to Fig3, there is a small difference in the free CKD2 levels between the low-level and high-level DNA damage conditions due to the difference in the amount of p21 levels between the two conditions. Although there is a significant difference in the amount of p21 in the two different DNA-damage situations shown in Fig.2, only a part of p21 can bind to CycE/CDK2-P or CycA/CDK2-P to control free CKD2 in cells because the increased CycE/CDK2-P and CycA/CDK2-P near G1/S transition can promote the degradation of p27, resulting in releasing more free CycD/CDK4/6 that creates a competition for p21 binding with these two complex proteins (CycE/CDK2-P and CycA/CDK2-P).

Next, we focus on the effect of production rate of CKIs on CDK2 levels. Results revealed by the mathematical model show that the production rate of CKIs has little or no effect on the CDK2 concentration for low-level and high-level DNA damage situations (not shown). Finally, initial conditions of CKIs are used as the focus to analyse their relationship to CDK2. The analytical results show that only p27 initial condition has a significant effect on the variation of CDK2 under DNA damage situations as shown in Fig. 4. According to Fig.4, the variation of initial condition of p27 has a more significant effect on the CDK2 behaviour than that caused by the variation of degradation rate of p21 shown in Fig.3. Increased initial concentration of

p27 makes available more free p27 for binding with CycE/CDK-P complex to inhibit its activity after p27 associates with CycD/CDK4/6 during G1 phase. This results in inhibiting the activation of E2F, the critical protein in G1/S, that in turn inhibits the accumulation of CycE required for the completion of the G1/S progression. Inhibition of CycE also makes the rate of decrease of CDK2 become slower in response to increase in the initial concentration of p27. Thus, CDK2 can be lowered by adjusting initial conditions of p27 to trigger senescence early. Thus, the analysis of CKIs reveals that the degradation rate of p21 and initial level of p27 affect CDK2 significantly and can be targets for lowering the senescence bar.

According to the investigation of the effect of CKIs on the behaviour of CDK2 under two different DNA-damage situations from the model's simulation, results indicates that p21 degradation rate and p27 initial rate make contributions to inhibiting the activation of CDK2 in cells. These results qualitatively support the experimental findings and the biological theory that p21 and p27 as cell cycle Cip/Kip inhibitors prevent the cell cycle progression and suppress tumor growth by inhibiting the activation of CycE/CDK2 as well as CycA/CDK2 to regulate the level of CDK2 in the cells[10-12]. Additionally and importantly, an advantage of the model is that it reveals the spectrum of the behaviour of CDK2 as it relates to varying levels of p21 and p27 in the whole region of G1/S transition.

3.3 Effectiveness of CKIs alone and simultaneous variation of CDK2/CKIs on lowering senescence bar

Relying on the outcome of the analysis of the relationship between CDK2 and CKIs, the focus of this step is to analyse the percentage of damaged cells that can be prevented from passing G1/S by further lowering the senescence threshold through regulating the CKIs and the combination of CDK2 and CKIs. The value of β in response to individually changing CDK2 or CKIs (in our case, p21 degradation rate and p27 initial condition) based on the behavior of E2F for parameter range of ±30% is shown Fig. 5 which indicates that the increase in p27 initial condition makes the most significant contribution to reducing the probability of a damaged cell passing the G1/S for the two DNA-damage situations, followed by the reduced CDK2 level and the decreased p21 degradation rate, with the difference between the latter two being minor. Comparison of the 30% change of CDK2 or CKIs with their 50% counterpart indicates that 50% change of CDK2 or CKIs can reduce a larger percentage of damaged cells passing G1/S. These simulation results in response to the effectiveness of CKIs alone are in a good agreement with the experimental findings that the Skp2 inactivation leading to oncogenic-stress-driven senescence critically depends on p27, p21 and Atf4[4]. Lin et al.[4] pointed out that upregulation of p27, p21 or Atf 4 contributes to promoting cellular senescence upon Skp2 inactivation. Furthermore, Lin et al.[4] found that the concomitant upregulation of p27, p21 and Atf4 can be considered as a more required and powerful engine to trigger cellular senescence under Skp2 deficiency situation. The most important advantage of modelling is that,

while supporting the above biological findings, the model simulations allow us to ascertain the reduction of percentage of damaged cell passing G1/S checkpoint in response to different levels of CKIs.

After the analysis of separately changing CDK2 or CKIs, we also focus on the effect of the combination of CDK2 and CKIs on β for two different levels of their change (30% and 50%). For example, simultaneously reducing CDK2 and p21 degradation rate as well as simultaneously reducing CDK2 and increasing p27 initial condition. Fig.6 displays the results of the combination of CDK2 and CKIs for parameter range ±30%, which indicates that the combination of CDK2 and p27 initial condition makes a much greater reduction in the percentage of damaged cells passing G1/S than CDK2 and p21 combination. For 50% change to CDK2 and p27 levels, the percentage of damaged cell passing G1/S is almost equal to zero for the two different DNA-damage situations. Probing into the G1/S checkpoint pathway can shed light into these observations as follows. Taking the characteristics of the G1/S pathway into account, the accumulation of E2F is regulated directly by the activity of Rb and indirectly through the action of Cyclins and their dependent kinases (such as CycD/CDK4/6 to initiate the phosphorylation of Rb, CycE/CDK2-P and CycA/CDK2-P to further hypophosphorylate Rb for the release of E2F) at the G1/S transition. The reduction of CDK2 alone can decrease the concentration of CycE/CDK2-P or CycA/CDK2-P in cells to delay the release of E2F under no DNA-damaged condition. In response to CDK2- deficiency and DNA-damage situations,

the synthesis of p21 triggered by DNA-damage signal further inhibits the activation of CycE/CDK2-P or CycA/CDK2-P and results in further delay in the activation of E2F. For the combination of CDK2 and p27 under DNA-damage situations, p21 is already activated by DNA-damage signal. If the concentration of p27 is kept at the standard level, it still makes a further delay in the accumulation of E2F caused by the activated p21. If the concentration of p27 increases, more CycE/CDK2-P and CycA/CDK2-P are required to be associated with p27, which results in a longer delay in the accumulation of E2F. In terms of the combination of CDK2 and p21, the model only adjusts the levels of p21 and CDK2 in cells. This is why the combination of CDK2 and p27 makes a larger contribution to reducing the percentage of damage cells passing the G1/S transition. Thus the combined targeting of CDK2 and key CKIs are a powerful way to lower the senescence bar. As far as we know, the combined effect CDK2 and CKIs have not been studied experimentally. Therefore, the results from our analysis can suggest some novel biological experiments for validating the effectiveness of the combined targets in a practical setting.

According to the behavior of CycE for parameter range ±30% (see Fig.7 and 8), we get similar results to those revealed by E2F, except for the β for the case of individually changing CDK2 or CKIs. In this case, reduction in CDK2 level makes a larger contribution to decreasing the percentage of damaged cells passing G1/S checkpoint than increase in p27 initial condition The values of β in response to changing CDK2 or CKIS separately as well as in combination based on the PDF of

the two biomarkers for parameter range $\pm 50\%$ can be found in the Supplementary Materials. Results for the parameter range $\pm 50\%$ show similar trends for the behaviour of E2F and CycE as those revealed from parameter range $\pm 30\%$; however, the percentage of the damaged cell passing G1/S under $\pm 50\%$ parameter range is larger than that under $\pm 30\%$ parameter range.

4 Conclusions

We have demonstrated that the mathematical model incorporating G1/S checkpoint pathway and DNA damage signal transduction pathway supports the possibility of lowering the bar for cellular senescence. It shows that CDK2 and its CKIs (p21 and p27) can be targets for achieving this. Specifically, analysing the time to reach the maximum (PT) concentration of E2F and CycE as two critical proteins in the G1/S transition, we demonstrated that lowering CDK2 levels influences the PT of these critical proteins, which we used to assess the change in the probability β of a damaged cell passing the G1/S checkpoint. Then we investigated the relationship between CDK2 and its CKIs for the two DNA-damage signals as well as the effects of CKIs and the combination of CDK2 and CKIs on the probability of a damaged cell passing the check point.

The results of β based on the PDF of PT of the chosen biomarkers (E2F and CycE) for three reduced CDK2 levels (CDK2-10%, CDK2-30% and CDK2-50%) under two DNA-damage conditions reveal that reducing CDK2 levels can reduce the percentage of damage cells passing the G1/S checkpoint, indicating that CDK2 can be a target for

lowering the threshold for senescence to bring forward the entry of damage cells into an irreversible state of cell growth arrest and prevent the proliferation of cancerous cells. More specifically, 50% reduction of CDK2 can reach 65% reduction in the percentage of damage cells passing the checkpoint. These results point out that the model can highlight the possibility of lowering the bar for cellular senescence by lowering the CDK2 level.

In the search for other effective ways to bring forward cellular senescence, the model revealed some useful relationships between CDK2 and its CKIs (in terms of production rate, degradation rate and initial conditions) under DNA-damage situations. Specifically, results revealed that CDK2 has a strong relationship with p21 (degradation rate) and p27 (initial condition). Additionally, the model revealed the spectrum of the behaviour of CDK2 in terms of varying levels of p21 and p27 in the whole region of G1/S transition. This gives us some insights into how to further trigger cellular senescence in oncogenically primed cells through targeting these key CKIs individually or a combination of lowered CDK2 levels and reduced degradation rate of p21, and/or increased initial level of p27 before cells start proliferation. Individually, p27 appeared to be more than twice as effective as p21 in resisting damaged cells passing the G1/S, based on both E2F and CycE behaviour. The behaviour of the two biomarkers revealed a small discrepancy in the effect of individually varying CDK2 level in that CDK2 is slightly more effective than even p27 according to CycE, making CDK2 the most effective individual target.

Nonetheless, both E2F and CycE showed that the combination of CDK2 and p27 is much more effective than that of CDK2 and p21, with 50% change in the former arresting almost all damaged cells before passing G1/S.

Results revealed so far from the mathematical model are consistent with the current knowledge of biology and experimental observations, and adds a detailed view of the efficacy of important targets for lowering senescence bar. More importantly, the model investigation of the effect of the combination of CDK2 and CKIs on the percentage of damaged cells passing the G1/S transition may give us some ideas to explore a powerful and effective way to further trigger cellular senescence in oncogenically primed cells. Biologist may wish to verify these findings to validate the effectiveness of the targets in a practical setting. We hope that further analysis will help us better understand cellular senescence for exploiting its mechanisms for an effective cancer treatment by lowering its threshold.

Methods Summary

In this study, the developed mathematical model is used to investigate the behaviour of PT of the chosen biomarkers (E2F and CycE) of the G1/S transition in response to CDK2-deficient situations. The purpose of this is to confirm whether the developed model can highlight cellular senescence in response to lowering the critical point (CDK2 inhibition) for senescence. We use the same model and the parameter set as our earlier study[3] only reducing CDK2 levels for two DNA damage situations (Low-level and High-level), and calculate the probability β of a DNA-damaged cell passing

the G1/S transition. Then, we compare the values of β under the normal CDK2 level and low CDK2 levels. If β decreases with decreasing levels of CDK2, our model accurately represents cellular senescence and supports the hypothesis that lowering CDK2 is an effective means of promoting senescence in damaged/cancerous cells.

Secondly, we also interrogated the model to investigate whether it represents and supports the relationship between CDK2 and CKIs: increasing the expression of CKIs is an effective way to inhibit the activity of CDK2 under senescence-inducing stressors[4,5]. There are two different ways to validate the relationship between CDK2 and its CKIs under DNA damage situations using the mathematical model: one is to focus on what the behaviour of CKIs, such as p16, p21 and p27, is in response to reducing CDK2 levels; the other is to focus on what the behaviour of CDK2 itself is in response to increasing the expression of its CKIs by adjusting their corresponding kinetics parameters in the mathematical model in relation to: initial condition, production rate and degradation rate. Lastly, we study the effectiveness of simultaneously changing CDK2 and CKIs on lowering the senescence bar.

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Table 1 The probability (β) of damaged cells passing G1/S checkpoint

Parameter Range	*β (probability of a damaged cell passing G1/S as healthy)*			
	Low-Level DNA-damage		**High-Level DNA-damage**	
	E2F	**CycE**	**E2F**	**CycE**
Reference Values ± 10%	0.004	0.001	0.029	0.01
Reference Values ± 20%	0.218	0.147	0.365	0.272
Reference Values ± 30%	0.429	0.386	0.542	0.504
Reference Values ± 50%	0.639	0.574	0.689	0.658

Reference values indicate default normal values of parameters

Table 2 The probability of a damaged cell passing G1/S for reduced CDK2 levels (based on PT of E2F)

Parameter Range	β *(probability of a damaged cell passing as healthy)*					
	Low-Level DNA-damage			**High-Level DNA-damage**		
	CDK2-10%	**CDK2-30%**	**CDK2-50%**	**CDK2-10%**	**CDK2-30%**	**CDK2-50%**
Reference Values ± 10%	0.0018	0.0002	0	0.015	0.0019	0
Reference Values ± 20%	0.178	0.097	0.034	0.311	0.185	0.064
Reference Values ± 30%	0.39	0.287	0.159	0.5	0.389	0.229
Reference Values ± 50%	0.606	0.515	0.366	0.66	0.574	0.422

Table 3 The probability of a damaged cell passing G1/S for three reduced CDK2 levels (based on PT of CycE)

Parameter Range	β *(probability of a damaged cell passing G1/S checkpoint)*					
	Low-Level DNA-damage			**High-Level DNA-damage**		
	CDK2-10%	**CDK2-30%**	**CDK2-50%**	**CDK2-10%**	**CDK2-30%**	**CDK2-50%**
Reference Values ± 10%	0.00005	0	0	0.0013	0	0
Reference Values ± 20%	0.085	0.0166	0.0032	0.172	0.033	0.0037
Reference Values ± 30%	0.302	0.141	0.0453	0.411	0.202	0.059
Reference Values ± 50%	0.5136	0.362	0.203	0.57	0.413	0.233

Figure Legends

Fig.1 Schematic diagram of the structure of the G1/S checkpoint pathway involving DNA damage signal transduction pathway.

Fig.2 The behaviour of p21 in response to four different CDK2 levels for (a) low-level DNA damage and (b) high-level DNA damage (For reference purposes, p21 level for no DNA damage under normal CDK2 level (healthy cell) is also shown in the figure).

Fig.3 The behaviour of CDK2 in response to the variation of degradation rate of p21 for (a) low-level DNA damage and (b) high-level DNA damage (For reference purposes, CDK2 level for no DNA damage under normal degradation rate of p21 (healthy cell) is also shown in the figure)

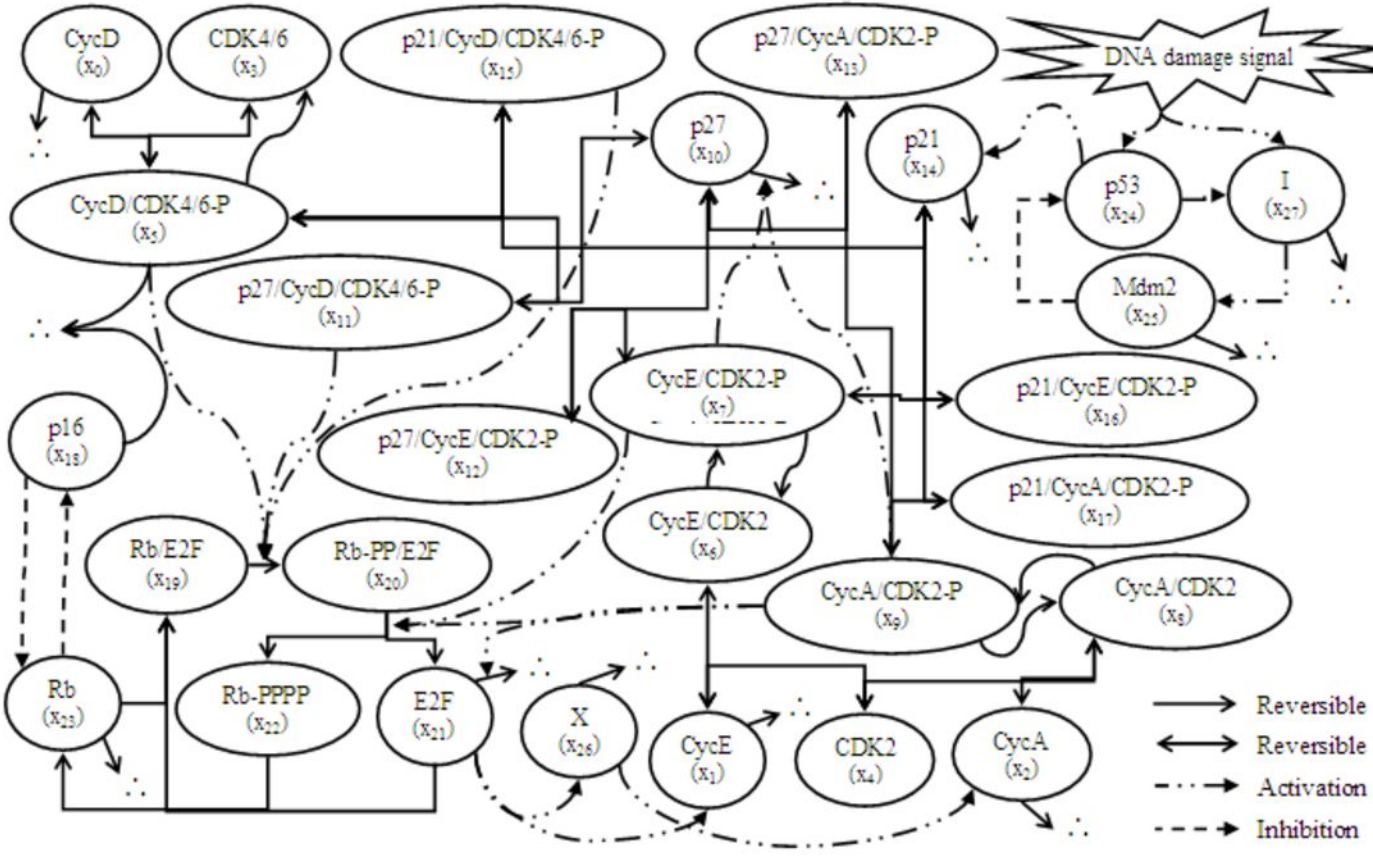
Fig.4 The behaviour of CDK2 in response to the variation of initial conditions of p27 for (a) low-level DNA damage and (b) high-level DNA damage (For reference purposes, CDK2 level for no DNA damage under normal initial condition of p27 (healthy cell) is also shown in the figure).

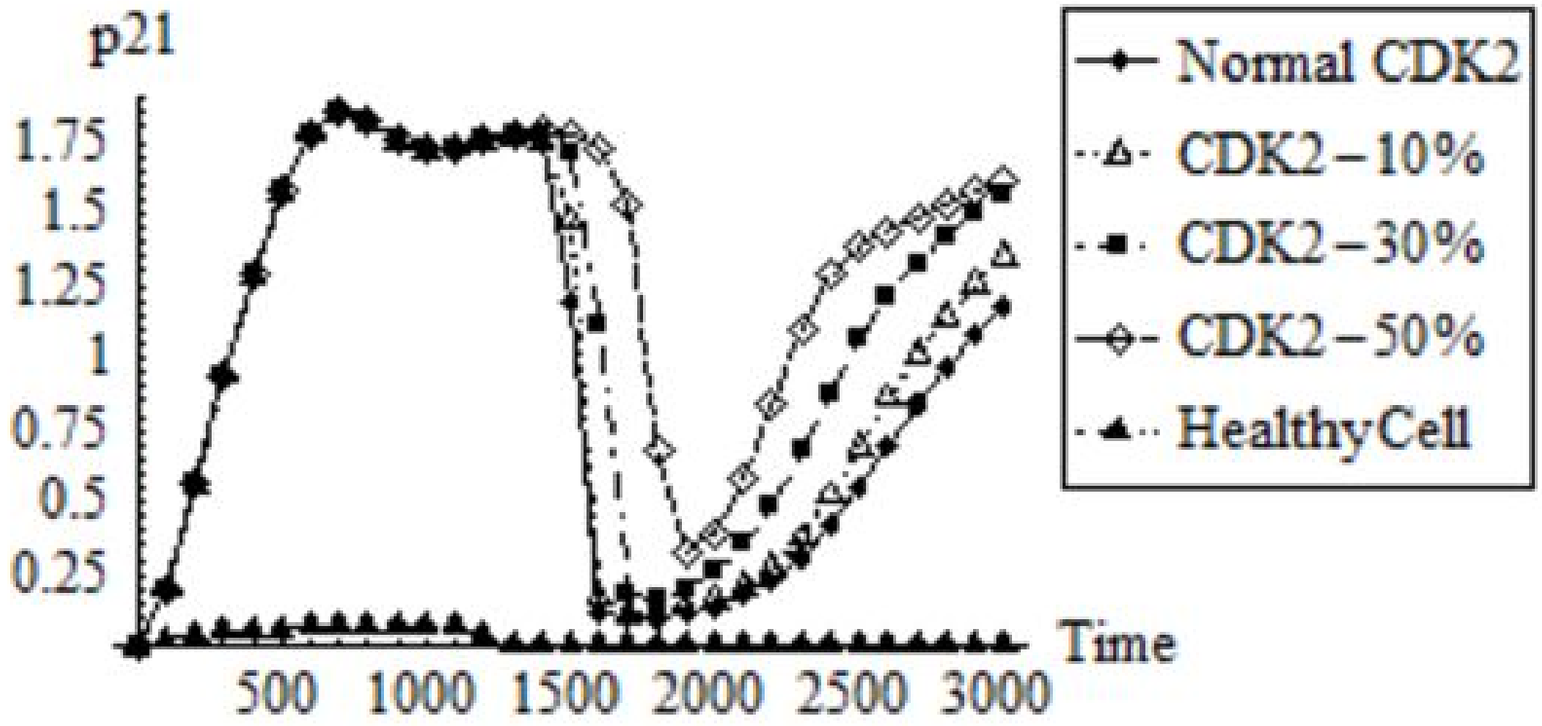
Fig.5 The probability β of a damage cell passing G1/S checkpoint in response to only changing CDK2 or CKIs (i.e. p21 degradation rate and p27 initial condition) for three different ranges of normal, 30% and 50% - specifically, CKD2 or p21 degradation rate reduced or p27 initial condition increased by 0%, 30% and 50%, respectively - using the behavior of E2F for parameter range ±30% under different DNA-damage conditions: low level DNA-damage and high level DNA-damage.

Fig.6 The probability β of a damaged cell passing G1/S in response to simultaneously changing CDK2 and CKIs (i.e. p21 degradation rate and p27 initial condition) for three different ranges of normal, 30% and 50% - specifically, CKD2 and p21 degradation rate both reduced by 0%, 30% and 50%, respectively, or CDK2 reduced and p27 initial condition increased by 0%, 30% and 50%, respectively- using the behavior of E2F for parameter range ±30% under two different DNA-damage conditions.

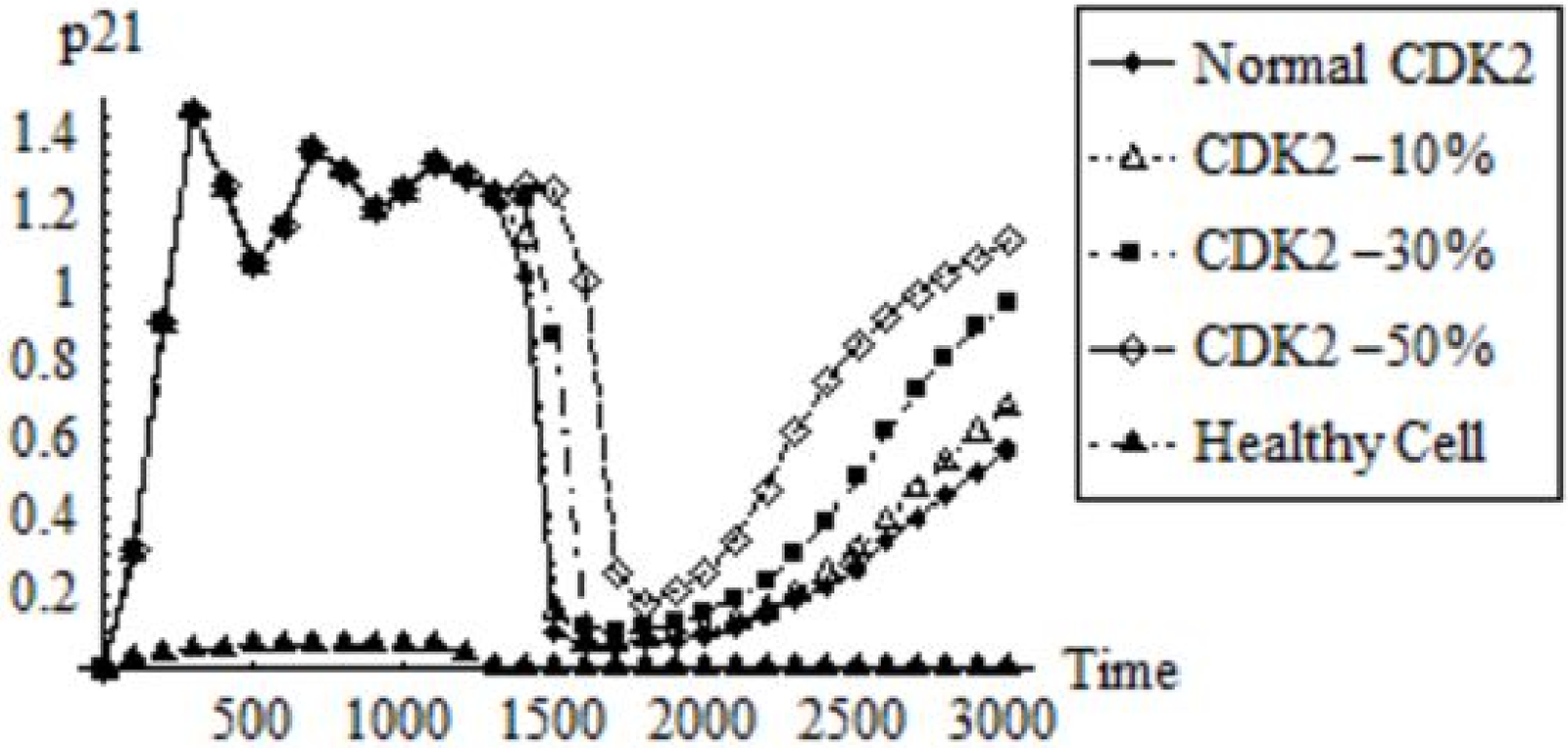
Fig.7 The probability β of a damaged cell passing G1/S checkpoint for only changing CDK2 or CKIs (i.e. p21 degradation rate and p27 initial condition) for three different ranges of normal, 30% and 50% - specifically, CKD2 or p21 degradation rate reduced, or p27 initial condition increased by 0%, 30% and 50%, respectively - using the behavior of CycE for parameter range ±30% under different DNA-damage conditions: low level DNA-damage and high level DNA-damage

Fig.8 The probability β of a damaged cell passing G1/S in response to simultaneously changing CDK2 and CKIs (i.e. p21 degradation rate and p27 initial condition) for three different ranges of normal, 30% and 50% - specifically, CKD2 and p21 degradation rate both reduced by 0%, 30% and 50%, respectively, or CDK2 reduced and p27 initial condition increased by 0%, 30% and 50%, respectively - using the behavior of CycE for parameter range ±30% under two different DNA-damage conditions.

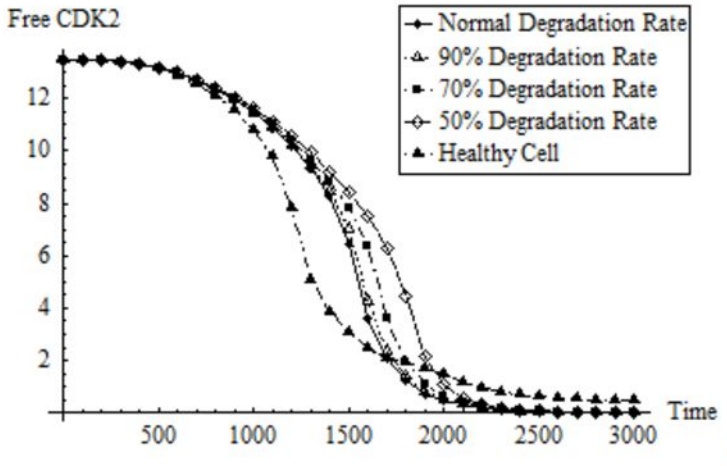

CycD
(x0)
CDK4/6
(x3)
p21/CycD/CDK4/6-P
(x15)
p27/CycA/CDK2-P
(x13)
DNA damage signal
p27
(x10)
p21
(x14)
p53
(x24)
I
(x27)
CycD/CDK4/6-P
(x5)
p27/CycD/CDK4/6-P
(x11)
Mdm2
(x25)
CycE/CDK2-P
(x7)
p21/CycE/CDK2-P
(x16)
p16
(x18)
p27/CycE/CDK2-P
(x12)
p21/CycA/CDK2-P
(x17)
CycE/CDK2
(x6)
Rb/E2F
(x19)
Rb-PP/E2F
(x20)
CycA/CDK2-P
(x9)
CycA/CDK2
(x8)
Rb
(x23)
Rb-PPPP
(x22)
E2F
(x21)
X
(x26)
CycE
(x1)
CDK2
(x4)
CycA
(x2)
Reversible
Reversible
Activation
Inhibition


p21
1.75
1.5
1.25
1
0.75
0.5
0.25
500
1000
1500
2000
2500
3000
Time
Normal CDK2
CDK2 – 10%
CDK2 – 30%
CDK2 – 50%
HealthyCell

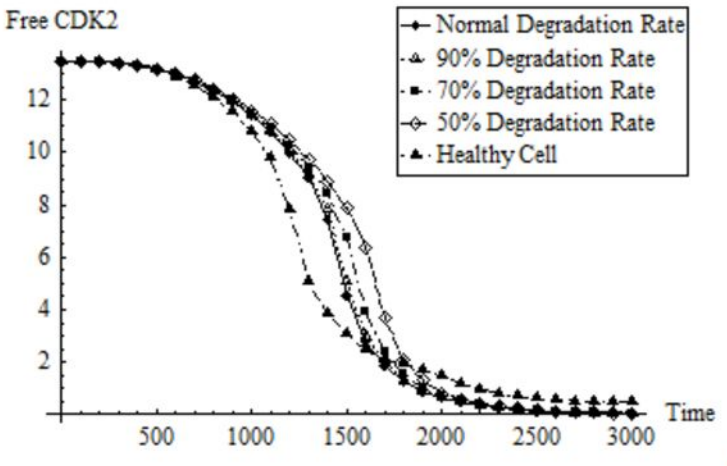
(a)


p21
1.4
1.2
1
0.8
0.6
0.4
0.2
500
1000
1500
2000
2500
3000
Time
Normal CDK2
CDK2 –10%
CDK2 –30%
CDK2 –50%
Healthy Cell

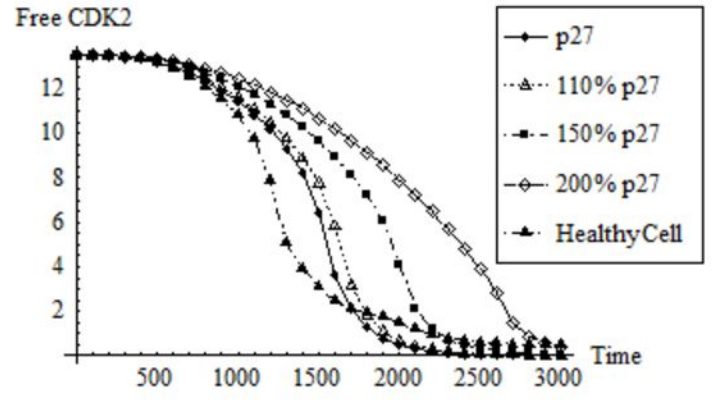
(b)


Free CDK2
Normal Degradation Rate
90% Degradation Rate
70% Degradation Rate
50% Degradation Rate
Healthy Cell
12
10
8
6
4
2
500
1000
1500
2000
2500
3000
Time

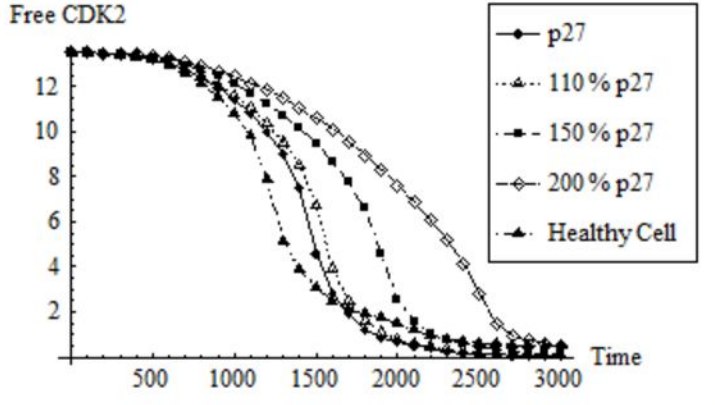
(a)


Free CDK2
Normal Degradation Rate
90% Degradation Rate
70% Degradation Rate
50% Degradation Rate
Healthy Cell
12
10
8
6
4
2
500
1000
1500
2000
2500
3000
Time

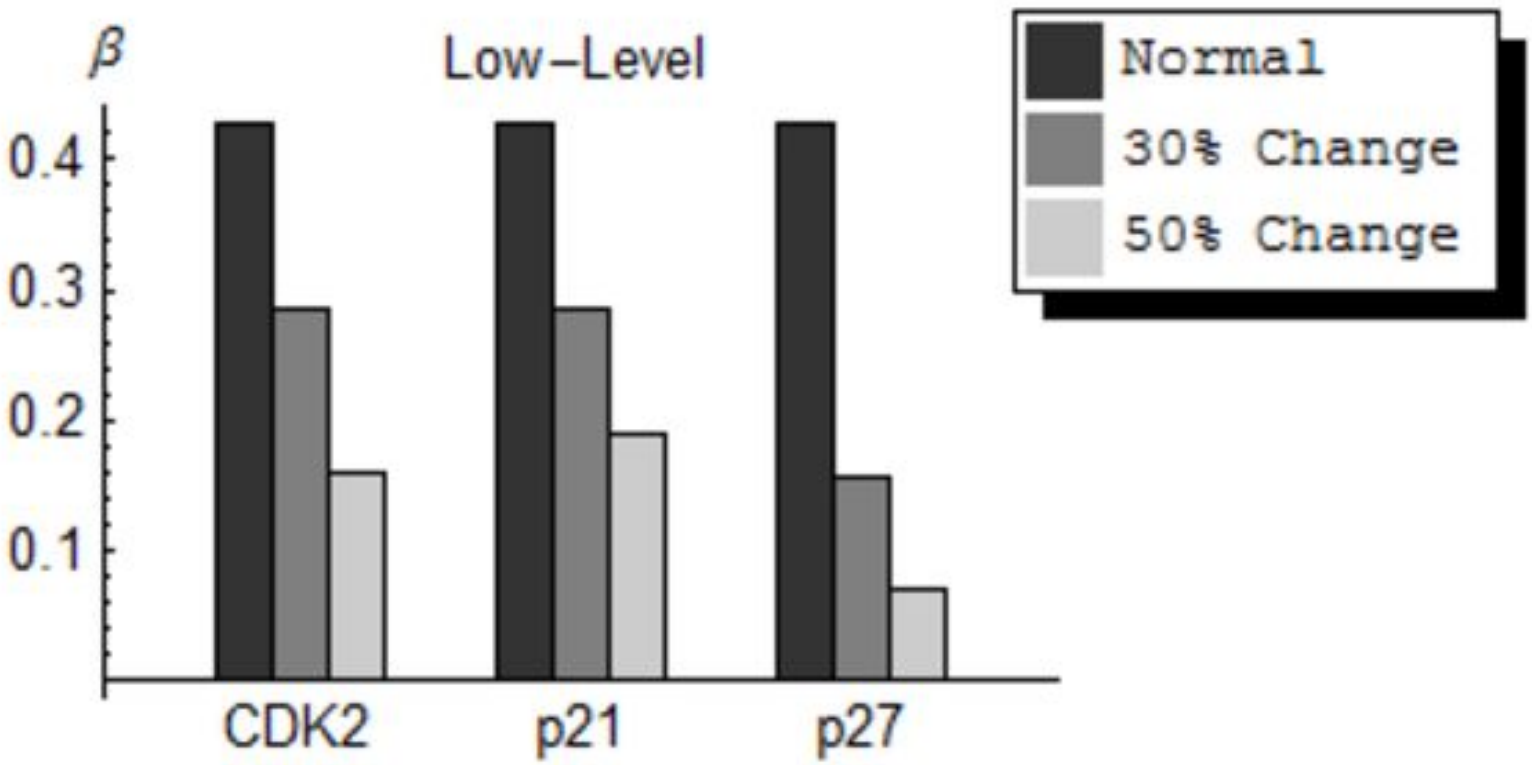
(b)

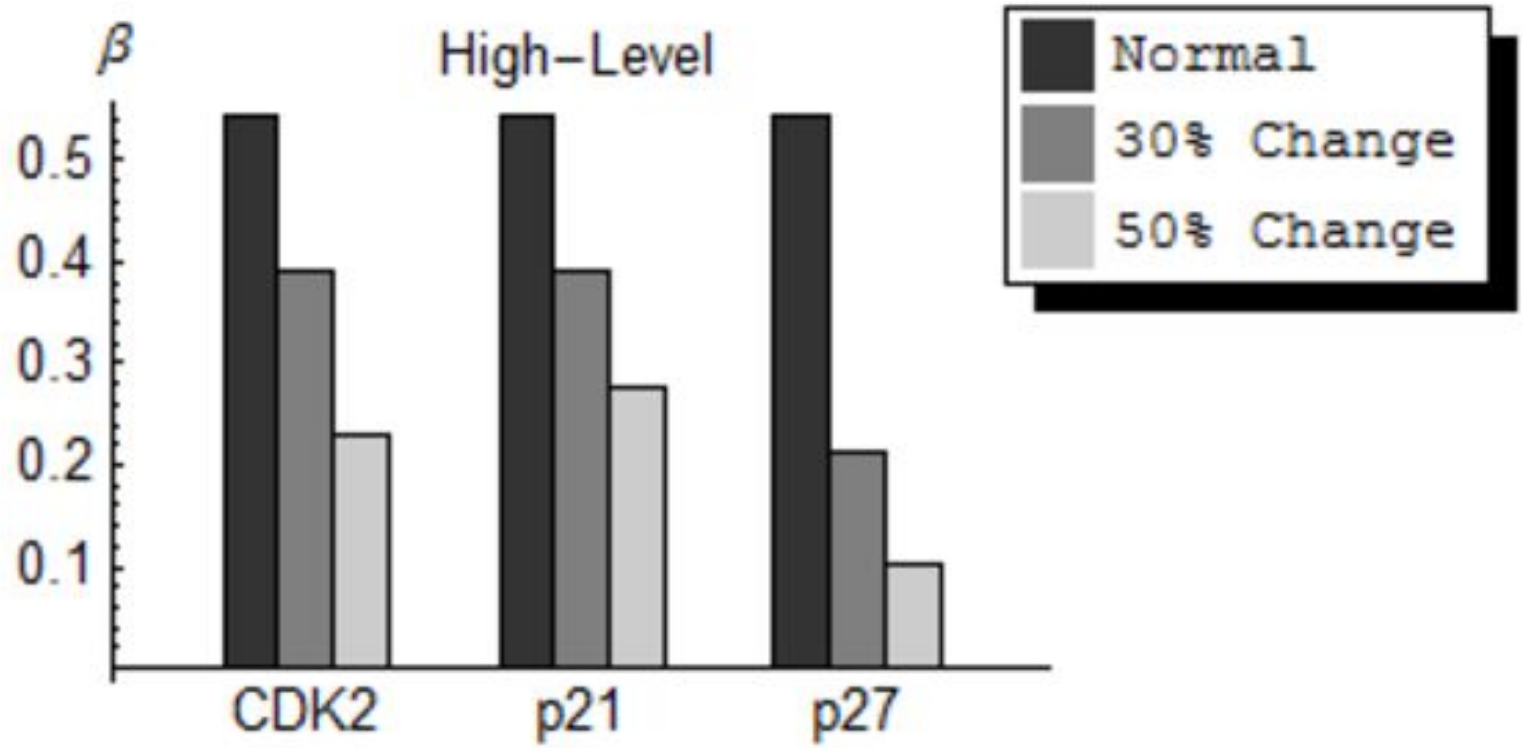

Free CDK2
p27
110% p27
150% p27
200% p27
Healthy Cell
12
10
8
6
4
2
500
1000
1500
2000
2500
3000
Time

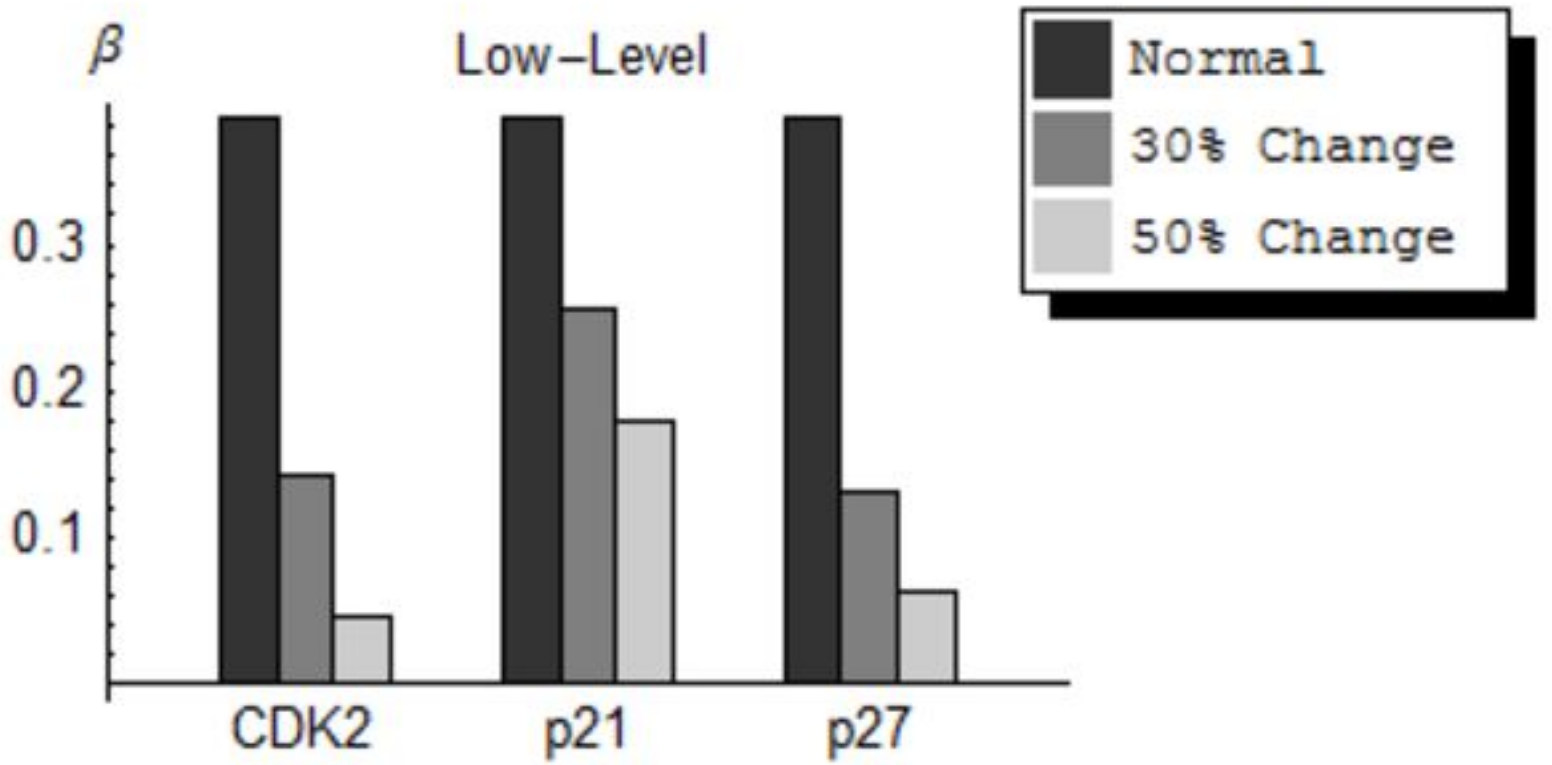
(a)

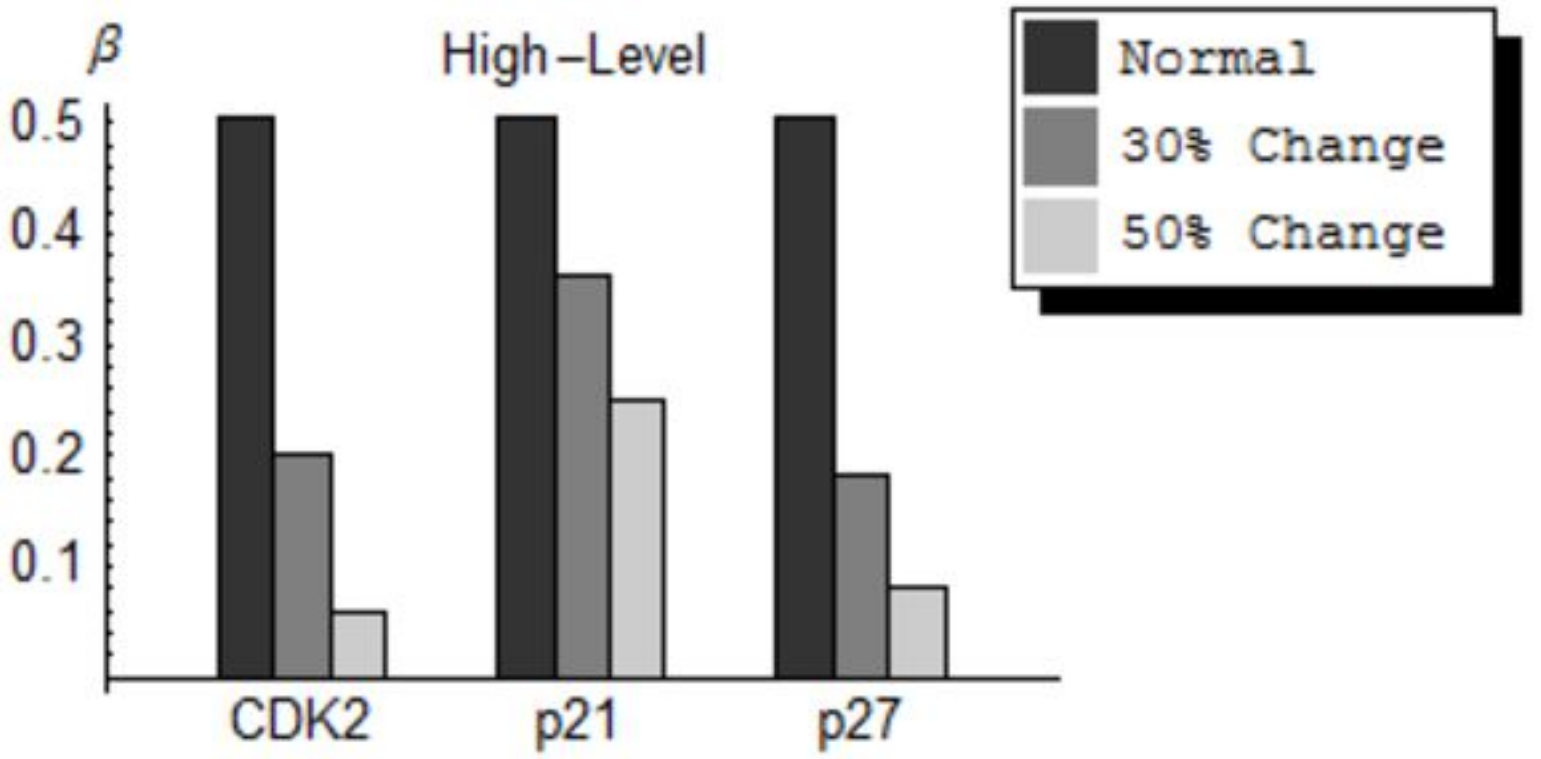

Free CDK2
p27
110% p27
150% p27
200% p27
Healthy Cell
12
10
8
6
4
2
500
1000
1500
2000
2500
3000
Time

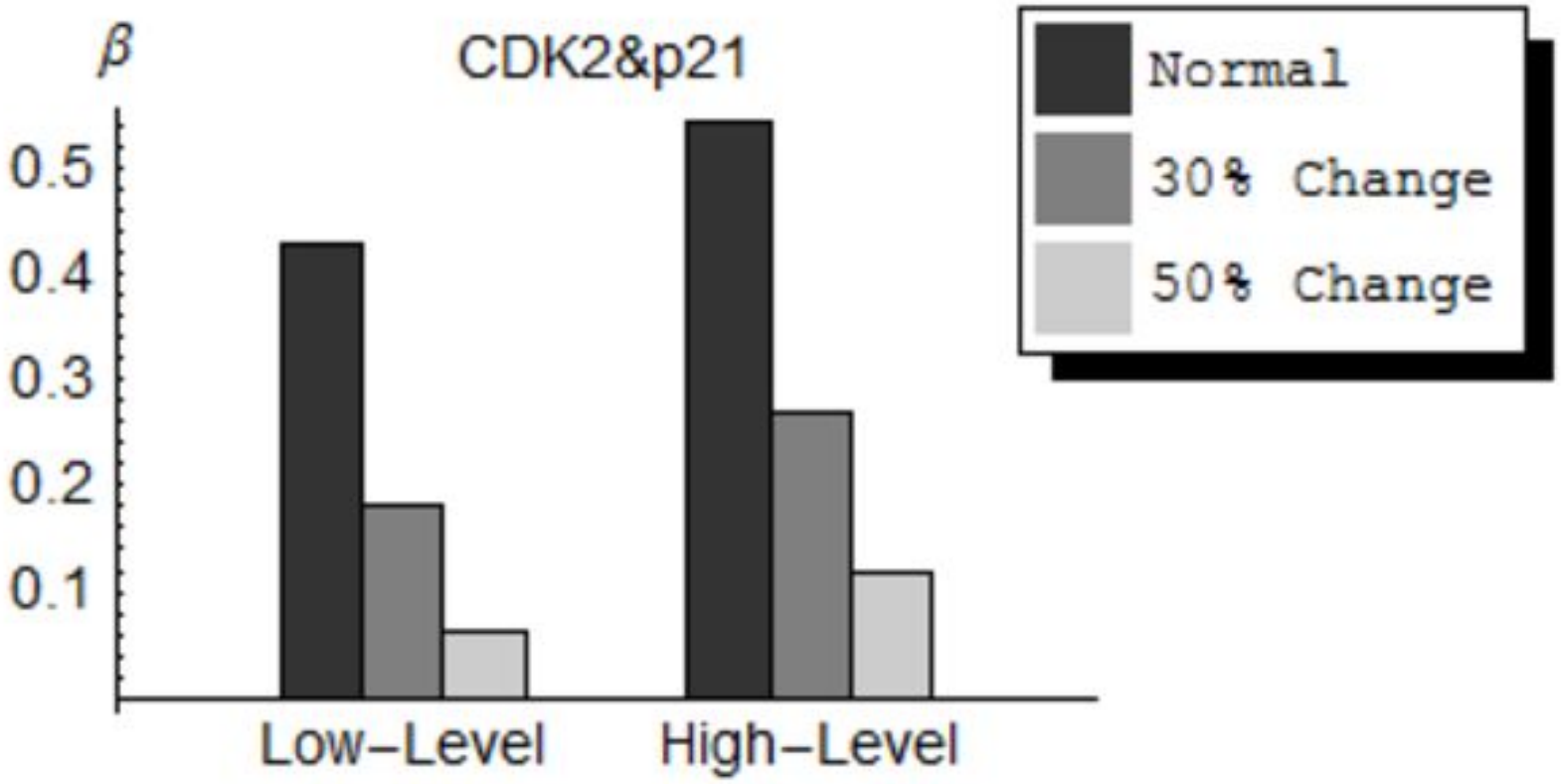
(b)

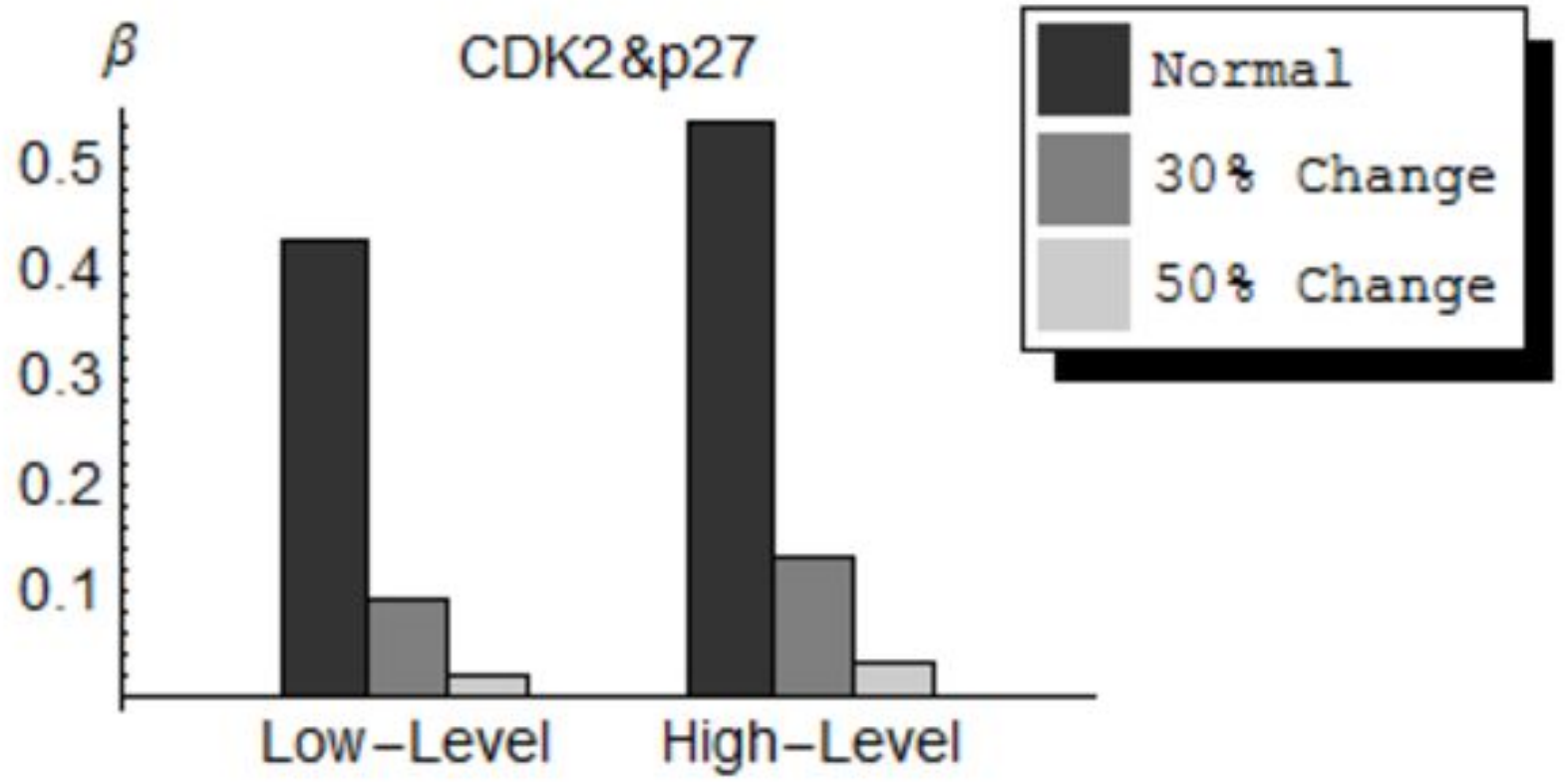

β
Low−Level
0.4
0.3
0.2
0.1
CDK2
p21
p27
Normal
30% Change
50% Change

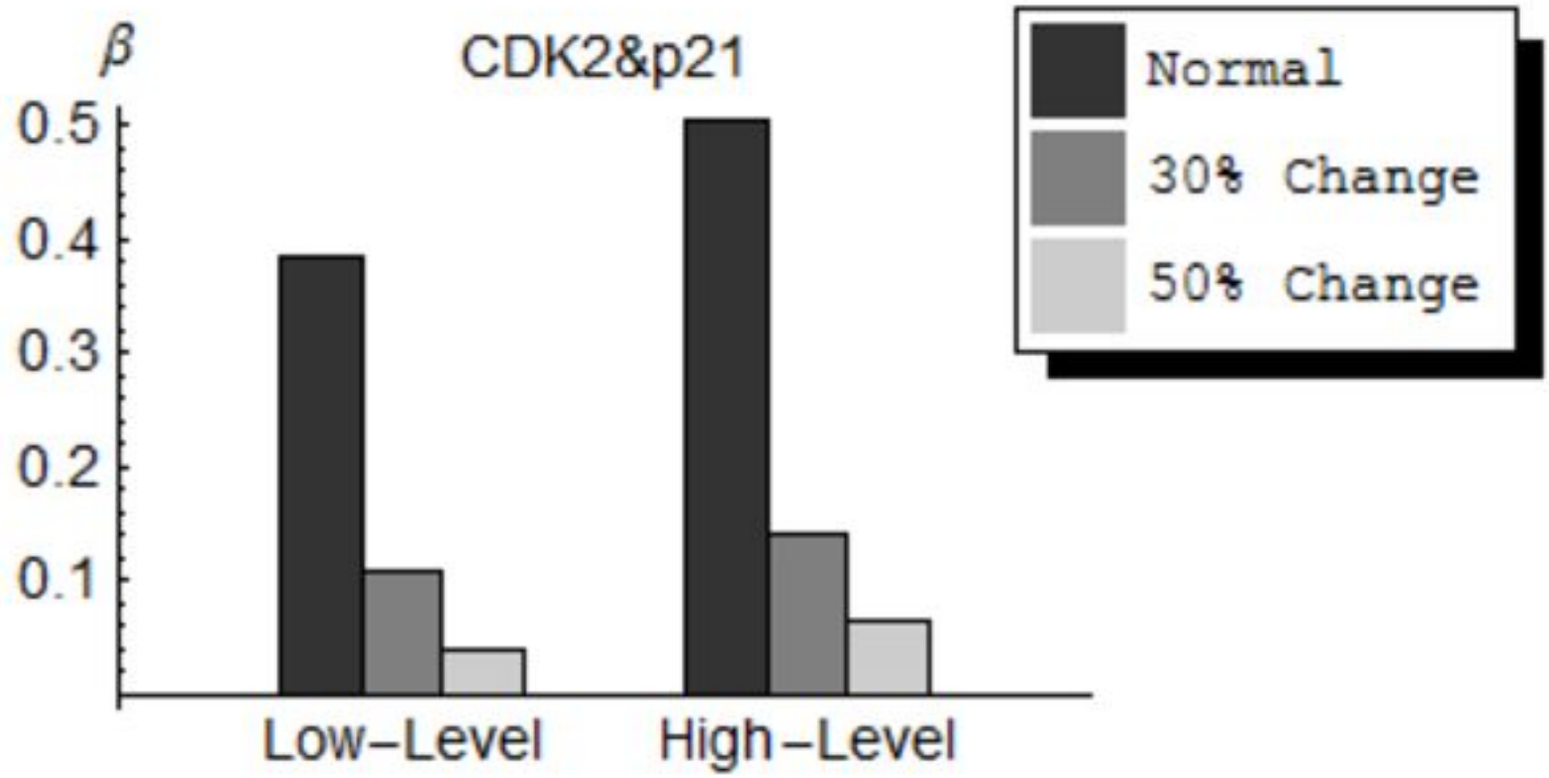

β
High−Level
0.5
0.4
0.3
0.2
0.1
CDK2
p21
p27
Normal
30% Change
50% Change

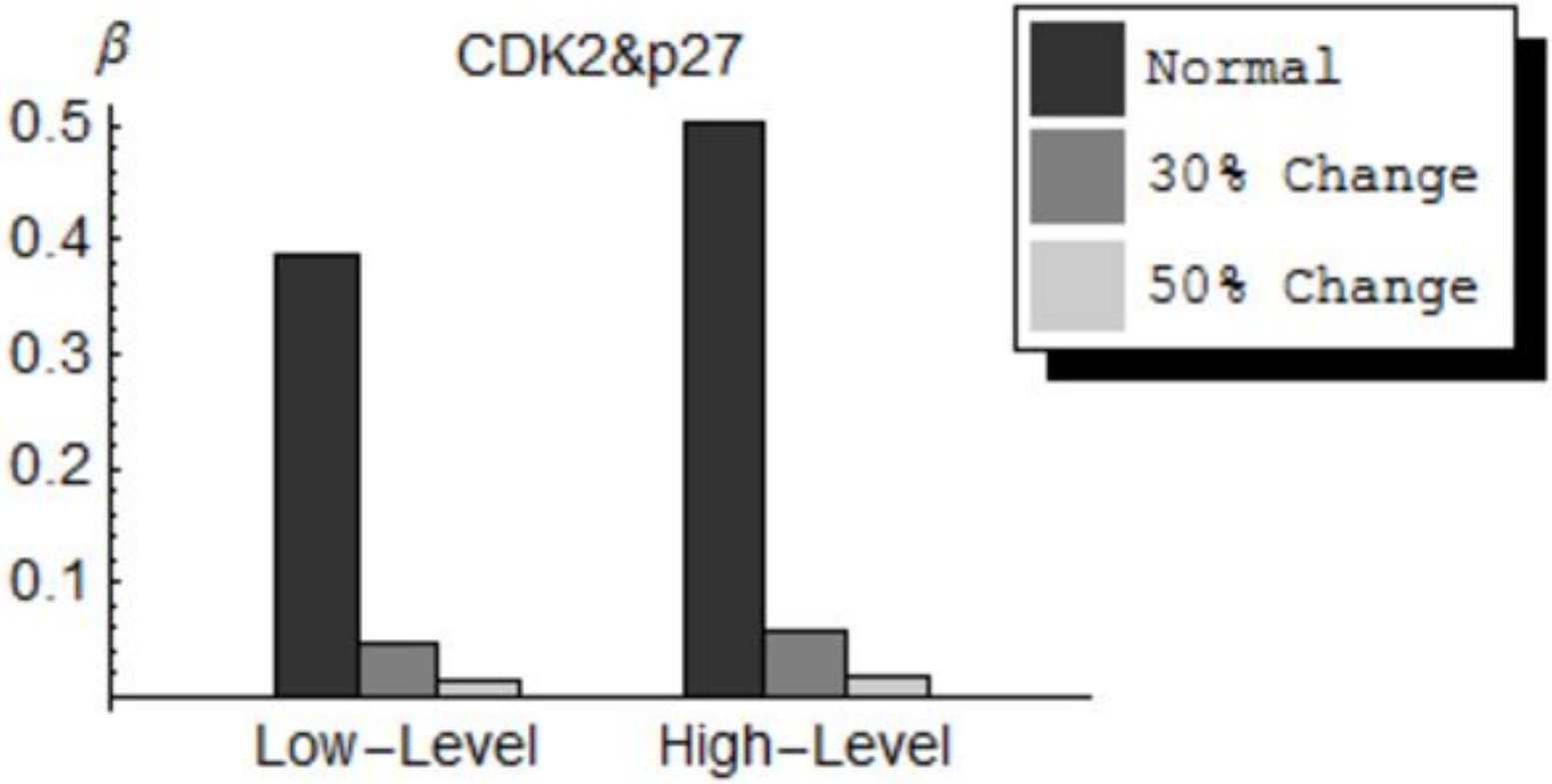

β
Low−Level
0.3
0.2
0.1
CDK2
p21
p27
Normal
30% Change
50% Change


β
High−Level
0.5
0.4
0.3
0.2
0.1
CDK2
p21
p27
Normal
30% Change
50% Change


β
CDK2&p21
0.5
0.4
0.3
0.2
0.1
Low−Level
High−Level
Normal
30% Change
50% Change


β
CDK2&p27
0.5
0.4
0.3
0.2
0.1
Low−Level
High−Level
Normal
30% Change
50% Change


β
CDK2&p21
0.5
0.4
0.3
0.2
0.1
Low−Level
High−Level
Normal
30% Change
50% Change


β
CDK2&p27
0.5
0.4
0.3
0.2
0.1
Low−Level
High−Level
Normal
30% Change
50% Change