Robustness of CDK2 in Triggering Cellular Senescence Based on Probability of DNA-Damaged Cells Passing G1/S Checkpoint

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***Abstract*—Recent experiments have shown that cellular senescence, a mechanism employed by cells for thwarting cell proliferation, plays an important role in protecting cells against cancer; therefore, a deeper understanding of cellular senescence can lead to effective cancer treatment. Inhibition of CDK2 is thought to be the critical trigger for cellular senescence. In this study, we first implement a mathematical model of G1/S transition involving the DNA-damage pathway and show that cellular senescence can be achieved by lowering CDK2. The robustness of CDK2 in triggering cellular senescence is determined from the probability (β) of DNA-damaged cells passing G1/S checkpoint for normal CDK2 and CDK2-deficient situations based on different thresholds of the peak time of two important biomarkers, CycE and E2F. The comparison of the values of β under the normal CDK2 and lower CDK2 levels reveals that reducing CDK2 levels can decrease the percentage of damaged cells passing G1/S checkpoint; more importantly, 50% reduction of CDK2 achieves 65% reduction in the percentage of damaged cells passing the G1/S checkpoint. These results point out that the developed model can highlight the possibility of lowering the bar for cellular senescence by reducing CDK2 levels. The results of investigation of β for the different thresholds of the peak times of other biomarkers show that β is insensitive to these perturbations of the peak time indicating that CDK2 activity is robust in lowering the senescence bar for low and high levels of DNA-damage. Furthermore, a mathematical formulation of robustness indicates that the robustness of CDK2 -triggered senescence increases with decreasing levels of CDK2, and is slightly greater for low-level DNA damage condition.**

Keywords: Robustness; Cellular Senescence; G1/S Checkpoint Pathway; Cancer; Systems Biology

I. INTRODUCTION

Cellular senescence was first described by Hayflick and Moorhead [4] more than four decades ago. They showed that the normal cells entered an irreversible state of cell growth arrest in response to the uncontrolled proliferative capacity of normal cells. According to our current knowledge of cell cycle, cellular senescence can be considered as a physiological mechanism employed by cells for thwarting the proliferation of tumor cells [11]. Serrano [11] aptly points out that "Encouraging cancer-prone cells to senesce might therefore be a way to nip this disease in the bud". Most human and mouse tumour cells stop proliferation and undergo senescence at the pre-malignant stage [3] - the second stage of tumorigenesis- where a non-invasive tumour is formed, indicating that the senescence-inducing signals reach sufficient intensity to be effective only in this stage. This also suggests that much of cells carrying oncogenes are allowed to proliferate in the pre-tumoral stage – the first stage of tumorigenesis- with little or no senescence. This is a response adopted by the organism to deal with the fact that at any given time there are probably millions of cells primed to become cancerous and catching all these at the pre-tumoral stage would be an exhausting task. Therefore, eliminating these cells that tend to become cancerous (pre-malignant stage) is an intelligent response adopted by cells to efficiently address the problem. 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 plays an important role in exploiting this route for effective cancer treatment.

Both Lin et al. [8] and Campaner et al. [1] found that the inhibition of activity of the 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, inhibition of CDK2 activity is a critical factor for lowering the bar for 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. Although there is some experimental evidence pointing out that the inhibition of CDK2 can be the critical trigger for senescence, currently, there aren't any mathematical models developed to highlight the cellular senescence under DNA damage situations in the literature. This phenomenon results in a more exciting question: can a mathematical model highlight the cellular senescence and formulate scenarios for adjusting the threshold for senescence to evaluate its efficacy, outcomes and robustness? This paper is the first attempt to address this question.

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The robustness of biochemical pathways is crucial to the very existence of healthy cells, and the concept of robustness of living organisms has been discussed in many papers (see Kitano [6] and Stelling et al. [12], for example). We define the robustness accordingly as an emergent systemic property reflecting the ability of a system to sustain the functionality amidst internal and external perturbations and uncertainties. The robustness is a broader concept than stability and homeostasis; and the robustness of subsystems is essential for homeostasis of the whole system [6]. Therefore, any investigation into the robustness of complex checkpoint pathways as subsystems for the systemic robustness may begin with the identification of a performance measure(s) and a limited number of key proteins (biomarkers), which are the major indicators of proper functioning of the system, in a biologically meaningful manner. By perturbing the kinetic parameter space associated with the biomarkers and investigating their effects on the performance measures would give us information on the robustness of the system. However, this approach would be very difficult to implement *in vivo* or *in vitro* justifying the use of mathematical models for the purpose.

The purpose of this paper is to elucidate an approach to answer the question of lowering senescence and its robustness based on a mathematical model of G1/S cell cycle checkpoint pathway incorporating DNA-damage signal transduction. More specifically, we compute the probability (β) of DNA–damaged cells passing the G1/S phase transition in the presence of various levels of perturbations to the key kinetic parameters associated with the model in response to reduced CDK2 levels to ascertain if the CDK2 can lower the threshold for senescence. Furthermore, we analyse the robustness of the CDK2 in lowering senescence bar according to the investigation of β under different thresholds (perturbations) of the peak time of the biomarkers; and more importantly, a mathematical formulation is generated to simulate the robustness of CDK2 in triggering cellular senescence.

II. METHODOLOGY

The latest mathematical model of the G1/S phase transition was published in 2008 by Iwamoto et al. [5], which simulates the G1/S transition incorporating the DNA damage signal transduction pathway based on the biological findings through effectively combining Tashima et al.[13] G1/S phase transition model and Lev Bar-or et al.[7] DNA damage signal transduction model. Tashima et al. [13] focus on the chemical interactions among the biochemical species of the G1/S transition and their dynamic behaviour, while Lev Bar-or et al. model [7] focusses on the dynamic behaviour of the DNA signal transduction mechanism, which is mainly based on the damped oscillation of p53 and Mdm2. In Iwamoto et al. [13] 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). Each ordinary differential equation represents the change of concentration of individual molecular species over time

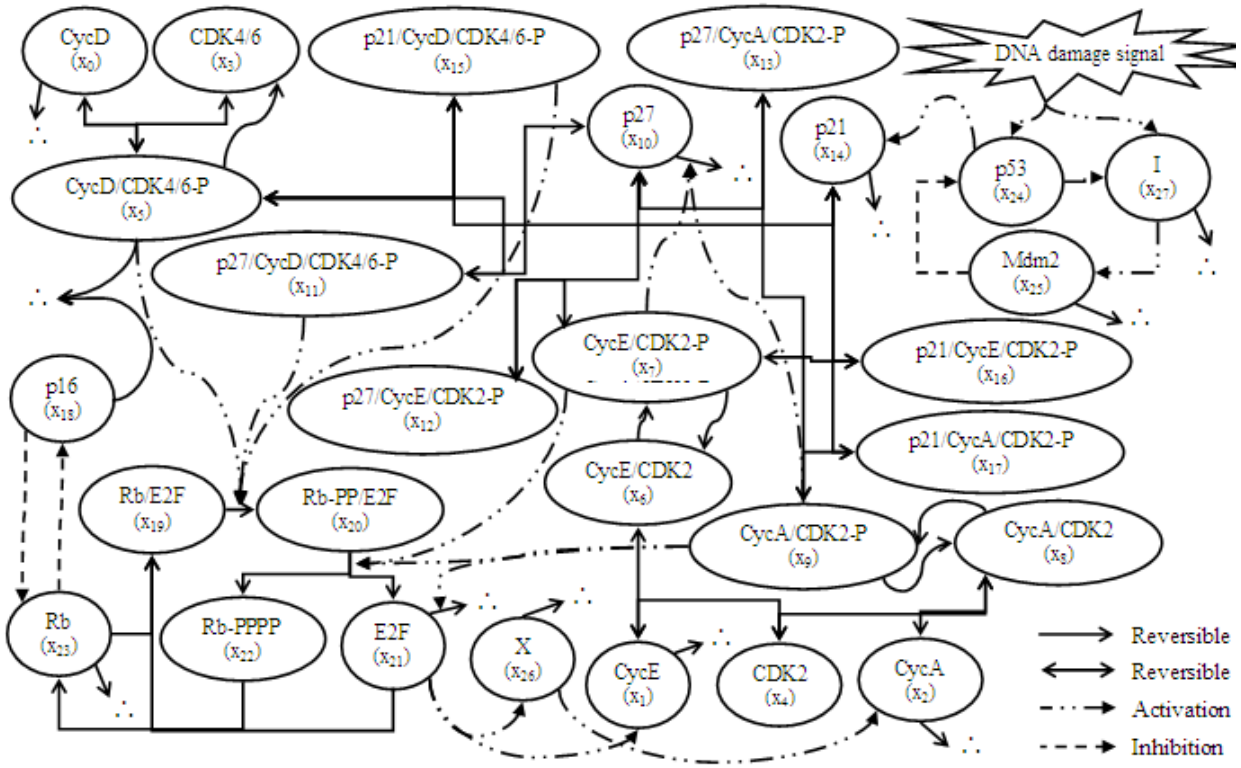

Fig. 1 Schematic diagram of the structure of the G1/S checkpoint pathway involving the DNA damage signal transduction pathway. The molecular components in the systems and their corresponding mathematical symbols used in the equations are shown in our earlier work (Ling et al. [9]).

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based on chemical kinetic theory. For example, the change of concentration of CDK4/6 can be written as:

$$\frac{dx_3}{dt} = k_4 x_5 + k_{13} x_5 - (k_3 x_0 x_3) \quad (1)$$

where x_0, x_3, x_5 represent CycD, CDK4/6 and CycD/CDK4/6, respectively; k_3 is association rate of CycD/CDK4/6; k_4 is dissociation rate of CycD/CDK4/6 and k_{13} is the rate of CDK4/6 production through CycD/CDK4/6. The change of concentration of the remainder of the participating molecular species in the G1/S transition can be written the same way as above. Under the DNA-damage situation, p53 is activated by the damaged signal through ATM, and then p53 promotes the synthesis of p21 to inhibit the activation of the complex Cyc/CDK by binding to these complexes for a blockage of cell cycle progression. In this study, we use the equation $signal = DDS \times \exp(-k_{72} t)$ to represent kinetics of the damage signal for p53 activation. We assume an initial pulse of signal DDS (for example: low and high-level damage) representing a short exposure of cells in response to DNA-damage, and then the signal is subsequently resolved by cellular mechanisms of DNA damage repair with a constant parameter k_{72} to keep the model as simple as possible. More importantly, all proteins crucial for triggering senescence are involved in the G1/S transition.

A. Effectiveness and Robustness of CDK2 in Triggering Cellular Senescence

In this study, we focus on the probability (β) of a DNA-damaged cell passing the G1/S phase transition in the presence of various levels of perturbation in the key kinetic parameters associated with the model in response to normal and CDK2-deficient (senescence triggering) situations. In order to achieve this, five important steps were followed:

(1) Choice of biomarkers and Local Sensitivity Analysis (LSA): we first decided on the important biomarkers for the G1/S transitions and selected E2F and CycE (In this case, we focus on the peak time of two selected biomarkers: the time for biomarkers at their maximum concentration. The maximum of E2F indicates the synthesis of CycE and CycA as two regulatory elements of the cell cycle, while the maximum of CycE is an important indicator for cells' progression into S phase); 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 LSA where the effect of each parameter was assessed while holding the other parameters at constant values (out of 75 parameters, we identified 8, 15 and 14 significant parameters for E2F, and 10, 17 and 16 significant parameters for CycE for the three DNA-damage conditions, respectively);

(2) Global Sensitivity Analysis (GSA): In real biochemical systems, rate constants of biochemical reactions *in vivo* seem to simultaneously vary based on the external environment. These factors indicate that GSA is more suitable and appropriate for sensitivity analysis of biochemical systems and reactions [10]. In terms of GSA in this investigation, we calculated the probability density function (PDF) of the peak time (PT) of the two biomarkers (E2F and CycE) in response to various levels of perturbations to the selected significant kinetic parameters through a GSA where these significant parameters for the two chosen biomarkers are varied simultaneously under different DNA damage situations;

(3) Evaluation of β: Statistical Hypothesis Testing with Type II Error: based on the PDF of PT from GSA, we computed β or power (1-β) using statistical hypothesis testing-*Type II error*, where β in this investigation indicates the probability of a damaged cell passing the G1/S checkpoint pathway (More details of steps 1-3 can be found in Ling et al. [9]).

(4) Analysis of the Effectiveness CDK2 in Lowering the Bar for Cellular Senescence: the subject of this paper begins with this aspect where we use the same parameter set to calculate the values of β only changing CDK2 levels (normal level and three reduced CDK2 levels: -10%, -30% and -50%) for two DNA damage situations (low and high) by repeating steps 2 and 3. 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 indicates the possibility of cellular senescence and supports the hypothesis that lowering CDK2 is an effective means of promoting senescence in damaged/cancerous cells.

(5) Robustness of CDK2 in Lowering the Bar for Cellular Senescence: the final part of the paper is concerned with the robustness of CDK2 in triggering senescence, and there are two different approaches to investigate robustness of CDK2:

i) Investigation of β for Different Thresholds of peak time (PTs ±10% ***and*** ±20%***) of Biomarkers (E2F and CycE):*** we calculate the values of β for the four different thresholds (perturbations) of PT of the two biomarkers under the given parameter perturbation regimes for two different DNA-damage situations in response to different reduced CDK2 levels. In general, PT of proteins is the focus of most research studies. However, in this study of robustness, the behaviour close to the PT of proteins is considered an important indicator of robustness of the CDK2 in lowering senescence bar; hence the reason for choosing different thresholds to perturb the PT. If β is insensitive to the perturbations of PT, this indicates that CDK2 activity is robust in lowering the senescence bar.

ii) Generating the Mathematical Formulation according to the discussion of Kitano [6], we define the robustness of CDK2 in triggering cellular senescence based on the probability of DNA-damaged cells passing G1/S checkpoint as follows:

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$$R = \sum_i \chi(\Delta p_i)(1-\beta_i)|\Delta p_i| \qquad (2)$$

where R is robustness, $\chi(\Delta p_i)$ is the probability of a particular perturbation occurrence, Δp_i is perturbation of i^{th} significant biomarker variable, and $(1-\beta_i)$ is the performance measure indicating the level of activation (effectiveness) of senescence through the reduced CDK2 levels. In our case, we have two biomarker variables – E2F and CycE (meaning that i is equal to two) – whose PTs were perturbed.

III. RESULTS AND DISCUSSION

A. *Effectiveness of CDK2 in Lowering the Bar for Cellular Senescence according to PTs of Biomarker*

TABLE 1 summarizes the probability of a damaged cell passing the G1/S checkpoint based on the PDF of PT of E2F and CycE under two DNA damage levels for the normal CDK2 level. The results show that the percentage of tumor cells passing G1/S increases with the level of DNA damage and the results for the two biomarkers show similar trends for a range of parameter perturbations. 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 ±50% perturbations to the key parameters. Our simulation results according to TABLE 1, in terms of the percentage of damaged cells that pass G1/S checkpoint, agree with Collado et al. [2] finding and Serrano's [11] assertion: a large number of damaged cells undergo proliferation without being caught at the 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 the cell division without having to wait for premalignant cellular senescence in order to catch damaged cells early in stage as happens normally. 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 for the behaviour of E2F and CycE under reduced and normal CDK2 levels, the probability of a damaged cell passing the G1/S checkpoint have decreased with inhibiting the 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.

Compared to TABLE1, TABLE 2 indicates that for the

TABLE 1 The probability (β) of damaged cells passing G1/S checkpoint for two DNA damage levels (Reference values indicate the standard parameter values)

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

TABLE 2 The probability of a damaged cell passing G1/S for three reduced CDK2 levels under different DNA-damage situation (based on PT of E2F)

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%	0.0018	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 under different DNA-damage situations (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

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same high-level DNA- damaged condition and parameter ranges, the percentage of damage cells passing G1/S based on E2F can be reduced from 68.9% to 42.2% by decreasing the CDK2 levels by 50%. This amounts to a reduction of 38.8%. For the low-level DNA-damage, lowering CDK2 by 50% amounts to 42.7% reduction in the damaged cells passing the G1/S checkpoint. The above observed trend is confirmed by the trends in the PDF of PT of CycE. 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 condition, 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' [1] 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 on the reduction of the percentage of damaged cells passing the G1/S checkpoint.

B. Robustness of the CDK2 Triggered Lowering of the Cellular Senescence Bar with respect to Different Thresholds (perturbations) (PT ±10% and ±20%) of Biomarkers

We also evaluated the probability (β) of damaged cells passing the G1/S checkpoint for four different thresholds of PT of the activity of the two biomarkers, E2F and CycE (PT ±10% and PT ±20%), under different DNA-damage situations in response to different reduced CDK2 levels. TABLE 4 displays the range of value of β based on the PDF for the four different thresholds of PT of E2F under different DNA-damage situations in response to different reduced CDK2 levels. According to TABLE 4, the results for the different thresholds are quite similar to each other, and similar to the results based on the PDF of PT of E2F in TABLE 2. As for β for the different thresholds of PT of CycE under different DNA-damage situations and different reduced CDK2 levels, results for β in TABLE 5 follow exactly the same trend as that shown by E2F: that β is not affected by the perturbation in PT up to ±20% indicating the robustness of lowering the senescence bar through reduced CDK.

C. Robustness of CDK2 in Triggering Cellular Senescence Based on the Mathematical Definition

Since the changes in threshold values of PTs of the two biomarkers would not change β_i significantly as shown in TABLES 4 and 5, we ignore the variations in PT. Therefore, we assume that the probability of a perturbation is equally likely (i.e. $\chi(\Delta p_i) = 1/2$). For example, if we have a total of n such perturbations, $\chi(\Delta p_i) = \frac{1}{n}$. The robustness, calculated according to the mathematical definition of robustness of CDK2 in triggering cellular senescence in the methodology section, for low- and high- level DNA-damage situations is shown in Fig. 2 Results in Fig. 2 indicate that the robustness

TABLE 4 The range of the probability (β) of a damaged cell passing G1/S based on the PDF for four different thresholds of PT (PT-20%, PT-10%, PT+10% and PT+20%) of E2F under different DNA-damage conditions in response to three reduced CDK2 levels

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%	0.0018~ 0.0019	0.0002	0	0.015~ 0.016	0.0019~ 0.002	0
Reference Values ± 20%	0.178~0.18	0.097~ 0.098	0.035~ 0.037	0.311~ 0.312	0.185~ 0.186	0.064
Reference Values ± 30%	0.39	0.287~ 0.288	0.159	0.5	0.389~0.39	0.229~0.23
Reference Values ± 50%	0.606	0.515	0.203	0.66	0.413	0.422

TABLE 5 The range of the probability (β) of a damaged cell passing G1/S based on the PDF for four different thresholds of PT (PT-20%, PT-10%, PT+10% and PT+20%) of CycE under different DNA-damage situations in response to three reduced CDK2 levels

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~ 0.087	0.0166~ 0.0169	0.0032	0.172~ 0.175	0.034	0.0037~ 0.0038
Reference Values ± 30%	0.301~ 0.302	0.14~ 0.141	0.0453~ 0.0456	0.41~0.43	0.202~ 0.203	0.059~ 0.06
Reference Values ± 50%	0.5137~ 0.514	0.362	0.203	0.57	0.413~ 0.414	0.233~ 0.234

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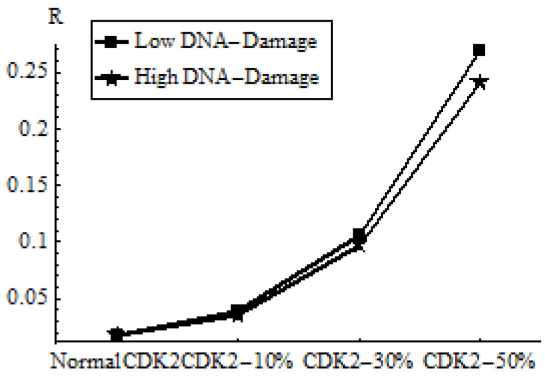

Fig. 2 Robustness of CDK2 in triggering cellular senescence based on the probability of DNA-damaged cells passing G1/S checkpoint

of CDK2 in triggering cellular senescence under low-level DNA-damage situation is slightly higher than that under high-level DNA-damage situation; especially for greater reductions in CDK2 levels. This means that the system under low-level of DNA damage is slightly robust than for high-level DNA-damage with regards to the reduced CDK2 level triggering cellular senescence against various levels of perturbations in the key kinetic parameters associated with the model. The possible reason is that senescence is mainly triggered under the low-level DNA damage situation not the high-level DNA damage, but this idea should to be validated by biological experiments. Fig. 2 indicates the possibility that if we further reduce CDK2 levels, difference in robustness of CDK2 in triggering cellular senescence between low-level and high-level DNA-damage could become larger. As expected, for both DNA-damage situations, the robustness of CDK2 in triggering cellular senescence increases with reduced CDK2 levels.

IV. 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 through reduced CDK2 levels. More importantly, we investigated robustness of the above CDK2 induced senescence by studying its influence on the PTs of the chosen biomarkers (E2F and CycE), which we used to assess the change in the probability β of a damaged cell passing the G1/S checkpoint. These results were then represented in a mathematical formulation of the robustness of CDK2-triggered lowering of senescence threshold.

The results of β based on the PDF of PTs of E2F and CycE in the presence of four predefined levels of parameter range under two DNA-damage situations in response to three reduced CDK2 levels (CDK2-10%, CDK2-30% and CKD2-50%) revealed that reducing CDK2 levels can reduce the percentage of damage cells passing the G1/S checkpoint, indicating that CDK2 can be the target for triggering cellular senescence to bring forward the entry of damage cells into an irreversible state of cell growth arrest and prevent proliferation of cancerous cells. More specifically, 50% reduction of CDK2 can reach 65% reduction in the percentage of damage cells passing the G1/S checkpoint. These results point out that the mathematical model can highlight the possibility of lowering the bar for cellular senescence by reducing CDK2 level.

In terms of the investigation on robustness of CDK2 in triggering cellular senescence, results of the values of β of damaged cells passing the G1/S checkpoint for four different thresholds of PT of the two chosen biomarkers revealed that β was not affected by the changes in PT up to ±20% indicating that CDK2 activity is robust in lowering the senescence bar. According to the mathematical definition of robustness, results pointed out that the robustness of CDK2 in lowering the cellular senescence bar increased with the reduced CDK2 levels for both DNA-damaged situations. However, the robustness of CDK2 in triggering cellular senescence under the low-level of DNA- damage is slightly higher than that under the high-level of DNA-damage.

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