



Research article

Dynamic behavior of the p53-Mdm2 core module under the action of drug Nutlin and dual delays

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Abstract: Nutlin is a family of p53-targeting drugs. It is able to bind to Mdm2, thereby accelerate the accumulation of p53 that is a prominent tumor suppressor. An integrated module of the Nutlin PBK and p53 pathway is composed of positive feedback mediated by Mdm2 mRNA as well as the drug Nutlin and negative feedback mediated by Mdm2 protein. The main research content of our paper is how the time delay of protein synthesis, response time delay of Nutlin drug, the degradation rate of Mdm2, the degradation rate of p53 depended on Mdm2 and the actual dose of Nutlin in the cell affect the oscillatory behavior caused by Hopf bifurcation in the integrated network system of Nutlin PBK and p53 pathways. The stability of the unique positive equilibrium point and the existence of Hopf bifurcation are studied by taking the time delays as the bifurcation parameters and applying bifurcation theory. Based on the normal form theory and central manifold theorem, explicit criteria to determine the Hopf bifurcation direction and stability of the bifurcated periodic solution are established. It is found that the time delays and key parameters in the integrated network system of Nutlin PBK and p53 pathways play an important role in the amplitude and period of p53 oscillation according to the results from the numerical simulation and theoretical calculation. These results may provide us with a better understanding of the biological functions of the p53 pathway and some clues for cancer treatment.

Keywords: p53 signal pathway; drug Nutlin; time delays; Hopf bifurcation

1. Introduction

p53 protein is one of the most important tumor inhibitors, whose mutation is contained in more than 50 percent of human cancer cells [1, 2]. It has been proved that the wild-type p53 is able to block the activation of oncogenes in vitro and inhibit the development of tumor cells [3]. Current evidence has suggested that the p53 signaling pathway is one of the promising and effective strategies to treat cancer [4]. In response to stress stimulation or DNA damage, the key functions of p53 protein are the promotion of DNA repair, cell cycle arrest, apoptosis to prevent the damage inherited to daughter

cells [1, 5]. In addition to activating the transcription of promoters containing homologous binding sites, wild-type p53 can also inhibit the activities of various promoters, and whose expression is positively correlated with the genes of cell proliferation or increased malignant tumor [6]. There are three mechanisms for Mdm2 to inhibit p53. First, activate Mdm2 and trans p53, inhibit the transcriptional activity of p53. Second, the combination of Mdm2 and p53 contributes to the degradation of the proteasome. Third, Mdm2 acts as a p53 ubiquitin ligase (E3), thus it down-regulate the level of p53 protein [2, 7–9]. The reason that we are concerned about Mdm2 is (a) Mdm2 deletion mutants are mortal in early mouse development, and (b) Mdm2 directly regulates the activity and stability of tumor suppressor p53 [2]. The p53 oscillation induced by undamped IR at the single-cell level consists of three subsystems: DNA damage repair module, ataxia-telangiectasia mutation (ATM) switch, and p53-Mdm2 oscillator [10]. Disrupting the interaction between p53 and Mdm2 is considered as a new strategy for the treatment of cancer without p53 mutation [11]. Low expression often occurs in tumors that carry wild-type p53 [12]. This phenomenon is caused by the over-expression of Mdm2, a major competitor of p53 [12]. For example, it has been observed in breast cancer, brain cancer, and lung cancer that when the p14 gene suppresses Mdm2 by isolating Mdm2 in the nucleus, low expression of p53 may occur in tumors that carry wild-type p53 [12]. Binding with viral proteins in infected cells also leads to low expression of p53 [12]. Tp53 mutates in half of all tumors, which is the most common mutation gene found in human tumors, and this also emphasizes the important anti-tumor effect of p53 [10]. Sometimes p53 is referred to as the gene "Guardian" [5, 13].

Nutlins are potent and selective small-molecule antagonists of Mdm2 that in the p53-binding pocket and activate the p53 pathway in cells with wild-type p53, thereby inhibiting the growth of cells and cause cell apoptosis or cell cycle arrest (including aging and quiescence) [14, 15]. It has been proved to be effective against a variety of wild-type p53 tumor cells, including neuroblastoma, osteosarcoma, retinocytoma, and leukemia [15]. Although Nutlin is more effective than RiRNA, this binding affinity cannot change the autoubiquitination effect of Mdm2 [4]. Nutlin has been taken as an activator of p53 in preclinical studies currently [11]. Some studies have shown that Nutlin can be activated independently of the p53 phosphorylation [14]. The structure of Nutlin is similar to SN15 peptide that is bound to Mdm2, which is a competitive inhibitor of p53-Mdm2 interaction [16]. Nutlin activates p53 by releasing p53 from negative control mediated by Mdm2, thus making up for the lack of the upstream signal pathway of p53 [8]. Nutlin is a p53 activator that can induce G1-S and G2-M detection points to protect cell proliferation, but it cannot protect the cells with mutant p53 from the toxicity of steviol cells [17]. However, stable tetraploid can be isolated to clone the cells treated by Nutlin, and clone diploid with higher drug resistance to apoptotic induced by ionizing radiation and cisplatin [18]. Due to understanding the new uses of existing drugs can provide valuable information for the molecular mechanism and also ensured safety, drug relocation has become a new strategy in drug discovery [16]. Nutlin-3a can show the ability to activate p53 in cell culture at a certain concentration (about 5 – 10 μ M) and inhibit tumor growth when oral administration, which confirms the interaction between Nutlin and p53- Mdm2 [19]. In conclusion, Nutlin has a significant anti-tumor effect regardless of the state of the p53 gene, which shows that it brings hope for the treatment of human tumors [15].

Recently, researchers believe that the dynamic system with time delay has more complex behaviors. For example, the time delay can lead to the loss of stability, various oscillations, and

periodic solutions induced by Hopf bifurcation [20]. Scientists have developed a variety of mathematical models, including continuous-time differential equations, discrete-time differential equations, time delays differential equations, and stochastic models [5]. It is worth noting that Lév Bär-Or et al. established an ordinary differential equations (ODEs) model, in which the negative feedback loop between p53 and Mdm2 is taken into account [5]. If the damage can be repaired, the DNA damage will be gradually repaired with the oscillation of p53. It needs to be aware that the form of oscillation is different between single-cell and multi-cell. Concretely, the oscillation in single-cell is basically limit cycle oscillation, while that in multi-cell is damped oscillation [21]. A four-dimensional model [4] is proposed by Häseeb et al, which is mainly integrated by Hünziker's model [2] and Püszynski's model [12]. However, in fact, both protein synthesis and drug response are time-consuming processes that are neglected in the above model. Here, we focus on the collaborative effect of time delays, drug doses, and important biochemical reaction rates on the p53 drug model.

To sum up, the innovation of this paper is mainly reflected in four aspects. First of all, the effect of time delays on the integrated model with Nutlin PBK and p53 pathway is considered. Secondly, the collaborative effects of drug dose of Nutlin and time delay on the dynamic behavior of p53 are studied. Thirdly, the stability of positive equilibrium and the Hopf bifurcation is studied theoretically and numerically by choosing time delays as the bifurcation parameters. Fourthly, according to the results of numerical simulation, the direction of Hopf bifurcation and the stability of the period are determined. Through numerical simulation, it is found that the time delay of protein synthesis can determine the period and amplitude of the oscillation. The dose of the drug and the time delay of Nutlin reaction also affect the p53 signal pathway.

2. Materials and methods

The integrated module of Nutlin PBK dynamics and p53 pathway dynamics is shown in Figure 1. The time delay of gene expression is marked in the diagram, with the red arrow representing degradation, the blue arrow representing generation and the orange arrow representing decomposition. Among them, Mdm2 is the main negative regulator of p53, which can inhibit the activity of p53 that can in turn induce the transcription and translation of Mdm2. In this way a negative feedback loop is formed, which can restrict p53 in an inactive state in the normal cells. Thus blocking-up the negative feedback loop between p53 and Mdm2 is a focus of treat disease. Upon the damage caused by extracellular stress, the activation of p53 plays a vital role by promoting cell cycle arrest or apoptosis of the damaged cell. Nutlin is a specific small molecule antagonist of Mdm2, which can bind to the binding site of Mdm2 and p53, so prevent the binding of Mdm2 and p53. It should be noted that the synthesis of Mdm2 protein is a complex and time-consuming process, which requires a certain amount of time to complete gene expression. Thus, there is always an inevitable time delay between the initiation of transcription and the emergence of fully functional Mdm2 proteins, represented by τ_2 (transcriptional delay) and τ_3 (translation delay), respectively. In addition, a certain amount of time is also need for the module to response to the drug Nutlin, which is denoted as τ_1 .

According to the interactions among the components, Häseeb et al propped the following differential equations (2.1) to design the drug dosage in order to revive p53 activity [4].

$$\begin{cases} \dot{p}(t) = \sigma - p(t)\alpha - k_f p(t)m(t) + k_b c(t) + c(t)\gamma, \\ \dot{m}_m(t) = k_t p^2(t) - m_m(t)\beta, \\ \dot{m}(t) = k_{tl} m_m(t) - k_f p(t)m(t) + k_b c(t) + c(t)\delta - m(t)\gamma - k_{a3} nm(t), \\ \dot{c}(t) = k_f p(t)m(t) - k_b c(t) - c(t)\delta - c(t)\gamma, \end{cases} \quad (2.1)$$

where $p(t)$, $m_m(t)$, $m(t)$ and $c(t)$ are denote the concentration of nuclear-p53, Mdm2 protein, Mdm2 mRNA and p53-Mdm2 complex, respectively.

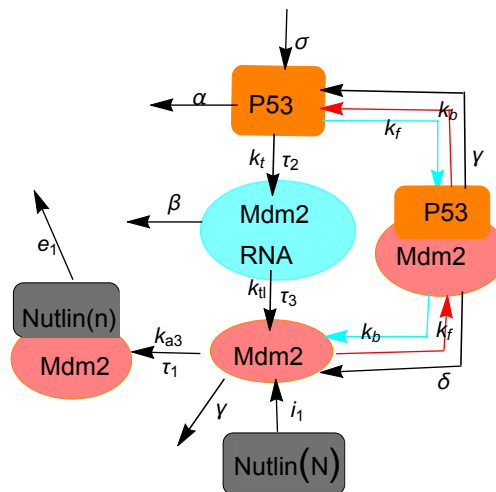


Figure 1. An integrated network diagram of Nutlin PBK and p53 pathway. The red arrows represent degradation, the blue arrows represent formation, and the orange arrows represent dissociation.

In this part, based on the model (2.1), the response time delay of Nutlin drug and the protein synthesis time delay of mdm2 are included in our new model. The new model (2.2) can be shown as follows:

$$\begin{cases} \dot{p}(t) = \sigma - p(t)\alpha - k_f p(t)m(t) + k_b c(t) + c(t)\gamma, \\ \dot{m}_m(t) = k_t p^2(t - \tau) - m_m(t)\beta, \\ \dot{m}(t) = k_{tl} m_m(t) - k_f p(t)m(t) + k_b c(t) + c(t)\delta - m(t)\gamma - k_{a3} nm(t - \tau_1), \\ \dot{c}(t) = k_f p(t)m(t) - k_b c(t) - c(t)\delta - c(t)\gamma, \end{cases} \quad (2.2)$$

The meaning and value of the parameters in our new model (2.2) are shown in Table 1.

The ubiquitous delay in protein synthesis and drug response has important consequences on dynamics of gene expression and consequently on downstream biological function [37]. In particular, such delay usually can result in oscillatory behavior and other more complex dynamics [29, 30]. However, so far the study of the impact of such delay on the integrated module of Nutlin PBK and p53 pathway has not been reported both experimentally and theoretically. Therefore, we focus on the effect of varying protein synthesis delay and the response delay of drug Nutlin on the stability and

amplitude and period of the p53 oscillations. Biologically speaking, the p53 protein is one of the most important tumor inhibitors, with mutations present in more than 50 percent of human cancer cells [1, 2]. The oscillation of p53 can promote the repair of damage [31]. If the damage cannot be completely repaired, p53 can also promote programmed cell death [32]. In this way, cells can avoid inaccurate genetic information inherited to the next generation of cells [33–35]. In general, there is an average transcriptional delay of 10–20 minutes between the action of a transcription factor on a gene promoter and the presence of the corresponding mature mRNA in the cytoplasm [36]. Similarly, the synthesis of a typical protein from mRNA requires a translation delay of about 1–3 minutes [36]. Therefore, the total Mdm2 protein synthesis time delay τ is estimated about 11–23 minutes, which can be observed in a real experiment. In our manuscript, we set it among 10–25 minutes. In addition, the critical value of Nutlin response time delay is calculated theoretically to be about 24 minutes [19]. In our paper, we estimate the Nutlin response time delay τ_1 is about 10–30 minutes that is within the biologically permissible range [14, 16–19].

Table 1. List of the meaning and value of parameters in the system (2.2).

Parameter	Description	value	Reference
σ	Production rate of p53	1000nM h^{-1}	[2]
α	Mdm2 independent deactivation/degradation of p53	0.1 h^{-1}	[2]
δ	Mdm2 dependent deactivation/degradation of p53	11 h^{-1}	[2]
k_t	Transcription of Mdm2	0.03 $nM^{-1}h^{-1}$	[2]
k_{tl}	Translation of Mdm2	1.4 h^{-1}	[2]
β	Degradation rate of Mdm2 mRNA	0.6 h^{-1}	[2]
γ	Mdm2 degradation/deactivation	0.2 h^{-1}	[2]
k_b	Dissociation of Mdm2-p53	7200 h^{-1}	[2]
k_f	Dissociation constant of Mdm2-p53	5000 $nM^{-1}h^{-1}$	[2]
k_{a3}	Nutlin-Mdm2 association rate	0.00006 sec^{-1}	[12]
τ	Translational and transported time delays	10 ~ 25min	estimate
τ_1	The time delays of the reaction of adding Nutlin	10 ~ 30min	estimate
n	The actual amount of Nutlin present in the cell	5 μM	[4]

3. Results

3.1. The effect of time delays on the dynamic behavior of the p53-Mdm2 core module under action of drug Nutlin

p53, as a transcription factor, regulates the expression of the Mdm2 gene, which is inevitably associated with the protein synthesis time delay. Due to the fact that the process of transcription, translation and translocation is slow, time delay inevitably appears in the process of gene regulation. The appearance of time delays often causes great changes in the dynamic properties of the network system. On account of the limitation of signal transmission speed, the effect of time delays on the system is an important factor that has to be considered. In consequence, it is of great significance to analyze the stability of gene regulatory networks with time delays [22, 23]. In this part, based on the results of numerical simulation, we will study how the time delays affect the dynamic behavior of the system (2.2). Special attention will be focused on the stability of positive equilibrium and the

existence of Hopf bifurcation by analyzing the distribution of the corresponding characteristic equation root. In addition, the properties of Hopf bifurcation are discussed by giving the judgment formulas of three indexes based on the standard form theory and the central manifold theorem for differential equations with time delays developed by Hässard et al. The detailed theoretical calculation can be seen in the Supplementary.

The numerical simulation will be carried out by using Mathematica 12.0 to verify the theoretical results. The parameters of system (2.2) are based on the previous experimental data [2, 12] and estimated on the biochemical constraints, and they are shown in Table 1.

$$\begin{cases} \dot{p}(t) = 1000 - 0.1p(t) - 5000p(t)m(t) + 7200c(t) + 0.2c(t), \\ \dot{m}_m(t) = 0.03p^2(t - \tau) - 0.6m_m(t), \\ \dot{m}(t) = 1.4m_m(t) - 5000p(t)m(t) + 7200c(t) + 11c(t) - 0.2m(t) - 0.216 * 5m(t - \tau_1), \\ \dot{c}(t) = 5000p(t)m(t) - 7200c(t) - 11c(t) - 0.2c(t). \end{cases} \quad (3.1)$$

According to the parameters in Table 1, it is easy to calculate that the system (2.2) has a positive equilibrium E^* .

$$E^* = (19.537, 19.085, 6.698, 90.731). \quad (3.2)$$

To explore the effect of time delays on the dynamic behavior of network integrated by Nutlin PBK and p53 pathways, numerical simulation are performed under different cases of time delays via Mathematica 12.0, as shown in Figures 2 and 3. The two sets of figures show that both the protein synthesis time delay τ and the Nutlin drug response time delay τ_1 can lead to oscillation of the Nutlin PBK and p53 pathway integration network. When there are no time delays or the time delays are too little, the system will trend to a stable state. Once the time delays exceed their thresholds, the system will lose stability and begin oscillation. Moreover, as the time delays continue to increase, the oscillation will be more obvious, which also can be observed from Figures 4 and 6. In addition, the amplitude and period increase with the increase of time delay, as shown in Figures 5 and 7.

The above simulation results also can be verified. To do this, the first step is to consider the dynamic behavior of the system (2.2) without time delays. Based on the Routh-Hurwitz criterion, it is verified that the positive equilibrium point of the system (2.2) is asymptotically stable when $\tau = 0h$ and $\tau_1 = 0h$.

In the second step, there are a pair of pure imaginary roots $\lambda = \pm\omega_0i$ as the characteristic roots of the system (2.2), where $\omega_0 = 1.94754$. Moreover, a set of critical time delays are obtained as follows.

$$\tau_j = 0.214 + 0.3.266j, \quad j = 0, 1, 2, \dots \quad (3.3)$$

Define $\tau_0 = \text{hour}(\tau_j) = 0.214$. According to the lemma in Supplementary (S1.2), the transversal condition is satisfied. Therefore, the positive balance E^* is gradually stable at $\tau < \tau_0 = 0.214h$, as shown in Figure 2(a),(b). With the increase of time delay, when the time delay $\tau > \tau_0 = 0.214h$, the positive equilibrium E^* would be unstable and Hopf bifurcation would occur, as shown in Figure 2(c),(d).

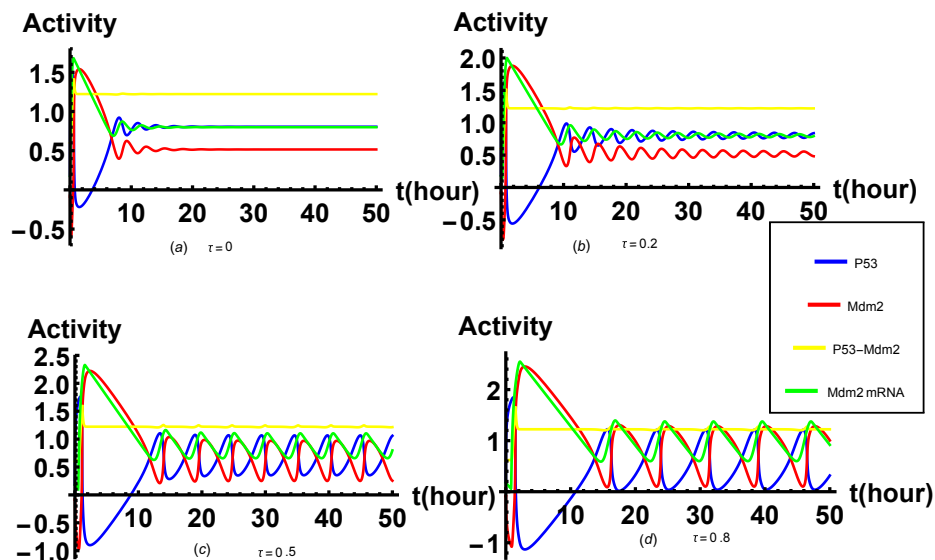


Figure 2. The effect of time delay τ on dynamic behavior of network integrated by Nutlin PBK and p53 pathways at $\tau_1 = 0h$. The blue, red, yellow and green lines respectively represent the concentrations of p53, Mdm2, p53-Mdm2 complex, Mdm2 mRNA. (a) The time histories of the system (2.2) when $\tau = 0h$. (b) The time histories of the system (2.2) when $\tau = 0.14h < \tau_0 = 0.214h$. (c) The time histories of the system (2.2) when $\tau = 0.5h > \tau_0 = 0.214h$. (d) The time histories of the system (2.2) when $\tau = 0.8h > \tau_0 = 0.214h$.

The third step is to investigate the effect of time delays on the properties of Hopf bifurcation and the bifurcated oscillations. Combined with the above discussion and the calculation process in the Supplementary S2, we get three indexes to characteristic the properties of the Hopf bifurcation and the bifurcated oscillations.

$$\begin{aligned}
 \mu_2 &= 331451.6547490089 > 0, \\
 T_2 &= 2.132144488497094 \times 10^{-6} > 0, \\
 \beta_2 &= -715228.8602363452 < 0.
 \end{aligned} \tag{3.4}$$

Similarly, for the Nutlin drug response time delay τ_1 , there are a pair of pure imaginary roots $\lambda = \pm\omega_{10}i$ as the characteristic roots of the system (2.2), where $\omega_{10} = 2.30206$. Moreover, a set of

critical values of time delay τ_1 are found as follows.

$$\tau_{j1} = 0.402 + 2.729j, \quad j = 0, 1, 2, \dots \quad (3.5)$$

Define $\tau_{10} = \text{hour}(\tau_{j1}) = 0.402$ and $\lambda'(\tau_{10}) = 1.071 + 0.100i$. The three indexes are also obtained.

$$\begin{aligned} \mu_2 &= 0.001168196819386769 > 0, \\ T_2 &= 0.0055866866227185819 > 0, \\ \beta_2 &= -0.0021632348152077793 < 0. \end{aligned} \quad (3.6)$$

According to the theorem in Supplementary (S2.1), μ_2 means that the system (2.2) has experienced a supercritical Hopf bifurcation at $\tau = \tau_0$ or $\tau_1 = \tau_{10}$. $\beta_2 < 0$ means that the bifurcated-period solution from the positive equilibrium $E^* = (19.537, 19.085, 6.698, 90.731)$ is stable in the central prevalence. In addition, $T_2 > 0$ means that the period of the periodic solution increases as τ or τ_1 increases.

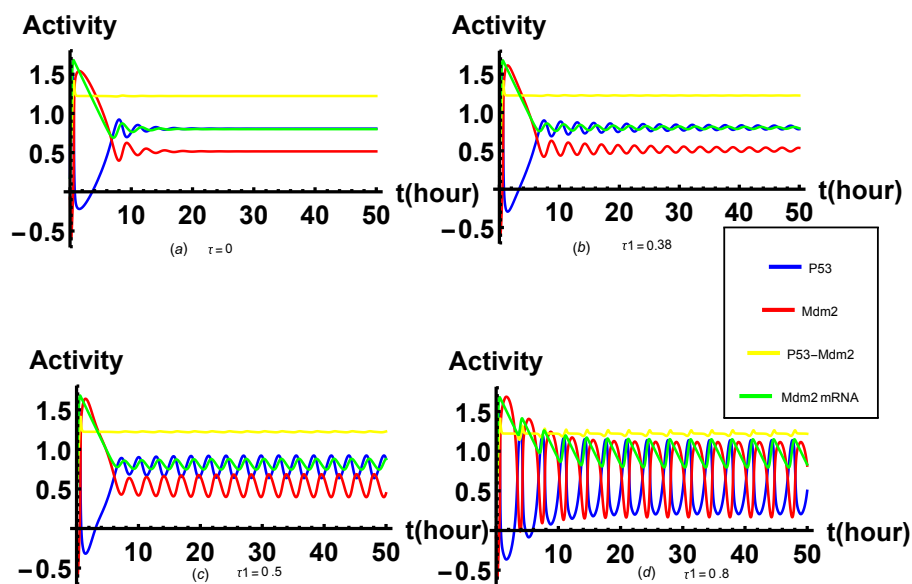


Figure 3. The effect of time delay τ_1 on dynamic behavior of network integrated by Nutlin PBK and p53 pathways at $\tau = 0h$. The blue, red, yellow and green lines respectively represent the concentrations of p53, Mdm2, p53-Mdm2 complex, Mdm2 mRNA. (a) The time histories of the system (2.2) when $\tau_1 = 0h$. (b) The time histories of the system (2.2) when $\tau_1 = 0.3h < \tau_{10} = 0.402h$. (c) The time histories of the system (2.2) when $\tau_1 = 0.5h > \tau_{10} = 0.402h$. (d) The time histories of the system (2.2) when $\tau_1 = 0.8h > \tau_{10} = 0.402h$.

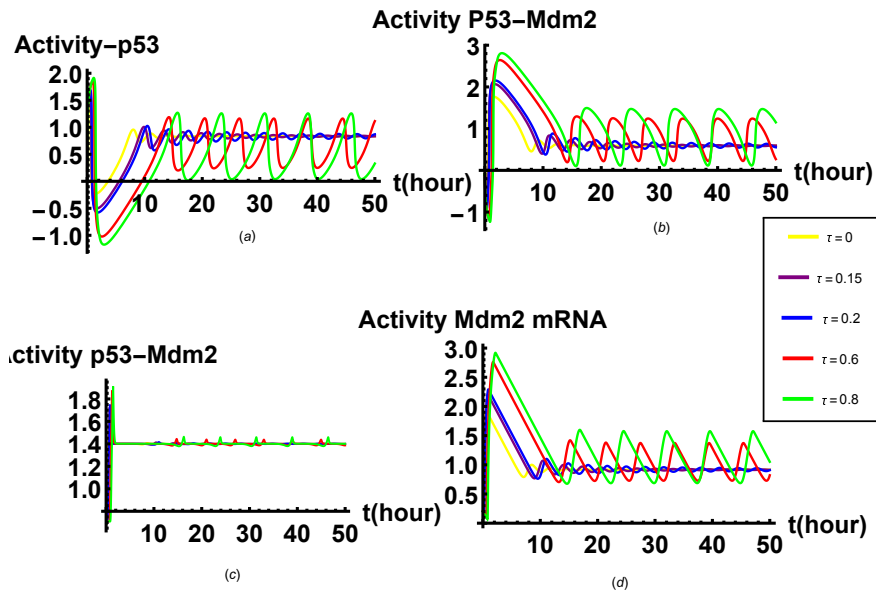


Figure 4. The effects of different time delays τ on the oscillation amplitude and period of the Nutlin PBK and p53 pathways at $\tau_1 = 0h$. The yellow, purple, blue, red and green lines indicate that the time delay is taken as $\tau = 0, 0.15, 0.2, 0.6, 0.8h$, respectively. (a) The time histories of the concentration levels of p53. (b) The time histories of the concentration levels of Mdm2. (c) The time histories of the concentration levels of p53-Mdm2 complex. (d) The time histories of the concentration levels of Mdm2 mRNA. The results show that as the time delay τ increases, the amplitude and period of the oscillation also increases.

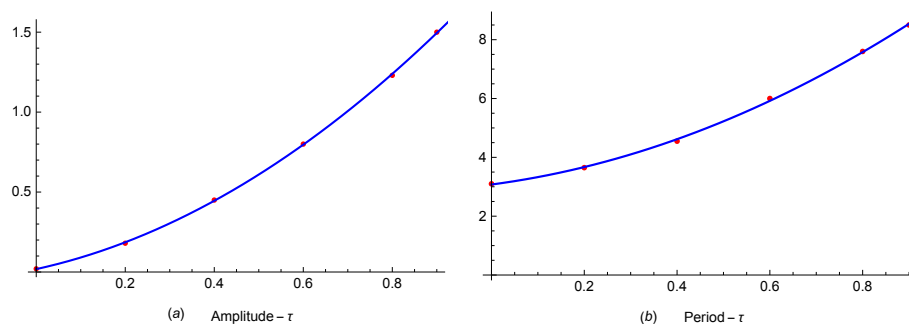


Figure 5. When $\tau_1 = 0h$, the changing trend of the properties of the oscillation as the time delay τ increases. (a) The changing trend of the amplitude as the time delay τ increases. (b) The changing trend of the period as the time delay τ increases.

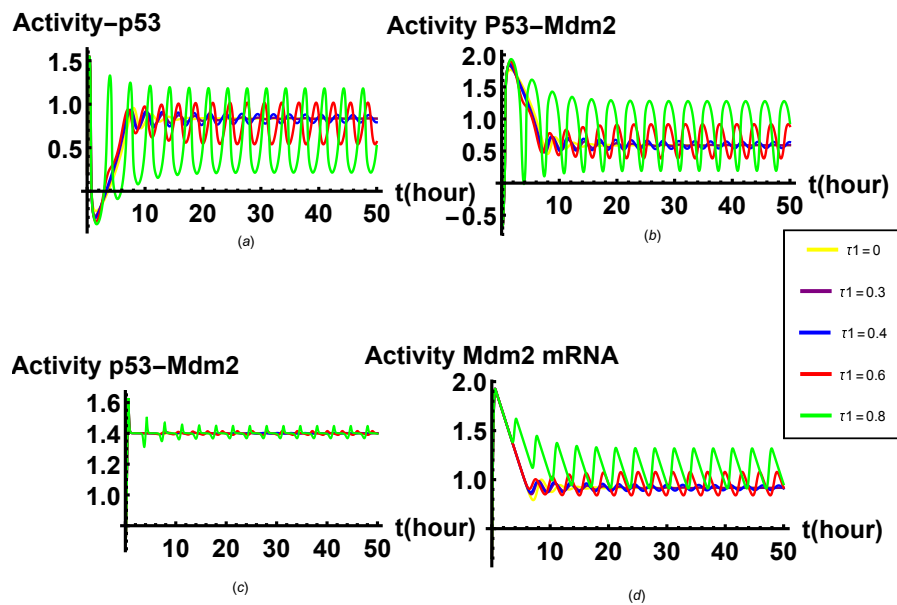


Figure 6. The effects of different time delays τ_1 on the oscillation amplitude and period of the Nutlin PBK and 53 pathways at $\tau = 0h$. The yellow, purple, blue, red and green lines indicate that the time delay is taken as $\tau_1 = 0, 0.3, 0.4, 0.6, 0.8h$. (a) The time histories of the concentration levels of p53. (b) The time histories of the concentration levels of Mdm2. (c) The time histories of the concentration levels of p53-Mdm2 complex. (d) The time histories of the concentration levels of Mdm2 mRNA. The results show that as the time delay τ_1 increases, the amplitude and period of the oscillation also increases.

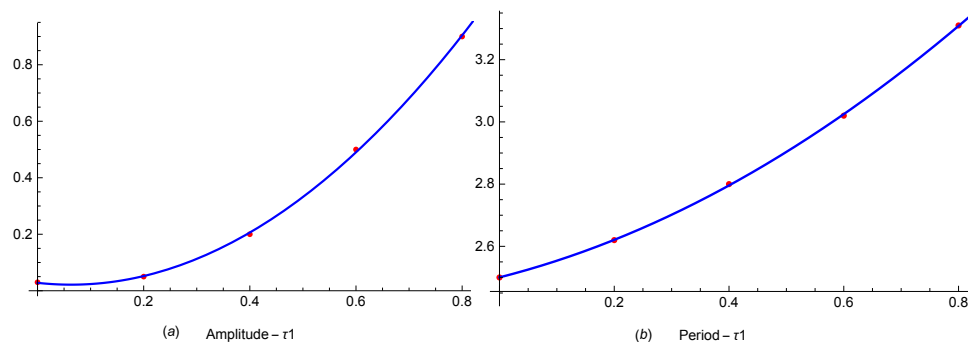


Figure 7. When $\tau = 0h$, the changing trend of the properties of the oscillation as the time delay τ_1 increases. (a) The changing trend of the amplitude as the time delay τ_1 increases. (b) The changing trend of the period as the time delay τ_1 increases.

3.2. The collaborative effects of important biochemical rates and time delays on the dynamic behavior of the Nutlin PBK and p53 pathways in integrated networks.

In order to further study the effect of time delays in system (2.2), the dynamic behaviors under two cases including fixed biochemical rates with different time delays and fixed time delays with different biochemical rates are discussed. Here, we mainly focus on the effects of three parameters on p53 oscillations, namely Mdm2 degradation rate γ , Mdm2 dependent p53 degradation rate δ , and the actual dose of Nutlin in the cell n .

As an effective inhibitor of p53, Mdm2 binds to the transcriptional activation domain of p53, and it also promotes the rapid degradation of p53 in other stable conditions [25]. To investigate the role of Mdm2-dependent degradation of p53 on the integrated network of Nutlin PBK and p53 pathways, the evolution processes of the system (2.2) are simulated under different Mdm2 degradation rates γ with time delay $\tau = 0.8h$ ($\tau_1 = 0.8h$). As shown in Figure 8 (a)–(d) (Figure 10 (a)–(d)). When $\gamma = 0.01$, the system in Figure 8 (a) maintains at a stable state, while the system in Figure 9 (a) is in a progressively stable state. With the increase of γ , oscillation occurs, and the amplitude of oscillation increases with the increase of γ . Due to the different meanings of the two time delays, the amplitude of oscillation is slightly different even under the same time delays and degradation rate. The results showed that Mdm2 degradation rate γ could cause Hopf bifurcation, and only a certain value of Mdm2 degradation rate could drive p53 oscillation. The changing trends of amplitude and period of the bifurcated oscillation are shown in Figures 9 and 11.

In the absence of time delays, as the degradation rate δ of p53 dependent on Mdm2 decreases, the system is always in an oscillating state [4]. In order to understand the collaborative effect of time delays and the degradation rate of p53 depending on Mdm2, the dynamic behaviors of the system (2.2) under different values of δ are studied. We select three groups of parameter levels, i.e. $\delta = 11$, $\delta = 30$, $\delta = 60$, then carry out the numerical simulation under three situations including without time delay and time delay is before and after the critical value, as shown in Figure 12. From (a)–(c), we can see that the system is always aesthetically stable regardless of $\delta = 11$, 30, and 60 when $\tau = 0h$. However, in the middle channel (d)–(f) of Figure 12, the time delay $\tau = 0.2h$ that is less than the critical value. When $\delta = 11$, the system (2.2) is asymptotically stable, while $\delta = 30$ or $\delta = 60$, the system (2.2) is stable. In Figure 12 (g)–(i), time delay is set as $\tau = 0.7h$ that is bigger than the critical value. When $\delta = 11$, the system (2.2) begins to oscillate significantly and the amplitude of oscillation increases significantly. When $\delta = 30$ and $\delta = 60$, the amplitude and period of oscillation of system (2.2) decrease with the increase of degradation rate δ . Similarly, when $\tau_1 = 0h$, the system is always asymptotically stable regardless of $\delta = 11$, 30, 60 as shown in Figure 14 (a)–(i). In Figure 14 (d)–(f), time delay is set as $\tau_1 = 0.4h$ that is less than the critical value. When $\delta = 11$, the system (2.2) is asymptotically stable. When $\delta = 30$ and $\delta = 60$, the system (2.2) is stable. However, when the time delay is larger than the critical value, for example $\tau_1 = 0.7h$ as shown in Figure 14 (g)–(i). When $\delta = 11$, the system (2.2) begins to oscillate significantly and the amplitude of oscillation increases significantly. When $\delta = 30$, the oscillation of system (2.2) still exists, but along with it, the amplitude is apparently fallen. When $\delta = 60$, the system (2.2) is in a state of damped oscillation. In other words, when the time delays are less than the critical value, whether the degradation rate is low, medium or high, the system (2.2) is asymptotically stable. When they are larger than their critical values, the amplitude and period of the oscillation are decreased obviously as the degradation rate increases. The changing trends of amplitude and period of the bifurcated oscillation are shown in Figures 13 and 15.

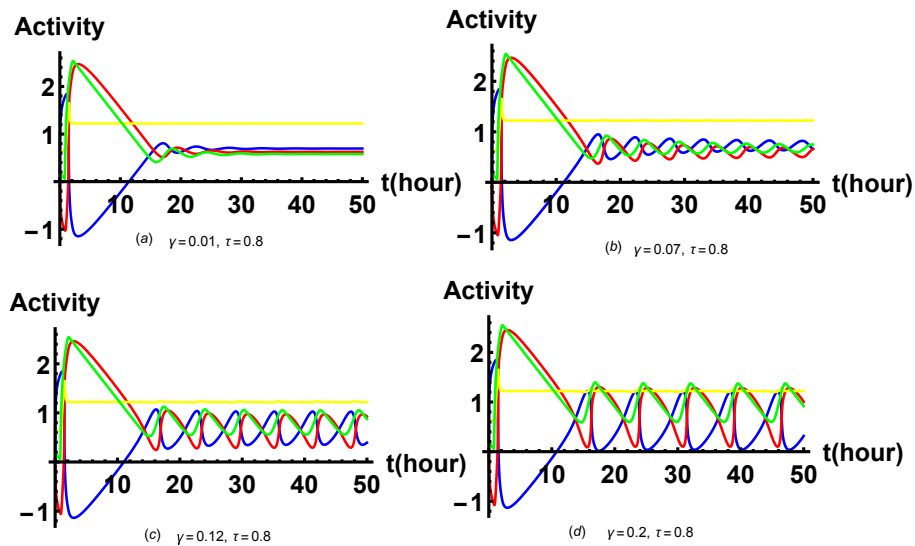


Figure 8. The evolution of the Nutlin PBK and p53 integrated network with time delays $\tau = 0.8h$ and $\tau_1 = 0h$. The blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(d) The evolution processes of system (2.2) when the Mdm2 degradation rates $\gamma = 0.01, 0.07, 0.12, 0.2$, respectively.

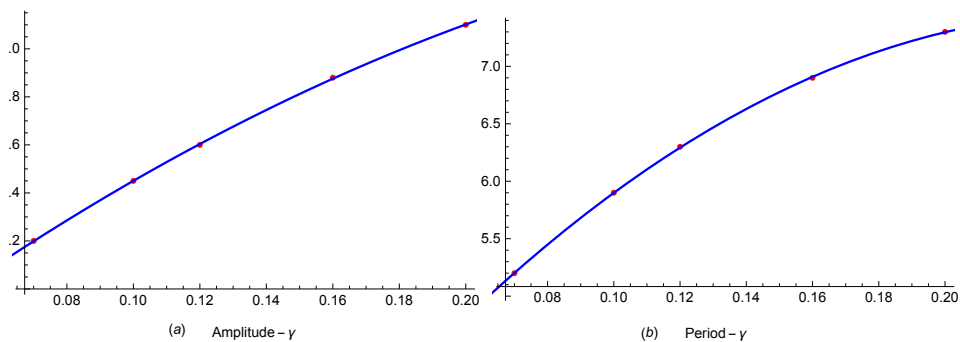


Figure 9. When $\tau_1 = 0h$, the changing trend of the oscillation properties as the Mdm2 degradation rates γ increases. (a) The changing trend of the amplitude as the Mdm2 degradation rates γ increases. (b) The changing trend of the period as the Mdm2 degradation rates γ increases.

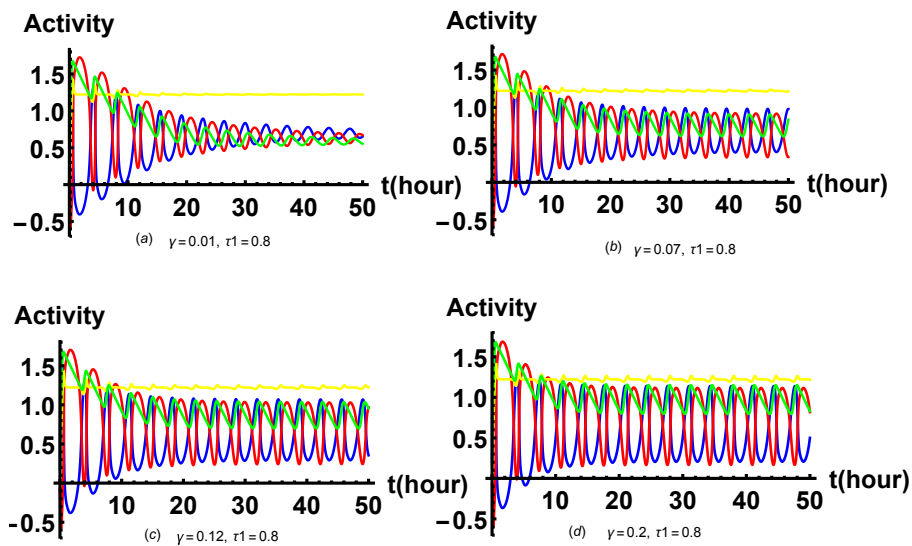


Figure 10. The evolution of the Nutlin PBK and p53 integrated network with time delays $\tau_1 = 0.8h$ and $\tau = 0h$. The blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(d) The evolution processes of system (2.2) when the Mdm2 degradation rates $\gamma = 0.01, 0.07, 0.12, 0.2$, respectively.

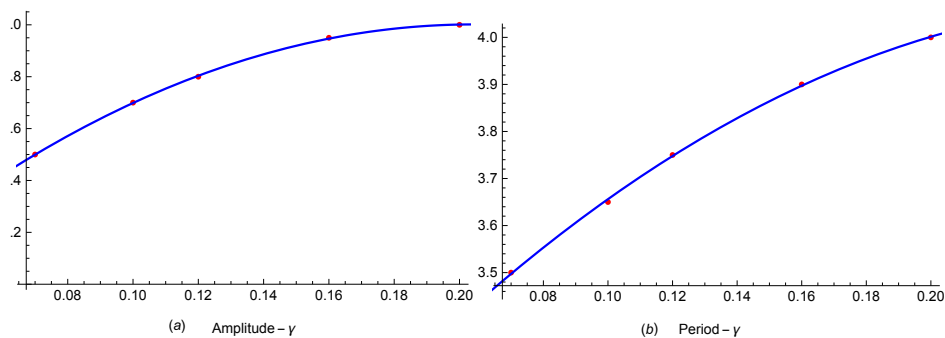


Figure 11. When $\tau = 0h$, the changing trend of the oscillation properties as the Mdm2 degradation rates γ increases. (a) The changing trend of the amplitude as the Mdm2 degradation rates γ increases. (b) The changing trend of the period as the Mdm2 degradation rates γ increases.

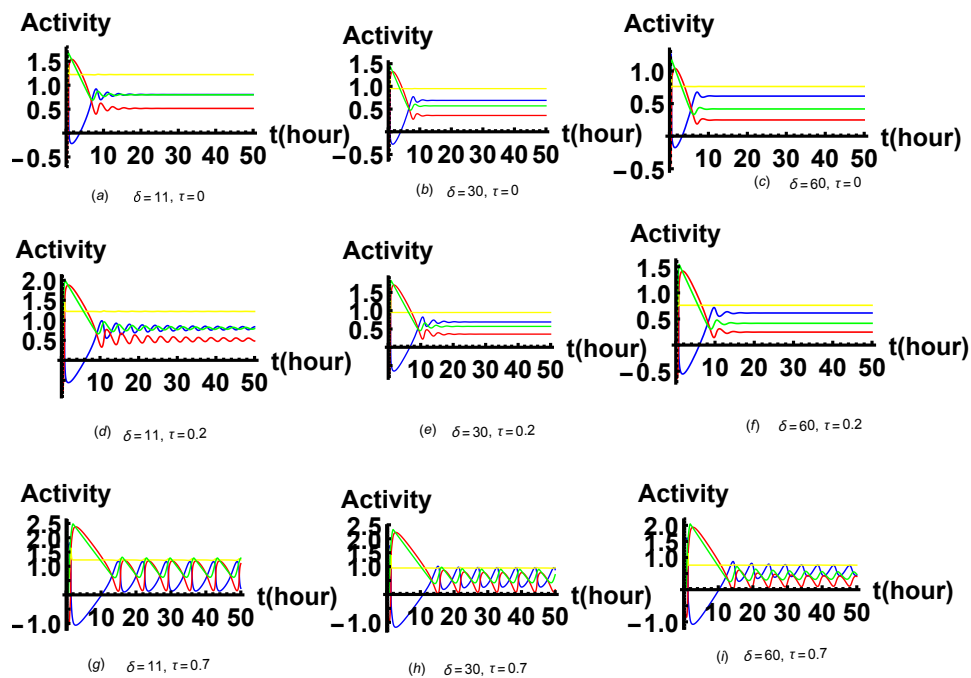


Figure 12. When $\tau_1 = 0h$, the influence of parameter δ and time delay τ on the dynamic behavior of the system (2.2). Blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(c) The evolution process of system (2.2) when $\tau = 0h$ and δ takes 11, 30, 60 in turn. (d)–(f) The evolution process of system (2.2) when $\tau = 0.2h$ and δ takes 11, 30, 60 in turn. (g)–(i) The evolution process of system (2.2) when $\tau = 0.7h$ and δ takes 11, 30, 60 in turn.

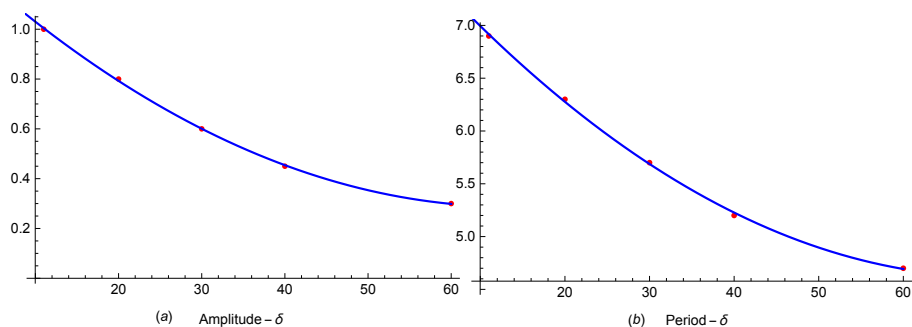


Figure 13. When $\tau_1 = 0h$, the changing trend of the oscillation properties as the degradation rate δ of p53 dependent on Mdm2 increases. (a) The changing trend of the amplitude as δ increases. (b) The changing trend of the period as δ increases.

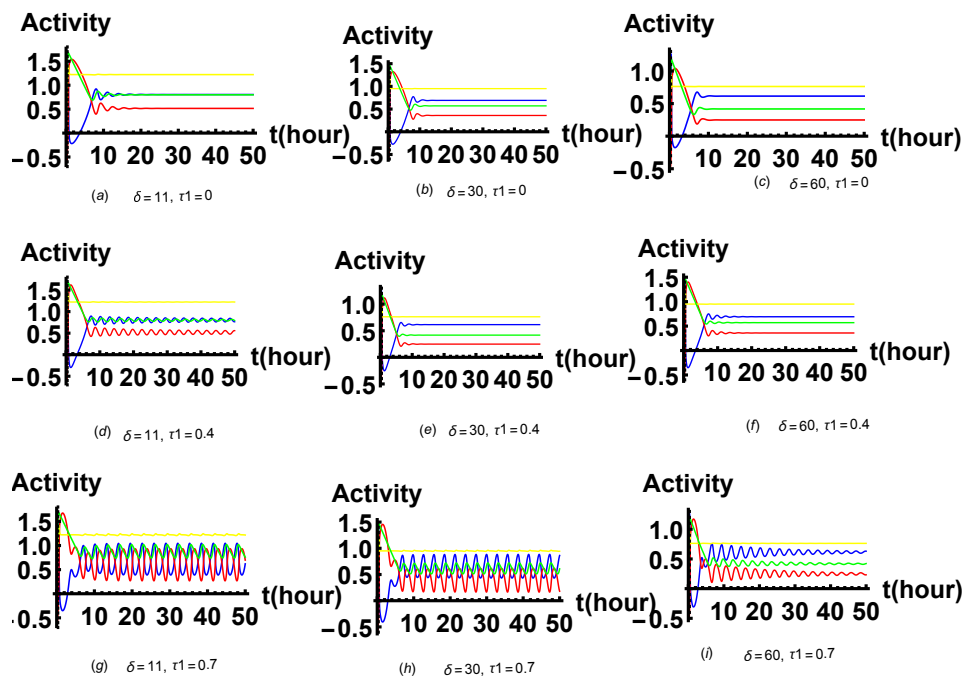


Figure 14. When $\tau = 0h$, the influence of parameter δ and time delay τ_1 on the dynamic behavior of the system (2.2). Blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(c) The evolution process of system (2.2) when $\tau_1 = 0h$ and δ takes 11, 30, 60 in turn. (d)–(f) The evolution process of system (2.2) when $\tau_1 = 0.2h$ and δ takes 11, 30, 60 in turn. (g)–(i) The evolution process of system (2.2) when $\tau_1 = 0.7h$ and δ takes 11, 30, 60 in turn.

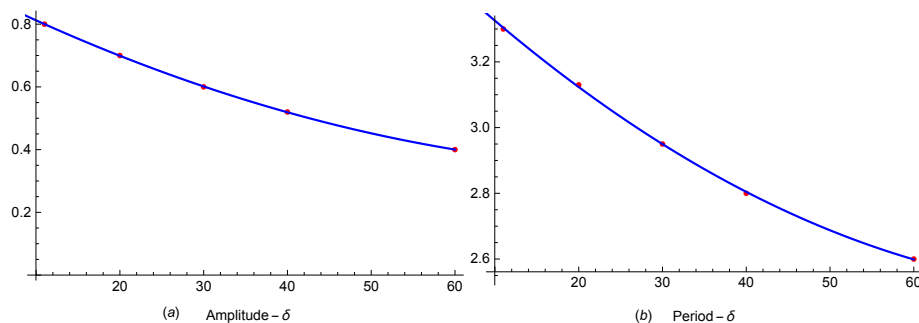


Figure 15. When $\tau = 0h$, the changing trend of the oscillation properties as the degradation rate δ of p53 dependent on Mdm2 increases. (a) The changing trend of the amplitude as δ increases. (b) The changing trend of the period as δ increases.

Blocking HDM2 by Nutlin may also restore the level of p53 [26]. In order to further study how Nutlin recovers the level of p53 in the presence of time delays, the time evolution processes are displayed by changing the actual amount n of Nutlin present in the cell under two cases of time delay $\tau = 0.2h$ and 0.5 . As shown in Figure 16, (a)–(c), when n takes 5, 8, 10 in turn, the system (2.2) is asymptotically stable when $\tau = 0.2$ regardless of the values of n . Figure 16 (d)–(f) shows the evolution processes of the system (2.2) when $\tau = 0.5h$. When $n = 5$, the system (2.2) oscillates. When $n = 8$ and $n = 10$, the amplitude and period of the oscillation is decreasing with the increase of n . In other words, when the time delay is less than the critical value, the system is asymptotically stable in spite of the value of n . When the time delay is larger than the critical value, the amplitude and period of the system decrease with the increase of n . As shown in Figure 18 (a)–(c), we can see that when $\tau_1 = 0.38h$, the oscillation mode are gradually distinct when n increases from 5 and 8 to 10. Figure 18 (d)–(f) depicted that oscillation always exists when $\tau_1 = 0.5h$, $n = 5$, $n = 8$, $n = 10$, and the amplitude of oscillation increases with the increase of n . The changing trends of amplitude and period of the bifurcated oscillation are shown in Figures 17 and 19.

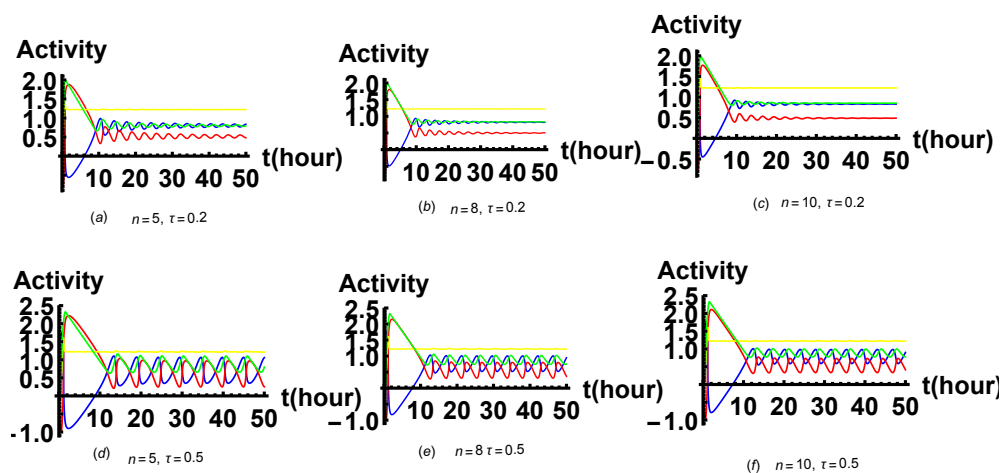


Figure 16. When $\tau_1 = 0h$, the influence of parameter n and time delay τ on the dynamic behavior of the system (2.2). Blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(c) The evolution process of system (2.2) when $\tau = 0.2h$ and n takes 5, 8, 10 in turn. (d)–(f) The evolution process of system (2.2) when $\tau = 0.5h$ and n takes 5, 8, 10 in turn.

To sum up, when the time delay τ is less than the critical value, the system is asymptotically stable. When it is larger than the critical value, the oscillation occurs, and the amplitude and period decrease with the increase of n as shown in Figure 16. In contrast, when the time delay τ_1 is less than its critical value, oscillation occurs with the increase of n . When it is larger than the critical value, the amplitude of system (2.2) increases with the increase of n . This also indicates that the time delay in protein synthesis, the time delay in Nutlin response, and the actual dose of Nutlin in the cell have effects on the dynamic behavior of the system, so it is reasonable and valuable to introduce the delay into the model.

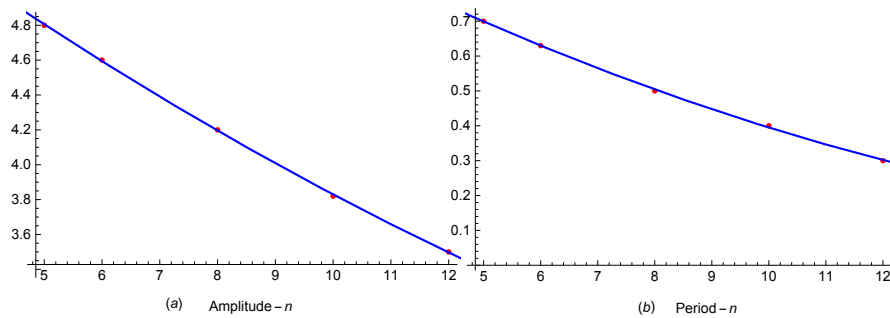


Figure 17. When $\tau_1 = 0h$, the changing trend of the oscillation properties as the actual amount n of Nutlin present in the cell increases. (a) The changing trend of the amplitude as n increases. (b) The changing trend of the period as n increases.

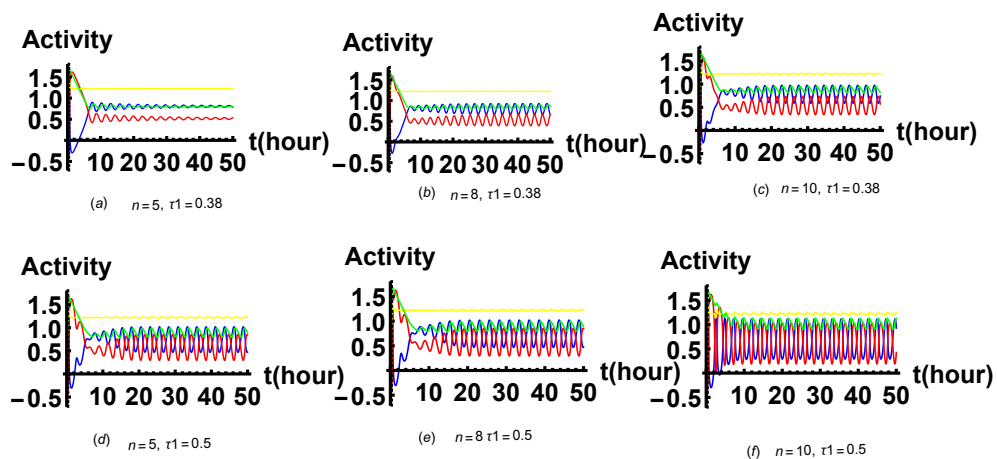


Figure 18. When $\tau = 0h$, the influence of parameter n and time delay τ_1 on the dynamic behavior of the system (2.2). Blue, red, yellow, green lines indicate the concentration levels of p53, Mdm2, p53-Mdm2, and Mdm2 mRNA, respectively. (a)–(c) The evolution process of system (2.2) when $\tau_1 = 0.38h$ and n takes 5, 8, 10 in turn. (d)–(f) The evolution process of system (2.2) when $\tau_1 = 0.5h$ and n takes 5, 8, 10 in turn.

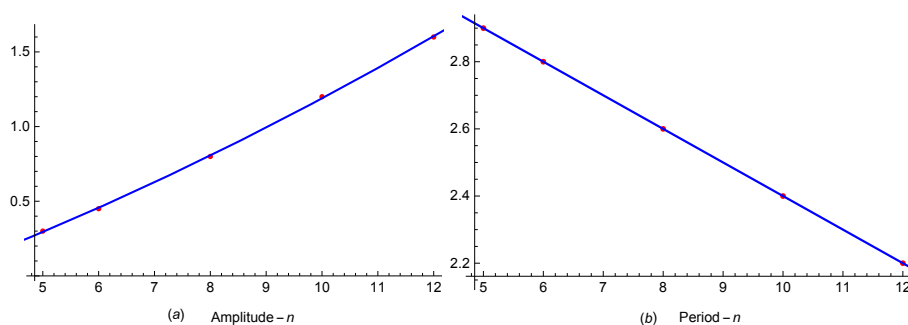


Figure 19. When $\tau = 0h$, the changing trend of the oscillation properties as the actual amount n of Nutlin present in the cell increases. (a) The changing trend of the amplitude as n increases. (b) The changing trend of the period as n increases.

4. Discussion and conclusions

In this paper, based on the model proposed by Häseeb et al., a model with the time delay of protein synthesis and the time delay of Nutlin drug response is developed. The stability and oscillation of the system (2.2) with and without time delays are analyzed. The dynamic behavior of the Nutlin PBK and p53 integrated network is studied by Hopf bifurcation theory and numerical simulation. By choosing time delays as bifurcation parameter, we find that they play a key role in inducing Hopf bifurcation and determine the amplitude and period of oscillation. Both the Mdm2 protein synthesis time delay τ and drug Nutlin response time delay τ_1 can control the appearance and disappearance of p53 oscillation. Moreover, the mode of the p53 oscillation, including the period and amplitude, is tightly relying on the Mdm2 protein synthesis time delay τ and drug Nutlin response time delay τ_1 . We can infer spontaneously that the p53 oscillation is regulated in a cooperative mode of the two time delays. In fact, changing the number of introns in a gene can bring the changes of protein synthesis time delay [27].

Biologically speaking, the p53 protein is one of the most important tumor inhibitors, with mutations present in more than 50 percent of human cancer cells [1, 2]. The oscillation of p53 can promote the repair of damage [31]. If the damage cannot be completely repaired, p53 can also promote programmed cell death [32]. In this way, cells can avoid inaccurate genetic information inherited to the next generation of cells [33–35]. Therefore, cell fate decision can be regulated by the Mdm2 protein synthesis time delay τ and drug Nutlin response time delay τ_1 through controlling the p53 oscillation. In addition to taking the time delays as the bifurcation parameter, we also consider the collaborative effect of several important parameters and time delays on the dynamic behavior of the Nutlin PBK and p53 pathways in the integrated network. p53 is a tumor suppressor, and Nutlin is a highly effective selective small molecule antagonist of Mdm2 found in clinical trials in recent years.

Nutlin is a p53 activator [28]. The results in this paper can not only help us to understand the dynamic behavior of the Nutlin PBK and p53 integrated networks, but also can provide theoretical evidence to select proper drug Nutlin and set the expected time delays to for the treatment of human tumors.

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Conflict of interest

The authors declare that they have no conflict of interest.

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