p53-Mdm2 Core Regulation Revealed by a Mathematical Model

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***Abstract*—p53 is a paramount protein in cancer studies. p53-Mdm2 interaction is the core regulation for most activities of p53 protein-related networks. In this paper a new mathematical model is proposed to reveal the mechanisms existing in this core regulation. This model is mainly based on the recent biological findings, as well as few reasonable hypotheses and approximations. The performance is in good accord with the experimental phenomenon in the literature. And a new threshold mechanism is also discussed, which gives more insights for experimental design and validation.**

***Index Terms*—p53-Mdm2 Core Regulation, Mathematical Model, Bifurcation Analysis, Systems Biology**

I. Introduction

The p53 tumour suppressor lies at the center of cellular pathways that sense DNA damage, cellular stress and oncogenetic stimulation [11]. p53 integrates such signals and, in response, induces growth arrest, triggers apoptosis (cell programmed death), blocks angiogenesis or mediates DNA repair, etc [4]. The critical role of p53 is experimentally evidenced by the presence of mutations found in almost 50% human tumours. Therefore, studies of p53 have attracted attentions of many researchers in life science for decades [1].

Different stresses will waken corresponding agents to modify the amino acid residues of p53. For example, when the cell is exposed to the DNA damage signals, such as ultraviolet (UV), ionizing radiation (IR), etc. ATM kinase will be activated to phosphorylate p53 at serine 15 within its N-terminal region. In addition, acetylation of lysine residues or phosphorylation of serine residues near the C-terminal region will induce the formational change which enhance the DNA binding ability of p53. With these post-translational modifications, the level of p53 is increased and the binding region is unfolded, thus the p53 is stabilized and activated. Then, p53 serves as a transcriptional activator to promote the target genes' expressions and the downstream products will repair the double-strand breaks (DSB) and ultimately mitigate the DNA damage.

The p53 network is normally "off". In normal cells p53 protein usually maintains at a low level and has a short half-life due to the degradation by ubiquitination and proteolysis. The inhibitor is Mdm2 protein which is a E3 ubiquitin ligase for p53 and also a target gene of p53 simultaneously. Apparently, there exists a negative feedback to maintain the low p53 level. The core regulation can be simply represented as p53 $\rightarrow$ Mdm2 $\dashv$ p53. Furthermore, the Mdm2-interacting region in p53 resides at the 1-42 amino acids within N-terminal region. As mentioned above, when the cell is stressed by IR, ATM will add phosphate group to the serine 15 which leads to the poor binding by Mdm2 to p53. Thus the p53 level will be raised and activated through C-terminal modification, then performs its major function: to bind to particular DNA sequences and activate the expression of adjacent genes. Meanwhile, ATM has another role to accelerate the transcription of target gene, including Mdm2, by phosphorylation of p53. The schematic diagram is referred to Fig. 1.

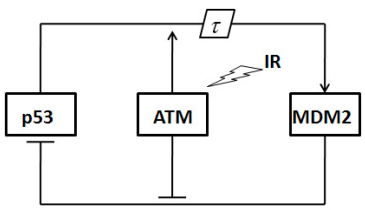

Fig. 1. Schematic diagram to illustrate p53-Mdm2 core regulation. Arrow represents activation, while arrow-bar means inhibition. IR is short for ionizing radiation. τ is the assumed time lag from p53 to Mdm2's translation.

In this paper, the main focus is to investigate this core regulation by a newly proposed mathematical model. ATM is involved to associate the DNA damage signal with this regulation. ATM's dynamics will appear in not only p53's degradation by Mdm2 but also transcriptional activities of p53. Moreover, through bifurcation analysis of p53 with respect to ionizing radiation, a threshold mechanism of radiation dose, which has never been discussed before, is found and prediction is brought along to give suggestion for experiment design.

The rest of the paper is organized as follows. Section II reviews the existing literature and compare various models. In section III, mathematical expressions will be derived one by one according to the biological bases and assumptions. Next, simulation results and bifurcation analysis are given to exploit the model. Discussion part is devoted to study ATM's role and advise experimental verification for model prediction. Finally, this paper will end with conclusion part.

II. Related Literature

Recently, two research groups found the oscillation phenomenon in p53-Mdm2 loop [7] [6]. Damped oscillatory behaviors in population of cells and undamped oscillatory behaviors in individual cells were observed after the ionizing radiation. Oscillatory expressions are actually observed in many other systems, such as Hes1 and NF-κB related networks [8]. Due to the lack of biological evidence and sufficient experimental data, the true mechanisms are still yet illustrated. So these oscillations motivate researchers' interest in this. Many works have been devoted to build a reasonable model to resemble this oscillatory phenomenon. It has been learned that oscillations can arise from negative feedback alone which is composed of at least three components. So in Lav Bar-Or's work [7], they resort to a putative intermediary in the negative feedback loop. They integrate the external stress signal in their model as well. After the theoretical modeling, they verify by the experimental results in both mouse fibroblasts NIH 3T3 cells and human breast cancer epithelial MCF-7 cells. They also explore the dependence of oscillations on different parameters. One important parameter k_{delay}, which represents the time lag from intermediary to Mdm2, inspires others' works which consider this time lag as an explicit parameter in the transcriptional and translational process of Mdm2.

As stated in central dogma of molecular biology, transcripts synthesized in the nucleus have to endure RNA processing and splicing, then be exported to cytoplasm to continue being translated to protein. Furthermore, Mdm2 must return back into nucleus to inhibit p53's transcriptional activity. It will take much more time lag to perform all these activities. Monk first considers the delayed feedback, and integrates all the time lags as one term in the formation process of Mdm2 in [8]. From then on, most consecutive works adopt this idea, such as [12] [9]. In [12], Wagner considers ATM's function in the formation of phosphorylated p53. But he sets ATM's concentration as a fixed value not influenced by the stress signal's evolvement. A fresh idea is brought in [2]. Tyson and his colleagues introduce a positive feedback mechanism besides the common negative feedback loop. The model also splits many different stages of the two major players– p53 and Mdm2. Finally, the authors propose experimental methods to verify the existence of positive feedback. Another good modeling work is also from the Alon's research group, who first observe undamped oscillations in individual cells [6]. In their later work [3], they give a long-term (up to 3 days) experimental data set. Moreover, they list six different model types for the dynamics of p53-Mdm2 network, among of which there is one model proposed by themselves. They compare the robustness of these models by introducing noise term in the parameters. In their own model, stress signal's evolvement is included and different states of p53 and Mdm2 are also considered. Their model can well explain the cell-cell variability in the system.

Among the existing works, some are not considering the dynamics of ATM [12]; some ignore the stress signal's effect [8]. Some are introducing so many putative components that makes the model redundant [2] [9]. For example, in [9], the authors discriminate plain p53 and phosphorylated p53. In their result and analysis part, they combine these two states together and investigate the temporal behavior of the combination all the time. One simplified model can be generated by adding these two states equations and consider the combined state variable only. The performance is verified not to be much different. The limitations of these models inspire the modeling work in this paper to overtake some disadvantages and hopefully bring along more insights of this popular regulation.

III. Formulation

Our model rests on prevailing evidences and widely accepted assumptions. For sake of simplicity, only the p53 and Mdm2 proteins are considered, rather than the messenger RNA of them. The reliability of this simplification will be verified by the later simulation result. And the delays happened in the transcription, translation and translocation processes are all merged as one delay term appearing explicitly in the Mdm2 dynamics. The selection of parameter is performed after scaling the original equations.

A. Model

First of all, p53 dynamics is evaluated.

$$\frac{\mathrm{d}p53}{\mathrm{d}t} = a_p - d_p \cdot p53 - deg(S(t)) \cdot \frac{p53}{p53 + K_p} \cdot Mdm2 \quad (1)$$

Here the coefficient a_p specifies the synthesis rate of the p53 protein. The second term reflects the Mdm2-independent p53 degradation. d_p is the basal degradation rate. The last term describes the Mdm2-induced p53 degradation. Mdm2 binds to p53 and labels it with ubiquitin. Finally, p53 is hydrolytically broken down by protease. Michaelis-Menten kinetics is applied to this process, consistent with an enzyme (Mdm2)-catalyzed degradation from a substrate (p53 protein). As for $deg(S(t))$, it is the degradation rate which is a function of the ATM, denoted by $S(t)$.

The expression for $deg(S(t))$ is as follows:

$$deg(S(t)) = d_0 \cdot (1 - \frac{S^n}{S^n + K_1^n}) \quad (2)$$

d_0 is the basal rate for Mdm2-dependent p53 degradation. As mentioned in the introduction part, when the cell is exposed to the ionizing radiation, ATM will weaken the binding ability of Mdm2 to p53. So this basal degradation rate will be reduced by the existence of ATM. It is assumed that the reduction follows Hill function with order, also called cooperativity, n. As shown in Fig. 2, Hill function has a sigmoidal character. And the bigger the Hill function order, the steeper the curve, i.e., the more sensitive the output with respect to independent variable.

Secondly, the dynamics of Mdm2 is described in the following equation:

$$\frac{\mathrm{d}Mdm2}{\mathrm{d}t} = a_m - d_m \cdot Mdm2 + agg(S(t)) \cdot \frac{p53^4(t-\tau)}{p53^4(t-\tau) + K_m^4} \quad (3)$$

The coefficients a_m and d_m give the basal rate of synthesis and degradation for Mdm2, respectively. The last term represents

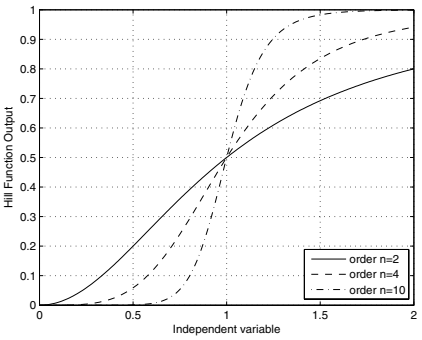

Fig. 2. Hill function curve. The general expression is $f(x) = \frac{x^n}{x^n+\theta^n}$, where $\theta = 1$ in this example.

the transcription activation of Mdm2 by p53. Here for the sake of simplicity, transcription product —Mdm2 messenger RNA is replaced by Mdm2 protein and phosphorylated p53 is replaced by p53 protein. The two forms of p53 will not be discriminated in this model. The phosphorylation by ATM kinase is expressed in the coefficient function $agg(S(t))$. To account for p53's preference for tetramerisation, P2 promoter's dependence on p53 is modeled as a Hill function with cooperativity 4 [13]. Time lag τ is utilized to represent all the duration cost in this process.

$agg(S(t))$ is formulated as follows:

$$agg(S(t)) = a_0 \cdot \frac{S^m}{S^m + K_2^m} \tag{4}$$

Another Hill function is introduced to describe the promotion of ATM for the transcription process.

Finally, ATM's kinetics is shown below:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = k \cdot IR - d_s \cdot S \tag{5}$$

The first term shows the ATM's dependence on the dose of ionizing radiation. The second term describes the degradation of ATM.

B. Selection of parameters

Dimensionless scaling helps to reduce the burden for selection of parameters. Introduce the new variables and scaling relationships as follows:

$$p\hat{5}3 = \frac{p53}{a_p}, M\hat{d}m2 = \frac{Mdm2}{a_m}$$

$$\hat{d_p} = d_p, \hat{d_m} = d_m$$

$$\hat{d_0} = \frac{a_m}{a_p} \cdot d_0, \hat{K_p} = \frac{K_p}{a_p}$$

$$\hat{a_0} = \frac{a_0}{a_m}, \hat{K_m} = \frac{K_m}{a_p}$$

Thus, the rescaled dynamics is expressed by the new variables in the following form.

$$\begin{aligned}
\frac{\mathrm{d}p\hat{5}3}{\mathrm{d}t} &= 1 - \hat{d_p} \cdot p\hat{5}3 - deg(S(t)) \cdot \frac{p\hat{5}3}{p\hat{5}3 + \hat{K_p}} \cdot M\hat{d}m2 \\
\frac{\mathrm{d}M\hat{d}m2}{\mathrm{d}t} &= 1 - \hat{d_m} \cdot M\hat{d}m2 + agg(S(t)) \cdot \frac{p\hat{5}3^4(t-\tau)}{p\hat{5}3^4(t-\tau) + \hat{K_m}^4} \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= k \cdot IR - d_s \cdot S \\
deg(S(t)) &= \hat{d_0} \cdot (1 - \frac{S^n}{S^n + K_1^n}) \\
agg(S(t)) &= \hat{a_0} \cdot \frac{S^m}{S^m + K_2^m}
\end{aligned}$$

To highlight the role of p53 in transcriptional activation, $\hat{a_0}$ should be selected much greater than 1, which is the unitized basal synthesis rate of Mdm2. The same selection criterion is applicable to the p53's degradation rates. Mdm2 makes the p53's proteolysis much faster compared with the basal degradation. So $\hat{d_0}$ is reasonably considered much greater than $\hat{d_p}$. While the basal degradation rate $\hat{d_p}$ is determined by the rough estimation of half-life time. So is $\hat{d_m}$. In most literatures, the basal synthesis rate of Mdm2 and degradation rate of p53 are sometimes neglected. Parts of parameters, such as $\hat{d_p}, \hat{d_m}$ the degradation are adapted from [12]. As for the Hill function's cooperativity, orders of 1 and 4 in Eq.(1) and Eq.(3) are according to the Michaelis-Menten kinetics and p53's tetramerisation. The orders of n and m used in Eq.(2) and Eq.(4) are determined by the sensitivity of the component.

Summarizing above, all the parameters are listed in Table I.

TABLE I
PARAMETER LIST OF DIMENSIONLESS KINETICS EQUATIONS

Parameter	Value	Parameter	Value
τ	1		
$\hat{d_p}$	0.2	$\hat{d_0}$	2
$\hat{K_p}$	0.2	$\hat{K_1}$	0.3
$\hat{d_m}$	1	$\hat{a_0}$	4
$\hat{K_m}$	0.5	$\hat{K_2}$	0.2
m	2	n	2
k	1	d_s	1

In most cases, it is not easy to define all the parameters due to the lack of reliable experimental data, which have to be faced by Systems Biology. So the parameters are mostly estimated by reasonable approximations and trial-and-error. Actually, one qualitative model is sufficiently good if it can explain and predict the phenomenon. In other words, in the modeling process, it is more important to determine which variables to be included and identify their relationships than the final product [14].

IV. Simulation and Analysis

A. Simulation Result

Our model exhibits sustained oscillation in response to increased radiation dose. As can be seen in Fig. 3, during the interval $0 \le t \le 15$, the cell stays in normal condition without exposure to ionizing radiation ($IR = 0$). p53 is maintained at low level due to the spontaneous inhibition by Mdm2. After $t > 15$, the cell is exposed to ionizing radiation ($IR = 0.5$). The oscillation is persisted until ionizing radiation is withdrawn at $t = 80$. Then the p53 and Mdm2 both return to the original states through a transient process. The difference between p53 and Mdm2 levels will not matter too much because of the scaling operation.

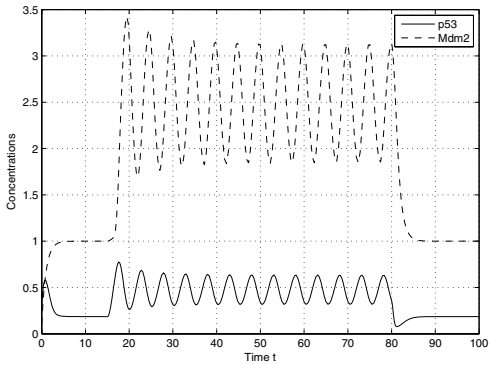

Fig. 3. Temporal performance of p53 and Mdm2. During $15 \le t \le 80$, $IR = 0.5$. For other durations, $IR = 0$. Other parameters are listed in Table I.

The first peak of p53 is earlier than Mdm2 after onset of IR. And the lag is about 1.8. The periods for both variables are the same. And the period is not influenced much by the radiation dose. (For $IR = 0.4$ case, the number of peaks keep constant as $IR = 0.5$. Data are not shown here). These qualitative performances fit to the experimental data in [7] [6] and previous simulation works. The difference resides on the scale, which is due to the parameters' selections. The evolvement agrees with the observed experimental phenomenon.

As evidenced by the experimental data shown in Fig.6b of [7], weak damage signal will slow the rise of steady state and no observable oscillations exists within the time frame of the experiment. To verify this point, IR is reduced to 0.2, and the result depicted in Fig. 4 shows the oscillation disappears and settling time is elongated compared to the previous case.

B. Bifurcation Analysis

According to the simulation results above, when $IR = 0.2$, p53 stays at a stable steady state. When IR is raised to 0.5, p53 will oscillate, meaning that the original fixed point changes the stability. Moreover, if IR is considered as a parameter, it will induce the bifurcation of the nonlinear systems expressed by Eq.(1)–(5). Specifically, this is Hopf Bifurcation, i.e. the stable

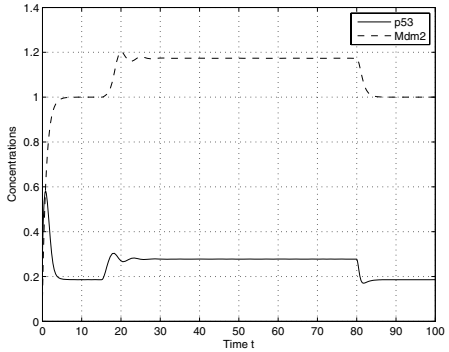

Fig. 4. Temporal performance of p53 and Mdm2. During $15 \le t \le 80$, $IR = 0.2$. For other durations, $IR = 0$. Other parameters are listed in Table I.

equilibrium point becomes unstable by a parameter change, and a limit cycle appears in the neighborhood [10].

Eq.(5) shows the independence on the p53 and Mdm2 dynamics. The solution of $S(t)$ is shown as below.

$$S(t) = e^{-d_s t} \cdot S(0) + k \cdot \int_0^t IR(\tau) e^{-d_s(t-\tau)} \mathrm{d}\tau$$

The settling time depends on the parameter d_s only. Given $k = 1, d_s = 1$, let

$$\frac{\mathrm{d}S}{\mathrm{d}t} = k \cdot IR - d_s \cdot S^* = 0 \tag{6}$$

then get $S^* = IR$.

Thus, it is convenient to study the bifurcation of reduced systems, which is comprised of only p53 and Mdm2 kinetics. Let the right hand sides of scaled p53 and Mdm2 equations equal zero and replace S with $S^* = IR$. (From afterward, omit the hat sigh above the variables and parameters in scaled equations without inducing misunderstanding and use P, M as abbreviations of p53 and Mdm2, respectively.)

$$1 - d_p \cdot P^* - deg(IR) \cdot \frac{P^*}{P^* + K_p} \cdot M^* = 0 \tag{7}$$

$$1 - d_m \cdot M^* + agg(IR) \cdot \frac{P^{*4}}{P^{*4} + K_m^4} = 0 \tag{8}$$

After arrangement, by using the same parameter set above, an implicit function of P^* with the parameter IR is derived below.

$$1 - 0.2 \cdot P^* - \frac{0.18}{IR^2 + 0.09} \cdot \frac{P^*}{P^* + 0.2} \left[1 + \frac{4 * IR^2}{IR^2 + 0.04} \cdot \frac{P^{*4}}{P^{*4} + 0.5^4} \right] = 0$$

Because the higher order existing in the implicit function, it is hard to get a close-form solution of P^*. By sampling IR's range $[0.1, 0.3]$ by interval of 0.02, perform symbolic solver in Matlab iteratively. Several pairs of coordinates can be obtained. As for the negative real roots and complex roots, they are filtered because of the reality consideration. When $IR = 0.2$, the steady state of p53 is approximately 0.277, same as the

Fig. 4, which can also be seen in bifurcation diagram shown below.

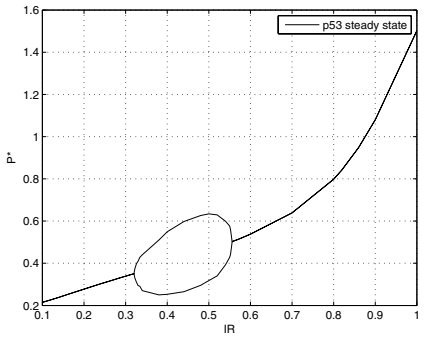

Fig. 5. Bifurcation diagram of p53's steady state with respect to IR. For the stable limit cycle, the maxima and minima are drawn. When IR is greater than 20, the steady state converges to 5. Data are not shown here.

According to the bifurcation diagram in Fig. 5, when $IR > 0.32$, the oscillation happens. And when $IR > 0.556$, the oscillation disappears and returns to the unique steady state again. Theoretically, it is because that, when IR is sufficiently large, ATM's level also becomes bigger. p53's degradation by Mdm2's ubiquitination is largely inhibited by ATM. That's to say, the third term of Eq.(1) can be neglected. So p53 level will be definitely raised. And Mdm2 is also aggregating due to the transcriptional activation of p53, leading the level higher than the basal level. Thus, p53 and Mdm2 will not be influenced by the ATM as much as in the oscillation region, and the core regulation is modified by the elimination of Mdm2's inhibition on p53. An example can be seen in Fig. 6. So far, there is no experimental data showing the response of big dose of ionizing radiation. The analysis based on this model predicts the retrieval of stable steady states in higher level.

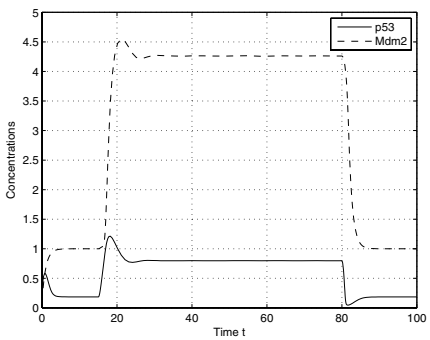

Fig. 6. Temporal performance of p53 and Mdm2. During $15 \leq t \leq 80$, $IR = 0.8$. For other durations, $IR = 0$. Other parameters are listed in Table I.

V. Discussion

In this model, ATM is involved to connect the DNA damage signal with the core regulation. ATM's dual function is treated as two dynamical factors influencing the degradation of p53 by Mdm2 and transcriptional activation of Mdm2. In Eq.(2) and Eq.(4), ATM is expressed as independent variable of Hill function. The selection of K_1 and K_2 plays an important role for the tuning effect of ATM. As can be seen in Fig. 2, when $x = \theta$, the output $f(\theta)$ is 0.5. This threshold θ determines the working range of x. For example, if θ is set small, $f(x)$ will exceed half easily through a lower threshold. If the cooperativity is big at the same time, the whole output is prone to approach 1 after a small variation. Thus the tuning effect of x is attenuated. Therefore, more attention should be paid on the threshold selection of these two Hill functions in order to give enough tuning range for ATM. Otherwise, ATM will become dispensable.

Through bifurcation analysis, the curve around the first critical point agrees with the simulation result. After the second critical point, the curve shows that the oscillation is eliminated, replaced with stable steady state. It is the first time to reveal threshold mechanism in p53's research. This finding is not verified by the current experiment data. A verifying experiment is suggested to conduct, being used for two scenarios. On the one hand, if a higher steady state of p53 is observed, which is in accord with the prediction from the model, the model is validated, revealing true mechanisms in the core regulation to some extend. On the other hand, if the observation shows that there are still sustained p53 oscillations or low level of stable steady state, some other facts, such as the downstream activities of p53, which are involved in cell cycle, apoptosis and DNA repair [4], should be considered to improve the model's persuasion, or, the role of ATM should be reevaluated to modify the interactions with the core regulation. Herein one of the most important concerns is the radiation dose limitation to avoid killing the cell.

VI. Conclusion

In this paper, a new mathematical model is proposed to explain the inherent mechanisms happened in p53-Mdm2 core regulation in response of DNA damage. The decision of the components and the relations among them is based on biological facts and reasonable approximations, helping to derive all the dynamical equations. Meanwhile, many a factor is not completely included, such as DNA repair mechanisms, the volume ratio of cytoplasm and nucleus where proteins and messenger RNAs are exported in and out, etc. The main structure is built in delayed differential equations. The simulation environment is in Matlab mainly using dde23 [5].

Selection of parameters and simplification assumptions are proven appropriate by the good agreement of simulation result with the experimental phenomenon. Furthermore, a more detailed investigation is performed to analyze the bifurcation of p53's concentration with respect to the dose of ionizing radiation to predict a new threshold mechanism used to explore this core regulation.

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