Proceedings of the 29th Annual International
Conference of the IEEE EMBS
Cité Internationale, Lyon, France
August 23-26, 2007.

A Dynamical Model on the Network of p53-Mdm2 Feedback Loop Regulated by p14/19ARF

Hui Liu ,Ying-Rong Han, Yong Zhan, Ya-Fei Chen, Yu-Hong Zhang, Hui Yu ,
Tong-Jun Zhao, Yi-Zhong Zhuo

Abstract—Base on new experimental results, we give a dynamical model to study the dynamical mechanism of the negative feedback loop composed of p53 and Mdm2 proteins regulated by p14/19ARF. The oscillatory behaviors for the activities of p53 and Mdm2 proteins regulated by p14/19ARF in individual of cells are described in our dynamical model. The results help us build a basal network about oscillatory behaviors among p53, Mdm2 and P14/19ARF. The dynamical model and its numerical results will help us understand the oscillatory behavior among other network of different proteins.

I. Introduction

The study of dynamics and variability of one of the proteinnetwork motifs that recurs across organisms: a negativefeedback loop is very important .p53 tumor suppressor protein plays a key role in preventing the development of cancer and is inactivated in many human malignancies. Mutations in the p53 tumor suppressor gene occur in about 50% of human tumors. In response to genomic stress, p53 activation may elicit cell-cycle arrest or apoptotic cell death, as well as contribute to DNA repair processes. Because some of the cellular effects of activated p53 can be irreversible, keeping p53 function under tight control in normal cells is critical. A key player in the regulation of p53 is the Mdm2 protein. This duality defines a negative feedback loop, which is widely recognized. P14/19ARF is another important new tumor suppressor protein, it has its own independent promoter .The p14/19ARF protein can increase the level of p53 by neutralizing Mdm2 which destabilize p53,ultimately play a role in suppressing cancer.

Recently, intensive studies have been devoted to the signal-response relation between DNA damage and gene expressions within living cells. The studies mainly carried out on the autoregulatory oscillatory dynamics of the expressions or activities of the common network motif composed of the tumor suppressor protein p53 and its transcriptional target Mdm2[1]–[5].

Manuscript received April 1, 2007. This project is supported by National Natural Science Foundation of China under Grant No.10645006,College Scientific Research Foundation of China under No. 20060080001, National Natural Science Foundation of Hebei Province under Grant No. No.A2007000001 , C2005000011and No.A2004000005.

Hui Liu is with School of Science, Hebei University of Technology, Tianjin, China. (phone: +86-22-60204802;e-mail:lhcell@163.com).
Ying-Rong Han, Yong Zhan, Ya-Fei Chen, Yu-Hong Zhang, Hui Yu, Tong-Jun Zhao are with School of Science, Hebei University of Technology, Tianjin, China.
Yi-Zhong Zhuo is with China Institute of Atomic Energy, Beijing, China.

Several simple theoretical models[1],[6] based on the p53-Mdm2 autoregulatory feedback between the transcription of p53 and Mdm2 proteins have been proposed to qualitatively describe the dynamical behaviors of average protein levels in population of cells. However, we are still far away from understanding the dynamical mechanism for the sustained oscillatory behaviors in individual level, and the relation between the damped oscillatory behaviors in population of cells and undamped oscillatory behaviors in individual cells[2],[5]. Moreover, an exponential function in time is generally used to express the signal response to the damage in the case of population of cells[2]. This response relation has far-reaching implication for our understanding of how cells respond to damage in different manner in individual and population cases.

An exciting finding is that the p53 pathway is intimately linked to other signal ransduction pathways that play a significant role in the origins of cancer. One of the first connections studied involves p14/19ARF and Mdm2. The p14/19 ARF protein binds to the Mdm2 protein and modulates down its ubiquitin ligase activity, increasing the levels of the p53 protein (Figure 1). The transcription of the p14/19 ARF gene is positively regulated by E2F-1 and beta-catenin and negatively regulated by p53 itself. In addition, the levels of p14/19 ARF protein are increased by Ras and Myc activities in a cell (Figure 1). The complexity of the regulation of p53 by p14/p19 ARF has been recently reviewed. The p14/19 ARF- Mdm2 complexes are often localized in the nucleolus of the cell due to the nucleolar localization signals present within p14/p19ARF. The nucleolus is the site of ribosomal biogenesis and p14/19 ARF activity itself can alter the rate of RNA processing of the ribosomal RNA precursor into mature ribosomal subunits. Thus, p14/19 ARF by controlling Mdm2 and p53 levels and coordinating this with ribosomal biogenesis plays an important role in cell cycle regulation. This has recently been reinforced by the demonstration that the p14/19 ARF protein can regulate Myc activity as well (and therefore cell size). The Mdm2 in the nucleolus is not, however, a passive entity. The Mdm2 protein has been shown to bind specifically to three large ribosomal subunit proteins L5, L11 and L23, and the binding of L5 or L11 to Mdm2 lowers its ubiquitin ligase activity. In addition, the ring- finger domain of Mdm2 binds specifically to an RNA sequence found in the large ribosomal RNA subunit. While all of these observations point to a central role for Mdm2 and p14/19 ARF in the regulation of

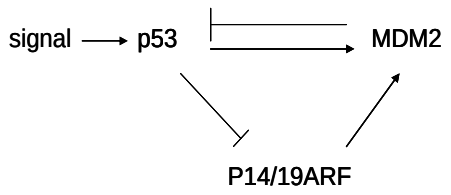

Fig.1.p53/Mdm2/p14/19 ARF loop. See text for details. Arrows denote stimulatory interactions, whereas horizontal bars instead of arrowheads indicate inhibitory influences.

ribosome biogenesis and the cell cycle, we do not understand how these observations come together to form this regulatory loop.

Exploiting autoregulatory negative feedback loop, in this paper, we add another important factor p14/19ARF into the dynamical model. we propose a dynamical model of negative p53-Mdm2 feedback system regulated by p14/19ARF with the aim to describe the dynamical behaviors of protein levels both in individual and populations of cells in a self-consistent and unified way. It should be emphasized that different from the previous models[1] [6], the dynamics of damage-derived signal is paid special attention in this paper in addition to taking account of all the knowledge of the biochemical mechanism of the system and to be simplified to the major components in the system, because the dynamics of damage-derived signal might play crucial role in describing the different dynamical activities of the system. At the cellular level, the signal is assumed as the form with abrupt transition ("on" or "off") as soon as signal strength passes forth and back across a threshold. The time duration when the signal is above the threshold mainly depends on the signal strength, the different manners for cells to response the damage and the repairing abilities of cells. For the case of population of cells, the activities of p53 protein will be the ensemble average of the individual cells, each of which responses damage with different time duration.Under abovementioned considerations, the experimental results[1] and [2] will be satisfactorily reproduced in this paper.

II. Dynamical Model

We now present our dynamical model of p53-Mdm2 feedback loop regulated by p14/19ARF. We assume that the concentration of p53 protein obeys the following kinetic equation:

$$\frac{dp53(t)}{dt} = S_{p53} - \alpha_{p53} \cdot Mdm2(t) \cdot p53(t) \cdot (1 - \gamma_p S(t)) - \beta_{p53} p53(t) \quad (1)$$

On r.h.s. in Eq. (1), the first term describes the synthesis rate of the p53 protein, the second one represents Mdm2 and signal-dependent degradation of p53 and the last one reflects an Mdm2-independent mechanism for p53 degradation. The coefficient α_{p53} represents the ability of Mdm2 to promote p53 degradation, and controls the basal levels of p53. S(t) is the damage-derived signal which is the key component as described above. The introduction of parameter γ_p is to take into account of that to what extent the damage-derived signal S(t) might inhibit the p53 degradation induced by the activation of Mdm2 protein.

Mdm2(t) represents the concentration of Mdm2 protein whose kinetic equation is given as:

$$\frac{dMdm2(t)}{dt} = S_{Mdm2} + \alpha_{Mdm2} \cdot T(t) - \beta_{Mdm2} \cdot Mdm2(t) \cdot p_{14/19ARF}(t) \quad (2)$$

Here the coefficient S_{Mdm2} denotes the rate of p53-independent Mdm2 transcription and translation, whereas the last term describes Mdm2 degradation. The coefficient α_{Mdm2} denotes the maximal initiation rate of Mdm2 transcript initiation up regulated by p53[6]. $T(t)$ in the second term is a Hill-type function and reads which takes into account the transcriptional and/or translational time delay, denoting as time τ , between the activation of p53 and the induction of Mdm2. The parameter K corresponds to some sort of threshold-foractivation for p53-protein concentration, and N is a Hill coefficient that determines the steepness of $T(t)$.

$$T(t) = \frac{\{p53(t-\tau)\}^N}{K^N + \{p_{53}(t-\tau)\}^N} \quad (3)$$

$P_{14/19ARF}(t)$ represents the concentration of p14/19ARF protein whose kinetic equation is given as:

$$\frac{dp_{14/19ARF}(t)}{dt} = S_{p14/19ARF} - \alpha_{p14/19ARF} \cdot p_{53}(t) \cdot p_{Mdm2}(t) \cdot p_{14/19ARF}(t) - \beta_{p14/19ARF} \cdot p_{14/19ARF} \quad (4)$$

On r.h.s. in Eq. (4), the first term describes the synthesis rate of the p14/19ARF protein, the second one represents p53, Mdm2 and signal-dependent degradation of p14/19ARF and the last one reflects an Mdm2-independent mechanism for p14/19ARF degradation. The coefficient $\alpha_{p14/19ARF}$ represents the ability of p53 and Mdm2 to promote p14/19ARF degradation.

Equations (1),(2),(3)and (4) describe how the nonlinear dynamics of the system depends on the parameters incorporated in the model.

It is not so easy to define all the model parameters. Most of the parameters can not be defined since the lack of reliable experimental data. Some of them can be roughly estimated

phenomenologically, for example, α_{p53} is taken to be small with respect to the Mdm2-dependent rate of p53 elimination, which reflects the fact that although other mechanisms for the degradation of p53 may exist, a large body of data points to Mdm2 as the key regulator of p53 stability[1]. The first order degradation rate of Mdm2 β_{Mdm2} could be chosen as 0.05/min, which corresponds to Mdm2 half-lives approximately 20-25min under basal condition. The sound experimental results at the cellular level[2] have also been considered to determine the parameters, which shows that the width of pulse was 350±160 min; the timing of the first pulse maximum was rather variable, 360±240 min after damage, but the time between the maxima of two consecutive pulses was more precise, 440±100 min. Mdm2 peaks with a time delay of $\tau \approx$ 100 min relative to p53 maximum. With the parameters used in this paper, the eigenvalues of the dynamical matrix of Eqs. (1)(2) and (4) are always negative and thus the solutions are expected to be always stable.

When cells are exposed to the damaging agents, such as UV or ionizing radiation, the signal S(t) will be derived which eventually activates an initial pulse of p53 concentration. From biological point of view, at cellular level, S(t) can be considered as switch "on" and will be with abrupt transition from "on" to "off" when signal is resolved, as the behavior of the p53-Mdm2 system evolves to give reasonably defined quanta of repair enzymes in response to stress[2]. S(t) might be defined in Eq. (5) as a step function in time.

$$s(t) = \phi(t - \tau_{th}) = \begin{cases} 1 & if \quad t \le \tau_{th} \\ 0 & if \quad t > \tau_{th} \end{cases} \qquad (5)$$

where $\tau_{th} = n\tau_{ch}$, and τch is the characteristic duration within which the signal stress is in the region of oscillatory response and a pulse is activated. $n\tau_{th}$ accounts for the total time scale of S(t). The value of τth used in this paper is $\tau_{th} \approx$ 410 min. which is obtained from the characteristic frequency of the solutions of Eqs. (1) and (2).

Figure2 shows the dynamical evolution of the concentration of p53 and Mdm2 proteins for the case in individual cells with S(t) defined in Eq. (5), which are scaled with their basal values $P_{53}(0)$ and Mdm2(0) . Under normal environment, the amount of p53 protein in the cell is kept low and tightly autoregulated by a genetic network built of Mdm2 and p53 itself. p53 is produced at an essentially constant rate and promotes the expression of the Mdm2 gene[7]. On the other hand, the Mdm2 protein binds to p53 and promotes its degradation[8], decreasing its concentration. When DNA molecule is damaged, a cascade of events causes phosphorylation of several serines in the p53 protein, which modifies its binding properties to Mdm2[9]-[11]. As a consequence, the cell experiences a sudden increase in the concentration of p53, which activates a group of genes responsible for cell growth arrest and apoptosis. The increase in p53 protein levels and the transcription activity of p53 lead, in turn, to increase the production of Mdm2. Mdm2 protein again promotes the rapid degradation of the p53 protein. So as the sustained oscillations occur. When the signal is completely resolved, the p53-Mdm2 loop return to normal case and the levels of p53 and Mdm2 to their basal values. The key features displayed in Fig. 2 are that the width of each pulse is 328 min; the time of first pulse maximum at 327 min; the time between first and second pulses 413 min; the time delay τ = 100 min and the peaking of second pulse at 720 min. All those features satisfactorily fit the experimental results reported in [2].

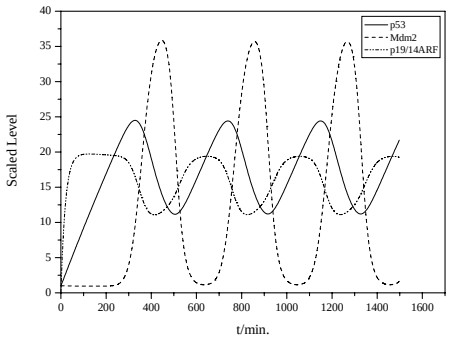

Fig.2.Concentration of p53 tumor suppressor protein and its transcriptional target Mdm2 and p14/19ARF relative to their basal levels with the parameters: S_{p53}= 0.5, α_{p53}= 1.8, γ_p= 0.996, β_{p53}= 0.00025, S_{Mdm2} = 0.00235 , α_{Mdm2}= 0.1 , β_{Mdm2}= 0.05, $S_{p14/19ARF}$=0.5, $\alpha_{p14/19ARF}$=0.00025 , $\beta_{p14/19ARF}$ =0.00025, K = 120, N = 10. The initial conditions of $P_{53}(t)$, Mdm2(t) and $P_{14/19ARF}(t)$ are defined by their basal values as $P_{53}(0)$ = 5.89, Mdm2(0) = 0.047 and $P_{14/19ARF}(0)$=0.5. The dynamics of signal S(t) is described by Eq. (5). The transcriptional time delay τ =100 minutes.

III. Discussion and Analys

We propose a dynamical model to study the dynamical mechanism of the negative feedback loop composed of p53 and Mdm2 proteins regulated by p14/19ARF. We also want to discuss another important problem , it is how the p14/19ARF regulate the negative feedback loop composed of p53 and Mdm2 proteins. So we studied an important parameter in the model that is $S_{p14/19ARF}$ describing synthesis rate of the p14/19ARF protein. In Fig.3 , we give the Limit Cycle of Mdm2 and p53 and how the parameter $S_{p14/19ARF}$ in the model regulate the negative feedback loop.

From the results in the Fig.3 we can see that the new factor we add to the negative feedback loop composed of p53 and p14/19ARF proteins place an very important function. We can see that the Limit Cycle of p14/19ARF and p53 will become smaller with the change of the parameter $S_{p14/19ARF}$. That result means that the model we have found can study the function of p14/19ARF in the protein network very well. Just as we see from the Fig.1 , when the parameter ---$S_{p14/19ARF}$ become larger , the first influence is take to Mdm2. the concentration of Mdm2 become lower . Because of the negative feedback loop composed of p53 and Mdm2, the concentration of p53 become larger . The same results we also can find from the Fig.4. In Fig.4, with the change of the

parameter $S_{p14/19ARF,}$ we can see the change of scaled level about p14/19ARF and Mdm2. when the parameter $S_{p14/19ARF}$

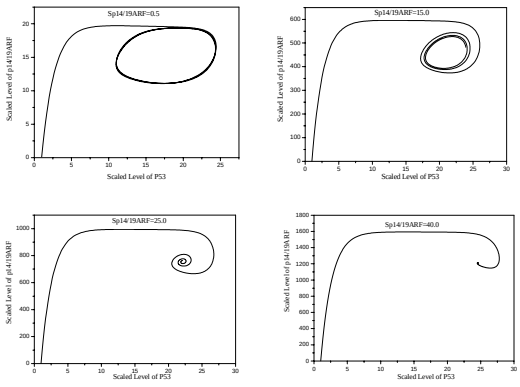

Fig. 3. Based on the dynamic model, we give the Limit Cycle of Mdm2 and p14/19ARF to study how the parameter $S_{p14/19ARF}$ in the model regulate the negative feedback loop. It also present the regulation of p14/19ARF to negative feedback loop composed of p53 and p4/19ARF.

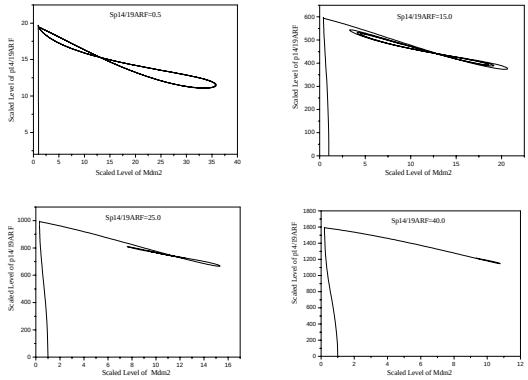

Fig.4. Based on the dynamic model, we give the scaled level of Mdm2 and p14/19ARF to study how the parameter $S_{p14/19ARF}$ in the model regulate the negative feedback loop. It also present the regulation of p14/19ARF to negative feedback loop composed of Mdm2 and p14/19ARF .

become larger, the scaled level about p14/19ARF become larger and the scaled level about Mdm2 become lower. In order to study the network using numerical results, in Fig.5 ,we give the average scaled level about p53 and Mdm2 witch changing with the parameter $S_{p14/19ARF}$.

IV. CONCLUSION

We present a dynamical model of the p53-Mdm2 feedback loop regulated by p19ARF both in individual cell and in population of cells. We attempt to capture the gross mechanisms of p53-Mdm2 interactions regulated by p14/19ARF, we have investigated numerically how different parameters can shape the types of behavior that the system can exhibit. In particular, we show that specific assumptions characterizing the interactions between p53,Mdm2 and p14/19ARF regulated by p14/19ARF lead to an oscillatory behavior of p53, Mdm2 and p14/19ARF protein levels after a sufficiently strong damage signal. Such oscillation may

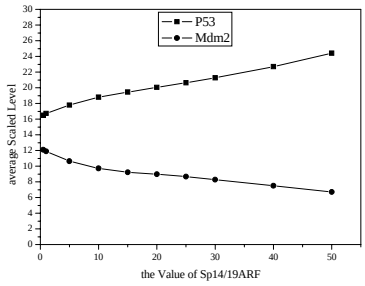

Fig.5. the average scaled level about p53 and Mdm2 witch changing with the parameter $S_{p14/19ARF}$.

enable more effective execution of a reversible p53 response. In agreement with this prediction, the levels of three proteins are proved to satisfactorily fit experimental results reported in lung cancer cells. The dynamical model of cancer-correlative p53-Mdm2 feedback loop regulated by p14/19ARF and its numerical results will help us understand the origin of cancer and the model may help to understand oscillations and variability in other regulatory systems [12],[13].

REFERENCES

[1] Lev Bar-Or, R., R. Maya, L. A. Segel, U. Alon, A. J. Levine, and M. Oren. Generation of oscillations by the p53-Mdm2 feedback loop:a theoretical and experimental study. Proc. Natl .Acad.Sci.USA. vol.97, Oct,2000,pp.11250-11255.

[2] Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB, Alon U. Dynamics of the p53-mdm2 feedback loop in individual cells. Nat Genet ,vol.36,Nov,2004,pp.147-150.

[3] Bray D. 1Protein molecules as computational elements in living cells. Nature .vol.376.Jul,2002,pp.307-312.

[4] JE Ferrell, Jr and W. Xiong, Bistability in cell signaling: how to make continuous processes discontinuous, and reversible processes irreversible. Chaos ,vol.11,Mar,2001, pp. 227-236.

[5] Ma L, Wagner J, Rice JJ, Hu W, Levine AJ, Stolovitzky GA.A plausible model for the digital response of p53 to DNA damage. Proc Natl Acad Sci USA .vol.102, Otc,2005, pp. 14266-14277.

[6] N.A.M. Monk, Oscillatory expression of Hes1, p53, and NF-κB driven by transcriptional time delays. Curr. Biol. Vol.13,Aug,2003, pp. 1409-1413.

[7] Haupt Y, Maya R, Kazaz A and Oren M . Mdm2 promotes the rapid degradation of p53. Nature .vol.387, May,1997,pp.296-299.

[8] Kubbutat MHG, Jones SN & Vousden KH. Regulation of p53stability by MDM2. Nature, vol.387,May,1997,pp.299-303.

[9] Unger T, Juven-Gershon T, Moallem E, Berger M, Vogt Sionov R, Lozano G , Oren M, and Haupt Y. Critical role for Ser20 of human p53 in the negative regulation of p53 by Mdm2. EMBO J . vol. 18. Jul, 1999 . pp.1805-1814.

[10] Volfson D, Marciniak J, Blake WJ, Ostroff N, Tsimring LS, Hasty J Origins of extrinsic variability in eukaryotic gene expression. Nature. vol. 439 ,Feb,2006,pp. 861-864.

[11] Harris SL, Levine AJ (2005) The p53 pathway: positive and negative feedback loops. Oncogene .vol.24, Nov, 2005,pp. 2899-2908.

[12] Naama G Z, Nitzan R. S., Itzkovitz R., Milo Alex S., Erez D.,l Talia Y., Yuvalal L., Paz P., Lahav G and Alon U, Oscillations and variability in the p53 system. Molecular Systems Biology .val.2.2006,2006.0033.

[13] Ogunnaike BA. Elucidating the digital control mechanism for DNA damage repair with the p53-Mdm2 system: single cell data analysis and ensemble modelling.J R Soc Interface. 3(6),175-84 (2006).