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Mathematical Modeling and Simulation of the P53 and SMAR1using their Biochemical Interactions

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Abstract

Recent studies suggest that Smar1, a MAR binding protein, also interacts with p53 by phosphorylating it at Serine-15 residue and stabilize it. This mechanism is important to increase the level of p53 by many folds, which is required for the cell cycle arrest. Pathway reconstruction suggests that p53 is maintained at lower level by Mdm2. When any DNA damage is induced in the cell the level shoots up which is of great importance to cause the cell cycle arrest. This procedure buys time in the cell cycle to carry out repair mechanism, but if the repair is not possible then p53 induces apoptosis in the cell. Simulating the interaction suggest that p53, phosphorylated p53 and acetylated p53 show the damped oscillation around the constant concentration when triggered with ionizing radiation. With the change in the amount of Ionizing radiation dose the mean of p53 in 30 hrs oscillates between 60 to 72 units of concentration affecting the counterparts as well. The behaviors of all the p53 forms are similar and reach the maximum concentration at almost same time. ATM and Smar1 control the p53 oscillation with the help of feedback loop formed by p53 and negative regulator, Mdm2.

Keywords: SMAR1, BANP, p^{53}, ODE (Ordinary Differential Equation), Simulation.

Introduction

Cell cycle is important clue mechanism of cancer cells [17]. Proto-oncogenes are activated to become oncogenes by mutations that cause the gene to be excessively

active in growth promotion. This involves the tumor suppressor genes which codes for anti-proliferation signals that suppress mitosis and cell growth. Tumor suppressor genes are transcription factors that are activated by cellular stress or DNA damage. The function of such, a gene is to arrest the progression of the cell cycle in order to carry out DNA repair, preventing mutations from being passed on daughter cells [17].

Tumor suppressor p53

Maintenance of the G1/S cell cycle checkpoints is dependent on the product of tumor suppressor gene TP53. This gene is mutated or deleted in over half of all cancers making genetic changes in TP53. The tumor suppressor p53 plays a critical role in preventing human cancer formation. p53 is a tumor suppressor protein has an important role in cell cycle control and apoptosis[3,4,9]. Defective p53 could allow abnormal cells to proliferate, resulting in cancer as many as 50% of all human tumors contain p53 mutants [11, 22]. In response to a variety of stress signals, often associated with the progression of neoplastic diseases, p53 becomes activated and induces cell cycle arrest and/or programmed cell death (apoptosis). Wild type of p53 binds to specific genomic site with consensus site at 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3'. P53 binds as tetramer and stimulates expression of downstream genes that negatively controls growth and apoptosis. In some cells type p53 results into apoptosis as means of eliminating damaged cells [12, 13, 20, 28]. In normal cells, p53 is maintained at low level by targeted degradation by its negative regulator MDM2. MDM2 functions as p53 ubiquitin ligase and triggers its degradation. Another potential mechanism of p53 is acetylation. Multiple lysine residues in p53 are acetylated by p300 or CBP, studies shows that these sites are acetylated in response to DNA-damaging agents [26]. Acetylation stimulates p53 DNA binding activity. DNA damages occur in the cell due to environmental stress causing DNS damage or by oncogene activation. Therefore, it decreases the activity of MDM2 and allows the accumulation of the p53 in the nucleus. Such DNA damage may activate different types of the protein kinases such as ATM, ATR, DNA-PK, CHK2 as well as SMAR1. All these protein kinases involved in the phosphorylation of p53 residues at particular site there by increases p53 levels. This activation of different types of protein kinases mainly depends upon the type of DNA damage agents that are UV radiation, IR, hypoxia, doxorubicin. Here we are interested in doxorubicin DNA damage which activates SMAR1 [18, 19, 21, 25].

SMAR1

SMAR1 is scaffold/matrix associated region 1 identified as the MAR (matrix attachment region) binding protein. SMAR1 exists in two alternatively spliced forms: $SMAR1^{L}$ and $SMAR1^{S}$, with deletion of 39 amino acids in the N-terminus in the later form. SMAR1 shows more than 99% identity with its human counterpart BANP. This again suggested that SMAR1 might have a role in breast tumorigenesis. SMAR1 is located at human chromosome 16q24.3 locus. This region harbours tumor suppressor genes involved in breast cancers. Hence tumor suppressor activity of p53 and SMAR1 results into cell cycle arrest. Doxorubicin DNA damage induces the production of the SMAR1 in particular case. Doxorubicin is a anti-cancer drug

therefore used to induce the DNA damage. Doxorubicin acts through the p53 activation and found that SMAR1 is induced upon doxorubicin treatment in the cell [5, 15, 16, 24, 25, 26].

Methods

Construction of network model is done from the above interactions. These interactions show that p53 is synthesised at constant rate of value, under normal conditions it gets bound to the MDM2 and then get degraded [14]. The synthesis of MDM2 is mainly depends upon the mdm2_mRNA expression. We assume that in normal conditions p53 is kept at low by MDM2 and p53 is transcriptionally activate when not bound with MDM2. Upon DNA damage it induces SMAR1 activation which gives the phosphorylation of p53 ata serine-15 residue and it destructs MDM2_p53 binding while, MDM2 is degraded and its level get decreased [12,13,17]. Here we construct the p53-MDM2-SMAR1 model with their expression pattern. Firstly, these interactions are organizing into map or wiring diagram.

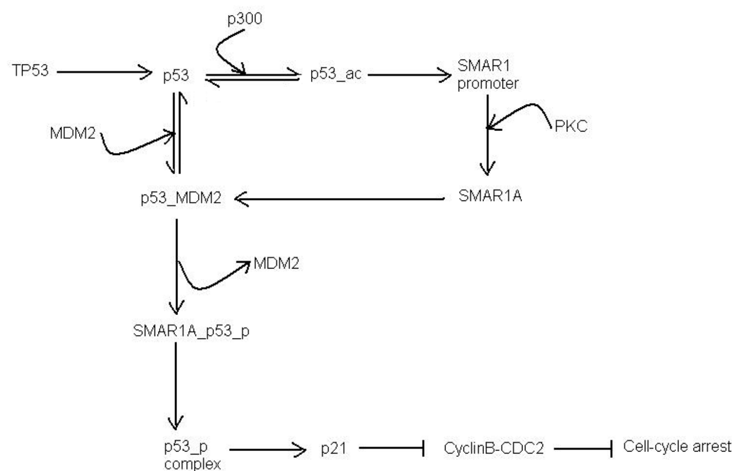


Figure : Wiring diagram of P53-MDM2-SMAR1 Interactions.

TP53 = Tumor protein 53 gene, P53_ac = Acetylated p53, SMAR1 = Scaffold/matrix associated region, PKC = Protein kinase family C, MDM2 = Murine double minute-2, SMAR1A = Activated SMAR1

We define these interactions by using biochemical reactions. The species involved in the reaction is quantified by using the parameter values. This biochemical reactions are need to converted into nonlinear ODEs [8]. These rate equations are written for each biochemical entity i.e. each species in the reaction. All the reactions are simulated in cell designer tool [1].

Experimental testing of model

A major reason to build a model is to make the novel predictions that informs

subsequent round of experimentation. By using such a mathematical model experiment can be simulated in silico by varying the values of the parameter in logical ways. This will gives the behavior of the model with respect to time. The interplay between the model and its experimentation is most valuable to design the novel and non- intuitive predictions [1, 25].

Modelling tools

The modeling tool is used to simulate all the reactions, these tools are necessary for model building, simulation of reactions, data fitting, and data management. Here, we use the Celldesigner [1] and COPASI (Complex Pathway Simulator) for the simulation of reaction [27].

Reactions

This model includes 20 reactions defined as below.

1. Source -> p53 mRNA
2. p53 mRNA -> Sink
3. Mdm2 mRNA –> Mdm2 mRNA + Mdm2 + mdm2syn
4. p53 –> p53+Mdm2 mRNA+Mdm2mRNAsyn
5. p53_P –> p53_P + Mdm2 mRNA + Mdm2mRNAsyn
6. Mdm2 mRNA –> Sink+Mdm2mRNAdeg
7. Mdm2 –> Sink+mdm2deg
8. p53 mRNA –> p53+p53 mRNA+p53syn
9. Mdm2 p53 –> Mdm2+p53deg
10. p53+Mdm2 –> Mdm2 p53
11. Mdm2 p53 –> p53+Mdm2
12. Doxor –> Doxor + damDNA
13. damDNA –>Sink
14. damDNA + P53_ac –> damDNA + SMAR1A
15. p53+SMAR1A –>p53_P+ SMAR1A
16. p53_P –> p53
17. Mdm2 + SMAR1A -> Mdm2_p + SMAR1A
18. Mdm2_p -> Mdm2
19. Mdm2_p -> sink + Mdm2deg
20. SMAR1A –> SMAR1I

List of parameter values and initial amount of species of biochemical reaction

Parameter Values

Id	Value	Unit
IR	0.000 10	Gy
ksynMdm2	$4.95 \cdot 10^{-4}$	s^{-1}
kdegMdm2	$4.33 \cdot 10^{-4}$	s^{-1}

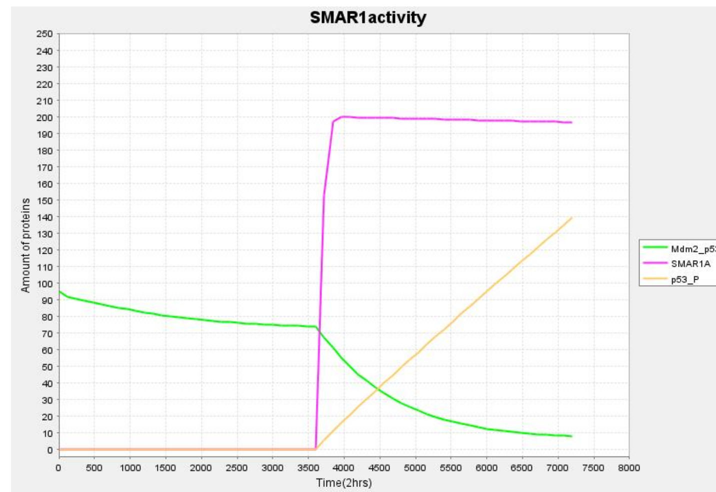
ksynp53	0.006	s−1
kdegp53	8.25 ·10−4	s−1
kbinMdm2p53	0.001	mol−1·s−1
krelMdm2p53	1.155 ·10−5	s−1
ksynMdm2mRNA	10−4	s−1
kdegMdm2mRNA	10−4	s−1
kactSMAR1	10−4	mol−1 ·s−1
kdegSMAR1Mdm2	4 ·10−4	s−1
kinactSMAR1	5 ·10−4	s−1
kphosp53	5 ·10−4	mol−1 s−1
kdephosp53	0.050	s−1
kphosMdm2	2.000	mol−1 ·s−1
kdephosMdm2	0.500	s−1
Kdam	0.080	mol · s−1
krepaDoxor	2 ·10−5	s−1
Kproteff	1.000	dimensionless
ksynp53mRNA	0.001	s−1
kdegp53mRNA	10−4	s−1

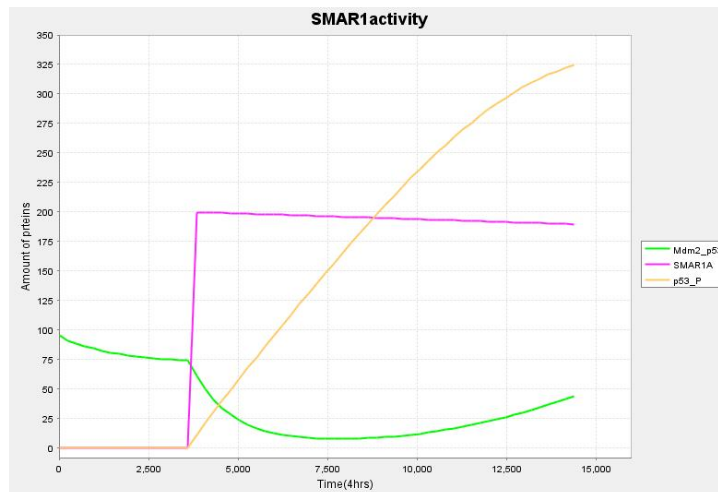
Initial Amount

Species	Initial Amount
Mdm2	5 mol
p53	5 mol
Mdm2_ p53	95 mol
Mdm2 mRNA	10 mol
p53 mRNA	10 mol
SMAR1A	0 mol
SMAR1	0 mol
P53_Ac	200 mol
p53 P	0 mol
Mdm2_P	0 mol
Doxor	0 mol
damDNA	0 mol
Sink	1 mol
Source	1 mol
p53deg	0 mol
p53syn	0 mol
mdm2deg	0 mol
mdm2syn	0 mol
Mdm2mRNAdeg	0 mol
Mdm2mRNAsyn	0 mol
totp53	0 mol
totMdm2	0 mol

By using above parameters and initial values behaviour of the system model can be checked. All these reactions are simulated in cell designer. We choose all default parameters for the model. After the DNA damage it shows increase in rate of p53, Mdm2 and SMAR1 results in reduction of oscillations. Increase in binding affinity of p53 and MDM2 results into degradation of the p53 this shows the decrease in rate value. P53 synthesis depends upon the transcription of p53 i.e. value of p53 depends upon the level of the p53_mRNA. Due DNA damage it gives the increase in the SMAR1gene. SMAR1 transcription depends upon the acetylated p53, which recruited on the SMAR1 promoter and leads to induce the expression of the gene, This results into formation of SMAR1 protein [24, 25, 28]. This SMAR1 protein acts on p53 shows increase in phosphorylated p53 which is separated from MDM2, this also gives decrease in the MDM2 reaction rate. Hence this phosphorylated p53 further activates p21 for G1 phase arrest in the cell cycle [23, 29].

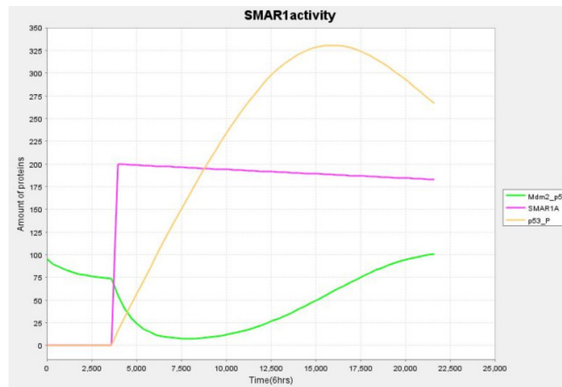
Results



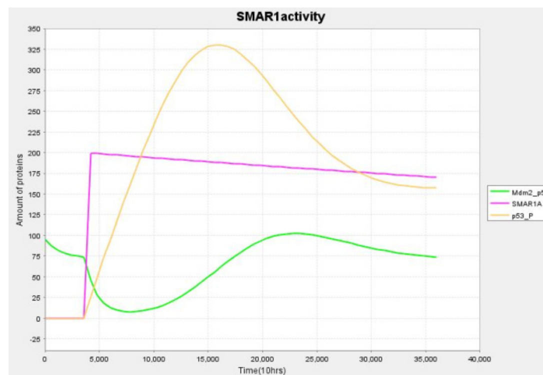


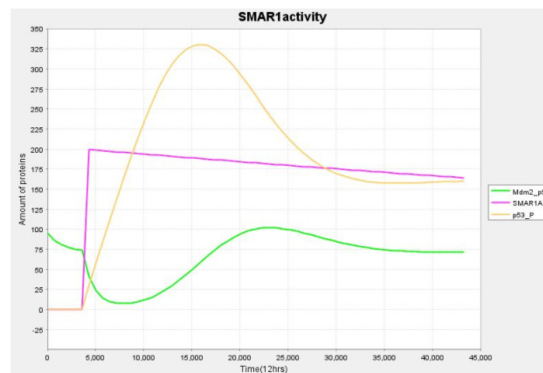
As shown in first graph for 1hr activated SMAR1 and phosphorylated p53 shows no increase while, MDM2_p53 decreases slowly. At the start of 2hr shows increase in phosphorylated p53 simultaneously increase in SMAR1. First graph shows gradual increase at the start of 2hr shows the effect of DNA damage agent [6, 7, 9, 19].

In second graph, decrease in MDM2_p53 relates with increase in phosphorylated p53 and SMAR1. This shows up to 4 fold increase in graph i.e. accumulation oh phosphorylated p53. This leads to cell cycle arrest [21].

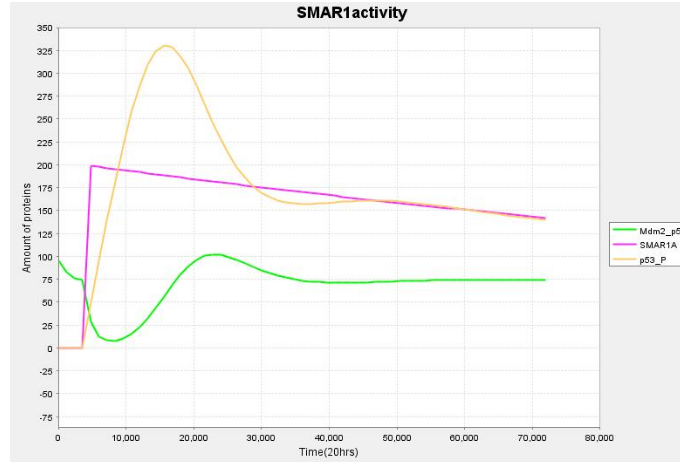


At the start of 6hrs phosphorylated p53 decreases with smaller increase in MDM2_p53 and slowly stabilization of SMAR1. [11]





In the graph of 10 hrs, at the point of 8 to 8.5 hrs shows decrease in the phosphorylated p53 and SMAR1 with smaller increase in MDM2-p53. While, within 12hrs SMAR1 get stabilized with smaller decrease in phosphorylated p53 [25]



Within 20hrs phosphorylated p53 and SMAR1 decreases slowly & get stabilized at same point. Also, MDM2_p53 increases & get stabilized. MDM2_p53 shows oscillatory behavior [21,22,23].

Discussions

We develop the simple mathematical model for activity of p53 and SMAR1 after DNA damage occurs in the cell. Our model shows the different mechanisms of the SMAR1, p53 and MDM2. Firstly we started with the network model that incorporate with the biochemical reactions and SBML converted this into stochastic model that can be simulated. All biochemical reaction rates are based on the mass action kinetics. Our model shoes that after DNA damage SMAR1 get induced which interferes with the p53_MDM2 binding. This SMAR1 phosphorylates the p53 at serine-15 residue so, it gives rise to p53 level which further leads to cell cycle arrest. This lowers the MDM2 level by degradation. If p53 is prevent from binding with MDM2 and cell cycle begins again. This prevention of binding MDM2_p53 after DNA damage shows the oscillatory behavior in the model. SMAR1 phosphorylates p53 which disrupts its activity to bind with MDM2 and causes to cell cycle arrest. Also our model shows the stabilization of p53. As per the literature, our model shows accurate behavior of p53, MDM2 and SMAR1 [2, 6, 5, 10, 11]. In addition to this phosphorylated p53 and MDM2_p53 binding shows their oscillatory behavior in the system at different interval of time. The variability of the graphs in the models are depend upon the reaction rate kinetics. This shows the agreement between model and experimental data. Most of the parameters are based on known values and the parameters for which we were least confident had the smallest effect on the model behavior [25].

Conclusion

We have developed the model of p53-MDM2-SMAR1 interactions to examine the cell cycle mechanism after DNA damage. From the literature, we consider that p53 is stabilised by the activation of the SMAR1. The model predicts the oscillations in the phosphorylated p53 and MDM2-p53 activity. The models were encoded in SBML that they can be easily modified and extended as more data become available. Our work illustrates the importance of systems biology approaches to understanding the complex role of p53 and SMAR1in cancer [25, 28].

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