Modeling amplified p53 responses under DNA-PK inhibition in DNA damage response

SUPPLEMENTARY DATA

MATERIALS AND METHODS

Model construction

The model consists of 11 species and 65 kinetic parameters. The schematic diagram is shown in Supplementary Figure 1A and the model equations are listed in Supplementary Table 2. For p53, MDM2 and WIP1, we incorporated both mRNA (italics) and protein. Total levels of class-IV phosphoinositide 3-kinase (PI3K)-related kinases (PIKK) named Ataxia-telangiectasia-mutated (ATM), ataxia telangiectasia and Rad3-related (ATR) and DNA-dependent protein kinase (DNA-PK) were set to be constant during the simulation [1]. Eq.1 and Eq.2 describe the production and degradation of p53 and mdm2 mRNA. The degradation rate of p53 (k_p) was estimated from [2]. The degradation rate constant for mdm2 (k_m) was adapted from [3]. Eq.2 further incorporated P53 induced mdm2 expression. It has been shown that P53 is present at mdm2 promoter even under unstressed conditions and mdm2 promoter is not tightly associated with nucleosomes [4, 5]. According to ‘anti-repression’ model, P53 is constitutively active but repressed by MDM2 [4]. Therefore, free p53 may transactivate a subset of genes such as MDM2. Activated p53 by posttranslational modifications may further transcriptionally upregulate downstream targets such as mdm2 and wip1 [6]. Therefore, Eq.3 describes wip1 turnover. The first two terms in Eq.4 show translation and degradation for P53. The combined P53 degradation rates (basal and MDM2-induced) were adjusted to be consistent with experiments [7]. ATM, ATR and DNA-PKcs can all phosphorylate P53 at Ser15 and activate P53 [4, 6]. For simplicity, DNA-PKcs was abbreviated as DNA-PK throughout the text. Previous reports also showed that WIP1 can dephosphorylate activated ATM, and phosphorylated forms of both P53 and MDM2 [8]. Eq.5 describes the conversion from P53 to activated forms and deactivation by WIP1. We also described MDM2 mediated degradation of activated p53 as previously suggested [9]. Eq. 6 shows mdm2 translation and MDM2 turnover. ATM could phosphorylate MDM2 at Ser395 and induce MDM2 self-degradation [10, 11]. Therefore, we assumed that MDM2 can perform self-degradation once it is phosphorylated in Eq. 6 and Eq. 7. Eq. 8 simply describes wip1 translation and WIP1 turnover. The first term in Eq. 9 represents DNA damage induced ATM activation. The second term shows ATM trans-autoactivation. WIP1 mediated ATM dephosphorylation was incorporated as described previously [12]. Eq. 10 also covers DNA damage induced ATR activation. It has been demonstrated that ATR can promote its own phosphorylation and activation via either autophosphorylation or activation via ATM mediated phosphorylation or autophosphorylation at several clusters [15] although recent findings discriminate these effects in further details [16]. Therefore, the second and third terms in Eq. 11 characterize ATM catalyzed DNA-PK phosphorylation and autophosphorylation, respectively for simplicity purpose. Basal deactivation rates are incorporated for PIKKs.

Noticeably, we assumed that DNA double strand breaks (DSBs) can directly activate PI3K-like kinases and subsequently induce p53 activation by phosphorylation (Supplementary Figure 1A and Supplementary Table 1). We also incorporated DNA-PK mediated ATM inhibition (Supplementary Table 1, where the ATM mediated activation was multiplied by a coefficient $\frac{1}{k_{13} \cdot [DNA-PK] + k_{14}}$ ) since some reports implied that the ATM kinase activity is inhibited by DNA-PK [17, 18]. Earlier events in DSB repair such as MRE11-RAD50-NBS1 (MRN) and mediator of DNA damage checkpoint protein 1 (MDC1) recruitment, phosphorylation of γH2AX foci, binding of the KU70 and KU80 heterodimers to sites of DNA breaks as well as detailed downstream repair events were not incorporated in our model for simplicity [19]. It has been well documented that ATM relays DNA damage signals through Chk2 while ATR and DNA-PK can coordinate activation of downstream effectors via Chk1 [20, 21]. We did not consider Chk1 and Chk2 in our current model for simplicity owing to their linear signaling transduction properties. Inhibition of DNA-PK catalytic subunit (DNA-PKcs) by specific inhibitors was simplified as DNA-PK inhibition or DNA-PKi. The numbers in Supplementary Figure 1A denote the parameters listed in Supplementary Table 2. Notably, numbers corresponding to basal deactivation rate of activated ATM, ATR and DNA-PK (31, 34 and 38) were not shown in the diagram. We further assumed that the inhibitors can completely block the activity of associated kinases. For treatment with ATM
inhibitor, five parameters ($k_{1,2}$, $k_{2,3}$, $k_{2,4}$, $k_{3,4}$, and $k_{3,6}$) were simultaneously set to be zero. For ATR inhibitor, three parameters ($k_{1,2}$, $k_{5,2}$, and $k_{5,4}$) were restricted to be zero. For DNA-PK inhibitors, four parameters $k_{5,2}, k_{5,3}, k_{5,4}$, and $k_{5,5}$ were taken to be zero. Transcriptional and translational delays are adapted from Ma et al. [9]. The kinetic equations are listed in Supplementary Table 1 while the parameters are provided in Supplementary Table 2.

**Stochastic DSB repair**

This module is a modification of Ma et al’s TLK model. According to Ma et al’s work, there exist two repair pathways (i.e. fast and slow) [9]. It has been shown that 1 Gy irradiation can generate 30 DSBs [22]. For stochastic simulations, we assumed that the generated DSBs obey a Poisson distribution with the mean 30$\chi$ where $\chi$ denotes the radiation dose. Ma et al’s TLK model focuses on p53 oscillations under stressed conditions. Ma et al assumed that 70% of the total DSBs are repaired by fast kinetics, while remaining breaks are repaired through slow kinetics. For example, if initial DSB is 100, then 70 DSBs are repaired by fast kinetics while the remaining 30 DSBs are fixed by slow kinetics.

Given relatively low number of repair proteins, the DNA repair process was implemented through stochastic simulation. In DSB repair module, each locus in which a DSB is created can be in one of three states corresponding to intact DSB (state 1), DSB in complex with repair proteins (state 2), and (correctly or incorrectly) fixed DSB to intact DSB (state 1), DSB in complex with repair proteins (state 2), and (correctly or incorrectly) fixed DSB (state 3) (Supplementary Figure 1). At time step $k$, the number of DSBs in corresponding states (i.e., 1, 2, or 3) are represented by $D(k)$, $C(k)$, and $F(k)$, respectively. By using subscripts ‘1’ and ‘2’ to differentiate simple DSBs (fast kinetics) and complex DSBs (slow kinetics), we have $D(k) = D_1(k) + D_2(k)$, $C(k) = C_1(k) + C_2(k)$ and $F(k) = F_1(k) + F_2(k)$. The total number of repair proteins (RP) is assumed to be 20 as previously indicated [9]. At any time, variable repair proteins are free to bind to DSBs. To implement step size control, we chose a relatively small step size ($\Delta t = 0.2$, which is smaller than the shortest $\tau$ in $\tau$-leap method in our simulation). That means, even the smallest $\tau$ can cover more than one $\Delta t$ in $\tau$-leap simulation (Supplementary Figure 1). We updated the DNA repair module by consecutive $\Delta t$ (i.e. $n\Delta t$, where $n = \text{mod} \Delta t$, ‘mod’ denotes modulus after division). To retain compatibility with $\tau$-leap size, the last step size within the current ‘$\tau$’ was set to be $\tau$-$n\Delta t$. We simply modified the fixation rate of double strand breaks ($k_{fix1}$) as: $k_{fix1} = k_{fix1}^\tau \cdot (ATM + ATR + [DNA-PK]^3)/3$ as activated ATM, ATR and DNA-PK can participate in the DSB repair process by various ways. The Monte Carlo algorithm for the evolution of DSBs during time $[0, t_{final}]$ is as follows:

1. Set the initial conditions. The initial total number of DSBs ($DSB_{i1}$) was generated by a Poisson distribution.

Set $t=0$. We further set $D_1(0) = 0.7 \cdot DSB_{i1}$, $D_2(0) = 0.3 \cdot DSB_{i1}$, $C_1(0) = C_2(0) = 0$ and $F_1(0) = F_2(0) = 0$. Given that initially all repair proteins are free, the simulation starts with total $RP=20$. There is no DSB until intrinsic DSBs are encountered. If a new DSB occurs, we assume that it is in state 1 (i.e. intact DSB).

2. Increment time. Set $t=t+\Delta t$ (if not specified). Let $k= t/\Delta t$.

3. Update the states for each of the damage sites controlled by fast repair. Compute the transition probabilities as follows:

   - From state 1 to state 2,
     
     $P_{D1\rightarrow C1} = RP [k_{h1} + k_{c1}(D_1(k-1)+D_2(k-1))] \Delta t$

   - From state 2 to state 1,
     
     $P_{C1\rightarrow D1} = k_{h1} \Delta t$

   - From state 2 to state 3,
     
     $P_{C1\rightarrow F1} = k_{h1} \Delta t$

   For each DSB, generate a value $X$ from a uniform distribution with 0 and 1. If the DSB is in state 1, a transition to state 2 occurs if $0 \leq X < P_{D1\rightarrow C1}$, while it stays in state 1 if $P_{D1\rightarrow C1} \leq X \leq 1$. If the damage is in state 2, a transition to state 1 occurs if $0 \leq X < P_{C1\rightarrow D1}$, or a transition to state 3 occurs if $P_{C1\rightarrow D1} \leq X < P_{C1\rightarrow F1} + P_{C1\rightarrow D1}$, or no transition occurs if $P_{C1\rightarrow D1} + P_{C1\rightarrow F1} \leq X \leq 1$. If the damage is in state 3, it stays in state 3 (i.e., state 3 is absorbing). Set $RP = RP - 1$ if transition from state 1 to state 2 occurs; set $RP = RP + 1$ if transition from state 2 to state 1 occurs; otherwise $RP$ remains the same. Count the number of fast repaired breaks at time $t$ in states 1, 2, and 3.

4. Update the states for each of the damage sites controlled by slow repair. Compute the transition probabilities as follows:

   - From state 1 to state 2,
     
     $P_{D2\rightarrow C2} = RP [k_{h2} + k_{c2}(D_1(k-1)+D_2(k-1))] \Delta t$

   - From state 2 to state 1,
     
     $P_{C2\rightarrow D2} = k_{h2} \Delta t$

   - From state 2 to state 3,
     
     $P_{C2\rightarrow F2} = k_{h2} \Delta t$

   For each DSB, generate a value $X$ from a uniform distribution with 0 and 1. If the damage in state 1, a transition to state 2 occurs if $0 \leq X < P_{D2\rightarrow C2}$, while it stays in state 1 if $P_{D2\rightarrow C2} \leq X \leq 1$. If the damage is in state 2, a transition to state 1 occurs if $0 \leq X < P_{C2\rightarrow D2}$ or a transition to state 3 occurs if $P_{C2\rightarrow D2} \leq X < P_{C2\rightarrow F2}$ or no transition occurs if $P_{C2\rightarrow D2} + P_{C2\rightarrow F2} \leq X \leq 1$. If the damage is in state 3, it stays in state 3 (i.e., state 3 is absorbing). Set $RP = RP - 1$ if transition from state 1 to state 2 occurs; set $RP = RP + 1$ if transition from state 2 to state 1 occurs; otherwise $RP$ remains the same. Count the number of fast repaired breaks at time $t$ in states 1, 2, and 3.

5. Let $D(k) = D_1(k) + D_2(k)$, $C(k) = C_1(k) + C_2(k)$, and $F(k) = F_1(k) + F_2(k)$.

6. Repeat steps 2-5 until $t = t_{final}$. 

Summarizing the binomial τ-leap stochastic simulation

For a system with \( N \) molecular species, the vector \( X(t) = (X_1(t), \ldots, X_N(t)) \) denotes the number of molecules \( X_i \) at time \( t \), \( 1 \leq i \leq N \) (\( N = 11 \) in our model). Each species is involved in \( M \) chemical reaction channels (\( M = 36 \) in our model) represented by \( R_j, j=1, 2, \ldots, M \) with a propensity function \( a_j \). Then, \( a_j dt \) denotes the probability that one reaction \( R_j \) will happen in the time interval \([t, t + dt]\) and the \( v_{ij} \) is the stoichiometric coefficient of species \( S_i \) in reaction \( R_j \).

In binomial τ-leap method, each of the \( M \) reactions is allowed to fire in a given order in \([t, t + \tau]\). The maximum firing number \( k_{\text{max}} \) is determined by the limiting substrates, i.e. the species which would be completely depleted if the reaction went to completion. Identification of the limiting reactants is the critical step for implementation of the algorithm.

During the stochastic simulations, the parameters should be rescaled by a factor \( \Omega \). For kinetic parameters describing zero-order reaction (e.g. with unit \( \mu \text{M} \cdot \text{min}^{-1} \)), corresponding parameters in stochastic simulations should be multiplied by \( \Omega \). For second order reaction parameters (e.g. with unit \( \mu \text{M}^{-1} \cdot \text{min}^{-1} \)), the corresponding parameters should be divided by \( \Omega \). The first order parameters are unchanged (See Supplementary Table 2 for parameters).

All reactants are multiplied by \( \Omega \). \( \Omega = 6 \times 10^4 \) in the model.

Briefly, the binomial τ-leap algorithm is represented as follows:

1. Obtaining the stoichiometric coefficients \( v_{ij} \), the initial number of all species \( X(0) \) and the rate constants.
2. Setting the initial time, \( t_0 \).
3. Repeat step 4-6 until the final time \( t_{\text{final}} \) has been reached.
4. Obtaining the propensity function \( a_j(X(t)) \) using the current population \( X(t) \).
5. Compute \( \tau \) and update \( t = t + \tau \). \( \tau \) is defined as follows:

\[
\tau = \frac{f}{\sum_{j=1}^{M} a_j}
\]

Where \( f \) denotes a coarse-grained factor and is set to be \( 10^4 \) as suggested [23].

6. For \( j = 1 \) to \( M \) reactions,
   
   (a) Computing \( k_{\text{max}}^{(j)} = \min(v_{ij} < 0)(\text{int}(X'_i/v_{ij})) \), where \( X' \) denotes the updated \( X \) before execution of any reaction step and \( \text{int}(\cdot) \) is the largest number of integer function.
   
   (b) Compute \( p_j = a_j \tau k_{\text{max}}^{(j)} \), sample \( k_j \) from the binomial distribution as defined below:

\[
P_{\text{BD}}(k_j; p, k_{\text{max}}^{(j)}) = \frac{k_{\text{max}}^{(j)}!}{k_j!(k_{\text{max}}^{(j)} - k_j)!} p_j^{k_j} (1 - p_j)^{k_{\text{max}}^{(j)} - k_j}
\]

BD denotes binomial distribution. If \( a_j \tau > k_{\text{max}}^{(j)} \), we set \( p = 1 \).

   (c) Set \( X_i = X'_i + v_{ij} k_j \) for \( i = 1, \ldots, N \) if \( v_{ij} < 0 \).

7. Go to step 6a.
8. Update \( X_i = X'_i + v_{ij} k_j \), for \( i = 1, \ldots, N \).
9. Go to step 3.

The reactions in stochastic simulations were listed in Supplementary Table 3.

The stoichiometric matrix \( S = [v_{ij}] \) is provided below:

\[
\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
\]

For detailed algorithm, please refer to refs [23, 24].
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Supplementary Figure 1: Representation of the DSB module. A. Two Lesion Kinetic (TLK) model for DSB repair. Each locus can be in one of three states corresponding to intact DSB (state 1), DSB in complex with repair proteins (state 2), and fixed DSB (state 3). At time step k, the number of DSBs in corresponding states (i.e. 1, 2 or 3) are represented by D(k), C(k), and F(k), respectively. Subscripts ‘1’ and ‘2’ differentiate simple DSBs (fast kinetics) and complex DSBs (slow kinetics), respectively. B. Compatibility between Monte Carlo time steps and τ-Leap size. Δt represents Monte Carlo step size while τ denotes τ-Leap size. The smallest leap size τ is larger than Δt (Δt = 0.2).
Supplementary Figure 2: Temporal dynamics of p53 and DSB. A. *In silico* stochastic simulation of p53 dynamics in cells either untreated (violet) or treatment with all PIKK inhibitors (green). IR = 10 Gy. Three stochastic simulation runs are shown for each condition. B. Temporal evolution of DSB repair under 10 Gy irradiation. 100 representative plots were shown.
Supplementary Figure 3: Statistical results of Supplementary Figure 2. The estimated changes of the mean pulse width (A. red) and amplitude (B. blue) for the first p53 pulses under indicated conditions. Quantification of the changes in pulse width C. and amplitude D. in cells untreated or treated with DNA-PK inhibitors under 2.5 Gy, 5 Gy and 10 Gy conditions. Error bars represent 95% confidence intervals.
**Supplementary Figure 4: Statistical results of Figure 3.** The estimated changes of the mean FWHM (A. red) and amplitude (B. blue) for the first p53 pulses under the circumstances when the PIKK inhibitors were used in combination or alone. The error bars denote 95% confidence intervals. The groups with PIKK inhibitors were compared with untreated groups.
Supplementary Figure 5: Simulation of p53, MDM2 and activated ATM with or without DNA-PK inhibition. Temporal dynamics of total p53 A, MDM2 B, and activated ATM C, within first 5 hours post irradiation as determined in Finzel et al.’ work [18] with (blue) or without (red) DNA-PK inhibitor treatment. Data were averaged from 1000 simulations.
Supplementary Figure 6: Relative changes of pulse amplitude and width under DNA-PK inhibitor treatment and wild type conditions. The difference for pulse amplitude (left) and period (right) between DNA-PKi and WT conditions were evaluated and normalized to the values under WT conditions.
Supplementary Table 1: Dynamic equations for the model

\[ \frac{dp53}{dt} = k_1 \cdot p53 \]

Eq.1

\[ \frac{dm2}{dt} = k_3 - k_4 \cdot m2 + k_5 \cdot (P53(t - \tau) + P53p(t - \tau)) / \left[ (P53(t - \tau) + P53p(t - \tau))^2 + K_2^2 \right] \]

Eq.2

\[ \frac{dwip1}{dt} = k_6 \cdot wip1 + k_4 \cdot P53p(t - \tau) \]

Eq.3

\[ \frac{dP}{dt} = k_9 \cdot P53 - k_10 \cdot P53 - k_1 \cdot MDM2 \cdot P53 \cdot \left( \frac{P53 + K_6}{P53 + K_6} \right) - k_15 \cdot [DNA - PK]^* \cdot P53 \]

Eq.4

\[ \frac{dMDM2}{dt} = k_{21} \cdot m2(t - \tau) - k_{20} \cdot MDM2 \cdot \left( \frac{ATM^*}{MDM2 + K_9} \right) \]

Eq.6

\[ \frac{dATM}{dt} = k_{24} \cdot DSB \cdot ATM + k_{26} \cdot ATM^* \cdot ATM \left( \frac{ATM + K_3}{ATM + K_3} \right) \]

Eq.9

\[ \frac{dATR}{dt} = k_{32} \cdot DSB \cdot ATR + k_{33} \cdot ATM^* \cdot \left( \frac{ATR + K_5}{ATR + K_5} \right) \]

Eq.10

\[ \frac{d[DNA - PK]}{dt} = k_{35} \cdot DSB \cdot [DNA - PK] + k_{36} \cdot ATM^* \cdot [DNA - PK] / \left( [DNA - PK] + K_{16} \right) \]

Eq.11

\[ ATM + ATM^* = 1, \ ATR + ATR^* = 1, \ [DNA - PK] + [DNA - PK]^* = 1 \]

The equations are formulated with ordinary differential equations and delay differential equations. The italics denote mRNA species.
Supplementary Table 2: Model parameters and description

See Supplementary File 1
**Supplementary Table 3: The reaction number in stochastic simulation**

| No. | Reactions |
|-----|-----------|
| 1   | $k_i$     |
| 2   | $k_{2;p53}$ |
| 3   | $k_3$     |
| 4   | $k_{7;mdm2}$ |
| 5   | $k_7(P53p+P53)^+[(P53p+P53)^+ + K_{7;}]$ |
| 6   | $k_8$     |
| 7   | $k_{7;wip1}$ |
| 8   | $k_8P53p^+/(P53p^+ + K_{8;})$ |
| 9   | $k_9P53$ |
| 10  | $k_{10;P53}$ |
| 11  | $k_{10;P53-MDM2}/(P53 + K_{10;})$ |
| 12  | $k_{12;P53-ATM^*}/(P53 + K_{12;})$ |
| 13  | $k_{12;P53-ATM^*}/(P53 + K_{12;})$ |
| 14  | $k_{13;P53p-WIP1}/(P53p + K_{13;})$ |
| 15  | $k_{14;P53p-MDM2}/(P53p + K_{14;})$ |
| 16  | $k_{15;mdm2}$ |
| 17  | $k_{16;MDM2}$ |
| 18  | $k_{17;ATM^*-MDM2}/(MDM2 + K_{17;})$ |
| 19  | $k_{18;WIP1-MDM2p}/(MDM2p + K_{18;})$ |
| 20  | $k_{20;MDM2p}$ |
| 21  | $k_{21;WIP1}$ |
| 22  | $k_{22;WIP1}/(MDM2p + K_{22;})$ |
| 23  | $k_{23;ATR^*-P53}/(P53 + K_{23;})$ |
| 24  | $k_{24;ATM^*}/(P53 + K_{24;})$ |
| 25  | $k_{25;ATM^*}/(1-ATM^*)/(1-ATM^* + K_{25;})$ |
| 26  | $k_{26;ATM^*}/(1-ATM^*)/(1-ATM^* + K_{26;})$ |
| 27  | $k_{27;ATM^*}/(P53 + K_{27;})$ |
| 28  | $k_{28;ATM^*}/(P53 + K_{28;})$ |
| 29  | $k_{29;ATM^*}/(P53 + K_{29;})$ |
| 30  | $k_{30;ATM^*}/(P53 + K_{30;})$ |
| 31  | $k_{31;ATM^*}/(P53 + K_{31;})$ |
| 32  | $k_{32;ATM^*}/(P53 + K_{32;})$ |
| 33  | $k_{33;ATM^*}/(P53 + K_{33;})$ |
| 34  | $k_{34;ATM^*}/(P53 + K_{34;})$ |
| 35  | $k_{35;ATM^*}/(P53 + K_{35;})$ |
| 36  | $k_{36;ATM^*}/(P53 + K_{36;})$ |