Modeling the interplay between DNA-PK, Artemis, and ATM in non-homologous end-joining repair in G1 phase of the cell cycle

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Abstract

Modeling a biological process equips us with more comprehensive insight into the process and a more advantageous experimental design. Non-homologous end joining (NHEJ) is a major double-strand break (DSB) repair pathway that occurs throughout the cell cycle. The objective of the current work is to model the fast and slow phases of NHEJ in G1 phase of the cell cycle following exposure to ionizing radiation (IR). The fast phase contains the major components of NHEJ; Ku70/80 complex, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and XLF/XRCC4/ligase IV complex (XXL). The slow phase in G1 phase of the cell cycle is associated with more complex lesions and involves ATM and Artemis proteins in addition to the major components. Parameters are mainly obtained from experimental data. The model is successful in predicting the kinetics of DSB foci in 13 normal, ATM-deficient, and Artemis-deficient mammalian fibroblast cell lines in G1 phase of the cell cycle after exposure to low doses of IR. The involvement of ATM provides the model with the potency to be connected to different signaling pathways. Ku70/80 concentration and DNA-binding rate as well as XXL concentration and enzymatic activity are introduced as the best targets for affecting NHEJ DSB repair process. On the basis of the current model, decreasing concentration and DNA binding rate of DNA-PKcs is more effective than inhibiting its activity towards the Artemis protein.

Keywords Mathematical modeling · NHEJ repair · ATM · Artemis · DNA-PKcs

Abbreviations

ATM	Ataxia telangiectasia mutated
CC	Correlation coefficient
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DSB	Double-strand break

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HR	Homologous recombination
IR	Ionizing radiation
Ku	Ku70/80 complex
MEF	Mouse embryonic fibroblast
NHEJ	Non-homologous end joining
PIKK	Phosphatidylinositol 3-kinase-related kinase
XLF	XRCC4-like factor
XXL	XLF/XRCC4/ligase IV complex

1 Introduction

Ionizing radiation (IR) is both a risk factor and an approved therapy regarding cancer. The most important cellular target of IR is the DNA molecule. IR can induce several types of DNA damage including base damage, single-strand break, and double-strand break (DSB). DSB is considered the most important type of damage, and DSB accumulation can lead to apoptotic or mitotic cell death [1]. Following induction of DSBs by IR, the target cell tries to survive by arresting the cell cycle and performing DSB repair. Activated DSB repair is considered one of the mechanisms of cell resistance to IR, and targeting DSB repair is proposed as a potent strategy for radiosensitizing cancer cells [2]. Having a quantitative and comprehensive understanding of DSB repair is essential for such a strategy.

The most important DSB repair processes are homologous recombination (HR) and non-homologous end joining (NHEJ). HR needs a homologous template and therefore occurs in late-S and G2 phases of cell cycle, is error-free, and involves RAD51 family of proteins including RAD52, RAD54, BRCA1, and BRCA2. NHEJ is the process of joining the adjacent damaged ends and takes place in every phase of the cell cycle. It may involve short deletion but is the predominant form of DSB repair in higher eukaryotes [3]. During NHEJ, a complex of Ku70 and Ku80 proteins (Ku) recognizes and forms a ring around the broken end [4]. The Ku complex recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) that pushes the Ku complex inward [5]. The complex of the DNA molecule, Ku, and DNA-PKcs is named DNA-PK. When located within the DNA-PK complex, DNA-PKcs protein becomes active and phosphorylates several targets including DNA-PKcs itself. It is suggested that this autophosphorylation is necessary for dissociation of DNA-PKcs from the ends [6]. Both Ku and DNA-PK complexes are capable of recruiting a complex composed of XRCC4-like factor (XLF), XRCC4, and ligase IV (XXL complex) [7]. XLF and XRCC4 increase stability and activity of ligase IV, which is required for ligation of the broken ends [8, 9].

DSB repair is composed of fast and slow phases. The fast component is dependent on the major NHEJ proteins mentioned above. However, about 10 to 20% of DSB lesions are repaired slowly. Evidence exists that such lesions are complex or occur in heterochromatin regions of DNA [10, 11]. If a cell is in late S or G2 phases of the cell cycle, HR repairs DSBs that remain unrepaired for a long time. If a cell is in G0 or G1 phases or if HR is defective at its initial steps, the slow phase of DSB repair is performed by NHEJ [12]. The slow NHEJ is dependent on proteins including ataxia telangiectasia mutated (ATM) and Artemis, besides major NHEJ components [10]. It is proposed that ATM and Artemis proteins are required for performing end processing before ligation of the two ends [10, 13]. Although a small number of DSBs are repaired during the slow phase of NHEJ, failure in ATM and Artemis function significantly decreases survival of cells within G1 phase of the cell cycle [10, 13, 14].

Studies on DNA repair have identified the proteins involved; however, the dynamics of these proteins working together as a whole still needs more investigation. Mathematical modeling is a useful tool for filling such gaps. In the field of DNA repair, mathematical models have been used for modeling mismatch repair, base excision repair, nucleotide excision repair, single-strand annealing (SSA), and HR [15–21]. NHEJ is modeled by Monte Carlo and kinetics approaches. Friedland et al. used Monte Carlo simulation for prediction of fraction of residual DSBs after different types of radiation [22, 23]. Among kinetics models, Cucinotta et al. modeled the slow phase of DSB repair by decreasing DNA-PKcs phosphorylation and ligase binding rates for complex DSBs [24]. Li and Cucinotta showed theoretically that recruitment order is not crucial for NHEJ [25]. Taleei and Nikjoo modeled NHEJ after exposure to low-energy electrons, ultrasoft X-rays, and 20–80 Gy X-rays using Monte Carlo codes for track structure. The slow phase of NHEJ was modeled by its dependence on Artemis protein, which was not regulated by other proteins involved in the model [26–28]. Using major NHEJ components, Dolan et al. indicated that DNA-PK-dependent NHEJ was important for the fast component of DSB repair and back-up NHEJ was critical for the slow repair in fibroblast cells that were exposed to higher doses of gamma irradiation [29]. Taleei and Nikjoo modeled DSB repair in G1 and early S phases of the cell cycle by three major routes. Artemis and ATM proteins were considered necessary for end processing and relaxation of DSBs located in heterochromatin regions of DNA without being regulated by other NHEJ proteins [30]. In another post-IR NHEJ modeling performed by Li et al., simple DSBs were repaired by Ku and XRCC4/ligase IV complex and not by DNA-PKcs. DNA-PKcs together with Artemis were involved in repairing complex DSBs. In this model, ATM inhibition was applied by decreasing the rates of end processing and synapse formation and the rate of Ku and DNA-PKcs release for complex DSBs [31]. Belov et al. made a model for DSB repair in mammalian cells following exposure to IR of a wide range of linear energy transfer. The model included NHEJ, HR, SSA, microhomology-mediated end joining (MMEJ), and PARP-dependent alternative NHEJ pathways. Complex DSBs, produced by high LET radiation, were suggested to be dependent on HR repair and a limited fraction of such DSBs were repaired by MMEJ and alternative NHEJ. DNA-PKcs/Artemis complex recruitment hindered homology-mediated repair pathways by preventing strand degradation. The complex was also involved in bridging of the two ends during the next steps [32].

In the current study, the NHEJ process is modeled by a biochemical kinetic approach and Michaelis–Menten equation is used for enzymatic activities. The model contains both fast and slow components of DSB repair during G1 phase of the cell cycle. A few parameters are obtained by fitting the model to experimental data and most parameters are obtained directly from quantitative experiments. ATM is entered in the model as a protein necessary for Artemis phosphorylation and activation in company with activated DNA-PKcs. Artemis is considered as a separate protein and its level and activity are included. The model is verified by γ -H2AX foci kinetics in normal, ATM-deficient, and Artemis-deficient cell lines exposed to 0.02 to 2-Gy therapeutic doses of IR. Six different human or mouse fibroblast cell lines (MRC-5, HSF2, 48BR, 1BR3, MEF1, and MEF2) are used to verify the model in the normal non-mutated condition [10, 14, 33]. An ATM-knockout mouse embryonic fibroblast (MEF) cell line and two ATM-deficient primary human skin fibroblast cell lines, AT1BR and AT7BI, are used to compare the computed results in the absence or shortage of ATM with experimental observations [10, 14]. Foci kinetics of four Artemis-deficient human cell lines, CJ179, DB333, F01–240, and F02–385, are compared with the model's results in the absence of Artemis [10, 33].

On the basis of the model's output, it is suggested that DNA-PKcs has a facilitating role in the fast phase and cannot be disregarded in this repair phase. In other words, DNA-PKcs is involved in the repair of simple ends in addition to repair of complex DSBs. Although simple ends can be repaired by Ku and XXL complexes alone, addition of DNA-PKcs facilitates the ligation. Finally, the best points in which the NHEJ process can be targeted in experiment are proposed. It is suggested that decreasing Ku, DNA-PKcs, and ligase IV concentrations and inhibiting ligase IV activity are more effective than decreasing ATM and DNA-PKcs activities toward Artemis. Besides, DSB repair is influenced by Artemis's level and activity although less than by Ku, DNA-PKcs, and ligase IV. The current detailed model provides a useful setting for studying the effect of other signaling pathways that affect any of the proteins involved in NHEJ repair and thereby influence the response of a cell to ionizing radiation.

2 Methods

2.1 Model development

The NHEJ kinetic model used in this study is represented schematically in Fig. 1. Both slow and fast phases of DSB repair are considered. The law of mass action and the Michaelis–Menten equation are utilized in determining reaction rates. This model consists of the Ku complex, composed of Ku70 and Ku80 proteins; DNA-PKcs; ATM; Artemis; and the XXL complex that is composed of ligase IV, XRCC4, and XLF proteins. Ku70 and Ku80 proteins bind together to make a dimer. This dimer has a high affinity to the ends of DNA breaks [4]. Therefore, in the current model they are considered together as one Ku complex (Ku). On the other hand, at the end of NHEJ, ligation is performed by DNA ligase IV in complex with a XRCC4 homodimer

Fig. 1 Schematic diagram of the NHEJ model. The reactions are depicted by *open arrows*. Species located on open arrows or connected to them by thin arrows catalyze that reaction by their enzymatic activity. The conversion of C1L complex to C2L, that is, binding of DNA-PKcs to ligatable ends is permitted in model 1 and prevented in model 2

[8]. XLF or Cernunnos homodimer binds to XRCC4/ligase IV complex and facilitates ligase IV activity [9]. Therefore, at each DSB, a complex containing ligase IV, XRCC4, and XLF proteins attaches the two ends together. These proteins are considered as a whole and the complex is called XXL in the model. In the next paragraphs, equations and their underlying biological evidence are explained in detail. The rate of reaction i is depicted by v_i and concentrations are displayed by brackets.

In the model, the number of initial DSBs is set as a constant fold of the incident IR dose as observed in experiment [34]. It is assumed that the dose is received within a short period and no repair is performed during the radiation time. Therefore, we have:

$$\begin{aligned} \emptyset &\xrightarrow{IR} DSB, \\ v_1 &= c \times IR \text{ dose} \end{aligned} \quad (1)$$

Although Mre11 or Mre11/Rad50 could not displace Ku from the DNA in humans, the Ku complex competes away this complex at each end of DSB after induction of DSB [35]. The resultant complex that is composed of a DSB bound to two Ku complexes is denoted by C1 in the model; 80–90% of IR-induced DSBs can be repaired with the main NHEJ proteins, Ku complex, DNA-PKcs, and XXL complex. The remaining 10–20% of repair is also dependent on other proteins including ATM and Artemis [10, 13]. Therefore, complexes containing DSB and Ku are divided into two groups: ligatable complexes that can be ligated by the main proteins of NHEJ (C1L) and unligatable complexes that cannot be ligated by the main NHEJ proteins (C1U). In Eqs. (2–4), which describe binding of Ku and DNA-PKcs to the broken ends, the reaction rates are determined by the law of mass action.

$$\begin{aligned} DSB + 2Ku &\rightarrow 0.85C1L + 0.15C1U, \\ v_2 &= Ku_DSB \text{ binding rate} \times [Ku]^2 \times [DSB] \end{aligned} \quad (2)$$

DNA-PKcs is recruited to Ku complex bound to DSB and cause Ku complex to move inward from the ends [5]. Complexes composed of a DSB, two Ku complexes, and two molecules of DNA-PKcs are denoted by C2 in the model. These complexes can be ligatable (C2L) or unligatable (C2U) if they are produced from C1L or C1U, respectively. When DNA-PKcs binds to Ku complex and DNA, it becomes active and phosphorylate its targets including DNA-PKcs itself [36]. Therefore, we consider that C2 complexes contain autophosphorylated DNA-PKcs.

$$\begin{aligned} C1L + 2DNA-PKcs &\rightarrow C2L, \\ v_3 &= DNA-PKcs_C1L \text{ binding rate} \times [C1L] \times [DNA-PKcs]^2 \end{aligned} \quad (3)$$

$$\begin{aligned} C1U + 2DNA-PKcs &\rightarrow C2U, \\ v_4 &= DNA-PKcs_C1U \text{ binding rate} \times [C1U] \times [DNA-PKcs]^2 \end{aligned} \quad (4)$$

In Eqs. (2–4), the reactions are considered irreversible. In fact, the backward rates are negligible compared with the forward rates [37]. The remaining reactions (5–9) are dependent on the enzymatic activity of ligase IV, ATM, DNA-PKcs, and Artemis proteins. Therefore, the reaction rates are described by the Michaelis–Menten equation:

$$v = (k_{cat} \times [E] \times [S]) / (K_m + [S])$$

in which v is the reaction rate. $[E]$ and $[S]$ are the enzyme and substrate concentrations, respectively. k_{cat} is catalytic rate or turnover number that is the maximum rate of product formation by each catalytic site. K_m is the substrate concentration in which the reaction rate reaches half of the maximum rate.

Ku complex can recruit XRCC4/ligase IV complex [38, 39] as well as XLF [40] to DNA ends in a DNA-PKcs-independent manner. It is also observed that some DSBs such as simple [41] and blunt [9] DSBs are repaired by Ku and XXL complexes independent of DNA-PKcs. These DSBs should be in a ligatable form and are repaired by enzymatic activity of ligase IV in XXL complex. At the same time the two bound Ku complexes would be released:

Like C1L, C2L complexes can also be repaired by the XXL complex. DNA-PKcs protein facilitates recruitment of the XXL complex [42, 43]. To consider this fact in the model, the XXL's K_m value for C2L complex is set lower than that value for C1L complex. Ku complexes and DNA-PKcs proteins are released during ligation.

C2U complexes- (unligatable complexes consisting of DSB, Ku, and DNA-PKcs) should become ligatable in order to be repaired. This change is considered as the rate-limiting step of the model. It is mentioned that the slow component of DSB repair is dependent on Artemis nuclease activity [10, 44]. In the model, the C2U complex is altered to C2L by Artemis nuclease activity. Ionizing radiation induces rapid Artemis phosphorylation from a basically phosphorylated form to a hyperphosphorylated state denoted by ArtemisPP in the model [45]. Some studies indicate that this phosphorylation is necessary for Artemis endonuclease activity [44, 46]. Because each C2U complex contains two DNA ends that should undergo processing, the concentration of ArtemisPP's substrate is considered twice as much as C2U concentration.

Hyperphosphorylation of Artemis is dependent on phosphatidylinositol 3-kinase-related kinases (PIKKs); DNA-PK, ATM, and ATR; activated following induction of DNA damage [44, 47]. In the case of DSB, DNA-PK-dependent phosphorylation of Artemis is necessary for its endonuclease activity [44, 48]. Cells deficient in ATM or Artemis protein are defective in slow phase of DSB repair and have similar DSB repair kinetics [10]. Therefore, in the model, it is assumed that ATM-dependent phosphorylation of Artemis is also necessary for its endonuclease activity.

DSBs are firstly recognized by the Mre11/Rad50/Nbs1 sensor complex. This complex activates the ATM protein [49]. As mentioned previously, in the case of DSBs Ku complex competes away the Mre11/Rad50 complex and facilitates recruitment of DNA-PKcs [35]. Therefore, it is assumed that first ATM and then DNA-PKcs phosphorylate the Artemis protein

to convert the basically phosphorylated Artemis to hyperphosphorylated forms, denoted by ArtemisP and ArtemisPP, respectively.

DNA-PKcs is active when it is in complex with Ku and DNA [50]. Each DSB end contains one active DNA-PKcs molecule. Hence, the concentration of active DNA-PKcs is considered to be twice as much as the total concentration of C2L and C2U complexes.

It is mentioned that Artemis dephosphorylation correlates with cell cycle progression to mitosis and is not correlated with DSB repair [45]. Because Artemis dephosphorylation is not regulated by the amount of DNA repair, Artemis dephosphorylation is not considered in current DSB repair model and therefore Artemis remains hyperphosphorylated within a cell until the end of simulation. Based on the described equations and reaction rates, ordinary differential equations are defined as follow:

$$\begin{aligned} 1) \quad & \frac{d[\text{DSB}]}{dt} = v_1 - v_2 = c \times \text{IRdose-Ku_DSB binding rate} \times [\text{Ku}]^2 \times [\text{DSB}] \\ 2) \quad & \frac{d[\text{Ku}]}{dt} = -2v_2 + 2v_5 + 2v_6 = -2 \times \text{Ku_DSB binding rate} \times [\text{Ku}]^2 \times [\text{DSB}] \\ & + 2 \times \frac{k_{cat} \text{ XXL for C1L} \times [\text{XXL}] \times [\text{C1L}]}{K_m \text{ XXL for C1L} + [\text{C1L}]} + 2 \times \frac{k_{cat} \text{ XXL for C2L} \times [\text{XXL}] \times [\text{C2L}]}{K_m \text{ XXL for C2L} + [\text{C2L}]} \\ 3) \quad & \frac{d[\text{C1L}]}{dt} = 0.85v_2 - v_3 - v_5 = 0.85 \times \text{Ku_DSB binding rate} \times [\text{Ku}]^2 \times [\text{DSB}] \\ & - \text{DNA-PKcs_C1L binding rate} \times [\text{C1L}] \times [\text{DNA-PKcs}]^2 \\ & - \frac{k_{cat} \text{ XXL for C1L} \times [\text{XXL}] \times [\text{C1L}]}{K_m \text{ XXL for C1L} + [\text{C1L}]} \\ 4) \quad & \frac{d[\text{C1U}]}{dt} = 0.15v_2 - v_4 = 0.15 \times \text{Ku_DSB binding rate} \times [\text{Ku}]^2 \times [\text{DSB}] \\ & - \text{DNA-PKcs_C1U binding rate} \times [\text{C1U}] \times [\text{DNA-PKcs}]^2 \\ 5) \quad & \frac{d[\text{DNA-PKcs}]}{dt} = -2v_3 - 2v_4 + 2v_6 = \\ & -2 \times \text{DNA-PKcs_C1L binding rate} \times [\text{C1L}] \times [\text{DNA-PKcs}]^2 \\ & -2 \times \text{DNA-PKcs_C1U binding rate} \times [\text{C1U}] \times [\text{DNA-PKcs}]^2 \\ & + 2 \times \frac{k_{cat} \text{ XXL for C2L} \times [\text{XXL}] \times [\text{C2L}]}{K_m \text{ XXL for C2L} + [\text{C2L}]} \\ 6) \quad & \frac{d[\text{C2L}]}{dt} = v_3 - v_6 + v_7 \\ & = \text{DNA-PKcs_C1L binding rate} \times [\text{C1L}] \times [\text{DNA-PKcs}]^2 \\ & - \frac{k_{cat} \text{ XXL for C2L} \times [\text{XXL}] \times [\text{C2L}]}{K_m \text{ XXL for C2L} + [\text{C2L}]} + \frac{k_{cat} \text{ ArtemisPP} \times [\text{ArtemisPP}] \times 2 \times [\text{C2U}]}{K_m \text{ ArtemisPP} + 2 \times [\text{C2U}]} \end{aligned}$$

$$\begin{aligned}
& \frac{d[C2U]}{dt} = v_4 - v_7 \\
7) & \frac{DNA-PKcs_C1U \text{ binding rate} \times [C1U] \times [DNA-PKcs]^2}{k_{cat} \text{ ArtemisPP} \times [ArtemisPP] \times 2 \times [C2U]} \\
& \quad \frac{K_m \text{ ArtemisPP} + 2 \times [C2U]}{d[Repaired DSB]} = v_5 + v_6 \\
8) & \frac{dt}{k_{cat} \text{ XXL for C1L} \times [XXL] \times [C1L]} + \frac{k_{cat} \text{ XXL for C2L} \times [XXL] \times [C2L]}{K_m \text{ XXL for C1L} + [C1L]} \\
9) & \frac{d[Artemis]}{dt} = -v_8 = -\frac{k_{cat} \text{ ATM} \times [ATM] \times [Artemis]}{K_m \text{ ATM} + [Artemis]} \\
10) & \frac{d[ArtemisP]}{dt} = v_8 - v_9 = \frac{k_{cat} \text{ ATM} \times [ATM] \times [Artemis]}{K_m \text{ ATM} + [Artemis]} \\
& \quad \frac{k_{cat} \text{ DNAPKcs} \times 2 \times ([C2L] + [C2U]) \times [ArtemisP]}{K_m \text{ DNAPKcs} + [ArtemisP]} \\
11) & \frac{d[ArtemisPP]}{dt} = v_9 = \frac{k_{cat} \text{ DNAPKcs} \times 2 \times ([C2L] + [C2U]) \times [ArtemisP]}{K_m \text{ DNAPKcs} + [ArtemisP]}
\end{aligned}$$

The introduced model contains both fast and slow phases of DSB repair for ligatable and unligatable ends, respectively. The ligatable ends can undergo fast ligation by XXL in the presence of Ku complexes alone or after association of DNA-PKcs with Ku complexes. Association of DNA-PKcs with Ku complexes decreases the K_m value of XXL complex and thus facilitates the ligation. Unligatable ends need further processing before ligation and therefore make the slow component of DSB repair. ATM, DNA-PKcs, and Artemis proteins are involved in this end processing step.

It is suggested that DNA-PKcs has a facilitating but nonessential role in repairing damages that do not need Artemis-dependent end processing [10, 51]. Such damages are considered as ligatable ones in our model. We tested whether our model is successful in showing the facilitating effect of DNA-PKcs for repairing ligatable ends. We performed this checking by omitting the binding of DNA-PKcs to Ku-bound ligatable complexes (Eq. 3), i.e., by preventing the conversion of C1L to C2L complex (Fig. 1). We named the first unchanged model “model 1” and the derivative model “model 2”.

2.2 Derivation of constant parameters

Constant parameters used in the model are summarized in Table 1. All parameters are computed for a single cell nucleus and the average volume of a cell nucleus is considered $400 \mu\text{m}^3$ [52]. The Brenda database is used for determining k_{cat} and K_m of ArtemisPP; a range is obtained for each parameter based on k_{cat} and K_m values for enzyme families that have endonuclease activity [53]. Specific values are assigned to k_{cat} and K_m of ArtemisPP so that the number of damaged foci at different time points after receiving 2 Gy IR in our model is similar to that in normal MRC-5 fibroblast cells (Figure 1B from [10]). K_m values of the PIKK proteins ATM and DNA-PKcs are considered equal and determined so that the Artemis hyperphosphorylation rate would be the same as experimental observations. It is reported that Artemis is almost completely hyperphosphorylated 15 to 30 min after exposure of cells to 10 Gy IR [45].

Table 1 Values of parameters used in NHEJ models 1 and 2

Parameter	Value	Unit	Reference
c (DSB induction)	35	Molecule/Gy	[34]
Ku DSB binding rate	9.6×10^{-5}	$1/(\text{molecule}^2 \times \text{second})$	[37]
DNA-PKcs CIL binding rate	5.8×10^{-4}	$1/(\text{molecule}^2 \times \text{second})$	[37]
DNA-PKcs CIU binding rate	5.8×10^{-4}	$1/(\text{molecule}^2 \times \text{second})$	[37]
k_{cat} XXL for CIL	6.67×10^{-4}	1/s	[54]
k_{cat} XXL for C2L	6.67×10^{-4}	1/s	[54]
K_m XXL for CIL	48,000	Molecule	[55]
K_m XXL for C2L	10,000	Molecule	[42, 43, 55]
k_{cat} ArtemisPP (model 1)	3×10^{-5}	1/s	[10, 53]
k_{cat} ArtemisPP (model 2)	10	1/s	[10, 53]
K_m ArtemisPP (model 1)	240	Molecule	[10, 53]
K_m ArtemisPP (model 2)	0.024	Molecule	[10, 53]
k_{cat} ATM	0.19	1/s	[53]
K_m ATM (model 1)	10,000	Molecule	[45]
K_m ATM (model 2)	5000	Molecule	[45]
k_{cat} DNA-PKcs	0.19	1/s	[53]
K_m DNA-PKcs (model 1)	10,000	Molecule	[45]
K_m DNA-PKcs (model 2)	5000	Molecule	[45]

The initial concentration of each species is mentioned as number of molecules of that species per cell nucleus (Table 2). The initial concentrations of Ku and DNA-PKcs used in the model are related to cultured human cells. However, previous studies indicate that DNA-PK complex is much less abundant (50–100 fold) in rodent and insect cell lines than in primates [56]. ATM molecules are in the inactive dimer form in the absence of DNA damage. Following DSB induction, the two ATM subunits phosphorylate each other. This phosphorylation leads to monomerization and activation of ATM molecules. Activation of ATM molecules by IR occurs very fast; well beyond 50% of ATM molecules become phosphorylated 5 min after exposure to 0.5 Gy of IR. Phosphorylation is maximal at this time point and remains stable and detectable for at least 24 h. Besides, ATM phosphorylation is highest at 0.4 Gy and does not increase with increasing dose up to 9 Gy [57]. Therefore, we consider the amount of ATM molecule 0 in the absence of IR and a constant value in the presence of IR. The number of Artemis molecules per cell nucleus is very small [58] and is set to 50 in the model.

2.3 Model simulation and analysis

Simulations are performed using ode15s (stiff/NDF) solver of MATLAB software that is a stiff deterministic solver of differential-algebraic equations (DAEs) based on numerical backward differentiation formulas (BDFs) or Gear's method. In our simulations, the remaining DSB is calculated by subtracting the number of repaired DSBs from the initial number of DSBs in each step of simulation. Induction of DSB in living cells leads to phosphorylation of histone H2AX on serine 139, so-called

Table 2 Initial number of species per cell nucleus used in the NHEJ models 1 and 2

Species	Initial concentration	Reference
Ku	400,000	[59]
DNA-PKcs	80,000	[56]
XXL	3000	[60]
ATM	140	[61]
Artemis	50	[58]

γ -H2AX, in about several Mb around the DSB and thus formation of γ -H2AX foci [62]. It is demonstrated that the number of γ -H2AX foci is a direct indicator of number of unrepaired DSBs, especially at low doses of IR [34]. Therefore, we compare the kinetics of remained DSB in our simulation with the kinetics of γ -H2AX foci in experiment following exposure to ionizing radiation both for fitting and for verifying our model. The t test and correlation coefficient (CC) are used to compare simulated and experimental data. For t test, P values closer to 1 represent higher similarity.

Sensitivity analysis is performed with the same solver to compute the time-dependent sensitivity of repaired DSB states with respect to all species' initial conditions and all parameter values of the model, that is, the time-dependent derivatives [63] with full dedimensionalization.

3 Results

3.1 Comparing models

To check whether model 1 can show the facilitating role of DNA-PKcs in fast repair of ligatable ends, we compared model 1 with model 2, in which the binding of DNA-PKcs to C1L complexes before the ligation step is prevented (Fig. 1). k_{cat} and K_m of hyperphosphorylated Artemis (ArtemisPP) and K_m of ATM and DNA-PKcs are set within the experimentally acceptable range [45, 53] so that the best fit is obtained between the in vitro (Figure 1B from [10]) and in silico data (Fig. 2; Table 1). The kinetics of simulated remained DSB is compared with γ -H2AX foci kinetics in MRC-5 fibroblasts following exposure to 2 Gy IR from 3 min to 14 days after exposure to IR. The repair kinetics is simulated by model 1 ($P > 0.99$ in all time periods) more precisely than by model 2 ($P > 0.64$ up to 14 days and $P > 0.45$ up to 8 h). Therefore, model 1 can successfully simulate the facilitating property of DNA-PKcs for repair of ligatable ends and indicates that the role

Fig. 2 Comparison of data obtained from simulated models (models 1 and 2) with γ -H2AX foci kinetics in the normal human lung fibroblast cell line MRC-5 following exposure to 2 Gy X-rays (Figure 1B from [10]). P values for model 1 and 2 up to 4 days (96 h) following radiation are more than 0.99 and more than 0.62, respectively

of DNA-PKcs cannot be disregarded at early times following radiation. Besides, model 2 shows that increased Artemis endonuclease activity (about 3×10^5 fold increase in k_{cat} and 10^4 fold decrease in K_m of Artemis, as well as a twofold decrease in K_m of ATM and that of DNA-PKcs towards Artemis) cannot compensate for the DSB repair defect in the absence of DNA-PKcs–CtL interaction in the fast phase of DSB repair. Model 1 is further verified in the next steps.

3.2 DSB repair kinetics

The number of computed remained DSBs is compared with the number of γ -H2AX foci in various cell lines at different time points ranging from 3 min to 14 days following exposure to different doses of low LET IR. Normal, ATM-deficient, and Artemis-deficient cell lines are used to verify our main model (model 1).

The kinetics of DSB repair simulated by the model is compared with kinetics of γ -H2AX foci in normal human fibroblast cell lines; MRC-5, HSF2, 48BR, and 1BR3; and mouse embryonic fibroblast cell lines; MEF1 and MEF2; at 2, 1.3, 0.2 and 0.02 Gy of IR [10, 14, 33] (Fig. 3). Simulation data is in good agreement with experimental observations with $P > 0.81$, $P > 0.98$, $P > 0.72$, and $P > 0.82$ at 2 Gy, 1.3 Gy, 0.2 Gy, and 0.02 Gy, respectively. The correlation coefficients between experimental and simulation data are more than 0.97 in human fibroblast cell lines and more than 0.88 in MEFs.

Kinetics of DSB repair in ATM^{-/-} MEFs within 3 days following 2 Gy radiation [10] is compared with simulated DSB repair kinetics in which concentration of ATM is set zero (Fig. 4a). Simulation data agrees well with the experimental data ($P > 0.96$, $CC > 0.78$).

Some ATM-deficient cell lines still have a residual amount of ATM protein [64] and the amount of ATM mRNA is not zero in AT1BR cell line [65]. Therefore, for simulating DSB repair in ATM-deficient AT1BR and AT7BI skin fibroblast cell lines we set the amount of ATM one molecule/cell. Comparison of simulated and experimental results for 2, 1.3, 0.2, and 0.02 Gy of IR [10, 14] shows that there is a good similarity between in silico and in vitro data with P values more than 0.88, 0.94, 0.75, and 0.92 and CCs more than 0.97, 0.98, 0.99, and 0.93, respectively (Fig. 4b–e).

A simulation is also performed in 0 concentration of ATM. The discrepancy between the model and the experimental data at different doses of IR is higher in 0 concentration of ATM than in the presence of 1 molecule per cell, especially at time points later than 2 days following IR (data not shown). The most prominent effect is observed in AT1BR cell line from two different studies [10, 14] in which the P value for data during 14 days following IR is more than 0.6 in the absence of ATM and more than 0.88 in the presence of 1 molecule of ATM per cell.

The patient-derived CJ179, DB333, F01–240, and F02–385 cell lines have genomic deletions in Artemis [10, 33]. To compare DSB repair kinetics between these Artemis-deficient cell lines and the model, concentration of Artemis is set zero. Kinetics of DSB repair obtained by the simulation is in accordance with experimental observations at 2 and 1.3 Gy with P values and CCs more than 0.87 and 0.95, respectively (Fig. 5).

3.3 Sensitivity analysis

Sensitivity of the amount of repaired DSBs to all species' initial concentrations and all parameter values is determined in a time-dependent manner. The maximum absolute values of sensitivities are depicted in Fig. 6. In the model, Ku concentration and its DNA binding rate, XXL concentration and activity, DNA-PKcs concentration and binding rate to ligatable ends, and finally Artemis concentration and enzymatic activity are the prominent parameters for DSB repair via NHEJ

Fig. 3 Comparison of model 1 with experimental observations in normal cell lines after receiving 2, 1.3, 0.2, and 0.02 Gy doses of IR. The number of γ -H2AX foci at different time points in mouse embryo fibroblasts MEF1 and MEF2 (Figure 2B from [10]), human lung fibroblast MRC-5 (Figures 4D, 6A from [14]), and human skin fibroblasts HSF2 (Figure 1B from [10]), 1BR3 (Figure 1C from [10]), 48BR (Figure 5A from [14]), and 48BR containing an empty vector (48BR vector) (Figure 2A from [33]) is compared with kinetics of remained DSBs in model 1-based simulations. **a** At 2 Gy P values are more than 0.93, 0.95, 0.91, 0.93, and 0.81 and correlation coefficients are more than 0.98, 0.98, 0.88, 0.98, and 0.99 for MRC-5, HSF2, MEF1/2, 48BR, and 48BR vect, respectively. **b** At 1.3 Gy P value and CC are higher than 0.98 and 0.97, respectively. **c** At 0.2 Gy P value and CC are higher than 0.72 and 0.97, respectively. **d** At 0.02 Gy P value and CC are higher than 0.82 and 0.99, respectively

pathway in the order of decreasing importance. Concentrations and parameter values of most species are effective at the initial time of simulation. While XXL's k_{cat} and K_m values for C1L ends are effective at the time of radiation, XXL concentration and its k_{cat} and K_m values for C2L ends affect repair of DSBs up to about 12 h following IR. The concentration and kinetic parameters of ATM and kinetic parameters of DNA-PKcs have a very low effectiveness compared with other parameters that peaks at about 3 h post-irradiation and continues up to more than 4 days. Artemis's initial concentration and k_{cat} and K_m of ArtemisPP affect repaired DSBs up to 7 days following IR and have highest effectiveness at about 1 day following radiation.

4 Discussion

In this work, NHEJ is modeled using kinetic rates based on the law of mass action and Michaelis–Menten equation. The fast and slow phases of DSB repair are included and ATM and Artemis proteins are inserted as crucial proteins for repair of DSBs during the slow phase.

Fig. 4 Comparison of simulation data with experimental data in ATM-deficient cell lines. Number of γ -H2AX foci in ATM knocked out MEF and AT1BR and AT7BI ATM-deficient human skin fibroblast cell lines is compared with the number of remained DSBs computed in our simulation at different time points following different doses of IR. **a** In ATM-knocked out MEF exposed to 2 Gy IR (Figure 2B from [10]), P value and CC are more than 0.96 and 0.78, respectively. **b** In ATM-deficient AT1BR (Figure 1B from [10] and Figure 4D from [14]) and AT7BI cell lines (Figure 5A from [14]) exposed to 2 Gy IR, P values and CCs are more than 0.88 and 0.97, respectively, up to 14 days following radiation. **c** In ATM-deficient AT7BI cells exposed to 1.3 Gy IR (Figure 1C from [10]), P value and CC are more than 0.94 and 0.98, respectively. **d** In ATM-deficient AT1BR cells exposed to 0.2 Gy IR (Figure 6A from [14]), P value and CC are more than 0.75 and 0.99, respectively. **e** In ATM-deficient AT1BR cells exposed to 0.02 Gy IR (Figure 6A from [14]), P value and CC are more than 0.92 and 0.93, respectively

The NHEJ process is considered step by step and in each step concentrations of species and parameters related to enzyme activity or protein binding are obtained from experimental observations. Such a step-by-step model provides better understanding of cell fate and

Fig. 5 Comparison of simulation data with experimental data obtained from Artemis-deficient cell lines. Number of γ -H2AX foci in patient-derived Artemis-deficient human skin fibroblast cell lines is compared with the number of remained DSBs computed in the simulation at different time points following 2 Gy and 1.3 Gy doses of IR. **a** In human fibroblast cell line CJ179 (Figure 1B from [10]) and CJ179 containing an empty vector (CJ179 vector) (Figure 2A from [33]) exposed to 2 Gy IR, P values and CCs are more than 0.87 and 0.98, respectively. **b** In CJ179, DB333, F01-240, and F02-385 Artemis-deficient cell lines exposed to 1.3 Gy IR (Figure 1C from [10]), P value and CC are more than 0.88 and 0.95, respectively

responses after receiving low LET ionizing radiation. It should be emphasized that all this analysis and modeling relates to non-cycling G0/G1 cells, which are the predominant cells in adults. Therefore, this modeling is distinct from models that encompass asynchronous cycling cells such as the work done by Belov et al. [32].

Fig. 6 Sensitivity of repaired DSB concentration to the species' initial concentrations or parameter values

The ATM protein activates several signaling pathways after induction of DSBs. These pathways include cell cycle checkpoint activation, HR and NHEJ DSB repair, stress-response pathways, telomere maintenance, senescence, and apoptosis [66]. Hence, considering ATM in the model is invaluable in connecting NHEJ repair with other pathways affected by ATM protein and providing more comprehensive models in future.

The proposed model has reasonable predictions about kinetics of DSB reduction in several normal mammalian cell lines radiated with 0.02 to 2 Gy doses of low LET IR (Fig. 3). As for low LET radiations a linear relationship exists between dose of IR and number of DSBs and the rate of simple to complex DSBs is constant up to several hundred Gy of IR [34], this model can also be used for higher doses of low LET ionizing radiation.

In our model, ATM affects Artemis phosphorylation and activation and thereby end processing during the slow phase of DSB repair. DNA-PKcs is involved not only in Artemis phosphorylation and activation but also in facilitating XXL activation during early DNA repair. These considerations can appropriately interpret the existing experimental observations on DSB repair kinetics.

Correlation coefficient between simulation data and experimental data is higher than 0.88 and 0.78 in mouse embryo fibroblast cells in normal and in ATM^{-/-} conditions, respectively and is higher than 0.93 for all human cell lines in normal, ATM-deficient, and Artemis-deficient situations (Figs. 3, 4, and 5). Because the components of DNA-PK complex (Ku70, Ku80, and DNA-PKcs) are 50 fold lower in rodents than in human cell lines [56], we checked the model with 50-fold lower concentrations of Ku and DNA-PKcs, i.e., 8000 and 1600 molecules per cell, respectively. There was no difference in DSB repair kinetics in that situation. Therefore, model 1 in its current status is applicable to rodent cells as well as human cell lines. Interestingly, although rodent and human cells have 50-fold different DNA-PK level, they have similar radiosensitivity [56].

The current model proposes that a minimal ATM activity in ATM-deficient cell lines can decrease DSBs several days following radiation, as reported in experiments [10, 14], and is

enough to fulfill repair of all DSBs if the cell survives for a long time after radiation. However, this late repair might be performed by other repair pathways or by proteins not included in the model.

According to Fig. 4b–e, at later time points, as the dose increases the number of DSBs in ATM-deficient cells remains higher than the one predicted by the model. Because higher doses of IR lead to denser DNA damage, the higher doses of IR in Fig. 4 might form DNA lesions that are repaired with a rate slower than the one expected by the model. These lesions might be repaired by other repair system(s) with slower kinetics compared with NHEJ, or there might be proteins that make these lesions ready to be repaired by ATM and Artemis at later time points.

The amount of DSBs at time points exceeding 48 h is lower in Artemis-deficient cells than in the model (Fig. 5a). This observation points to the fact that either there might be a little amount of Artemis remained in these cells, or there are other repair processes that can compensate for Artemis deficiency in cells that survive several days following radiation.

There is a correlation between the amount of decreased γ -H2AX foci up to 24 h after radiation and the surviving fraction at 2 Gy IR [67]. In other words, remained DSBs at 24 h can be an indicator of the amount of cell survival after radiation. Therefore, by estimating the number of γ -H2AX foci at 1 and 24 h following radiation, the model is capable of providing a qualitative tool for comparing cell survival in cells with different modifications in proteins involved in NHEJ.

In the field of radiotherapy, it is very advantageous to target the DSB repair pathways to increase radiosensitivity [2]. NHEJ is the main DSB repair pathway performed during all phases of cell cycle in mammalian cells. Besides, mutations in NHEJ proteins have dramatic effects in cancer predisposition via mutation accumulation [68]. Hence, it is important to know which proteins are more critical for NHEJ and therefore are better choices to be targeted or investigated.

Based on time-dependent sensitivity analysis, if we want to target NHEJ repair it is fine to target Ku and DNA-PKcs concentrations and DNA binding rates, and XXL concentration and activity before radiation and to target Artemis concentration and activity prior to 1 day following IR. Our model indicates that DSB repair is highly affected by Ku and DNA-PKcs concentrations and binding rates. Decreasing DNA-PKcs's protein level, e.g., by decreasing its gene transcription, increasing protein degradation, or using siRNA, or designing molecules that target DNA-PKcs/Ku or DNA-PKcs/DNA interface, is more efficient than decreasing DNA-PKcs's kinase activity on Artemis. Similar to our finding it is shown that DNA-PKcs activity is not correlated with the amount of DSB repair [69]. On the other hand, sensitivity analysis indicates that the amount of DSB repair is influenced by binding of DNA-PKcs to simple ends and not to complex ones. These results indicate that DNA-PKcs mainly influences the fast component of DSB repair although its deficiency does not seize the repair of simple ends. In this manner, sensitivity analysis again emphasizes the facilitating role of DNA-PKcs for the fast component of DSB repair.

On the basis of sensitivity analysis, designing specific inhibitors for ligase IV that can be targeted to the site of interest can have an outstanding anti-repair and radiosensitizing effect and inhibitors of Artemis would be of the second importance. In the model, XRCC4 and XLF accompany ligase IV and participate in XXL complex. Therefore, another strategy for targeting NHEJ might be targeting these proteins at the level of expression or activity. Although previous experimental observations have pointed to the significance of Ku complex, DNA-PKcs, and ligase IV in DSB repair process [10, 51], our model provides suggestions about what parameters related to such proteins should be targeted, that is, concentration and binding rate of Ku and DNA-PKcs as well as concentration and activity (k_{cat} and K_m) of the XXL complex.

A few studies indicate that Artemis's phosphorylation is not necessary for its endonuclease activity or for DSB repair [70, 71]. However, these studies focus on SQ phosphorylation sites of Artemis that are probably not the main phosphorylation targets of DNA-PKcs [46]. It is mentioned that the C-terminal region of Artemis may have an autoinhibition property that can be overcome by DNA-PK-dependent phosphorylation at 11 non-SQ sites [46]. Although in our model it is assumed that ATM and DNA-PKcs affect the slow phase of DSB repair by phosphorylating Artemis protein, according to the result of sensitivity analysis, the kinase activity of ATM and DNA-PKcs toward Artemis protein hardly affects the amount of repaired DSB. Another possibility proposed by Goodarzi et al. is that DNA-PKcs autophosphorylation make the DNA conformation ready for endonucleolytic activity of Artemis [71]. In that case there is no need for ArtemisP in our model to be converted to ArtemisPP by DNA-PKcs and ArtemisP can convert unligatable ends to ligatable ones.

5 Conclusions

In summary, we simulate NHEJ DSB repair pathway in G1 phase of the cell cycle following exposure to low LET IR by a biochemical kinetics model. The majority of parameters of the model are obtained from experimental observations. This model is capable of predicting number of remained DSBs post-IR in each cell after exposure to low LET IR in normal, ATM-deficient and Artemis-deficient human and mouse fibroblast cell lines. The amount of residual DSBs at different time points, especially at 24 h after radiation, is valuable for prediction of survival of cells following radiotherapy. It is proposed that involvement of ATM in NHEJ is through phosphorylation of Artemis protein as a prerequisite for phosphorylation and activation of Artemis by DNA-PKcs, necessary for changing complex ends to simple ones. Involvement of ATM and DNA-PKcs proteins in the model provides the opportunity to connect NHEJ with other signaling pathways to obtain a better estimation of cell's response to ionizing radiation. We plan to extend the model by making such connections between NHEJ and other signaling pathways.

Because the model includes different proteins involved in NHEJ process, we can suggest which proteins' parameters at what times are of most importance in NHEJ. Ku protein's level and binding rate to DSB, XXL complex's level and enzymatic activity, DNA-PKcs protein's level and binding rate to simple ends, and Artemis protein's level and enzymatic activity are the most important parameters in determining the amount of residual DSBs after radiation in the order of decreasing importance. These parameters would be suitable targets for enhancing the efficiency of radiotherapy in cancer patients.

Further experimental observations can help us to determine model's parameters more accurately and the model can be modified based on new information. Our model is fitted to and verified by the data related to thirteen mammalian fibroblast cell lines. Future experimental data, containing DSB repair kinetics especially number of γ -H2AX foci after receiving IR in nonfibroblast cells can help us to extend our model to a more comprehensive one.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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