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Crosstalk between p53 and NF- κ B systems: pro- and anti-apoptotic functions of NF- κ B

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Running title: Crosstalk between p53 and NF- κ B systems

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Abstract

Nuclear factors p53 and NF- κ B control many physiological processes including cell cycle arrest, DNA repair, apoptosis, death, innate and adaptive immune responses, and inflammation. There are numerous pathways linking these systems and there is a bulk of evidence for cooperation as well as for antagonisms between p53 and NF- κ B. In this theoretical study, we use our earlier models of p53 and NF- κ B systems and construct a crosstalk model of p53 – NF- κ B network in order to explore the consequences of the two-way coupling, in which NF- κ B upregulates transcription of p53, while in turn p53 attenuates transcription of NF- κ B inhibitors I κ B α and A20. We consider a number of protocols in which cells are stimulated by TNF α (that activates NF- κ B pathway) and/or gamma irradiation (that activates p53 pathway). We demonstrated that NF- κ B may have both anti- and pro-apoptotic roles. TNF α stimulation, preceding DNA damaging irradiation, makes cells more resistant to irradiation-induced apoptosis, while the same TNF α stimulation, when preceded by irradiation increases the apoptotic cell fraction. Our finding suggests that diverse roles of NF- κ B in apoptosis and cancer could be related to the dynamical context of activation of p53 and NF- κ B pathways.

Keywords: signaling pathways, positive and negative feedbacks, mathematical modeling, stochastic simulations, apoptosis, p53, NF- κ B.

1 Introduction

1.1 Biological background

p53 and NF- κ B are potent transcription factors that regulate hundreds of genes, products of which control many physiological processes including cell cycle arrest, DNA repair, apoptosis, death, innate and adaptive immune responses and inflammation. Levels and activities of p53 and NF- κ B are tightly regulated by both positive and negative feedback loops.

The p53 regulatory pathway is composed of hundreds of genes and their products that respond to a variety of stress signals (reviewed in [1],[2]). The input signals that include DNA damage, oncogene activation, heat and cold shock, and others, are transmitted by the upstream mediators. They influence p53 level and its transcriptional activity by several posttranslational modifications. The action of p53/Mdm2 core is controlled by a number of positive and negative feedbacks [3]. The downstream events, which are mediated by groups of genes and their products, are regulated by the p53 protein, most commonly by transcriptional activation but in some cases by protein-protein interactions. The cellular outputs of these downstream events include cell cycle arrest, DNA repair and apoptosis or cellular senescence, and often result in extensive communication with other transduction pathways, as reviewed in [4]. Relevant to the current model is the p53 activation in the response to DNA damage. In healthy cells, the level of p53 remains typically low under the control of Mdm2, which is responsible for p53 ubiquitination leading to its rapid degradation [5]. In turn, synthesis of Mdm2 transcript is controlled by p53 [6], which defines the negative feedback. DNA damage activates the checkpoint proteins, which destabilize Mdm2 and trigger p53 phosphorylation elevating its stability and transcriptional activity (reviewed in [7]). This disturbs homeostatic balance between Mdm2 and p53 leading to oscillations and/or rise of the p53 level. Activated p53 triggers transcription of groups of genes, products of which are responsible for cell cycle arrest and DNA repair and, if the last fails or takes too long, for initiation of apoptosis. One of the key p53 regulators is the p53 responsive phosphatase PTEN that mediates, via phosphatidylinositol 3-phosphate (PIP3) and Akt/PBK kinase, the positive feedback loop allowing p53 to rise to a high level and initiate apoptosis ([8]). This places PTEN

among the most commonly lost tumor suppressors in human cancer. Mutations (or deletions) of PTEN, which occur during tumor development, block its enzymatic activity leading to increased cell proliferation and reduced cell death (reviewed in [9]).

Nuclear factor κ B (NF- κ B) regulates genes important for pathogen or cytokine inflammation, immune response, cell proliferation and survival (reviewed in [10], [11]). In mammals, the NF- κ B family of transcription factors contains five members, but the ubiquitously expressed p50-RelA heterodimer constitutes the most common inducible NF- κ B binding activity. In resting cells, p50-RelA heterodimers (referred herein to as NF- κ B) are sequestered in the cytoplasm by association with the members of another family of proteins termed I κ B. This family includes several proteins but most of the I κ B family inhibitory potential is carried by I κ B α , whose synthesis is controlled by a highly NF- κ B-responsive promoter, generating autoregulation of NF- κ B signaling ([12] also studied in [13]). Activation of NF- κ B requires degradation of I κ B α , which allows NF- κ B to translocate into the nucleus, bind to κ B motifs present in promoters of numerous genes and upregulate their transcription. NF- κ B activating signals converge on the cytoplasmic I κ B kinase (IKK), a multiprotein complex that phosphorylates I κ B α leading to its ubiquitination and then to its rapid degradation by the proteasome, reviewed in [14]. Activation of IKK kinase is induced by various extracellular signals including tumor necrosis factor- α (TNF α) and interleukin-1 (IL-1) via transduction pathway involving IKK kinases referred herein to as IKKK. IKK inactivation is controlled by the zinc finger protein A20, which similarly as I κ B α , is strongly NF- κ B responsive and generates a second autoregulatory loop in NF- κ B signaling ([15] also studied in [16]). Mice deficient in A20 develop severe inflammation and cachexia, are hypersensitive to TNF α , and die prematurely [17].

There is bulk of evidence that p53 and NF- κ B collectively control cell responses to stress that include apoptosis, cellular senescence and cell cycle arrest. In last two decades, numerous cross-regulations of these two systems have been identified (reviewed in [18], [19]). We list here some of them;

- NF- κ B is a transcription factor for p53 [20].
- NF- κ B is essential in p53-mediated cell death. Inhibition of MEK1 blocks activation of NF- κ B

by p53 and abrogates p53-induced cell death [21].

- p53 upregulates cFLIP, inhibits transcription of NF- κ B -regulated genes and induces caspase-8-independent cell death [22].
- p53 expression stimulates the serine/threonine kinase ribosomal S6 kinase 1, which in turn phosphorylates the p65 subunit of NF- κ B [23].
- NF- κ B up-regulates p53 expression and increases the stability and DNA binding activity of p53; expression of p53 downstream target genes p21^{waf1} and PUMA is NF- κ B-dependent [24].
- Tax inhibits p53 activity through the Ser 536 phosphorylation of p65/RelA subunit of NF- κ B [25].
- Activated p53 induces NF- κ B DNA binding but suppresses its transcriptional activation through inhibition of IKK and histone H3 kinase [26].
- RelA, the component of NF- κ B and p53 mutually repress each other's ability to activate transcription and compete for coactivator CBP [27], [28].
- Phosphorylation of CBP by IKK α promotes cell growth by switching the binding preference of CBP from p53 to NF- κ B [29].

In addition, TNF α is known to regulate the opposing pro-apoptotic – pro-survival pathways, as reviewed e.g. in [30]. TNF α binding to ubiquitously expressed TNFR1 receptors leads to binding of the adaptor protein TRADD that then serves as a assembly platform for TRAF2 and RIP (complex I). In a second step, TRADD and RIP associate with FADD and cell-death-initiation caspase 8 (complex II), which then activates executioner caspase 3. When NF- κ B is activated by complex I, complex II also harbors the caspase inhibitor FLIP-L (long form of FLIP) and the cell survives; otherwise cell undergoes apoptosis, [31]. In addition to FLIP, NF- κ B targets several anti-apoptotic factors c-IAP, IEX-1L, Bfl-1/A1, XIAP and Bcl-Xl, [32]. As a result, the direct cytotoxic effect of TNF α is only manifested when NF- κ B activation is blocked. Particularly, NF- κ B inducible c-IAP inhibits apoptosis by specifically binding to activated caspase-3 and thus preventing DNA fragmentation [33], [34]. The interplay between these two opposing survival and apoptotic pathways initiated by TNF α has been modeled recently by Rangamani and Sirovich [35].

The current study is concentrated on the interplay between p53 and NF- κ B systems, and TNF α is considered as a "general" NF- κ B activator (such as IL-1) and its pro-apoptotic actions via FADD-caspase 8 complex are not taken into account, assuming it is balanced by NF- κ B activation as discussed by Jin and El-Deiry [30].

1.2 p53 and NF- κ B models

NF- κ B and p53 systems have been actively modeled in last years, as reviewed in [36] and in [37] for NF- κ B and [38] for p53 system. The crosstalk model of NF- κ B and p53 pathways, proposed here, is based on our two earlier stochastic models of these systems [39, 40], described shortly below.

NF- κ B model [39] is focused on TNF α signaling, a process initiated by binding of TNF α to the ubiquitous receptor TNFR1, Fig. 1. In short, the action of the regulatory pathway may be summarized as follows: Binding of TNF α trimer initiates receptor TNFR1 trimerization and formation of an active receptor complex in a multistep process involving binding of RIP and TRAF2. The active receptor complex activates the IKKK kinase (transformation from IKKK_n to IKKK_a). Active kinase IKKK_a phosphorylates and activates the IKK kinase (transformation from IKK_n to IKK_a). Active IKK_a kinase transiently binds to the cytoplasmic (NF- κ B|I κ B α) complex and phosphorylates I κ B α initiating its degradation. Released NF- κ B enters the nucleus to induce transcription of inhibitors I κ B α and A20 genes. The first negative feedback loop involves the I κ B α protein, which is rapidly resynthesized, enters the nucleus and recaptures NF- κ B back into the cytoplasm. In the continued presence of IKK_a, however, the resynthesized I κ B α is continuously degraded, which results in continued nuclear NF- κ B translocation. A second level of negative autoregulation occurs with the resynthesis of A20, a ubiquitin ligase which controls IKK activity. A20 initiates the degradation of RIP, the key component of TNFR1 receptor complex, attenuating the activity of receptors and directly associates itself with IKK_a, enhancing its conversion to catalytically inactive IKK_i. Inactive kinase IKK_i spontaneously converts back to IKK_n through the intermediate form IKK_{ii}. Similarly, active kinase IKKK_a rapidly converts to the inactive form IKKK_n. The stochasticity of NF- κ B regulation is introduced at the levels I κ B α and A20 of gene expression and TNFR1 activation and inactivation.

The p53 system model [40] involves two feedback loops: The negative, coupling p53 with its immediate downregulator Mdm2, and the positive, that involves PTEN, PIP3 and Akt. Existence of the negative feedback assures homeostasis of healthy cells and oscillatory responses of DNA-damaged cells, which are persistent when DNA repair is inefficient and the positive feedback loop is broken as observed by Geva-Zatorski et al. [41]. The positive feedback destroys the negative coupling between Mdm2 and p53 by sequestering most of Mdm2 in cytoplasm, so it may not longer prime the nuclear p53 for degradation. It works as a clock, providing the cell with time for DNA repair. However, when DNA repair is inefficient, the active p53 rises to a high level and triggers transcription of proapoptotic genes. As a result, small DNA damage may be repaired and the cell may return to its initial "healthy" state, while extended damage results in apoptosis. Stochasticity of p53 regulation, introduced at the levels of gene expression and DNA damage and repair, leads to the high heterogeneity of cell responses and causes cell population to split after irradiation into subpopulations of apoptotic and surviving cells, with fraction of apoptotic cells growing with the irradiation dose.

As far as we know, this study is the first attempt to integrate NF- κ B and p53 systems in one mathematical model.

2 Crosstalk model formulation

As already said, the crosstalk model combines our two recent stochastic models of NF- κ B and p53 systems [39], [40]. The structure, and most of parameters, of these models are left unchanged, see *Online Supplementary Material*. Among numerous suggested couplings of p53 and NF- κ B systems we selected two that are well documented experimentally.

(1) NF- κ B is the transcription factor for p53.

Wu and Lozano showed that in HeLa cells NF- κ B activates p53 and that this activation is inducible by TNF α [20]. By co-transfection experiments they showed that the p65 subunit of NF- κ B could bind and activate the p53 promoter. The activation of the p53 promoter by p65 ranged from 3- to 7-fold the control level. Then Kim et al. [42] (studying rabbit articular chondrocytes)

demonstrated that nitric oxide-induced activation of the p38 kinase leads to activation of NF- κ B, which in turn induces transcription of the p53 gene. Fujioka et al. [24] showed that activated NF- κ B up-regulates p53 expression and increases its stability and DNA binding activity.

Based on these to these findings we will assume that p53 transcription is a sum of NF- κ B dependent and independent terms. We will assume that binding of NF- κ B to the p53 promoter increases its transcription twofold.

(2) Activated p53 attenuates transcription of NF- κ B inhibitors I κ B α and A20.

As demonstrated by Wadgaonkar et al. [27], Webster and Perkins [28] and later by Ikeda et al. [43], the RelA component of NF- κ B, and p53, mutually repress each other's ability to activate transcription and compete for transcriptional coactivator proteins p300 and CBP (CREB-binding protein) for maximal activity. Bartke et al. [22] found that NF- κ B translocation measured by EMSA analysis was not significantly affected by p53 over a wide range of TNF α concentrations, whereas TNF α -induced transcription of NF- κ B driven reporter gene was drastically reduced. DLD-1 cells carrying mutations in their endogenous p53 alleles but stably transfected with p53-wt showed about four times lower reporter gene expression (at 2ng/ml or 20 ng/ml TNF α stimulation) than cells transfected with p53-mut. Recently, Kawauchi et al. [26] showed that ectopically expressed p53 enhances the DNA-binding activity of NF- κ B by acetylation and phosphorylation at Ser 536 of p65, but suppresses its transcriptional activity. The amount of transcriptionally activated phosphorylation of histone H3 was markedly decreased when accompanied by p53 binding, suggesting that activated p53 inhibits NF- κ B on the chromatin.

Following these finding we assume that I κ B α and A20 transcription rates are proportional to

$$\frac{h_1}{h_1 + P53_{pn}} \quad (1)$$

where $P53_{pn}$ is the amount of active nuclear p53. To avoid nested feedback loops, we will neglect the potential negative influence of p53 on its own NF- κ B controlled transcription. Instead we assume that binding of NF- κ B to the p53 promoter augments its transcription only twofold, i.e. less than estimated by Kim et al. [42].

Introduction of these two cross-regulations dynamically connects the p53 and NF- κ B systems

and adds a new regulatory positive feedback loop to the resulting system: NF- κ B upregulates transcription of p53, which in turn downregulates transcription of its inhibitors A20 and I κ B α . The resulting system is thus controlled by five feedback loops. The second positive feedback loop, mediated by PTEN, PIP and Akt acts on a slower time scale. It is responsible for terminating p53|Mdm2 oscillations by capturing Mdm2 in cytoplasm, and thus protecting nuclear p53 from Mdm2 driven degradation. The three negative loops couple transcription factors with their inhibitors.

System activation: As already said, the p53 and NF- κ B systems respond to various stress signals and their activation is mediated by numerous kinases. Here we focus on p53 activation in response to DNA damage resulting from gamma irradiation and NF- κ B activation due to TNF α stimulation.

The irradiation leads to about 40 double strand breaks (DSBs) per Gy [44]. The damage is sensed by the damage-dependent kinases, particularly ATM, that signal DNA damage to p53 and Mdm2. As a result, p53 is phosphorylated at several serine residues, which leads to its stabilization and enhances its transcriptional activity [45], [46]. Simultaneously, Mdm2 is phosphorylated, which results in its accelerated degradation [47]. In the model, it is assumed that p53 may exist only in two forms: active and inactive. Only the active form has transcriptional activity and simultaneously a lower (Mdm2 dependent) degradation rate. We assume that irradiation leads to DNA damage in a Poisson process. Next, we assume that DNA damage influences p53 activation and Mdm2 degradation and that the strength of this influence follows a Hill function of the number of DSBs.

TNF α stimulation leads to activation of TNFR1 receptors, also in the stochastic process with the rate proportional to ligand concentration. Activated receptors transmit the signal to IKKK - IKK kinase cascade; in turn activated IKK leads to I κ B α degradation and NF- κ B nuclear entry.

Transcription and translation: Following our earlier models we assume that each of p53 dependent genes (i.e. Mdm2 and PTEN) or NF- κ B dependent genes (i.e. I κ B α , A20, p53) has two copies. The state of each gene may be described by the discrete variable $G \in \{0, 1, 2\}$ (we will consider also the deterministic approximation in which the state of the gene is continuous variable $G \in [0, 2]$), which is equal to the number of gene copies being activated by the transcription factor.

We will assume that p53 dissociation is spontaneous, while NF- κ B dissociation rate is controlled by its inhibitor I κ B α , which may bind NF- κ B and remove it from the promoter. Transcription rate is in general equal to the sum of basal transcription (independent of gene activation) and induced by the transcription factor. The protein synthesis rate is proportional the amount of the corresponding transcript.

DNA damage and repair: We assume that DNA damage (measured by number N of DSBs) arises due to gamma irradiation in a Poisson process, but also due to the action of the *apoptotic factor*, synthesis of which is controlled by active p53, and which initiates the process of DNA fragmentation, a hallmark of apoptosis. There are several pro-apoptotic factors of Bcl-2 family proteins that can be activated by p53 leading to cascade of processes terminating in apoptosis. Specifically, as demonstrated by Nakano and Vousden [48], p53 induces synthesis of Puma, which binds to Bcl-2 and localizes to the mitochondria to induce mitochondrial outer membrane permeabilization and cytochrome C release. The cytochrome C released from mitochondria activates caspase 9, which in turn activates caspase 3, as reviewed in [49]. Finally, caspase-3 activates nucleases DFF (DNA Fragmentation Factor) [50] and CAD (caspase-activated deoxyribonuclease) [51], the enzymes that cleaves DNA.

The apoptotic decision switch has been recently modeled by Chen and colleagues [52]. The core module exhibits bistability. When the level of *enabler* (as the authors term it) or *apoptotic factor* rises above the given threshold, the level of active Bax (proapoptotic member of the Bcl-2 family) jumps up leading to mitochondrial membrane permeabilization and an irreversible cascade of processes leading to apoptosis.

In agreement with biological data, the model of Chen et al. [52], and others apoptotic decision models ([53],[54],[55]) we will assume that once the level of *apoptotic factor* rises above the threshold, DNA fragmentation starts at a high rate, so that DNA damage overtakes DNA repair. As a result the apoptotic decision is irreversible - the number of DNA breaks tends to infinity (in the model), and the levels of active p53, and other variables, stabilize. Such idealized model has its limitations. In reality the extended DNA damage stops mRNA synthesis, and activation of executioner caspases creates an expanding cascade of proteolytic activity causing protein levels to quickly fall down.

Appearance of new DSBs adds further to p53 activation, and thus gives rise to the additional positive feedback loop. However, since DNA fragmentation starts at the time when the cell is already at the "point of no return", existence of this positive loop may not change the cell fate. Since DNA is fragmented first into kilobase-size fragments [56] and then completely digested into multiples of nucleosomal size fragments, it may not be repaired.

Numerical Simulations: We have performed two types of simulations; deterministic (all reactions are modeled by ODEs) and stochastic (following numerical algorithm used in [57]) in which reactions are separated into slow (stochastic) involving small number of molecules, and fast involving large number of molecules described by ODEs. The slow reactions are these of gene activation/inactivation, receptor activation/inactivation and DNA damage and repair (creation and healing of DSBs).

The model equations, parameters and details of the numerical procedure are listed in the *Online Supplementary Material*.

3 Results

First, we analyzed responses of the crosstalk model (performing both deterministic and stochastic simulations) to $\text{TNF}\alpha$ stimulation and gamma irradiation separately, and then to gamma irradiation combined with $\text{TNF}\alpha$ stimulation. We found that for cells that are stimulated by $\text{TNF}\alpha$ or gamma irradiation separately, the p53 and NF- κ B systems coupling, which was introduced in the crosstalk model plays only a minor role, see in Figs. 2 and 3. In response to 4 hour long, 10 ng/ml $\text{TNF}\alpha$ stimulation, cells exhibit quasiperiodic oscillations of the NF- κ B and $\text{I}\kappa\text{B}\alpha$ levels, similar to those observed in simulations of NF- κ B model alone [39]. NF- κ B activation is not sufficient to trigger p53 activity, which remains under the control of its inhibitor Mdm2, Fig. 2. In turn, in response to 2 Gy irradiation, nuclear NF- κ B and $\text{I}\kappa\text{B}\alpha$ exhibit irregular, aperiodic oscillations only (with low nuclear level of NF- κ B) similar to those observed in unstimulated cells in our NF- κ B model (see [57] Fig. 3). The coefficients controlling DNA repair and apoptosis are chosen in the way, that in the deterministic approximation the critical gamma dose above which cell dies is slightly smaller

than 2 Gy (1.993 Gy). Similarly as for our p53 system model [40] irradiation of 2 Gy causes in most of cells the p53 level to exhibit one or two oscillations, after which the population of cells splits into a subpopulations of surviving cells, with a low p53 level and apoptotic cells with a high p53 level. The fraction of apoptotic cells at that dose is about 0.6, and the life or death decision is taken within 24 hours after the gamma irradiation.

Next, we considered the combined stimulation protocol in which cells are exposed to 4 hours long, 10 ng/ml, TNF α stimulation started 3 hours prior to the 2 Gy gamma irradiation, Fig. 4. One can observe that p53 activation attenuates the transcription of NF- κ B inhibitors and disturbs NF- κ B oscillations (Fig. 4 versus Fig. 2). After irradiation, the nuclear NF- κ B settles on the high plateau, which causes that the level of p53, which is NF- κ B responsive is also elevated. As a result, DNA repair may proceeds faster and for such stimulation protocol (4 hours long, 10 ng/ml, TNF α stimulation started 3 hours prior to irradiation) the critical gamma dose (determined in deterministic simulations) equals 2.240 and is somewhat larger than without TNF α stimulation.

The effect of TNF α stimulation is opposite if the stimulation starts 8 hours after irradiation, Figs. 5 and 7. In this case, NF- κ B upregulates the synthesis of p53 in the moment, in which the positive feedback mediated by PTEN suppresses nuclear entry of the p53 inhibitor Mdm2. Subsequently, in most cells, p53 rises to the high level triggering apoptosis. As a result the critical irradiation dose equals 1.249 and is much smaller without TNF α stimulation.

By performing deterministic simulations we explained how the critical irradiation dose depends on the time span T between TNF α stimulation and irradiation, Fig. 6. In general, TNF α stimulation which starts before irradiation causes slight increases of the critical gamma dose, while TNF α stimulation that starts after irradiation makes the critical gamma dose substantially smaller. In accordance, after performing three series of 10000 stochastic simulations, Fig. 7, we found that at the 2 Gy dose, the fraction of apoptotic cells decreases if the TNF α stimulation precedes irradiation, but substantially grows if the TNF α stimulation takes place 8 hours after the DNA damaging irradiation.

Since the process of DNA damage is a an important source of stochasticity in the p53 regulation,

we performed additional simulations in order to verify if our conclusions would remain valid after approximating the DNA damage process by a deterministic process. As shown in Fig. 8, the removal of this source of stochasticity makes the effect of $\text{TNF}\alpha$ stimulation (both prior and after irradiation) even more pronounced. In the case of stochastic DNA damage the numbers of apoptotic cells were: *No TNF*: 6225; *TNF before IR*: 5476; *TNF After IR*: 9811. Assuming that irradiation leads to in each cell to creation of precisely 80 DSBs, the corresponding numbers were: *No TNF*: 6405; *TNF before IR*: 5475; *TNF After IR*: 9974. One should expect that removal of other stochasticity sources would cause the fraction of apoptotic cells would tend to 0 for *TNF before IR* protocol and tend to 1 for *TNF After IR* protocol, that for irradiation dose of 2 Gy, since the corresponding critical irradiation values obtained in purely deterministic simulations are 2.240 Gy and 1.249, respectively..

4 Discussion

The crosstalk model of p53 and NF- κ B systems proposed in this study explores two of the numerous couplings existing between these two potent transcription factors systems. In the model, NF- κ B controls p53 transcription, which rises twofold after NF- κ B binds the p53 promoter. In turn, p53 attenuates transcription of the NF- κ B inhibitors A20 and I κ B α . As a result, the crosstalk system dynamics is controlled by five feedback loops; three negative coupling transcription factors with their inhibitors, and two positive that switch off the negative feedback loops.

The introduced coupling of p53 and NF- κ B systems has only a negligible effect on cell dynamics in the case when cells are separately stimulated by irradiation or by $\text{TNF}\alpha$, but the effect of crosstalk of these two systems becomes pronounced when these two stimuli are combined. Cells exposed to $\text{TNF}\alpha$ exhibit oscillations in NF- κ B nuclear level, which are terminated by gamma irradiation, Figs. 2 and 4. Activation of p53 by DNA damage triggers the positive coupling between p53 and NF- κ B, and causes the nuclear NF- κ B settle on a high level.

Our main finding is that the NF- κ B can have either anti-apoptotic or pro-apoptotic influence on irradiated cells. As NF- κ B activation preceding DNA damage protects cells from apoptosis, NF- κ B activation following irradiation increases the fraction of apoptotic cells. Interestingly, in both cases

NF- κ B elevates p53 level. The only difference is in timing; in the first hours after radiation, the elevated p53 level causes DNA repair to proceed faster. However, if NF- κ B is activated at later times, upregulation of p53 transcription takes place at the same time, when the signal passing through the slow positive feedback loop mediated by PTEN suppresses the activity of p53 inhibitor Mdm2. As a result the effects of the two positive feedbacks adds up, so that p53 is stabilized at a high level triggering apoptosis.

The crosstalk model predictions must be considered with caution: First, the proposed model, although based on experimentally established interactions, awaits for experimental verification. Second, as said already, we account only for two of the numerous couplings of p53 and NF- κ B systems. Third, even this "minimal" crosstalk model has a very rich dynamics, which was not fully explored in the current study. Moreover the strength of particular feedback loops is possibly cell type-dependent, and thus it is difficult to expect that dynamics predicted by our model will be universal. Nevertheless, our theoretical finding sheds some new light on the pro- and anti-apoptotic roles of NF- κ B [21], [58], [59], [19], [60]. Particularly, our colleagues found that pretreatment with TNF α markedly reduces the frequency of UV-induced apoptosis in p53 proficient cells, while in p53-deficient cells the fraction of apoptotic cells was even higher compared to cells only irradiated [60]. Although, from the cancer therapy perspective, the anti-apoptotic action of NF- κ B is always undesirable, for an organism that was "by chance" exposed to DNA damaging agents, both pro- and anti-apoptotic actions of NF- κ B could be helpful; early NF- κ B activation helps damaged cells to recover, while the NF- κ B activation at later times triggers apoptosis in cells in which DNA repair is impossible or too slow.

Acknowledgments

The programs written to perform the simulations will be available at our website:

<http://www.ippt.gov.pl/~tlipnia/> after the paper is published. The model equations, parameters and numerical implementation can be found in the *Online Supplementary Material*. This work was supported by Polish Ministry of Science and Higher Education Grants 4 T07A 001 30, N N519 319535 and PBZ-MNiI-2/1/2005, and NIH Grant GM086885.

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5 Figure captions

Fig. 1. Schematic of the p53-NF- κ B model. Dotted lines with arrow-heads and hammer-heads denote, respectively, positive and negative regulation. The couplings between NF- κ B and p53 systems considered in the crosstalk model are marked in red.

Fig. 2. Response of the crosstalk system to 10 ng/ml, 4 hours long TNF α stimulation. Upper Panel: The deterministic approximation; Active p53, nuclear Mdm2, the number of DSBs, nuclear NF- κ B and total I κ B α . Middle and lower Panels: shown are 30 single cells stochastic simulations. Middle Panel: Active p53, nuclear Mdm2, and the number of DSBs. Lower Panel: nuclear NF- κ B and the total I κ B α .

Fig. 3. Response of the crosstalk system to irradiation; The irradiation phase lasted 1 hour, and the total dose was 2 Gy. Upper Panel: The deterministic approximation; Active p53, nuclear Mdm2, the number of DSBs, nuclear NF- κ B and total I κ B α . Middle and lower Panels: shown are 30 single cells stochastic simulations. Middle Panel: Active p53, nuclear Mdm2, and the number of DSBs. Lower Panel: nuclear NF- κ B and the total I κ B α .

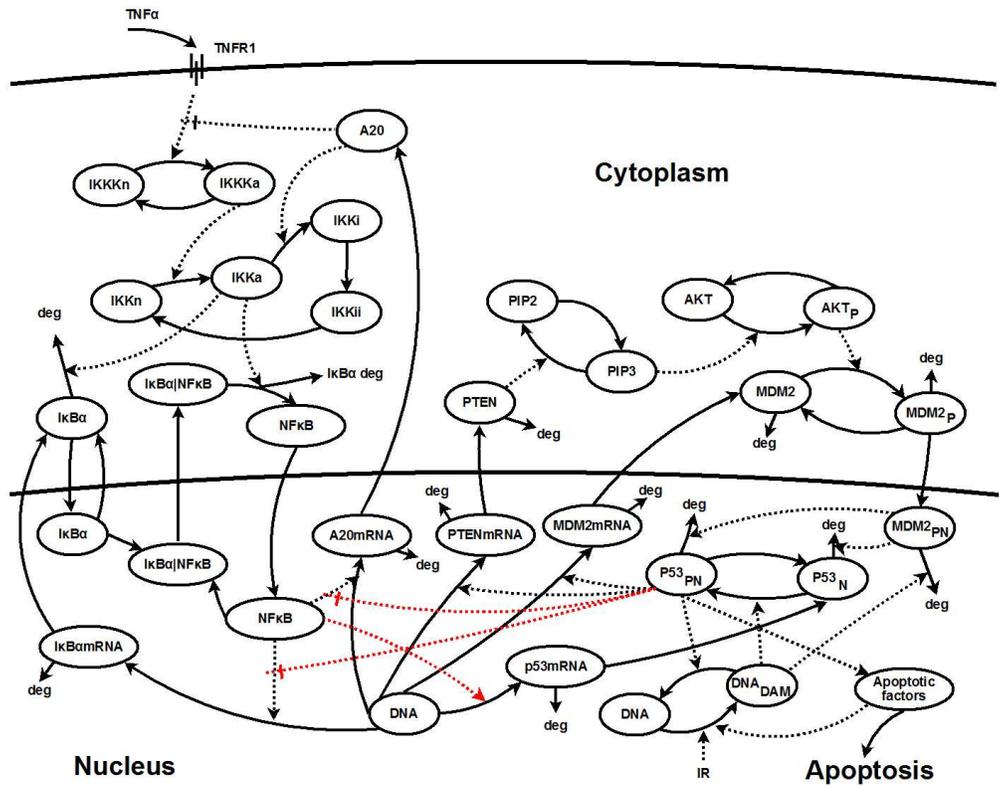
Fig. 4. Response of the crosstalk system to costimulation by TNF α and 2 Gy irradiation. 10 ng/ml TNF α stimulation started 3 hours prior to irradiation and lasted for 4 hours, the irradiation phase lasted 1 hour. Upper Panel: The deterministic approximation; Active p53, nuclear Mdm2, the number of DSBs, nuclear NF- κ B and total I κ B α . Middle and lower Panels: shown are 30 single cells stochastic simulations. Middle Panel: Active p53, nuclear Mdm2, and the number of DSBs. Lower Panel: nuclear NF- κ B and the total I κ B α .

Fig. 5. Response of the crosstalk system to costimulation by TNF α and 2 Gy irradiation. 10 ng/ml TNF α stimulation started 8 hours after irradiation and lasted for 4 hours, the irradiation phase lasted 1 hour. Upper Panel: The deterministic approximation; Active p53, nuclear Mdm2, the number of DSBs, nuclear NF- κ B and total I κ B α . Middle and lower Panels: shown are 30 single cells stochastic simulations. Middle Panel: Active p53, nuclear Mdm2, and the number of DSBs. Lower Panel: nuclear NF- κ B and the total I κ B α .

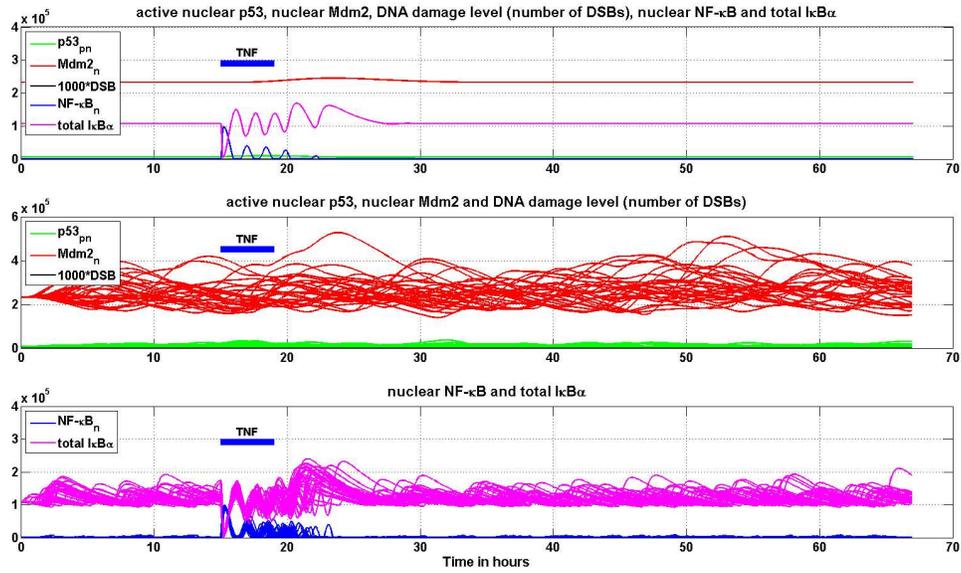
Fig 6. Critical irradiation dose as a function of time span T between beginnings of 4 hours long $\text{TNF}\alpha$ stimulation and irradiation; negative T means that $\text{TNF}\alpha$ stimulation starts before irradiation.

Fig. 7. Distribution of active p53 and active Akt in populations of 10000 cells 48 hours after the irradiation and/or $\text{TNF}\alpha$ stimulation ends. The 4 hours long 10ng/ml $\text{TNF}\alpha$ stimulation started 3 hour before irradiation (total dose 2 Gy) reduces the apoptotic cell fraction, while the same stimulation started 8 hours after irradiation resulted in the increase of the apoptotic cell fraction.

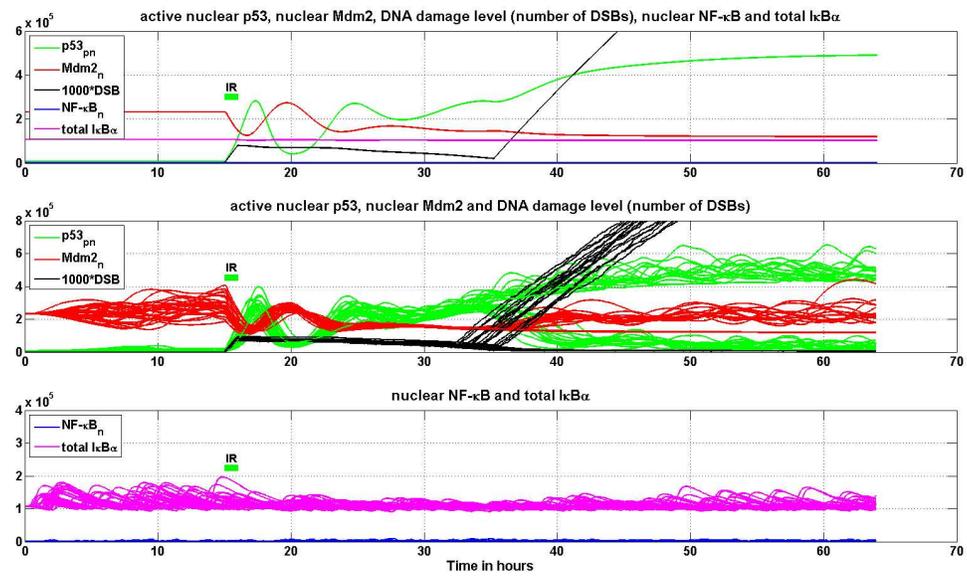
Fig. 8. Distribution of active p53 and active Akt in populations of 10000 cells 48 hours after the 2 Gy irradiation and/or $\text{TNF}\alpha$ stimulation. The simulation protocols are identical as for Fig 7, but DNA damage process caused by irradiation is considered in the deterministic approximation, i.e. in all stimulated cells precisely 80 DSBs are introduced during 1 hour of irradiation.



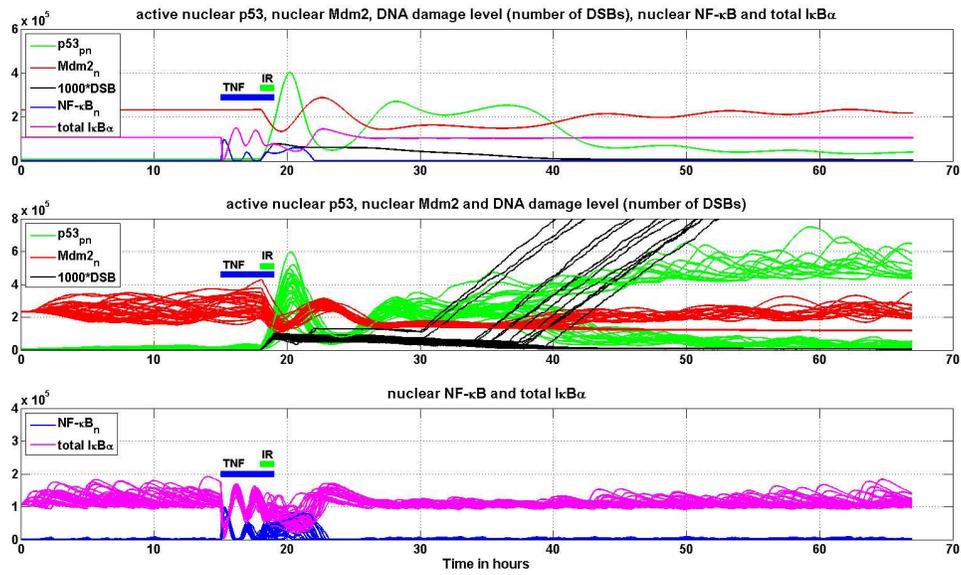
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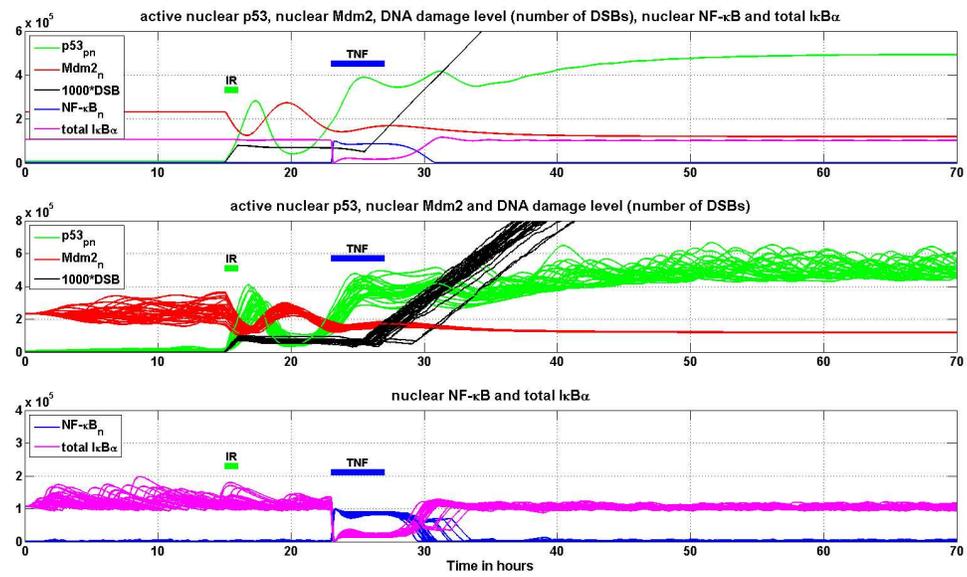
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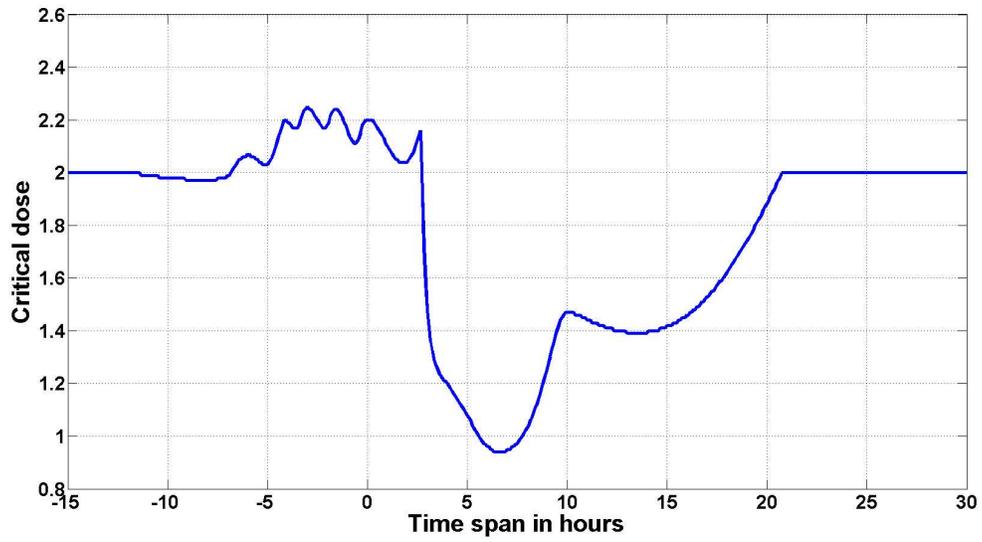
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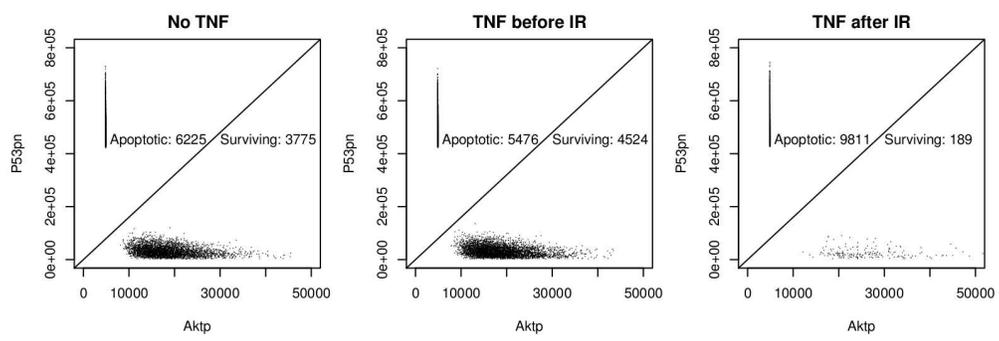
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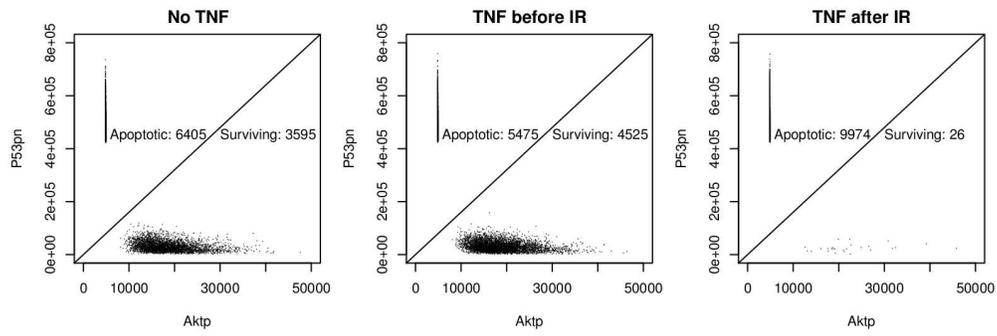
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Online Supplementary Material for

Crosstalk between p53 and NF- κ B systems: pro- and anti-apoptotic action of NF- κ B

Krzysztof Puszynski, Roberto Bertolusso and Tomasz Lipniacki

1 Model parameters

In the model computations, amounts of all the substrates are expressed in the numbers of molecules. Since we use the ODE's to describe most of the model kinetics, amounts of molecules are not integer numbers, but since these numbers are in most cases much greater than 1, such description is reasonable. To translate concentrations of the substrates to the numbers of molecules, one can assume that the cell volume is $2000\mu\text{m}^3$ (typical volume of human fibroblast cells) and that the ratio of the cytoplasm to nucleus is 5. In such a cell, cytoplasmic concentration of 1nM corresponds to 1000 molecules, while the same nuclear concentration corresponds to 200 molecules.

Table 1. Parameters of the NF- κ B part of the crosstalk model

Parameter	Description	Value
$k_v=V/U$	cytoplasmic to nuclear volume	5
k_b	receptor activation rate	$4 \times 10^{-6}/s$
k_f	receptor inactivation rate	$6 \times 10^{-4}/s$
q_{1n}	NF- κ B binding at I κ B α , A20 and p53 sites	$1.5 \cdot 10^{-7}/s$
q_{2n}	I κ B α mediated NF- κ B dissociation from I κ B α ,A20 and p53 sites	$10^{-6}/s$
KN	Total number of IKKK molecules	10^4
KNN	Total number of IKK molecules	2×10^5
M	Total number of TNFR1 receptors	10^3
NF- κ B $_{tot}$	Total number of NF- κ B molecules	10^5
k_a	IKKK activation rate	$10^{-4}/s$
k_i	IKKK inactivation rate	$10^{-2}/s$
c_{1n}	NF- κ B inducible A20 and I κ B α mRNA transcription	$10^{-1}/s$
c_{3n}	A20 and I κ B α mRNA degradation	$7.5 \times 10^{-4}/s$
c_{4n}	A20 and I κ B α translation rate	$5 \times 10^{-1}/s$
c_{5n}	A20 degradation rate	$5 \times 10^{-4}/s$
k_{a20}	A20 TNF block	10^4
k_2	IKKa inactivation due to A20	10^4
k_1	IKKn activation rate	$5 \times 10^{-6}/s$
k_3	IKKa inactivation rate	$3 \times 10^{-3}/s$
k_4	IKKii transformation	$5 \times 10^{-4}/s$
a_{1n}	I κ B α -NF κ B association	$5 \times 10^{-7}/s$
a_{2n}	IKKa mediated I κ B α phosphorylation	$10^{-7}/s$
a_{3n}	IKKa mediated (NF κ B I κ B α) phosphorylation	$5 \times 10^{-7}/s$
tp	degradation of P-I κ B α and P-I κ B α bounded to NF- κ B	$10^{-2}/s$
c_{5a}	degradation of I κ B α	$10^{-4}/s$
c_{6a}	degradation of I κ B α bounded to NF- κ B	$2 \times 10^{-5}/s$
i_1	NF- κ B nuclear import	$10^{-2}/s$
e_{2a}	I κ B α NF- κ B nuclear export	$5 \times 10^{-2}/s$
i_{1a}	I κ B α nuclear import	$2 \times 10^{-3}/s$
e_{1a}	I κ B α nuclear export	$5 \times 10^{-3}/s$

Table 2. Parameters of the p53|Mdm2 part of the crosstalk model

Parameter	Description	Value
a_0	spontaneous $P53_n$ phosphorylation rate	$1 \times 10^{-4}/s$
a_1	DNA damage induced $P53_n$ phosphorylation rate	$1 \times 10^{-3}/s$
a_2	PIP activation rate	$5 \times 10^{-5}/s$
a_3	AKT activation rate	$2 \times 10^{-9}/s$
a_4	MDM phosphorylation rate	$7.5 \times 10^{-9}/s$
c_0	PIP_p dephosphorylation rate (by $PTEN$)	$2.5 \times 10^{-9}/s$
c_1	AKT_p inactivation rate	$2 \times 10^{-4}/s$
c_2	MDM_p dephosphorylation rate	$1 \times 10^{-4}/s$
c_3	spontaneous $P53_{pn}$ dephosphorylation rate	0
d_0	Mdm2 spontaneous deg. rate (all Mdm2 forms)	$3 \times 10^{-5}/s$
d_1	DNA damage induced Mdm2 deg. rate (all Mdm2 forms)	$1.5 \times 10^{-4}/s$
d_2	$PTEN$ degradation rate	$5 \times 10^{-5}/s$
d_3	spontaneous $P53_n$ degradation rate	$1 \times 10^{-4}/s$
d_4	MDM_{pn} -induced $P53_n$ degradation rate	$1 \times 10^{-13}/s$
d_5	spontaneous $P53_{pn}$ degradation rate	$1 \times 10^{-4}/s$
d_6	MDM_{pn} -induced $P53_{pn}$ degradation rate	$1 \times 10^{-14}/s$
d_7	MDM_t degradation rate	$3 \times 10^{-4}/s$
d_8	$PTEN_t$ degradation rate	$3 \times 10^{-4}/s$
e_0	MDM_{pn} nuclear export	0
i_0	MDM_p nuclear import	$5 \times 10^{-4}/s$
s_0	MDM_t transcription rate	$6 \times 10^{-2}/s$
s_1	$PTEN_t$ transcription rate	$6 \times 10^{-2}/s$
t_0	MDM translation rate	$5 \times 10^{-1}/s$
t_1	$PTEN$ translation rate	$1 \times 10^{-1}/s$
h_0	Michaelis const. for $P53_n$ activation and Mdm2 degradation (all Mdm2 forms)	7
q_0	spontaneous activation of Mdm2 and PTEN genes	$1 \times 10^{-4}/s$
q_1	$P53_{pn}$ -depended activation of Mdm2 and PTEN genes	$5 \times 10^{-13}/s$
q_2	Mdm2 and PTEN genes inactivation rate	$3 \times 10^{-3}/s$
N_{SAT}	saturation coefficient in DNA repair	3
d_{DAM}	DNA damage rate	40/Gy
d_{REP}	DNA repair rate	$2 \times 10^{-14}/s$
AKT_{tot}	total number of Akt molecules ($AKT + AKT_p$)	2×10^5
PIP_{tot}	total number of PIP molecules ($PIP + PIP_p$)	1×10^5
a_6	max. DNA damage rate (induced by the apoptotic factor)	$1 \times 10^{-2}/s$
d_9	apoptotic factors degradation rate	$2 \times 10^{-4}/s$
p_1	max. synthesis rate of apoptotic factor	200/s
q_3	coefficient in apoptotic factor synthesis	$2.5 \times 10^{-11}/s$
q_4	coefficient in apoptotic factor synthesis	1/s
Th	threshold for apoptotic death	0.65

Table 3. Parameters of the crosstalk part of the model

Parameter	Description	Value
d_{10}	p53 mRNA degradation	$2 \times 10^{-4}/s$
s_2	NF- κ B inducible p53 mRNA transcription	$5 \times 10^{-2}/s$
s_3	independent p53 mRNA transcription	$5 \times 10^{-2}/s$
t_2	p53 translation rate	$5 \times 10^{-1}/s$
h_1	Michaelis constant at A20 and I κ B α transcription	6×10^4

2 Definitions of the variables

- AKT_n - inactive form of Akt
- AKT_p - active form of Akt
- MDM_t - Mdm2 transcript
- MDM - cytoplasmic Mdm2
- MDM_p - phosphorylated cytoplasmic Mdm2
- MDM_{pn} - phosphorylated nuclear Mdm2
- $P53_n$ - inactive form of nuclear p53 dimers
- $P53_{pn}$ - active form of nuclear p53 dimers
- $P53_t$ - p53 transcript
- $PTEN_t$ - PTEN transcript
- $PTEN$ - (cytoplasmic) PTEN
- PIP_n - inactive form of PIP (PIP2)
- PIP_p - active form of PIP (PIP3)
- N - number of DSBs
- B - number of active receptors
- R - irradiation intensity in Gy/s
- TNF - TNF α concentration in ng/ml,
- A - level of apoptotic factor
- $IKKn$ - neutral form of IKK kinase,
- $IKKa$ - active form of IKK,
- $IKKi$ - inactive form of IKK,
- $IKKii$ - inactive intermediate form of IKK,
- $IKKKn$ - amount of neutral form of IKKK,
- $IKKKa$ - amount of active form of IKKK,

- $I\kappa B$ - cytoplasmic amount of $I\kappa B\alpha$,
- $I\kappa B_n$ - nuclear $I\kappa B\alpha$,
- $I\kappa B_t$ - $I\kappa B\alpha$ mRNA transcript,
- $I\kappa B_p$ - phosphorylated cytoplasmic $I\kappa B\alpha$
- $A20$ - A20 protein
- $A20_t$ - A20 transcript
- $NF\kappa B$ - free cytoplasmic NF- κ B
- $NF\kappa B_n$ - free nuclear NF- κ B
- $NF\kappa B|I\kappa B$ - cytoplasmic (NF- κ B| $I\kappa B\alpha$) complexes
- $NF\kappa B|I\kappa B_p$ - phosphorylated cytoplasmic $I\kappa B\alpha$ complexed to NF κ B
- $(NF\kappa B|I\kappa B)_n$ - nuclear (NF- κ B| $I\kappa B\alpha$) complexes
- G_{Mdm2} - state of Mdm2 gene, in the stochastic simulations - a discrete variable, $G_{Mdm2} \in \{0, 1, 2\}$; in the deterministic simulations - a continuous variable, $G_{Mdm2} \in [0, 2]$
- G_{PTEN} - state of PTEN gene, in the stochastic simulations - a discrete variable $G_{PTEN} \in \{0, 1, 2\}$; in the deterministic simulations - a continuous variable, $G_{PTEN} \in [0, 2]$
- G_{p53} - state of p53 gene, in the stochastic simulations - a discrete variable $G_{p53} \in \{0, 1, 2\}$; in the deterministic simulations - a continuous variable, $G_{p53} \in [0, 2]$
- $G_{I\kappa B}$ - state of $I\kappa B\alpha$ gene, in the stochastic simulations - a discrete variable, $G_{I\kappa B} \in \{0, 1, 2\}$; in the deterministic simulations - a continuous variable, $G_{I\kappa B} \in [0, 2]$
- G_{A20} - state of A20 gene, in the stochastic simulations - a discrete variable, $G_{A20} \in \{0, 1, 2\}$; in the deterministic simulations - a continuous variable, $G_{A20} \in [0, 2]$
- $r_{NF\kappa B}^b, r_{NF\kappa B}^d$ - activation and inactivation propensities of NF- κ B responsive genes (stochastic simulations)
- r_{p53}^b, r_{p53}^d - activation and inactivation propensities of p53 responsive genes (stochastic simulations)
- r_r^b, r_r^d - receptors activation and inactivation propensities (stochastic simulations)
- r_{DNA}^n, r_{DNA}^r - DNA damage and repair propensities (stochastic simulations)

3 Equations

The deterministic approximation of the model is defined by the system ODEs (1-38).

(Cytoplasmic) PTEN, $PTEN$: The first term describes $PTEN$ synthesis and the second one describes its degradation

$$\frac{d}{dt}PTEN(t) = t_1 PTEN_t(t) - d_2 PTEN(t). \quad (1)$$

Inactive form of PIP, PIP_n : First term describes PIP dephosphorylation by $PTEN$ and the second one its phosphorylation (activation)

$$\frac{d}{dt}PIP_n(t) = c_0 PTEN(t) PIP_p(t) - a_2 PIP_n(t). \quad (2)$$

Active form of PIP, PIP_p : The first term describes PIP phosphorylation (activation) and the second one describes its dephosphorylation by $PTEN$

$$\frac{d}{dt}PIP_p(t) = a_2 PIP_n(t) - c_0 PTEN(t) PIP_p(t). \quad (3)$$

Inactive Akt, AKT_n : The first term describes AKT_p inactivation and the second one describes AKT_n activation catalyzed by PIP_p

$$\frac{d}{dt}AKT_n(t) = c_1 AKT_p(t) - a_3 AKT_n(t) PIP_p(t). \quad (4)$$

Active Akt, AKT_p : The first term describes AKT_n activation by PIP_p and the second one describes AKT_p inactivation

$$\frac{d}{dt}AKT_p(t) = a_3 AKT_n(t) PIP_p(t) - c_1 AKT_p(t). \quad (5)$$

Cytoplasmic Mdm2, MDM : First term describes MDM synthesis, the second term describes MDM_p dephosphorylation, the third term describes MDM phosphorylation catalyzed by AKT_p , while last one stands for MDM degradation

$$\begin{aligned} \frac{d}{dt}MDM(t) &= t_0 MDM_t(t) + c_2 MDM_p(t) \\ &\quad - a_4 MDM(t) AKT_p(t) - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)} \right) MDM(t). \end{aligned} \quad (6)$$

Cytoplasmic phosphorylated Mdm2, MDM_p : The first term represents MDM phosphorylation catalyzed by AKT_p , the second term describes MDM_p dephosphorylation, the third and the fourth term represent MDM_p nuclear import and export, and the last one stands for MDM_p degradation

$$\begin{aligned} \frac{d}{dt}MDM_p(t) &= a_4 MDM(t) AKT_p(t) - c_2 MDM_p(t) - i_0 MDM_p(t) \\ &\quad + e_0 MDM_{pn}(t) - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)} \right) MDM_p(t). \end{aligned} \quad (7)$$

Nuclear phosphorylated Mdm2, MDM_{pn} : The first and the second term represent MDM_{pn} nuclear import and export, while the last term describes its spontaneous and DNA damage induced degradation

$$\frac{d}{dt}MDM_{pn}(t) = i_0 MDM_p(t) - e_0 MDM_{pn}(t) - \left(d_0 + d_1 \frac{N^2(t)}{h_0^2 + N^2(t)} \right) MDM_{pn}(t). \quad (8)$$

Inactive (nuclear) p53, $P53_n$: The first term describes $P53_n$ synthesis, the second one its spontaneous and DNA damage induced activation, the third one inactivation of $P53_{pn}$, and last one spontaneous and MDM_{pn} induced degradation

$$\begin{aligned} \frac{d}{dt} P53_n(t) = & t_2 P53_t(t) - \left(a_0 + a_1 \frac{N^2(t)}{h_0^2 + N^2(t)} \right) P53_n(t) + c_3 P53_{pn}(t) \\ & - (d_3 + d_4 MDM_{pn}^2(t)) P53_n(t). \end{aligned} \quad (9)$$

Active (nuclear) p53, $P53_{pn}$: The first term represents spontaneous and DNA damage induced $P53_n$ activation, the second term represents $P53_{pn}$ inactivation, and last one its spontaneous and MDM_{pn} induced degradation

$$\begin{aligned} \frac{d}{dt} P53_{pn}(t) = & \left(a_0 + a_1 \frac{N^2(t)}{h_0^2 + N^2(t)} \right) P53_n(t) - c_3 P53_{pn}(t) \\ & - (d_5 + d_6 MDM_{pn}^2(t)) P53_{pn}(t). \end{aligned} \quad (10)$$

Mdm2 transcript, MDM_t : The first term represents MDM_t transcription, while the second one describes its degradation

$$\frac{d}{dt} MDM_t(t) = s_0 G_{Mdm2} - d_7 MDM_t(t). \quad (11)$$

PTEN transcript, $PTEN_t$: The first term represents $PTEN_t$ transcription, while the second one describes its degradation

$$\frac{d}{dt} PTEN_t(t) = s_1 G_{PTEN} - d_8 PTEN_t(t). \quad (12)$$

p53 transcript, $P53_t$: The first and the second term represent NF- κ B independent and dependent $P53_t$ transcription. The last term describes $P53_t$ degradation

$$\frac{d}{dt} P53_t(t) = 2s_3 + s_2 G_{p53} - d_{10} P53_t(t). \quad (13)$$

IKKK in active state $IKKKa$: the first term describes activation of $IKKKn$ by active receptors $B(t)$, which activity is attenuated by $A20$. The second term represents $IKKKa$ spontaneous inactivation

$$\frac{d}{dt} IKKKa(t) = k_a \frac{k_{a20}}{k_{a20} + A20(t)} B(t) IKKKn(t) - k_i IKKKa(t). \quad (14)$$

IKKK inactive state $IKKKn$: the first term represents $IKKKa$ spontaneous inactivation and the second term describes $IKKKn$ kinase activation.

$$\frac{d}{dt} IKKKn(t) = k_i IKKKa(t) - k_a \frac{k_{a20}}{k_{a20} + A20(t)} B(t) IKKKn(t). \quad (15)$$

IKK in the natural state $IKKn$: The first term describes $IKKn$ recovery from the intermediate form $IKKii$, while the second one depletion of $IKKn$ due to its activation by $IKKKa$

$$\frac{d}{dt}IKKn(t) = k_4 IKKii(t) - k_1 IKKKa(t) IKKn(t). \quad (16)$$

IKK in the active state $IKKa$: The first term represents activation of $IKKn$ to $IKKa$ mediated by $IKKKa$, while the second one represents depletion of $IKKa$ due to its transformation into inactive form $IKKi$ mediated by $A20$

$$\frac{d}{dt}IKKa(t) = k_1 IKKKa(t) IKKn(t) - k_3 IKKa(t) \frac{k_2 + A20(t)}{k_2} \quad (17)$$

IKK in the inactive state $IKKi$: The first term represents the $A20$ mediated inactivation of $IKKa$, while the second term describes $IKKi$ transformation to $IKKii$

$$\frac{d}{dt}IKKi(t) = k_3 IKKa(t) \frac{k_2 + A20(t)}{k_2} - k_4 IKKi(t). \quad (18)$$

IKK in the second inactive state $IKKii$: The first represents $IKKi$ transformation to $IKKii$, while the second term describes $IKKii$ transformation to $IKKn$

$$\frac{d}{dt}IKKii(t) = k_4 IKKi(t) - k_4 IKKii(t). \quad (19)$$

Phospho- $I\kappa B\alpha$ $I\kappa B_p$: The first term describes $I\kappa B\alpha$ phosphorylation by $IKKa$, while the second one describes catalytic degradation of $I\kappa B\alpha_p$

$$\frac{d}{dt}I\kappa B_p(t) = a_{2n} IKKa(t) I\kappa B(t) - t_p I\kappa B_p(t). \quad (20)$$

Phospho- $I\kappa B\alpha$ complexed to NF- κB $NF\kappa B|I\kappa B_p$: The first term describes $I\kappa B\alpha$ phosphorylation (in complexes with $NF\kappa B$) by $IKKa$, while the second one describes catalytic degradation of phosphorylated $I\kappa B\alpha$ ($NF\kappa B$ is recovered)

$$\frac{d}{dt}(NF\kappa B|I\kappa B_p)(t) = a_{3n} IKKa(t) (NF\kappa B|I\kappa B)(t) - t_p (NF\kappa B|I\kappa B_p)(t). \quad (21)$$

Free cytoplasmic NF- κB $NF\kappa B$: The first two terms represent liberation $NF\kappa B$ due to degradation of $I\kappa B\alpha$ in $NF\kappa B|I\kappa B\alpha$ complexes and its depletion due to formation of these complexes. The third term accounts for liberation of $NF\kappa B$ due to degradation of phosphorylated $I\kappa B\alpha$. The last term describes transport of free cytoplasmic $NF\kappa B$ to the nucleus

$$\begin{aligned} \frac{d}{dt}NF\kappa B(t) &= c_{6a} (NF\kappa B|I\kappa B)(t) - a_{1n} NF\kappa B(t) I\kappa B(t) \\ &+ t_p (NF\kappa B|I\kappa B_p)(t) - i_1 NF\kappa B(t). \end{aligned} \quad (22)$$

Free nuclear NF- κB $NF\kappa B_n$: The first term describes transport of free cytoplasmic $NF\kappa B$ to the nucleus. The second term represents depletion of free nuclear $NF\kappa B$ due to the association with nuclear $I\kappa B\alpha$ (the reaction rate is adjusted to the smaller nuclear volume resulting in a larger concentration by multiplying the synthesis coefficient a_1 by $k_v = V/U$)

$$\frac{d}{dt}NF\kappa B_n(t) = i_1 NF\kappa B(t) - a_{1n} k_v NF\kappa B_n(t) I\kappa B_n(t). \quad (23)$$

A20 protein $A20$: The first term describes $A20$ synthesis and its constitutive degradation

$$\frac{d}{dt}A20(t) = c_{4n} A20_t(t) - c_{5n} A20(t). \quad (24)$$

A20 transcript $A20_t$: The first term describes $NF\kappa B$ inducible transcription of $A20_t$, while the second term describes degradation of $A20$ transcript

$$\frac{d}{dt}A20_t(t) = c_{1n} \frac{h_1}{h_1 + P53_{pn}} G_{A20} - c_{3n} A20_t(t). \quad (25)$$

Free cytoplasmic $I\kappa B\alpha$ protein $I\kappa B$: The first term describes $I\kappa B\alpha$ phosphorylation by $IKKa$, the second one accounts for $NF\kappa B - I\kappa B\alpha$ binding. Next two terms describe $I\kappa B\alpha$ synthesis and its constitutive degradation. The last two terms represent transport into and out of the nucleus

$$\begin{aligned} \frac{d}{dt}I\kappa B(t) = & -a_{2n} IKKa(t) I\kappa B(t) - a_{1n} NF\kappa B(t) I\kappa B(t) \\ & + c_{4n} I\kappa B_t(t) - c_{5a} I\kappa B(t) \\ & - i_{1a} I\kappa B(t) + e_{1a} I\kappa B_n(t). \end{aligned} \quad (26)$$

Free nuclear $I\kappa B\alpha$ protein $I\kappa B_n$: The first term accounts for formation of $NF\kappa B|I\kappa B\alpha$ nuclear complexes (the reaction rate is adjusted to the smaller nuclear volume resulting in a larger concentration by multiplying the synthesis coefficient a_1 by $k_v = V/U$), while the last two terms represent the $I\kappa B\alpha$ transport to and out of the nucleus,

$$\frac{d}{dt}I\kappa B_n(t) = -a_{1n} k_v NF\kappa B_n(t) I\kappa B_n(t) + i_{1a} I\kappa B(t) - e_{1a} I\kappa B_n(t). \quad (27)$$

$I\kappa B\alpha$ transcript $I\kappa B_t$: The first term describes $NF\kappa B$ inducible transcription of $I\kappa B\alpha$, while the second term describes degradation of $I\kappa B\alpha$ transcript

$$\frac{d}{dt}I\kappa B_t(t) = c_{1n} \frac{h_1}{h_1 + P53_{pn}} G_{I\kappa B} - c_{3n} I\kappa B_t(t). \quad (28)$$

Cytoplasmic $NF\kappa B|I\kappa B\alpha$ complexes $NF\kappa B|I\kappa B$: The first two terms account for formation of $NF\kappa B|I\kappa B\alpha$ complexes and their degradation. The third term represents phosphorylation of $I\kappa B\alpha$ in $NF\kappa B|I\kappa B\alpha$ complexes due to the catalytic activity of $IKKa$. The last term represents transport of the complex from the nucleus

$$\begin{aligned} \frac{d}{dt}(NF\kappa B|I\kappa B)(t) = & a_{1n} NF\kappa B(t) I\kappa B(t) - c_{6a} (NF\kappa B|I\kappa B)(t) \\ & - a_{3n} IKKa(t) (NF\kappa B|I\kappa B)(t) + e_{2a} (NF\kappa B|I\kappa B)_n(t). \end{aligned} \quad (29)$$

Nuclear $NF\kappa B|I\kappa B\alpha$ complexes $(NF\kappa B|I\kappa B)_n$: The first term accounts for formation of $NF\kappa B|I\kappa B\alpha$ nuclear complexes (the reaction rate is adjusted to the smaller nuclear volume resulting in a larger concentration by multiplying the synthesis coefficient a_1 by $k_v = V/U$). The second term describes their transport out of the nucleus

$$\frac{d}{dt}(NF\kappa B|I\kappa B)_n(t) = a_{1n} k_v NF\kappa B_n(t) I\kappa B_n(t) - e_{2a} (NF\kappa B|I\kappa B)_n(t). \quad (30)$$

Apoptotic factor A: We assume that p53 regulates synthesis of some apoptotic factor. For simplicity, we skip its mRNA transcription, and assume that it is produced in a deterministic manner being regulated by active p53. In the below equation the first term stands for apoptotic factors production while second one describes its degradation

$$\frac{d}{dt}A(t) = p_1 \frac{q_3 P53_{pn}^2(t)}{q_4 + q_3 P53_{pn}^2(t)} - d_9 A(t). \quad (31)$$

State G_{Mdm2} of p53 dependent gene Mdm2: The first term describes the p53 independent and dependent Mdm2 gene activation, while the second term describes the spontaneous Mdm2 gene inactivation

$$\frac{d}{dt}G_{Mdm2}(t) = (q_0 + q_1 P53_{PN}^2(t)) (2 - G_{Mdm2}(t)) - q_2 G_{Mdm2}(t). \quad (32)$$

State G_{PTEN} of p53 dependent gene PTEN: The first term describes the p53 independent and dependent PTEN gene activation, while the second term describes the spontaneous PTEN gene inactivation

$$\frac{d}{dt}G_{PTEN}(t) = (q_0 + q_1 P53_{PN}^2(t)) (2 - G_{PTEN}(t)) - q_2 G_{PTEN}(t). \quad (33)$$

State $G_{I\kappa B}$ of NF- κ B dependent gene I κ B: The first term describes the NF- κ B dependent I κ B α gene activation, while the second term describes the I κ B α dependent I κ B α gene inactivation (I κ B α may bind NF- κ B and remove it off DNA)

$$\frac{d}{dt}G_{I\kappa B}(t) = q_{1n} NF\kappa B_n(t) (2 - G_{I\kappa B}(t)) - q_{2n} I\kappa B_n(t) G_{I\kappa B}(t), \quad (34)$$

State G_{A20} of NF- κ B dependent gene A20: The first term describes the NF- κ B dependent A20 gene activation, while the second term describes the I κ B α dependent A20 gene inactivation

$$\frac{d}{dt}G_{A20}(t) = q_{1n} NF\kappa B_n(t) (2 - G_{A20}(t)) - q_{2n} I\kappa B_n(t) G_{A20}(t). \quad (35)$$

State G_{p53} of NF- κ B dependent gene p53: The first term describes the NF- κ B dependent p53 gene activation, while the second term describes the I κ B α dependent p53 gene inactivation

$$\frac{d}{dt}G_{p53}(t) = q_{1n} NF\kappa B_n(t) (2 - G_{p53}(t)) - q_{2n} I\kappa B_n(t) G_{p53}(t). \quad (36)$$

Number of active receptors B : The first term describes TNFR1 receptors activation by TNF α , while the second one their spontaneous deactivation. M is the total number of the cell receptors

$$\frac{d}{dt}B(t) = k_b (M - B(t)) TNF(t) - k_f B(t). \quad (37)$$

Number of DSBs N : First two terms describes DSBs creation by radiation of intensity R and by action of apoptotic factor, while second one describes DSBs sealing induced by $P53_{pn}$

$$\frac{d}{dt}N(t) = d_{DAM}R(t) + a_6 (\text{sign}(A(t)) \frac{d_9}{p_1} - Th) + 1 - d_{REP} \frac{N(t)}{N(t) + N_{SAT}} P53_{pn}^2(t) \quad (38)$$

The rules for the stochastic simulations

In the stochastic model simulations the gene states, the number of active TNFR1 receptors and the number of DSBs are considered as stochastic variables, while the remaining variables follow the systems of ODEs (1-31) given above.

Propensities of receptors and genes activation and inactivation

The TNFR1 receptors are activated and inactivated independently with activation propensity $r_r^b(t)$ proportional to the $TNF\alpha(t)$ concentration and constant inactivation propensity r_r^d

$$r_r^b(t) = k_b TNF(t), \quad r_r^d = k_f. \quad (39)$$

We assume that all genes have two homologous copies that may be independently activated by NF- κ B (A20, I κ B α and p53) or p53 (Mdm2 and PTEN). Additionally, we assume some basal gene activation of p53 responsive genes. The NF- κ B dependent genes are inactivated due to the I κ B α mediated removal of NF- κ B molecules off DNA, while inactivation of p53 dependent genes is spontaneous. The activation and inactivation propensities $r^b(t)$ and $r^d(t)$, respectively, for the p53 dependent genes are:

$$r_{p53}^b(t) = q_0 + q_1 P53_{pn}^2(t), \quad r_{p53}^d(t) = q_2, \quad (40)$$

and for NF- κ B dependent genes:

$$r_{NF\kappa B}^b(t) = q_{1n} NF\kappa B_n(t), \quad r_{NF\kappa B}^d(t) = q_{2n} I\kappa B\alpha_n(t), \quad (41)$$

where $P53_{pn}$ is the nuclear amount of phosphorylated p53 dimers. The state of gene copy G^i ($i = 1, 2$) is $G^i = 1$ whenever transcription factor is bound to the promoter, and $G^i = 0$ when the promoter site is unoccupied. As a result the gene state $G = G^1 + G^2$ can be equal to 0, 1 or 2.

DNA damage and repair

We assume that DNA damage (measured by the number of DSBs) arises due to gamma irradiation and the action of the apoptotic factor, which may destroy the integrity of DNA. DSBs appears with propensity equal $r^n(t)$

$$r_{DNA}^n(t) = d_{DAM} R(t) + a_6 \left(\text{sign}(A(t)) \frac{d_9}{p_1} - Th \right) + 1, \quad (42)$$

where p_1/d_9 is the maximum number of the apoptotic factor molecules following from Eq. (31), a_6 is a measure of their DNA damaging efficiency, $R(t)$ is the irradiation intensity and $d_{DAM} = 40/\text{Gy}$ is the experimentally determined DNA damage coefficient. Since in any individual cell apoptosis is *Yes* or *NO* event we introduce the apoptotic death threshold $Th = 0.65$, which assures that when the level of the apoptotic factor is low it does not have any effect on the DNA integrity.

We do not include any mechanism of DNA repair explicitly in our model. Instead, since p53 regulates transcription of numerous genes involved in DNA repair, we assume that the rate of DSBs sealing is governed by the active p53 level. Since the probability of the p53 dependent genes activation depends on square of $P53_{pn}(t)$ the propensity $r^r(t)$ of DSBs sealing is equal

$$r_{DNA}^r(t) = d_{REP} \frac{N(t)}{N(t) + N_{SAT}} P53_{pn}^2(t). \quad (43)$$

4 Numerical implementation

The implemented numerical scheme for the stochastic model is the following:

(1) At simulation time t , for given states of all genes, receptors and fixed number of DSBs we calculate the total propensity function $r(t)$ of occurrence of any of the activation and inactivation reactions or new DSB creation or healing,

$$\begin{aligned}
 r(t) = & r_r^b(M - B) + r_r^d B + r_{NF\kappa B}^b(t)(2 - G_{A20}) + r_{NF\kappa B}^b(t)(2 - G_{I\kappa B}) \\
 & + r_{NF\kappa B}^b(t)(2 - G_{p53}) + r_{p53}^b(t)(2 - G_{Mdm2}) + r_{p53}^b(t)(2 - G_{PTEN}) + r_{DNA}^n(t) \\
 & + r_{NF\kappa B}^d(t)G_{A20} + r_{NF\kappa B}^d(t)G_{I\kappa B} + r_{NF\kappa B}^d(t)G_{p53} + r_{p53}^d(t)G_{Mdm2} \\
 & + r_{p53}^d(t)G_{PTEN} + r_{DNA}^r(t).
 \end{aligned} \tag{44}$$

(2) We select two random numbers p_1 and p_2 from the uniform distribution on $[0, 1]$.

(3) Using the fourth order ode23tb MATLAB solver or the Dormand–Prince five order method (see below) we evaluate the system of ODEs (1-31) accounting for fast reactions, until time $t + \tau$ such that

$$\log(p_1) + \int_t^{t+\tau} r(s)ds = 0. \tag{45}$$

(4) In this step we determine which one of the 14 potentially possible reactions occurs at time $t + \tau$ using the inequality

$$\sum_{i=1}^{k-1} r_i(t + \tau) < p_2 r(t + \tau) \leq \sum_{i=1}^k r_i(t + \tau), \tag{46}$$

where $r_i(t + \tau)$, $i = 1, \dots, 14$ are individual reaction propensities and k is the index of the reaction to occur.

(5) Finally time $t + \tau$ is replaced by t , and we go back to the point (1).

Both deterministic and stochastic simulations are started from the steady state of the deterministic approximation of the system in the absence of any stimulation (in the case of stochastic stimulation the real numbers describing steady state are rounded to integers).

The simulations were performed using our codes written in MATLAB and also the much faster (R-C) code, which is compiled in C, as an extension function to be called from R (see Bertolusso and Kimmel, Bioinformatics 2009 - submitted). This faster code enabled us to perform series of 10000 stochastic simulations overnight on PC. In both codes the deterministic simulation give almost identical trajectories and values of the three critical gamma doses (corresponding to the three discussed simulation protocols - see main text) agrees satisfactorily; *No TNF*: 1.993 (MATLAB) versus 1.993 (R-C code); *TNF before IR*: 2.240 (MATLAB) versus 2.241 (R-C code); *TNF after IR*: 1.249 (MATLAB) versus 1.256 (R-C code).