Application of bifurcation theory and siRNA-based control signal to restore the proper response of cancer cells to DNA damage

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Emilia Kozłowska, Krzysztof Puszynski*

Institute of Automatic Control, Silesian University of Technology, Akademicka 16, 44-100 Gliwice, Poland

HIGHLIGHTS

- We show how the bifurcation theory and siRNA can be used for cancer cells treatment.
- Two cancer-specific abnormalities were investigated: Mdm2 over-expression and PTEN silencing.
- Bifurcation theory can reveal significant differences in normal and cancer cells dynamics.
- These differences can be overcome by siRNA-based control signals.
- We show that this can lead to the sensitization of the cancer cells to IR. Additionally healthy cells can be immunized to IR.

ARTICLE INFO

ABSTRACT

Many diseases with a genetic background such as some types of cancer are caused by damage in the p53 signaling pathway. The damage changes the system dynamics providing cancer cells with resistance to therapy such as radiation therapy. The change can be observed as the difference in bifurcation diagrams and equilibria type and location between normal and damaged cells, and summarized as the changes of the mathematical model parameters and following changes of the eigenvalues of Jacobian matrix. Therefore a change in other model parameters, such as mRNA degradation rates, may restore the proper eigenvalues and by that proper system dynamics. From the biological point of view, the change of mRNA degradation rate can be achieved by application of the small interfering RNA (siRNA). Here, we propose a general mathematical framework based on the bifurcation theory and siRNA-based control signal in order to study how to restore the proper response of cells with damaged p53 signaling pathway to therapy by using ionizing radiation (IR) therapy as an example. We show the difference between the cells with normal p53 signaling pathway and cells with abnormalities in the negative (as observed in SJSA-1 cell line) or positive (as observed in MCF-7 or PNT1a cell lines) feedback loop. Then we show how the dynamics of these cells can be restored to normal cell dynamics by using selected siRNA.

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1. Introduction

Endogenous processes and exogenous agents can cause DNA damage; for example, radiation can lead to double strand breaks (DSB). Inefficient DNA repair process or unrepairable DNA damage induces genetical diseases, among them the most lethal one—cancer. Maintaining genomic integrity is an important task of living cells (Olivier et al., 2010). The p53 signaling pathway plays a main role in this task by controlling genomic stability, thus, the p53 protein is called 'the guardian of the genome'. Its role is to stop the cell cycle and initiate the DNA repair process after the

DNA damage detection. If the cell is unable to maintain genome integrity, the p53 signaling pathway triggers apoptosis (Zilfou and Lowe, 2009) or senescence (Lindgren et al., 2015). From the molecular point of view, the core of p53 signaling pathway functions as follows: in normal cells the p53 level is kept low by its inhibitor Mdm2, which is transcriptionally dependent on p53. It defines negative feedback loop. On the other hand, p53 regulates PTEN transcription, which through PIP3 and Akt blocks the Mdm2 phosphorylation which is necessary for its nuclear entry and p53 inhibition. This defines the positive feedback loop. DNA damage results in p53 phosphorylation which protects p53 from Mdm2 (p53 inhibitor) and simultaneously enhances Mdm2 degradation (Puszynski et al., 2008). The p53 protein concentration increases. When the p53 concentration crosses the first threshold defined by Kracikova et al. (2013), the cell cycle is arrested to prevent transmission of the damage to daughter cells. Unsuccessful DNA repair

* Corresponding author.
E-mail addresses: emilia.kozlowska@helsinki.fi (E. Kozłowska), krzysztof.puszynski@polsl.pl (K. Puszynski).

during the time defined by the initiation of the positive feedback causes Mdm2 blockade in the cytoplasm and further increase of the nuclear p53 level. Finally, when p53 level crosses the second threshold, apoptosis or senescence is initiated.

The mechanism of maintaining genome integrity is, however, disabled in many cancers through mutations of the p53 protein itself or abnormalities in the p53 signaling pathway (Muller and Vousden, 2013). Such alterations can be observed for example in many sarcomas. Strong Mdm2 over-expression caused e.g. by strong Mdm2 gene amplification up to 25 × in SJSA-1 cells (Tovar et al., 2006) leads to very strong p53 inhibition and its inability to cross even the first threshold. This could be the reason of the sarcomas resistance to ionization radiation therapy. Another malfunction of mechanisms to protect cells genome integrity is observed in the MCF-7 cells (breast cancer) where PTEN gene methylation (Krawczyk et al., 2007) disables the positive feedback loop. PTEN loss was observed also in the PNT1a cells (prostate cancer) (Mak et al., 2012) and some of the subtypes of diffuse large B-cell lymphoma (DLBCL) (Pfeifer et al., 2013). Consequently, increased p53 level is followed by the Mdm2 production and p53 degradation. Hence, in these cells we observe sustained p53-Mdm2 level oscillations below the apoptotic/senescence threshold level.

Radiation and other classical treatments of patients with cancer cause significant apoptotic death or senescence of tumor cells, as well as damage to surrounding healthy tissues. Hence, the treatments may induce serious morbidity and potentially fatal outcomes. What is more, one hallmark of tumor cells is resisting programmed cell death (Hanahan and Weinberg, 2011). It means that tumor cells are prone to escaping from apoptosis or senescence e.g. by the mechanisms described above. Therefore, a mechanism which sensitizes tumor cells to radiation is of great interest. Despite the technological advances in the field of radiation oncology, overcoming tumor cells' radio-resistance remains a therapeutic challenge (Ding et al., 2013).

Sensitization of cancer cells to irradiation could be achieved by introduction of some artificial molecules such as those based on interfering RNA (Davis, 2009) or chemical compounds (Vassilev et al., 2004). From these possibilities, the RNA interference (RNAi) based silencing mechanism is the most reliable method, because it additionally allows avoiding severe side effects which often accompany such methods. These features attracted the attention of researchers in many fields ranging from basic science studies to clinical medicine, aiming to provide new insights into the methods of treatment and cancer prevention, viral infections and auto-immune disorders (Dorsett and Tuschl, 2004; Hannon and Rossi, 2006; Ryther et al., 2005).

RNA interference (RNAi) is a process of sequence-specific gene silencing discovered by Andrew Fire and colleagues in 1998 (Fire et al., 1998). They observed that the introduction of external double-stranded RNAs can initiate sequence-specific degradation of mRNAs which are homological to the dsRNA. RNAi governs key resistance mechanisms in cells to endogenous and exogenous nucleic acids, as well as regulates gene expression. With increasing progress in understanding the molecular mechanisms of the RNAi pathway and discovery of miRNA and siRNA (effector molecules of the RNA interference), the phenomenon has been applied in various areas: nanomedicine (Tokatlian and Segura, 2010), functional genomics (Kiger et al., 2004) and cancer biology (Fuchs and Borkhardt, 2007).

In contrast to miRNA, siRNA do not naturally exist in human cells and have higher specificity and the ability to almost entirely silence the expression of a single target gene. It makes them especially useful as a controlling agent in therapeutic studies

(Bumcrot et al., 2006). The biggest disadvantage of the siRNA compared to miRNA is the fact that only negative regulation is available through the direct influence of siRNA. Positive regulation is only achievable indirectly through double negation, such as the blockage of target gene repressor. In case of the miRNA, gene silencing is related to over-expression of miRNA, and over-expression of target mRNA is caused by knockdown of miRNA.

Another problem arises in the in vivo research. The efficiency of the iRNA transport into the cell is one of the most crucial elements in efficiency of siRNA or miRNA based therapy. siRNA/miRNA can be introduced to the cells in their mature form directly or with the help of transporting agents such as polymer-based nanoparticles. Kim et al. (2004) reported high efficiency of local and systematic delivery of naked siRNA in animal models. What is more, Shim and Kwon (2010) proved that targeting agents can significantly increase siRNA's effectiveness. Another group of methods involve transfection of small RNA-expressing constructs with the help of viral or plasmid vectors, which results in their intercellular production with long term effect (Makinen et al., 2006).

Besides the biological discoveries, another effort to better understand cancer and determine possible treatment is taken by system biologists. Through the mathematical model development and its analysis, they try to understand how the biological processes are governed by signal transduction pathways, and explain the complex interactions between the proteins, mRNAs, siRNAs or miRNAs, and the dynamics of such interactions (Bartlett and Davis, 2006; Groenenboom and Hogeweg, 2008; Puszynski et al., 2012). Systems biology is not only able to integrate information from multiple sources into a coherent quantitative model, but also through computational and mathematical methods such as bifurcation and sensitivity analysis to provide the key information useful for therapy planning and drug development; Young and Michelson, 2011). For example, bifurcation analysis could provide information concerning the differences between the dynamics of intracellular processes in normal and cancer cells, and sensitivity analysis can reveal the key points in the signaling pathway which would be the best targets for therapy development. For example, mathematical description of small RNA has proven its usefulness providing precious pharmacodynamics simulation data used in various clinical trials including the design of CALAA-01 dosage system, successfully tested on patients with solid tumors in a phase I clinical trial (Davis et al., 2010).

The term *Bifurcation* was introduced by Henri Poincare in 1885 (Poincare, 1885). Bifurcation in the system occurs when small change of one or more of its parameters causes qualitative change in the system's behavior, such as destabilization, oscillations born or chaos initiation. From the mathematical point of view, such a change is related to the change of equilibrium point type of our system or equilibria position change and their collision which can be observed as change in the eigenvalues of Jacobian matrix of the examined model. The parameter which causes system behavior change is called bifurcation parameter, and the value of that parameter at which the change occurs is named bifurcation point.

Examining bifurcation diagrams has contributed toward understanding of how observed biological response arises out of signaling pathway topology and parameters. Bifurcation theory (see Kuznetsov, 2004 for overall introduction) has two main applications in biology (Lu et al., 2006). The first one is to locate regions in parameter space exhibiting biologically meaningful bifurcation type behavior by mapping parameter space to the bifurcation diagram. It is called forward problem and is useful for understanding which possible behaviors may arise out of the given mathematical model. For instance, Tyson et al. performed bifurcation analysis to explain dynamics of growth and reproduction

of fission yeast (Tyson et al., 2002). The second application is to unravel causes for observed or desired effects by mapping bifurcation diagram to parameter space (inverse problem). It can be used to explain in the form of biochemical parameters how model dynamics arise. For instance, Lu et al. (2006) applied inverse bifurcation analysis of check points in mammalian cell phase transition.

Our previous results (Puszynski et al., 2012) show possibilities of p53 pathway regulation by siRNA. We demonstrated that silencing Mdm2 by siRNA can increase sensitivity to irradiation of tumor cells with aberrations in the p53 pathway. Based on a simulation study we established that problems of radiotherapy dosage limitations can be avoided by using a siRNA based control mechanism. Here, we extended the studies by proposing a general mathematical framework, based on bifurcation theory, to determine model parameters which have to be changed by siRNA application and the value of this change in order to restore proper cell functioning. Additionally, we wanted to explore the possibility of immunization of the healthy cell to ionization radiation. The idea was to imitate the cancer-specific immunization which, as mentioned, is through the Mdm2 over-expression or PTEN silencing. Since we wanted to use siRNA as the input we chose to use PTEN targeted siRNA to reduce its expression. SiRNA inputs can significantly improve IR based therapy or other therapies based on DNA damage induction by sensitization of the cancer cells with simultaneous immunization of the healthy ones. It should lower IR or other damaging agent doses and decrease of the side effects.

2. Materials and methods

The aim of our work was to investigate the possibility of sensitization of the cancer cells and immunization of the healthy ones to the IR by using siRNA targeted for Mdm2 and PTEN mRNA respectively. To test it, we developed a mathematical model which is modification of our newest p53 signaling pathway model presented in Puszynski et al. (2014). The modification includes removal of the Nutlin control signal and addition of the IR and siRNA influence on the system (see Fig. 1). The whole model is described in detail in the Supplementary Materials. Here we describe only the equations crucial for our research. Eqs. (1) and (2) describe Mdm2 and PTEN genes state change in time. The first term stands for p53 independent and dependent gene activation while the second for spontaneous gene deactivation. Power 2 in the first term reflects the fact that main transcription factor role is played by p53 tetramers while normally it is present in cell as a dimer. Parameters n_{MDM} and n_{PTEN} state for the Mdm2 and PTEN functional gene number. In normal cells we have two functional copies of each gene so $n_{MDM} = n_{PTEN} = 2$.

In many cancers Mdm2 over-expression is related to Mdm2 gene amplification caused by the chromosome mutation. Usually amplification ranges between 2- and 10-fold (Momand et al., 1998) (up to 25 in SJSA-1 cell line, Tovar et al., 2006). To imitate cancer cells with Mdm2 gene amplification we set $n_{MDM} = 4$.

The second type considered was breast (MCF-7 cell line) or prostate (PNT1a cell line) cancer with characteristic PTEN gene blockade. To imitate this we set $n_{PTEN} = 0$
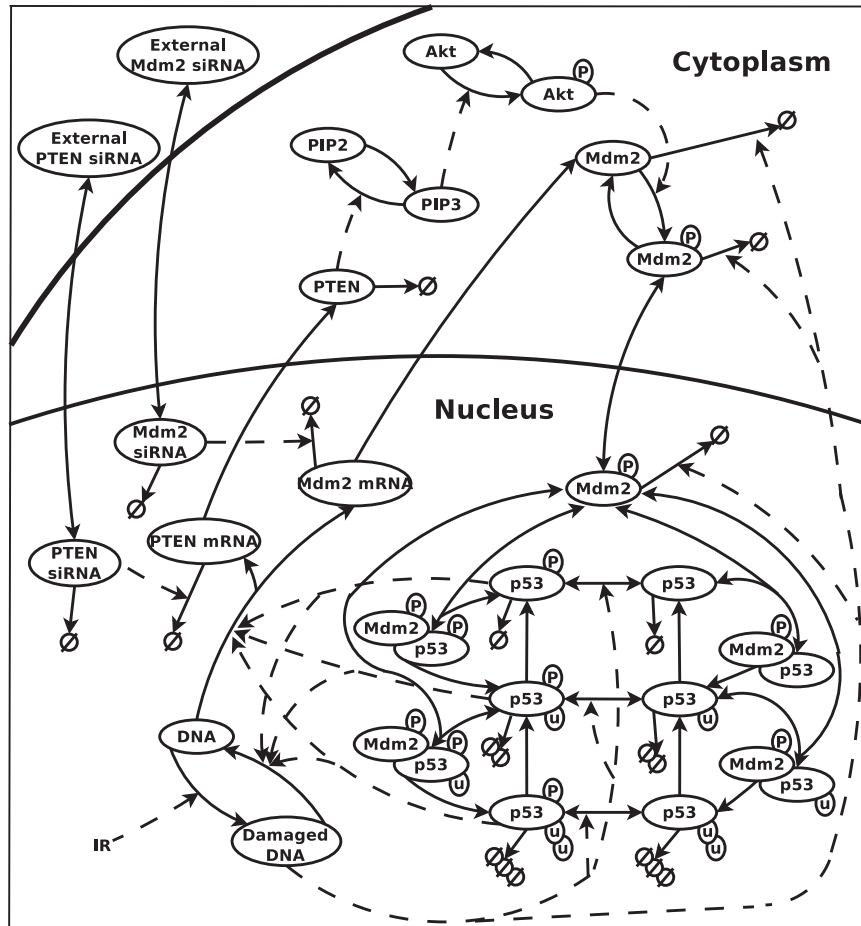
Fig. 1. Model scheme. Ellipses stands for model variables, solid lines for transitions while dashed for influences. Crossed circle symbolize degradation, circle with "P" means phosphorylation while circle with "u" means ubiquitination. Please notice the model inputs: IR and external siRNA (Mdm2 and PTEN); negative feedback loop p53-Mdm2 and positive feedback loop p53-PTEN-PIP3-Akt-PTEN-Mdm2.

$$\frac{d}{dt}GMdm2(t) = \left(q^a_{MDM_0} + q^a_{MDM_1}P53^2_{pn}(t)\right)\left(n_{MDM} - GMdm2(t)\right)$$
$$- q^d_{MDM}GMdm2(t). \tag{1}$$

$$\frac{d}{dt}GPTEN(t) = \left(q^a_{PTEN_0} + q^a_{PTEN_1}P53^2_{pn}(t)\right)\left(n_{PTEN} - GPTEN(t)\right)$$
$$- q^d_{PTEN}GPTEN(t). \tag{2}$$

Eqs. (3) and (4) describe MDM2 and PTEN mRNAs amount changes in time. The first term stands for mRNA production which depends on the number of active genes, the second term stands for spontaneous and the third for siRNA dependent mRNA degradation. Saturation term in these equations reflects the known biological fact that mRNA silencing caused by siRNA cannot reach 100%. In fact we were able to reach 90% maximum in our wet-lab (data not shown).

$$\frac{d}{dt}MDM_{RNA}(t) = s_0G_{MDM}(t) - d_6MDM_{RNA}(t) - sd_1\frac{SIRMDM}{SIRMDM + sh_1}$$
$$MDM_{RNA}(t). \tag{3}$$

$$\frac{d}{dt}PTEN_{RNA}(t) = s_1G_{PTEN}(t) - d_7PTEN_{RNA}(t) - sd_2\frac{SIRPTEN}{SIRPTEN + sh_2}$$
$$PTEN_{RNA}(t). \tag{4}$$

Model simulations and analysis were performed in Matlab environment.

3. Results

We performed the analysis of our model for the "normal" cells, which means the cells with properly functioning p53 module such as RKO cells, then cells with amplification in the negative feedback loop such as SJSA-1 cells and in the last case the cells with malfunction of the positive feedback loop such as MCF-7 or PNT1a cells. The difference between these cell types is the number of Mdm2 or PTEN alleles. Normal cells have two copies of both Mdm2 and PTEN alleles. Cells with amplification in the negative feedback loop have four copies of Mdm2 and two copies of PTEN allele while cells with not-functional positive feedback loop have two copies of Mdm2 and 0 copies of PTEN alleles (see Table 1).

The bifurcation diagram of the cells with fully functioning p53 pathway reveals three regions of the system response to the various dose of IR (see Fig. 2). In the first region, low dose of IR (<1.9 Gy) causes small number of DSBs which are rapidly repaired. As a result, all cells in population repair their DNA so they go to "proliferation" equilibrium point in which we have low level of p53. In the second region (1.9 < IR < 3.2 Gy), we have two equilibria: "proliferation" and "apoptosis". Because of the heterogeneity and stochasticity of the intracellular processes such as gene activation/deactivation the cells population will split on two subpopulations. Some cells will be able to repair their DNA and go to

Table 1
Parameter values for three different model types: "normal", with amplification in negative feedback loop and damaged positive feedback loop. Rest of the parameters as well as model equations are presented in supporting materials.

| Cell type	No. MDM2 alleles	No. PTEN alleles
"Normal" cells	2	2
Cells with amplified negative feedback loop	4	2
Cells with damaged positive feedback loop	2	0
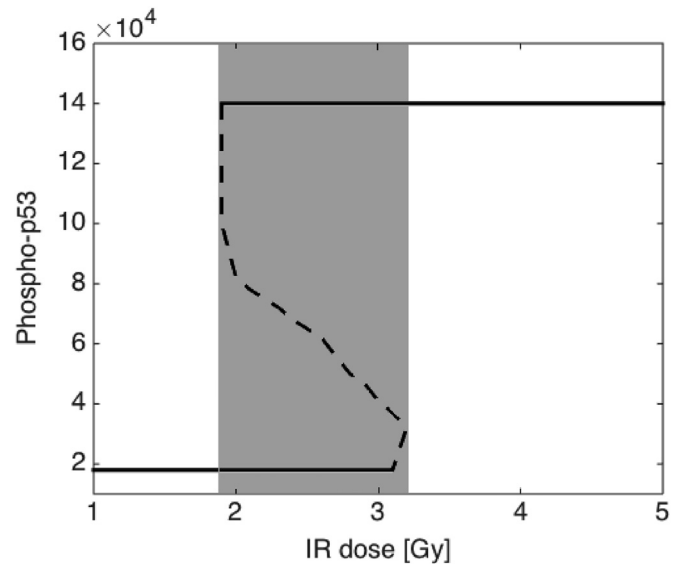
Fig. 2. Bifurcation diagram of the presented model. For the IR dose <1.9 Gy we have only one equilibrium point with low dose of p53 which we call "proliferation" equilibrium. For the IR dose >3.2 Gy we also have one equilibrium point but with high p53 level which is called "apoptotic". Between these two regions we have region of bistability where both solution exist and thus depending on the initial conditions and/or stochastic processes realization (in stochastic case) cells could choose proliferation or apoptosis.

the "proliferation" equilibrium while others will not and chose "apoptotic" equilibrium. For large enough IR (> 3.2 Gy) the DNA damage caused is so extensive that all cells in population die.

This is also visible on the p53-Mdm2$_{nuc}$ phase plane (Fig. 3 upper row). As expected the abnormalities of the feedback loops typical for cancers have impact on the cells behavior and thus their bifurcation and phase-plane diagrams. For all cells type for the IR=1 Gy, as expected, we have only one stable equilibrium point (Fig. 3 column A) with low amount of p53 so it is "proliferation" equilibrium. For the IR=2 Gy the "normal" cells exhibit two stable equilibria one "proliferation" with low level of p53 and second "apoptotic" with high level of p53, while for IR=5 Gy we can observe only one "apoptotic" equilibrium point with high level of p53 (Fig. 3 first row, columns B and C). Amplification in the negative feedback loop causes bifurcation point to shift from 1.9 Gy to 21 Gy, so the doses of 2 and 5 Gy are not enough to cause apoptosis and only one "proliferation" equilibrium exists for these cells (Fig. 3 second row, columns B and C). Similar effect can be observed in cells with inactive positive feedback loop although the reason behind is different. In these cells, because of the positive feedback loop malfunction, the negative feedback cannot be switched off and thus the "apoptotic" solution does not exist even for extensive DNA damage (Fig. 3 last row, columns B and C).

In both considered cancer types the p53 signaling pathway malfunction results in the elevated nuclear Mdm2 level and thus low p53 level in time when the apoptotic decision has to be made. To overcome this malfunction we propose to use Mdm2 targeted siRNA. Our goal was to restore the proper cell response to the DNA damage, which may be considered as the restoration of the "apoptotic" equilibrium point type and location. We used bifurcation analysis in the iterative way to determine the optimal siRNA dosage for both malfunction type cells (Fig. 4). We found that "apoptotic" equilibrium point location and type will be the same as in "normal" cells when we tread cells with amplification in negative feedback with dose of 5.2 nM of Mdm2 targeted siRNA while cells with malfunction in positive feedback with dose 4.2 nM (Fig. 4 left and right panels respectively).

As expected, introduction of the optimal siRNA doses to the
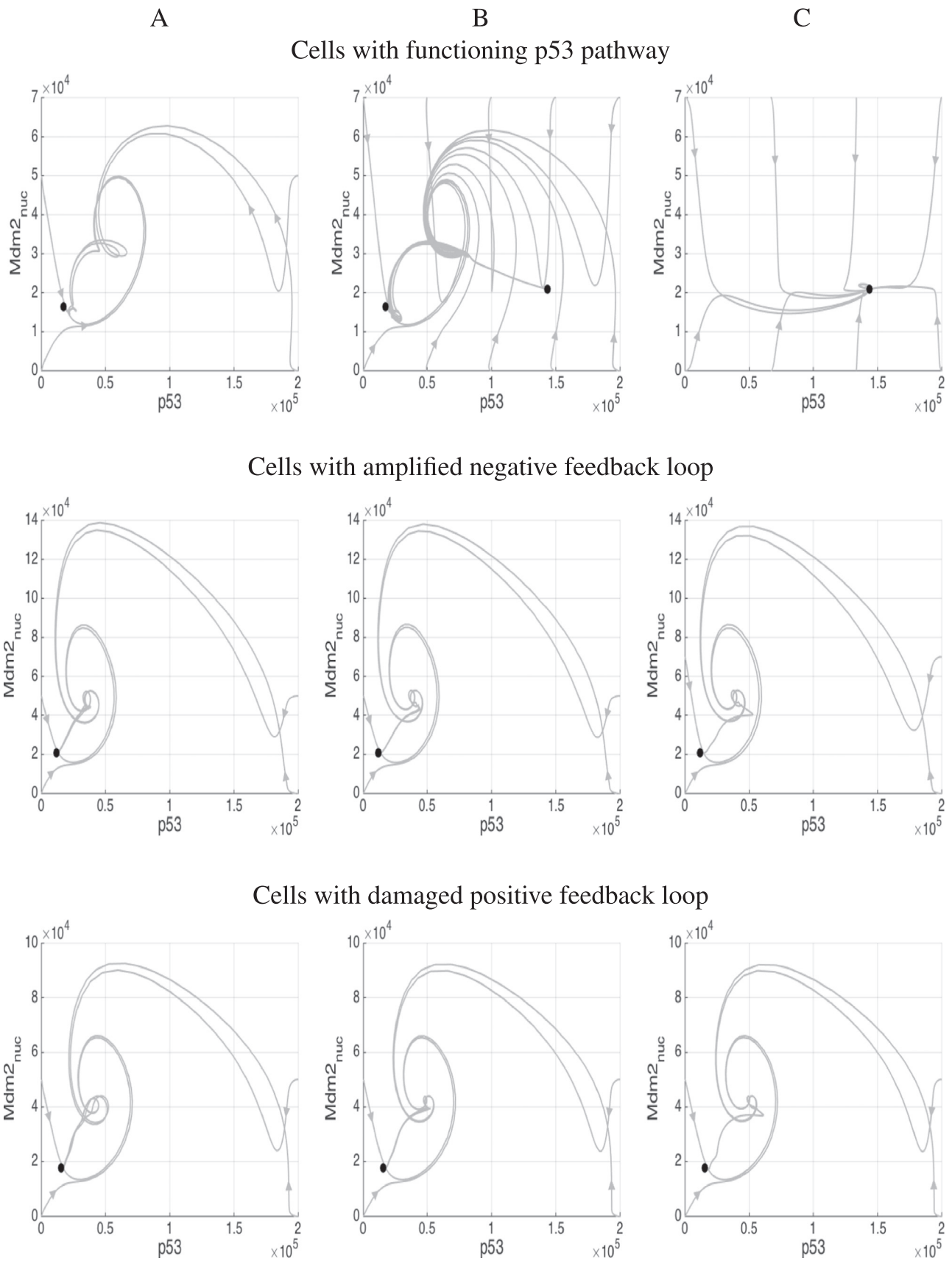
Fig. 3. p53-Mdm2 phase plane for different doses of IR and cell types in case without siRNA treatment. Black dots present equilibrium points location while grey lines sample trajectories. Column A: IR dose 1 Gy, column B: IR dose 2 Gy, column C: IR dose 5 Gy. Normal cells (first row) have one "proliferation" equilibrium point for IR dose 1 Gy, one "apoptotic" for IR dose 5 Gy and both of them for dose 2 Gy. Cancer cells with malfunction in negative (second row) or positive (third row) feedback loop have only "proliferation" equilibrium and thus are unable to die due apoptosis.
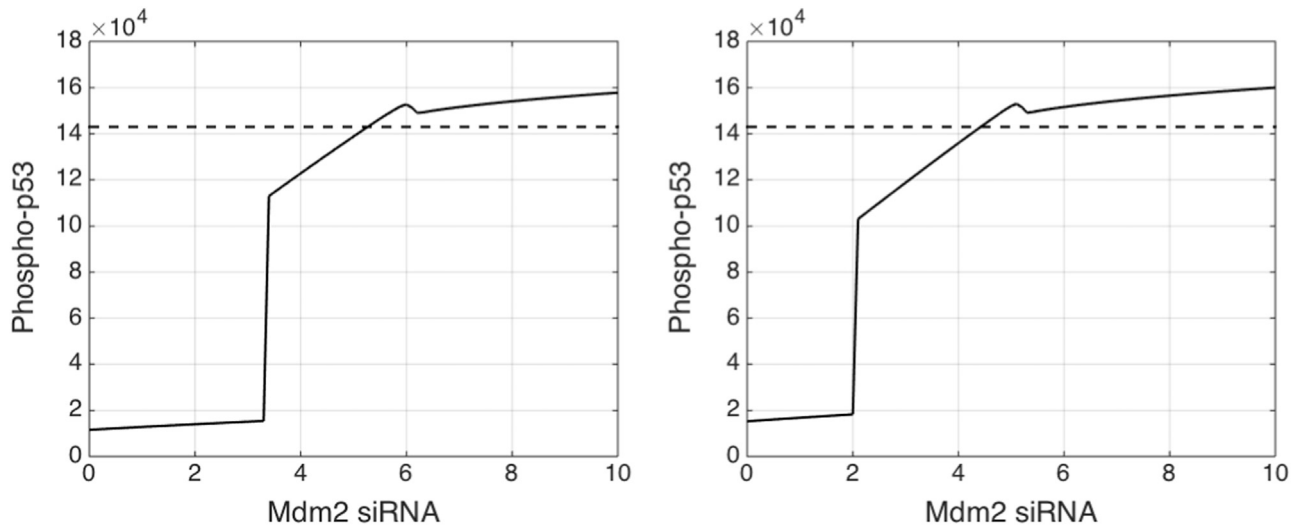
Fig. 4. Determination of the optimal siRNA dose to restore proper response of the cells with malfunction in feedback loop. Dashed lines represent the location of the "apoptotic" equilibrium point in normal cells irradiated with dose 5 Gy. Solid lines represent the location of the equilibrium in cells with amplification in the negative feedback loop (left panel) and cells with damaged positive feedback loop (right panel).

malfunctioned systems restored their proper behavior in response to DNA damage (Fig. 5). All cell types respond in the same, correct way to the various doses of IR. 1 Gy of IR results in the existence of one "proliferation point" (Fig. 5 column A). With 2 Gy we have "proliferation" and "apoptotic" equilibria (Fig. 5 column B) while for high dose of 5 Gy only "apoptotic" equilibrium point exist. (Fig. 5 column A).

One of the major disadvantages of the IR based therapy are the side effects caused by the radiation taken by the healthy cells. The technical limitations do not allow to deliver the radiation only to the cancer cells, usually it has to travel through the healthy cells before it reaches tumor. Hence, it could be useful to immunize the healthy cells to radiation and thus limit the side effects. As we showed before the cells can be immunized to IR e.g. by amplification in the negative feedback loop or by switch off of the positive feedback of the p53 signaling pathway. Therapeutic extortion of the negative feedback amplification can be challenging or even impossible while silencing of the positive feedback is possible through proper siRNA. Complete silence of the selected gene by siRNA is usually impossible but with proper dose we can silence it to around 10% (Puszynski et al., 2012). To explore the possibility of "normal" cell immunization to IR we checked how the bifurcation points location depends on the siRNA dose (Fig. 6). One can notice strong dependence between them. The higher siRNA dose the higher IR and thus more extensive DNA damage is required to push the cells to apoptosis.

4. Discussion

In our work we present that "normal" cells can choose between two possible stable equilibrium points: proliferation or apoptosis. In case of low or medium DNA damage level, these equilibria are reached by the damped oscillations manner. When DNA damage is too high cells quickly die and no oscillations are observed. This damped oscillations behavior reproduced in our model may be due the dominant role of PTEN feedback which, with sufficient time, push cells to the apoptotic solution, or more likely due to deterministic approach (required by bifurcation analysis), while stochastic model reproduce sustained oscillations (see Liu et al., for more details). In some papers, authors reported sustained oscillations of p53 and Mdm2 levels after DNA damage. Such behavior was observed for in vitro cells by Uri Alon group

(Geva-Zatorsky et al., 2006) and Lahav group (Batchelor et al., Chen et al., 2016) and for in vivo cells by Hamstra et al. (2006). Presence of the sustained or damped oscillations may depend on the studied cell type, so the model adjustments (e.g. lowering the strength of PTEN influence) may be required each time particular cells type is considered. In the case of sustained oscillations, instead of trajectories aiming at the equilibrium points, we could observe trajectories orbiting equilibrium points. However, existence and location of these equilibrium points still could be determined and changed by the siRNA administration.

Please notice that our siRNA dosage was based on the information provided by commercial siRNA manufacturers that the siRNA concentration of 30 nM outside the cell results in 75% silencing efficiency. When the siRNA sets of different efficiency are used, the parameters responsible for mRNA degradation by siRNA in the model need to be adjusted.
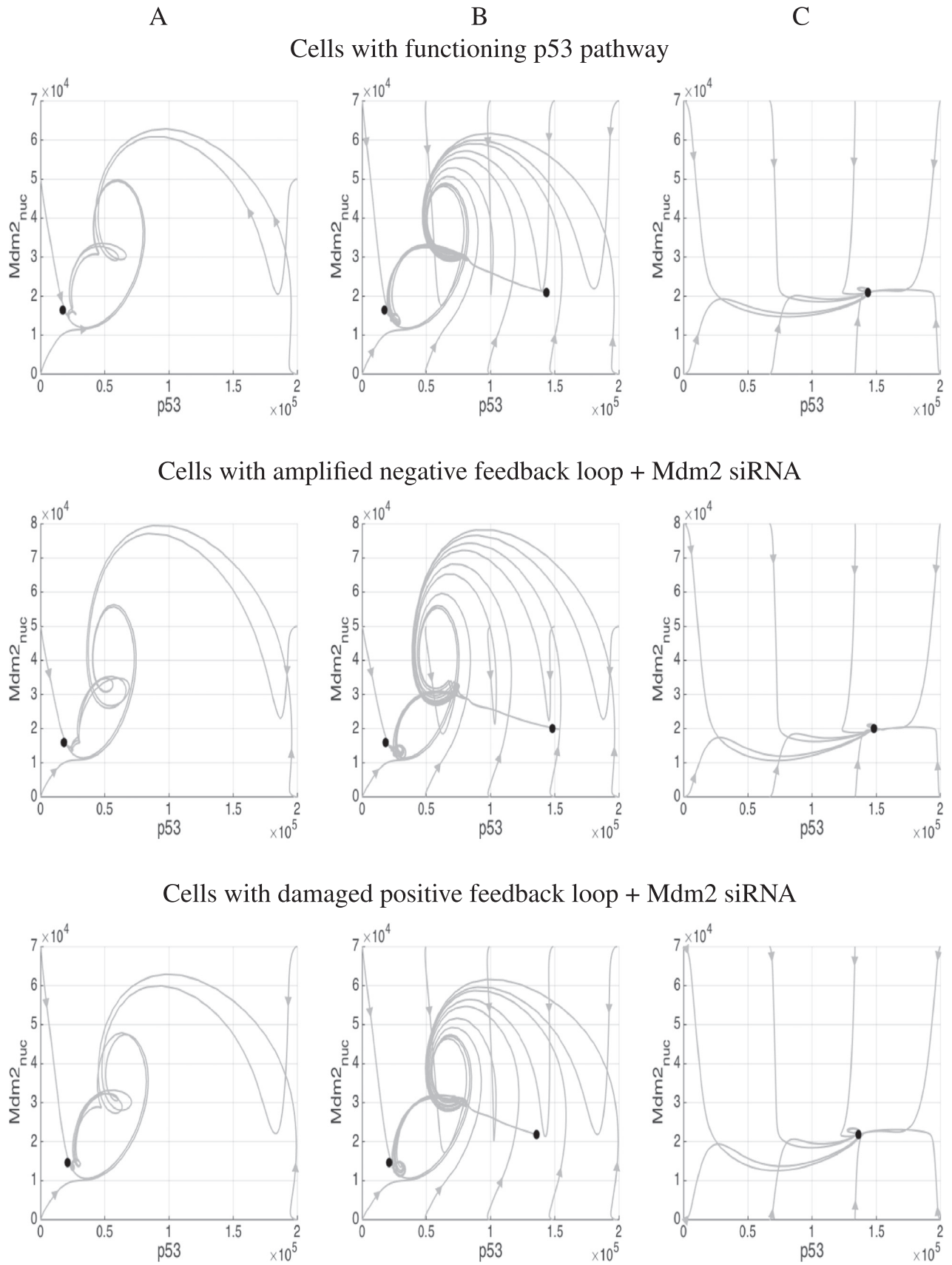
5. Summary and conclusions

In this work, we presented a general mathematical framework based on the bifurcation theory that allows to:

- visualize the differences in the dynamics of various types of cells (for example cells with normal and damaged p53 signaling pathway),
- determine the difference in equilibrium points and their location between various cells,
- determine the parameters which have to be changed to restore proper bifurcation diagrams and equilibria type and location,
- determine of the dose of additional control signals, such as siRNA, whose role is to provide the necessary changes in the model parameters e.g. change of the mRNA degradation rate.

Although in the paper we often refer to IR as input signal in fact the only impact of IR we consider is to cause DNA damage which activates the p53 pathway. Therefore our results should be correct also for other agents as long as only the DNA damage is considered.

In some cells, such as MCF-7, because of other than the p53-core damage, even the restoration of the proper p53-core functioning may not be enough to cause p53-dependent apoptosis. However, in these cells elevated p53 level can cause their

A   B   C

Cells with functioning p53 pathway

Cells with amplified negative feedback loop + Mdm2 siRNA

Cells with damaged positive feedback loop + Mdm2 siRNA

Fig. 5. p53-Mdm2 phase plane for different doses of IR and cell types in case with siRNA treatment. Black dots present equilibrium points location while grey lines sample trajectories. Column A: IR dose 1 Gy, column B: IR dose 2 Gy, column C: IR dose 5 Gy. As one can notice proper dosage of siRNA restored the type and location of the "apoptotic" equilibrium point in the cells with amplification in negative feedback (second row) or damaged positive feedback (third row) to the type and location of the equilibrium in normal cells (first row).
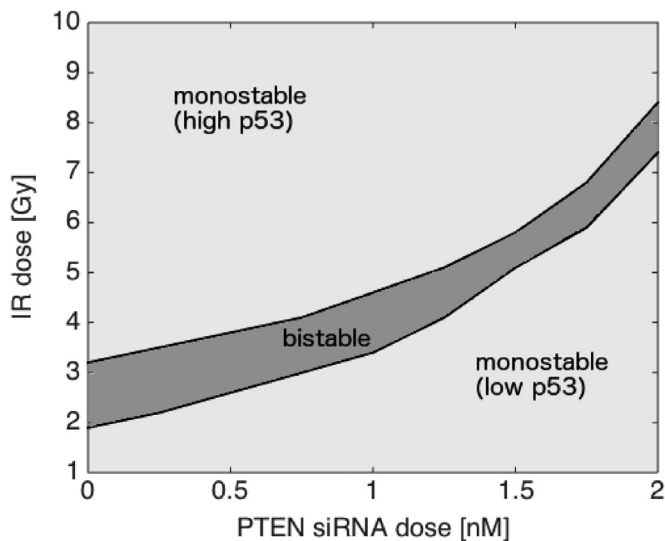
Fig. 6. Bifurcation plot for cells with normally functioning p53 pathway as a function of irradiation and PTEN targeted siRNA. One can notice that the higher dose of siRNA targeted to PTEN the higher IR dose is required to switch the cells from low p53 region (proliferation) to high p53 region (apoptosis). This proves that PTEN targeted siRNA can immunize cells to IR.

senescence, i.e. permanent cell cycle arrest, which also blocks the cancer development and may be considered as a therapy goal.

One has to remember that the purpose of this article was not the comprehensive modeling of the p53 signaling pathway but presentation of the methodology based on bifurcation analysis and siRNA application.

We used p53 the signaling pathway as an example because its structure is well known and the parameters are determined for many cell lines. The model parameters are gained from the in vitro experiments and the siRNA influence on the cells is also considered as in the in vitro experiments, e.g., we assumed a constant siRNA concentration outside the cells.

The real application of our approach in patient treatment requires a few additional modifications. First of all, the model parameters should be taken from the patient's cells. It can be done through the biopsy of the cancer and healthy surrounding tissue. It will allow to determine the difference between the healthy and cancer cells from the system dynamics point of view. Then, the best target for siRNA based therapy should be determined. If there is more than one possible target, sensitivity analysis can be applied to provide parameter ranking where the higher place means better therapy target (Puszynski et al., 2012).

Another major problem with the in vivo application of our method is the delivery system. Oral and intravenous administration of the drug usually leads to its presence in all body tissues. This can lead to serious side effects, which can lead to more harm from the therapy than benefits. Therefore, it could be useful to provide a targeted delivery system, such as proposed by Davis (2009) or by Shim and Kwon (2010). Considering in vivo application of our approach, it is also necessary to model the delivery system and the pharmacokinetics of the siRNA. It can be done by using the time plots of the siRNA concentration in the tissues as we did in Puszynski et al. (2014). As shown in Puszynski et al. (2016), also the stochasticity of gene switching cannot be neglected when pharmacodynamics of the drug such as siRNA is considered.

Our approach can be used also for the sensitization to IR of the cells with properly functioning p53 pathway. It will allow to lower the IR dose and side effect or receive the higher apoptotic fraction of the cancer cells while keeping the IR dose. On that way the

problem of radiotherapy dosage limitations can be avoided.

With the growing number of the publications discovering various signaling pathways structure and parameters, and by growing power of the computational systems and improvements in the targeted delivery systems, it becomes possible to provide truly personalized therapy. In this system, bifurcation theory can provide the information on the target and dose, while siRNA can be used as one of the controlling signal.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jtbi.2016.08.017.

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