Chapter 1

Dynamics of p53 and Cancer

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Additional information is available at the end of the chapter

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Abstract

Cancer is a multifactorial disease in which cell types lost their capability to regulate growth, proliferation, and cell death pathways, causing the uncontrolled proliferation of tumor cells. Cell death pathway is supported by the operation of the p53–Mdm2-negative feedback loop that has a central role to prevent the development of tumor cells. Under severe DNA damage, this loop takes the control of the apoptotic pathway and activates Bax, which, in turn, activates the caspase cascade to produce the death of the injured cell. However, events like Mdm2 overexpression or the suppression of caspase-9 gene can block the transmission of the death signal to the caspase cascade allowing the survival of the mutated cell. In this chapter, a mathematical model that explores the effect of Mdm2 overexpression and the suppression of caspase-9 on the control of death by the p53–Mdm2 loop is presented. From the model, two strategies for tumor cell survival are indentified, showing how mutations that affect the death pathway allow the survival of transformed cells. The model suggests that the combination of different simultaneous treatments against these mutations can be a suitable strategy against cancer.

Keywords: Cancer, p53–Mdm2 feedback loop, Mdm2 overexpression, suppression of caspase-9 gene

1. Introduction

Cancer has become one of the most important chronic diseases around the world [1]. A great number of studies on cancer have been focused on the identification for the genetic factors responsible of the predisposition for it. All these works have reported that a wide range of

genes is implicated, as well as a great number of environmental factors that increase cancer risk [1]. From a dynamical point of view, cancer is a complex robust system, in which many of the feedback motives that assure the homeostasis of cells have been altered, allowing a perturbed state of the cell in which it growths and proliferates at an increased rate.

The complexity of cancer lies on the high number of punctual DNA mutations (~105 per cell), which are grouped in a small number of signaling circuits, which seems to be the same recurring pathways [2]. In malignant tumors, mutations comprise oncogenes and tumor suppression genes like Mdm2, which takes part in the negative p53–Mdm2 feedback loop that controls the entrance of the cell to apoptosis [3]. Mdm2 is an E3 ubiquitin ligase that maintains low p53 levels in normal cells. However, under stress of genotoxic signals, Mdm2 inhibition on p53 is released, and several different responses are activated, including cell arrest and apoptosis.

The importance of the p53–Mdm2 loop in the tumor suppression is reflected in the fact that almost 50% of malignant tumors present a mutation in the p53 gene [4]. In cells without p53 mutation, the tumor suppression function of the p53 transcription factor can be stopped by overexpression of Mdm2, blocking the entrance of the cell to apoptosis. Another mechanism to override p53 tumor suppression function is the uncoupling of the caspase cascade from the intrinsic apoptosis pathway; mutations in caspase-3 and caspase-9 can be responsible for this uncoupling.

This chapter explores the dynamics of the p53 tumor suppression system under normal and stress conditions in order to understand how overexpression of Mdm2, and mutations in the components of the caspases cascade, alters the form in which p53 controls the entrance of the cell to apoptosis. The dynamic features of the ordinary and altered operation conditions of p53–Mdm2 feedback loop are explored with a mathematical model that links it with the caspase cascade. The biological implications of the results obtained from the model are discussed in deep to understand how the deregulation of the intrinsic apoptotic pathway leads the modified cells to escape from death.

2. Molecular biology of the p53 pathway

2.1. The p53 pathway

Activation of the p53 pathway starts under the command of a complex feed forward structure implied in DNA damage detection, which is formed by proteins like the histone acetyl transferase Tip60 and the MRN complex. These proteins induce the activation of the ATM kinase [5–7]. In the next step, ATM phosphorylates and activates histone H2AX and other proteins with a BRCT domain (such as Nbs1, 53BP1, and MDC1) [7, 8]. These proteins will bind to the broken segment of DNA in order to stop the spread of damage [8]. Similarly, ATM activates Chk2 kinase, partially responsible for promoting cell cycle arrest [8, 9]. Chk2 also promotes the activity of the transcription factor E2F-1 [9], which regulates the expression of Chk2 and proapoptotic proteins ASPP [10] (Figure 1).

The active nuclear form of ATM assembles a complex with NEMO, which is ubiquitinated and transported to the cytoplasm where induces the release of the transcription factor NF-κB [11], a factor that enters the nucleus and promotes the expression of various genes [12, 13], including BCL-XL [14, 15]. Simultaneously, in the nucleus, active ATM phosphorylates the p53 store, and Mdm2 becomes less effective to recognize p53 delaying its degradation. In addition, ATM phosphorylates Mdm2 and induces its self-ubiquitination and degradation in the proteosome, allowing the increase of p53 nuclear concentration over time [16].

On the other hand, active Chk2 phosphorylates p53 to stabilize its structure [8]. At the end of this initial cascade of phosphorylation, p53 can interact either with accessory proteins ASPP1, ASPP2, or with MUC1 [17, 18] (Figure 1). In response to genotoxic stimuli, the transmembrane glycoprotein Musin-1 (MUC1) is cleaved, and the cytoplasmic segment is targeted to the nucleus, allowing it to bind to phosphorylated p53, inducing cell cycle arrest [19–24]. However, if there is severe DNA damage, p53 binds mainly to ASPP proteins, enhancing the proapoptotic function of p53 [25]. Thus, according to the intensity of the stimulus, p53 can be oriented either to transcribe proapoptotic genes or antiapoptotic genes, depending on the accessory proteins to which it is attached.

When the DNA damage signal is weak, nuclear concentration of MUC1 increases and enhances its binding to p53. After that, p53/MUC1 interacts with p300/CBP-associated factor (PCAF) to form a new complex [18, 26]. PCAF acetylates p53 on lysine 320, allowing it to recognize specifically the promoters of genes related with DNA damage repair and cell cycle arrest, as p21WAF1/cip1 [18]. MUC1 blocks Bax dimerization and the Bax-mediated release of cytochrome *c* [27]. MUC1 is capable of enhancing the expression of ARF, resulting in Mdm2 repression [28]. When p53/MUC1 complex starts its nuclear functions, the deacetylase SIRT1 opposes the PCAF-dependent acetylation of p53 by removing the acetyl group, blocking its transcriptional activity when the cell does not have enough energy to express proteins [29–31]. However, SIRT1 is not the only control point of p53. As it was discussed above, active ATM, along with NEMO, turns on NF-κB, which promotes the expression of antiapoptotic genes like BCL-XL [15].

NF-κB competes with p53 for the cofactors acetyltransferases p300, CBP, and PCAF, which are required for the binding of p53 to DNA [32, 33]. The effect of such interaction leads to the indirect repression of the transcriptional activity of p53 because the concentration of the cofactors remains relatively constant [11, 32, 34] (Figure 1).

Once nuclear p53 is activated, it starts the transcription of sensor genes like the phosphatase Wip1 and Mdm2 (in its p90 isoform) in order to activate the negative feedback control of the pathway. Wip1 function is to dephosphorylate Chk2, ATM, activated p53, and phosphorylated Mdm2. This action leads to inactivation of all of these proteins, except Mdm2 that is activated [25, 33, 35–38]. The dephosphorylated form of Mdm2, together with activated Wip1 and new synthesized Mdm2, inhibits p53 and reduces its nuclear concentration, avoiding its transcriptional activity [39]. Because of Wip1 function, the signal generated by ATM and Chk2 blinks while DNA damage is not repaired since ATM activator proteins remain linked to broken DNA [33, 40, 41]. When DNA damage is repaired, the nuclear topology is restored and ATM activating proteins stops the signal. In this case, Wip1 definitely inactivates ATM and its

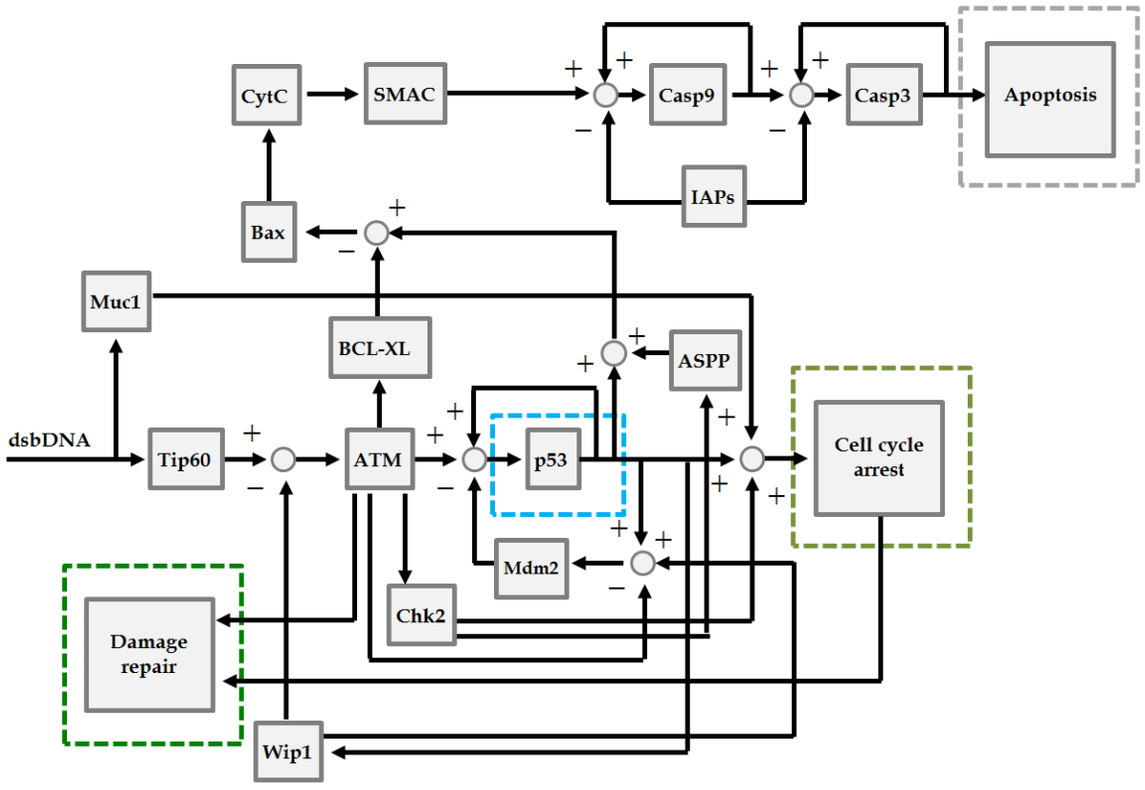

Figure 1. The p53 pathway.

effector molecules like Chk2. This encourages Mdm2 (p90 isoform) to suppress efficiently p53 activity, allowing the cell to return to its normal state. The levels of p90Mdm2 and Wip1 also return to their basal value [38] (Figure 1). If the signal of DNA damage is either too strong or persistent, and the cell has not enough energy to repair the damage, p53 binds to ASPP proteins and forms a complex with the histone acetyltransferase p300/CBP to acetylate p53 on lysine 373 [26, 42].

Another regulatory interaction is held by proteins p90 and Tip60. The interaction of p90 with p53 allows Tip60 to transfer an acetyl group to Lysine 120 of p53 [43]. Together, these chemical modifications permit the selective recognition of the promoter sites of proapoptotic genes like PUMA, Bak, and Bax by p53 [43, 44]. It is noteworthy that the formation of the p53/p300 complex is reversible, and p300 is separated from the complex by the deacetylation of p53 [30]. The enzyme responsible for this step is the deacetylase SIRT1 [30, 45]. This enzyme uses NAD +, from the cellular metabolism, as a cofactor; typically, SIRT1 activity can delay the apoptotic intrinsic pathway when there is no sufficient metabolic energy and whether DNA damage is not enough to activate apoptosis directly; nonetheless, this enzymatic regulation has no importance when DNA damage is severe [30].

In the cytoplasm, p53 forms a complex with the antiapoptotic protein BCL-XL in order to create a cytoplasmic reserve of p53 [16, 46]; in parallel to this, the excess of free BAX is neutralized in the cytoplasm by forming a complex with the antiapoptotic protein BCL2 [3]. Once the cell has chosen to die, p53 enhances the expression of proapoptotic genes like Bax, PUMA, NOXA,

BID, p53AIP, DR5, caspase-6, PERP, and FAS [47]. As a result, PUMA cytosolic concentration increases and enables its interaction with the p53/BCL-XL complex, replacing p53; in turn, free cytoplasmic p53 interacts with complex BCL2/BAX in order to form a new complex BCL2/p53, releasing BAX into the cytoplasm [46–48]. Free BAX monomers will interact with other Bak and Bax monomers to form the mitochondrial apoptosis-induced channel (MAC), and those complexes will perforate mitochondrial outer membrane, allowing the release of cytochrome *c*, SMAC/DIABLO, and Omi/HTRA [47, 49], triggering the commitment step in the apoptotic intrinsic pathway. Free cytochrome *c* will interact with apoptotic protease activating factor 1 (Apaf-1) and procaspase-9 to form the apoptosome. At the same time, cytosolic SMACs block the repression of inhibitor of apoptosis proteins (IAPs) on the initiator and executer caspases, ensuring apoptosis effectiveness [50–57]. Then the apoptosome cleaves the inactive procaspase-9 to active initiator caspase-9, and then it will activate executer caspase-3 [58]. Once this protein is activated, it starts a positive feedback loop for its self-activation as well for the activation of initiator caspases [59]. Caspase-3 starts the degradation of the cytoskeleton and other important cellular components, triggering the exposure of phosphotidylserine on the outer leaflet of the apoptotic cells, allowing the noninflammatory phagocytic recognition of these cells [47, 60] (Figure 1).

3. Nonlinear dynamics

3.1. Cells are complex networks

Cells are formed by several types of molecules (nodes) that are linked by the interactions between them forming a complex and hierarchical network. This network consists of a series of subnetworks that controls cellular processes like signaling, metabolism, apoptosis, transcription, translation, and DNA repairing and modification. The links between nodes can be of several types, including protein–protein, protein–DNA (transcription factors), small molecule–DNA (acetylation, methylation, etc.), small molecule–protein (posttranslation modifications), and metabolic [12]. Furthermore, these interactions are organized in clusters, with specific motives such as feedforward structures, single-input modules, or feedback modules [12]. This modular architecture of the cell's network is regulated by global regulators or "hubs" such as p53, mTOR, or AMPK [61].

3.2. The control principles of biology

A remarkable property of the cellular network is that all of its connections respond to specific and well-defined stimuli; in other words, such interactions are controllable. In this form, the modular structure of cells allows them to control several processes simultaneously, depending of the received inputs. Control is exerted by specific regulatory structures or motives like the negative feedback loop.

In order to understand why the negative feedback loop is so important for the control of the cellular processes, the block diagram of Figure 2A shows the functioning of this motive when a reference signal (or input) r is transformed into an output signal y. From this diagram,

$$y = rG + rG\Delta + \delta - yG - yG\Delta \tag{1}$$

where G corresponds to a system, Δ is the intrinsic noise proper of the system, and G and δ is the extrinsic noise due to external factors.

Working Equation (1) algebraically, we obtain

$$y = \frac{rG(1+\Delta)}{1+G(1+\Delta)} + \frac{\delta}{1+G(1+\Delta)} \tag{2}$$

That is equivalent to

$$y = \frac{r}{1/G(1+\Delta)+1} + \frac{\delta}{1+G(1+\Delta)} \tag{3}$$

According to Equation (3), if the regulation of the system G tends to be stronger (i.e., $G \to \infty$), then the input signal will be totally transformed into the output, regardless the presence of intrinsic and extrinsic noise. In other words,

$$y = r \tag{4}$$

In this form, negative feedback loops are able to neutralize interferences due to extrinsic and intrinsic noise. This characteristic is known as *robustness*, and it confers to the system the capability to function in a changing environment like the intracellular medium [62, 63], which explains why negative feedback motives are so common in cell biology. In regard to robustness, it was thought that *redundancy* was its obligated synonym because if the cell loses a determined connection, and there are redundant interactions, then the cell can compensate the absence of such a connection. However, one system can be redundant but not robust depending on the type of connections that are redundant. At the molecular level, redundant interactions contribute to enhance the regulation of a biological process, which is a sine qua non condition to reject all effects of intrinsic and extrinsic noises [62].

Besides redundant connections, the cell also has contradictory interactions in which a biological molecule simultaneously activates and represses one process. These motives are better known as feedforward loops, and sometimes they are called *incoherent* feedforward motives [64]. In order to understand why the cell needs to activate and to repress one process at the same time, from Figure 2B, the block diagram can be written as follows:

$$y = -rCAP - rC\Delta + rAP + r\Delta + yCAP + yC\Delta + \delta \tag{5}$$

where P is the process to be controlled (e.g., transcription or translation), A is the molecule that physically modifies and executes the process (e.g., RNA polymerase), and C is a biological controller unit (e.g., a signaling circuit).

Working this algebraic expression, we obtain

$$y = \frac{-rC\left(AP+\Delta\right)}{1-C\left(AP+\Delta\right)} + \frac{r\left(AP+\Delta\right)}{1-C\left(AP+\Delta\right)} + \frac{\delta}{1-C\left(AP+\Delta\right)} \tag{6}$$

That is equivalent to

$$y = \frac{-r}{\left(C\left(AP+\Delta\right)\right)^{-1}-1} + \frac{r\left(AP+\Delta\right)}{1-C\left(AP+\Delta\right)} + \frac{\delta}{1-C\left(AP+\Delta\right)} \tag{7}$$

Once again, if the control of the system (Equation 7) tends to be stronger (i.e., $C \to \infty$), then we obtain Equation (4), which means that this structure also neutralizes the effects of intrinsic and extrinsic noises. Thus, this apparently incoherent motive has robustness as well as negative feedback loops. Nevertheless, in order to exert their full robustness properties, these motives must be faster than the negative feedback ones during the regulation of a process, and their robustness is given by the fact that they can prevent a disturbance before it occurs [65]. For such reasons, feedforward motives are useful to control complex systems like positive feedback loops or nonlinear processes like splicing.

However, none of these cellular motives could be effective without biological controllers. In fact, such devices are specifically designed for operating a determined control motive such as negative feedback loops, feedforward loops, or open loops (i.e., without feedback, Figure 2C) [66].

However, there is a general structure for assembling controllers (Figure 3A), which consists in coupling a comparator and a control action module (the core of controller) with an amplifier [66]. The comparator is a device that collects and contrasts the reference signal (input) with the signals produced by either a sensor (device that measures the output of the system) or a timer (in open-loop systems). The difference between these signals is known as the error signal ($e(t)$), and it is used by the core of the controller to activate its inner mechanism in order to produce a control signal. Then the amplifier augments the potency of the control signal for regulating the entire system [66].

There are many types of controller cores, but the simplest are on–off cores (all or nothing control). Mathematically, this controller core is described by the following equation:

$$u\left(t\right) = \begin{cases} 1, & e\left(t\right) \geq \alpha \\ 0, & e\left(t\right) < \alpha \end{cases} \tag{8}$$

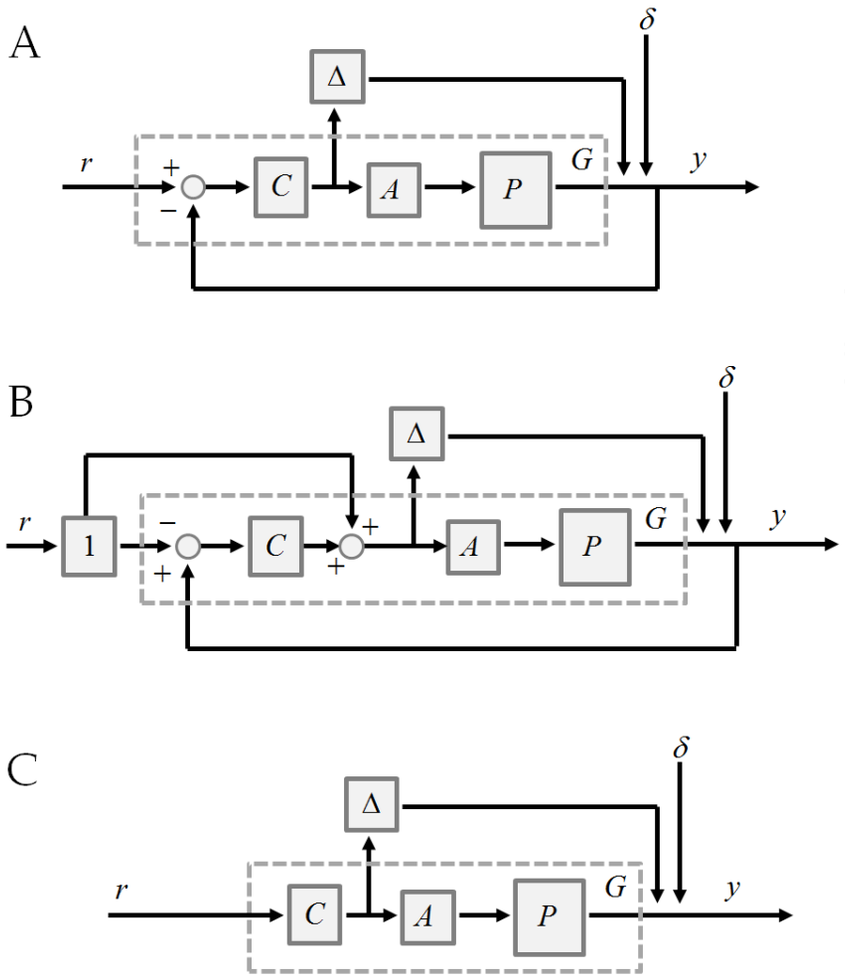

Figure 2. Control motives. (A) Negative feedback loops with the presence of intrinsic (Δ) and extrinsic noises (δ). (B) Feedforward motives with intrinsic and extrinsic noises. (C) Open-loop motives. In all cases, C is the controller, A is the effector, P is the plant or process to be controlled, and G represents the whole system.

It means that the controller core will produce a control signal only if the magnitude of error signal is higher than a threshold value (α) [66]. In turn, this signal will be interpreted by the system as an order for turning on its functioning.

However, if the error signal magnitude is below the threshold value, the controller core will turn off the system. It is important to remark that such controller cores could be problematic when the error signal has an irregular behavior as shown in Figure 3B. To deal with that error signals, there is a special variant of on–off cores that includes a memory range (Figure 3C). This type of core is known as on–off with hysteresis [66] and generically is described by the following equation:

$$u(t) = \begin{cases} 1, & e(t) \geq \alpha \\ 0, & e(t) < -\alpha \end{cases} \tag{9}$$

It means that the core will produce a control signal only when the magnitude of the error signal is higher than a threshold value (α), and it will stop when the error signal is below another threshold value (- α). With this modification, the controller core does not change unexpectedly because of fluctuations in the error signal [66]. In cell biology, we can find this controller core almost everywhere. For example, in molecular switches (Figure 3D) at transcriptional level, one gene cannot be fully transcribed unless there is enough concentration of its inducer [63, 67].

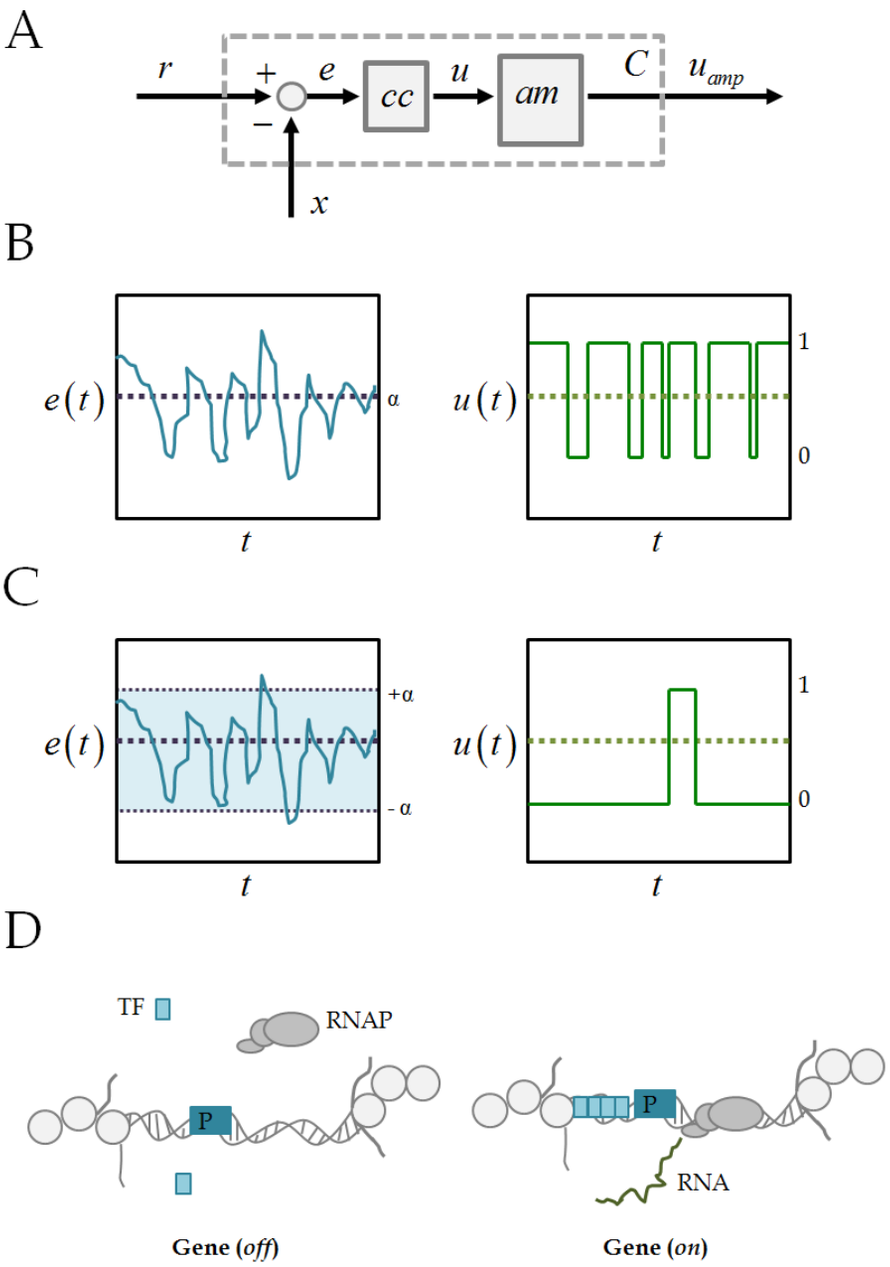

Figure 3. Biological controllers. (A) The canonical structure of a controller. (B) Temporal response of a core controller *all or nothing*. (C) Temporal response of a core controller *all or nothing with hysteresis*. (D) Biological equivalence to a core controller *all or nothing with hysteresis*.

3.3. Control principles on the p53 pathway

An example of control principles applied to biology can be observed during the evaluation of the DNA damage (Figure 1). This process is performed by some specialized kinases such as ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3 related (ATR), as well as some DNA binding proteins like the MRN complex (Mre11, Rad50, and Nbs1) [8]. If these proteins do not detect any DNA damage, the cell cycle will continue normally [68]. But if so, they will activate a feedforward signaling structure in order to induce the cell cycle arrest through effector molecules such as Chk2 and p21Waf1/cip1 [69]. Furthermore, when DNA damage is severe, the cell will turn on apoptosis through p53 transcriptional activity [68].

The p53 transcription factor also regulates the expression of genes responsible for the cell cycle arrest, repair of DNA damage, senescence, apoptosis, and other signaling pathways [70, 71]. The expression of p53 gene is activated by many inputs from transcriptional factors that include the p50 subunit of NF-κB, C/EPEβ-2, Ets-1, Pitx1, p73, and p53 itself [72–75]. Cytoplasmic p53 concentration is regulated in a negative feedback motive that starts when a fraction of p53 interacts with the anti apoptotic protein BCL-XL to generate a heterodimer [46, 76], and the remaining fraction of p53 is marked in its carboxyl-terminus with a nuclear import signal (NLS1) to be subsequently transported into the nucleus, where it interacts with its natural repressor: the ubiquitin ligase Mdm2 (Hdm2 in humans).

On the other hand, the negative regulator Mdm2 is an example of a biological sensor because its expression is a consequence of the p53 transcriptional activity, which allows the formation of a negative feedback loop. However, many studies suggest that p53–Mdm2 interactions are more complicated than it was thought; mainly because the Mdm2 gene has two promoters (P1 and P2) that can generate at least two isoforms of Mdm2 [35]. The first one isoform (p90Mdm2) is responsible of the p53 inhibition, but the second one (p76Mdm2) promotes the translation of p53 mRNA [35, 77]. Under ordinary conditions, p76Mdm2 expression is greater than p90Mdm2 [78], generating basal levels of p53 throughout the cell cycle. However, under cellular stress conditions, p53 induces the expression of p90Mdm2 in detriment of the p76Mdm2 isoform [77]. Remarkably, it was reported that other transcriptional factors can activate the Mdm2 gene in order to increase the regulation of p53, which is a clear example of the enhancement of biological robustness due to redundant interactions [78].

3.4. Nonlinear dynamical systems

All previous concepts are useful for understanding how the cell coordinates its molecular processes. However, it is important to introduce some concepts from dynamical systems prior to analyze how cells make operative decisions.

Most of the cellular processes can be represented in mathematical terms with nonlinear differential equations, which together with a set of initial conditions define a dynamical system. In this form, if the state of a system at time t is determined by the set of variables $\{x_1(t), x_2(t), ..., x_n(t)\}$, then the respective dynamical system is the set of nonlinear differential equations $x_j(t) = f_j(x_1(t), x_2(t), ..., x_j(t), ..., x_n(t))$, $j = 1, 2, ..., n$, subject to the initial conditions $\{x_{1o}(0), x_{2o}(0), ..., x_{no}(0)\}$. The set of variables $\{x_1(t), x_2(t), ..., x_n(t)\}$ defines the phase space of the

dynamical system in which the motion of the system occurs. As time goes on, the point $\mathbf{x}(t)=\langle x_1(t),\ x_2(t),\ ...,\ x_n(t)\rangle$ moves along the phase space and defines a curve o trajectory of the system for each initial condition. Thus, the objective is to analyze the dynamical system in order to know the complete set of trajectories of the system in the phase plane (the phase portrait), and to discern the behavior of the trajectories in the neighborhood of the equilibrium o fixed points of the dynamical system. Equilibrium or fixed points are the points of the phase plane in which all the derivates vanish. As an example, in Figure 4, the phase portrait of the two-dimensional dynamical system $\dot{x}=\sin(y),\ \dot{y}=x-x^3$ is shown:

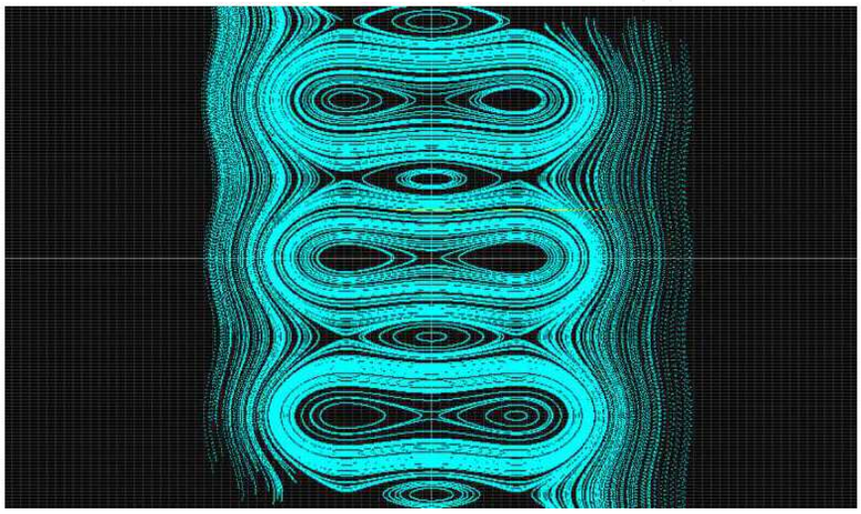

Figure 4. Portrait phase of the system $\dot{x}=\sin(y),\ \dot{y}=x-x^3$.

Nonlinear dynamical systems cannot be analytically solved; however, there are mathematical tools that can be used to know the qualitative behavior of the system. One of these tools is the linearization around a fixed point.

Let $\mathbf{x}^o=\left(x_1^o,\ x_2^o\right)$ be a fixed point of the two-dimensional dynamical system:

$$\begin{aligned}\dot{x}_1 &= f_1\left(x_1, x_2\right)\\ \dot{x}_2 &= f_2\left(x_1, x_2\right)\end{aligned} \tag{10}$$

If fluctuations δx_1 and δx_2 perturb the fixed point $\mathbf{x}^o$, the system is displaced to a new state $\delta\mathbf{x}^o=\left(x_1^o+\delta x_1,\ x_2^o+\delta x_2\right)$, and the trajectory that emerges from this point of the phase plane can either bring closer to the original equilibrium or go far away from it. If the trajectory tends to the fixed point in an asymptotic form, then $\mathbf{x}^o$ is stable, and it is an attractor. If the trajectory moves away from the fixed point, then $\mathbf{x}^o$ is unstable, and it is a repeller.

The dynamical behavior of the fluctuations determines the stability of the system, and it is essential to establish a form to analyze the evolution of the fluctuation along time. In order to

achieve this goal, it is necessary to assume that the behavior of the fluctuations in the neighborhood of the fixed point is linear, i.e., the nonlinear terms can be neglected.

From Equation (10),

$$\begin{aligned} f_1\left(x_1^o+\delta x_1, x_2^o+\delta x_2\right) &\approx f_1\left(x_1^o, x_2^o\right)+\left.\frac{\partial f_1}{\partial x_1}\right|_{\left(x_1^o, x_2^o\right)} \delta x_1+\left.\frac{\partial f_1}{\partial x_2}\right|_{\left(x_1^o, x_2^o\right)} \delta x_2+O\left(\delta x_1^2, \delta x_2^2, \delta x_1 \delta x_2\right) \\ f_2\left(x_1^o+\delta x_1, x_2^o+\delta x_2\right) &\approx f_2\left(x_1^o, x_2^o\right)+\left.\frac{\partial f_2}{\partial x_1}\right|_{\left(x_1^o, x_2^o\right)} \delta x_1+\left.\frac{\partial f_2}{\partial x_2}\right|_{\left(x_1^o, x_2^o\right)} \delta x_2+O\left(\delta x_1^2, \delta x_2^2, \delta x_1 \delta x_2\right) \end{aligned} \tag{11}$$

Neglecting the nonlinear terms and taking into a consideration that the derivates vanish in the fixed point, Equation (11) can be written as

$$\begin{aligned} \delta \dot{x}_1 &= \left.\frac{\partial f_1}{\partial x_1}\right|_{\left(x_1^o, x_2^o\right)} \delta x_1+\left.\frac{\partial f_1}{\partial x_2}\right|_{\left(x_1^o, x_2^o\right)} \delta x_2 \\ \delta \dot{x}_2 &= \left.\frac{\partial f_2}{\partial x_1}\right|_{\left(x_1^o, x_2^o\right)} \delta x_1+\left.\frac{\partial f_2}{\partial x_2}\right|_{\left(x_1^o, x_2^o\right)} \delta x_2 \end{aligned} \tag{12}$$

which can be written in a matrix form as

$$\begin{bmatrix} \delta \dot{x}_1 \\ \delta \dot{x}_2 \end{bmatrix}=\left[\begin{array}{cc} \dfrac{\partial f_1}{\partial x_1} & \dfrac{\partial f_1}{\partial x_2} \\ \dfrac{\partial f_2}{\partial x_1} & \dfrac{\partial f_2}{\partial x_2} \end{array}\right]_{\left(x_1^o, x_2^o\right)} \begin{bmatrix} \delta x_1 \\ \delta x_2 \end{bmatrix} \tag{13}$$

Equation (13) represents the dynamics of the fluctuations around the fixed point when the nonlinear terms are neglected. The stability of the fixed point is then determined by the matrix $\mathbf{J}\left(\mathbf{x}^o\right)=\left[\begin{array}{cc} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} \end{array}\right]_{\mathbf{x}^o}$, which is the Jacobian of the dynamical system. In two-dimensional dynamical systems, the evolution of the trajectories in the phase plane is settled on by the roots of the characteristic equation of the Jacobian: $\lambda^2+tr(\mathbf{J})\lambda+\det(\mathbf{J})=0$, where $tr(\mathbf{J})$ is the trace of the matrix and $det(\mathbf{J})$ its determinant. This characteristic equation has two roots, known as eigenvalues, that can be real, imaginary, or complex numbers. In all cases, if both λs are negative real numbers or complex numbers with a negative real part, the fixed point is stable, and it is an attractor. If at least one of the roots is a positive real number or has a positive real part, the system is unstable. Figure 5 summarizes the properties of eigenvalues for any system. In the general case of an n-dimensional dynamical system, there are n eigenvalues, and if all of them

are negative real numbers or are complex numbers with negative real part, the fixed point is an attractor. Otherwise, if a least one eigenvalue is a positive real number or a complex number with a positive real part, the fixed point is unstable.

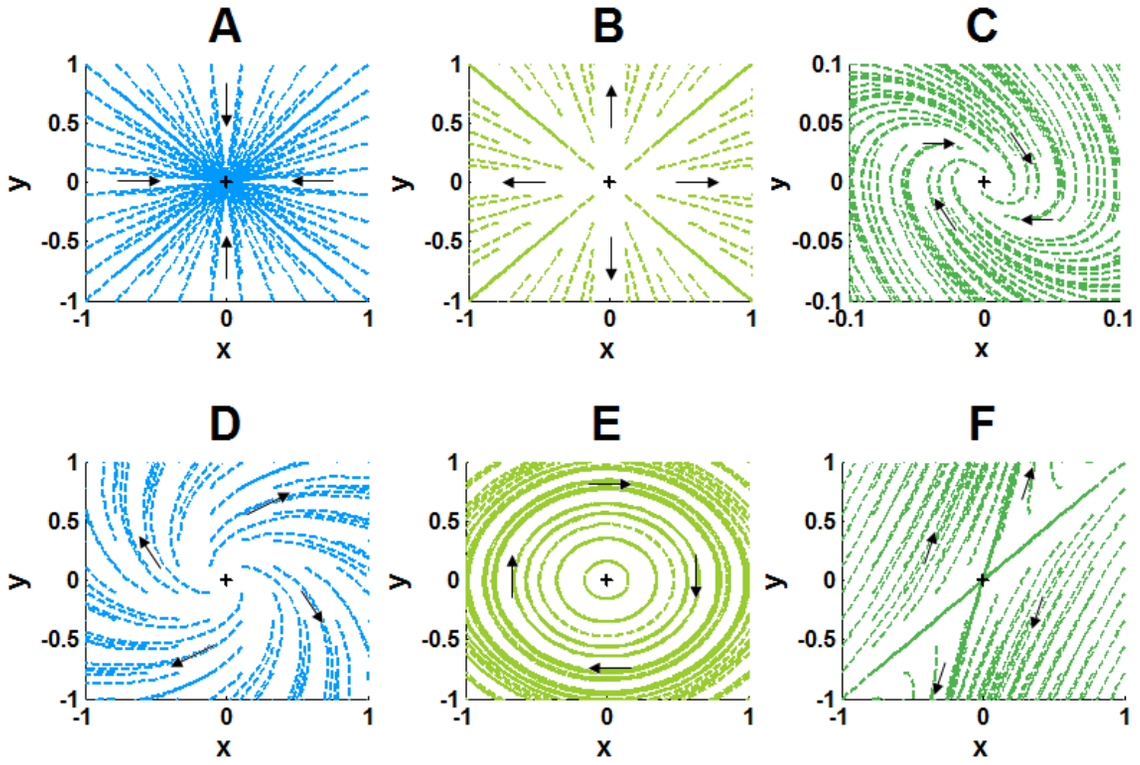

Figure 5. Portrait phases for different eigenvalues. (A) The portrait phase of one system is described by a stable node (sink) when $\mathrm{Re}(\lambda_i) < 0$, $i = 1,2$, and there is no imaginary part. (B) On the other hand, if $\mathrm{Re}(\lambda_i) > 0$, $i = 1, 2$, the portrait phase will show an unstable node (source). (C) Similarly, when $\mathrm{Re}(\lambda_i) < 0$, $i = 1, 2$, and there is an imaginary part, the system presents a stable spiral (sink). (D) In case of $\mathrm{Re}(\lambda_i) > 0$, $i = 1, 2$, and if there is an imaginary part, the system will present an unstable spiral (source). (E) When $\mathrm{Re}(\lambda_i) = 0$, $i = 1, 2$, and there is an imaginary part, the portrait phase of the system will show a center (it is stable, but not asymptotically stable like a sink). (F) If the system has real eigenvalues with different signs, for instance, $\lambda_1 > 0$ and $\lambda_2 < 0$, the system will present a saddle point. In all cases, the origin is the equilibrium and is marked with a black cross, and the arrows show the direction of trajectories.

If $\mathrm{Re}(\lambda_{1,2}) \neq 0$, the fixed points are hyperbolic points, which are robust points that cannot be altered by the small nonlinear terms. Fixed points like stable nodes and stable spirals are hyperbolic points. The Hartman–Grobman theorem affirms that the phase portrait in the neighborhood of a hyperbolic fixed point of a nonlinear system is topologically equivalent to the phase portrait of the linearization, i.e., the phase portrait of the nonlinear system is an invertible deformation of the phase portrait of the linearized system. A phase portrait whose topology cannot be altered by small nonlinear perturbations is structurally stable.

The phase portrait of a dynamical system can be modified as some parameter of the system varies, giving rise to qualitative changes in its structure. Such dynamical transitions are known as bifurcations. They generally occur in a one-dimensional subspace, and the remaining dimensions of the phase plane are affected as a consequence of the trajectories that can be

attracted or repelled from this subspace. Bifurcations can lead to the rich qualitative behavior characteristic of nonlinear systems that includes bistability, biological switches, circadian rhythms, bursting, and traveling waves, among others.

Considering the imaginary plane, we can roughly classify bifurcations into two cases: (1) the eigenvalues of the Jacobian matrix are both real and bifurcations occur along the real axis as certain parameter changes. This kind of bifurcation comprises the saddle-node bifurcation, the transcritical bifurcation, and subcritical and supercritical pitchfork bifurcation. (2) The eigenvalues of the Jacobian matrix are complex conjugated. Bifurcations occur crossing the imaginary axis as certain parameter changes. This kind of bifurcation comprises the supercritical and subcritical Hopf bifurcation.

The formation of the complex by fibroblast growth factor (FGF) and its receptor and the subsequent homodimerization of the complexes can be taken as an example of a dynamical system. The biochemical reaction is

$$\begin{aligned} &\mathrm{FGF} + \mathrm{FGFR} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{FGF} - \mathrm{FGFR} \\ &2\left(\mathrm{FGF} - \mathrm{FGFR}\right) \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \left(\mathrm{FGF} - \mathrm{FGFR}\right)_2 \end{aligned} \tag{14}$$

The corresponding dynamical system is

$$\begin{aligned} &\frac{\mathrm{d}c}{\mathrm{d}t} = k_1\left(\mathrm{FGF}_\mathrm{T} - c\right)\left(\mathrm{FGFR}_\mathrm{T} - c\right) - k_{-1}c, \\ &\frac{\mathrm{d}c_2}{\mathrm{d}t} = k_2 c^2 - k_{-2}c_2, \\ &c\left(0\right) = c_o \quad c_2\left(0\right) = c_{2o}. \end{aligned} \tag{15}$$

where FGF_T is the total amount of the agonist in the medium, FGFR_T is the total amount of FGF receptor in the cell's membrane, c is the amount of the complex agonist–receptor FGF-FGFR, c_2 is the amount of the homodimer complex $(\mathrm{FGF\text{-}FGFR})_2$, and k_1, k_{-1}, k_2, and k_{-2} are the rate constants. For the following particular values of parameters and constants, FGF_T = 1 μM, FGFR_T = 0.5 μM, $k_1 = 1\ \mu\mathrm{M}^{-1}\mathrm{s}^{-1}$, $k_{-1} = 0.004\ \mathrm{s}^{-1}$, $k_2 = 2\ \mu\mathrm{M}^{-1}\mathrm{s}^{-1}$, and $k_{-2} = 1.8\ \mathrm{s}^{-1}$; the fixed points are A(0.4652, 0.24) and B(1.0747, 1.282). The linearized system is

$$\begin{bmatrix} \delta\dot{c} \\ \delta\dot{c}_2 \end{bmatrix} = \begin{bmatrix} 2c - 1.54 & 0 \\ 4c & -1.8 \end{bmatrix} \begin{bmatrix} \delta c \\ \delta c_2 \end{bmatrix} \tag{16}$$

and the Jacobian of the linearized system is

$$\mathbf{J}\left(\mathbf{x}^o\right) = \begin{bmatrix} 2c - 1.54 & 0 \\ 4c & -1.8 \end{bmatrix}_{\mathbf{x}^o} \tag{17}$$

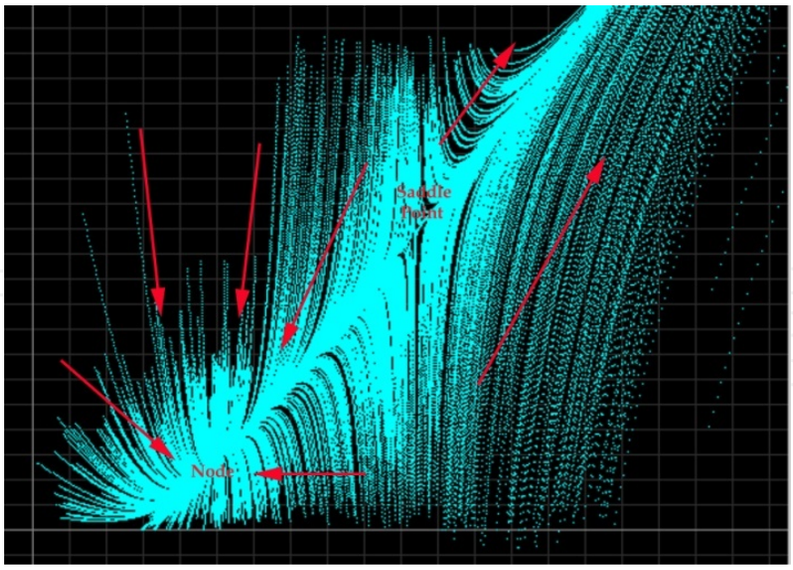

Figure 6. Portrait phase of the FGF model.

Evaluating the Jacobian (Equation 17) in each fixed point, the eigenvalues for the fixed point A are both real negative, and for the case of point B, one eigenvalue is real negative while the other is real positive. Thus, the fixed point A is a stable node, while the fixed point B is a saddle point. In Figure 6, the phase portrait of the dynamical system is shown, in which the saddle point is an energetic barrier that separates the dynamics of the system around the node from the dynamics of the outer region of the phase space, which is filled of trajectories that continually diverge. Both fixed points are hyperbolic and the phase portrait is structurally stable.

4. Modeling apoptosis

4.1. Mathematical modeling of the p53 pathway

p53 has a central role in the control of apoptosis and in the induction of death in cells after chemotherapy or radiotherapy. One of the main characteristics of the control of apoptosis by p53 is the existence of oscillations in the p53–Mdm2 negative feedback loop whose frequency and duration determine the entrance of the cell to apoptosis. These oscillations arise through a supercritical Hopf bifurcation in response to DNA damage signals [37, 79].

Many attempts have been done to understand how the negative p53–Mdm2 feedback loop sustains the observed p53–Mdm2 oscillations. These models have addressed that oscillations can arise in systems in which there is a sustained input signal and a single negative feedback loop with a delay term. However, models of this type are unable to produce multiple oscillations and are necessary to introduce a positive feedback in the core of equations to reproduce a wide range of oscillatory responses experimentally observed [80]. Nevertheless, the dynamical features of this kind of models are not robust against changes in the value of the parameters, and new attempts have been done to correct this problem by introducing additional feedback

motives in the model that improve robustness but cannot explain the observed variability in the p53 and Mdm2 oscillations in cell populations [81]. Stochastic models have been proposed in order to overcome these limitations of deterministic models. In this kind of modeling, random variables like noise or DNA damage are taken into consideration in the core of the equations giving rise to a series of oscillations with variable amplitude in concordance with the experimental observations [80].

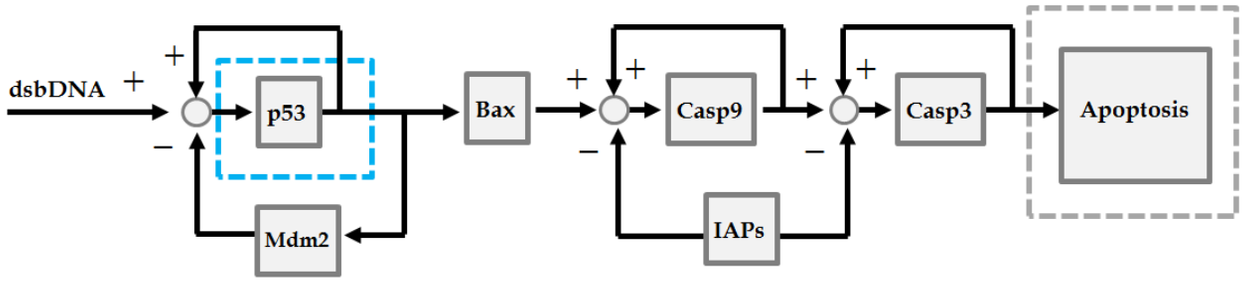

Figure 7. Minimal model of the p53 pathway.

Proctor and Douglas [81] proposed two stochastic mechanistic models of the p53–Mdm2 feedback loop. The first model explores the inhibitory effect of p14ARF on Mdm2, which leads to the stabilization of p53; the second model explores the effect of the phosphorylation of both Mmd2 and p53 by ATM. From both models, oscillations p53 and Mdm2 with high variability are obtained; however, the ATM model exhibits more variability. Both models are robust for a wide range of parameter values, indicating that stochasticity could be a basic component of the cell's response to DNA severe damage. Liu et al. [82] proposed a complete stochastic model of p53 and apoptosis with three-coupled modules. The first module represents the p53–Mdm2 feedback loop dynamics; the second module represents cytochrome *c* release from the mitochondria, and the third module represents caspase activation. Results from this model indicate that the major determinants of cell fate are the strength of p53 transcriptional activity together with its coupling to mitochondrial opening. This model indicates that p53 oscillations are a necessary but not sufficient condition for the onset of apoptosis. In this point, the Bax molecules located at the surface of the outer mitochondrial membrane have a central role in driving apoptosis, indicating that the possible role of the p53–Mdm2 system is to control the cytoplasmic levels of activated Bax.

Bistability is a broad dynamical feature of nonlinear biochemical systems, in which a saddle-point separates two stable nodes. The transition between nodes requires energy to overcome the high energetic barrier between them, leading to a saddle-node bifurcation. Generally, once the transition from one steady point to the other occurs, return to the original state needs a different pathway with different energy requirements, forming a hysteresis loop. Molecular switches are the common examples of this type of dynamics [83].

Bagci et al. [60] studied the role of Bax, Bcl2 synthesis, and degradation rates in mitochondria-dependent apoptosis, finding the existence of a saddle-node bifurcation in the caspase-3-Bax degradation rate diagram. This bifurcation drives the system either to a stable point with a

high concentration of caspase-3 (cell death) or to a stable point with a low concentration of caspase-3 (cell survival). Out of this region, the systems dynamics is settled on by the existence of a single stable fix point that determines cell surviving independently of the concentration of caspase-3. An important conclusion from this work is that an abnormal state, like cancer, arises in cells when Bax degradation rate is above a threshold value, giving rise to a stable cell survival dynamics, i.e., cells do not die.

In order to explore the dynamics of the caspase cascade when coupled to the p53 pathway by Bax switch, a model of ordinary differential equations (ODEs) is proposed (Figure 7). However, this model takes into account only the most representative nodes of the pathway (Section 2.1): p53, Mdm2 and its mRNA, Bax, IAPs, active initiator caspase-9, and the active executioner caspase-3 (Figure 7B). Besides its simplicity, this model reproduces the oscillatory dynamics of the p53–Mdm2 and its effects on the activation of caspase-3. In this model of the p53 pathway, the concentration of p53 is given by

$$\frac{\mathrm{d}[\mathrm{p53}]}{\mathrm{d}t} = \alpha_{\mathrm{act}}[\mathrm{p53}] + k_1[\mathrm{p53}][\mathrm{Mdm2}] - \delta_1[\mathrm{p53}] \tag{18}$$

where α_{act} is the rate of p53 synthesis, k_1 is the affinity of Mdm2 to p53, and δ_1 is the p53 rate of decay. In a similar form, the concentration of the Mdm2 mRNA is given by

$$\frac{\mathrm{d}[\mathrm{mRNA}]}{\mathrm{d}t} = \alpha_1[\mathrm{p53}] - \delta_2[\mathrm{mRNA}] \tag{19}$$

where α_1 is the rate of mRNA synthesis due to p53 activity and δ_2 is the mRNA rate of decay. Continuing with the model, the concentration of Mdm2 is given by the following equation:

$$\frac{\mathrm{d}[\mathrm{Mdm2}]}{\mathrm{d}t} = \alpha_2[\mathrm{mRNA}] + k_1[\mathrm{p53}][\mathrm{Mdm2}] - \delta_3[\mathrm{Mdm2}] \tag{20}$$

In this equation, α_2 is the rate of Mdm2 synthesis, and δ_3 is the Mdm2 rate of decay. The next equation represents the fraction of free Bax protein:

$$\frac{\mathrm{d}[B^*]}{\mathrm{d}t} = k_2([B]_T - [B^*])[\mathrm{p53}] - \delta_4[B^*] \tag{21}$$

where k_2 is the rate of Bax release due to p53 activity, $[B^*]_T$ is the total concentration of Bax protein and δ_4 is its rate of decay. The following equation represents the decrease of IAPs activity due to free Bax:

$$\frac{\mathrm{d}[I]}{\mathrm{d}t} = -k_3[B^*][I] \tag{22}$$

Finally, we have the equation for the activation of initiator caspase-9:

$$\frac{\mathrm{d}[C9^*]}{\mathrm{d}t} = k_4\left([C9]_T - [C9^*]\right)\left([B^*] + [C3^*]\right) - \left(\delta_5 + k_5[I]\right)[C9^*] \tag{23}$$

Moreover, the equation for the activation of executioner caspase-3 is as follows:

$$\frac{\mathrm{d}[C3^*]}{\mathrm{d}t} = k_6\left([C3]_T - [C3^*]\right)\left([C9^*] + [C3^*]\right) - \left(\delta_6 + k_5[I]\right)[C3^*] \tag{24}$$

where k_4 and k_6 are the rates of activation, δ_5 and δ_6 are the rates of caspases decay, and k_5 is the rate of repression due to IAPs activity. All parameters of the model are reported in Table 1. The initial concentrations (μM) of the variables are

$$[\mathrm{p53}]_0 = 0.01 \qquad [I]_0 = 1 \qquad [\mathrm{mRNA}]_0 = [\mathrm{Mdm2}]_0 = [B^*]_0 = [C9^*]_0 = [C3^*]_0 = 0$$

Parameter	Value	Biological meaning
α_{act}	0.15 s^{-1} Low damage 0.2 s^{-1} Medium damage 0.3 s^{-1} Strong damage	Rate of p53 synthesis
k_1	1 $\mu M^{-1} s^{-1}$	Rate of p53 degradation due to Mdm2 repression
δ_1	0.01 s^{-1}	Basal degradation of p53
α_1	1 s^{-1}	Rate of Mdm2 RNA synthesis due to p53
δ_2	0.1 s^{-1}	Basal degradation of Mdm2 RNA
α_2	0.1 s^{-1}	Rate of synthesis of Mdm2
δ_3	0.5 s^{-1}	Basal degradation of Mdm2
k_2	0.1 $\mu M^{-1} s^{-1}$	Rate of Bax releasing due to p53 activity
δ_3	0.1 s^{-1}	Basal degradation of Bax
k_3	0.02 s^{-1}	IAPs inhibition due to Bax activity
k_4	0.01 $\mu M^{-1} s^{-1}$	Rate of procaspase-9 activation
δ_5	0.1 s^{-1}	Basal degradation of caspase-9
k_5	10 $\mu M^{-1} s^{-1}$	Inhibition of caspase-9 due to IAPs activity
k_6	0.1 $\mu M^{-1} s^{-1}$	Rate of procaspase-3 activation
δ_6	0.1 s^{-1}	Basal degradation of caspase-3
$[B]_T$	1 μM	Total concentration of Bax
$[C9]_T$	1 μM	Total concentration of caspase-9
$[C3]_T$	1 μM	Total concentration of caspase-3

Note: all parameters were estimated for this work.

Table 1. Model parameters

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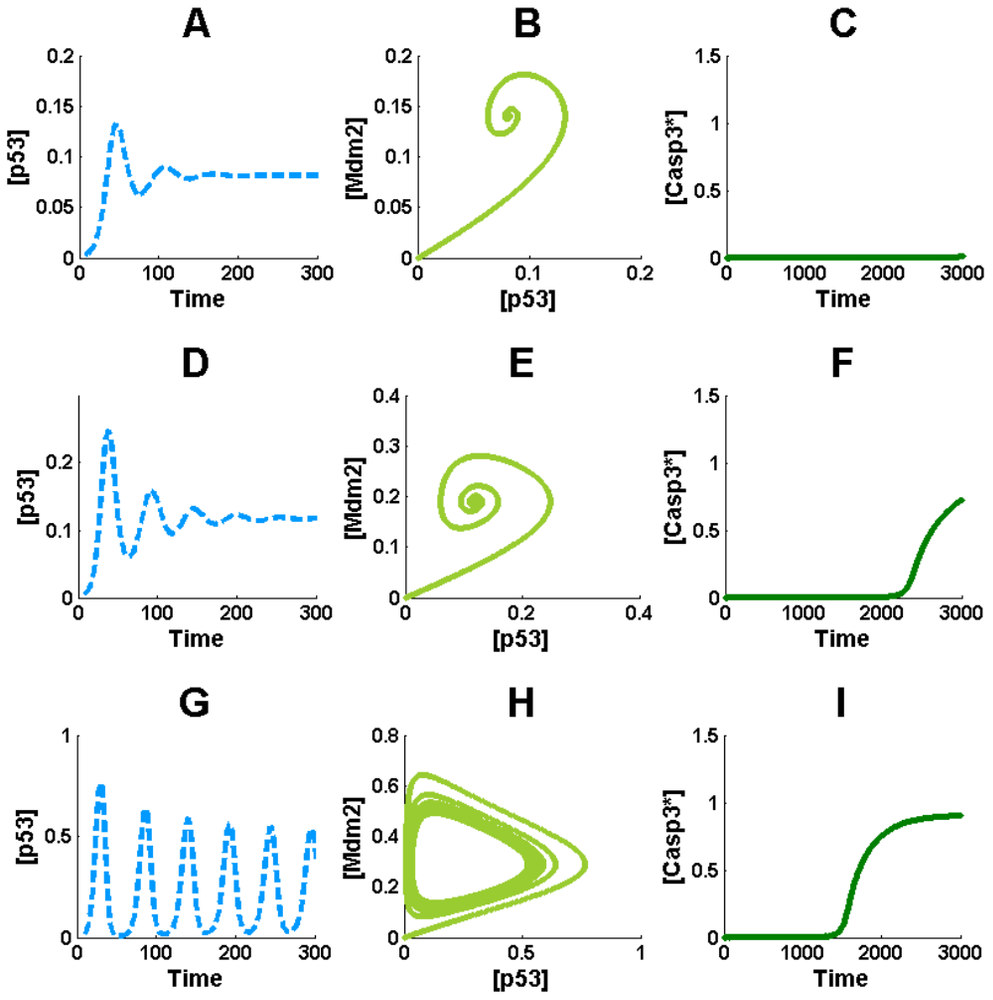

Figure 8. Dynamics of a simplistic model of p53 pathway and apoptosis. When the cell receives a soft damage on DNA, a few damped oscillations on p53 concentration (A) appear, which correspond to a stable spiral (B), and there is no increase in active caspase-3 concentration (C). When the damage on DNA is stronger, p53 exhibits stronger damped oscillations (D), and after a certain time, apoptosis starts (F). Finally, when DNA damage is severe, p53 oscillations are not damped (G), and a limit cycle is settled on (H), leading to a faster start of apoptosis (I). For all simulations, the time scale is in minutes, and concentrations are in micromolar units.

4.2. Dynamical features of the p53 pathway

From the above model, it is possible to visualize the main molecular events in the p53 pathway, as well as the activation of apoptosis. In the model, soft damage in DNA is interpreted as an increase of p53 synthesis. In this scenario, the p53 concentration presents damped oscillations (Figure 8A), which are represented by a stable spiral in the p53-Mdm3 phase plane (Figure 8B). With this level of DNA damage, the system does not activate apoptosis because the caspases-3 concentration remains zero (Figure 8C). When DNA damage reaches a medium level, the p53 concentration exhibits more pronounced damped oscillations, i.e., the stable spiral has a higher initial amplitude and a larger relaxation time (Figure 8D). However, if the DNA damage persists for long time, the active caspase-3 concentration will augment until it starts apoptosis irreversibly (Figure 8F). Moreover, when DNA damage is severe, the p53

pathway undergoes a supercritical Hopf bifurcation (reviewed in [83]), which means that the stable spiral (Figure 8E) becomes a stable limit cycle through an unstable spiral (Figure 8G). During the bifurcation process, the caspase-3 activation is faster, which implies that the cell has no option but to die (Figure 8I). Thus, p53 concentration acts as a biological timer and allows the cell to response in a specific way for certain perturbations, such as DNA damage intensity and duration. In addition, the activation of caspase-3 is governed by a control action of an "all or nothing" scheme, which avoids the random activation of apoptosis.

4.3. Over expression of Mdm2

In the nature, there are many factors that can permanently perturb the p53 network functioning, such as genetic alterations like deletions or constitutive overexpression due to polymorphisms [84]. A classical example of this point is the overexpression of Mdm2, which is present in more than forty different types of cancer, taking a central role in cancer development [85].

In order to know how the overexpression of Mdm2 perturbs apoptosis, Equation (20) of the model (see Section 5.1) can be modified as follows:

$$\frac{\mathrm{d}[\mathrm{Mdm2}]}{\mathrm{d}t} = \alpha_2[\mathrm{mRNA}] + \varepsilon_{\mathrm{onco}} + k_1[\mathrm{p53}][\mathrm{Mdm2}] - \delta_3[\mathrm{Mdm2}] \tag{25}$$

where $\varepsilon_{\mathrm{onco}}$ is a parameter and represents the external factors that induce the Mdm2 overexpression.

From a dynamical point of view and according to the model results, Mdm2 overexpression disrupts the basal dynamics of the p53-pathway and of apoptosis. From Figures 9A to 9C, clearly the normal response of the system to strong DNA damage is a series of oscillations of p53 concentration, i.e., a limit cycle dynamics that induces the activation of caspases-3 if the oscillations last for time enough. However, Mdm2 overexpression suppresses the p53 oscillations (Figures 9D to 9F) and avoids cascapse-3 activation (see Section 2.1), allowing the survival of cells with strong DNA damage. This could be one of the possible dynamical forms in which cells with intense DNA damage loss the control of apoptosis and survive, leading to a malignant tumor.

4.4. Suppression of caspase-9

Caspase-9 is a key protease that is constitutively expressed in a variety of fetal and adult tissues. Previous studies on cancer risk [58] show that polymorphism of caspase-9 is strongly correlated with lung cancer development in light smokers, reflecting an intense gene-environment interaction at this level of the apoptotic pathway. The loss of this caspase interrupts apoptosis, leading to abnormal proliferation of cells with severe DNA damage.

In the model of the p53 pathway, the missing of caspase-9 gene is modeled, assuming that

$$\frac{d[C9^*]}{dt} = 0 \tag{26}$$

Results from the minimal model presented in Section 5.1, modified with Equation (26), show that deletions on the caspase-9 gene suppress the activation of the effector caspase-3, uncoupling the p53 dynamics from the caspases activation module. Although the limit cycle in the p53–Mdm2 phase plane remains, the absence of caspase-9 avoids the activation of caspase-3 and apoptosis even if the DNA damage is severe.

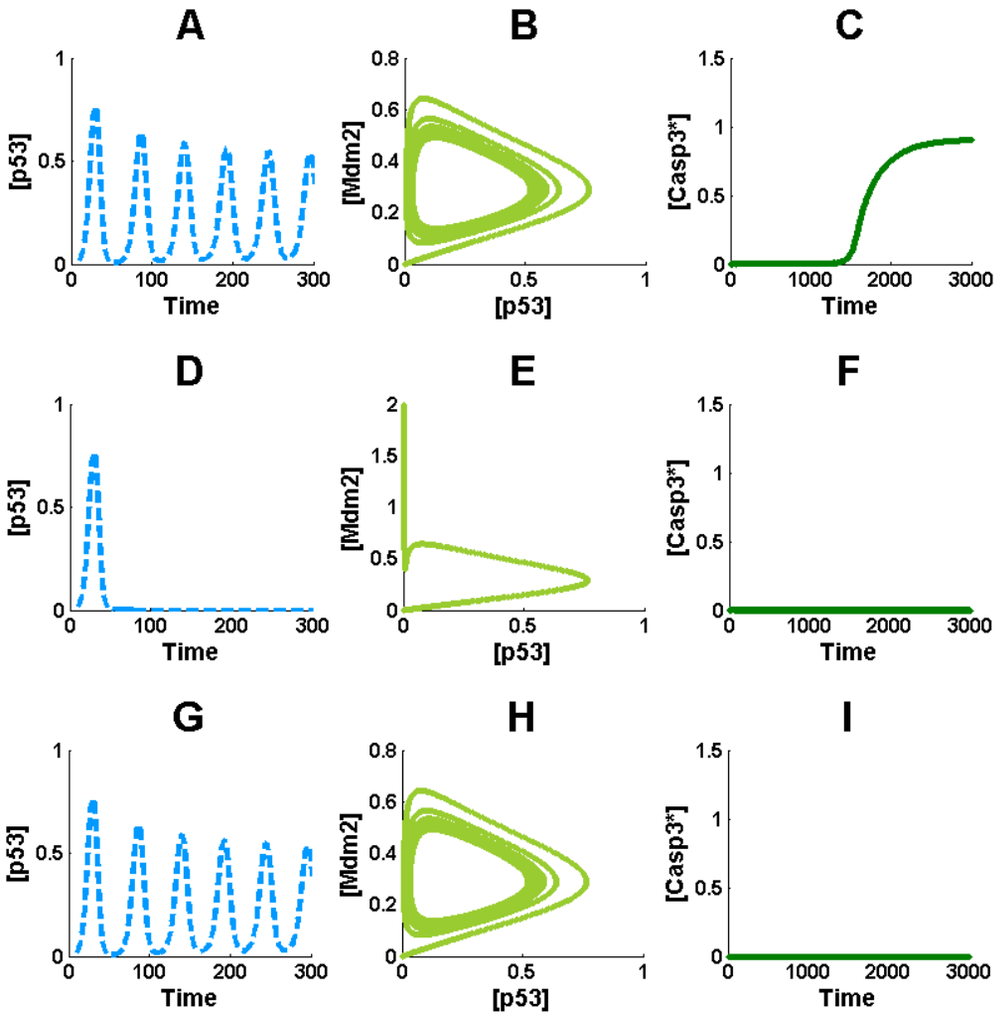

Figure 9. Oncogenic perturbations on the p53 and apoptosis pathways. Normally, when the cell receives severe DNA damage, the p53 concentration oscillates (A) following a limit cycle dynamics (B), which eventually starts apoptosis (C). However, overexpression of Mdm2 abrogates the p53 oscillations (D), dampens the normal dynamics (E), and avoids the caspase-3 activation (F). On the other hand, deletions on the caspace-9 gene simply abrogate the activation of effector caspase-3 (I). For all simulations, the time scale is in minutes and concentrations are in micromolar units.

4.5. Survival of malignant cells

These two examples of genetic perturbations show that there are two possible dynamical mechanisms implicated in the deregulation of apoptosis in cancerous cells. Such mechanisms are (1) uncoupling between the p53–Mdm2 limit cycle (pacemaker) and the caspase cascade

and (2) suppression of p53 activity by overexpression of a negative regulator (like Mdm2) that leads to the not activation of caspase-3.

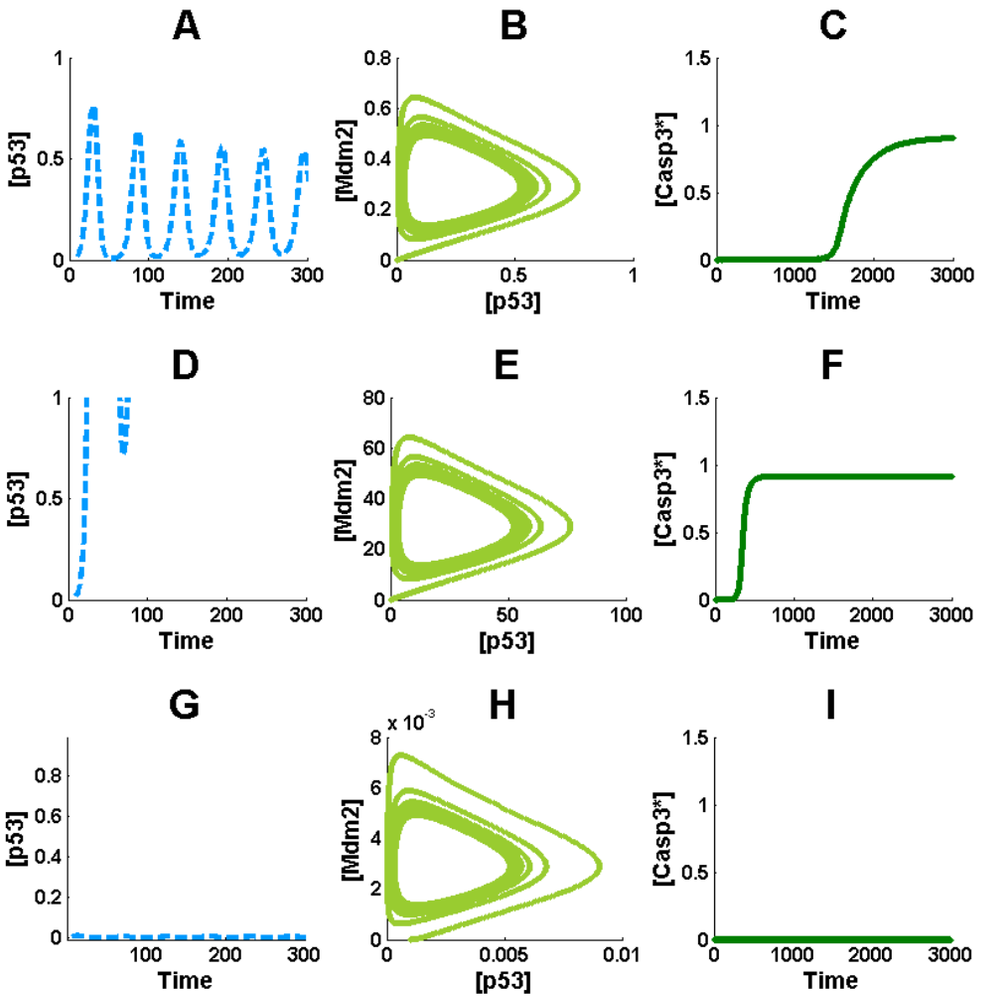

Figure 10. Variation of the rate of p53 degradation due to Mdm2 repression (k_1). Compared with the normal cell response to severe DNA damage (A, B, and C), the effect of decreasing in two orders the rate of p53 degradation due to Mdm2 k_1 augments the magnitude of p53 oscillations (D), preserving its limit cycle (E) and starts quicker apoptosis (F). On the other hand, the effect of increasing k_1 in two orders reduces the magnitude of p53 oscillations (G), avoiding caspase activation (I) but preserves the limit cycle (H).

In fact, p53 inhibition decreases miR-34a and BTG3 levels, releasing the inhibition on the activator E2fs and promoting the proliferation of abnormal cells [4]. Thus, a perturbed functioning of the network may give rise to the malignant transformation of damaged cells.

Besides genetic predisposition, there are other factors that contribute in transforming defective cells, such as viral and bacterial infections, metabolic disorders, and exposure to toxic substances. These exogenous agents modify the global behavior of the p53 network by targeting strategic interactions like (1) p53 repression due to Mdm2 (represented by k_1), (2) the rate of Mdm2 degradation (represented in the model by δ_3), (3) the rate of Bax releasing (represented

by k_2), and (4) the rate of caspase-9 activation (represented by k_3). In order to observe the effect of alterations on these interactions, the corresponding parameters were varied in two orders of magnitude, and then the stability of the net was studied.

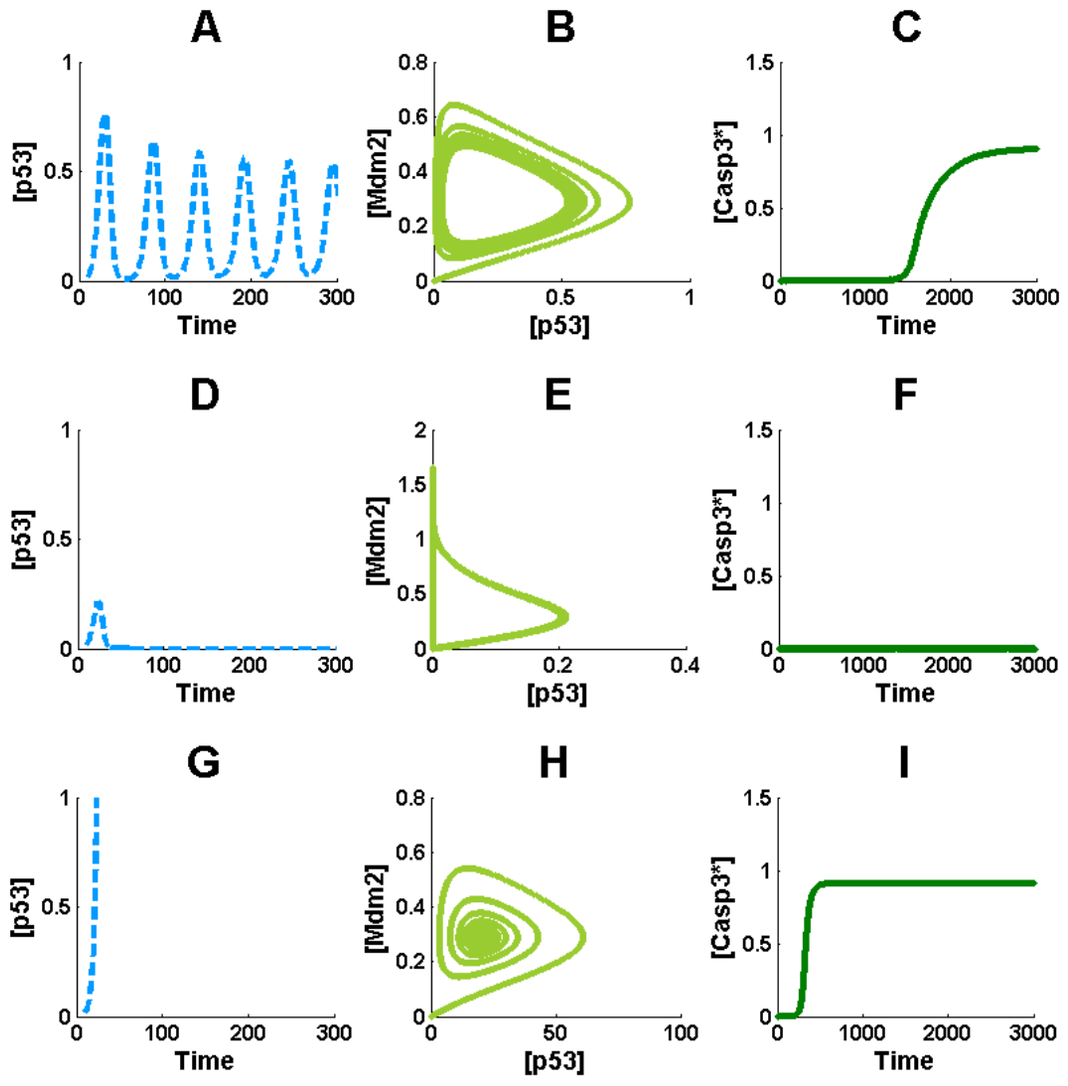

Figure 11. Variation of the basal degradation of Mdm2 (δ_3). Compared with the normal cell response to severe DNA damage (A, B, and C), the effect of increasing in two orders the basal degradation of Mdm2 δ_3 abrogates p53 oscillations (D), as well as its limit-cycle (E), avoiding apoptosis (F). However, the effect of decreasing δ_3 in two orders augments the magnitude of p53 concentration (G), making quicker the activation of apoptosis (I) but destroys the limit cycle dynamics (H). For all simulations, the time scale is in minutes and concentrations are in micromolar units.

The model shows that decreasing k_1 allows a faster induction of apoptosis in damaged cells (Figures 10D to 10F). In biological terms, this effect can be produced when damaged cells are treated with nutlin-3 [86] and proteins of the influenza A virus [87]. Moreover, augments on k_1 avoid apoptosis triggering (Figures 10G to 10I). In the nature, blocking such an interaction is a common strategy used by pathogens like Epstein–Barr virus [88] and Chlamydia [89] in order to ensure their replication inside the host.

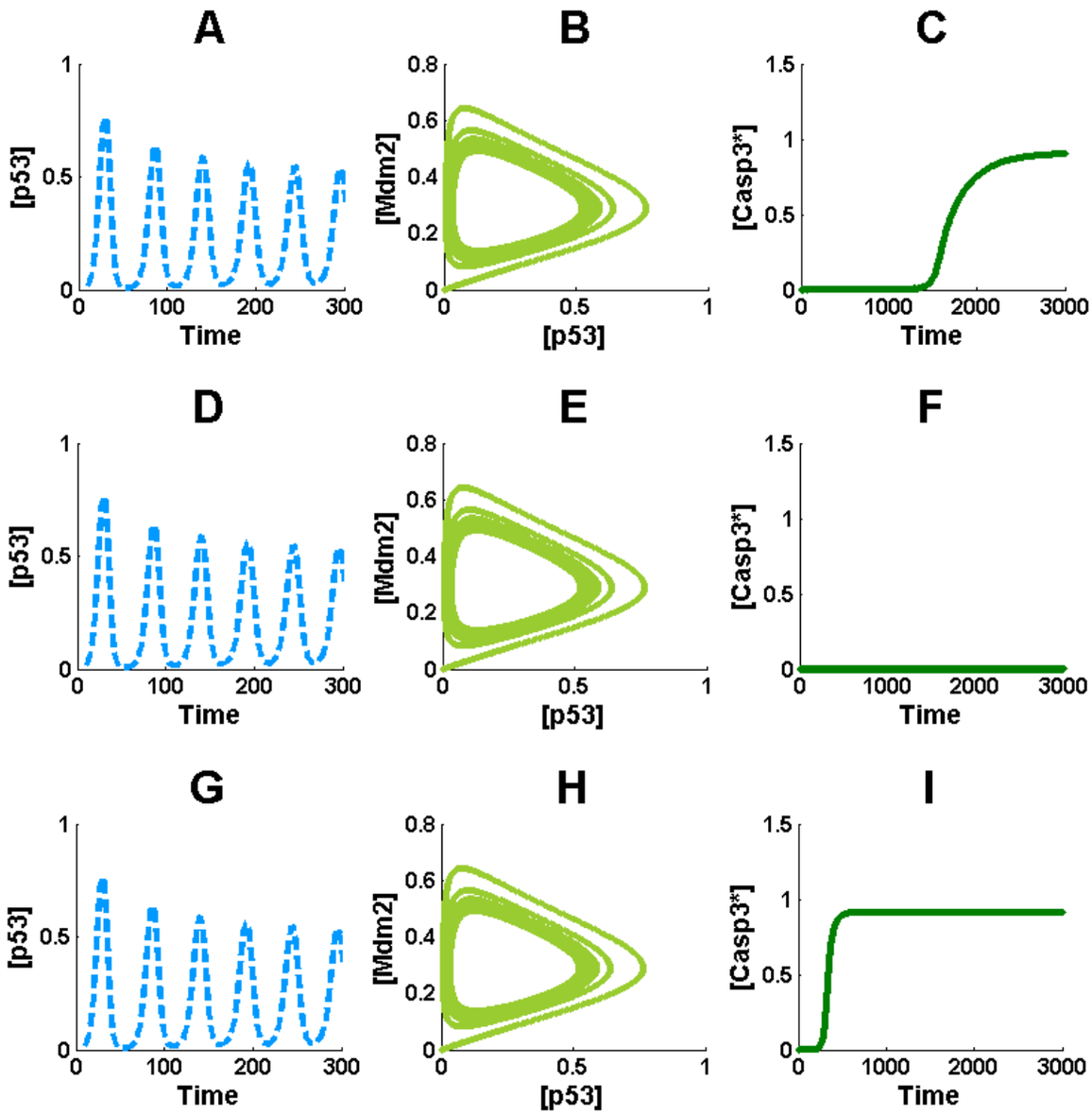

Figure 12. Variation of the rate of Bax releasing due to p53 activity (k_2). Compared with the normal cell response to severe DNA damage (A, B, and C), the effect of decreasing in two orders the rate of Bax releasing due to p53 activity k_2 does not interfere with the normal behavior of p53 (D), as well as its limit-cycle (E), but avoids apoptosis activation (F). Furthermore, the effect of increasing k_2 in two orders only accelerates the activation of apoptosis (I). For all simulations, the time scale is in minutes and concentrations are in micromolar units.

According to previous results, the increase of δ_3 makes the cell more sensitive to DNA damage, allowing a quicker activation of apoptosis (Figures 11D to 11F). At the molecular level, this effect can be obtained by augmenting p14ARF activity [90], using substances like capsaicin [91], or viral proteins like hepatitis C [92]. Another way to achieve this effect is destabilizing Mdm2 protein by affecting NEDD-4-1 functioning [93]. On the other hand, reducing δ_3 destroys Hopf bifurcation on p53–Mdm2 interaction, blocking definitively the initiation of apoptosis (Figures 11G to 11I).

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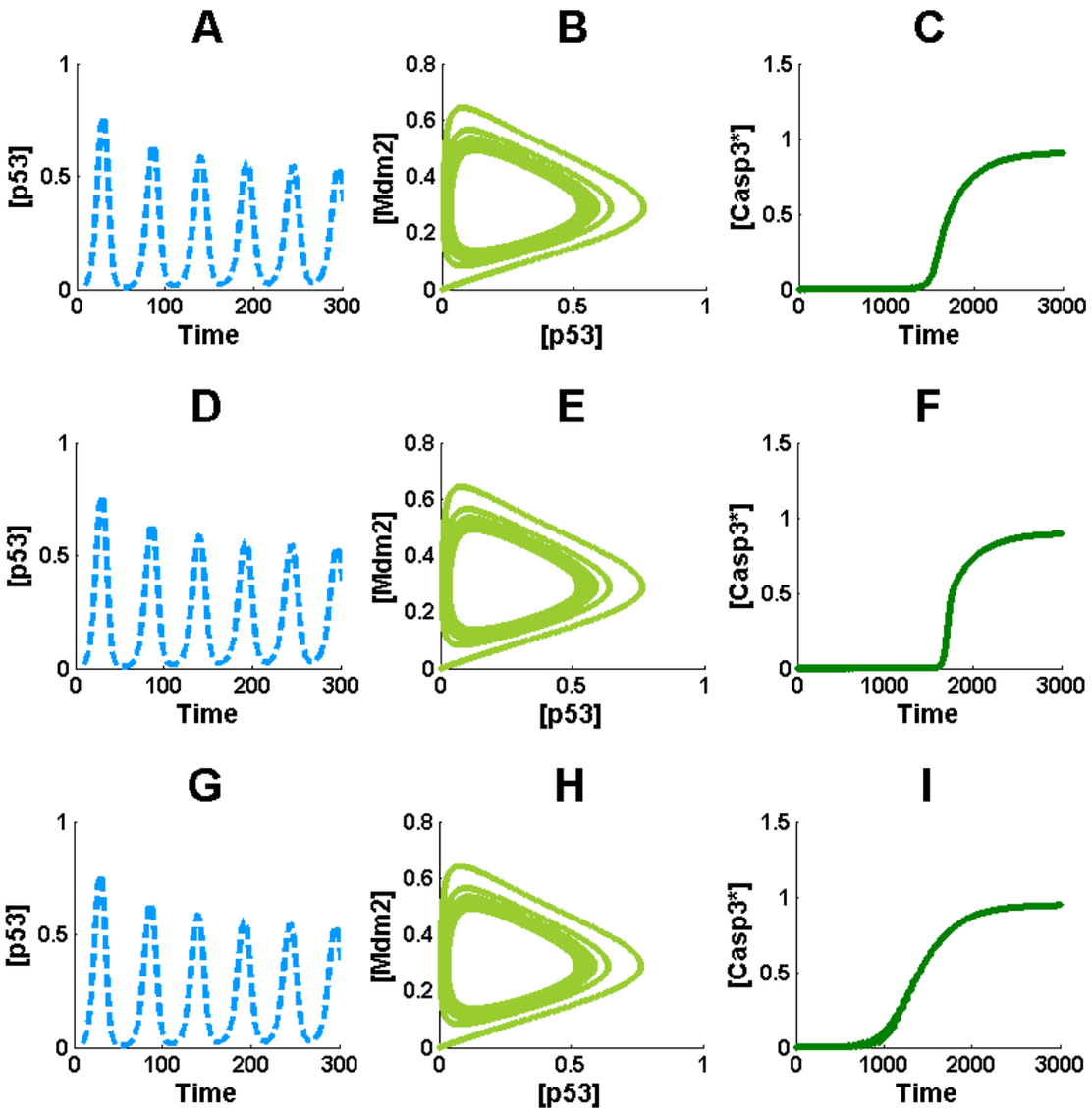

Figure 13. Variation of the rate of procaspase-9 activation (k_3). Compared with the normal cell response to severe DNA damage (A, B, and C), the effect of decreasing in two orders the rate of procaspase-9 activation k_3 does not interfere with the normal behavior of p53 (D), its limit-cycle (E), and apoptosis activation (F). However, the effect of increasing k_3 in two orders only increases slightly apoptosis activation (I). For all simulations, the time scale is in minutes, and concentrations are in micromolar units.

According to the model, when the rate of Bax releasing (k_2) increases, the cell is more sensitive to genotoxic damage as is observed in cells that overexpress Bax [94] (Figures 12G to 12I). However, when k_2 is reduced, the cell lacks its damage responsiveness (Figures 12D to 12F). Paradoxically, decreasing in k_3 has no significant effect on caspase-3 activation (Figures 13D to 13F). This phenomenon can be explained by the fact that there are many bistable switches on caspase cascade, sustained by positive feedback loops [59]. Thus, when those alterations are maintained for long time, the cell tends to accumulate several mutations that destabilize its genome, which eventually allows its transformation into a tumor cell.

5. Therapeutics approaches against cancer

Treatments against all types of cancer aim to interfere the functioning of transformed cells. Traditionally, this interference was induced by using drugs that block the cell growth together with radiotherapy [95, 96]. This approach has been the most successful; however, produces serious collateral effects on patients.

For this motive, in recent years, the use of more selective drugs for inducing apoptosis in cancerous cells has been proposed. These drugs comprise inhibitors of IAPs, BCL2, Mdm2, p53, and constitutive activators of death receptors [97–100]. Similarly, another new type of drugs derived from extract of *Emilia sonchifolia* has been used to activate caspases through the induction of reactive oxygen species (ROS) [101]. Polyphenolic curcumin is another promising natural compound that is capable to trigger apoptosis by increasing the expression and clustering of FasR in tumor cells. Another approach is based on the construction of nanoparticles that transport anticancerous compounds to tumor cells [102]. Moreover, more revolutionary approaches to treat cancer use phototherapy, which consists in administrate of nontoxic drugs known as "photosensitizers" to cancerous cells. After that, cells receive posterior illumination of visible light at specific wavelength, triggering the activation of photosensitizers that produce high amounts of ROS to eliminate the tumor cells by apoptosis [103].

Another novel approach to treat cancer is gene therapy, which uses many genes to alter the functioning of transformed cells blocking survival mechanisms and triggering apoptosis [1, 96, 104]. Other original strategies related with gene therapy use micro-RNA specifically designed to avoid the overexpression of oncogenes like BCL2 family or p53 in cancerous cells [105, 106].

In addition to gene therapy, the use of cytokines to treat cancer as an effective approach was proposed because there are reports indicating that the cancer cell lines treated with TNFα have an increase in DR5 as well of interferon-γ and interferon-α [95]. It has been suggested that the utilization of some hormones like estrogen in controlled doses can be used as a treatment because it was reported that cancer breast cells exposed to high doses of estrogen died by apoptosis [107].

The multiple origins of cancer are the main obstacle for all of these approaches. In order to show the importance of this point on cancer treatment, the model of apoptosis for simulating two groups of cancerous cells is used. Both groups overexpress IAPs, but the second group also has an important deletion on caspase-3 gene. Mathematically, for both groups, Equation (24) of model 5.1 are modified by adding an oncogenic perturbation parameter ($\varepsilon_{\text{onco}}$) to overexpress IAPs [108] as follows:

$$\frac{\mathrm{d}[I]}{\mathrm{d}t} = \varepsilon_{\text{onco}} - k_3[B^*][I] \tag{27}$$

http://dx.doi.org/10.5772/60916

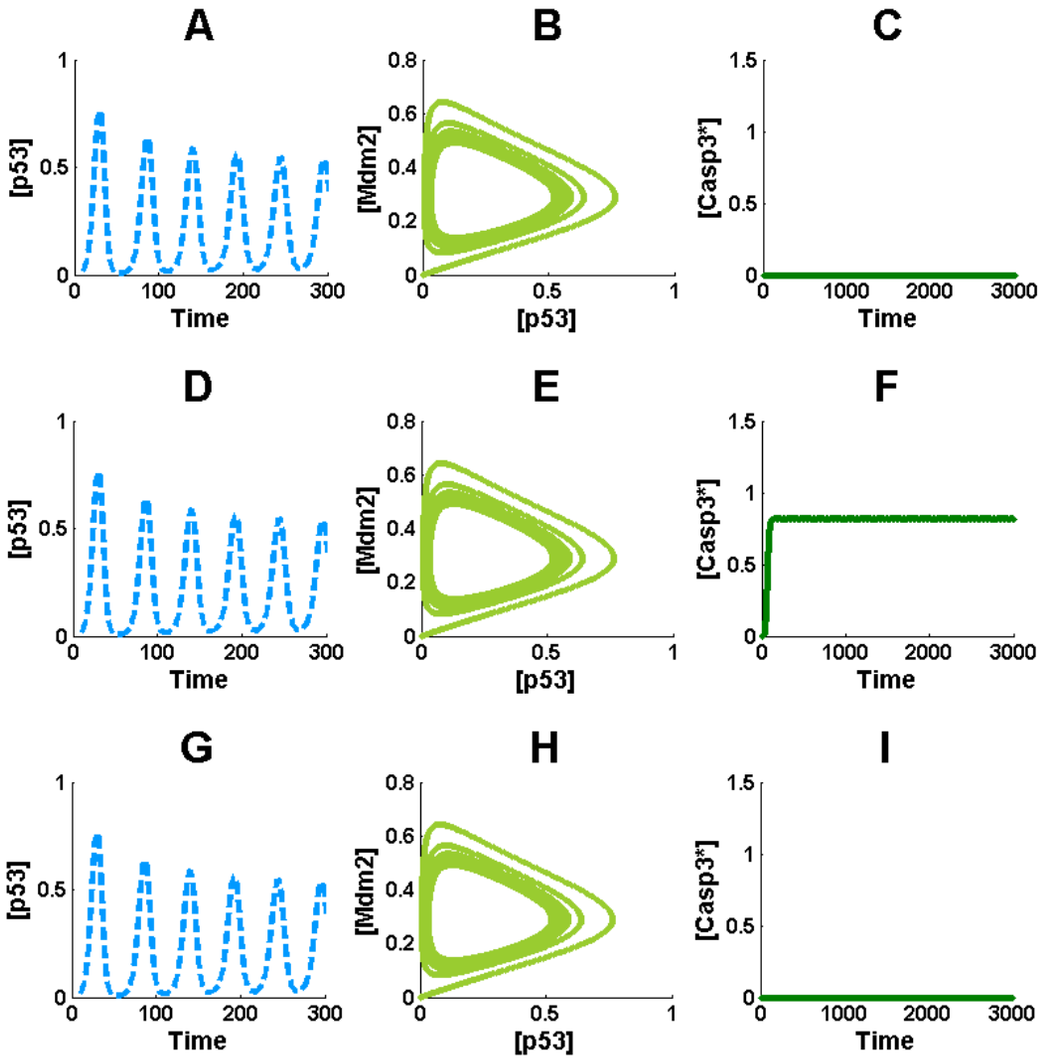

Figure 14. The subclonal heterogeneity as a therapeutics challenge. Cancerous cells that have deletions on the caspase-3 gene and/or overexpress IAPs present the same phenotype, and dynamically such mutations do not affect the p53 behavior when the cell receives severe DNA damage (A, B) but avoid apoptosis initiation (C). When cancerous cells that overexpress IAPs are treated with an inhibitor of IAPs activity and radiotherapy, the apoptosis starts normally (F). However, this approach does not work with cells that lack caspase-3 gene (I). For all simulations, the time scale is in minutes and concentrations are in micromolar units.

For simulating caspase-3 deletion in the second group of cancerous cells, Equation (24) must be modified as follows:

$$\frac{d[C3^*]}{dt} = 0 \tag{28}$$

With respect to their phenotype, both groups of cells look very similar, and neither the oncogenic perturbation nor the deletion of caspase-3 interferes with the Hopf bifurcation on p53–Mdm2 feedback loop (Figures 14A to 14C).

The effect of treatments on both cells groups with drugs that inhibit IAPs together with radiotherapy is represented as follows:

$$\frac{\mathrm{d}[I]}{\mathrm{d}t} = \varepsilon_{\mathrm{onco}} - k_3[B^*][I] - k_d[D][I] \tag{29}$$

where k_d is the rate of IAPs repression due to inhibitor drugs, represented by [*D*]. After the theoretical treatment, the model shows that the first type of cancerous cells is successfully eliminated by apoptosis (Figures 14D to 14F) but not the second one (Figures 14G to 14I). Clinically, this problem is known as subclonal heterogeneity [109] and is very common to several types of tumors, in which transformed cells have many different damages, and thus, they do not response equally to treatments.

One way to overcome this problem is testing hypothesis and planning strategies to manipulate the cell functioning with mathematical models. For example, nowadays, it is possible designing genetic programs with specific functions like self-regulatory systems to attack cancerous cells [110].

6. Conclusions

The p53–Mdm2 module has a central role to prevent the development of tumor cells. This module has different modes of operation, depending upon the degree o DNA damage by pathogens, chemicals, or genotoxic signals. When DNA damage is severe, p53–Mdm2 takes the control of the apoptotic pathway by activating Bax, which in turn switches on the caspase cascade. From a dynamical point of view, this process implies the coupling of a supercritical Hopf bifurcation produced in the nucleus, with a saddle-node bifurcation produced in the cytoplasm that induces bistability in Bax [111], and caspases activation [59]. Thus, even if the DNA damage signal is intense, the p53–Mdm2 oscillatory dynamics must sustain enough time to increase Bax concentration to a threshold value that initiates apoptosis in an irreversible form; otherwise, the damaged cell can survive.

Mdm2 overexpression disrupts the p53–Mdm2 limit cycle in the nucleus blocking the transmission of the death signal to the caspase cascade, allowing the survival of the mutated cell. The suppression of caspase-9 gene causes the uncoupling of the p53–Mdm2 limit cycle and the caspase cascade, leading to the survival of the mutated cell despite that the DNA damage signal activates the p53 mechanism of damage suppression. Accordingly, mutated cells can escape from apoptosis when mutations in cells either disrupt the p53–Mdm2 limit cycle or uncouple it from the caspase cascade.

As a consequence, tumor cells can arise when they can overcome the apoptotic pathway by different mechanisms, leading to different strategies for their survival [110]. This variety in the causes that can originate and allow the survival of transformed cells is one of the obstacles to find a definitive cure for cancer. The combination of treatments seems to be the best strategy

against cancer at the moment. Mathematical models of different aspects of cancer can be a guidance to find betters strategies to optimize and design novel treatments against cancer.

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