Oncogenic alterations in the p53 pathway abolish oscillatory competence

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Abstract

The tumor suppressor p53 displays concentration oscillations in response to DNA damage, a behavior that has been suggested to be essential to its anti-cancer function. Many genetic alterations in the p53 pathway have been shown to be oncogenic, whether by experiment or by clinical associations with various cancers. These oncogenic alterations include somatic mutations, copy number variations and inherited polymorphisms. Using a differential equation model of p53-Mdm2 dynamics, we employ Hopf bifurcation analysis to show that all of the oncogenic perturbations have a common effect, to abolish the oscillatory competence of p53, thereby impairing its tumor suppressor function. In this analysis, these diverse genetic alterations, widely observed in human cancers, have a unified mechanistic explanation.

Keywords: p53 oscillations, DNA damage response, tumor suppression, oncogenic alterations, Hopf bifurcation

Significance In human cancers, the p53 tumor suppressor pathway is frequently altered by diverse genetic changes. An integrated understanding of these oncogenic alterations is currently lacking. We show that all oncogenic alterations in the p53 pathway abolish the oscillatory competence of p53, a property that is essential for cell cycle arrest upon stress, for effective DNA damage response and for maintaining genome integrity. This unified dynamical explanation of distinct cancer driver events that converge on a key cancer hallmark pathway has practical implications for anti-cancer therapies.

1 Introduction

The tumor suppressor p53 plays important roles in safeguarding the genome against genotoxic and other stresses, thereby preventing cancer (P. Chen, Chen, Bookstein, & Lee, 1990; Finlay, Hinds, & Levine, 1989; A. J. Levine, 1997). p53 has several inhibitors, including Mdm2 (Haupt, Maya, Kazaz, & Oren, 1997; Momand, Zambetti, Olson, George, & Levine, 1992). Oren, Levine and colleagues showed that a simple model of p53-Mdm2 negative feedback predicted oscillations in p53 protein concentration, which they confirmed by observing one cycle of the p53-Mdm2 oscillation after ionizing radiation in cultured cells (Lev Bar-Or et al., 2000). Lahav and colleagues found that γ -irradiation triggered multiple oscillations in p53 and Mdm2 in single cells, with a period of about 5.5 hours (Lahav et al., 2004); they also developed an explanatory mathematical model (Geva-Zatorsky et al., 2006).

Oscillations in the p53-Mdm2 system have been suggested to have functional roles, as a mode of intracellular communication to coordinate cell cycle arrest and DNA repair, which crucially determine cell fate after DNA damage (Lev Bar-Or et al., 2000; Purvis et al., 2012). As a result, impaired p53 oscillations would compromise genome integrity and promote cancer.

Genetic alterations in the p53 pathway are frequently oncogenic, including somatic mutations, copy number variations and inherited polymorphisms affecting p53 and its regulators such as Mdm2, Mdm4 and ATM. Here, we show that each of these oncogenic genetic changes, when biophysically modeled in the p53-Mdm2 negative feedback system, acts to abolish the oscillations upon DNA damage, thus providing an insight into the common mechanism of their oncogenic effect: a loss of the oscillatory competence of p53.

2 Results

The mathematical model. To study the effect of individual oncogenic alterations on the oscillatory behavior of the p53-Mdm2 system, we developed a mathematical model of the core p53-Mdm2 negative feedback system. Based on previous studies (Batchelor, Mock, Bhan, Loewer, & Lahav, 2008; Geva-Zatorsky et al., 2006; Heltberg et al., 2019), our model reflects the transcription factor p53 enhancing the production of its own inhibitor Mdm2 (Fig. 1A):

$$\begin{aligned} p53 : \frac{dX}{dt} &= \alpha - \beta Y \frac{X}{\gamma + X} \\ MDM2 \text{ transcript} : \frac{dR}{dt} &= \sigma \frac{X}{\kappa + X} - \epsilon R \\ Mdm2 : \frac{dY}{dt} &= \phi R - \delta Y \end{aligned}$$

The key features of the mathematical model are: (i) p53 is produced at a constant rate (α) and is degraded upon binding to Mdm2 through a saturated degradation process (β , γ), where β represents the maximum rate of Mdm2-induced degradation of p53 and γ the p53 concentration for half-maximum degradation; (ii) *MDM2* transcript is produced upon p53 transactivation through a saturated induction process (σ , κ) and degraded through a first-order decay process (ϵ), where σ represents the maximum rate of p53-dependent transcription of *MDM2* and κ the p53 concentration for half-maximum *MDM2* transcription; (iii) Mdm2 is produced proportional to the *MDM2* transcript level (ϕ) and degraded through a first-order decay process (δ).

We chose appropriate values for each biophysical parameter based on previous calibrations (*Methods*). Numerical simulation of the mathematical model recapitulated the oscillatory behavior of p53 during DNA damage response, and agreed with experimental observations that p53 protein level oscillates with a characteristic period of 5-6 hours, and that both *MDM2* transcript and Mdm2 protein levels oscillate during DNA damage (Fig. 1B-C) (Batchelor et al., 2008; Geva-Zatorsky et al., 2006; Hanson, Porter, & Batchelor, 2019; Heltberg et al., 2019; Lahav et al., 2004; Purvis et al., 2012).

We evaluated known oncogenic alterations in the p53 pathway for their effect on the biophysical parameters of the p53-Mdm2 system (Table 1). They affect three essential aspects of p53-Mdm2 dynamics: production of p53 (α), p53-Mdm2 interactions (β and σ), and Mdm2 degradation (δ).

The oscillatory behavior of p53 is sensitive to parameter values. We studied the transient response of the p53-Mdm2 system by performing a series of single-parameter sensitivity analyses for α , β , σ and δ (*Methods*). We found that lowering α reduced the amplitude of initial p53 pulses in response to DNA damage (Fig. 1E); a 50% reduction of α resulted in markedly reduced initial amplitudes and damped oscillations in both p53 and Mdm2 (Fig. 1D). Decreasing δ had a similar effect (Fig. 1E). Lowering β or σ dramatically enhanced initial p53 pulses and shortened their latency. Further lowering of β and σ increased the oscillatory period and ultimately caused a loss of the oscillatory response. Increasing β or σ , on the other hand, reduced initial p53 pulses and diminished the oscillatory behavior of the system (Fig. 1E).

The long-term behavior of the system displayed sustained p53 oscillations only when α was sufficiently large (Fig. 1F). For β , σ and δ , however, we noted that robust p53 oscillations first emerged and then disappeared as parameter values varied (Fig. 1F), consistent with previous numerical studies on related models (Ciliberto, Novak, & Tyson, 2005; Lev Bar-Or et al., 2000; Ma et al., 2005; Wagner et al., 2005). That is, p53 oscillations were viable only within a range of parameter values, a “Goldilocks” effect that is explained by these results. Each one of these biophysical parameters can affect the oscillatory competence of the p53-Mdm2 system.

Figure 1: The oscillatory behavior of the p53-Mdm2 system during DNA damage response is sensitive to parameter values. (A) Schematic of the proposed model. (B) Numerical simulation of the model in response to DNA damage, showing simulated p53 protein level (blue), *MDM2* transcript level (yellow), and Mdm2 protein level (red). (C) The long-term behavior of the system approaches a limit cycle attractor that is stable under perturbations (the blue and red trajectories). (D) p53 pulses in response to DNA damage are dampened when α is halved. (E) Simulated p53 concentrations over time (the horizontal axes) are shown as heatmaps for varying parameter values of α , δ , β and σ . (F) One-dimensional bifurcation diagrams of the p53 system against single-parameter variation in α , δ , β and σ . The steady-state p53 concentration is shown as a solid line when it is stable and dotted when unstable; the amplitudes of the limit cycle oscillations are indicated by the blue curves.

Table 1: **Impact of oncogenic alterations on model parameters.**

Parameter	Oncogenic Alteration	Effect	Reference(s)
α	Truncating mutations in <i>TP53</i>	$\alpha \downarrow$	X. Wang and Sun (2017) Donehower et al. (2019)
	Allelic deletion of p53	$\alpha \downarrow$	Livingstone et al. (1992) Venkatachalam (1998)
	Inherited cancer risk polymorphism in <i>TP53</i>	$\alpha \downarrow$	Macedo et al. (2016) P. Zhang et al. (2019)
β	Hotspot mutations in <i>TP53</i> (e.g., residue R175 and R273)	$\beta \downarrow$	Peng, Chen, Li, Lu, and Chen (2001) Li, Marchenko, Schulz, et al. (2011) Li, Marchenko, and Moll (2011) Alexandrova et al. (2015)
	Loss of ATM	$\beta \uparrow$	Shieh, Ikeda, Taya, and Prives (1997) Banin et al. (1998) Cheng, Chen, Li, Lane, and Chen (2009)
	Overexpression of Mdm4	$\beta \uparrow$	Gu et al. (2002)
σ	Viral Oncoprotein (HPV E6)	$\beta \uparrow$	Werness, Levine, and Howley (1990)
	Missense mutations in <i>TP53</i>	$\sigma \downarrow$	Park et al. (1994) Kato et al. (2003)
	Overexpression of Mdm4	$\sigma \downarrow$	Pei, Zhang, and Zheng (2012)
	Viral Oncoprotein: SV40 T-antigen	$\sigma \downarrow$	Linzer and Levine (1979) Lane and Crawford (1979)
	Adenovirus E1A/E1B-SSKd		Sarnow, Ho, Williams, and Levine (1982)
	HBV HBx		X. W. Wang et al. (1994)
	HCV NS5A		Lan et al. (2002)
	HTLV-1 Tax		Pise-Masison et al. (1998)
	EBV EBNA3C		Yi et al. (2009)
	Inherited polymorphism in <i>MDM2</i>	$\sigma \uparrow$	Bond et al. (2004) Post et al. (2010)
	Genetic amplification of <i>MDM2</i>	$\sigma \uparrow$	Momand et al. (1992)
	Loss of ATM	$\delta \downarrow$	Khosravi et al. (1999) Maya et al. (2001)

Parametric conditions for sustained p53 oscillations. Next, we sought to understand how the system would respond to simultaneous changes in multiple parameters. A single oncogenic event can alter more than one biophysical parameter, such as p53 hotspot mutations and the loss of ATM (Table 1). Also, multiple oncogenic events can co-occur in tumors, for instance, p53 mutations and loss of heterozygosity on chromosome 17p (Donehower et al., 2019).

Moreover, while simulations can reveal the presence of periodicity, it is important to ask whether a true qualitative change has occurred, leading to the presence of a *limit cycle attractor*, with a preferred frequency and, importantly, stable under a variety of perturbations and changes of initial conditions. These are all highly desirable properties for the cell's DNA damage response system (for this point see also Wagner et al. (2005)).

We therefore looked for limit cycle attractors in our model using Hopf Bifurcation Theory (*Methods*). There is only one equilibrium point of the p53-Mdm2 system at which p53, *MDM2* transcript and Mdm2 levels are all positive. By evaluating the eigenvalues of the Jacobian matrix at this equilibrium point, we derived the criteria for the emergence of a limit cycle attractor:

$$p < 0, \\ p^2 - 4q < 0.$$

Here, $-p/2$ is the real part of the pair of complex conjugate eigenvalues; $p^2 - 4q < 0$ is required for the eigenvalues to be complex conjugate; p and q are functions of model parameters.

Note that p53 oscillations emerge by subcritical bifurcations (Fig. 1F), meaning that they emerge full-blown at a finite amplitude, rather than growing slowly from zero. This is also desirable for the DNA damage response system, as was pointed out by Ciliberto et al. (2005). Two types of bifurcation have this property: subcritical Hopf bifurcation and saddle-node-loop bifurcation. Saddle-node-loop bifurcation is ruled out here because there is only one equilibrium point at all parameter value combinations we examined.

Loss of ATM jeopardizes p53 oscillations upon DNA damage. We used the criteria for the emergence of a limit cycle attractor to study how ATM regulates the p53-Mdm2 system in response to DNA damage. When cells are non-stressed, p53 concentration is maintained at very low levels. Following DNA double strand breaks (DSBs), p53 and Mdm2 exhibit oscillatory behavior, which can last for several days (Batchelor et al., 2008; Geva-Zatorsky et al., 2006). Upon DSBs, ATM activates p53 by inhibiting Mdm2-mediated p53 degradation (primarily lowering β (Banin et al., 1998; Cheng et al., 2009; Shieh et al., 1997)) and mediating fast degradation of Mdm2 (increasing δ (Khosravi et al., 1999; Maya et al., 2001; Stommel & Wahl, 2004)) (Fig. 2A). To examine the joint effect of these two biophysical processes, we performed Hopf bifurcation analysis using β and δ as the bifurcation parameters, while keeping other parameters constant. Intriguingly, the conditions for Hopf bifurcation are satisfied within an ‘island’ in β - δ parameter space (Fig. 2C; Fig. S1A, B).

We therefore suggest that the action of ATM in creating the oscillatory response upon DSBs can be explained as a Hopf bifurcation induced by ATM’s impact on lowering β (Mdm2 inhibition of p53) and increasing δ (degradation of Mdm2), representing a shift of system state in β - δ parameter space (the solid arrow in Fig. 2C), from a non-oscillatory region (the green circle in Fig. 2C) to the critical region where oscillation is self-sustained (the gray region in Fig. 2C). The best-fit parameter combination (the red dot in Fig. 2C) resides inside, but near the boundary of the critical region. It is plausible that reduced levels of ATM, due to mutations or copy number loss, is insufficient to carry the system into the critical region after DNA damage (dashed arrows in Fig. 2C), rendering the p53-Mdm2 system incapable of oscillation. And indeed, ATM inhibitors dampen p53 oscillation upon treatment with the radiomimetic drug neocarzinostatin in multiple cancer cell lines with wild type p53 (Stewart-Ornstein & Lahav, 2017). On the contrary, DNA-dependent protein kinase inhibitors that *hyperactivate* ATM increase p53 abundances but do not trigger oscillations (Stewart-Ornstein & Lahav, 2017), possibly due to over-transiting the system beyond the critical region (the dotted arrow in Fig. 2C; Fig. 2B).

In contrast to the dual effect of ATM on model parameters, a horizontal shift leftward from the green region in the parameter space alone would bring the system closer to the Hopf bifurcation zone, to a region where the system has a damped oscillatory response (Fig. 2C). It would also result in an increase in p53 levels (Fig 2B). These two changes combine to produce a prolonged pulse of high amplitude (see Fig 1E for low values of β), as seen in ATR-mediated p53 response to ultraviolet radiation (Batchelor, Loewer, Mock, & Lahav, 2011).

Figure 2: ATM mediates the onset of p53 oscillations. (A) The impact of ATM on the p53-Mdm2 system in response to DNA DSBs. (B) The equilibrium concentration of p53 as a function of parameters β and δ , describing a surface over the β - δ plane. (C) Hopf bifurcation analysis identifies the critical region in the β - δ parameter space, where the p53-Mdm2 system displays a stable oscillatory response to DNA damage. The gray region specifies the parameter subspace where p53 oscillations are self-sustained ($p < 0$ and $p^2 - 4q < 0$); while p53 concentration goes to equilibrium in the white region. The green circle denotes a hypothetical region where the p53-Mdm2 system operates under non-stressed conditions. The red dot denotes the best-fit combination of β and δ for DSB conditions. ATM activates p53 by shifting the state of the system into the critical region (the solid arrow). Loss of ATM would compromise this transition (dashed arrows); hyperactive ATM results in over-transition (the dotted arrow). DSBs: double strand breaks.

Oncogenic alterations abolish p53 oscillations. The remaining oncogenic alterations affect the parameters α , β and σ (Table 1). Considering that β and σ co-occur as a product throughout the analytic calculation (*Methods*), we let α and $\beta\sigma$ be the bifurcation parameters for the Hopf bifurcation analysis while keeping the other parameters constant. The equilibrium point of this system (Fig. 3A) was stable when the values of α or $\beta\sigma$ were relatively small. Importantly, we identified an area in the α - $\beta\sigma$ plane where the parametric conditions of Hopf bifurcation were fulfilled, ensuring robust p53 oscillation (Fig. S2). Similarly, Hopf bifurcation analysis on α , $\beta\sigma$ and δ revealed a Hopf subspace (Fig. 3C). Note that the best-fit parameter combination (the red sphere) is located within the Hopf subspace, but fairly near the boundary, where a modest shift in parameter values could result in a qualitative change in the dynamic behavior of the p53-Mdm2 system, resulting in a loss of p53 oscillations.

In this dynamical systems framework, there are four routes for diverse oncogenic alterations to impair p53 oscillations during DNA damage response (Fig. 3C; Movie S1).

First, p53 missense mutations reduce the p53-dependent *MDM2* transcription rate (lowering σ (Kato et al., 2003; Park et al., 1994); p53 hotspot mutations also reduce the Mdm2-dependent p53 degradation rate (lowering β (Alexandrova et al., 2015; Li, Marchenko, & Moll, 2011; Li, Marchenko, Schulz, et al., 2011; Peng et al., 2001)), pushing the system out of the Hopf subspace (Fig. 3C). We observed that small values of $\beta\sigma$ result in high steady-state p53 levels (Fig. 3A), which explains the accumulation of mutant p53 in tumor-derived cells as well as the elevated p53 levels in cells transformed by tumor viruses such as SV40 and adenovirus type 5 (Table 1). For extremely small values of $\beta\sigma$, p53 levels grow without bound (Fig. 3B), reconciling the observation that the Mdm2 inhibitor Nutlin-3, known to block Mdm2-dependent p53 degradation, potentially abrogates p53 oscillations and increases p53 levels greatly (Purvis et al., 2012; Stewart-Ornstein & Lahav, 2017). In addition, given the span of the Hopf subspace for lower $\beta\sigma$ values (Fig. S2; Movie S1), we reason that p53 missense mutations that moderately reduce the transactivation activity of p53 would be tolerated and have limited pathological consequences, as has been reported in functional screens of p53 mutants (Giacomelli et al., 2018; Kotler et al., 2018). In these cases, an additional loss of p53 would assist to compromise the oscillatory competence of the p53-Mdm2 system, plausibly accounting for the prevalent loss of heterozygosity in tumors with mutant p53 (Donehower et al., 2019).

Second, a loss of p53, due to inherited cancer risk polymorphisms, truncating mutations or allelic deletion in *TP53*, decreases the production rate of p53 (lowering α (Donehower et al., 2019; Livingstone et al., 1992; Macedo et al., 2016; Venkatachalam, 1998; X. Wang & Sun, 2017; P. Zhang et al., 2019)), dropping the system out of the Hopf subspace (Fig. 3C). Phenotypically, the loss of p53 oscillations due to germline mutations in *TP53* could account for the contribution of genotoxic therapies to the development of subsequent primary tumors among individuals with Li-Fraumeni syndrome (Bougeard et al., 2015; Kasper et al., 2018).

Third, genetic amplification or a certain inherited polymorphism increases *MDM2* transcription rate (elevating σ (Bond et al., 2004; Momand et al., 1992; Post et al., 2010)), readily disrupting p53 oscillations upon DNA damage (Hu et al., 2007). Overexpression of Mdm4 due to inherited polymorphisms or copy number gain has a net effect of increasing $\beta\sigma$ (Table 1; *SI Appendix*). Of note, the viral oncoprotein HPV E6 enhances p53 degradation (increasing β (Scheffner, Werness, Huibregtse, Levine, & Howley, 1990; Werness et al., 1990)). These alterations all bring the system out of the Hopf subspace (Fig. 3C).

Lastly, we showed that ATM mediates the onset of p53 oscillations through its impact on β and δ (Fig. 2C), thus a loss of ATM keeps the system out of the Hopf subspace during DNA damage response (Fig. 3C).

Together, despite that oncogenic alterations take multiple routes to alter key biophysical parameters of the p53-Mdm2 system, they have the common effect: p53 oscillations are abolished.

Figure 3: Oncogenic alterations take multiple routes to abolish p53 oscillations. (A) The equilibrium concentration of p53 is a function of parameter α and $\beta\sigma$, which describes a surface over the α - $\beta\sigma$ plane. (B) For small values of $\beta\sigma$, the equilibrium concentrations of p53 are shown in a heatmap. The p53-Mdm2 system loses its stable equilibrium point when $\beta\sigma$ is extremely small (white region), and the p53 concentration grows unbounded in time during DNA damage. (C) Hopf bifurcation analysis, by calculation of eigenvalues, indicates that oscillatory behavior of the p53-Mdm2 system is self-sustained in a parameter subspace of α , $\beta\sigma$ and δ , enclosed by the gray surface and annotated as "Hopf" ($p < 0$ and $p^2 - 4q < 0$). The red sphere marks the best-fit combination of parameter values. The arrows indicate the impact of broad-category oncogenic alterations on parameter values.

3 Discussion

Physiological processes were long thought to be governed by static equilibria. But that concept, the doctrine of homeostasis, is giving way to a more dynamical view of physiological processes, many of which are characterized by oscillatory modes of operation (Boiteux, Hess, & Sel'Kov, 1980; Ferrell, Tsai, & Yang, 2011; J. H. Levine, Lin, & Elowitz, 2013). The emerging picture is that health consists of the successful coordination of many oscillatory processes; conversely, disease may often manifest as a loss of these oscillatory processes and/or a loss of their coordination (Buhr, Yoo, & Takahashi, 2010; Glass, 2001; Noble, 2006; Tu, Kudlicki, Rowicka, & McKnight, 2005).

Our results, together with previous findings, suggest that this may well be the case for p53, since, in our model, the functional response to DNA damage is to generate oscillations in the p53-Mdm2 system, whereas oncogenic alterations all disrupt p53-Mdm2 oscillations, as predicted by Hopf bifurcation theory. Thus, genetic alterations related to cancer, which were largely established through correlation or experimental manipulation, can now be understood mechanistically: diverse oncogenic alterations in the p53 pathway drive cancer by abolishing p53-Mdm2 oscillations.

Hypothesis: A Role For ARF. Besides coordinating DNA damage response, p53's role as a tumor suppressor also manifests in response to oncogenic signaling such as the overexpression of Myc, E1A, or E2F-1 (D. Chen, Shan, Zhu, Qin, & Gu, 2010; Efeyan, Garcia-Cao, Herranz, Velasco-Miguel, & Serrano, 2006). Upon oncogenic activation, the tumor suppressor ARF triggers p53-dependent cell cycle arrest and/or apoptosis (Kamijo et al., 1997). Mice lacking ARF are highly prone to tumor development (Kamijo, Bodner, van de Kamp, Randle, & Sherr, 1999), and deficiency in ARF underlies multiple cancer types in humans (Knijnenburg et al., 2018; Sherr, 1998). ARF activates p53 by inhibiting Mdm2-mediated degradation of p53 and accelerating Mdm2 degradation (Pomerantz et al., 1998; Weber, Taylor, Roussel, Sherr, & Bar-Sagi, 1999; Y. Zhang, Xiong, & Yarbrough, 1998). Since this is also the mechanism of ATM-induced p53 oscillations during the DNA damage response (Fig. 2), it is likely that ARF triggers p53 oscillations in response to oncogenic activation. If true, a loss of ARF could also abolish the oscillatory competence of the p53-Mdm2 system, thus promoting oncogenesis.

Therapeutic Implications. Small molecules APR-246 and COTI-2 can reactivate missense-mutant p53 by reversing its oncogenic effect of lowering σ , thereby bringing the p53-Mdm2 system back to the oscillatory regime (Bykov et al., 2002; Lambert et al., 2009; Salim, Maleki Vareki, Danter, & Koropatnick, 2016). Their therapeutic efficacy is currently being tested in clinical trials (Bykov, Eriksson, Bianchi, & Wiman, 2018). Progress has also been made to reactivate nonsense-mutant *TP53*. For example, induced translational readthrough of *TP53*^{R213X} has been shown to reverse the oncogenic effect of lowering α due to a truncating mutation (Bidou, Bugaud, Belakhov, Baasov, & Namy, 2017; Floquet, Deforges, Rousset, & Bidou, 2011). Our analysis suggests that concepts like “stabilizing” and “unleashing” p53 must be interpreted in terms of p53’s oscillatory competence, not just its steady-state levels. In this regard, our unified framework of diverse oncogenic alterations in the p53 pathway could guide future therapeutic development to restore p53 oscillations.

Apart from the four parameters considered above, we observed that the remaining parameters (γ , κ , ϕ and ϵ) could also modulate the dynamic behavior of the p53-Mdm2 system (Fig S3). Although these parameters are not affected by oncogenic alterations, they are potentially modifiable by pharmacological interventions.

4 Methods

Mathematical modeling. Analytic calculations and numerical simulations of the mathematical model were performed in MATLAB (MathWorks). The parameter values we used are shown in Table S1. The justification for these values is as follows: α , β , γ : taken from Tsabar et al. (2020); σ : taken from Mönke et al. (2017), rescaled to agree with Tsabar et al. (2020); κ : taken from Mönke et al. (2017); the parameters σ , ϵ , ϕ and δ form a group, all affecting Mdm2 dynamics. As a reality check, we verified that the chosen set of parameters produced oscillations in p53 protein levels with the characteristic period of 5-6 hours Lahav et al. (2004), as well as oscillations in *MDM2* transcript and Mdm2 protein levels that are consistent with experimental observations (Hanson et al., 2019). We tested three different sets of parameter values and found that the qualitative behavior of the model was robust under all three sets of parameter values (Table S2; Fig. S4).

Hopf bifurcation analysis. In dynamical systems theory, a qualitative change in system behavior as a parameter varies is called a bifurcation, and the emergence of a stable limit cycle attractor from a stable equilibrium point as parameters vary is called Poincare-Andronov-Hopf bifurcation, usually shortened to *Hopf bifurcation* (Garfinkel, Guo, & Shevtsov, 2017).

The mathematical signature of Hopf bifurcation is given by linear stability analysis of the equilibrium point(s) of a system, that is, by the eigenvalue structure of the equilibrium point. The Hopf Bifurcation Theorem tells us that when a pair of complex conjugate eigenvalues change from negative real part to positive real part, the equilibrium point will become unstable, and a new stable oscillation, a limit cycle attractor, will be created. Therefore, we can analytically calculate the critical regions for oscillation in parameter space by calculating how the eigenvalue structure depends on those parameters. We used these tools of dynamical systems theory to evaluate the Hopf bifurcation behavior of the p53-Mdm2 model, and to derive the parametric conditions under which p53 oscillations can be sustained.

Location of Equilibrium Points. First, we looked for equilibrium points of the p53-Mdm2 system at which p53 (X_{EP}), *MDM2* transcript (R_{EP}) and Mdm2 (Y_{EP}) levels are all positive.

At an equilibrium point, we have $\frac{dX}{dt} = \frac{dR}{dt} = \frac{dY}{dt} = 0$. That is,

$$\begin{aligned} \frac{X_{EP}^2}{(\gamma + X_{EP})(\kappa + X_{EP})} &= \frac{\alpha\epsilon\delta}{\beta\sigma\phi}, \\ R_{EP} &= \frac{\sigma}{\epsilon} \frac{X_{EP}}{\kappa + X_{EP}}, \\ Y_{EP} &= \frac{\sigma\phi}{\epsilon\delta} \frac{X_{EP}}{\kappa + X_{EP}}. \end{aligned}$$

Hence, the equilibrium solutions are:

$$X_{EP} = \frac{-\alpha\epsilon\delta(\gamma + \kappa) \pm \sqrt{(\alpha\epsilon\delta)^2(\gamma + \kappa)^2 - 4(\alpha\epsilon\delta - \beta\sigma\phi)(\alpha\epsilon\delta\gamma\kappa)}}{2(\alpha\epsilon\delta - \beta\sigma\phi)}.$$

This equation gives an analytic solution of the steady state p53 concentration as a function of the eight model parameters.

Stability of Equilibria. Next, we performed linear stability analysis of the system at the equilibrium point in order to derive conditions for the oscillations in the p53-Mdm2 system. The Jacobian matrix evaluated at the equilibrium point has the form:

$$J = \begin{pmatrix} -\frac{\alpha\gamma}{X_{EP}(\gamma+X_{EP})} & 0 & -\frac{\beta X_{EP}}{\gamma+X_{EP}} \\ \frac{\sigma\kappa}{(\kappa+X_{EP})^2} & -\epsilon & 0 \\ 0 & \phi & -\delta \end{pmatrix}.$$

The eigenvalues (λ) of the Jacobian matrix are roots to the characteristic (cubic) equation:

$$\lambda^3 + \left(\frac{\alpha\gamma}{X_{EP}(\gamma+X_{EP})} + \epsilon + \delta\right)\lambda^2 + \left(\frac{\alpha\gamma(\epsilon+\delta)}{X_{EP}(\gamma+X_{EP})} + \epsilon\delta\right)\lambda + \alpha\epsilon\delta\left(\frac{\gamma}{X_{EP}(\gamma+X_{EP})} + \frac{\kappa}{X_{EP}(\kappa+X_{EP})}\right) = 0.$$

A cubic equation must have at least one real-valued solution. If we denote the real-valued solution as λ_{real} , we can factor the cubic equation as the following:

$$(\lambda - \lambda_{real})(\lambda^2 + p\lambda + q) = 0.$$

Then the other two roots are: $\lambda_{1,2} = \frac{-p \pm \sqrt{p^2 - 4q}}{2}$. By expanding the factored cubic equation, we derive:

$$p = \frac{\alpha\gamma}{X_{EP}(\gamma+X_{EP})} + \epsilon + \delta + \lambda_{real},$$

$$q = -\frac{\alpha\epsilon\delta}{\lambda_{real}}\left(\frac{\gamma}{X_{EP}(\gamma+X_{EP})} + \frac{\kappa}{X_{EP}(\kappa+X_{EP})}\right),$$

where both X_{EP} and λ_{real} are functions of the eight model parameters.

At a Hopf bifurcation point, a pair of complex conjugate eigenvalues passes from having a negative real part to having a positive real part. It follows that a limit cycle attractor is created when the following two conditions are satisfied:

p changes from $p > 0$ to $p < 0$ (the real part becomes positive), and

$p^2 - 4q < 0$ (the imaginary part exists).

Further details are provided in *SI Appendix*.

All data and code are available at: https://github.com/LingyunXiong/p53_oscillations.

Competing interests

Authors declare no competing interests.

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