RESEARCH

Modelling the influence of TSC and PTEN on mTOR pathway for cancer treatment

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Abstract

In most instances of tumor development, cancer cell populations develop features to respond to selection barriers, such as physical constraints or immune responses, and rapidly adapt to an always changing environment. In the present paper a particular feature is studied, the capacity of sustaining its proliferation over time. By means of Michaelis Menten dynamics, the mTOR cascade, involved in cell proliferation is studied and a model around its interactions is built. Bifurcation diagrams are analyzed in order to understand the behavior of the cascade and its interactions when cancer comes into play. The results show a wider affection of PTEN molecule (a tumor suppressor) on cancer than TSC (a kinase that aims to regulate mTOR activity by means of mutual inactivation).

Keywords: Cancer; mTOR; TSC; PTEN; Proliferative Signaling; Hyteresis

1 Introduction

The Hallmarks of Cancer are six capabilities acquired during the development of human tumors. These characteristics are the ones that enable cancer cells to proliferate involving huge dynamic changes in the genome. Douglas Hanahan and Robert A. Weinberg proposed these characteristics back in 2000 [1] which consisted of sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis.

In the present paper we are going to focus on the first and most studied Hallmark, sustaining proliferative signaling. The authors believe that this is the most fundamental characteristic of cancer cells, because it is based on the fact that cancer cells have an abnormal growth. Normal tissues have an accurate control of their own growth in order to ensure homeostasis and maintain their architecture and function. Cancer cells can deregulate the signals that regulate the progression through the cell cycle as well as cell growth. This capability is acquired in different ways. Producing growth factor ligands themselves, sending signals to normal cells that can also be found in the tumor stroma, elevating the levels of receptor proteins at their surface (making the cell hyperresponsive) and also the making the activation of components constitutive, such as the Ras signal transducer, that recapitulates a subset of the regulatory instructions transmitted by an activated receptor.

For normal behavior of pathways, there exists an extreme importance of negative feedback loops that normally operate to dampen various types of signaling and thereby ensure homeostatic regulation of the flux of signals coursing through the intracellular circuitry [3] [4] [5]. Defects in these feedback mechanisms are capable of enhancing proliferative signaling. We are going to study a very specific pathway that disrupts two tumor suppressor genes, in such a way that makes the cell proliferation increase. This pathway is the one that involves mTOR kinase, a coordinator of cell growth and metabolism that lies both upstream and downstream of the PI3K pathway.

mTOR belongs to the atypical kinase family of phosphotidylinsoitol-3-kinase-related kinases. The diseases in which mTOR has been implicated include cancer, obesity and diabetes, diseases of the eye, neurodegenerative disorders and cognitive dysfunction. mTOR is a highly conserved serine-threonine protein kinase that belongs to the 6-member PIKK family of proteins and has a high degree of homology to the lipid kinase PI3K. Yeast have two TOR genes, TOR1 and TOR2, both of which participate in two functionally distinct complexes that are known as TORC1 and TORC2, respectively.

The two mTOR complexes have distinct interactions with their respective substrates and regulators, leading

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to the diverse functional roles of mTOR in processes such as nutrient, energy and amino acid sensing, the initiation of transcription, the regulation of protein translation, cell proliferation and autophagy. Thus, every single function listed can be directly related with the cell proliferation, and in consequence, any disruption of this pathway leads to an increase of it.

The activation of mTOR by the PI3K-Akt axis has been thoroughly investigated. In response to binding insulin, IGFR activates an intracellular signaling cascade mediated by PI3K. The lipid phosphatase PTEN (phosphatase and tensin homolog) negatively regulates PI3K signaling and is frequently deleted in tumors. An important target of PI3K signaling is the protein kinase Akt, which is involved in a variety of biological functions, including survival, growth and apoptosis. Tuberous sclerosis complex (TSC), which consists of TSC1 and TSC2, is an inhibitor of mTOR. Akt phosphorylates and inactivates TSC, thereby relieving the inhibition of mTOR by TSC.

Our approach is grounded in the formulation of a straightforward hypothesis: both tumor suppressors have a direct affection to cell proliferation, but the processes in which they are involved are different, due to the nature of this differences we expect different dynamical behaviors. We are aware about the complexity related to these genetic pathways, they are involved in signaling cascades, so more species are recognized creating intermediate loops or collateral inhibition or amplification. Our aim is to detect the principal pathway that gives the major dynamical response and show, both mathematically and numerically, how connected are these two endogenous tumor suppressors (PTEN and TSC) to the signalling cascade related with cell proliferation. Since they are well studied processes in the cell there are estimated parameters related with rates or basal concentrations that will help us to do accurate models, and subsequently, to identify the most interesting pharmaceutical target between them.

2 Model Description

We have developed a model for the cascade in Figure 1, which is a simplified version of the one explained by S. Sudarsanam and D. E. Johnson [2]. We aim to mimic and understand cancer dynamics in this particular cascade, which is involved in many pathways as explained in the introduction section. Since this cascade consists basically of enzyme interactions, we are going to model it though Michaelis-Menten dynamics, taking into account activation and deactivation dynamics as in figure 2.

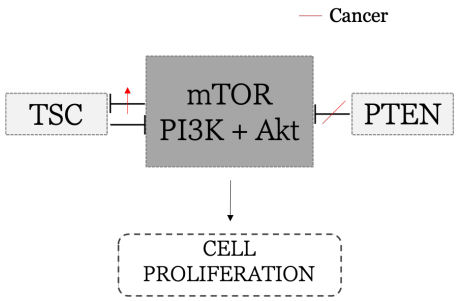

Figure 1 Basic scheme of the model analyzed in this paper. The main complex, mTOR(PI3K+Akt) is the main pathway for cell proliferation. In order to control it, there are two main molecules, TSC, which interacts negatively forward and backward mTOR(PI3K+Akt) and PTEN, which inactivates the complex directly

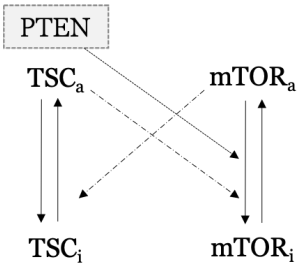

Figure 2 In this figure, PTEN acts as the protein rising from its tumor suppressor precursor gene. TSC and mTOR have kinase activity, for this reason, they can be either in its active or inactive states.

We can consider a system of three equations that interact in a way that each one has activation and inactivation dynamics over the others. Let us assume that we are in a "quasi" steady-state situation (only valid when the substrate concentration is greater than the enzyme concentration, and for steady state conditions).

We have assumed that PTEN depends only on itself, since their dynamics would be a simple equation of growth and decay because the mTOR complex does not interact with it. We have decided to model it through a parameter (α_{pten}), that can be changed depending on the existence of a neoplastic situation. PTEN's biologic function consist of inhibiting the PI3K+Akt complex.

2.1 Equations

The concentration of mTOR is not constant and it depends on its entry by the previous stages of the cascade (S). Moreover, mTOR is deactivated by PTEN in

normal situations in order to control excessive cell proliferation. Its interactions with TSC enzymes produce deactivations and activations considered in its equations (either activated x_a and x_i). The inactive version of mTOR (x_i) does not have cascade income, since it is assumed that all the mTOR entrying is activated. On the other hand, we considered TSC (y) as a constant molecule, with the same levels of activation and inactivation produced by mTOR (x_a). Then, the amount of TSC is defined by $Y_T = Y + Y_{inact}$.

$$\frac{\mathrm{d}x_a}{\mathrm{d}t} = -\alpha_{pten}x_a - \delta_a x_a - \frac{\beta_a y x_a}{k_a + x_a} + \frac{\beta_i x_i}{k_i + x_i} + S \quad (1)$$

$$\frac{\mathrm{d}x_i}{\mathrm{d}t} = \alpha_{pten}x_a - \delta_{xi}x_i + \frac{\beta_{xa} y x_a}{k_{xa} + x_a} - \frac{\beta_{xi}x_i}{k_{xi} + x_i} \quad (2)$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = \frac{\beta_{ya} y x_a}{k_y + y} - \frac{\beta_{yi}(y_T - y)}{k_y + (y_T - y)} \quad (3)$$

Eq. (1) belongs to the active version of mTOR, and it has several parameters that need to be explained. The inactivation carried out by PTEN is modelled though the constant α_{pten} that specifies the strength of the interaction and can be modified in cancer situations as Figure 1 shows in red. Moreover, it has its own degradation (not constant concentration) and its interactions of deactivation (affected by TSC (y)) and activation, modelled by β_a and β_b defining the strength of the events and the Michaelis constants k_a and k_i, which are defined as the substrate concentration with which the enzymatic reaction rate reaches a value equal to half the maximum speed.

Eq. (2) is the contrary version of Eq. (1), where all the concentration lost due to inactivation is its input (e.g. PTEN inactivates mTOR). As a consequence, its output are the activation terms.

The last equation (3) of our system corresponds to TSC activity, which is slightly different than the previous cases. Now, there is no creation or destruction of molecules because it is considered to be in abundance, therefore it has constant production and decay. For this reason, only the Michaelis terms are considered with their respective constants.

2.2 Parameters

In order to simulate the model the most realistic as possible, we have searched in the literature some parameters for growth, decay and different interactions (Michaelis contstants, activation constants, etc). In Table 1 it is possible to find the parameters used for the healthy situation.

Parameter	Value
α_{pten}	0.3
δ_a^*	0.1
β_a^*	10
k_a	1
β_i	0.01
k_i	1
δ_i	0.1
S^*	1.2
β_{ya}^*	0.02
k_y	1
β_{yi}^*	0.017762
k_y	1
y_T	0.5

Table 1 Parameter values. The * values are taken from [7], the others have been discovered experimentally, testing our model.

3 Results

The system is studied from a healthy environment scope, where cancer has no effect on the parameters. Then, we will use modifications that according to our model lead to cancer and see the main affection in order to discuss which is the best target to overcome the hallmark related with sustaining proliferative signaling. Our analysis is addressed to know the stability of solutions of the lately exposed differential equations and trajectories of the described dynamical systems under small perturbations of initial conditions, realistic rates and estimated parameters. We have modelled everything with *Python* [1].

3.1 Integration

In order to study the dynamics of the system, the evolution of the healthy environment as a function of time is studied. To integrate the ordinary differential equations described in Eq. (1), Eq. (2) and Eq. (3), *Scipy Python Library Odeint* [2] is used. The evolution of time has been represented using a vector from 0 to 500 units, in steps of 0.5. The initial concentration introduced for the three variables (x_a, x_i, y) is 0.1, since negative concentration would not have a real biological meaning and starting from 0 is not possible due to mathematical reasons.

3.2 Bifurcation Diagrams

The affection of PTEN concentration in mTOR activation is evaluated using a bifurcation diagram (Figure 4). The PTEN variable was assumed to be modified

[1] Python 3 - `https://www.python.org`

[2] Scipy Odeint - `https://docs.scipy.org/doc/scipy/reference/generated/scipy.integrate.odeint.html`

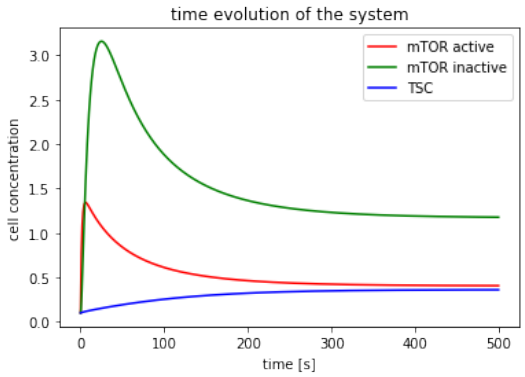

Figure 3 Evolution in time of the healthy system.

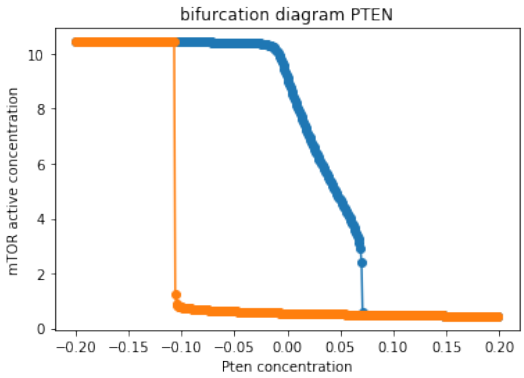

Figure 4 Bifurcation diagram of active mTOR depending on PTEN concentration.

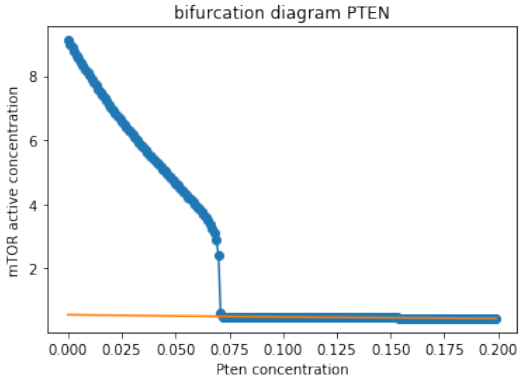

Figure 5 Bifurcation diagram of active mTOR depending on PTEN concentration, with the biological meaning (i.e., no negative concentrations).

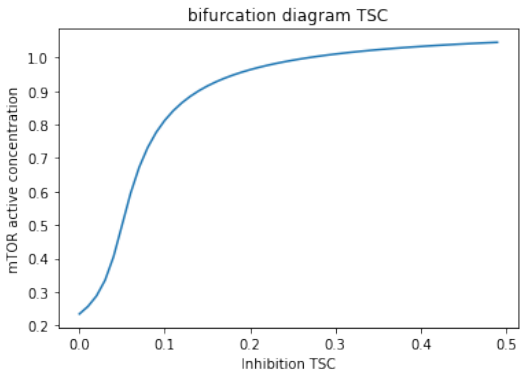

Figure 6 Bifurcation diagram of active mTOR depending on growing values of TSC inhibition

by changing its constant growing (α_{pten}), which was defined using a vector from -0.3 to 0.2 units of concentration, in steps of 0.01, to see the whole behavior. For each concentration, the steady state of the system was calculated using the same procedure as in *3.1. Integration*. Initial conditions for *odeint* in the first steady state (lowest number of α_{pten}) were also the same considered in *3.1. Integration*; however, for further α_{pten} values, initial conditions where defined as the steady state values for the step before, in order to have an optimized coding and computational velocity.

TSC effect on mTOR active was studied trough two different bifurcation diagrams (Figure 7, Figure 6).

On Figure 7, active mTOR steady state is plotted depending on growing values of TSC activation. That is to say, growing values of β_{ya}. This parameter was defined using a vector from 0.0 to 0.2 in steps of 0.01.

On Figure 6, mTOR active behavior is modified using growing values of TSC inhibition β_{yi}. It is described as a vector from 0 to 0.5, in steps of 0.01.

In both cases, initial conditions are the ones used in *3.1. Integration* for the lowest value of x-axes. For further steps, initial conditions used are the steady point of the step before, for the same reason mentioned in the case of PTEN.

4 Validation and Discussion

Our goal of producing an accurate and reliable model led us to the need of validation and verification of our simulation models. The idea was to study and explore new and more efficient targets in order to counteract the proliferation in cancer. To do so, it is important to verify that the behavioral approximation obtained is correct and consistent with the reality.

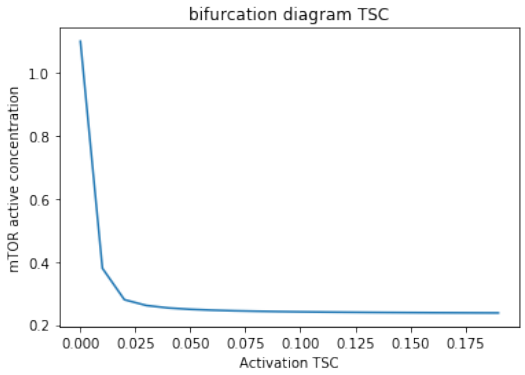

Figure 7 Bifurcation diagram of active mTOR depending on growing values of TSC activation

4.1 Validation

The validation of the simulations starts after functional specifications have been documented and initial model development has been completed. Our model is an approximate imitation of a real process and it does not exactly mimic the whole cascade's dynamics. Therefore, the model will be validated with different biological pathways in which our species are involved, but the biological meaning is completely different.

4.1.1 Validation of TSC-mTOR relation

The first verification is focused on the study of TSC-mTOR relation based on [3] from Xinsheng Gao et al. In the article they show that Tsc1 and Tsc2 can physically associate with TOR and function upstream of TOR genetically. In the experiments they decreased Tsc1 and Tsc2 obtaining as a result a TOR-dependent increase of S6K activity, a product of mTOR. Their hypothesis is that Tsc1–Tsc2 complex antagonizes the TOR-mediated response to amino acid availability. When the cell starts a proliferative process it is required the presence of amino-acids in order to produce new proteins.

These studies suggest that Tsc1-Tsc2 operates on a parallel pathway to insulin. Also that the TOR-dependent amino acid signalling pathway converges on the insulin one at a similar position prompted them to examine a possible relationship between Tsc1–Tsc2 and TOR.

The only available assay that accurately reflects the physiological regulation of TOR relies on measuring the activity of its downstream effectors, such as S6K2. Thus, to examine the potential regulation of TOR by Tsc1–Tsc2, they carried out RNA interference (RNAi) to specifically knockout Tsc1 or Tsc2 in Drosophila S2 cells and analysed S6K activity using a phospho-specific antibody that detects activated S6K.

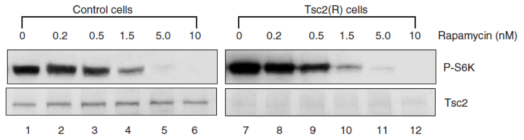

Figure 8 Obtained result from the studied article [3]. It is observable that as we increase TSC concentration, the analysed S6k quantity is decreased.

4.1.2 Validation of PTEN-mTOR relation

On the other hand, PTEN-mTOR verification is centered in the study [8] from Xihui Xu et al. The article takes into account the studied interaction between PTEN and mTOR downregulation. They hypothesize that the loss of PTEN is capable of promoting hyperactivation of mTOR in animal models and patients. It is widely conceived that mTOR orchestrates cardiac hypertrophy given its role in governing protein synthesis, then aberrant activation of mTOR has been shown to contribute to the onset and development of cardiac hypertrophy.

Although cardiomyocyte-specific knockout of PTEN did not affect survival in mice, it prompted a hypertrophic cardiomyopathy phenotype in adult mice. Echocardiography analysis depicted that CM-PTENKO overtly increased left ventricular (LV) wall thickness, LV end diastolic diameter, and LV end systolic diameter.

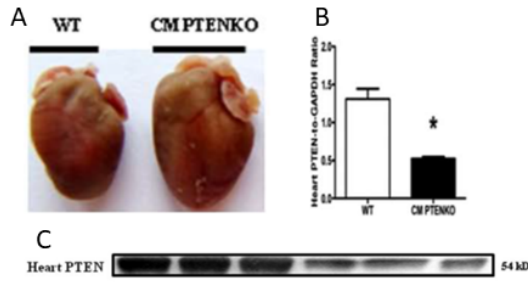

Figure 9 Obtained from [8]. **(A)** Representative gross images of hearts from WT and CM-PTENKO mice. **(B)** Hypertrophic cardiomyopathy in CM-PTENKO mice. Echocardiographic indices for wall thickness.**(C)**Representative gel blots depicting protein levels of PTEN in heart,

4.2 Discussion

4.2.1 Integration

We focused our attention in mTOR active species in order to discuss the results obtained in figure 3 to understand its relation with other species. Both active and inactive mTOR show the same dynamics because there is no mutual regulation between them. Every time mTOR active suffers any regulation, the result is the increase of mTOR inactive since they are the same species. As a result, it is coherent to see a greater concentration of mTOR inactive than active, since in healthy cases, cell proliferation is regulated.

Both mTOR active and inactive have an initial sudden increase according to the low concentration of TSC, an inactivator of mTOR active. Then, as TSC rises, the concentrations reach a stable situation, meaning that the system is balanced (i.e., healthy conditions).

The dynamics of mTOR active have a maximum value of concentration of 1.342 at the very initial steps and, after that, reaches its steady state value of 0.238. On the other hand, inactive mTOR reaches 3.156 just after the concentration of mTOR active starts to decrease and, at step 200 (approximately), the steady state is accomplished with a value of 1.177. TSC concentration starts from 0 increasing to its saturation at 0.361.

4.2.2 Bifurcation diagrams

The obtained results in figure 4 show hysteresis-like behavior. Hysteresis is the dependence of the state of a system on its history, that is, the initial conditions. In our case, with the selected parameters from table 1, we can distinguish to different behaviors; monostability and bistability.

When focusing on monostability, which corresponds to the extremes of figure 4, it only depends on the initial PTEN concentration. If it is more than 0.075, the mTOR concentration will be really low, corresponding to a healthy state. The left part would correspond to a negative concentration of PTEN, which has no biological meaning and, therefore, out of the scope of our study.

The most analyzable part of the diagram is the bistability. The singularity of these type of behaviors is that the stability of the fix points not only depends on the initial concentration of PTEN, but also, on the initial concentration of mTOR. What really interests us is the relation of this bistability and the consequences for cancer. For this reason, we focus on figure 5, where negative concentrations are not taken into account. In cancer PTEN concentration are reduced, so following the blue curve it is observable that in a certain value there is an abrupt phase transition into high rate production of mTOR, therefore our model describes the change from healthy state to cancer. In this diagram it is only observable the stable state, due to the methodology we used. However we do not see the unstable state we are aware of its existence, driving us to the conclusion that it is a saddle-node bifurcation.

From the obtained results in figure 6 it is observable a monostable state which describes the effect by increasing the inhibitory strength from mTOR to TSC. We are interested in the observable change from healthy state to cancer, which is due to the initial low inhibitory effect of mTOR and a continuous increasing of $beta_{yi}$. Even thought, if we compare the maximum concentration of mTOR after both modifications, a decreased PTEN concentration and increased inhibitory effect of mTOR to TSC, we see that PTEN has a grater impact in proliferation. Specifically, around the order of 8 times more.

Figure 7 shows the representation of the mTOR behavior depending on the activation of TSC, in order to see how it affects cell proliferation. The ideal pharmacological treatment for this pathway would be the increase of active TSC in order to have more inhibitor to reduce mTOR activity. What happens is that this activity is not highly reduced, possible due to the constant interaction between TSC and mTOR, action that does not happen with PTEN.

In the light of these results, we conclude that the best treatment that can rise from our model is the action over PTEN, because it is the one with biggest affection in controlling cell proliferation.

5 Future work

We have studied the behavior of cancer concerning to the emergence of cancer in a cell and its consequences. As seen in the results, we have obtained an hysteresis loop, but actually its significance appears only with positive concentrations of PTEN (figure 5).

Taking this into account, this behavior reflects only a transition from healthy to cancer state, but a possible further development of the model is the study of new parameters to move the whole diagram towards the positive concentrations. This way, we could be able to analyze a transition from cancer state to healthy, which would correspond to a recovery.

6 Bibliography

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