Mathematical Models in Cancer Therapy

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Abstract

In this article, deterministic mathematical models are derived from biochemical models within a human cell in two distinct cases, for comparison: healthy cell and cancerous cell. The former model is based in the cell cycle model by Novak and Tyson and its adaptation by Conradie, and makes use of the MAPK cascade pathway and the PI3K/AKT pathway for signalling transduction, to create a wider updated model for the regulation of a healthy cell. The latter model, for the cancer cell, is derived from the healthy cell model by altering specific pathways and interpreting the outcome in the light of literature in cancer. This last study is done in two approaches: simulation of common deregulations and specific cancer simulation, colon cancer. After studying both models, we propose targeting therapies and simulate their consequences. We thus explore mathematical modeling efficacy and usefulness in providing enough information from which to derive ideas for therapies. The purpose is to validate mathematics, once again, as a powerful tool with which one can model the underlying nature of chaotic systems and extract useful conclusions to real-life problems.

I. Introduction

Cancer is one of the most deadly diseases among humanity in great part due to the large amount of variables which have to be taken into account in its development and dynamics, making it particularly difficult to approach therapeutically. The understanding of how cancer mechanism works starts with understanding how a healthy cell behaves, since the differences between cancer dynamics and healthy tissue dynamics are a reasonable object of analysis in cancer theory.
When a single mammalian cell fails to stop cell cycle when it needs to, proceeding to replicate and originate offspring with anomalies, it can quickly develop a tumor whose priority is to grow and divide uncontrollably, selfishly wearing all resources in its environment, destabilizing its neighbouring healthy cells in the tissue and, consequently, the whole organism. The study of individual healthy and cancerous cells dynamics is therefore an understandable approach for cancer therapy development and is the one we discuss in this paper.

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II. Preliminaries

i. The Cell Cycle Regulation

The Cell Cycle of Eukaryotic cells can be divided in two main events: replication of DNA, known as S phase, and Mitosis, known as M Phase, followed by cytokinesis [6]. Between S phase and M phase the cell enters G_1 and G_2 phase, in which different concentrations of biomolecules change. When it is not in any of these phases, it means it is in quiescence state, the so called G_0 phase, or is preparing itself for apoptosis, i.e. programmed cell death.
The biomolecules that regulate this cycle are the Cyclins (Cyc's) and the Cyclin Dependent Kinases (CDK's), proteins and enzymes, respectively. To enter the cycle from G_0 phase, some external signal must be transducted through the cell's cytosol reaching the nucleus and

promoting transcription of CycD and CDK4,6. During the transition between G_1 and S phase, CycE/CDK2 complexes increase their concentration in the cytosol, allowing for the transcription of CycA and CDK2, which, in the form of complex, promotes the movement to the G_2 phase of the cycle, where CycA/CDK1 complexes are predominant, leading to the passage to Mitosis, where, in turn, CycB/CDK1 complexes are in abundance. This completes the cycle of concentrations of Cyclin/CDK complexes, right before the cytokinesis event, that divides the cell in two daughter cells.
Activation of the anaphase-promoting complex (APC) by binding of cell-division cycle protein 20 (CDC20) and cadherin 1 (CDH1) is necessary for exit from mitosis.

ii. The Restriction Point Regulation

The previous section resumed what is known about the Cell Cycle regulation. The dances of Cyclins and CDK's are the mechanism that promote the advance of the cell through the cycle. However, at some specific point in the cycle, the cell no longer needs extracellular signals to proceed. In the late G_1 phase there is some device that allows the cycle to continue regardless of mitogenic activity at the membrane. This point, called *Restriction Point*, was set between the 3^{rd} and the 4^{th} hour of G_1 phase [7].
In human eukaryote cells, the Retinoblastoma Protein, Rb, whose transcription is done from a genetic sequence found in chromosome 13 [42], plays an important role in regulating the restriction point along with E2F transcription factor. Active E2F migrates to the nucleus of the cell where it promotes DNA replication, initiating S phase. Active Rb binds to E2F, deactivating E2F and thus inhibiting the passage to S phase. Rb is activated in its hypophosphorylated form, and is deactivated in its phosphorylated form. CycD-CDK4/6 complexes inhibit active Rb, phosphorylating it partially, leading to a partial activation of E2F. PP1 phosphatase dephosphorylates Rb, increasing the concentration of active Rb and thus promoting the inhibition of E2F. Along with CycE/CDK2 complexes, E2F promotes the passage through the G_1-S phases frontier, hence leading to DNA replication, independent of further mitogenic signals. At this point, the cell enters in automatic program.

iii. Cell cycle arrest and Apoptosis

The cell cycle can be disrupted by the cell itself if something is not according to the regulation we summarized in the previous sections, and in some cases this conduces the cell to a specific fate called apoptosis, i.e., programmed cell death. Apoptosis is a mechanism of defense developed to protect multicellular organisms from malformations in cell development and/or activity, for it conduces the cell to destroy itself without damaging neighboring cells. It does so by shrinking, condensing, tearing up its outer layers and breaking the DNA into fragments [6].
When DNA is damaged, the ATM ("ataxia-telangiectasia mutated") kinase is activated, culminating in p53 concentration increase, which in turn gives place to a sequence of events that turn on Caspase-9 and ultimately induces apoptosis. The details on this mechanism are far too extensive for the purpose of our model, which is why we kept the apoptotic dynamics fairly simple, as we explain in the updated model section.

iv. The p53 pathway

The p53 gene, found in 1979 by separate groups of investigators [5-8], and set to be a *tumor supressor gene* in 1989 ([13], [14]), expresses the p53 protein, a central biomolecule in cancer research, specifically in the study of pathways within the cell. This is due to the fact that virtually all cancers exhibit some sort of mutation of p53 gene or modifications to its pathway. The study of p53 pathway revealed the core of its regulation as well as several links that it establishes between other major pathways, such as the one of Rb protein, E2F and Ras. The concentration of p53 protein within an unstressed cell is low, however it has a fast turnover when the cell is under stress.

The core regulation of p53 protein is co-protagonized by the protein Hdm2 (Mdm2 in the mouse) that inhibits p53 protein by binding to it directly. P53 protein promotes the transcription of Hdm2, defining a negative feedback loop between p53 protein and Hdm2 [15], [16]. $p14^{ARF}$ ($p19^{ARF}$in the mouse) in turn inhibits Hdm2 and its activity is inhibited by p53 protein. The transcription factor E2F also plays a role in p53 regulation, by sustaining a negative feedback loop with $p14^{ARF}$ by inducing it while being inhibited by it [5].
The core regulation of p53 protein leads to several different downstream events that culminate in different fates of the cell: *cell cycle arrest*, *apoptosis*, *inhibition of angiogenesis* and *metastasis*, and *DNA repair*.
In [17], these downstream events were explored in distinct pathways, as well as useful positive and negative feedback loops for P53 protein, which are fairly easy to model.
Let us resume the main downstream event triggered by p53 protein activity which culminates in cell cycle arrest: the p21 gene product, a Cyclin Dependent Kinase Inhibitor (CKI), that inhibits CycE/CDK2 complex is a relevant molecule in p53-mediated G_1-S phase arrest. Its transcription is induced by p53 protein activity. There is also CDC25 inhibited by 14-3-3-sigma, and CDC2 induced by CDC25 and CycB, the latter inhibited by Gadd45. CDC2 promotes Cell Cycle arrest between G_2-S phase. This last pathway is not of our interest, as it concerns another checkpoint in the cell cycle, not the restriction point. The cell cycle arrest pathway in which we focused our attention was the one concerning the checkpoint during G_1-S phase transition, and is obviously of the most relevant for studying the regulation of the Restriction Point.
The p53 pathway that culminates in apoptosis is triggered by ATM and induces Caspase-9, an essencial protease of apoptosis. (see model diagram 1).

v. The MAPK cascade pathway

MAPK Cascade signalling pathway (Mitogenic-Activating-Protein Kinase Cascade), is a main mechanism for protein synthesis motivated by extracellular signals. It depends on MAPKKK, MAPKK and MAPK whose phosphorylated form is the activated form.

Extracellular signals, also called Ligands, such as *Growth Factors*, bind to transmembrane receptors, whose cytosolic domain may be allosterically altered, enabling its phosphorylation, inducing the binding of Growth factor receptor-bound protein 2 (GRB2) molecule, activating it. Active GRB2 activates *Son of Seven-less* (SOS), which in turn phosphorylates Ras-GDP complex to Ras-GTP complex. The latter can then activate Raf (MAPKKK) by binding. Raf will proceed the mechanism by phosphorylating MEK (MAPKK). Activated MEK promotes the phosphorylation of ERK (MAPK). Finally, active ERK promotes the activation of transcription factors and subsequent migration to the nucleus where it will bind to DNA transcription sites, leading to protein synthesis [3]. Phosphorylated ERK promotes cell growth [19]. Important transcription factors are the *Early Response Genes* (ERG) *c-Fos*, part of the Fos family of transcription factors, the protein *c-jun* and the protein *Myc*. This cascade would continue indefinitely while Ras-GTP complex continues active. This is why this cascade also induces the transcription of GAP (GTPase-Activating Proteins) regulatory proteins, which act like a switch off button, phosphorylating Ras-GTP complex back to Ras-GDP complex, inhibiting the rest of the cascade and thus stopping the synthesis of the specific proteins that the mitogenic signals triggered. It has been documented that the silencing of GAP proteins is related to some human cancers [21], since it leaves the regulation of Ras protein to chance, resulting in the deregulation of the concentration of Ras, and consequently of the whole Cascade.
Overall, this signalling pathway needs to be well regulated to avoid cancers, because if one of the biomolecules involved in it were to be

mutated, it could imply the consistent transcription of proteins necessary for deregulated growth and division, and thus it is only natural the study of drugs that reverse the "on" or "off" states of these biomolecules for cancer treatment, such as in [23].

vi. PI3K-AKT-mTOR pathway

Another important intracellular transduction pathway is the PI3K (phosphatidylinositol-4,5-bisphosphate 3-Kinase)-AKT-mTOR (mechanistic target of rapamycin) pathway. This pathway is not as well studied as the MAPK cascade, however there are relevant dynamics that are sufficiently well documented, as the ones we describe in this section.

The regulatory subunit of PI3K, binds to phosphotyrosine peptide motifs in receptor protein tyrosine kinases (RTK's) or the insulin receptor substrate 1 (IRS-1). This activates PI3K, which converts PIP2 (Phosphatidylinositol 4,5-bisphosphate)to PIP3 (Phosphatidylinositol (3,4,5)-trisphosphate), a reaction that is counter-acted by PTEN. PIP3 binds to AKT (Protein kinase B), forming the PIP3/AKT. PDK1 interveins to phosphorylate this complex, fully activating it. Active PIP3/AKT phosphorylates TSC2 (Tuberous sclerosis 2), deactivating it. TSC1 (Tuberous sclerosis 1) and active TSC2 form TSC1/TSC2 complex, which inhibit Rheb (Ras homolog enriched in brain) activity. In turn, Rheb promotes mTORC1 (mammalian target of rapamycin complex 1) activation. A feedback control in this PI3K/AKT pathway is the inhibition caused by mTORC1 in RTK dynamics, by phosphorylating IRS1, and also inhibiting EGF receptor, ERB2 and IGF1 receptor, therefore not allowing for interaction with PI3K and subsequent activation and downstream of the pathway [24].

vii. Novak and Tyson Mathematical Model (2004)

Novak and Tyson constructed a mathematical model for the regulation of the Restriction Point based on the Cyclin/CDK complexes, the Rb-E2F interaction and the signal-transduction pathway, Growth Factors - Early response genes - Delayed response genes (GF-ERG-DRG) [1]. They assumed rapid message turnover for mRNA, that is, steady-state for mRNA transcription from the point of view of the other reactions, which occur in a longer timescale.

The GF-ERG-DRG is a brief pathway resuming the MAPK signalling Cascade that transducts extracellular signals through the cell's membrane and across the cytosol, reaching the nucleus and promoting the transcription of proteins. In this case, GF-ERG-DRG is going to be the triggering system that controls the CycD synthesis. CycD then binds to CDK4,6, also assumed to be fast enough to be in steady-state quickly, forming complexes CycD/CDK4,6 that phosphorylate Rb with the help of Cyclins A and B, inhibiting its action on E2F inhibition, therefore releasing free E2F. PP1 phosphatase promotes the dephosphorylation of Rb and is inhibited by CycE/CDK2, CycB/CDK1 and CycA/CDK2 complexes. CycE/CDK2 synthesis is in turn induced by E2F, and it can bind to Kip1, leading to the inhibition of CycE activity. This inhibiton by Kip1 is also applied to CycA. Kip1 degradation is mediated by CycB/CDK1 and CycA/CDK2. The former is mediated by Cdh1, that targets CycB for degradation. CycB, along with APC, promotes the deactivation of Cdh1, therefore establishing a mutual antagonism. Finally PPX mediates the synthesis of an intermediary enzyme, IE, whose phosphorylated form promotes the activation of CDC20. Active CDC20 and APC induce the degradation of CycA.

One of the most important features of this model is the growth and division simulation of the cell. The rate of mass is determined by the level of "General machinery", GM, controlled by the concentration of Rb and the absence of growth factors.

viii. Conradie Model

In the paper "*Restriction Point Control of the Mammalian Cell Cycle via the CycE/CDK2/p27*

complex", [4], the authors constructed a new framework in the RP model of Novak and Tyson, focusing on the CycE/CDK2/p27 complex. This model includes the dynamics of p27 and excludes the dynamics of Kip1 and Cyclins/Kip1 complexes. In this way, protein p27 becomes the cyclins' activity inhibitor – for every time p27 is active, the cyclins must be inactive. It does so by binding directly to the cyclins A,D,E complexes and keeping them inactive for the appropriate time in the cell cycle.

ix. Kholodenko (2000)

The quantitative computational model done in [2] was performed around the MAPK cascade pathway, studying negative feedbacks, ultrasensitivity and emergent oscillations which simulate the nature of cellular biochemical pathways.
In this model, MKKKK (Ras) phosphorylates MKKK (Raf) to MKKK-P (Raf-P). In turn, MKKK-P phosphorylates MKK (MEK) to MKK-P (MEK-P) and MKK-P to MKK-PP (MEK-PP) which, finally, phosphorylates the bottom layer of the cascade, i.e., MAPK (ERK) to MAPK-P (ERK-P) and MAPK-P to MAPK-PP (ERK-PP). In this model, MKKKK (Ras) is not used as a variable, as its activity is simulated recurring to the negative feedback loop established by the downstream activity of ERK-PP. In this way, ERK-PP directly influences the concentration of MKKK (Raf).
It makes use of Michaelis-Menten kinetics, described originally in [41], as main rate functions.

III. Updated Mathematical Model

The previous sections resumed the theory on which we based the construction of a wider updated mathematical [Figure 1], compared to that of Novak and Tyson.
In this new model, the MAPK signalling cascade was constructed as in [2], except for the fact that active ERK acts upon Ras-GDP phosphorylation, promoting it, instead of promoting Raf phosphorylation. This way the cascade has in its structure the dynamics of Ras, and maintains the feedback loop, even though Kholodenko used ERK's influence on the activation of Raf [2]. Indirectly, this is still the case, as ERK is activating Ras that in turn activates Raf. The purpose of this was to propagate the oscillations created by the ERK feedback loop to Ras as well. Thanks to this, it was possible to add another negative feedback, through the inclusion of GAP protein, whose transcription is induced by the cascade and whose inhibition on Ras-GTP decreases the flux of the cascade. The activity of the cascade culminates in the regulation of ERG, whose influence on DRG models the transcription of CycD [1].
The PI3K-AKT pathway was build inside another negative feedback loop - the inhibition of RTK by active mTORC1 against the activation of the latter by active Rheb. The joint concentration of active and inactive mTORC1 is not considered constant, as there is continuous synthesis and degradation of active mTORC1 (see equations (46) and (57)). Activity of AKT includes the formation of PIP3/AKT complex and the synthesis of CycD, which allows a simple connection to the cell cycle. There are several cross-talks between MAPK and PI3K-AKT pathways [reference], and here we have focused only on two major influences acting upon PI3K-AKT pathway: activation of PI3K by active Ras and inhibition of TSC1/TSC2 complex by active ERK.
E2F promotes transcription of $p14^{ARF}$, suggesting another obvious link between models. As for P53, its concentration is maintained low through normal cell cycle by inhibition caused by Hdm2. The Hdm2 protein is downregulated by Rb, CycE/Cdk2 complex and p14, following [17]. The apoptosis and cell cycle arrest events were added with the features of the software used for the simulations, COPASI [44]. This is explained in more detail in the next section (also, see notes on equations).
We based the core of our model, that is, the regulation of the Cyclins, in [4].

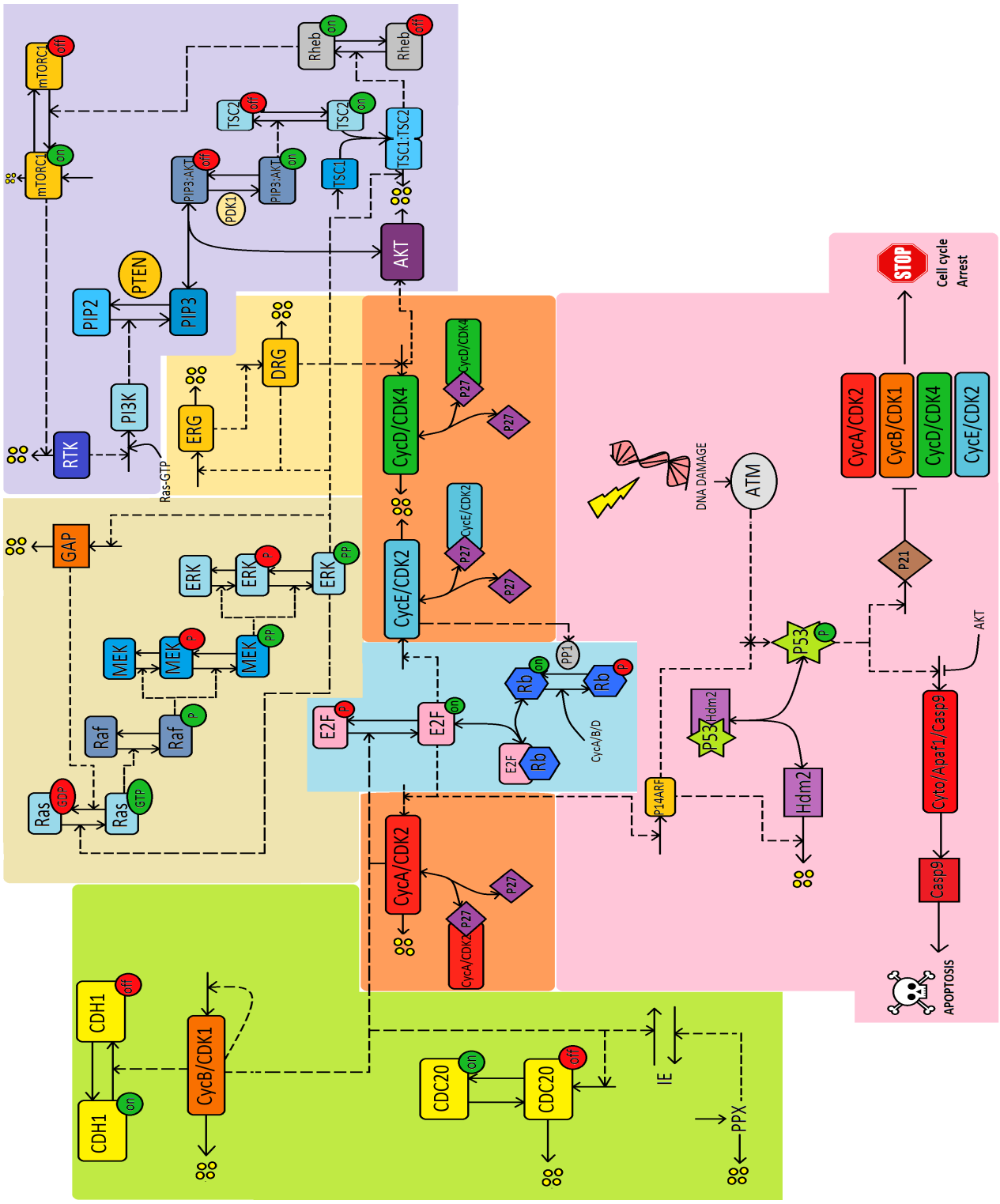

Figure 1: *Diagram of cell cycle dynamics divided according to specific functions in the model: generation of cell cycle oscillations through CDC20 and CDH1 reactions (green background); MAPK cascade (grey background), connection between MAPK cascade and production of Cyclin D through ERG and DRG synthesis (yellow background); PI3K/AKT pathway (light purple background); cyclin A/D/E and p27 interactions (orange background); restriction point regulation through E2F and Rb interactions (blue background); regulation of cell fate by p53 tumor supressor (pink background).*

IV. Units of concentrations

The time dimension frequently used in intracellular activities is the hour, even though some reactions take longer than others. Taking hour as the time unit, any reaction much faster than one hour (suppose a reaction of the order of seconds), can be seen as instantaneous, i.e., always in a steady state. For example, the time of messenger RNA (mRNA) turn over (passage from RNA to protein) is much faster than protein reactions time. From the time perspective of protein reaction, mRNA keeps roughly the same concentration throughout time, and therefore $d[mRNA]/dt \approx 0$,which does not really help much in the system of differential equations unless the constant [mRNA] is used in a parameter somewhere in the equations. However, in this paper the parameters are calibrated according to already mentioned previous models and not directly from real-life dynamics. To follow the modelling of Conradie (and also to be able to pick up the cell cycle model from Tyson and Novak) we assumed the concentration variables are scaled in order to have dimensionless Michaelis Menten parameters and rate constants with units hour. The concentration units are in μM, where M = mol/L.

V. Simulation of healthy cell

As in [1], we implement in the model the cell cycle division, regulated by "mass", whose concentration drops to half periodically (see notes on equations), as shown in the third graphic (left to right, up to bottom) of [Figure 2]. The mass is the indicator of the current cell cycle phase. This is ought to depend on external factors, such as growth factors, but the whole simulation is going to be performed as if the healthy cell were in a stable environment, receiving periodical stimuli, and the cancer cell in a proper environment for its development. Therefore, we exclude external inputs for the model of both healthy and cancer cell, and can then focus on the internal regulation of cell. This takes us to the second link: ERG/DRG dynamics are an isolated system in Novak and Tyson model. As we discussed in the MAPK Cascade section, ERG activity is induced by transcription factors at the bottom of the MAPK Cascade, thus opening a hole in ERG/DRG isolation. For the sake of simplicity, we just assumed that the biphosphorylated form of ERK has a positive impact in ERG concentration. Inspired by Kholodenko [2], the model includes the MAPK cascade with a feedback created by the influence of active ERK on the phosphorylation of Ras-GDP. This establishes a loop around the MAPK cascade, generating one of the three sources of oscillation within this model - the others being the dynamics of CDC20 and CDH1 (second graphic of Figure 2), and the negative feedback loop in PI3K-AKT pathway (fifth graphic of Figure 2), which regulate the mass, which, in turn, regulates the cyclins (first graphic of Figure 2). The Rb and E2F dynamics function as expected, following the cell cycle (sixth graphic of Figure 2).

When it comes to the regulation of Hdm2, we assumed a constant flux of synthesis plus the induction by phosphorylated P53. The flux of synthesis is larger than the dependency on phosphorylated P53. Here the tumor supressor is playing the role of regulating apoptosis and cell cycle arrest. Its concentration is low when Hdm2 is present, binding directly to it and increasing the concentration of Hdm2/P53 complex.

Simulating Cell Cycle Arrest implies a steady behaviour of cyclins concentration and cell volume for the time scale we are dealing with. It was therefore added a switch-like parameter in the rate functions of Cyclins, General Machinery, CDH1 and mass, regulated by the condition of having a minimum amount of p21.

The programmed cell death can be triggered in two ways: from within the cell or through extracellular signals. Intracellular induction of apoptosis is the only one we concerned this project about, and therefore the intracellular pathway is the mechanism on which the simulation of apoptosis of the model is based on.

For the simulation of intracellular induction

of apoptosis, it was added the conditions on minimum amounts of ATM which, if crossed, will trigger the increase in P53 dependency of Caspase-9. This induces a rapid construction of this protein, which will dismount the cell from within by degrading cyclins and leading the mass to zero in a switch-like way. This control of apoptosis and cell cycle arrest can be seen as a very simple Boolean system.
All parameters in common to the mentioned models were maintained or only slightly changed. New necessary parameters were chosen according to the desired oscillation output and links between pathways by trial-and-error, allowing the concentrations to sustain negative or positive feedback loops and at the same time establish smooth connections between distinct pathways of the cell cycle.

VI. Simulation of common deregulations

After setting the model of a healthy cell, we can now proceed to simulate the cancer cell. Since cancer is a set of diseases rather than just one easily generalizable disease, we will approach this simulation in two different ways: first, making use of the relevant information on MAPK cascade and PI3K/AKT pathway, on the p53 protein and its pathway, as well as the Retinoblastoma protein and the E2F transcription factor, to execute alterations on the model so as to simulate the beginning of a random cancer, which will deregulate some or several pathways. Second, starting in a specific type of cancer - colon cancer -, we will alter the relevant pathways to then proceed to logical therapies described in literature.

i. MAPK cascade signalling pathway in cancer

As a first approach to simulate cancer, the MAPK cascade dynamics in the model were altered, as it is a reasonable target for cancer study [3]. Starting by assuming mutated Ras or Raf proteins, such that its phosphorylating activity on Raf or MEK proteins, respectively, continues. For mutated Ras the binding to GTP is still ongoing but the binding to GAP protein and inactivation by active ERK ceases to be possible - and thus Ras dephosphorylated form becomes rare in the cascade. For mutated Raf, its phosphorylated form becomes dominant and therefore the oscillation effect inherited by the Ras stage of the cascade is lost due to this overactivation of Raf. Both inefficient Ras-GTP hydrolysis and inefficient Raf dephosphorylation result in the same deregulation of the ERG production and therefore DRG as well [Figure 3]. The cyclins' periodicity overall does not suffer from this change, except that of CycD, whose amplitude diminishes, as we see in [Figure 4].
For severe dephosphorylation inhibition of both phosphorylated forms of ERK or severe dephosphorylation inhibition of biphosphorylated MEK, the effect on ERG, DRG and CycD are the same as with deregulation induced by Ras or Raf mutations [Figure 3]. In other words, as shown in figure 3, the same downstream result is obtained in one or more of the above mentioned deregulations. The impact is not as strong for the analogous change on monophosphorylated MEK forms [Figure 5]. Thus, according to this simulation, it seems that ERK, compared to MEK, is a more sensitive component in the MAPK cascade, since any type of dephosphorylation inhibition on ERK suffices to loose the oscillation, while with MEK only if the biphosphorylated form suffers from severe phosphorylation inhibition.
A possible deregulation to explore in the MAPK cascade is the GAP synthesis, which inhibits directly active Ras. Increasing the GAP-dependency in the inactivation of Ras (which was done by increasing the parameter k_{gap} on equation (43)) and turning the production of GAP faster at the same time yields an increase in the number of waves of the MAPK cascade components' concentration [Figure 6] indicating a faster feedback. Since active ERK's concentration drops at the bottom of the cascade, there is a subsequent inhibition of the cell cycle, seen in Figure 7 (cyclins'

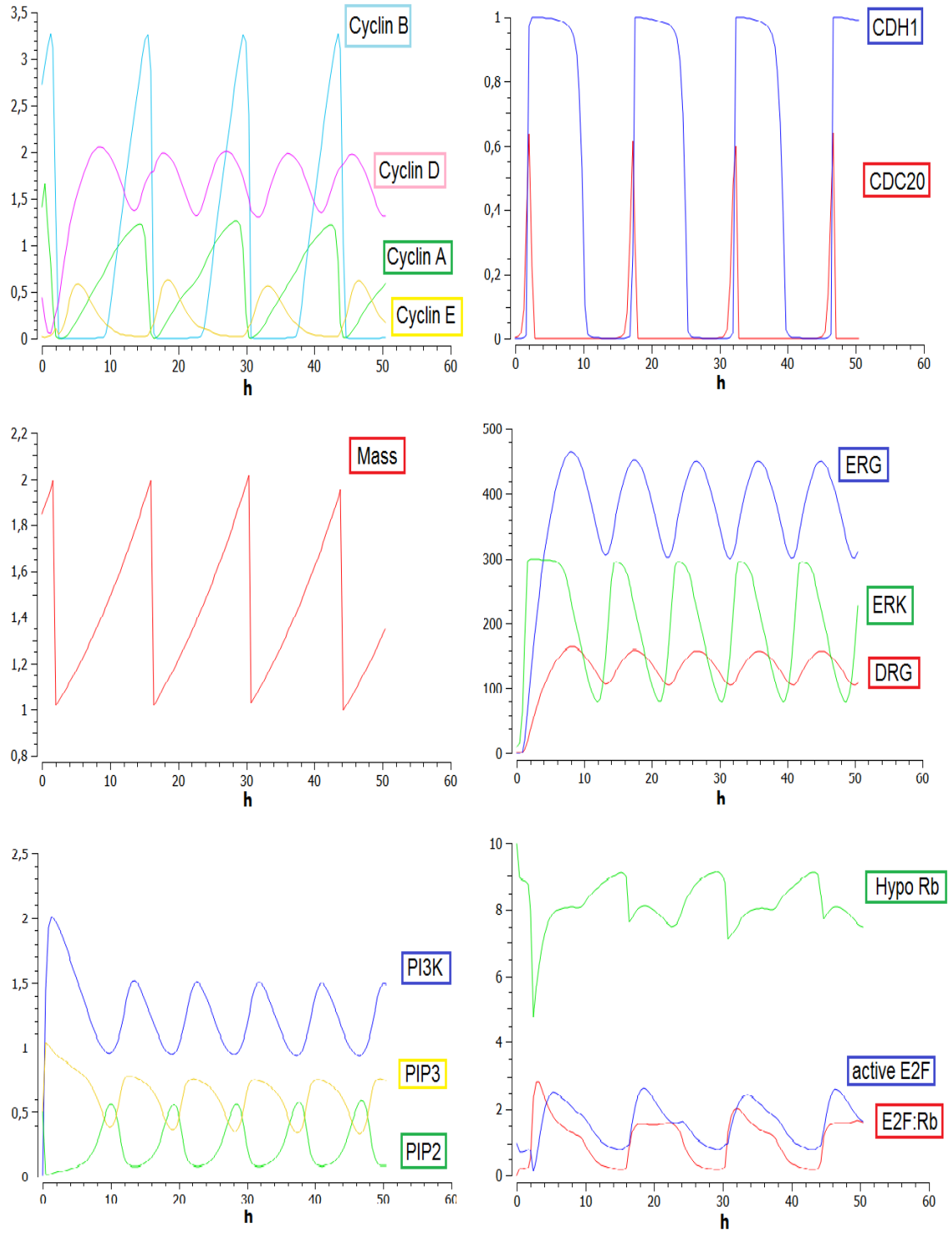

Figure 2: *Healthy cell dynamics (details in text). Concentration unit: µM; Time unit: hour.*

concentrations are bellow 0.5 μM, which is quite low compared to healthy behaviour [see first graphic of figure 2]). p_{27} complexes are also affected by having its concentration frequency follow that of the MAPK cascade components (bottom graphic of Figure 8), in contrast to the healthy case (upper graphic of Figure 8).

ii. PI3K/AKT pathway deregulations

The PI3K pathway is commonly altered in colon cancer [36]. PI3K enhancement and PTEN loss are just some of the common deregulations in this transduction pathway. AKT hyperactivity is another possible deregulation. For PI3K overexpression, synthesis was enhanced, leading to an ever-increasing concentration of PI3K and loss of oscillation pattern in Rheb, mTORC1 and TSC1-2 concentrations [Figures 9-11]. Deregulations that are caused by the MAPK cascade, through cross-talks, are simulated in the colon cancer section below, due to their importance in this cancer.

iii. CDH1 deregulation

Complete loss of CDH1 is implicated in 84% of lobular breast cancer [22], which may imply that the presence of CDH1 is a strong factor against lobular breast cancer. In the model, in absence of CDH1, i.e., with no synthesis whatsoever of CDH1, the wave length of the cycles in CDC20, the cyclins, p27 complexes, E2F and $p14^{ARF}$ are shortened (see first four plots of Figure 12). As the mass only engages in division whenever CDH1 concentrations surpasses a certain threshold, the overall volume of the cell increases with no type of regulation (see last plot of Figure 12). Although it may seem a positive feature of a deficient-CDH1 cell not having auto-induced division, like in cancer cells, this only happens due to the lack of cancer cell mechanism to induce its own division in this model. One can imagine that after CDH1 is removed completely from the cell, cell division is guided by another pathway, that is, a pathway which does not depend on

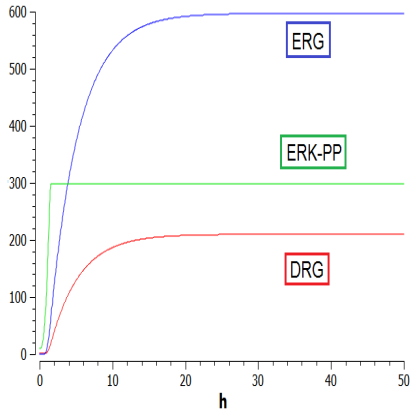

Figure 3: *Deregulations: inefficient Ras-GTP hydrolysis; inefficient Raf dephosphorylation; inefficient dephosphorylation of both forms of ERK; inefficient dephosphorylation of MEK-PP. See text for details.*

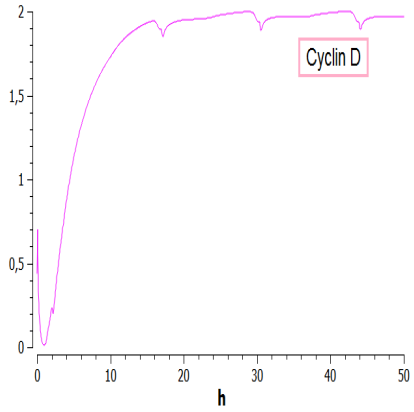

Figure 4: *Deregulation: inefficient Ras-GTP hydrolysis*

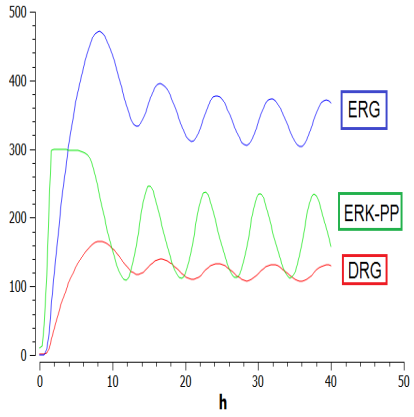

Figure 5: *Deregulation: inefficient dephosphorylation of MEK-P*

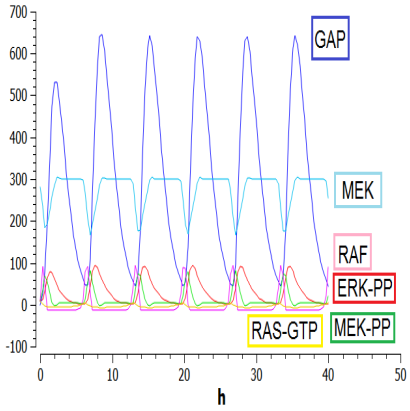

Figure 6: *Deregulation: strong dependency on and fast production of GAP.*

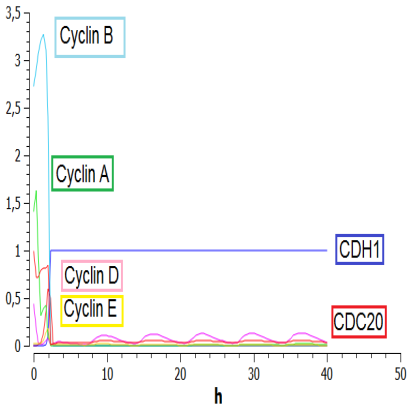

Figure 7: *Deregulation: strong dependency on and fast production of GAP.*

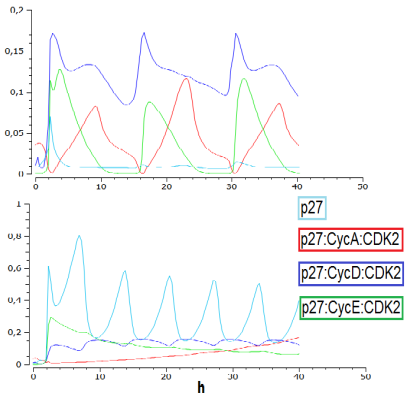

Figure 8: *Healthy case (upper graphic) and deregulation: strong dependency on and fast production of GAP (bottom graphic).*

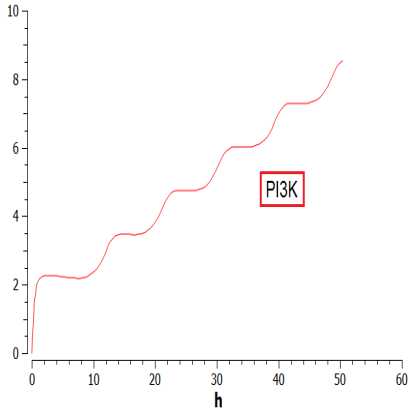

Figure 9: *Overexpressed PI3K (see text for details).*

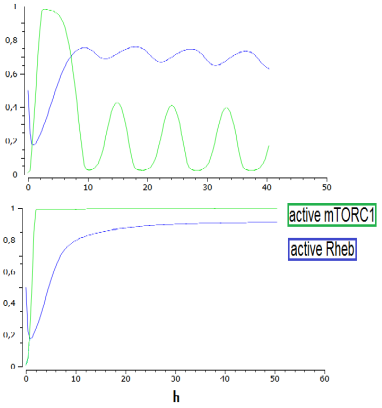

Figure 10: *Healthy case (upper graphic) and deregulation: overexpressed-PI3K (bottom graphic).*

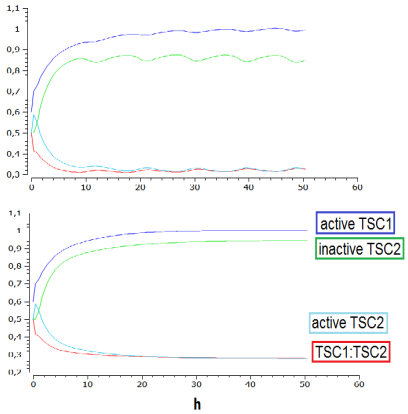

Figure 11: *Healthy case (upper graphic) and deregulation: overexpressed-PI3K (bottom graphic).*

CDH1 concentration nor CDC20 synthesis to induce cell division. Another outcome of this simulation is that p53 is not turned on (see fifth plot of Figure 12), which means there is no apoptosis nor cell cycle arrest, and this combined with the already mentioned hidden cancerous mechanism for self-regulation of division becomes the perfect combination for the birth of a cancer cell.

iv. Retinoblastoma mutation

If mutated, Rb can lose its ability to connect with E2F, not inhibiting its activity in proper time during the cell cycle, and consequently enabling E2F to migrate to the nucleus inducing the motion to S-phase in the cycle, moving past the restriction point. To simulate this, the chemical reaction of active Rb binding to E2F forming the complex E2F:Rb is shut off. This originated an increase in concentration of free E2F [Figure 13] and also an anticipation of the cell cycle, as the phase of the oscillation gets shifted [Figure 14], showing that the cell commits to an extra cell cycle earlier than in the healthy case. As a result of that, the general machinery does not have the proper time to build up, leading to an ever-decreasing mass in each cycle [Figure 15].

v. P53 deregulated pathway

Any damage to the DNA is ought to induce the production of ATM, promoting p53 synthesis, which can trigger events for apoptosis or cell cycle arrest, the latter also involved in eventual repair mechanisms. If synthesis of p53 is deregulated, arising the appearance of mutated forms of p53, the ability of the cell to induce apoptosis or cell cycle arrest might be compromised. If mutated p53 is not able to promote p21 synthesis or Caspase-9 synthesis, then the cell does not have any defense mechanism, and will eventually continue through the cycle, passing errors in DNA to the daughter-cells, leading to unrepairable cases of tumorigenesis.

To simulate this, the modifying role played by p53 in p21 and Caspase-9 synthesis was unintensified and the level of ATM was increased. The purpose is to simulate DNA damage in a mutated p53 environment. The result is clear [Figures 16-18]: the cell could not proceed to cell cycle arrest nor apoptosis and therefore the division continued. The concentrations of Hdm2 quickly reaches a constant level, since it has not enough supply of p53 to bind with.

VII. Colon cancer

According to the World Cancer Research Fund International, colorectal cancer, or colon cancer, is the third most common type of cancer in the world and nearly 95% of colorectal cancers are adenocarcinomas, i.e., abnormal growth of epithelial tissue with glandular origin and/or characteristics [46]. The molecular pathways our model simulates are reasonable targets for therapy of this type of cancer. The Ras/Raf/MEK/ERK cascade is deregulated in approximately 30% of all cancers, Ras alone being mutated in 36% [38] and B-Raf (a specific type of Raf) found mutated in 10% to 15% of colorectal cancers [37]. The PI3K catalytic subunit alpha (PI3KCA) mutations are implicated in about 32% of colorectal cancers [38]. Hyperactivation of AKT has been linked to tumorigenic development, increasing cell survival, and was proved to be vital for colon cancer stem cells [39]. The regulatory system of the cell cycle is also afflicted by Ras mutations, which come with raised ERK activity [38].
On the other hand, Raf inhibitors have shown to be promising in certain cancers, with clinically manageable effects [40].

i. Simulation of colon cancer

Ras hyperactivity in colon cancer was seen as a straight-forward approach. As in the previous section on common deregulations, Ras dephosphorylation rate was diminished sufficiently to affect the rest of the MAPK cascade and PI3K/AKT pathway. There is a

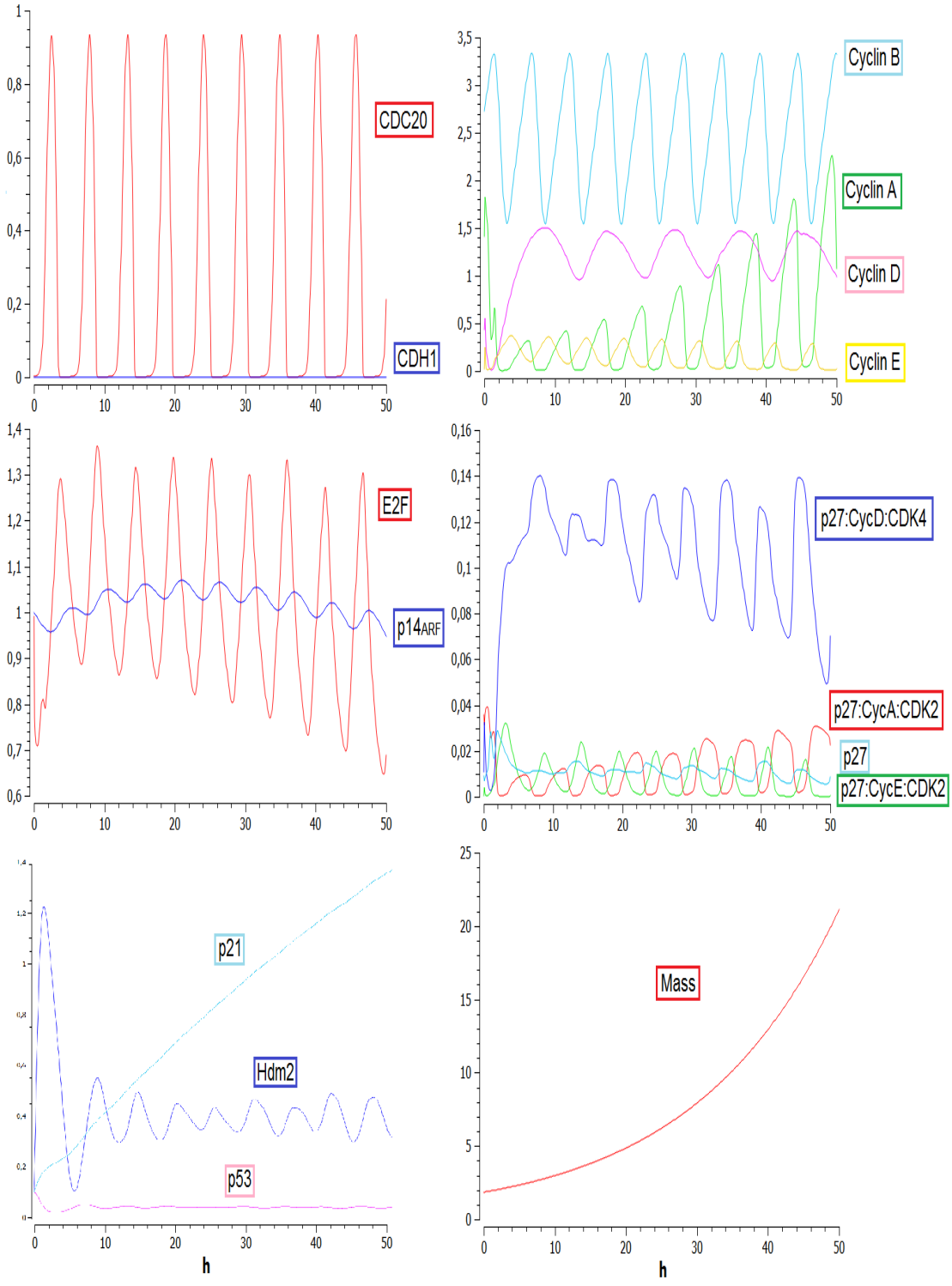

Figure 12: *Deregulation: CDH1-deficiency, i.e., no synthesis of CDH1 whatsoever. See text for more details.*

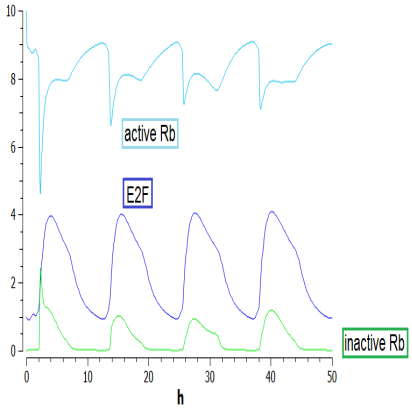

Figure 13: *Deregulation: Rb-mutated cell*

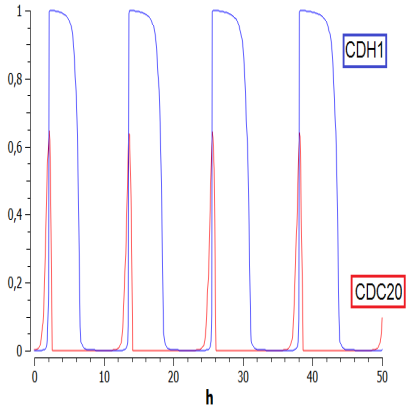

Figure 14: *Deregulation: Rb-mutated cell*

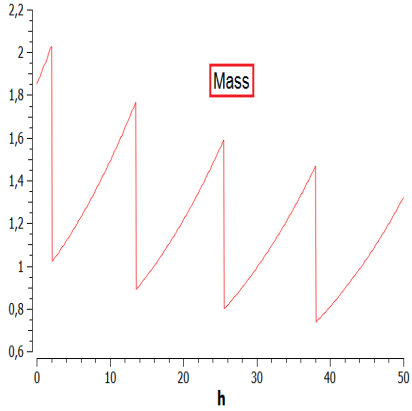

Figure 15: *Deregulation: Rb-mutated cell*

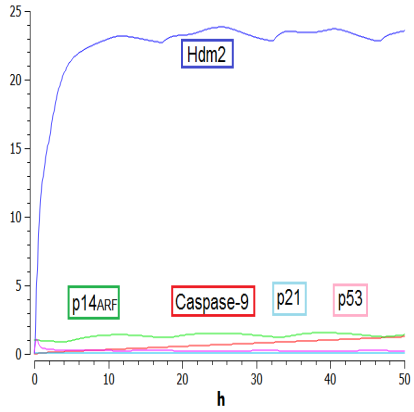

Figure 16: *Deregulation: mutated p53 such that it can no longer activate p21 synthesis*

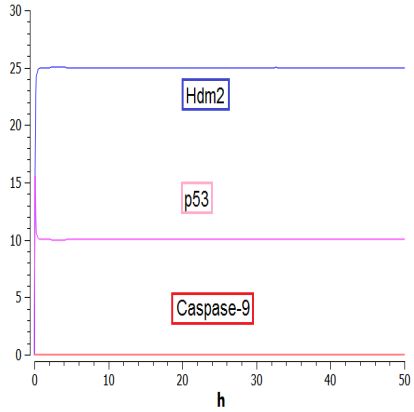

Figure 17: *Deregulation: mutated p53 such that it can no longer activate Caspase9 synthesis*

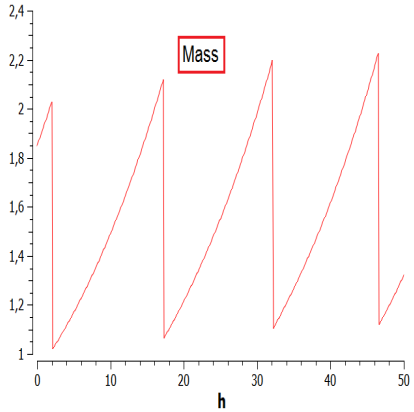

Figure 18: *Deregulation: p53-mutated cell*

subsequent over-activation of PI3K, leading to a fast increase and posterior stagnation of PI3K/AKT pathway active components in a high concentration level. DRG and ERG concentrations frequency increase notoriously, following active ERK overactivation [Figure 19]. This contributes to shorter wave-lengths on CDH1 and CDC20 concentrations [Figure 20]. Hence the restriction point is overcome one more time than in healthy cell conditions. Uncontrolled division, yet, as mentioned previously, is not sufficient to create a cancer cell, according to the hallmarks of cancer [35], and thus, Ras hyperactivity, along with P53 mutation, was performed in this simulation, in this way clearly avoiding the Boolean switch of apoptosis or cell cycle arrest, generated by overproduced Caspase-9 or p21, respectively. AKT hyperactivity affects mainly PI3K/AKT pathway, and doesn't seem to interfere with cyclins A,B and E, however it is still troublesome for it stimulates the overall production of CycD. TSC1 and TSC2 inherit the hyperactivity and raise, losing oscillation, while PIP3 lowers significantly and PIP3:AKT raises [Figure 21], both in active and inactive forms. PI3K, RTK and mTORC1 concentration's amplitude increases. Cell cycle is advanced, as with Ras activation [Figure 20].

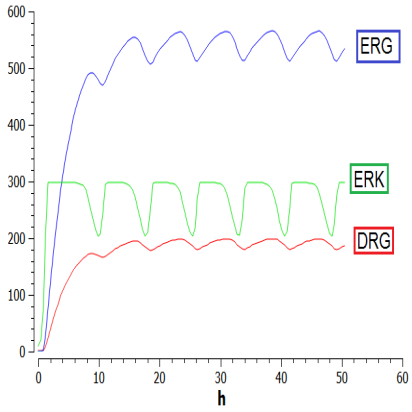

Figure 19: *Deregulation: hyperactive-Ras cell model*

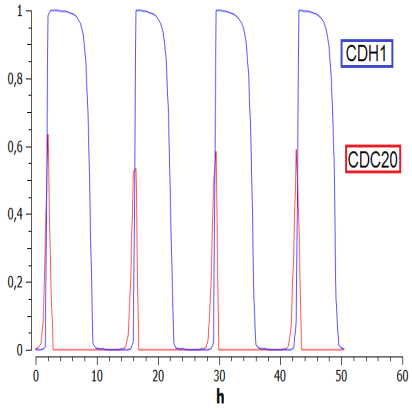

Figure 20: *Deregulation: hyperactive-Ras or hyperactive-AKT cell model*

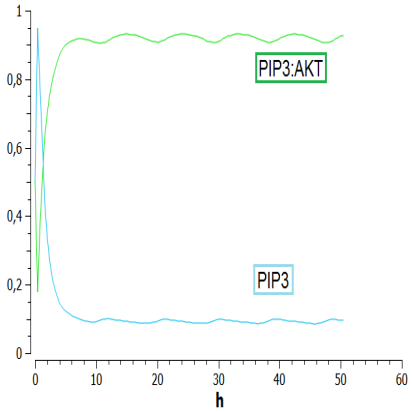

Figure 21: *Deregulation: hyperactive-AKT cell model*

VIII. Therapies

The simulations performed wouldn't fulfil their purpose if one couldn't derive therapeutical intervention from them. Specifically, from the results it is possible to extract ideas for biomolecular-based therapies or, at least, to detect pre-cancerous cells. MAPK cascade pathway's deregulation yields promising terrain for detecting possible cell cycle corruption: constant levels of active Ras, active Raf or active ERK can be measured indirectly through ERG and DRG concentrations [Figure 3] or Cyclin D concentration [figure 4]; as the increase in GAP-dependency leads to cyclin inhibition [Figure 7], targeting GAP in a previously-identified cancer cell for its overexpression is a possible

way to inhibit cell growth.
Complete loss of CDH1 consequently induces the cell to a faster passage through the restriction point, whose regulation is done by E2F/Rb dynamics, meaning that a possible treatment for CDH1-deficient cells could be the artificial introduction of fair amounts of Rb or Rb-like biomolecules to create a delay that could ultimately compensate the fast pace of S-phase commitment; the same idea could be applied to Rb-mutated cells, since there are no biomolecules to hold the transcription factor activities of E2F in those cases.
Since p53 is mutated or its pathway is altered in virtually every cancer, therapies directly turned towards its activity in the cell could imply groundbreaking approaches to fight cancer. The pathway described in the model presented in this paper is not complex enough to allow one to easily derive therapies, but still some appear naturally: adding p53-like biomolecules to a p53-deficient cell, inducing p21 or Caspase-9 synthesis in cancerous cell or even targeting Hdm2 for destruction to indirectly increase the concentration of p53 are just some, rather simplistic, approaches. However, p53 pathway is not target-like, being extremely difficult to derive practical therapies from its deregulation.
It is also useful to make use of the altered model to find consequences of ideal therapies. With this in perspective, some simulation of therapies were performed, based in the common deregulation in colon cancer explored in previous sections. For Ras hyperactivation, MEK and Raf inhibition yield similar results, although the inhibitions were performed in different intensities: strong inhibition of Raf or inhibition of MEK induces cell cycle arrest without use of the p53 defense mechanism, starting with CDH1 and CDC20 stability [Figure 22]. The cell signalling transduction ceases to operate, leading to stagnation of CycD production, and adding the arrest of CDH1 and CDC20, the cycle stops [Figure 23]. For this same deregulation, ERK inhibition delays cell cycle [Figures 24 and 25], although not restoring completely MAPK cascade feedback and leading to faster stationarity in PI3K/AKT dynamics. The delay seems to help increase the mass of the cell, since the first cell cycle takes longer to occur and the mass reaches higher values in the same period. As for PI3K inhibition, it does not seem very promising for Ras hyperactivity, because the cell cycle continues unharmed, even though PI3K/AKT components take longer to reach stationarity.

IX. Conclusions

This paper uses a deterministic mathematical model to study dynamics in healthy cell and cancerous cell. The model for healthy cell is based on the p53 gene network, cell cycle control mechanisms, the MAP kinase pathway and PI3K/Akt pathway. The cancer cell model is realized by the deregulation in the model of healthy cell. In addition, potential therapy targets are predicted by using mathematical simulation. This model has been done entirely within an *in silico* research work. The theoretical foundations with which we constructed the differential equations for the quantitative model are strongly connected with biochemistry fields for many years, and not only allow one to apply mathematics to biology but also do it in a low-budget and time-saving manner. With information from literature, we have derived an updated and extended, but still simple, version of an important model for systems biology, [1], changing some of its pathways in a experimental way and mimicking already known deregulations to reflect on the outcomes. This resulted in a broader simulation of the cell cycle. The extension of the model to the MAPK and PI3K/AKT pathways showed consistency with much of the information in biomolecular and cancer literature, maintaining the restriction point core of the original model. It also shows flexibility, as it is easy to manipulate the input reactions, even though the parameters might be dificult to adjust.
The therapies we have mentioned are constrained to the biochemical applicability in real-life scenarios. Still, the ideas that can be brought about when this kind of approach is

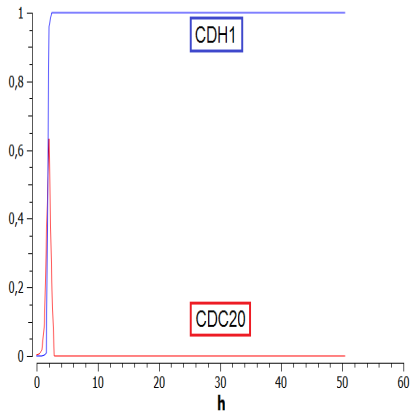

Figure 22: *Deregulation: hyperactive-Ras cell; Treatment: Raf or MEK inhibition*

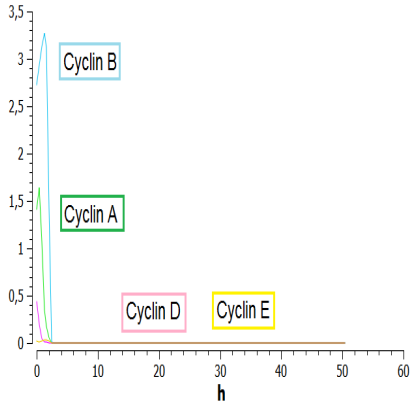

Figure 23: *Deregulation: hyperactive-Ras cell; Treatment: Raf or MEK inhibition*

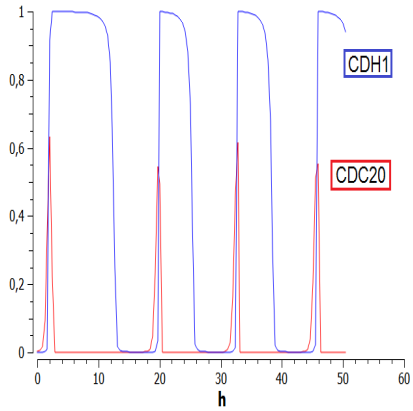

Figure 24: *Deregulation: hyperactive-Ras cell; Treatment: ERK inhibition*

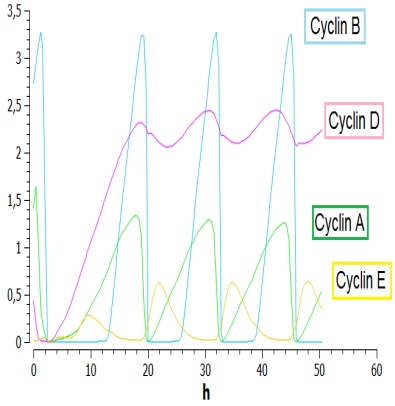

Figure 25: *Deregulation: in hyperactive-Ras cell; Treatment: ERK inhibition*

applied might conduce to breakthroughs in molecular biology.

The study of the colon cancer, and other types of cancer, as it keeps on being reviewed and updated, will help on the fitness and prediction power of our model.

X. Further work

We are aware of the limitations of the proposed model, not only because it is a gross simplification of the complexity of large scale regulatory dynamics of the cell but also because it is not completely up-to-date. However, it is an easily approachable in the sense that increasing its complexity or updating it is as simple as adding or removing more species and/or reactions. Particularly, cross-talks between the pathways considered, such as MAPK cascade and PI3K-AKT, should be object of a more detailed research, as well as the PI3K pathway itself. Completely updating the model, or at least bringing it closer to the most recent discoveries, might open the possibility of expansion to inter-cellular interactions, as well as enhancing it with stochastic modelling.

XI. Aknowledgments

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References

[1] Béla Novák, John J. Tyson, 2004. *A model for restriction point control of the mammalian cell cycle*. Journal of Theoretical Biology 230, 563-579.

[2] Boris N. Kholodenko, 2000. *Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades*. Eur. J. Biochem. 267, 1583-1588.

[3] AS Dhillon, S Hagan and W Kolch. *MAP kinase signalling pathways in cancer*. Oncogene (2007) 26, 3279-3290.

[4] Riaan Conradie, Frank J. Bruggeman, Andrea Ciliberto, Attila Csikász-Nagy, Béla Novák, Hans V. Wsterhoff, Jacky L. Snoep, 2009. *Restriction point control of the mammalian cell cycle via the cyclin E/Cdk2:p27 complex*. FEBS Journal 277, 357-367.

[5] Charles J. Sherr, Frank McCormick, 2002. *The RB and p53 pathways in cancer*. Cancer Cell 2, 103-12

[6] Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

[7] Zatterberg, A., Larsson, O., 1995. *Cell cycle progression and growth in mammalian cells: kinetic aspects of transition events*. In: Hutchison, C., Glover, D.M. (Eds.), Cell Cycle Control. Oxford University Press, Oxford, pp. 206-227.

[8] Peña, C., García, J.M., Silva, J., García, V., Rodríguez, R., Alonso, I., Millán, I., Salas, C., de Herreros, A.G., Muñoz, A., Bonilla, F. Hum. Mol. Genet. 2005, *E-cadherin and vitamin D receptor regulation by SNAIL and ZEB1 in colon cancer: clinicopathological correlations*. Hum Mol Genet, 14(22):3361-70

[9] Kress M, May E, Cassingena R, May P. 1979, *Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum*. Virol J 31: 472-483

[10] Lane DP, Crawford LV. 1979, *T antigen is bound to a host protein in SV40-transformed cells*. Nature 278: 261-263

[11] Linzer DI, Levine AJ. 1979, *Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells*. Cell 17: 43-52

[12] DeLeo AB, Jay G, Appella E, Dubois GC, Law LW, Old LJ. 1979 *Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse*. Proc Natl Acad Sci USA 76: 2420-2424

[13] Baker SJ et al. 1989 *Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas*. Science 244: 217-221

[14] Takahashi T, Nau MM, Chiba I, Birrer MJ, Rosenberg RK, Vinocour M, Levitt M, Pass H, Gazdar AF, Minna JD (1989) *p53: a frequent target for genetic abnormalities in lung cancer*. Science 246: 491-494

[15] Momand J., Zambetti GP, Olson DC, George D., Levine AJ. 1992, *The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation*. Cell 69(7):1237-45

[16] Picksley SM, Lane DP. 1993, *The p53-mdm2 autoregulatory feedback loop: a paradigm*

for the regulation of growth control by p53? Bioessays 15(10):689-90

[17] Sandra L. Harris, Arnold J. Levine, 2005. *The p53 pathway: positive and negative feedback loops*. Oncogene 24: 2899-2908

[18] Juven-Gerston and Oren, 99

[19] Meloche S., Pouysségur J. 2007, *The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G_1 to S-phase transition*. Oncogene 26(22):3227-39

[20] Ameyar, M; Wisniewska, M; Weitzman, JB. 2003, *A role for AP-1 in apoptosis: the case for and against*.. Biochimie. 85 (8): 747-52.

[21] Jin, Hongchuan et al. *Epigenetic Silencing of a Ca2+-Regulated Ras GTPase-Activating Protein RASAL Defines a New Mechanism of Ras Activation in Human Cancers*. Proceedings of the National Academy of Sciences of the United States of America. 2007, 104: 12353-12358.

[22] De Leeuw WJ; et al. 1997. *Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ*. J Pathol. 183 (4): 404-411.

[23] Orton RJ, Sturm OE, Vyshemirsky V, Calder M, Gilbert DR, Kolch W (Dec 2005). *Computational modelling of the receptor-tyrosine-kinase-activated MAPK pathway*. The Biochemical Journal. 392 (Pt 2), 249-61

[24] Christoph Wagener, Carol Stocking, Oliver Müller, "*Cancer Signaling: From Molecular Biology to Targeted Therapy*". Wiley VCH, 2016.

[25] C. Elizabeth Caldon, Roger J. Daly, Robert L. Sutherland, and Elizabeth A. Musgrove, 2006, *Cell Cycle Control in Breast Cancer Cells*, Journal of Cellular Biochemistry 97:261-274

[26] Malaney S, Daly RJ. 2001. *The ras signaling pathway in mammary tumorigenesis and metastasis*. J Mammary Gland Biol Neoplasia 6:101-113.

[27] Schlessinger J. 2000. *Cell signaling by receptor tyrosine kinases*. Cell 103:211-225.

[28] Sutherland RL, Musgrove EA. 2004. *Cyclins and breast cancer*. J Mammary Gland Biol Neoplasia 9:95-104

[29] Musgrove EA, Davison EA, Ormandy CJ. 2004. *Role of the CDK inhibitor p27 (Kip1) in mammary development and carcinogenesis: Insights from knockout mice*. J Mammary Gland Biol Neoplasia 9:55-66.

[30] Bearss DJ, Lee RJ, Troyer DA, Pestell RG, Windle JJ. 2002. *Differential effects of p21(WAF1/CIP1) deficiency on MMTV-ras and MMTV-myc mammary tumor properties*. Cancer Res 62:2077-2084.

[31] Musgrove EA, Lee CS, Buckley MF, Sutherland RL. *Cyclin D1 induction in breast cancer cells shortens G1 and is sufficient for cells arrested in G1 to complete the cell cycle*. Proceedings of the National Academy of Sciences of the United States of America. 1994;91(17):8022-8026.

[32] Arnaud Guille, Max Chaffanet and Daniel Birnbaum, 2013, *Signaling pathway switch in breast cancer*, Cancer Cell International, 13:66

[33] Fresno Vara JA1, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M., 2004, *PI3K/Akt signalling pathway and cancer*. Apr;30(2):193-204

[34] Camillo Porta,1, Chiara Paglino, and Alessandra Mosca, 2014, *Targeting*

PI3K/Akt/mTOR Signaling in Cancer, Front Oncol. 2014; 4: 64

[35] Hanahan D1, Weinberg RA., 2000, *The hallmarks of cancer*. Cell. 2000 Jan 7;100(1):57-70.

[36] Cathomas G. PIK3CA in Colorectal Cancer. Frontiers in Oncology. 2014;4:35. doi:10.3389/fonc.2014.00035.

[37] Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer discovery. 2012;2(3):227-235.

[38] Jing Yuan Fang, Bruce C Richardson, 2005. *The MAPK signalling pathways and colorectal cancer* . Lancet Oncol2005; 6: 322-27

[39] Y.K.Wang, Y.L.Zhu, F.M.Qiu, T.Zhang, Z.G.Chen, S.Zheng and J.Huang. *Activation of Akt and MAPK pathways enhances the tumorigenicity of CD1331 primary colon cancer cells*.Carcinogenesis vol.31 no.8 pp.1376-1380, 2010

[40] Libero Santarpia, Scott M Lippman and Adel K El-Naggar, 2012. *Targeting the MAPK-RAS-RAF signaling pathway in cancer therapy, Expert Opinion on Therapeutic Targets*, 16:1, 103-119

[41] L. Michaelis and Miss Maud L. Menten, 1913. *The Kinetics of Invertase Action*, translated by Roger S. Goody and Kenneth A. Johnson. Biochemistry, 2011, 50 (39), pp 8264-8269

[42] Rb: `https://www.ncbi.nlm.nih.gov/gene/5925`

[43] Conradie BioModels: `https://www.ebi.ac.uk/biomodels-main/BIOMD0000000265`

[44] Software used in simulations: `http://copasi.org/About/Team/`

[45] p53: `http://www.bioinformatics.org/p53/introduction.html`

[46] Colon cancer: `http://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/colorectal-cancer-statistics`

XII. Supplementary information

System of differential equations for healthy cell model

$$\frac{d[Cdc20]}{dt} = \frac{k_{13}(-[Cdc20]+[''inactiveCdc20''])[''phosphorylatedIEP'']}{J_{13}-[Cdc20]+[''inactiveCdc20'']} - \frac{k_{14}[Cdc20]}{J_{14}+[Cdc20]} - k_{12}[Cdc20] \quad (1)$$

$$\frac{d[Cdh1]}{dt} = \frac{(k^{'}{}_3 + k_3[Cdc20])(1-[Chd1])}{1+J_3-[Cdh1]} - \frac{V_4 CycleArrest[Cdh1]}{J_4+[Cdh1]} \quad (2)$$

$$\frac{d[CycA]}{dt} = k_{25r}[p27:CycA:Cdk2] + V_6[p27:CycA:Cdk2] + \epsilon k_{29} CycleArrest[mass][E2F] - k_{25}[CycA][p27] - k_{25}[CycA][Kip1] - k_{25r} - k_{30}[Cdc20][CycA] \quad (3)$$

$$\frac{d[CycB]}{dt} = \epsilon CycleArrest\left(k_{1'} + \frac{k_1[CycB]^2}{{J_1}^2(1+\frac{[CycB]^2}{J_{12}})}\right) - V_2[CycB] \quad (4)$$

$$\frac{d[CycD]}{dt} = V_6[p27:CycD:Cdk2] - k_{10}[CycD] + \epsilon CycleArrest k_9[DRG] + k_{akt}[AKT] - k_{24}[CycD][p27] + k_{24r}[p27:CycD:Cdk2] - k_{24}[CycD][Kip1] + k_{24r}[CycD:Kip1] + V_6[CycD:Kip1] + k_{10}[CycD:Kip1] \quad (5)$$

$$\frac{d[CycE]}{dt} = k_{25r}[p27:CycE:Cdk2] - V_8[CycE] + V_6[p27:CycE:Cdk2] + \epsilon(k_{7'}+k_7[E2F]) - k_{25}[CycE][p27] - k_{25}[CycE][Kip1] + k_{25r}[CycE:Kip1] + V_6[CycE:Kip1] + V_8[CycE:Kip1] \quad (6)$$

$$\frac{d[DRG]}{dt} = \epsilon\left(\frac{k_{17}[DRG]^2}{{J_{17}}^2(1+\frac{[DRG]^2}{{J_{17}}^2})} + k_{17}^{'}[ERG]\right) - k_{18}[DRG] \quad (7)$$

$$\frac{d[E2F]}{dt} = k_{20}(\lambda_A[CycA]+\lambda_B[CycB]+\lambda_D([P27:CycD:Cdk2]+[CycD])+\lambda_E[CycE])[E2F:Rb] + k_{26r}[E2F:Rb] - (k^{'}{}_{23}+k_{23}([CycA]+[CycB]))[E2F] + k_{22}[''phosphorylatedE2F''] - k_{26}[E2F][Rb] \quad (8)$$

$$\frac{d[E2F:Rb]}{dt} = k_{26}[E2F][Rb] + k_{22}[''phosphorylatedE2F:Rb''] - k_{20}(\lambda_A[CycA]+\lambda_B[CycB]+\lambda_D([p27:CycD:Cdk2]+[CycD]) + \lambda_E[CycE])[E2F:Rb] - k_{26r}[E2F:Rb] - (k^{'}{}_{23} + k_{23}([CycA]+[CycB]))[E2F:Rb] \quad (9)$$

$$\frac{d[ERG]}{dt} = \frac{[ERG]k_{ERK}[''ERK-PP'']}{kk_{34}+[ERG]} + \frac{\epsilon k_{15}}{1+\frac{[DRG]^2}{{J_{15}}^2}} - k_{16}[ERG] \quad (10)$$

$$\frac{d[GM]}{dt} = CycleArrest k_{27}[mass] r31switch - k_{28}[GM] \quad (11)$$

$$\frac{d[Rb\_hypo]}{dt} = k_{20}(\lambda_A[CycA] + \lambda_B[CycB] + \lambda_D([p27:CycD:Cdk2] + [CycD]) + \lambda_E[CycE])([E2F:Rb] + [''phosphorylatedE2F:Rb''] + [Rb]) - (k_{19}PP1A + k_{19'}(PP1T - PP1A))[Rb_hypo] \quad (12)$$

$$\frac{d[''inactiveCdc20'']}{dt} = \epsilon(k_{11}{}' + k_{11}[CycB]) - k_{12}[''inactiveCdc20''] \quad (13)$$

$$\frac{d[mass]}{dt} = \epsilon \cdot CycleArrest \cdot DeathSwitch\, \rho[GM] \quad (14)$$

$$\frac{d[p27]}{dt} = (k_{25r} + V_8)[p27:CycE:Cdk2] + (k_{25r} + k_{30}[Cdc20]) \cdot [p27:CycA:Cdk2] + (k_{10} + k_{24r})[p27:CycD:Cdk2] - (V_6 + k_{25}([CycE] + [CycA]) + k_{24r}[CycD])[p27] + \epsilon k_5 \quad (15)$$

$$\frac{d[p27:CycA:Cdk2]}{dt} = k_{25}[CycA][p27] - (k_{25r} + V_6 + k_{30}[Cdc20])[p27:CycA:Cdk2] \quad (16)$$

$$\frac{d[p27:CycD:Cdk2]}{dt} = k_{24}[CycD][p27] - (V_6 + k_{10} + k_{24r})[p27:CycD:Cdk2] \quad (17)$$

$$\frac{d[p27:CycE:Cdk2]}{dt} = k_{25}[CycE][p27] - (k_{25r} + V_6 + V_8)[p27:CycE:Cdk2] \quad (18)$$

$$\frac{d[''phosphorylatedE2F'']}{dt} = (k_{20}(\lambda_A[CycA] + \lambda_B[CycB] + \lambda_D([p27:CycD:Cdk2] + [CycD]) + \lambda_E[CycE] + k_{26r}))[''phosphorylatedE2F:Rb''] + k'_{23} + k_{23}([CycA] + [CycB])[E2F] - (k_{22} + k_{26}[Rb])[''phosphorylatedE2F''] \quad (19)$$

$$\frac{d[''phosphorylatedE2F:Rb'']}{dt} = -k_{20}(\lambda_A[CycA] + \lambda_B[CycB] + \lambda_D([p27:CycD:Cdk2] + [CycD]) + \lambda_E[CycE] - (k_{26r} + k_{22}))[''phosphorylatedE2F:Rb''] + k_{26}[''phosphorylatedE2F''][Rb] + (k'_{23} + k_{23}([CycA] + [CycB]))[E2F:Rb] \quad (20)$$

$$\frac{d[''phosphorylatedIEP'']}{dt} = \frac{k_{31}[CycB](1 - [''phosphorylatedIEP''])}{1 + J_{31} - [''phosphorylatedIEP'']} - \frac{k_{32}[PPX]([''phosphorylatedIEP''])}{J_{32} + [''phosphorylatedIEP'']} \quad (21)$$

$$\frac{d[PPX]}{dt} = \epsilon k_{33} - k_{34}[PPx] \quad (22)$$

$$\frac{d[Rb]}{dt} = -(k_{20}(\lambda_A[CycA] + \lambda_B[CycB] + \lambda_D([p27:CycD:Cdk2] + [CycD]) + \lambda_E[CycE] + k_{26r}))[Rb] + (k_{19}PP1A + k_{19}{}'(PP1T - PP1A))[Rb\_hypo] + k_{26r}[E2F:Rb] - k_{26}[E2F][Rb] + k_{26r}[''phosphorylatedE2F:Rb''] - k_{26}[''phosphorylatedE2F''][Rb] \quad (23)$$

$$\begin{aligned}
\frac{d[Kip1]}{dt} &= \epsilon k_5 - V_6[Kip1] - k_{24}[CycD][Kip1] + k_{24r}[CycD:Kip1] \\
&+k_{10}[CycD:Kip1] + -k_{25}[Kip1]([CycE]+[CycA]) \\
&k_{25r}([CycE:Kip1]+[CycA:Kip1]) + V_8[CycE:Kip1] \\
&+k_{30}[Cdc20][CycA:Kip1] - ((k_{25}([CycA]+[CycE]) + k_{24}[CycD])[Kip1] \\
&-k_{25r}([CycA:Kip1]+[CycE:Kip1]) - k_{24r}[CycD:Kip1] \\
&-V6([CycA:Kip1]+[CycD:Kip1]+[CycE:Kip1]) \\
&-k_{30}[Cdc20][CycA:Kip1] - V8[CycE:Kip1] - k_{10}[CycD:Kip1]) \qquad (24)
\end{aligned}$$

$$\begin{aligned}
\frac{d[CycA:Kip1]}{dt} &= k_{25}[CycA][Kip1] - k_{25r}[CycA:Kip1] - V_6[CycA:Kip1] \\
&-k_{30}[Cdc20][CycA:Kip1] \qquad (25)
\end{aligned}$$

$$\begin{aligned}
\frac{d[CycE:Kip1]}{dt} &= k_{25}[CycE][Kip1] - k25r[CycE:Kip1] \\
&-V_6[CycE:Kip1] - V_8[CycE:Kip1] \qquad (26)
\end{aligned}$$

$$\begin{aligned}
\frac{d[CycD:Kip1]}{dt} &= k_{24}[CycD][Kip1] - k24r[CycD:Kip1] - \\
&V_6[CycD:Kip1] - k_{10}[CycD:Kip1] \qquad (27)
\end{aligned}$$

$$\begin{aligned}
\frac{d[Hdm2]}{dt} &= k_{37}[''p53-P''] + k_{38} - (k_{p14}[p14ARF] + k_{Rb}[Rb_hypo] \\
&+k_{cycE}[CycE]) - \left(k_{39}[Hdm2][''p53-P''] - \frac{k_{40}[p53:Hdm2]}{kk_1+[p53:Hdm2]}\right) \\
&\qquad (28)
\end{aligned}$$

$$\frac{d[p14ARF]}{dt} = k_{41}([E2F]-[p14ARF]) \qquad (29)$$

$$\frac{d[GAP]}{dt} = k_{ERK}[''ERK-PP''] - k_{42}[GAP] \qquad (30)$$

$$\frac{d[''p53-P'']}{dt} = K_{43}[ATM] - k_{39}[Hdm2][''p53-P''] + \frac{k_{40}[p53:Hdm2]}{kk_2+[p53:Hdm2]} \qquad (31)$$

$$\frac{d[p53:Hdm2]}{dt} = k_{39}[Hdm2][''p53-P''] + \frac{k_{40}[p53:Hdm2]}{kk_2+[p53:Hdm2]} \qquad (32)$$

$$\frac{d[p21]}{dt} = \frac{k_{44}[''p53-P'']}{k_{45}+[''p53-P'']} + \frac{k_{46}[PIP33:AKT\_On]}{kk_3+[PIP3:AKT\_On]} - k_{47}[p21] \qquad (33)$$

$$\begin{aligned}
\frac{d[CytoC:Apaf1:Caspase9]}{dt} &= ''Caspase9flux''[''p53-P''] \\
&-k_{48}[AKT][CytoC:Apaf1:Caspase9] \qquad (34)
\end{aligned}$$

$$\frac{d[Raf]}{dt} = \frac{k_{49}[''Raf-P'']}{kk_4+[''Raf-P'']} - \frac{k_{50}[''Ras-GTP''][Raf]}{kk_4+[Raf]} \qquad (35)$$

$$\frac{d[''Raf-P'']}{dt} = \frac{k_{50}[''Ras-GTP''][Raf]}{kk_4+[Raf]} - \frac{k_{49}[''Raf-P'']}{kk_4+[''Raf-P'']} \qquad (36)$$

$$\frac{d[MEK]}{dt} = \frac{k_{51}[''MEK-P'']}{kk_5+[''MEK-P'']} - \frac{k_{52}[''Raf-P''][MEK]}{kk_5+[MEK]} \tag{37}$$

$$\begin{aligned}\frac{d[''MEK-P'']}{dt} &= \frac{k_{52}[''Raf-P''][MEK]}{kk_5+[MEK]} - \frac{k_{52}[''Raf-P''][''MEK-P'']}{kk_5+[''MEK-P'']} \\ &+\frac{k_{51}[''MEK-PP'']}{kk_5+[''MEK-PP'']} - \frac{k_6[''MEK-P'']}{kk_{15}+[''MEK-P'']}\end{aligned} \tag{38}$$

$$\frac{d[''MEK-PP'']}{dt} = \frac{k_{52}[''Raf-P''][''MEK-P'']}{kk_5+[''MEK-P'']} - \frac{k_{51}[''MEK-PP'']}{kk_5+[''MEK-PP'']} \tag{39}$$

$$\frac{d[ERK]}{dt} = \frac{k_{53}[''ERK-PP'']}{kk_5+[''ERK-P'']} - \frac{k_{52}[''MEK-PP''][ERK]}{kk_5+[ERK]} \tag{40}$$

$$\begin{aligned}\frac{d[''ERK-P'']}{dt} &= \frac{k_{52}[''MEK-PP''][ERK]}{kk_5+[ERK]} + \frac{k_{53}[''ERK-PP'']}{kk_5+[''ERK-PP'']} \\ &-\frac{k_{52}[''MEK-PP''][''ERK-P'']}{kk_5+[''ERK-P'']} - \frac{k_{53}[''ERK-P'']}{kk_5+[''ERK-P'']}\end{aligned} \tag{41}$$

$$\frac{d[''ERK-PP'']}{dt} = \frac{k_{52}[''MEK-PP''][''ERK-P'']}{kk_5+[''ERK-P'']} - \frac{k_{53}[''ERK-PP'']}{kk_5+[''ERK-PP'']} \tag{42}$$

$$\begin{aligned}\frac{d[''Ras-GDP'']}{dt} &= k_{gap}[GAP] + \frac{k_{54}[''Ras-GTP'']}{kk6+[''Ras-GTP'']} \\ &-\left(k_{mass}[mass] + \frac{k_{55}[''Ras-GDP'']}{\left(1+\left(\frac{[''ERK-PP'']}{ki}\right)^n\right)(k_{56}+[''Ras-GDP''])}\right)\end{aligned} \tag{43}$$

$$\begin{aligned}\frac{d[''Ras-GTP'']}{dt} &= k_{mass}[mass] + \frac{k_{55}[''Ras-GDP'']}{(1+(\frac{[''ERK-PP'']}{ki})^n)(k_{56}+[''Ras-GDP''])} \\ &-(k_{gap}[GAP] + \frac{k_{54}[''Ras-GTP'']}{kk6+[''Ras-GTP'']})\end{aligned} \tag{44}$$

$$\frac{d[RTK]}{dt} = k_{57} - k_{58}[RTK] - \frac{k_{59}[RTK][mTORC1]}{[RTK]+kk7} \tag{45}$$

$$\frac{d[PI3K]}{dt} = \frac{k_{Ras}[''Ras-GTP''][PI3K]}{kk8+[PI3K]} - \frac{k_{60}[PI3K]}{kk8+[PI3K]} \tag{46}$$

$$\begin{aligned}\frac{d[mTORC1]}{dt} &= \frac{k_{61}[mTORC1\_Off][Rheb\_On]}{kk8+[mTORC1\_Off]} + \frac{V3_{PI3K}(1-[mTORC1])}{kk8-[mTORC1]+1} \\ &-\frac{k_{62}[mTORC1]}{kk8+[mTORC1]} - \frac{k_{63}[mTORC1]}{kk9+[mTORC1]}\end{aligned} \tag{47}$$

$$\frac{d[PIP2]}{dt} = \frac{k_{63}[PI3K][PTEN]}{kk9+[PIP3]} - \frac{k_{63}[PI3K][PIP2]}{kk9+[PIP2]} \tag{48}$$

$$\begin{aligned}\frac{d[PIP3]}{dt} &= \frac{k_{63}[PI3K][PIP2]}{kk9+[PIP2]} \\ &+k_{64}[PI3K:AKT\_Off] - \frac{k_{63}[PIP3][PTEN]}{kk9+[PIP3]} - k_{65}[PIP3][AKT]\end{aligned} \tag{49}$$

$$\frac{d[PIP3:AKT\_Off]}{dt} = k_{65}[PIP3][AKT] - k_{64}[PI3K:AKT\_Off] - \frac{k_{63}[PDK1][PIP3:AKT\_Off]}{kk9+[PIP3:AKT\_Off]} + \frac{k_{63}[PIP3:AKT\_On]}{kk9+[PIP3:AKT\_On]} \quad (50)$$

$$\frac{d[PIP3:AKT\_On]}{dt} = \frac{k_{63}[PDK1][PIP3:AKT\_Off]}{kk9+[PIP3:AKT\_Off]} - \frac{k_{63}[PIP3:AKT\_On]}{kk9+[PIP3:AKT\_on]} \quad (51)$$

$$\frac{d[TSC2\_Off]}{dt} = \frac{k_{66}[''ERK-PP''][TSC2\_On]}{kk9+[''ERK-PP'']} - \frac{k_{64}[PIP3:AKT\_On][TSC2\_On]}{kk9+[TSC2\_On]} - \frac{k_{64}[TSC2\_Off]}{kk9+[TSC2\_Off]} \quad (52)$$

$$\frac{d[TSC2_On]}{dt} = \frac{k_{64}[TSC2\_Off]}{kk9+[TSC2\_Off]} + \frac{k_{67}[''ERK-PP''][TSC1:2]}{kk9+[''ERK-PP'']} - \frac{k_{66}[''ERK-PP''][TSC2\_On]}{kk9+[''ERK-PP'']} - \frac{k_{64}[PIP3:AKT\_On][TSC2\_On]}{kk9+[TSC2\_On]} - k_{67}[TSC1][TSC2\_On] \quad (53)$$

$$\frac{d[TSC1]}{dt} = k_{68} + \frac{k_{67}[''ERK-PP''][TSC1:2]}{kk9+[''ERK-PP'']} - k_{67}[TSC1][TSC2\_On] - k_{68}[TSC1] \quad (54)$$

$$\frac{d[TSC1:2]}{dt} = k_{67}[TSC1][TSC2\_On] - \frac{k_{67}[''ERK-PP''][TSC1:2]}{kk9+[''ERK-PP'']} \quad (55)$$

$$\frac{d[Rheb_Off]}{dt} = \frac{k_{69}[TSC1:2][Rheb\_On]}{kk9+[Rheb\_On]} - \frac{k_{70}[Rheb\_Off]}{kk9+[Rheb\_Off]} \quad (56)$$

$$\frac{d[Rheb\_On]}{dt} = \frac{k_{70}[Rheb\_Off]}{kk9+[Rheb\_Off]} - \frac{k_{69}[TSC1:2][Rheb\_On]}{kk9+[Rheb\_On]} \quad (57)$$

$$\frac{d[mTORC1\_Off]}{dt} = \frac{k_{64}[mTORC1]}{kk9+[mTORC1]} - k_{64}[mTORC1\_Off] - \frac{k_{68}[mTORC1\_Off][Rheb\_On]}{kk9+[mTORC1\_Off]} \quad (58)$$

$$\frac{d[AKT]}{dt} = k_{71} - k_{72}[PIP3][AKT] - k_{73}[AKT] + k_{64}[PIP3:AKT\_Off] \quad (59)$$

$$\frac{d[PDK1]}{dt} = k_{67}(1-[PDK1]) \quad (60)$$

$$\frac{d[PTEN]}{dt} = K_{74}(1-[PTEN]) \quad (61)$$

Steady-state equations

$$PP1A = \frac{[PP1T]}{1+K_{21}(\phi_E([CycE]+[CycA])+\phi_B[CycB])} \quad (62)$$

$$V_2 = k_2'(1-[CDH1]) + k_2[CDH1] + k_2''[Cdc20] \quad (63)$$

$$V_4 = k_4(\gamma_A[CycA] + \gamma_B[CycB]) \quad (64)$$

$$V_6 = k'_6 + k_6(\eta_E[CycE] + \eta_A[CycA] + \eta_B[CycB]) \quad (65)$$

$$V_8 = k'_8 \frac{k_8(\psi_E([CycE] + [CycA]) + \psi_B[CycB])}{J_8 + [CYCET]} \quad (66)$$

$$CYCET = [CycE : Kip1] + [CycE : Kip1] + [CycE] \quad (67)$$

$$CYCDT = [CycD : Kip1] + [p27 : CycD : Cdk2] + [CycD] \quad (68)$$

$$CYCAT = [p27 : CycA : Cdk2] + [CycA] + [CycA : Kip1] \quad (69)$$

$$P27T = [p27 : CycA : Cdk2] + [p27 : CycD : cdk2] + [p27 : CycE : Cdk2] + [p27] \quad (70)$$

$$V1_{PI3K} = \frac{[RTK]VM1_{PI3K}}{[RTK] + kc_{PI3K}} \quad (71)$$

$$V3_{PI3K} = [PI3K]VM3_{PI3K} \quad (72)$$

Notes on Equations:

(1) The mass of the cell drops to half, [mass] $\rightarrow$ [mass]/2, every time [Cdh1] crosses 0.2 from bellow.
(2) To simulate cell cycle arrest, whenever $[p_{21}] > 20$, $[CDH1] = [GM] = [CycA] = [CycB] = [CycD] = [CycE] = 0$. To do so, *CycleArrest* binary parameter was put in equations:

$$CycleArrest = \begin{cases} 0, & \text{if } [p21] > 20 \\ 1, & \text{in all other cases} \end{cases}$$

(3) As an event to prepare apoptosis, whenever $[ATM] > 20$, the synthesis rate of Caspase-9 increases with *"Caspase9 flux"* parameter:

$$"Caspase9flux" = \begin{cases} 5, & \text{if } [Cyto : Apaf1 : Caspase9] > 10 \text{ and } [ATM] > 20 \\ 10, & \text{if } [ATM] > 20 \end{cases}$$

(4) To simulate apoptosis, whenever $[Cyto : Apaf1 : Caspase9] > 10$ and $[ATM] > 20$, $[mass] = [GM] = 0$, using *DeathSwitch*:

$$DeathSwitch = \begin{cases} 0, & \text{if } [Cyto : Apaf1 : Caspase9] > 10 \text{ and } [ATM] > 20 \\ 1, & \text{in all other cases} \end{cases}$$

(5) The same assumptions done in [1] were used in this system. The parameter *r31switch* works as a two steps Heaviside function:

$$r31switch = \begin{cases} 1, & \text{if } \frac{[Rb]+[E2F\_Rb]+["phosphorylatedE2F:Rb"]}{[Rb\_hypo]+[Rb]+[E2F:Rb]+["phosphorylatedE2F:Rb"]} < 0.8 \\ 0, & \text{in all other cases} \end{cases}$$

Parameters:

$n = 1$, $k_1 = 0.6$, $k'_1 = 0.1$, $k_3 = 140$, $k'_3 = 7.5$, $k_5 = 20$, $k_6 = 100$, $k'_6 = 10$, $k_7 = 0.6$, $k'_7 = 0$, $k_8 = 2$, $k'_8 = 0.1$, $k_9 = 0.05$, $k_{10} = 5$, $K_{10} = 3.8$, $k_{11} = 1.5$, $k'_{11} = 0$, $k_{12} = 1.5$, $k_{13} = 5$, $k_{14} = 2.5$, $k_{15} = 0.25$, $k_{16} = 0.25$, $k_{17} = 10$, $k'_{17} = 3.5$, $k_{18} = 10$, $k_{19} = 20$, $k'_{19} = 25$, $k_{20} = 10$,

$k_{22} = 1$, $k_{23} = 1$, $k^{'}_{23} = 0.005$, $k_{24} = 1000$, $k_{24r} = 10$, $k_{25} = 1000$, $k_{25r} = 10$, $k_{26} = 10000$, $k_{26r} = 200$, $k_{27} = 0.2$, $k_{29} = 0.05$, $k_{30} = 20$, $k_{31} = 0.7$, $k_{32} = 1.8$, $k_{33} = 0.05$, $k_{34} = 0.05$, $k_{35} = 5000$, $k_{36} = 10$, $k_{37} = 25$, $k_{38} = 2$, $k_{39} = 1$, $k_{40} = 0.5$, $k_{41} = 0.1$, $k_{42} = 1$, $k_{43} = 5$, $k_{44} = 100$, $k_{45} = 10$, $k_{46} = 10$, $k_{47} = 1$, $k_{48} = 0.1$, $k_{49} = 50$, $k_{50} = 100$, $k_{51} = 77.75$, $k_{52} = 2.855$, $k_{53} = 53$, $k_{54} = 1000$, $k_{55} = 1000$, $k_{56} = 10$, $k_{57} = 1$, $k_{58} = 0.5$, $k_{59} = 0.82$, $k_{60} = 0.15$, $k_{61} = 0.01$, $k_{62} = 1$, $k_{63} = 10$, $k_{64} = 0.5$, $k_{65} = 2$, $k_{66} = 0.7$, $k_{67} = 5$, $k_{68} = 0.1$, $k_{69} = 12$, $k_{70} = 4$, $k_{71} = 0.1$, $k_{72} = 2$, $k_{73} = 0.4$, $k_{74} = 1$, $k_i = 9$, $k_{AKT} = 0.5$, $k_{erk} = 0.5$, $k_{gap} = 0.1$, $k_{CycE} = 0.3$, $k_{p14} = 0.3$, $k_{Ras} = 0.6$, $k_{Rb} = 0.3$, $kk = 1$, $kk1 = 5$, $kk2 = 5$, $kk3 = 10$, $kk4 = 15$, $kk5 = 15$, $kk6 = 8$, $kk7 = 0.2$, $kk8 = 0.005$, $kk9 = 0.05$, $J_1 = 0.1$, $J_3 = 0.01$, $J_4 = 0.005$, $J_8 = 0.1$, $J_{13} = 0.005$, $J_{14} = 0.005$, $J_{15} = 0.1$, $J_{17} = 0.3$, $J_{31} = 0.01$, $J_{32} = 0.01$, $\epsilon = 1$, $\lambda_A = 3$, $\lambda_B = 5$, $\lambda_D = 3.3$, $\lambda_E = 5$, $\psi_E = 10$, $\psi_B = 0.5$, $\mu_E = 0.5$, $\mu_A = 0.5$, $\mu_B = 0.1$, $PP1T = 1$.