Discrete Modeling of the MAPK-mTor Pathway Connection in Cancer

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Abstract

Cancer is a complex disease where the deregulation of pathways that are involved in cellular processes such as apoptosis, angiogenesis or proliferation needs to be analyzed. Constructing mathematical models, systems biology aims at describing the dynamic behavior of a network in order to gain a better understanding of the biologal mechanisms and functions.

One of the best described signaling pathways is the Mitogen-activated protein kinases (MAPK) cascade which is a role model for complex dynamics in cellular systems. Investigations revealed that MAPK and mammalian Target of Rapamycin (mTOR) signaling pathways show a high occurance of mutations in cancer cells, resulting in extensive development of drugs for blocking these mechanisms.

However, creating a mathematical model of both pathways is challenging, since data about crosstalk is sparse. Therefore, discrete models are used which only require qualitative information about the system processes and deliver an overview about the capable dynamics of a network using state transition graphs.

In this work a discrete model of the MAPK-mTor signaling is constructed in order to test observations extracted from literature to find plausible connections in this cancer system. Thereby an approach for explaining contradictory results from recent studies is given and possible steps for further model refinement are proposed.

Introduction

During the last decades, knowledge of molecular cell biology increased drastically. Deciphering cellular functions and their highly complex regulation is an ongoing process, especially in the context of diseases. In cancer, small differences in cell signaling lead to changes in the regulation of complex processes, such as proliferation, angiogensis and cell death. Though these changes can be identified biochemically, the understanding of the mechanisms is still incomplete [5]. In particular, the interplay between pathways needs to be understood, requiering a system level view on the involved processes.

There are different modeling methods to capture signaling and regulation mechanisms, depending on available information and the desired level of detail [9]. In a first step, the system structure, i.e. the particpating components and dependencies between them, are depicted in an interaction graph. Obviously, the structure is generally not able to fully reflect the behavior of the network, thus dynamical models are of great interest.

Traditionally, network dynamics are simulated by ordinary differential equations (ODEs) that provide quantitative information about physical rates. However, this method is only applicable if a sufficient amount of experimental data is available to approximate parameters and validate the outcome of the simulations [9]. An alternative method is discrete modeling where the characteristics of the system are formulated using logical functions based on qualitative information of the network. Herein, a finite number of activity levels for components are defined, e.g. 0 and 1, where logical functions describe how regulators influence a component. By applying these functions, state transitions of the system are generated that represent the behavior over time [7].

In some cases the applicabilty of discrete modeling is limited, e.g. when intersted in dose-dependent reactions of the network or determining durations of processes. [7]. However, despite the high level of abstract, a logical model carries interesting information and is furthermore amenable to efficient analysis using e.g. methods from graph theory [11]. Of particular interst in the analysis is the asymptotic behavior, where e.g. fixpoints or a cyclic attractors can be related to experimental observations.

In this work, the mTor and MAPK signaling system is modeled aiming at finding a simple network

structure to simulate and analyze the complex behavior of the network. A discrete approach is chosen since the MAPK and the mTor pathway are known to be connected via crosstalk, but the exact information about interactions are sparse and unclear [1]. Mutations in these pathways are very prominent in tumors, therefore the networks have already been modeled by different groups [8]. Both pathways, MAPK and mTor, are known to regulate cell proliferation, therefore a promising approach is to induce apoptosis in tumor cells by blocking them. However, experimental investigations lead to contradictory results, where some cells were sensitive and others resistant to the same inhibitor [3].

Discrete modeling

Over the years, different discrete modeling methods have been developed (see e.g. [11]). Generally, the structure of a model is determined by the *interaction graph* of the network. This is a directed graph, where the nodes represent the components of the network and the edges indicate the interactions between the components. Let $C = \{c_1, ..., c_n\}$ be a set of components. An edge defines the influence of a source node on its target node. The edge is labelled as either positive (activation) or negative (inhibition), in Fig. 1 and 2 positive edges have an arrows as head and negative edges are T formed.

For simulating the dynamical behavior of the pathways, logical functions need to be created that define how signals of incoming edges are processed reflecting the biological mechanisms. Each component $c_i \in C$ is a Boolean variable, i.e. the value 0 signifies the component being inactive and 1 being active. A *state* of the system X is defined by a vector with n elements, where each node is assigned to a value x_i. The dynamics of the system are modeled as logical functions $f : \{0,1\}^n \to \{0,1\}^n$, $f = (f_1, .., f_n)$, where each coordinate function f_i describes the regulation of component c_i by its predecessors in the interaction graph. The nature of regulation is described using logical operators AND ($\wedge$), OR ($\vee$) and NOT (!).

From the function f we derive the state transitions of the system. Different approaches can be employed for that, e.g. synchonous update, where all nodes are updated simultaneously, which means that each state of the system has exactly one following state $x \to f(x)$. In a biological context this strategy is rather unrealistic since cellular processes work on many different time scales. For this reason, Thomas et al. [11] introduced an ansynchronous update strategy, where only one component is changed at a time $x \to x' = \{x'_1, ..., x'_n\}$ with $x'_i = f_i(x) \neq x_i$ for some i and $x_j = x'_j$ for all $i \neq j$. In case a component c_i has only one predecessor, its state is $f_i(x) = \{x_j, 1 - x_j\}$ for activation or inhibition, respectively. Input nodes are modeled with a self loop to simulate a permanent signal $f_i(x) = x_i$. This generally results in a non-deterministic behavior, since a state can have several sucessors. Complexity of the analysis increases, but the representation includes more realistic state transitions (see [11] for further discussion).

The complete behavior of the system is then given by the state transition graph (STG), where nodes represent all possible states of the model and edges represent transitions between these states. The trajectories of the system are encoded as paths in the graph, whose strongly connected components that no trajectory can leave are called attractors. They correspond to e.g. fix points and limit cycles and are of particular interest [11]. Given such a model one can now easily analyze the effect of a signal on the asymptotic behavior, i.e. the attractors of the system.

The discrete modeling approach is supported by various software tools. In this work the software GINsim [1] is mainly utilized, developed by the group of Chaouiya et al. [4].

Results

Applying this mathematical method to the MAPK and mTor signaling, the crosstalk and its impact on apoptosis is seeked. In a first naive step, the network with all possible connection from the literature were implemented, but the results were too complex to relate mechanisms and functions. Thus, both pathways are modeled seperately, the dynamics are validated and then possible crosstalk is added and tested.

Model building

The mTor signaling promotes protein synthesis, cell growth and proliferation. The protein mTor is active in two complexes that regulate different downstream

[1] http://gin.univ-mrs.fr/

pathways. The complexes are controlled by the tuberous sclerosis complex (Tsc), which regulates mTorC1 via Rheb (Ras homolog enriched in brain) negatively and mTorC2 positively. Tsc itself is repressed by active Akt, which is dualy phosphorylated by mTorC2 and PDK1 [3]. The latter component recieves its phosphate from Phosphatidylinositide 3-kinases (PI3K), which also activates mTorC2. Since PDK1 is only passing the phosphate and therefore brings no new function, it is left out of the model. Moreover a tumor-suppressor-gene, the Phosphatase and tensin homolog (Pten), is added as a negative regulator PI3K and whose loss-off-function is oberserved in many tumors [2].

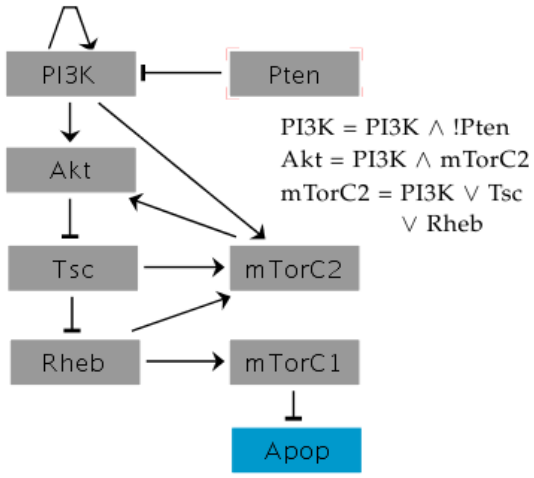

Figure 1: Interaction graph of mTor mechanism (grey), the output apoptosis (blue) and functions of the nodes PI3K, Akt and mTorC2.

The output node apoptosis represents a whole pathway, which is a cascade activated by mTorC1. Since only the final effect, namely whether the cell dies or not, is commonly observed across studies, this simplification is reasonable. The resulting interaction graph is shown in Fig. 1.

The second pathway, the MAPK cascade, is involved in many cellular responses leading to proliferation and cell cycle progress. This pathway is a simple cascade transfering phosphates via kinases to target proteins with negative feedback, thus its dynamics can be captured with three components Raf, Mek and Erk, whereas each component stands for a class [8]. Both pathways are depicted in Fig. 2.

For observing the dynamics of the network, Boolean functions are created for every component as described before. Functions with more than one regulator are shown in Fig. 1, whose connection is deduced from biological information.

Analysis

Using these functions, both pathways are implemented in GINsim and the STG is analyzed identifying all possible attractors.

The STG of the mTor mechanism shown in Fig. 1 splits up in two bassins for attractors of the system. Either apoptosis is active, in case PI3K is inactive resulting from absent input or repression through Pten, or apoptosis is inactive for active PI3K. This result is reasonable since the pathway induces proliferation, therefore should activation prevent apoptosis [3]. Moreover, Chen et al. [2] observed that Pten is necessary for inducing apoptosis.

The MAPK cascade also has two attractors, where active Raf leads to a cyclic attractor with oscillations in all components. This behavior has been shown by various experiments and also using ODE modeling [9]. In case Raf is inactive, the pathway remains in its trivial fixpoint, where all components are consequently inactive.

Crosstalk

In several studies it was observed that inhibition of the mTor pathway components like PI3K or Akt is not able to clearly induce apoptosis as the model in Fig. 1 suggests [3]. Moreover, it was shown that Erk is able to regulate mTorC1 similarly to Akt, whereas a possible connection is identified as Tsc [12]. Many different interactions between mTor and MAPK signaling were suggested, which varied in terms of cancer type and experiment [10].

Mathematically, by adding a single edge to a model, the dynamics of the system can change completely. When coupling two validated models it is therefore important to make sure that the validated behavior is preserved. Here, two model extensions are made for investigating the effect of crosstalk. Besides adding connections between the pathways, a common input is defined. Both mTor and MAPK are activated by receptor Tyrosine kinases (RTK), which is added as input on both PI3K and Raf. Besides the inhibitory edge from Erk to Tsc, it was investigated that Rheb supresses the Raf dimerisation, which is necessary for its activity [6]. Also Akt was shown to form a complex with Raf molecules and thereby

preventing its kinase acitivity. The resulting network is shown in Fig 2.

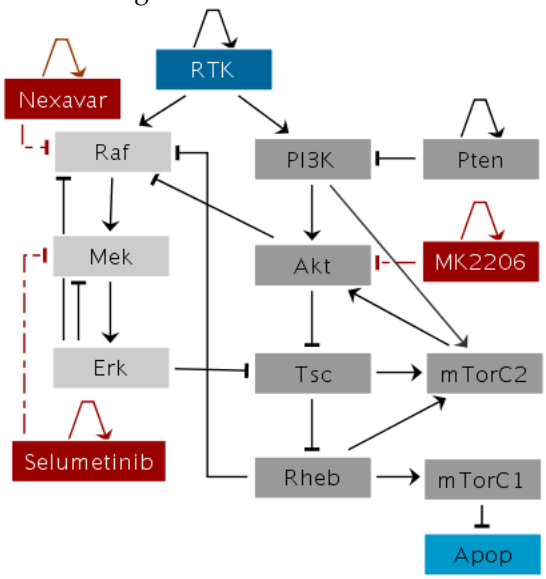

Figure 2: Model of MAPK (light grey) and mTor (grey) signaling with crosstalk, inhibitors (red) and two functions are expanded: $Raf = RTK \vee !Rheb \vee !Akt \vee !Erk$ and $Tsc = !Akt \vee !Erk$.

The dynamics of the coupled network are shown in the first block of Table 1. Here, the STG splits up in three bassins consisting of two fixpoints from the mTor model and the limit cycle of the MAPK model. In detail, the network with inactive RTK causes a fixpoint with active apoptosis and active RTK with inactive Pten has a fixpoint with inactive apoptosis, which are the same fixponts than the mTor model. Thus, for these inputs the MAPK pathway does not influence the attractors of the network. However, for active RTK and Pten, the mTor signaling via PI3K is blocked and the MAPK pathway causes an oscillating attractor which is transferred to the output through Tsc.

Inhibitors

In order to test this model, three common cancer drugs for this pathway are added, namely the Raf blocker Nexavar, MEK blocker Selumetinib, and Akt blocker MK-2206. The inhibitors are added as additional inputs that block the respective target independent from other incoming signals. The effects of these drugs are investigated in many studies, but lacking of clarity. Chappell et al. [1] regarded their roles in cancer therapy, where they report that the effectiveness of a drug strongly depends on the cancer type. For comparing the literature results to the developed model, all combinations of inputs and drugs are tested for the capability to induce apoptosis, listed in Table 1.

Adding Nexavar or Selumetinib to the pathway blocks the MAPK path to inhibit Tsc. If additionally Pten is active, apoptosis is induced. Table 1 shows that both drugs individually and the combination of them are not sufficient to guarantee apoptosis, confirming experimental observations, where Nexavar was not able to eliminate the tumor on long term [2]. The Akt inhibtitor MK-2206 is also not able to produce a clear fixpoint with active apoptosis, since MAPK induces oscillations when RTK is active.

Table 1: Attractors of the system depending on input RTK, Pten and inhibitors (Nex = Nexavar, Sel = Selumetinib, MK = MK-2206).

RTK	Pten	Nex	Sel	MK	Apop
1	0	0	0	0	0
1	1	0	0	0	osci
0	0/1	0	0	0	1
1	0	1	0	0	0
1	1	1	0	0	1
0	0/1	1	0	0	1
1	0	0	1	0	0
1	1	0	1	0	1
0	0/1	0	1	0	1
1	0/1	0	0	1	osci
0	0/1	0	0	1	1
1	0	1	1	0	0
1	1	1	1	0	1
0	0/1	1	1	0	1
0/1	0/1	1	0	1	1
0/1	0/1	0	1	1	1

Experiments revealed that blocking both pathways parallelly induced apoptosis in any case, thus these pathways have an overlapping function with crosstalk causing robustness towards drugs [1]. The last block of Table 1 confirms these results, since supressing both mTor and MAPK signaling sucessfully switches apoptosis permenantly on independent from the input.

Discussion

In this work a discrete model for a coupled MAPK and mTor signaling network was created. Starting

with isolated network models, expected qualitative dynamics are simulated with Boolean functions. Subsequently, simple but effective crosstalk connections extracted from literature were introduced. The resulting model is able to reproduce known behavior of the uncoupled pathways, it reflects the influence of inhibitors on the network and possibly clearifies discrepancies from experiments.

Especially observations towards the effect of Nexavar on cancer cells are complicated to analyze, since experimental setups differ in cell types showing different mutations. Here the question arises, if such a model can be useful to resolve literatur discrepancies by considering model realization depending on e.g. cell type.

Morever, a future aim is to decipher the role of each interaction in the model regarding its influence on the dynamics, because so far only the input/output effects were investigated. Relating to this question, developing a theoretical approach for expanding and/or reducing the complexetiy of models in terms of preserving the dynamics is aspired.

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