Construction and Analysis of A Multicellular Model of the Pancreatic Cancer Microenvironment

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Motivation

Cancer research should not focus on cancer cells only.

"*In addition to cancer cells, tumors exhibit another dimension of complexity: they contain a repertoire of recruited, ostensibly normal cells that contribute to the acquisition of hallmark traits by creating the tumor microenvironment.*"

--Douglas Hanahan, and Robert A. Weinberg. Hallmarks of Cancer: The Next Generation. 2011

Pancreatic fibroblasts, as one type of essential cells insisting of the microenvironment of pancreatic cancer, contribute to all the stages of pancreatic tumor development.

This fact calls for a model which can capture the intracellular mechanisms in both cancer cells and fibroblasts, as well as the interactions between them.

Contributions

-A multicellular model including pancreatic cancer cells (PCCs) and pancreatic fibroblasts (or pancreatic stellate cells, PSCs) has been constructed and described in both Boolean Network, and rule-based formats. The former encoding enables to conduct Symbolic Model Checking, the later enables to run simulations. Both Model Checking and simulations are used to analyze the constructed model.

-A list of rules are given to show how to use rule-based modeling to model multicellular Biological systems. According to these rules, PCCs-PSCs model has been encoded in RuleBender, which is a simulator for rule-based models of Biological systems. Simulation results obtained are consistent with experimental results showed in literatures.

Introduction to related Biological knowledge

Part I: Intracellular signaling network of pancreatic cancer cells

Cell Functions in PCCs	Related components
Proliferation	-K-Ras mutation causes proliferation -HER2/neu mutation causes proliferation -EGF activates proliferation and enhances it through autocrine -bFGF activates proliferation and enhances it through autocrine
Apoptosis	-TGFbeta1 signaling initiates apoptosis -Ras mutation / HER2/neu mutation / EGF inhibits apoptosis
Autophagy	-Inhibition of autophagy through mTOR -Overexpression of antiapoptotic factors promote autophagy

Part II: Intracellular signaling network of pancreatic stellate cells

Cell Functions in PSCs	Related components
Activation	-PDGFBB induces the activation of pancreatic stellate cells -TGFbeta1 activates pancreatic stellate cells -TNFalpha contributes to the activation of pancreatic stellate cells -IFNGR and PPARγ inhibit the activation
Migration	-PDGFBB also contributes to the migration of PSCs -Equally important is the involvement of ERK/AP1 pathway
Proliferation	Another major function of intracellular signaling pathway - ERK/AP1 - located downstream of PDGF receptor is to initiate the proliferation of PSCs.

(The reason why cell death is not considered here for the fibroblast is that, according to experimental observations, death for the fibroblast is comparatively show.)

Part III: Interactions between pancreatic cancer cells and pancreatic stellate cells

Pancreatic cancer cells release mitogenic and fibrogenic stimulants, such as
-transforming growth factor β (TGFβ),
-platelet-derived growth factor BB(PDGFBB),
-sonic hedgehog,
-galectin 3,
-…

Stellate cells in turn secrete various factors, including
-PDGFBB,
-stromal-derived factor 1,
-epidermal growth factor (EGF),
-insulin-like growth factor 1 (IGF),
-fibroblast growth factor (FGF),
-…
all of which get involved in the interactions between PCCs and PSCs.

Among them, TGFbeta1, PDGFBB, and bFGF are main growth factors constructing the bridge between tumor cells and stellate cells.

Methodology

Part I: Use Boolean Network to encode the PCCs-PSCs model

Interactions in biological pathways can be represented as an edge-weighted interaction graph $G: (V, E)$. A node $v_i \in V$ represents a biological component such as a gene or a protein. An edge $e_{ij} \in E$ from node v_i to node v_j has a weight α_{ij}, corresponding to the effect of the component represented by v_i on the component represented by v_j. Activation is specified by a positive value of α_{ij}, inhibition by a negative value.

A Boolean network $B(C, F)$ consists of a set of components C and a list of Boolean functions F. Each component $c_i \in C$ has a state, which can take two values: $c_i = 1$ if the component is active and $c_i = 0$ if the component is absent or inactive.

We use k to represent the number of components ($k = |C|$). The state s of a Boolean network corresponds to the vector $(c_1, \ldots, c_k) \in \{0, 1\}^k$ of the states of all components of the Boolean network. A Boolean function $f_i \in F$ is assigned to every component. This function maps the current state of the system to a Boolean value representing the next state of the corresponding component ($f_i : \{0, 1\}^k \rightarrow \{0,1\}$).

Time in Boolean networks is represented by a discrete variable t. We use the notations $c_i(t)$ and $s(t)$ to represent the state of a component, respectively the state of the whole system, at time t. The value of each protein at the next time step is computed by applying the corresponding Boolean function to the current state of the system.

$c_i(t+1) = f_i(s(t))$, where

$$f_i(s) = \begin{cases} 0 & \text{if } \sum_{c_j \in s} \alpha_{ij}\, c_j < 0 \\ 1 & \text{if } \sum_{c_j \in s} \alpha_{ij}\, c_j > 0 \\ c_i & \text{if } \sum_{c_j \in s} \alpha_{ij}\, c_j = 0 \end{cases}$$

Part II: Model Checking technique is adopted to verify and refine the model as well as checking proposed hypotheses

Model checking is an automatic verification technique for finite state systems which are modeled by labeled state transition graphs. It consists of an exhaustive exploration of the state-space of a given system in order to verify that it always adheres to a set of requirements.

In the case of biological systems, requirements are derived from experimental observations.

With Model Checking technique, we can
1) verify the built model through checking whether it can satisfy the requirements. If so, it means that the constructed model can generate consistent behaviors with the experimental observations;
2) if not, refine the model according to the obtained counterexample until the properties can be satisfied;
3) also, if necessary, incorporate new hypotheses into the model to check whether they make sense. Then, pass those hypotheses satisfying existing requirements to Biologists to check them in experiments.

In a nutshell, Model Checking technique can help to promote the understanding of Biological systems through the above ways.

Part III: A novel application of rule-based modeling to multicellular level's models

Rule-based modeling aims at representing molecules as structured objects and molecular interactions as rules for transforming the attributes of these objects.

BioNetGen is a formalism used to specify rule-based models of Biochemistry systems in RuleBender, a tool simulating BioNetGen rule-based models. Originally, the BioNetGen language is designed to specify protein-to-protein reactions as illustrated in the following figure 1.

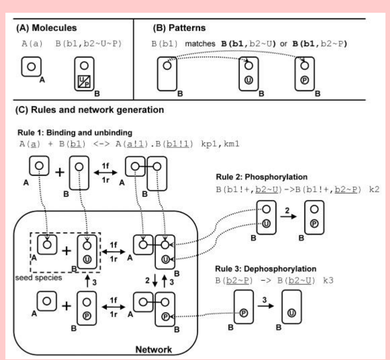

Figure 1: Rule-based modeling concepts and their encoding in BioNetGen Language (BNGL). (A) The basic building blocks are molecules, which may be assembled into complexes through bonds that link components of different molecules. (B) Patterns select particular attributes of molecules in species (shown in bold). (C) Rules specify the biochemical transformations that can take place in the system and may be used to build up a network of species and reactions.

In this work, BioNetGen is first used to specify cell-level multicellular models. The exampled encoding formats of cell-level systems are given as below.

The basic building blocks

The fundamental blocks can be either extracellular molecules or cells.

Patterns

Patterns are used to identify a set of species that share a set of features. Patterns used here are the same as original ones.

Rules

Rules	Example
Rule 1: Ligand-receptor binding	EGF+PCC(EGFR~F)->PCC(EGFR~T) r
Rule 2: Mutated receptors form a heterodimer	PCC(HER2~F,EGFR~F)->PCC(HER2~T,EGFR~T) r
Rule 3: Downstream regulation	PCC(MEK~T,ERK~T)->PCC(MEK~F,ERK~T) r PCC(MEK~T,ERK~F)->PCC(MEK~F,ERK~T) r
Rule 4: Secretion	PCC(TGFbeta1~T)->PCC(TGFbeta1~F)+PCCTGFbeta1 r
Rule 5: Mutation	PCC(RAS~F)->PCC(RAS~T) r (very high rate)
Rule 6: Degradation of extracellular molecules	EGF->Null() deg
Rule 7: Drug intervention	PCC(EGFR~T)->PCC(EGFR~F) interv
Rule 8: All kinds of cell functions	Autophagy: PCC(Aut~T)->EGF+TGFbeta1 autorate
	Apoptosis: PCC(Apo~T)->Null() apoprate
	Proliferation: PCC(Pro~T) -> PCC(Pro~F)+PCC(Pro~F,Apo~F,RAS~F,PI3K~F) prorate
	…

Results

Figure 2: Boolean Network version of PCCs-PSCs model

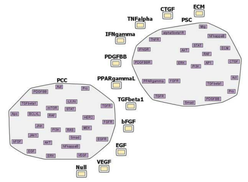

Figure 3: Rule-based version of PCCs-PSCs model

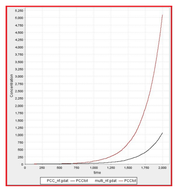

Figure 4: The existing of PSCs will promote the increase of proliferation of PCCs

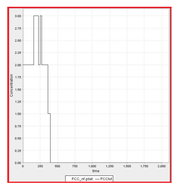

Figure 5: PCCs population without PSCs when proliferation is inhibited to some extent

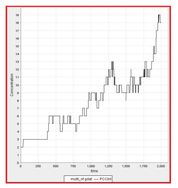

Figure 6: PCCs population with PSCs when proliferation is inhibited to some extent

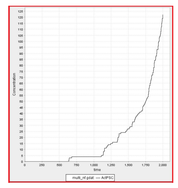

Figure 7: PSCs' activation and migration caused by PCCs

Future Work

From the aspect of the model of pancreatic cancer microenvironment,
1) Macrophages, which, together with cancer cells and fibroblasts, construct the main structure of pancreatic cancer microenvironment, will be studied and included;
2) More probable cell functions and underlying signaling pathways will be considered;
3) Multiple levels of concentration (instead of just low and high) will be described in future model.

From the aspect of computer tools used to analyze the model,
1) Explore formalisms and tools that can express multiple levels of concentration (e.g. Qualitative networks);
2) Extend existing formalisms in order to describe dynamic behaviors such as cell migration, cell death, and so on.

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