Studying prostate cancer as a network disease by qualitative computer simulation with Stochastic Petri Nets

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Abstract. Despite increased understanding of cancer pathogenesis, translating this knowledge into therapy remains challenging. Radical progress depends on utilizing molecular biology knowledge to understand processes by which genetic information is executed in response to microenvironmental perturbations. Understanding of the molecular machinery of the cell requires computer simulation of the complex network of molecular interactions and Petri net offers ideal framework. Lack of quantitative data about molecular amounts and transition rates necessitates development of qualitative methods providing useful insight with minimal knowledge on quantitative parameters. Here we show work in progress on the modeling of molecular interaction network involved in the evolution of prostate cancer. We use statistical model checking of qualitative model and show that the effects of genetic and pharmacological perturbations on prostate cancer evolution can be predicted by the number of token game trajectories reaching nodes representing proliferation and cell death events.

Keywords: Stochastic Petri net, statistical model checking, Prostate cancer

1 Introduction

Despite increased understanding of cancer pathogenesis, translating this knowledge into therapy remains challenging. Radical progress depends on utilizing advances in both DNA sequencing technology and molecular biology to understand processes by which genetic information is executed in response to microenvironmental perturbations. However, the expression of genetic information in response to environmental signals is performed by complex molecular machinery involving hundreds of thousands of components influencing each other through non-linear interactions. While the century of biochemistry and molecular biology resulted

in accumulation of voluminous data on molecular components and their interactions the size and complexity of the system makes it impossible to use this knowledge without computer models. This situation motivated intense research towards reconstruction or reverse engineering the network of interacting cellular components in the form of computer simulation capable of predicting effects of genetic and environmental perturbations on cellular behavior [1-4]. The major bottleneck in this effort is the lack of quantitative parameters describing molecular interactions. The existence and sign (activation, inhibition) of the molecular interaction are much more amenable to experimental studies than quantitative measurements of molecular amounts or transition rates. This encourages development of qualitative simulation approaches where valuable insight can be provided by analysis of molecular interaction network connectivity, with very limited quantitative data. In this contribution we present the Petri Net model of the molecular interaction network involved in the evolution of prostate cancer. We show that statistical model checking of the qualitative model, with discretized molecular activities and uniform transition rates provides valuable predictions of the effects of genetic and pharmacological perturbations on cancer progression.

2 Petri Net model of Prostate Cancer Network.

Molecular species were represented as places and interactions were represented by transitions. Edges and read edges were used to represent activation interactions whilst inhibitory interactions were represented by molecular state-change transitions rather than by inhibitory edges, as compiled from research data. Our model building started from interactions involved in the control of the PTEN gene. Subsequently, we have included transitions involving molecules controlling PTEN and proceeded until major inputs for environmental signals were covered. The model, which is still very much 'work in progress', currently includes growth factors, insulin, Wnt and androgen receptors activating the signaling networks of PI3K-PTEN-Akt, Ras-Raf_MEK-ERK, beta-catenin, c-Myc, mTor, p53 and p27. The intrinsic apoptotic pathway and a very basic representation of the cell cycle was included in the network. Two special places were introduced into the network to represent biological outcomes of cell death and proliferation. The model has been build using a Snoopy Petri Net tool [5] in Extended Petri Net mode. The final Petri Net model contains 251 nodes and 195 transitions.

3 Statistical Model Checking.

Cancer develops when the cell escapes the cell death program and starts proliferating out of the control of growth factor signals. Thus in our simulations we investigated reachability of the places representing cell death and proliferation. In particular, we were interested in whether the proliferation place is reached before the cell death place is reached. Since, we do not know molecular amounts and transition rates in the system we have attempted qualitative simulation.

We allowed the state of each place to vary between 0 and 2 tokens, thus representing absent, low activity and high activity states of molecular components. Even with this radical discretization the model was still too large to determine reachability of proliferation place by analysis of full reachability graph. We have therefore used a statistical model checking approach. For each simulation we have generated ensemble of token game trajectories starting from the same initial conditions and determined the number of trajectories in which the marking of Proliferation place changed from 0 to 1 while the marking of Cell Death place remained at initial value of 0. In short, we determined the number of token game trajectories in which proliferation occurred before cell death. We decided to use Gillespie algorithm [6] numerical simulation of Continuous Time Markov Chain dynamics to generate trajectory samples. Since, we have no knowledge of rate constants we have assumed that all transitions are equally likely to occur and set their rate constants to 1. This implies that we did not interpret Gillespie algorithm time as a real time, with physical time units. We used it exclusively to order the sequence of transitions and to limit the maximal trajectory length. The stochastic Petri net with Gillespie algorithm allowed perturbing the system by adjusting rates and thus altering probability of the occurrence of selected events. This is advantageous over similar approach previously used in biological context [7]. Trajectories were run until the cell death place changed its state or simulation time reached 100 arbitrary time units. For each of the numerical experiments we have run 10000 independent trajectories. The numbers of trajectories reaching proliferation before cell death were expressed as fractions and the 99% binomial probability confidence intervals (99% CI) were calculated to establish significance of the differences between simulation outcomes. The fractions which were outside of their 99% CIs were considered to be significantly different.

Simulations were performed by the extended version of SurreyFBA software [8], which allows statistical model checking in general molecular interaction networks. The Python script has been written to automatically convert Petri Nets in Snoopy file format to simulation software files. Confidence intervals were calculated by binconf() function of Hmisc R package using Wilsons method.

4 Results

We have first run 10000 token game trajectories for the original model. Within the simulation time of 100 arbitrary time units, the proliferation place has been reached before the cell death place in 4779 of trajectories. The 99% CI of the binomial probability was (0.4650524, 0.490777). Subsequently, we have inactivated p53 gene in the model by removing all tokens from the node representing p53 DNA. The number of token game trajectories in which proliferation was observed before cell death was 7223 and the 99% CI was (0.7106193, 0.7336859). Therefore, inactivation of p53 gene resulted in the significant rise of the proliferation. This is in agreement with voluminous experimental data on p53 gene

showing that inactivation of p53 results in the increased chances of proliferation and cancer. Motivated by this result we have investigated other perturbations. Simulation of the network where the state of testosterone input node was set to 0 tokens resulted in proliferation probabilities in the 99% confidence interval of (0.2349789, 0.2571581). Therefore, removal of testosterone input resulted in decreased chances of cell proliferation happening before cell death. This result is also in agreement with experimental data. Next, we tested whether our model reproduces the effect of GSK-3B enzyme inhibitors of potential use in cancer chemotherapy [9, 10]. As these drug decrease nuclear stability of an AR-GSK-3B complex and increase (deactivating) nuclear export of the AR, we modeled its effect by increasing the rate of transition removing tokens from the place representing the phosphorylated AR-GSK-3B nuclear complex. In the perturbed system the rate of inactivation transition was set to 1000, while all other transition rates were set to 1. The proliferation probability estimated by running 10000 trajectories was in the 99% CI of (0.3255304, 0.349885). Thus, the simulation reproduced the action of the drug. While numerical experiments shown above provide encouraging validation for the model, we have obtained quite disappointing results in simulation of the PTEN gene inactivation. The 99% CI of proliferation probability was (0.4678475, 0.4935781) i.e. there was no significant difference between the number of trajectories reaching proliferation in original wild type model and PTEN knock-out. There are cogent reasons why this may be the case because the network control of PTEN has been shown in another systems biology study to be different in the basal state than in the insulin-stimulated state [10] and so more sophisticated dynamic Petri net modeling is likely to clarify the situation, particularly as the present result is in contradiction to experimental data indicating an important role for loss of PTEN activity in the evolution of prostate cancer. The qualitative balance of PTEN versus PI3K sensitivity is another area that may also require adjustment in our model to fit data from the literature. Thus, although the simplified Petri net model of prostate cancer presented here highlights gaps in current knowledge and the requirement for meticulous incorporation of static and dynamic network behaviour from the literature, the success of this qualitative approach nevertheless demonstrates that it is useful to focus experimental effort on determination of a full set qualitative interactions, before proceeding to difficult quantitative experiments.

5 Conclusions

In this contribution we show preliminary results that demonstrate the applicability of Stochastic Petri nets for qualitative modeling of molecular interaction networks involved in cancer. We discretize molecular activities to 3 levels, set all transition rates to 1 and used Gillespie algorithm simulation to generate a sample of transition sequences leading to alternative biological outcomes of death and proliferation. We show that despite this radical qualitative approximation the method captures the effects of best studied perturbations on these key biological behaviours. The method is computationally efficient and allows accurate

determination of probabilities for large-scale models. Our test shows that it is possible to perform qualitative simulations of the numerous large-scale network reconstructions that were so far investigated exclusively by calculation of network connectivity statistics [1, 2], which does not allow prediction of perturbation effects. We believe that Stochastic Petri nets can be used to reconstruct genome scale models of molecular interaction networks and apply them to prediction of the effects of gene polymorphism and pharmacological interventions in complex network diseases such as prostate cancer.

References

1. Calzone, L., Gelay, A., Zinovyev, A., Radvanyi, F., Barillot, E.: A comprehensive modular map of molecular interactions in RB/E2F pathway. Molecular systems biology 4, 173 (2008)
2. Oda, K., Kitano, H.: A comprehensive map of the toll-like receptor signaling network. Molecular systems biology 2, 2006 0015 (2006)
3. Price, N.D., Reed, J.L., Palsson, B.O.: Genome-scale models of microbial cells: evaluating the consequences of constraints. Nature reviews. Microbiology 2, 886-897 (2004)
4. Kohn, K.W.: Molecular interaction map of the mammalian cell cycle control and DNA repair systems. Molecular biology of the cell 10, 2703-2734 (1999)
5. Rohr, C., Marwan, W., Heiner, M.: Snoopy–a unifying Petri net framework to investigate biomolecular networks. Bioinformatics 26, 974-975 (2010)
6. Gillespie, D.T.: Exact stochastic simulation of coupled chemical reactions. J. Phys. Chem 81, 2340-2361 (1977)
7. Ruths, D., Muller, M., Tseng, J.T., Nakhleh, L., Ram, P.T.: The signaling petri net-based simulator: a non-parametric strategy for characterizing the dynamics of cell-specific signaling networks. PLoS computational biology 4, e1000005 (2008)
8. Gevorgyan, A., Bushell, M.E., Avignone-Rossa, C., Kierzek, A.M.: SurreyFBA: a command line tool and graphics user interface for constraint-based modeling of genome-scale metabolic reaction networks. Bioinformatics 27, 433-434 (2011)
9. Schutz, S.V., Schrader, A.J., Zengerling, F., Genze, F., Cronauer, M.V., Schrader, M.: Inhibition of glycogen synthase kinase-3beta counteracts ligand-independent activity of the androgen receptor in castration resistant prostate cancer. PloS one 6, e25341 (2011)
10. Lequieu, J., Chakrabarti, A., Nayak, S., Varner, J.D.: Computational modeling and analysis of insulin induced eukaryotic translation initiation. PLoS computational biology 7, e1002263 (2011)