RESEARCH

Introduction to Focus Areas in Bioinformatics Project Week 7

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Abstract

Goal of the project: The aim of this project was to reproduce the results of a mathematical model for Epithelial to Mesenchymal Transition (EMT).

Main results of the project: We were able to successfully simulate the EMT in cancer cells.

Personal key learnings:
Sina: The etiology of metastasis
Christina: working with GINsim
Swetha: Understanding MaBoSS framework and software extension
Gokul: Working with WebMaBoSS

Estimation of the time:
Sina: 5 hours
Christina: 6 hours
Swetha : 6 Hours
Gokul: 7 Hours

Project evaluation: 3/5

Number of words: 1452

1 Scientific Background

Even though metastasis accounts for the majority of cancer deaths [1], the process itself is largely unknown [2]. The development of metastasis is most often viewed as a multi-step cascade [3]. First, the cells infiltrate into the adjacent tissue. For this purpose, cancerous cells need the ability to migrate, enabled by a morphological change. This process is called Epithelial to Mesenchymal Transition (EMT). It activates a switch from E-cadherin expression to N-cadherin expression [4], weakens cell-cell contacts, and with that enables the mutated cells to pass the basal membrane and enter surrounding tissue [5]. During the process, a complex transcription network is at play.

Cohen et al [6] models the molecular interactions involved in EMT regulation as a mathematical framework. They construct an influence network based on scientific articles. This means, identifying genes and proteins contributing to the process and connecting them depending on experimentally proven physical interactions. The network can be simplified into positive and negative influences and translated into a mathematical model using the Boolean formalism.

2 Model Building and Tools

Our goal was to reproduce the results in the paper by Cohen et al [6]. The influence network is available as a logical GINsim model [7].

To understand the function of organisms, the tool GINsim, which is short for Gene Interaction Network simulation, was developed for the modeling and the analysis of regulative networks [8]. It allows to reenact the stable states of a model, take a deeper look into the perturbations hen turning genes on and off and have a look at the phenotypes for the different states.

Additionally, we used MaBoSS, which is a software that allows to simulate individual cells and model stochastically the intracellular mechanisms that are deregulated in diseases. To do so, a regulatory network is illustrated by nodes that are linked using positive and negative influences. The regulatory network is transformed into a state transition graph using the logical rules, in such a graph each node is a vector of possible variables in the system. MaBoSS is based on the continuous-time Markov process applied on Boolean networks. The transition rates can be defined which helps to activate or inhibit a node. It provides a way to assign probabilities to each state of the model over time and quantifies the effect of a perturbation. That way, MaBoSS can simulate personalized models on single cell data. In MaBoSS any SBML format can be imported and model simulation and sensitivity analysis can be performed. In our case, WebMaBoSS was used, which is an online utility of the software. The tumor cell invasion and migration can be viewed with the help of heatmaps. Additionally, the output is provided in the form of time-dependent probabilities, for all biological entities (genes, proteins, phenotypes, etc.) of the model [9].

3 Validation and Analysis

3.1 Stable States and Phenotype

The stable states were determined with GINsim. First of all, the stable states for the wild type were calculated. As shown in Figure 5, there are nine stable states.

Afterwards, the stable states for a PTEN loss-of-function were identified. PTEN is not directly included in this model. However, according to the paper, a PTEN LoF can be simulated by activating the nodes AKT1 and AKT2 [6]. These perturbations gave back two stable states since the first row in Figure 6 is an initialization state.

For the perturbation with one random LoF and one random GoF, we decided to choose p53 for the loss-of-function and NICD for the gain-of-function. In Figure 7 can be seen that this combination had three stable states. The first stable state has the aggregated Metastasis phenotype, consequential to combining Migration, Invasion, and EMT. The second stable state has only EMT, while the third has EMT and Invasion.

3.2 Robustness

Once the model is imported, it can be visualized in the 'Overview tab' followed by the probability distribution for our model (Figure 1).

We can visualize the wild type model using various max time (10-1000) and sample count values (100-10000). Figure 2 shows different max time and sample values for the wild type model.

The final state distribution can be plotted automatically in WebMaBoSS as the probability trajectories for nodes (Figure 3).

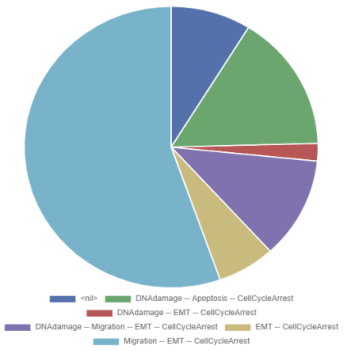

Figure 1: **Wild type model for the random initial conditions and parameters.**

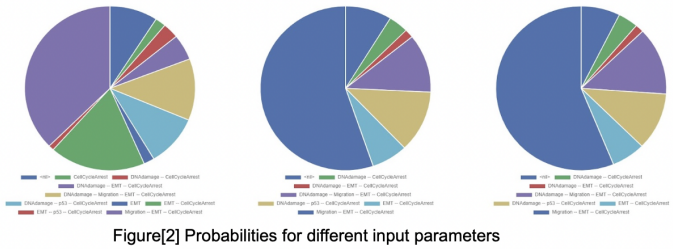

Figure 2: **Probabilities for different input parameters**

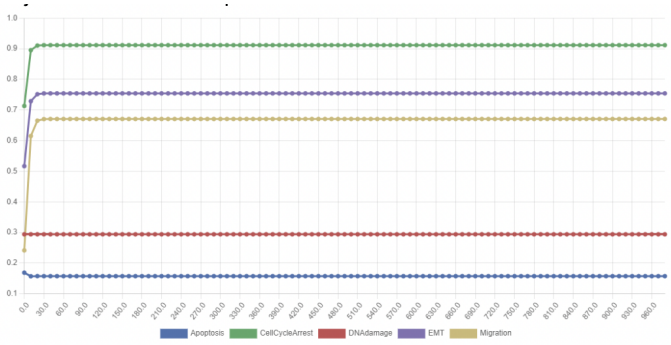

Figure 3: **Probability trajectories nodes for the Wild type model.**

3.3 Mutants

Followed by mutation settings, certain nodes were regulated up or down. Here first we can observe the upregulation of p53, NICD (single mutants), and P53/NICD (double mutant) followed by TWIST1, TGF Beta (single mutant), and TWIST1/TGF Beta (double mutant). This stage is useful for determining the impact of a gene or transcription factor on the different network states, as well as the impact of a node (Figure 4).

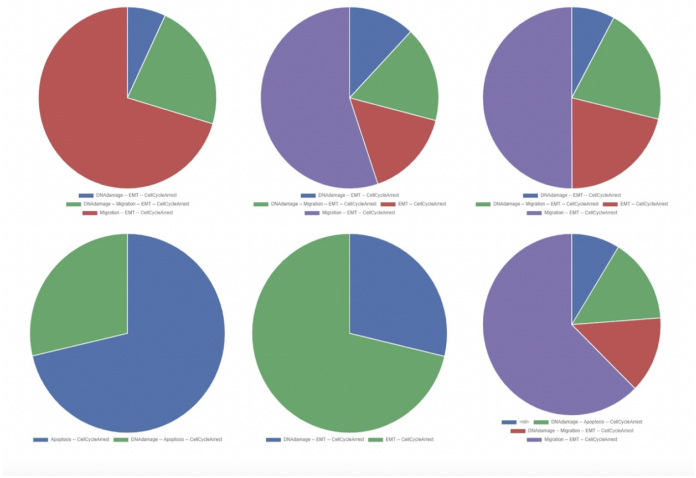

Figure 4: **Mutation settings to force up-regulation of nodes.**

4 Conclusion

In the first case, we simulated up-regulation of p53 which leads to the activation of Apoptosis-Cell cycle arrest and activation of NICD leads to activation of Migration. The double mutant of p53-NICD up-regulation resulted in activation of EMT-Cell cycle arrest. In the second case, we up-regulated TWIST1 and TGF Beta, both leading to activation of Migration, whereas their double mutant resulted in activation of Migration-EMT-CellCycleArrest.

5 Discussion

5.1 The Use of a Mathematical Model

The results from our computation and Cohen et al [6] were similar. According to Cohen et al [6] the model could be validated with experimental data. This proves the plausibility of the model. Such plausibility is a requirement for a model to be useful.

Since experimental data is often more expensive, more time-intensive, and less exact than mathematical models, a collaboration between modeling and experimentation is advantageous for biological research. This also applies in our specific case.

5.2 Framework by L. Calzone

With extensions of the used MaBoSS framework, it is possible to further analyze the process. L. Calzone suggests multiple such extensions.

The first extension is PROFILE [10]. It introduces the methodology to integrate the OMICS data into the generic models or patient-specific models. This enables the personalization of models that are built from biological knowledge. The framework uses a specific model for each patient which can be simulated for specific treatment. That way, a look at the patient-specific response is possible. This does not overlap with the goal of Cohen et al [6]. They aim to simulate the process of metastasis in combination with gene alterations. A model for individual patients is not useful, since they do not compare to patients but papers.

Another suggestion is EnsembleMaBoSS [11]. It simulates multiple non-interacting cells with similar behavior. We used a single-cell model based on gene expression. In our work, the initial model is simulated with random conditions in the "Editing tab". Those conditions are EMT, Apoptosis, DNA Damage, Migration, and Cell-Cyclearrest. The mutants were upregulated resulting in changes in the probability distribution of the Wild-type model.

There is also the possibility of simulating cell dynamics between interacting cells and updates in a cell population. For this purpose, the UPMaBoSS framework [12] was created. In the case of cancer cell invasion, this could imitate the interaction between multiple cancer cells or mutants and non-mutants. It would extrapolate from the problem of EMT and help with understanding the first step of metastasis formation more generally.

Lastly, PhysiBoSS [13] models the spatial organization of a dynamic population of interacting cells. It is an agent-based modeling tool (PhysiCell) that takes intracellular descriptions from MaBoSS into account. One possible use is the reproduction of different modes and mechanisms of invasion. A few parameters to consider while running PhysiBoSS are the activation and inactivation rate for every node, thresholds to control activation of boolean nodes, secretion rate for virus, cytokines, and speed for t-cell movements. This extension could be used for a broader approach to the problem discussed by Cohen et al [6]. For instance, the complete process of infiltration could be modeled. EMT is the change in regulation for single cells. It enables the next step of cancer cells to pass through the basal membrane. This process could be simulated with PhysiBoSS since it is based on the change of transcription in single cells and how that change influences the physical interaction with other cells.

Competing interests
The authors declare that they have no competing interests.

Author's contributions
Sina Glöckner wrote the report. Gokul Thothathri used WebMaBoss, analyzed and wrote the results. Swetha Rose Maliyakal Sebastian compared the paper to the talk by L. Calzone. Christina Kirschbaum worked with GINsim and wrote the results for this part.

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Figures

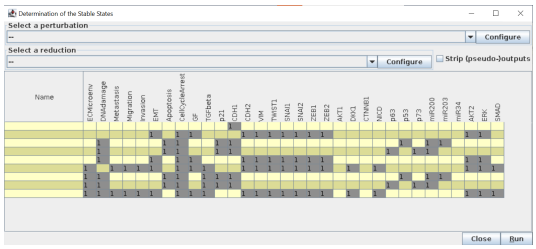

Figure 5: **Stable states wild type.** The screenshot shows the results from GINsim for the stable state determination for the wild type.

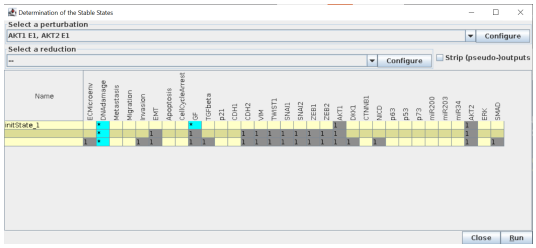

Figure 6: **Stable states PTEN LoF.** The screenshot shows the results from GINsim for the stable state determination for PTEN LoF.

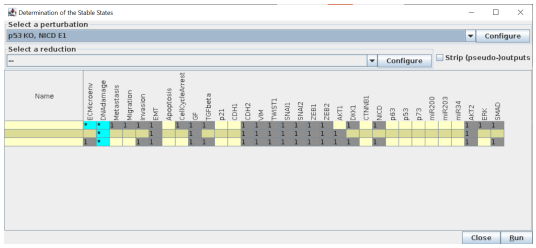

Figure 7: **Stable states p53 LoF & NICD GoF.** The screenshot shows the results from GINsim for the stable state determination for p53 LoF and NICD GoF.