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Modeling miRNA regulation in signaling networks: miR-34a regulation of the p53/Sirt1 module

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Abstract

Micro RNAs are a family of small regulatory RNAs whose function is to regulate the activity and stability of specific messenger RNA targets through post-translational regulatory mechanisms. We studied a signaling module composed by p53, Sirt1 and the small regulatory miRNA, miR-34a, based on the integration of experimental evidence and quantitative data, with mathematical modeling. We further used the model to investigate different possible designs of the silencing mechanisms exerted by miR-34a on Sirt1, and to simulate the dynamics of the system under pathological conditions of deregulation of its components.

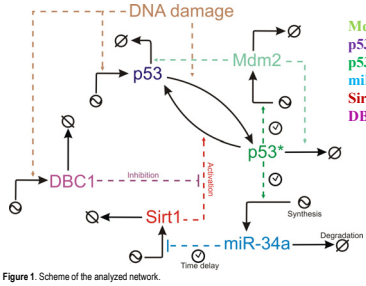

Figure 1. Scheme of the analyzed network.

Mdm2: Murine double minute.
p53: Deacetylated (inactive) p53.
p53*: Acetylated (active) p53.
miR-34a: Micro RNA 34a.
Sirt1: Silent Information Regulator 1.
DBC1: Deleted gene in Breast Cancer 1.

Modeling

We used ordinary differential equations (ODEs) to describe the interactions of the components in the network. We then characterized the parameter values in the model by using the following strategies: (i) we fixed some parameter values by using the known protein half-life; (ii) for the rest parameters, we constrained them in an biologically meaningful interval and estimated them by combing the experimental data published by Yamakuchi et al. (2008) and Suzuki et al. (2009). We used the experimental data generated by Yamakuchi and Lowenstein (2009) to check the validity of the model.

$$\frac{d}{dt}Mdm2 = k_{syn\_Mdm2} + k_{act\_Mdm2}\cdot p53^*(t-\tau_1) - k_{deg\_Mdm2}\cdot Mdm2$$

$$\frac{d}{dt}p53 = k_{syn\_p53} + k_{act\_p53}\cdot Dd + \frac{k_{tr\_p53^*}\cdot p53^*\cdot SIRT1}{DBC1} - k_{tr\_p53}\cdot p53\cdot Dd - k_{deg\_p53}\cdot p53\cdot Mdm2$$

$$\frac{d}{dt}p53^* = k_{tr\_p53}\cdot p53\cdot Dd - \frac{k_{tr\_p53^*}\cdot p53^*\cdot SIRT1}{DBC1} - k_{deg\_p53}\cdot p53^*\cdot Mdm2$$

$$\frac{d}{dt}miR34 = k_{syn\_miR34} + k_{act\_miR34}\cdot p53^*(t-\tau_2) - k_{deg\_miR34}\cdot miR34$$

$$\frac{d}{dt}SIRT1 = \frac{k_{syn\_SIRT1}}{miR34(t-\tau_3)} - k_{deg\_SIRT1}\cdot SIRT1$$

$$\frac{d}{dt}DBC1 = k_{syn\_DBC1} + k_{act\_DBC1}\cdot Dd - k_{deg\_DBC1}\cdot DBC1$$

Predictive Simulations

i. Analyzing the mechanism of miR-34a post-translational repression of Sirt1

We expanded the initial mathematical model by introducing four different mechanisms of miR-34a-mediated Sirt1 repression (see Fig. 2 Top). We then simulated the model in five different conditions to predict whether an experiment with such design could help discriminate between the different regulatory mechanisms (see Fig. 2 Bottom).

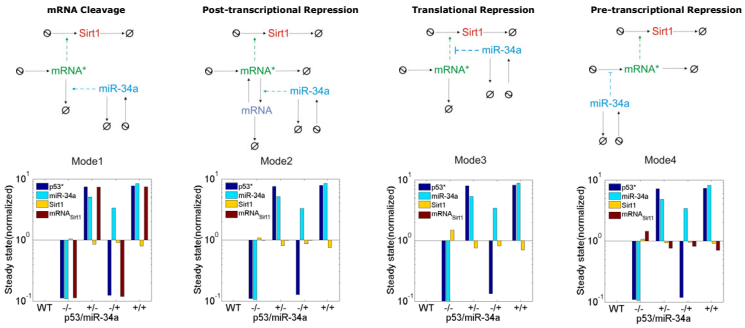

Figure 2. Top: The simplified scheme of four types of miRNA silencing mechanisms. Bottom: Simulation results of different miRNA silencing mechanisms under specific experimental condition. WT: wild type. +/-: up or down regulation of specific component. The model comparison suggests that total levels of mRNA would strongly differ in different experimental conditions for Mode 1 and 4, while they would keep similar in wild-type conditions for Mode 2 and 3. Moreover, the results also suggest that expression levels of Sirt1 are more affected in the simulated experimental conditions for the translational repression model (Mode 3).

ii. miR-34a silences Sirt1 through translational repression

By comparing the biological data published by Ford et al. (2008) with model predictions, we found that only Mode 3 can repeat the behavior of the experimental data (see Fig. 3). Thus, we concluded that miR-34a represses Sirt1 expression through translational repression rather than the other three defined mechanisms. Interestingly, our conclusion is also in agreement with the results generated by Yamakuchi and his co-workers (2008).

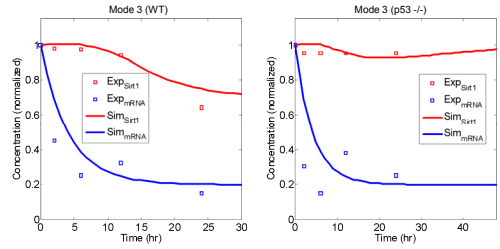

Figure 3. Prediction of miRNA silencing mechanism (Mode 3 in Fig. 2). Solid line: the model prediction. Square marker: biological data. Ford and colleagues measured mRNA and protein levels of Sirt1 after the stimulation with 5-FU in wild type (WT) or p53 mutated (p53 -/-) cell line.

iii. Different regulatory effects of miR-34a and DBC1

We further investigated different negative regulatory effects on Sirt1 caused by its two inhibitors (miR-34a and DBC1). Interestingly, the simulations suggested that, although the concentration of DBC1 and miR-34a are perturbed in the same normalized interval, changes in the expression of DBC1 induce bigger modifications in the levels of active p53 than miR-34a (see Fig. 4 Top), implying a stronger role for DBC1 as enhancer of active p53 compared to miR-34a. Furthermore, we asked whether the two negative regulators of Sirt1 could compensate the loss of active p53 associated to experimental values in which Sirt1 is over-expressed, a condition related in the literature with tumour progression. As we found, only intensive enhancement of DBC1 is still able to cancel out the loss of active p53 due to the extreme up-regulation of Sirt1 (see Fig. 4 Bottom).

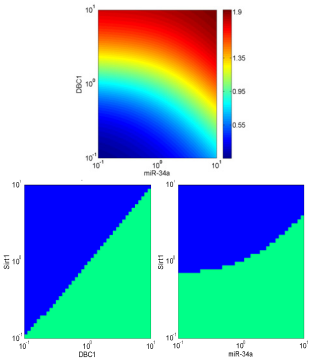

Figure 4. Top: Predictive simulation accounting for steady state level of active p53 under up and down-regulation of miR-34a and DBC1. Bottom: The ability of DBC1 and miR-34a to compensate the loss of active p53 for the abnormally high concentration of Sirt1 in cancerous cells. Active p53 is able (Green area) /unable (Blue area) to reach its basal level.

Summary

- We used a Systems Biology approach to set up a model accounting for the dynamical features of miRNA regulation in p53/Sirt1 signaling pathway.
- After qualitative validation of the model, we investigated two main features :
  - We modeled four different silencing mechanisms of miR-34 affecting on Sirt1 to predict the different observation that could be found in the specific experiment design.
  - By using a number of numerical computations, we compared the strength of the Sirt1's two negative regulators (DBC1 and miR-34a) and analyzed their impact on the status of active p53.
- Based on the analysis results, we concluded:
  - miR-34a silences Sirt1 through translational repression.
  - DBC1 is a more efficient negative regulator of Sirt1 than miR-34a.

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