Regulation of P53 Oscillations by MircoRNA-mediated Positive Feedback Loops

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***Short Abstract* — The tumor suppressor p53 oscillates in response to DNA double-strand breaks. We model a class of ubiquitous post-transcriptional regulators, termed microRNAs, which form positive feedback loops with the p53 regulatory network. Simulations reproduce the oscillation of p53 under DNA damage stimulus. Importantly, model analysis show that specific microRNA abrogation leads to loss of the wild-type phenotype. For evaluation, we perform microRNA-perturbation experiments in MCF7 breast cancer cells. Quantitative microscopy analysis confirms that the p53 oscillatory performance is compromised under specific microRNA perturbation. Our results provide evidence of the impact of microRNA-mediated positive feedback loops on the stress-induced p53 oscillations.**

I. Introduction

THE behavior of the tumor suppressor protein p53 could be significantly dynamical in response to stress signals [1]. Experiments demonstrate that DNA double-strand breaks trigger oscillations of p53 and its core antagonist MDM2 [2]. Previous studies have shown that the p53-MDM2 negative feedback loop is essential for the stress-induced p53 oscillations. However, the role of positive feedback loops in p53 oscillations remains largely elusive.

MicroRNAs are small noncoding RNAs serving as post-transcriptional regulators. Intriguingly, recent studies have revealed extensive crosstalk between the p53 network and microRNAs [3]. In this work, we investigate the role of microRNA-mediated positive feedback loops that interface with the p53 regulatory pathways.

We develop a mathematical model of a p53-MDM2-microRNA network that involves three different microRNAs forming positive feedback loops. We perform simulations and robustness analysis of p53 oscillations under abrogation of microRNA-mediated feedback loops. Specifically, bifurcation analysis is used to probe the system behavior under parametric variability in relationship to cellular noise. To experimentally evaluate our predictions, we introduce microRNA inhibitors in the MCF7 breast cancer cells, and perform time-lapse microscopy to track the p53 dynamics under drug-induced DNA double-stranded breaks. Our experimental results reveal that the three microRNA-mediated positive feedback loops confer different level of control to the stress-induced p53 oscillations.

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II. Results

A. MicroRNAs mediate positive feedback loops with p53

The miR-192 family, miR-34 family and miR-29 family are transactivated by p53. In turn, miR-192 inhibits MDM2. In addition, miR-34 inhibits SIRT1 and YY1, while miR-29 inhibits CDC42 and Wip1, which are direct or indirect negative regulators of p53. As a result, these microRNAs all form positive feedback loops with p53.

B. Modeling and analysis of p53-MDM2-miRNA network

A mass-action model of the p53-MDM2-miRNA network is developed based on our previous work [4], incorporating the three different microRNAs that form positive feedback loops. We simulate DNA damage-induced p53 oscillations under inhibition of each of the microRNAs. Furthermore, we perform bifurcation analysis to account for the significant cellular variability due to extrinsic noise in parameters. The results suggest that only the repression of miR-192 could effectively abrogate the p53 oscillations in single cells.

C. Experimental evaluation of microRNA abrogation on p53 oscillations

We experimentally track the p53 response in single MCF7 cells under wild-type and microRNA repressed conditions. By quantifying the percentage of oscillatory cells in a population, we confirm that cells transfected with the inhibitor of miR-192 show markedly decreased portion of p53-oscillating cells compared to the wild-type phenotype.

III. Conclusion

Using theoretical modeling in combination with single-cell experiments, we provide the evidence that microRNA-mediated positive feedback loops can control the robust manifestation of stress-induced p53 oscillations.

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