Mapping the regulatory structure between two key transcription factors in a breast cancer cell line

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Short Abstract — **Estrogen receptor alpha (ERα) expression is an important classifier for breast cancer patients. Therefore, understanding ERα regulation can be important for improving the efficiency of current therapeutic strategies for breast cancer. ERα and GATA3 were suggested to have mutual regulatory positive feedback loops, which may contribute to the ERα expression pattern. By measuring the response of the ERα-GATA3 regulatory network to various perturbations and fitting a set of quantitative gene regulation models to the data, we identified the regulatory structure between ERα and GATA3. Our results suggest possible regulatory modes that may exist between ERα and GATA3 in the T47D breast cancer cell line.**

***Keywords* — ERα, GATA3, Gene regulatory network model**

I. Introduction

Hormone receptors (such as ERα) act as master regulators of gene expression, controlling mammalian cell growth and differentiation as well as cancer cell proliferation. The expression level of ERα has become an essential criterion for breast cancer classification and assignment of hormonal therapy. Typically, ERα-positive breast cancers show better prognosis and response to hormone therapies than ERα-negative cancers. However, the molecular mechanisms controlling ERα expression are not well understood and no genetic mutation is known to be associated with ERα status in patients. Therefore, understanding the factors involved in ERα expression may improve therapeutic strategies for breast cancer. Another transcription factor, GATA3, was recently suggested to participate in mutual positive feedback loops with ERα. Therefore, the network module containing the mutually activating transcription factors ERα and GATA3 may serve as a control unit that plays a central role in determining ERα expression. However, a clear understanding of molecular mechanisms underlying the ERα-GATA3 regulatory structure is lacking. We examined by experimental perturbation and mathematical modeling the ERα-GATA3 regulatory structure in the T47D ERα-positive breast cancer cell line.

II. Methods and Results

We applied various cellular and biochemical perturbations individually or combined, including transcript-targeted knockdown by small-interfering RNA (siRNA), translation inhibition by cycloheximide (CHX), and overexpression from vectors carrying each of the two genes. We measured ERα and GATA3 expression at the population-average level (by Western blotting, RT-qPCR), at the single cell level (by flow cytometry) and at the single nucleus level (by immunofluorescence), to examine the response of the gene regulatory structure to these perturbations in the T47D ERα-positive breast cancer cell line. All experiments were performed in the absence of the ligand estrogen. In parallel, we developed a semi-phenomenological model of gene regulation. Fitting to these data the time course solutions of ordinary differential equations corresponding to the 81 possible regulatory scenarios (all combinations of positive, negative or no regulation of ERα and GATA3 on each other and themselves), we identified regulatory modes that may exist between ERα and GATA3, including a negative regulatory effect of ERα on GATA3.

III. Conclusion

We observed mutual gene regulation with compromised, and possibly negative regulatory effect of ERα on GATA3 in the T47D cell line. Therefore, our mathematical model and experimental data jointly support an asymmetric regulatory architecture between ERα and GATA3. These results provide a new approach for understanding gene regulation in breast cancer cells or other biological systems through experimentation combined with predictive mathematical modeling.

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