A cascade of bistable switches controls TGF-β induced epithelial-to-mesenchymal transition

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***Short Abstract* — Epithelial to mesenchymal transition (EMT) is essential for cell plasticity during development and plays important roles in cancer progression. Two mathematical models proposed different mechanisms on the regulation of TGF-β1 induced EMT. One model predicted that two bistable switches, governed by SNAIL1/miR-34 and ZEB/miR-200 double-negative feedback loops respectively, lead to epithelial, partial EMT, and mesenchymal phenotypes. Another model argued that the three phenotypes came from a tertiary switch formed by the ZEB/miR-200 loop. Our quantitative measurement unambiguously confirmed that a cascade of two binary switches governs TGF-β1 induced MCF10A EMT.**

***Keywords* — microRNA, bistability, flow cytometry**

I. Background

During epithelial to mesenchymal transition (EMT), cells gain the ability to invade and migrate through a loss of epithelial characteristics and acquisition of mesenchymal attributes. EMT plays important roles in cell development and disease [1,2]. In both physiological and pathological contexts, quantitative characterization of the EMT process is fundamental for resolving controversies on the molecular mechanisms, and potentially guiding prevention and treatment of cancer metastasis and organ-degenerative diseases.

Recently two competing mathematical models have been established to describe transforming growth factor beta (TGF-β) induced EMT. Through deterministic analyses and stochastic simulations, Tian *et al.* proposed a model of Cascaded Bistable Switches (CBS) [3]. In this model, EMT is a sequential two-step program, in which an epithelial cell first transits to the partial EMT then to the mesenchymal state, depending on the strength and duration of TGF-β stimulation. Mechanistically the system is governed by two coupled reversible and irreversible bistable switches. The SNAIL1/miR-34 double negative feedback loop is responsible for the reversible switch and regulates the initiation of EMT, while the ZEB/miR-200 feedback loop is accountable for the irreversible switch and controls the establishment of the mesenchymal state. Furthermore, an autocrine TGF-β/miR-200 feedback loop makes the second switch irreversible, modulating the maintenance of EMT.

Subsequently, Lu *et al.* proposed a different model of Ternary Chimera Switch (TCS) [4]. In this model, instead of acting as a binary switch, the SNAIL1/miR-34 motif is a monostable module and functions as a noise-buffering integrator of internal and external signals. A biological evidence they suggested is that SNAIL1 has negative auto-regulation. The ZEB/miR-200 module, with the help of yet-to-be-confirmed ZEB self-activation, forms a tertiary switch that can have three steady states corresponding to the three phenotypes.

II. Results

In this work we quantitatively measured the dynamics of TGF-β1 induced MCF10A EMT. Our measurements at RNA and protein levels verified the temporal and steady state dynamics show two-step behavior, and can be well represented by sequential Markovian transition among the three phenotypes, reversible transition between epithetial and partial EMT phenotypes, and irreversible transition between partial EMT and mesenchymal phenotypes. Furthermore, SNAIL1 shows both unimodal and bimodal distributions under different TGF-β1 concentration, indicating the bistable behavior of SNAIL1. The SNAIL1 behavior agrees with prediction of the CBS model, but disagrees qualitatively with that of the TCS model. Subsequently we refined our mathematical model using the experimental data.

III. Conclusion

Our integrated experimental and computational studies revealed molecular mechanism of EMT.

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