Unveiling Regulatory Network in T_H17 Cell Through Single-Cell mRNA Sequencing

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***Abstract*—In this article, I mainly introduce Prof. Nir Yosef's research over a specific immune cell: T_H17. I introduce their analysis of the single-cell mRNA sequencing of T_H17 cell during the whole process of differentiation. Based on the interaction between different regulators, they found that the T_H17 transcriptional network consists of two self-reinforcing, but mutually antagonistic modules. In another research, they identified SGK1 and constructed its surrounding network. They found that IL-23 stabilizes and enhances the T_H17 cell through SGK1, which illustrates the molecular mechanism of the stablization of T_H17.**

I. Introduction

In our course of *Introduction to Bioinformatics*, we talked extensively about network analysis of genome. Here I follow the work of Prof. Nir Yosef from UC Berkeley, which mainly focuses on how is the gene expression regulated in a particular immune cell: T_H17. His research applied the single-cell mRNA sequencing to finding out the mechanism of differentiation[1], stability[2] and pathogenicity[3] of T_H17.

CD4+ T helper cells are critical mediators of the cellular immune response. For many years, people thought that CD4+ T helper cells existed as a dichotomy of lineages name T_H1 and T_H2, due to the cytokine expression patterns of them. However, as it was more throughly studied, it was found that the T helper cell population was not limited to these two subsets[4].

It was shown that T cells could differentiate into IL-17-producing cells in vitro and in vivo independently of T_H1 and T_H2 lineage. T_H17 cells (interleukin-17 (IL-17)-producing helper T cells) are highly proinflammatory cells which are critical for clearing extracellular pathogens and inducing multiple autoimmune diseases. The anti-fungal immunity appears to be limited to particular sites with detrimental effects observed. Their main effector cytokines are IL-17A, IL-17F, IL-21, and IL-22. T_H17 cells mediate the regression of tumors in mice, but were also found to promote tumor formation induced by inflammation of the colon in mice. T_H17 cells, particularly auto-specific T_H17 cells, are associated with autoimmune disease such as multiple sclerosis, rheumatoid arthritis, and psoriasis.

Transforming growth factor beta (TGF-β), interleukin 6 (IL-6), interleukin 21 (IL-21) and interleukin 23 (IL-23) contribute to T_H17 formation in mice and humans. Key factors in the differentiation of T_H17 cells are, besides others, the signal transducer and the activator of transcription 3 (Stat3) and the retinoic acid receptor-related orphan receptors gamma (RORγ) and alpha (RORα). The T_H17 cells can alter their differentiation program ultimately giving rise to either protective or pro-inflammatory pathogenic cells.

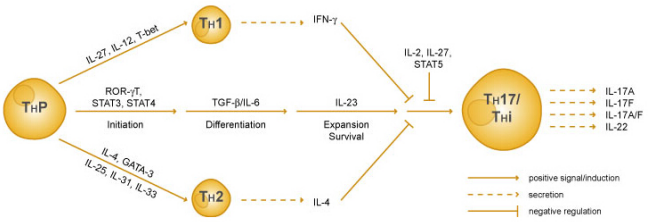

Fig. 1. T_H17 Cell Lineage Diagram

II. Regulatory Network Controlling T_H17 Cell Differentiation

Recent reconstructed regulatory networks mammalian cells have focused on short-term responses and relied on perturbation-based approaches which cannot be readily applied to primary T cells. In Prof. Nir Yosef's paper, they combined transcriptional profiling at high temporal resolution, novel computational algorithms and innovative nanowire-based perturbation tools to systematically derive and experimentally validate a model of dynamic regulatory network that controls the differentiation of mouse T_H17 cells.

A. Transcriptional Time Course of T_H17 Differentiation

They induced the differentiation of naive CD4+ T cells into T_H17 cells using TGF-β1 and IL-6, and measured the transcriptional profiles using microarrays at 18 time points along a 72 h time course.

They identified 1,291 genes that were differentially expressed specifically during T_H17 differentiation and partitioned them into 20 co-expression clusters by using k-means clusters.

The results show that there are mainly three main waves of transcription and differentiation: (1) Induction; (2) Onset of phenotype and amplification; (3) Stabilization and IL-23 signaling.

B. Substantial Regulatory Re-wiring During Differentiation

The active factors and interaction change from one network to the next. The majority of interaction are active only at some time windows. So on the basis of similarity in active interactions, they identified three network classes corresponding to

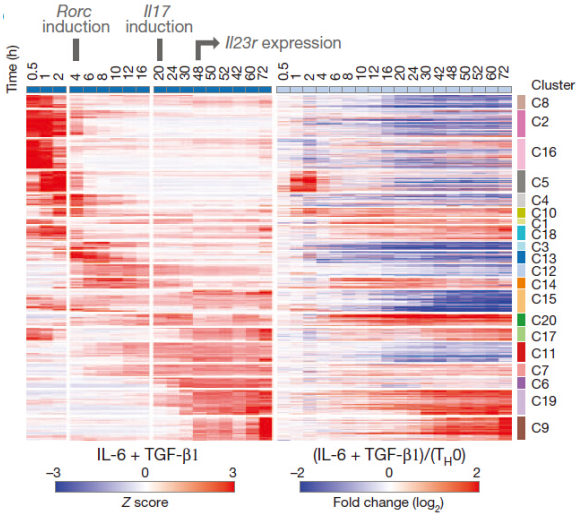

Fig. 2. Gene Expression Profiles During Differentiation.

three differential phases. They collapsed all networks into one model, which results in three consecutive network models.

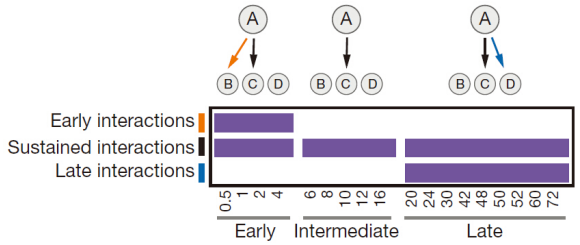

Fig. 3. Model of Dynamic Regulatory Network.

C. Nanowire-based Perturbation of Primary T Cells

In unstimulated primary T cells, viral and transfection-based siRNA delivery was nearly impossible because it either alters differentiation or cell viability. They therefore used a new delivery technology based on silicon nanowires. They perturbed 39 of the 65 selected genes – 29 regulators and 10 receptors.

By charaterizing each regulator by its effect on T_H17 signature genes, they found that, at 48 h, the network is organized into two antagonistic modules: a module of 22 "T_H17-positive factors", the perturbation of which decreased the expression of T_H17 signature genes, and a module of 5 "T_H17-negative factors", the perturbation of which had the opposite effect.

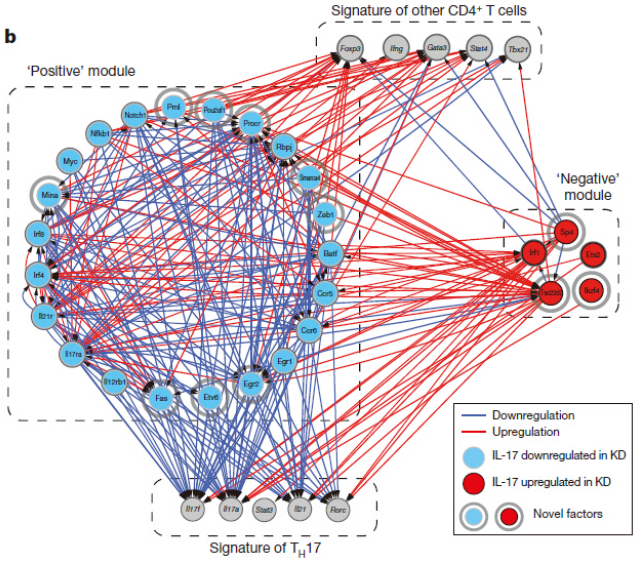

Fig. 4. Two Coupled and Opposing Modules.

We can find that each of the module is tightly interconnected through positive, self-reinforcing interactions between its members. However most inter-module interactions are negative.

III. IL-23 Stabilizes T_H17 Cell through SGK1

IL-23 has a critical role in stabilizing and reinforcing the T_H17 phenotype by increasing expression of IL-23 receptor (IL-23R) and endowing T_H17 cells with pathogenic effector functions. However, the precise molecular mechanism by which IL-23 sustains the T_H17 response and induces pathogenic effector functions has not been elucidated. Prof. Nir Yosef used transcriptional profiling of developing T_H17 cells to construct a model of their signalling network and nominate the major node.

A. Identification of High Network-Score Genes

They measured genome-wide mRNA expression profiles using microarrays along 18 time points over 72 h, which is followed by the exposure to TGF-β1 and IL-6. They added IL-23 at the last time points and monitored the transcriptional response in wild type and $Il23r^{-/-}$ cells.

They monitored the response in $Il23r^{-/-}$ cells, because it has been know that IL-23 reinforces the T_H17 cell by increasing the expression of IL-23 receptor.

By calculating the centrality score of genes, they identified top genes which is important for the cell maintenance. SGK1 is the one with largest centrality score.

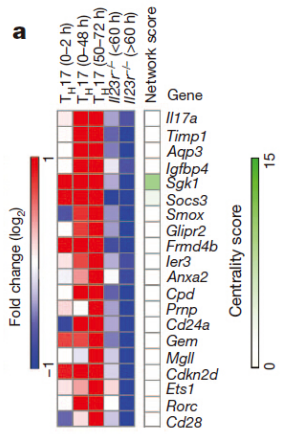

Fig. 5. Top Genes Ranked by Average Fold Increase in T_H17.

B. Downstream Network of SGK1

In order to understand better the molecular role of SGK1 in T_H17 cells, they conducted network analysis using PPI data to connect SGK1 to the tarnscription factors whose activity is disregulated in $Sgk1^{-/-}$ T_H17 cells.

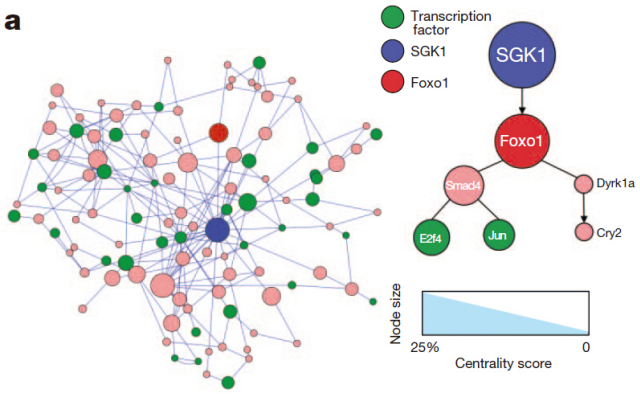

Fig. 6. SGK1 Signalling Promotes IL-23R Expression Through Phosphorylation of Foxo1.

From the analysis we can see that Foxo1 is one of the highest-ranking nodes downstream of SGK1. SGK1 stablizes T_H17 cell phenotype by deactivation of mouse Foxo1, which is a direct repressor of IL-23R expression.

C. Validation of SGK1 by Changing Nacl Concentration

Since SGK1 has been shown to govern Na^+ transport and salt homeostasis in other cells, the author designed the experiments to validate it.

They found that a modest increase in salt concentration induces SGK1 expression, which promotes IL-23R expression and enhances T_H17 cell differentiation. These provide a molecualr insight into a mechainsm by which an environment factor such as a high salt diet triggers T_H17 development and promotes tissur inflammation.

IV. Conclusion

In Prof. Nir Yosef's research, they analyzed high-throughput data of mRNA, which shed light into how is the gene regulation network like and identifying critical regulators in the differentiation period of T_H17 cells.

They have found that the T_H17 cell has a dynamic regulatory network which controls the cell differentiation. The network is composed mainly of two part: the "TH17-positive factors" and "T_H17-negative factors". Each part is tightly intraconnected through positive, self-reinforcing interactions between its members while the most inter-connected interactions are negative. They also found that IL-23 stablizes and enhances the T_H17 cell, by increasing the expression of SGK1, which is a downstream node of IL-23 signalling. SGK1 is critical for regulating IL-23R expression and stabilizing the T_H17 cell phenotype by deactivation of Foxo1.

Since life is a complex system, modeling the system using network is a reasonable way. In Traditional Chinese Medicine (TCM), we take a person as a system. When a person gets ill, it might be more helpful to look into the whole system, other than just one point.

The following research into the immune cells such as T_H17 cells is meaningful, because it helps us to understand how the network works and helps us to design better drugs. I believe singe-cell mRNA sequencing is a promising technique, which we may apply to more other T cells, and even B cells. They may show very different patterns of network.

Actually, Prof. Nir Yosef agreed with my idea and they are currently working in this direction, and they plan to generalize their research to other types of immune cells.

V. Aknowledgement

I would like to give my sincere thanks to Mr. Songpeng Zu and Dr. Shao Li, who provided us a comprehensive structure of computational biology in the past semester.

References

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