Removing the breaks: RBPJ ablation in T cells boosts iTreg differentiation, stability and function.

~Promising breakthrough therapy for autoimmune diseases~

Keywords: Regulatory T cell (Treg), FOXP3, RBPJ, CRISPR screening, single-cell RNA-seq (scRNA-seq)

Research Highlights

- A whole-genome CRISPR screen※1 was performed in human iTregs※2 and identified RBPJ※3 as a novel negative regulator of FOXP3※4 expression.
- Single-cell sequencing analyses with Perturb-icCITE-seq※5 catalogued the complex molecular network that governs FOXP3 expression.
- RBPJ knockout iTregs exhibited heightened FOXP3 and Treg signature gene expression※6 and enhanced functionality including immunosuppressive ability and FOXP3 stability.
- RBPJ associates with the NCOR complex※7 to directly suppress FOXP3 expression through histone deacetylation※8.
- RBPJ knockout enhances iTreg performance in vivo in mouse models, demonstrating its potential as a novel therapeutic approach for treating autoimmune diseases.

Summary

In a collaborative study, a research team led by Kelvin Chen and Shimon Sakaguchi at the Immunology Frontier Research Center (IFReC) of Osaka University, along with Tatsuya Kibayashi at Chugai Pharmaceutical Co., Ltd., discovered that deleting the transcription factor RBPJ in human CD4⁺ T cells enhances the differentiation, stability and functional capacity of in vitro-induced regulatory T cells (iTreg). This finding may help overcome a major hurdle limiting the success of iTreg-based cellular therapies in treating autoimmune diseases.

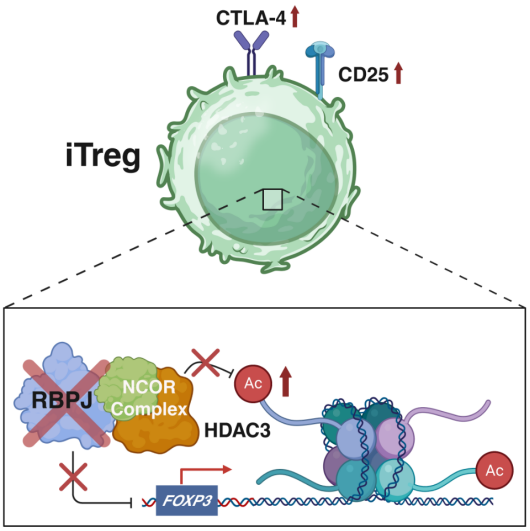

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Figure 1 Summary of the study.
RBPJ associates with the NCOR complex and binds to the FOXP3 region, repressing transcription through HDAC3-mediated histone deacetylation. Consequently, alleviation of this repression by RBPJ ablation increases FOXP3 expression in iTregs and elevates other Treg signature genes, such as CTLA-4 and CD25.

One of the major challenges in developing effective iTreg-based cellular therapies is that the same inflammatory environment they are meant to suppress can also destabilize them. Under such conditions, therapeutic iTregs often lose their suppressive function and, in some cases, even become pro-inflammatory, potentially worsening disease progression and harming the host. The researchers identified RBPJ as a novel regulator of FOXP3, which directly

influences its expression by interacting with the NCOR repressor complex to modulate local histone acetylation levels. By knocking out this single gene, the researchers created iTreg cells that not only exhibit enhanced immunosuppressive function but also remain stable under inflammatory conditions. Their findings are illustrated in Figure 1.

The study, “Genome-wide CRISPR screen in human T cells reveals regulators of FOXP3” was published in *Nature* at DOI: 10.1038/s41586-025-08795-5.

- **Research Background**

Tregs play a central role in establishing and maintaining immune self-tolerance and are crucial in regulating various immune responses, including autoimmunity, allergic reactions, transplant immunity, and tumor immunity. Induced Tregs (iTregs), generated in vitro from conventional $CD4^+$ T cells, mimic the function of naturally occurring Tregs and have garnered significant interest for their potential in modulating immune responses in cell-based therapies. However, challenges such as efficient iTreg induction, stability, functional potency, and in vivo persistence continue to hinder their clinical application. A key determinant of Treg function and identity is the transcription factor FOXP3, which not only defines the Treg lineage but is also essential for their suppressive activity. While previous CRISPR screens have successfully identified Foxp3 regulators in murine models, the factors that control FOXP3 expression in human iTregs remain largely unexplored.

- **Research content**

Using a CRISPR screen that allowed them to individually turn off each gene in the genome, the team identified hundreds of candidates that could either positively or negatively influence FOXP3 expression during the in vitro conversion of conventional CD4 T cells into iTregs. However, traditional methods, such as flow cytometry-based antibody staining, typically focuses on measuring a limited set of parameters, such as FOXP3 protein alone, which limits the ability to probe the broader phenotypic changes resulting from CRISPR knockouts.

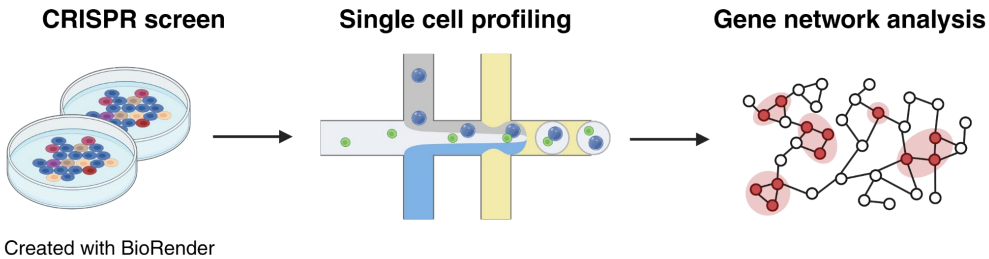

Figure 2 High-throughput phenotypic profiling with single-cell sequencing.
Thousands of knockout cells across hundreds of genes are generated through targeted CRISPR edits and profiled simultaneously using droplet microfluidics, allowing for high-resolution characterization of individual cells. By systematically linking specific genes to their effects on gene expression, Perturb-seq enables the mapping of complex gene regulatory networks, revealing how genes interact and influence cellular behavior. Using this method, the scientists mapped the gene network surrounding the regulation of FOXP3.

To overcome this limitation, the scientists turned to a novel single-cell RNA sequencing method, Perturb-icCITE-seq, which offers significant advantages in scalability and enables the direct measurement of both FOXP3 protein expression and transcriptional profiles for each knockout (Figure 2). The method enabeled for a deeper understanding of how each candidate regulates not only FOXP3 but also the nuanced phenotypic changes that occur during iTreg differentiation. This approach provided valuable insights into the complex regulatory networks that control iTreg development.

The team was particularly interested in a gene named *RBPJ*, as its knockout increased FOXP3 expression, and its known interacting partners were also identified as regulators, suggesting a possible cooperative mechanism for repressing FOXP3. By focusing on RBPJ, the scientists wanted to find out whether controlling expression of the gene in T cells might make them “better” iTregs.

The investigators generated iTregs with the RBPJ gene knocked out and found that the cells expressed higher amounts of FOXP3 than iTregs with a functioning RBPJ gene. They then compared the performance of these knockout cells to T cells with an intact RBPJ gene in a cell suppression assay to measure their ability to suppress immune responses in vitro, finding that the knockout cells outperformed their intact counterparts. When the cells were subjected to a stress test through repeated exposure to pro-inflammatory cytokine, the RBPJ knockout cells remained resilient, stably expressing FOXP3. The results were promising, but the findings raised an important question: How?

To address the mechanism behind RBPJ-mediated FOXP3 repression, the researchers conducted a series of experiments examining RBPJ’s chromatin occupancy profile[※9] and the resulting epigenetic landscape[※10] of RBPJ-modulated iTregs. They found that RBPJ directly binds to the FOXP3 locus, promoting chromatin repression through histone deacetylation, in cooperation with the NCOR complex. Ablation of RBPJ accompanied a local increase of histone acetylation at FOXP3, resulting in increased chromatin accessibility and FOXP3 expression.

Finally, to evaluate the clinical potential of their findings, the team transferred RBPJ-sufficient and -deficient human iTregs into an in vivo autoimmune disease model. They found that the RBPJ knockout cells were more effective at suppressing disease progression, suggesting that targeting RBPJ could enhance the therapeutic efficacy of iTreg-based cell therapies.

- **The impact of this research on society**

Despite the growing body of evidence implicating Tregs in the regulation of various immune responses, the development of effective cell-based therapies has faced significant challenges. The findings from

this study, particularly the identification of RBPJ and its role in controlling FOXP3 expression, deepen the understanding of FOXP3 regulation and open new avenues for improving immune interventions in autoimmune diseases.

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Glossary

※1 CRISPR screening
A method that allows researchers to turn off (knockout) every gene in the genome and see how each gene affects a specific biological process. This genome-wide scale approach helps researchers quickly identify which genes are potentially important for a particular biological function.

※2 In vitro-induced regulatory T cell (iTreg)
A type of CD4-positive helper T (Th) cell that actively promotes suppressive responses. In vitro-induced regulatory T cells (iTregs) are generated from conventional $CD4^+$ T cells under specific culture conditions, typically involving TGF-β and IL-2 stimulation. They express FOXP3 and contribute to immune suppression, mimicking the function of natural Tregs, though their stability and suppressive capacity may vary depending on the environment.

※3 RBPJ
A transcription factor known to be a key mediator of Notch signaling. RBPJ plays a crucial role in the hematopoietic system, primarily regulating the development and differentiation of T cells, B cells, and other immune cell lineages.

※4 FOXP3
The master transcription factor that defines Treg cell lineage and is critical for Treg differentiation and function. It regulates the expression of various genes and enables T cells to acquire immune suppressive capabilities.

※5 Perturb-seq/Perturb-icCITE-seq
Perturb-seq is a powerful technique that combines CRISPR gene editing with single-cell RNA sequencing, enabling the profiling of phenotypic consequences from gene knockouts in individual cells at scale. Perturb-icCITE-seq is an extension of Perturb-seq that adds the capability of profiling intracellular proteins, such as FOXP3.

※6 Treg signature gene expression

Refers to a specific set of genes that are turned on in Tregs and are responsible for their function and maintenance (e.g. FOXP3, CD25, CTLA-4).

※7 NCOR complex

A group of proteins that cooperate to repress gene expression of certain genes. The complex includes well-known repressor genes such as HDAC3 and NCOR1/2.

※8 Histone deacetylation

A process where acetyl groups are removed from histone proteins, leading to a reduction in gene accessibility and transcriptional repression. This process plays a critical role in regulating key biological functions, including cellular differentiation and gene expression.

※9 Chromatin occupancy profile

A chromatin occupancy profile maps where a specific protein – such as a transcription factor – binds to DNA across the genome.

※10 Epigenetic landscape

Refers to the chemical modifications on DNA and histone proteins that modulate gene activity. Modifications include DNA methylation, histone methylation and histone acetylation.