TAp63: A new protein drug target for rheumatoid arthritis

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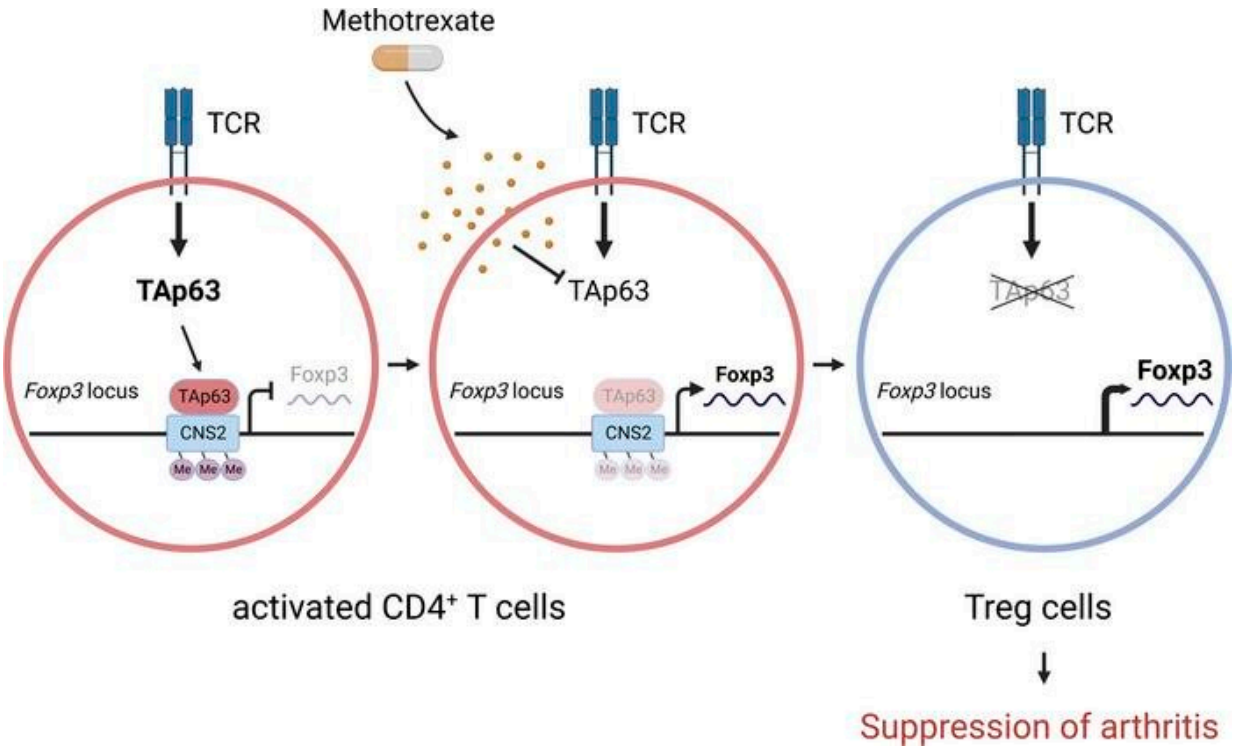

Graphical abstract. Credit: *JCI Insight* (2023). DOI: 10.1172/jci.insight.164778

Rheumatoid arthritis (RA) is an autoimmune disease characterized by joint deterioration. The clinical outcomes of patients with active RA can be improved using anti-rheumatic medications, such as methotrexate (MTX). Many patients rely on MTX to limit the destructive joint damage and functional disability typical of RA. Although the drug is a

folic acid antagonist, its precise mechanisms in RA patients are largely unknown.

Previous research suggests that MTX also affects a type of white blood cell called $CD4^+$ T cells. These cells are believed to play a role in the development of RA—specifically, the balance between the activation of interleukin-17-producing helper T (Th17) cells and $CD4^+$ regulatory T (Treg). Researchers suspect that MTX affects $CD4^+$ T cells by suppressing T cell activity and increasing Treg cells, but its specific effects besides folate metabolism remain unclear.

Recently, a group of researchers discovered that MTX targets tumor protein p63 (TP63) in $CD4^+$ T cells. Their findings were published online on May 22, 2023, in the *JCI Insight* journal. The team was led by Dr. Akiro Suto, an Associate Professor at the Department of Allergy and Clinical Immunology at the Graduate School of Medicine, Chiba University, and the Institute for Advanced Academic Research at Chiba University.

It also included Dr. Kensuke Suga, Dr. Shigeru Tanaka, and Dr. Hiroshi Nakajima of the Department of Allergy and Clinical Immunology at Chiba University and Dr. Osamu Ohara of the Department of Applied Genomics at the Kazusa DNA Research Institute.

"We were keen to profile gene expression before and after MTX treatment since the drug likely targets $CD4^+$ T cells, and little is known about its influence on gene expression in patients with active RA," explains Dr. Suto when discussing the team's motivation to pursue the research.

The researchers employed DNA microarray profiling of human $CD4^+$ T cells from RA patients to understand how MTX influences gene expression. They also used gene knockdown—a molecular technique to

suppress a target gene—and RNA sequencing (RNA-Seq) to validate gene function. The researchers found that TAp63, a protein isoform of TP63, was highly expressed in human and mouse Th17 cells.

Dr. Suto says, "Patients who received MTX treatment had significantly lower TAp63 messenger RNA expression in their $CD4^+$ T cells. MTX also suppressed TAp63 proteins in human and mouse Th17 cells. TAp63 suppression in mouse Th17 cells resulted in the improvement of autoimmune arthritis in mice."

The RNA-Seq and gene knockdown data revealed that another gene, FOXP3, the master regulator of Treg cells, was targeted by TAp63. When TAp63 was "knocked down" in Treg cells, Foxp3 protein expression increased. By performing a reporter assay, the researchers confirmed that TAp63 was bound to the FOXP3 enhancer and suppressed it.

Together, these findings suggest that TAp63 is intricately linked to the balance of Th17 and Treg cell differentiation. Thus, inhibiting TAp63 could enhance the suppressive function of Treg cells and limit autoimmune RA.

These findings reveal a robust mechanism for MTX action and show how Treg cells can be preserved in RA. They also demonstrate the potential of TAp63 as a new therapeutic target for RA.

More information: Kensuke Suga et al, TAp63, a methotrexate target in CD4+ T cells, suppresses Foxp3 expression and exacerbates autoimmune arthritis, *JCI Insight* (2023). DOI: 10.1172/jci.insight.164778