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Structure-Function Analysis of Clec12A, an Inhibitory Receptor Associated with Chronic Inflammatory Diseases

Objective(s): Clec12A is an inhibitory receptor that is involved in the pathogenesis of gout and rheumatoid arthritis (RA). Gout is one of the most painful types of arthritis caused by recurring inflammatory episodes in which neutrophils play a key role. When neutrophils come into contact with MSU, the expression of Clec12A diminishes. The decrease in Clec12A causes an enhanced neutrophil response. Although Clec12A contributes to the immunopathogenesis of gout, we still do not know how Clec12A signals.
The overall aim of the project is to characterize the Clec12A signaling pathway.

Method(s): We stably transfected 293T cells with HA-tagged, wild-type Clec12A and a Clec12A mutated in its ITIM motif. We developed an antibody against the phosphorylated form Clec12A's ITIM motif to track its phosphorylation status, (R-94P). Standard cell biology approaches were used to study the subcellular localisation of Clec12A and its signaling.

Result(s): The 293 cells stably expressing Clec12A can signal through this receptor. Phosphorylated Clec12A was detected with R-94P after incubation with pervanadate. Moreover, SHP-2 is recruited to the phosphorylated ITIM of Clec12A. Receptor engagement induces the translocation of Clec12A to detergent-resistant membrane domains (DRMs) where it becomes phosphorylated and internalised.

Conclusion(s): We developed an in vitro system and new antibody to study how Clec12A signals. Our findings reveal that Clec12A signals within DRMs. We also identified proteins involved in the Clec12A signaling pathway. Together, our observations provide insight into the pathways involved in gout and the molecular mechanism through which Clec12A dampers inflammation.