# The Role of the Deubiquitinase *ZRANB1*/TRABID in Inflammation and Bone Formation in Ankylosing Spondylitis

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**Background:** Inflammation and new bone formation are important disease mediators in ankylosing spondylitis (AS). Recent studies in mice show that the deubiquitinase TRABID epigenetically controls the expression of IL12/23 through JMJD2D (Jumonji Domain-Containing Protein 2D). Furthermore, TRABID also upregulates EZH2 (Enhancer of zeste homolog 2), a molecule that has been implicated in pro-osteogenic pathways. This study aims to study the functional and clinical relevance of TRABID in AS pathogenesis.

**Methods:**

1) TRABID, EZH2 and JMJD2D protein and gene expression was evaluated in AS and control tissue (mouse and human) by immunohistochemistry/immunofluorescence and qPCR.
2) THP-1 cells (monocyte cell line) and splenocytes from 16-week-old SKG mice were incubated with lipopolysaccharide (LPS) to stimulate cytokine production and varying concentrations of NSC112200, a small molecule inhibitor of TRABID. Enzyme linked immunosorbent assay (ELISA) was used to detect IL23, TNFa and IL1b production after 24 hours in the supernatant. Finally, western blot was used to evaluate expression of EZH2 and JMJD2D.
3) TRABID was knocked down in Saos-2 cells (osteoblast cell line) using siRNAs. Cells were then incubated in an osteogenic induction media for 14 days. RNA was extracted every 3 days to evaluate osteogenic gene expression by qPCR and RNA sequencing. At day 14, calcium mineralization levels were evaluated by Alizarin Red staining.

**Results**:

1) Our results showed that TRABID is significantly upregulated in human and mouse AS tissue (sFig 1). TRABID+ cells and gene expression were found to be upregulated in human AS gut ($n=20$/group, $p<0.0001$), bone marrow ($n=5$/group, $p<0.0001$) and synovium ($n=10$/group, $p<0.0001$). TRABID was preferentially expressed by CD68+ macrophages in the synovial lining. EZH2 and JMJD2D were also found to be significantly upregulated in the inflamed synovium.
2) THP-1 cells treated with NSC112200 and LPS together showed a dose dependent inhibition of TNFa, IL1b and IL23 production when compared with cells treated with only LPS ($n=6$, $p>0.0001$) (sFig 2). Furthermore, TRABID inhibition showed a significant downregulation of both JMJD2D and EZH2 protein levels.
3) SKG mouse splenocytes incubated with NSC112200 dose dependently showed decreased levels of TNFa when compared with cells treated with LPS alone after 24 hours ($n=3$, $p=0.0043$).
4) TRABID knockdown by siRNA showed significant suppression of pro-osteogenic genes SP7, Runx2 and alkaline phosphatase. Moreover, osteoblasts knocked down for TRABID failed to mineralize as evidenced by diminished alizarin red staining. RNA sequencing confirmed pathways related to TGFb signaling, bone remodeling and endochondral ossification were suppressed while negative regulation of the immune system was upregulated (sFig 3). Lastly,

osteogenic induction media significantly upregulated TRABID expression over time, compared with cells treated with unconditioned growth media ($p=0.0027$).

**Conclusions**: Our results show a role for TRABID in both cytokine production and osteogenesis. Future work will address downstream mechanisms of TRABID in these processes and evaluate TRABID inhibition in animal models of AS.