Mid1 is a novel mediator of subchondral bone resorption in antigen-induced arthritis

Cellular and molecular mediators of subchondral bone destruction in arthritis
Nina Lukač
Mentor: prof. dr. sc. Nataša Kovačić
Department of Anatomy & Laboratory for Molecular Immunology, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

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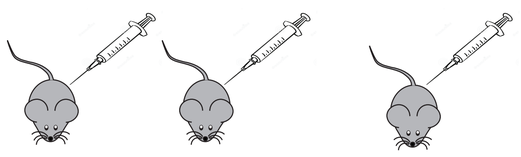
INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune joint disease characterized by subchondral bone destruction, irreversible by currently available therapeutics. We have shown that mice deficient for Fas gene (Fas-/-) are protected from local bone resorption in antigen-induced arthritis (AIA), a murine model of RA, lack accumulation of synovial myeloid cells, which down-regulate Mid1 gene.

AIM OF THE STUDY

The objective of the study was to evaluate the expression and function of Mid1 during AIA.

MATERIALS AND METHODS



12 week, C57BL6 (B6) and Fas-/- mice

day 0 — **AIA:** mBSA + CFA s.c. **ctrl:** mBSA + CFA s.c. **NI:** PBS s.c.

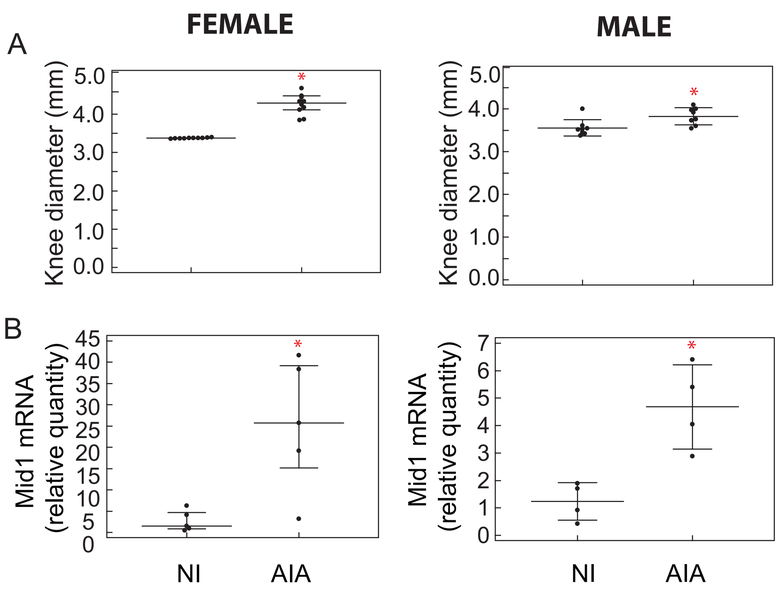
day 7

day 21 — **AIA:** mBSA i.a.. **ctrl:** PBS i.a. **NI:** PBS i.a.

day 24/31 — arthritis assesment & sample collection

knee diameter
visual score
µ-CT
Flow cytometry
FACS
Affymetrix St 2.0 microarrays
q-PCR

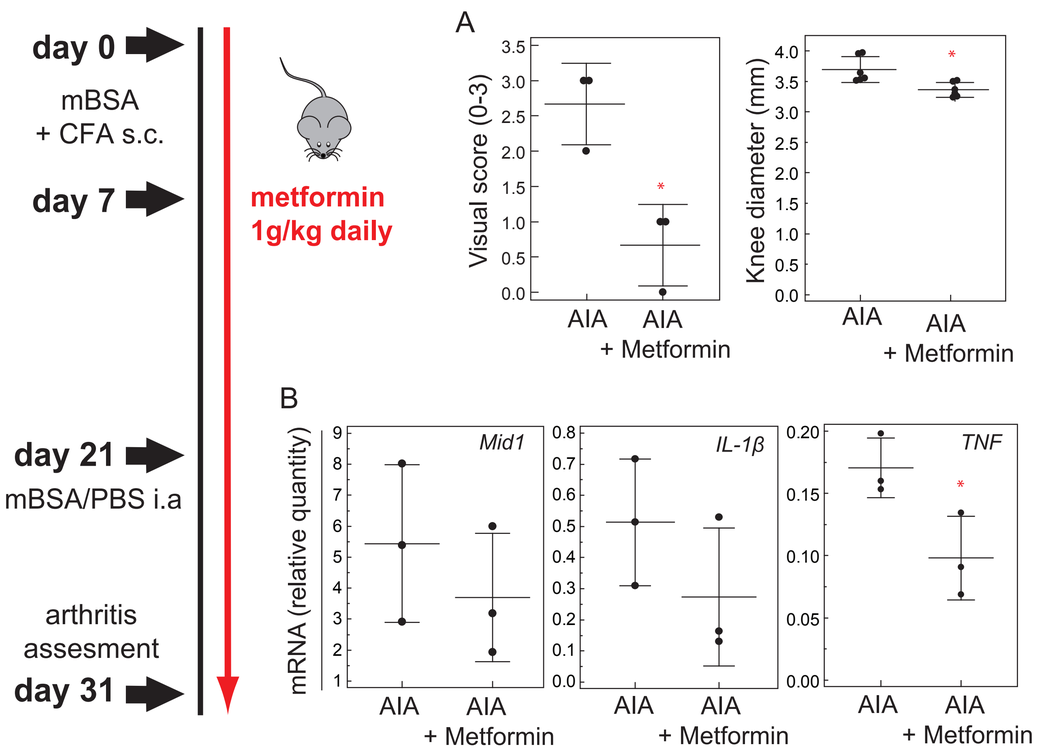
3. Upregulation of *Mid1* in B6-AIA is not sexually dimorphic



(A) AIA was induced in female and male mice and its severity was assesed by knee diameter. (B) Despite its position on X chromosome *Mid1* expression in joints is not sexually dimorphic and was up-regulated in B6-AIA in both female mice (FC=9.66, p=0.04, Welch-test) and male mice (FC=3.79, p=0.006, T-test).

*statistically significant difference, p<0.05, Welch- or T-test

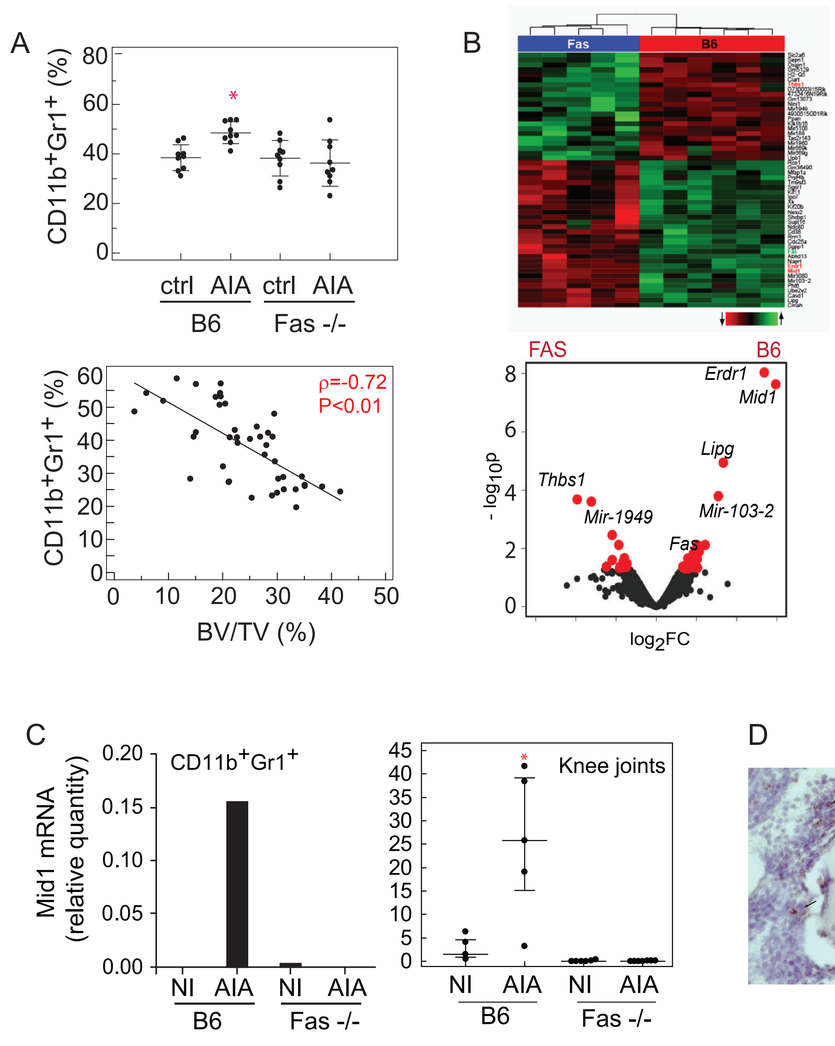
4. Metformin, which inhibits Mid1-PP2A interaction, ameliorates arthritis severity in B6-AIA mice



B6 AIA mice were treated with metformin daily during the immunization protocol to inhibit Mid1 proinflammatory effects by blocking Mid1-PP2A interaction. On d10 of arthritis, (A) knee diameter was measured, visual score was assessed and (B) Mid1 and proinflammatory cytokine expression was measured in knee joints by q-PCR.
* statistically significant difference, p<0.05, T-test

RESULTS

1. *Mid1* gene is upregulated in synovial CD11b$^+$Gr1$^+$ cells and bulk joint tissue in resorptive AIA in B6 mice

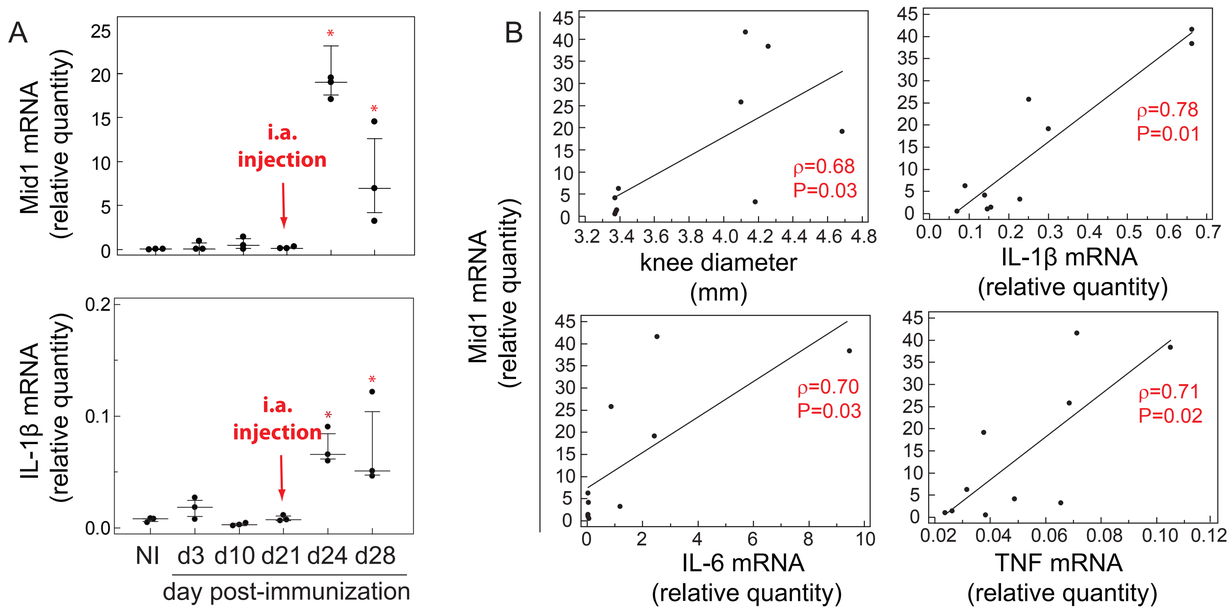


Up regulated in B6	p-value	q-value Bon
mitotic cell cycle process	2.45E-19	7.61E-16
mitotic cell cycle	1.29E-18	4.02E-15
cell cycle	4.42E-16	1.38E-12
nuclear chromosome segregation	2.01E-15	6.25E-12
cell division	2.71E-10	8.44E-07
sister chromatid cohesion	6.74E-10	2.10E-06
microtubule cytoskeleton organization	1.49E-08	4.63E-05
regulation of proteasomal ubiquitin-dependent protein catabolic process	2.00E-04	6.21E-01
regulation of cellular protein catabolic process	2.00E-04	6.23E-01
Up regulated in Fas -/-	**p-value**	**q-value Bon**
negative regulation of cellular biosynthetic process	7.29E-08	1.73E-04
defense response	6.19E-07	1.47E-03
positive regulation of apoptotic process	2.51E-06	5.95E-03
positive regulation of programmed cell death	2.74E-06	6.50E-03
negative regulation of nucleobase-containing compound metabolic process	4.50E-06	1.07E-02
positive regulation of cell death	4.83E-06	1.15E-02
inflammatory response	6.00E-06	1.43E-02
regulation of MAPK cascade	8.22E-06	1.95E-02

(A) Fas-/- mice with non-resorptive AIA lack accumulation of synovial CD11b$^+$Gr1$^+$ cells which are negatively associated with subchondral bone volume. (B) Microarray analysis of genes differentially expressed between B6 and Fas-/- synovial CD11b$^+$Gr1$^+$ cells. Significantly changed genes are shown on a heat map and are marked red on volcano plot (logFC>1.5, p(BH-adjusted)=0.05). B6 synovial myeloid cells upregulate *Mid1* gene (logFC=2.01, p(BH-adjusted)=0.0003, limma + BH-adjustment) and genes responsible for progression of cell cycle and mitosis and downregulate genes involved in regulation of inflammatory response.

(C) q-PCR analysis of *Mid1* expression in sorted synovial CD11b$^+$Gr1$^+$ cells and knee joint tissue homogenates in non-immunized (NI) and arthritic (AIA) B6 and Fas -/- mice and (D) *Mid1* RNA in situ hybridization on frontal sections of femora of B6 and Fas-/- mice with arthritis. *Mid1* is up-regulated in synovial CD11b$^+$Gr1$^+$ cells and bulk joint tissue of B6-AIA mice. Up-regulation is confirmed on paraffin sections. * statistically significant difference p<0.05, one-way ANOVA

2. *Mid1* expression is upregulated early after arthritis induction and correlates with local proinflammatory cytokine expression



(A) *Mid1* and *IL-1β* expression in knee joints incresases early after arthritis induction (d24) and remains elevated in a first week post-induction (d28). * statistically significant difference from NI, p<0.05, one-way ANOVA
(B) *Mid1* expression in knee joints is positively associated with joint diameter, expression of IL-1β, TNF, and IL-6 on d10 of arthritis. ρ Spearman`s correlation coefficient

CONCLUSIONS

- **Inflammatory response in resorptive AIA is marked by higher myeloid proliferation potential**
- ***Mid1* expression is upregulated in synovial myeloid cells and bulk knee joint tissue of B6 mice with resorptive arthritis**
- **Mid1 expression is upregulated early after arthritis induction and correlates with expression of local markers of inflammation**
- **Despite its position on X chromosome Mid1 expression in joints is not sexually dimorphic**
- **Metformin, which inhibits proinflammatory effects of Mid1 by interfering with Mid1-PP2A interaction, ameliorates arthritis severity**
- **Mid1 inhibition might present a new therapeutic target for inflammation-mediated joint destruction**

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