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### Extracellular 14-3-3 $\eta$ : A Novel Mediator of Inflammation Associated with Selective Activation of Intracellular Pathways

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**Background/Purpose:** Rheumatoid arthritis (RA) results from the interaction and convergence of mediators that contribute to pathological processes. The 14-3-3 family members are ubiquitously expressed intracellular chaperones. We previously demonstrated that the  $\eta$  isoform is specifically present extracellularly in the synovial fluid and matched serum of patients with inflammatory arthritis and that its expression significantly correlated with levels of MMP-1 and MMP-3. Recent data also indicates that serum 14-3-3 $\eta$  is highly specific for RA. We aimed to examine the role of extracellular 14-3-3 $\eta$  in the pathogenesis of RA by investigating its effects on 1) the activation of RA-relevant signaling cascades, 2) induction of pro-inflammatory mediators and 3) the effects of selectively targeting 14-3-3 $\eta$  with monoclonal antibodies.

**Method:** Cells of the monocytic lineage (THP-1) were stimulated with 12.5ng/ml of recombinant human 14-3-3 $\eta$  and activation of relevant signaling targets was assessed by immunoblot analysis using phosphospecific antibodies. Activation of HL-60, PCS-201-010, Jurkat and Daudi cells was assessed by evaluating the phosphorylation status of MAPK/ERK following 15 minutes of stimulation with 12.5ng/ml. mRNA levels of IL-1 $\beta$ , IL-6, IL-8, CCL2/MCP-1, CCL4/MIP-1 $\beta$ , MMP-1, MMP-9, TNF $\alpha$ , RANKL were assessed in THP-1 cells following 18h incubation with a dose range of 0.10 to 100ng/ml of recombinant human 14-3-3 $\eta$  reflecting in vivo concentrations. For antibody targeting studies, recombinant human 14-3-3 $\eta$  was co-incubated with antibody (0.2 to 20mg/ml) for 18h and levels of transcripts were assessed.

**Result:** Stimulation assays showed that while monocytic, myeloid, and fibroblast cell lines were activated in response to 14-3-3 $\eta$ , T and B cell lines were not. Extracellular 14-3-3 $\eta$ , at concentrations found in RA patient serum (median 1.12ng/ml and range of 0.12 to 20ng/ml) activated key intracellular signalling cascades that regulate cell proliferation (MAPK/ERK), survival (AKT), inflammation (JAK-STAT), and tissue remodelling (SAPK/JNK). These cell stimulatory effects were specific yet distinct from other reported endokines/extracellular factors since neither the activation of p38MAPK nor NF $\kappa$ B was not observed with 14-3-3 $\eta$  stimulation. Furthermore, extracellular 14-3-3 $\eta$  at levels approximating median serum levels behaved as a potent inducer of IL-1 $\beta$ , IL-8, CCL2/MCP-1, CCL4/MIP-1 $\beta$ , MMP-1, MMP-9 and RANKL transcripts. Higher levels of 14-3-3 $\eta$ , though within the range found in vivo, were required for induction of IL-6 and TNF $\alpha$ . Targeting 14-3-3 $\eta$  with monoclonal antibody compounds attenuated these effects.

**Conclusion:** Extracellular 14-3-3 $\eta$  is a novel mediator that induces expression of several factors associated with the pathogenesis of RA. In contrast to other endokines that activate both p38MAPK and NF $\kappa$ B as well as up-regulate pro-inflammatory factors, 14-3-3 $\eta$  acts through alternate signaling pathways. Targeting 14-3-3 $\eta$  with monoclonal antibody compounds attenuates its inducing effects underscoring its novelty as a potential therapeutic target.