

## Presented at EULAR 2011

Submission #EULAR11-3786, Program #OP0219

### EXTRACELLULAR 14-3-3 ETA: A NOVEL THERAPEUTIC TARGET FOR INFLAMMATORY ARTHRITIS

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**Background:** In inflammatory arthritis, a range of cytokines and extracellular factors contribute to the activation of the innate and adaptive immune systems that converge on pathways that drive joint damage. 14-3-3 proteins represent a family of ubiquitously expressed intracellular chaperonins consisting of seven different highly conserved isoforms. We previously demonstrated that the  $\eta$  isoform is specifically present extracellularly in the synovial fluid and matched serum of patients with inflammatory joint disease and that levels significantly correlated with levels of MMP-1 and MMP-3. A recent biomarker study in 265 subjects further demonstrated that 14-3-3 $\eta$  is differentially present in RA patients versus controls. These intracellular proteins, when specifically released outside of the cell following an inflammatory insult, could participate in perpetuating the immune response.

**Objectives:** To examine 14-3-3 $\eta$ 's role in the inflammatory arthritis disease process by investigating its effects on 1) the activation of RA-relevant signalling cascades, 2) the production of pro-inflammatory cytokines and joint damage factors and 3) whether selective 14-3-3 $\eta$  targeting with monoclonal antibodies can attenuate its inducing effects.

**Methods:** THP-1 monocytic cells were stimulated with 12.5ng/ml of recombinant human 14-3-3 $\eta$  and activation of diverse signalling cascades was assessed by immunoblot analysis using phosphospecific antibodies. We also analyzed mRNA levels of IL-1 $\beta$ , IL-6, TNF $\alpha$ , MMP-9, MMP-1 and RANK Ligand following 18h incubation with a dose range of 0.10 to 100ng/ml of recombinant human 14-3-3 $\eta$  or vehicle. For antibody targeting studies, recombinant human 14-3-3 $\eta$  was pre-incubated with antibody at a molar ratio of 1:5 for 2h prior to stimulation.

**Results:** 14-3-3 $\eta$  at concentrations found in serum of 135 patients with RA (median 1.12ng/ml and range of 0.12 to 20ng/ml) behaves as a potent extracellular ligand activating key intracellular signalling cascades that regulate cell proliferation (MAPK/ERK), survival (AKT), inflammation (JAK-STAT), cellular stress and tissue remodeling (SAPK/JNK and PKC). No activation of p38MAPK was observed underscoring the selective effects of 14-3-3 $\eta$  on these pathways. Furthermore, extracellular 14-3-3 $\eta$  induces various transcripts relevant to inflammation (IL-1 $\beta$ , IL-6, TNF $\alpha$ , MMP-9, and RANK Ligand) with a 50% increase in IL-1 $\beta$  and MMP-9 transcripts observed at 0.5 and 0.25ng/ml, respectively. Targeting 14-3-3 $\eta$  with monoclonal antibody compounds attenuates its inducing effects on specific pathways and immunological transcripts.

**Conclusions:** Extracellular 14-3-3 $\eta$  is a novel factor that may play an important role in the inflammatory arthritis disease process. Monoclonal antibody compounds that target extracellular 14-3-3 $\eta$  attenuate its potentiating effects *in vitro* indicating that this extracellular protein may represent a rational drug target for further investigation in inflammatory arthritis.