20 HISTONE DEACETYLASE INHIBITORS PREVENT INFLAMMATION-MEDIATED INACTIVATION OF THE FORKHEAD BOX CLASS O TRANSCRIPTION FACTOR FOXO1 IN RHEUMATOID ARTHRITIS

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Background and objectives Forkhead box O (FoxO) transcription factors, regulated by phosphatidlyinositol 3-kinase and reversible acetylation, integrate environmental signals to orchestrate inflammatory responses, cell cycle and apoptosis. Here, the authors examined the relationship between inflammation and FoxO expression and activity in rheumatoid arthritis (RA) synovial tissue, determined the effects of histone deacetylase inhibitors (HDACi) on FoxO expression and activity in RA fibroblast-like synoviocytes (FLS), and identified genes regulated by FoxO1 in RA FLS.

Materials and methods Total RNA was isolated from synovial biopsies obtained by arthroscopy from 20 RA patients. FoxO1, FoxO3a, FoxO4, TNFα, MMP-1 and IL-6 expression were measured by quantitative PCR (qPCR) in synovial tissue and RA and osteoarthritis (OA) FLS. RA FLS were stimulated with IL-1β or TNFα, in the absence or presence of the HDACi trichostatin A, and FoxO family member expression and FoxO1 DNA binding activity were measured by qPCR and ELISA-based assays, respectively. RA FLS were transduced with adenovirus encoding control GFP or constitutively active FoxO1ADA to examine the effects on RA FLS gene expression using low density qPCR arrays.

Results Negative correlations were observed between RA synovial tissue expression of FoxO1 and the levels of serum C reactive protein (CRP) ($R=-0.771$, $p=0.0008$), erythrocyte sedimentation rate (ESR) ($R=-0.739$, $p=0.0003$), and disease activity score 28 (DAS28) ($R=-0.575$, $p=0.01$). A strong negative correlation was also observed between synovial FoxO1 and IL-6 mRNA levels ($R=-0.628$, $p=0.004$), but not TNFα or MMP-1. FoxO1, FoxO3a and FoxO4 mRNA were each detected in RA and OA FLS, and FoxO1 mRNA levels were significantly reduced in RA FLS compared to OA FLS ($p<0.05$). IL-1β and TNFα significantly suppressed FoxO1 DNA binding activity in RA FLS, and selectively downregulated FoxO1 mRNA expression in a time-dependent manner. HDACi reversed IL-1β-mediated reduction of FoxO1 DNA binding and mRNA expression. Overexpression of FoxO1ADA in RA FLS suppressed expression of antiapoptotic Bcl-XL, and enhanced expression of proapoptotic Bim and the cell cycle inhibitor $p27^{Kip1}$. FoxO1ADA also suppressed IL-1β-mediated induction of inflammatory mediators, including CCL2, CXCL6, PDGF and ICAM1.

Conclusions The authors demonstrate that inflammatory stimuli decrease FoxO1 expression and DNA binding activity, effects reversed by exposure of RA FLS to HDACi. Restoration of FoxO1 activity suppresses a subset of genes regulated by HDACi during FLS activation, identifying the enhancement of FoxO1 function as a novel mechanism by which HDACi might mediate their anti-inflammatory effects in RA synovial cells.