a therapeutic potential in broadly, rather than specifically, targeting closely related Ras proteins.

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SILENCING EXPRESSION OF RAS FAMILY GTPASE HOMOLOGUES DECREASES INFLAMMATION AND JOINT DESTRUCTION IN EXPERIMENTAL ARTHRITIS

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Purpose Changes in expression and activation of Ras proteins are thought to contribute to the pathological phenotype of stromal fibroblast-like synoviocytes (FLS) in rheumatoid arthritis (RA). Here we examined the expression of each Ras protein in the synovial tissue of patients with RA and disease controls and the contributions of H-, K- and N-Ras homologues to FLS activation in vitro and experimental arthritis in vivo.

Methods Synovial Ras homologue expression was determined by immunohistochemistry and digital imaging analysis in patients with RA, inflammatory osteoarthritis and reactive arthritis, as well as a second cohort consisting of patients with RA and psoriatic arthritis (PsA). Ras protein and mRNA expression was examined in RA and PsA FLS. The activation status of Ras homologues in RA FLS following stimulation with tumour necrosis factor (TNF) and interleukin 1 (IL1) was determined by affinity precipitation and immunoblotting. RA FLS were transfected with active mutants of H-, K- and N-Ras and Ras protein expression was specifically or broadly silenced using locked nucleic acids (LNA) and the effects on basal and IL1-dependent cytokine and matrix metalloproteinase 3 (MMP-3) production was assessed. The potential therapeutic effects of broad pan-Ras silencing using pan-Ras and control LNA (1mg/kg, three times weekly intraperitoneal, eight mice per group) were studied in murine collagen-induced arthritis.

Results Similar levels of each Ras homologue were expressed in RA and disease control synovial tissue. Each Ras protein was also expressed in RA FLS and activated by TNF and IL1. H-Ras, but not other Ras homologues, was sufficient and required for spontaneous FLS MMP-3 production ($p<0.05$). However, each Ras homologue contributed to IL1-induced IL6 production ($p<0.05$). In vivo, pan-Ras LNA decreased the clinical severity of collagen-induced arthritis in mice compared with control LNA ($p<0.005$), as well as cartilage destruction ($p<0.05$), bone erosion ($p<0.05$) and the ratio of anticollagen IgG2a/IgG1 autoantibodies ($p<0.01$).

Conclusions Overlapping contributions of Ras homologues to global inflammatory parameters of RA FLS activation suggest