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INFLAMMATORY TISSUE DAMAGE IN CHRONIC DESTRUCTIVE ARTHRITIS IS REGULATED BY FHL2

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Purpose Four-and-a-half LIM 2 (FHL2) is a mediator of protein interactions involved in cellular processes that are of relevance for the activation of mesenchymal cells. It interacts with integrins, focal adhesion- and mitogen-activated kinases, transcription factor AP-1 and is involved in TRAF6-dependent signalling. We analysed the function of FHL2 in chronic inflammatory arthritis.

Methods The expression of FHL2 in synovial tissues from patients with rheumatoid arthritis (RA) and osteoarthritis (OA) and in destructive arthritis of human tumour necrosis factor transgenic (hTNFtg) mice was analysed by immunohistochemistry. Fibroblast-like synoviocytes from patients with RA (RA-FLS), from hTNFtg mice and appropriate controls were isolated and FHL2 levels were determined by immunoblot after stimulation with cytokines. Knock down of FHL2 was performed by RNA interference and the expression of matrix metalloproteinases (MMPs) was determined by western blot analysis and ELISA. The invasiveness of FLS was analysed using our established matrix-associated transepithelial resistance invasion (MATRIN) assay. FHL2-mediated signalling pathways were also studied. hTNFtg mice were crossbred with FHL2^{-/-} mice and clinical parameters of arthritis as well as histomorphometric parameters of joint destruction and MMP expression in wild-type, hTNFtg, FHL2^{-/-} and FHL2^{+/-}/hTNFtg animals were analysed.

Results Although there was a significantly higher expression of FHL2 in RA than in OA, only transforming growth

factor β (TGF β) but not TNF α induced the expression of FHL2 in RA-FLS. Analysis of FHL2 expression in hTNFtg mice together with further in vitro studies confirmed these findings by showing an early TGF β -dependent induction of FHL2 that was followed by a TNF α -mediated suppression of FHL2. However, the TNF α -mediated suppression of FHL2 in hTNFtg mice was incomplete and the levels of FHL2 were still significantly higher in hTNFtg mice than in wild-type controls. In vitro analyses revealed a prolonged phosphorylation of p38 and MAPKAP kinase 2 along with significantly elevated MMP levels in murine FHL2 $^{-/-}$ cells as well as in small interfering RNA-treated RA-FLS compared with controls. We also found an increased invasiveness of RA-FLS after FHL2 knock down. FHL2 $^{+/-}$ /hTNFtg mice had increased paw swelling and reduced grip strength compared with hTNFtg mice. FHL2 $^{+/-}$ /hTNFtg mice had increased joint destruction with markedly elevated levels of MMPs in situ.

Conclusions Our results suggest that, through modulating p38 activation, FHL2 is involved in the limitation of cytokine-induced MMP expression and constitutes an important regulator of inflammatory tissue damage in arthritis. While early tissue damage leads to an upregulation of FHL2 in affected tissues, in the frame of a healing attempt, chronic exposure to TNF α suppresses FHL2, leading to unbalanced and chronically progressing joint destruction.