TRANSFORMING GROWTH FACTOR-BETA INDUCES PERSISTENT SYNOVIAL FIBROSIS WHILE CONNECTIVE TISSUE GROWTH FACTOR-INDUCED FIBROSIS IS REVERSIBLE. ROLE FOR LYSYS HYDROXYLASE

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Purpose: The main characteristics of osteoarthritis are cartilage damage, osteophyte formation and synovial fibrosis. Synovial fibrosis can contribute to the loss of the function of an OA joint. TGF-β is considered one of the main players in fibrotic diseases. However, CTGF, which can be induced by TGF-β, has been reported to be an important player as well, stated to contribute to maintenance of fibrotic tissue. We investigated the underlying mechanism in that leads to the observed difference in fibrosis persistence between TGF-β- and CTGF-induced fibrosis.

Methods: We injected C57Bl/6 mice intra-articularly with Ad-TGF-β and Ad-CTGF. After 3, 7 and 21 days knee joint synovial tissue was isolated for RNA isolation or whole knee joints for histology. With Q-RT-PCR relative mRNA levels were determined of matrix molecules, proteases, protease inhibitors, and growth factors. To analyze the enzymes involved in collagen cross link formation, mRNA expression of lysyl hydroxylase (LH) was determined. CTGF-levels induced by TGF-β were measured by ELISA in 24 hour patella-synovial wash-outs of C57Bl/6 mice intra-articularly injected with Ad-TGF-β (3, 7 and 14 days).

Results: TGF-β and CTGF induced synovial fibrosis in murine knee joints as shown by histology (as observed by an increase in synovial width). However, TGF-β-induced fibrosis was very persistent, whereas CTGF-induced fibrosis resorbed by day 28. TGF-β induced elevated collagen type I and very high levels of aggregan expression and hardly any changes in collagen type II and III. CTGF induced no clear changes in collagen type I, II and III. CTGF even decreased aggregan expression (day 21 only). CTGF induced no changes in MMP-3, -9, -13, ADAMTS-4 or -5, whereas TGF-β increased MMP-3, -13 and ADAMTS4. Thus elevated levels of matrix degrading enzyme expression cannot explain the less persistent CTGF-induced fibrosis. TIMP1 (associated with matrix accumulation in fibrotic disorders), was highly up regulated by TGF-β (all days) and only slightly up regulated by CTGF (day 7). TGF-β also induced elevated mRNA levels of TGF-β1 and CTGF.

Only TGF-β induced high levels of LH expression, especially LH2b, which has been implicated in hard-to-degrade collagen linkage and was found up regulated in fibrotic lesions. As TGF-β can induce CTGF and up regulated CTGF mRNA in our experiments, we measured CTGF levels in 24 hour patella-synovial wash outs and found that TGF-β induced 64 ng CTGF/ml after 3 days and that CTGF levels were still elevated by day 14.

Conclusions: TGF-β induced increased collagen type I and aggregan mRNA levels in synovial tissue, but also high levels of degrading enzymes. The latter does not explain the persistent nature of TGF-β versus CTGF-induced synovial fibrosis. Lack of collagen type I mRNA induced by CTGF corresponds to findings of other groups. Despite this fact, fibrosis is found. Only TGF-β induced high levels of TIMP1 expression, which was previously found associated with strain dependent fibrosis sensitivity in mice and implicated in accumulation of ECM in fibrotic disorders. TGF-β, not CTGF, induced high levels of LH which are crucial for ECM cross linking. Moreover, LH2b was reported by van der Slot et al. to induce harder-to-degrade crosslinks compared to LH2a. The 2b splicing variant was highly expressed upon stimulation with TGF-β. Since TGF-β clearly up regulated MMP expression a dominant role for TIMP appears unlikely. The strong induction of LH2b by TGF-β compared to CTGF, and consequently harder to degrade cross links, appears to be the most likely cause of the induction of irreversible fibrosis by TGF-β.