

Collagen degradation and the expression of proteases involved in collagen metabolism in the contraction of collagen gels by Dupuytren's disease-derived fibroblasts

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HYPOTHESIS An imbalance of collagen deposition and degradation contributes to Dupuytren's disease pathology. The major collagen-degrading enzymes at neutral pH are from the matrix metalloproteinase (MMP) family, whilst related enzymes from the ADAMTS (a disintegrin and metalloproteinase domain with thrombospondin motifs) are procollagen propeptidases involved in collagen synthesis. Our hypothesis was therefore that these enzymes (MMP-1, -8, -13 and -14 and ADAMTS-2, -3 and -14) would contribute to contraction of the collagen-rich matrix which underlies disease pathology.

METHODS Fixed fibroblast populated collagen lattice (FPCL) assays were established using fibroblasts derived from the palmar fascia of Dupuytren's disease patients undergoing fasciectomy. Collagen lattices were allowed to develop tension over 48 hours prior to release and their subsequent contraction was followed over 24 hours using Image J software. Gels were harvested under tension at 24 and 48 hours, then at 3 hours and 24 hours after release by immediate immersion in Trizol. RNA was extracted, reverse transcribed and qRT-PCR was used to quantify gene expression. Taqman Low Density Arrays were employed to measure expression of MMPs, ADAMTSs and also the tissue inhibitors of metalloproteinases (TIMPs). In parallel, a hydroxyproline assay was performed on the lattice conditioned media to quantify collagen degradation. Statistical analysis utilised one way ANOVA with Tukey's multiple comparison test.

RESULTS Collagen lattices from six Dupuytren's fibroblast lines contracted similarly after release. Hydroxyproline assay clearly demonstrated that collagen break down occurs concomitant with gel contraction. MMP1 and MMP13 expression increased in collagen gels compared to monolayer culture and this expression further increased as tension developed and then decreased with release. MMP14 expression was minimally altered by tension, but increased upon release of the lattice. MMP8 was not significantly expressed. ADAMTS2, ADAMTS3 and ADAMTS14 were also regulated across this model with ADAMTS3 particularly increasing in expression under tension. It should also be noted that TIMP1 expression was high in these cells and increased with tension.

SUMMARY The expression of a number of proteases involved in collagen metabolism is regulated during the process of collagen gel contraction and release. Collagen degradation takes place despite apparent high expression of inhibitors in a similar manner to we previously described in the Dupuytren's palmar fascia itself (Johnston et al. 2007). We are currently using an siRNA approach to dissect the function of individual enzymes in this model of cell-mediated collagen contraction.

Johnston P, Chojnowski AJ, Davidson RK, Riley GP, Donnell ST and Clark IM (2007) *J Hand Surg [Am]* **32** 343-351. "A complete expression profile of matrix-degrading metalloproteinases in Dupuytren's disease"