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Title: Insulin-like Growth Factor Binding Protein (IGFBP)-6: A mediator of myofibroblast differentiation in Dupuytren's Disease?

Hypothesis:
Down-regulation of *IGFBP6*, encoding Insulin-like growth factor binding protein-6, leads to decreased IGFBP-6 levels, in turn increasing IGF-II availability in DD, to promote differentiation of myofibroblasts and the deposition of collagen into the ECM.

Methods:
Primary cells derived from Dupuytren's Disease (DD) cord tissue or phenotypically normal palmar fascia from DD patients (PF) are being assessed for proliferation (WST-1 assays) and myofibroblast differentiation and contractility (Stressed Fibroblast Populated Collagen Lattice assay, sFPCL) with or without the addition of exogenous recombinant IGFBP-6 alone or in combination with transforming growth factor (TGF)-β. α-smooth muscle actin levels are assessed by immunoblotting to confirm myofibroblast differentiation. Chromatin Immunoprecipitation (ChIP) assays are used to assess TGF-β-induced β-catenin /TCF/LEF transcription complex binding to the *IGFBP6* promoter. T tests and ANOVA analyses are performed using SPSS.

RESULTS:
Microarray analyses indicate a decrease in *IGFBP6* mRNA expression in DD cord tissue relative to patient-matched palmar fascia. TGF-β treatment of primary DD cells induced an increase in cytoplasmic β-catenin levels and repression of *IGFBP6* mRNA levels. PF cells were less sensitive than patient-matched DD cells to TGF-β-induced repression of *IGFBP6* transcription. ChIP assays to confirm TGF-β-induced β-catenin /TCF/LEF transcription complex binding to the *IGFBP6* promoter are underway. Preliminary data indicate that exogenous IGFBP-6 treatment has no discernable effect on DD or PF cell proliferation, however exogenous addition of IGFBP-6 inhibits TGF-β-induced contractility of DD and PF cells in sFPCLs. The effects of exogenous IGFBP-6 on DD and PF cell *COLIAI* and *IGF2* mRNA expression are currently being assessed using Real Time PCR.

Conclusions:
TGF-β induces β-catenin accumulation in DD and PF cells and induces the repression of *IGFBP6* expression, potentially through β-catenin /TCF/LEF transcription complex interactions with the *IGFBP6* promoter. As the primary function of IGFBP-6 is to sequester IGF-II, repression of IGFBP-6 may increase IGF-II availability in DD. As combinatorial interactions between TGF-β and IGF-II have been shown to result in myofibroblast differentiation and increased collagen production in other systems, it is plausible that decreased *IGFBP6* expression is specifically targeted to DD to facilitate myofibroblast differentiation and collagen deposition in this disease.