ADAM12 effects on Dupuytren's Disease cell morphology and cytoplasmic beta catenin accumulation require Type I IGF receptor tyrosine kinase activity

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Background: Dupuytren's disease (DD) is a debilitating fibroproliferative disease of the palmar fascia in the hand that results in the formation of a collagenous disease cord and permanent contraction of affected fingers. Our laboratory has previously documented increased levels of cytoplasmic beta-catenin, a signalling molecule involved in cell proliferation, in DD. The signalling pathway regulating beta-catenin accumulation in DD is not known. Affymetrix microarray analysis of surgically resected DD tissue performed in our laboratory indicates that A Disintegrin And Metalloprotease (ADAM) 12 is highly expressed in this fibrosis. This extracellular matrix-associated protease has been shown to increase Type I Insulin-like Growth Factor Receptor (IGFRI) tyrosine kinase activity, promote adherens junction disruption, and increase cytoplasmic levels of beta-catenin in other systems. The purpose of this study was to determine if ADAM12 affects beta-catenin accumulation in DD and, if so, whether this was dependent on IGFRI tyrosine kinase activity. **Methods:** Cells derived from DD patients were cultured on Type-1 collagen and treated with exogenous ADAM12 / vehicle in the presence or absence of NVP-ADW742-NX-7, a specific inhibitor of IGFRI tyrosine kinase activity. To more closely replicate the in vivo disease environment, a collagen co-culture method was employed. Briefly, primary DD cells were cultured on insert wells coated with collagen containing ADAM12 / vehicle, in co-culture with normal PF or DD cells cultured on untreated collagen. Immunofluorescence microscopy and Western blotting were used to analyze cell morphology and beta-catenin levels. **Results:** The addition of exogenous ADAM12 to DD cells, but not normal PF cells, resulted in marked changes in cellular morphology including condensed actin stress fibres and cytoplasmic beta-catenin accumulation. These effects of ADAM12 on DD cells were strongly inhibited by NVP-ADW742-NX-7, an IGFRI-specific tyrosine kinase inhibitor. Further, DD cells grown on collagen in co-culture with DD cells grown on collagen containing ADAM12 displayed increased beta-catenin levels relative to controls. **Conclusions:** This study demonstrates for the first time that inhibition of IGFRI tyrosine kinase activity markedly inhibits changes in cell morphology and that beta-catenin accumulation is induced by ADAM12, consistent with IGFRI signalling being an essential component of beta-catenin accumulation in DD.
Keywords: Dupuytren's Disease, ADAM12, IGF, tyrosine kinase

Caspase substrates screening by diagonal gel approach and study on caspase-1 substrates on glycolytic pathway

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Apoptosis is executed by caspase-mediated cleavage of various proteins. Elucidating the consequence of substrate cleavage provides us insight into cell death and other biological processes. In this study, we applied the diagonal gel approach, a proteomic strategy, to screen for caspase-1 and –3 substrates. Our results showed significant overlap between caspase-1 and –3 substrates obtained by the diagonal gel approach. Substrates for both caspase-1 and caspase-3 are implicated in common cellular functions, such as maintenance of the cytoskeleton, chaperons, translation, glycolysis, bioenergetics, signaling and trafficking. An important finding is that many glycolysis enzymes are targeted by caspase-1 in the diagonal gel approach. Cleavage of these glycolysis enzymes was confirmed by cleaving in vitro transcribed and translated substrates with recombinant caspase-1. Point mutation of GAPDH blocks its cleavage by caspase-1. This provides a direct link between apoptosis and glycolysis.
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