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EXPLORING THE INVOLVEMENT OF COLVI-NG2 AXI$AXIS IN THE GENERATION OF
CONTRACTURES AFFECTING UCMD PATIENTS

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Mutations of collagen VI (Col VI) cause Ullrich Congenital Muscular Dystrophy (UCMD), a severe disease leading patients also to suffer from invalidating contractures. Increasing evidences suggest tendon and muscle fascia dysfunction as a major cause. Joint contractures are currently treated with surgery with scarce success. Understanding the biologic mechanism of contractures is a poorly explored area. This study has the potential to bring substantial advances in finding a treatment to reduce progression of joint contractures which highly impact life quality of these patients.

On the basis of our previous findings we hypothesized that altered Col VI binding with the cell membrane may affect proper collagen I fibrils formation in tendons and muscle fascia cells because:
- NG2 proteoglycan mediates Col VI binding with the cell membrane
- inhibiting Col VI-NG2 binding impairs cell migration and response to mechanical stress
- mutations in COL6A2 gene causing UCMD affect NG2 expression
- degradation of NG2 by MMP2 or its deregulation by TGFbeta1 disrupt the integrity of Col VI-NG2 axis in UCMD.

We observed a significant number of cultured tendon and muscle fascia cells and evaluated the transcription level of NG2 proteoglycan, MMP2 and TGFB1. We also evaluated the effect of MMP2 and TGFbeta1 on normal tendon and fascia cells to explore the role of these molecules in determining UCMD cell phenotype. Finally, we treated in vitro UCMD cells with inhibitors of MMP2 activity and TGFbeta1 signalling to pave the way to future therapeutic applications.
Tendon biopsies from UCMD1, BM1 and BM2 patients showed irregular profiles and diameter variability of collagen fibrils with “cauliflower” (in UCMD) or irregular (in BM1 and in BM2) appearance. The fibril diameter distribution was shifted toward smaller values in UCMD1 and BM1 (loss of fibrils >150 nm). Conversely, a shift of the mean diameter toward larger values was detected in BM2 tendon, with reduced fiber population <60-100 nm and increase in population between 200 and 250 nm. Mean diameters did not vary. These data indicate tendon involvement in patients with Col VI genes mutations. The variability in size and the alterations in the fibrils profile (“cauliflower-like” or other) represent the hallmark of defective fibrillogenesis, and indicate that Col VI plays a pivotal role in the organization and function of the tendon ECM.

In UCMD1 and BM2 cell cultures, Col VI was reduced and showed an altered organization (spot-like aspect). Rotary shadowed replicas confirmed this finding. Western blot analysis of cells and conditioned media of BM2 and UCMD1 showed reduction of the α5(VI) chain in the cell layer but its increase in media of UCMD1, suggesting dissociation of the cell-bound ECM. Immuno-histochemical study revealed COL I and COL XII disorganization in UCMD1 and BM2 cultures, while fibronectin displayed abnormal parallel arrangement.

In UCMD tendon cell cultures, NG2 staining was barely detectable while Col VI was not associated with the cell surface. In BM2 cultures, NG2 did not co-localize with the (anomalous) Col VI spots. These data indicate that the organization of endogenous NG2 by tendon cell is affected in the presence of Col VI mutations, thus influencing the organization of Col VI-based pericellular matrix. A reduction of NG2 is present in UCMD1 cells but not in BM2. In UCMD1, NG2 expression was not downregulated at transcript level, suggesting the reduced protein amount is due to regulatory mechanism. In BM2 and UCMD1 tendons and myotendinous junctions, expression of NG2 regulators TGFbeta and MMP2 was not modified. In scratch wound assays, tendon fibroblasts of UCMD1 and BM2 patients showed a significant increase of incorrectly polarized cells. Cyclic stress revealed alteration in recovery of primary cilia after strain in UCMD1 cells with Col VI deficiency.

Publication

Poster

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