Tendon mineralization due to trauma and aging 
is linked to tenocyte matrix attachment and driven by mechanical stimuli

Daniel Kronenberg1, Philipp Michel2, Eva Hochstrat1, Momina Hasseb1, Uwe Hansen1 and Richard Stange1
1Department of Regenerative Musculoskeletal Medicine, Institute for Musculoskeletal Medicine, University of Münster;
2Department of Trauma-, Hand-, and Reconstructive Surgery, Münster, University Hospital, Münster, Germany
3Department of Molecular Medicine, Institute for Musculoskeletal Medicine, University of Münster, Münster, Germany
daniel.kronenberg@ukmuenster.de

INTRODUCTION: Tendinopathy describes a complex multifaceted pathology of the tendon, clinically characterized by activity-related tendon pain, decline in function and restricted mobility and disability, finally prone to tendon ruptures altogether leading to a high socio-economic burden. Integrin α2β1 is a transmembrane protein and one of the major collagen receptors. It is involved in ECM-cell attachment, signal transduction and expressed in all fibroblastic cells. We have recently investigated the role of integrin α2β1 in tendon biology demonstrating that tenocytes lacking this protein are able to produce more collagen type I. Additionally integrin α2 deficient tendons are containing more collagen I degrading MMP-2. In consequence, these tendons undergo a faster remodeling. This misbalance in collagenous/non-collagenous ECM proteins leads to a biomechanical weakness of the tendon. As differences in cellular attachment already led to these pronounced phenotypes in tendons of younger animals, we now investigated the outcome under traumatic and aging conditions in wild type and integrin KO animals.

METHODS: Animal experiments were conducted as approved by local authorities (Ref.No: 81-02.05.50.20.002/ 84-02.04.2015.A310). Tendon healing model: 12 weeks old male C57BL/6 and Integrin α2−/− mice were used (N=5, group). The left Achilles’ tendon was tenotomized and the leg was immobilized using a tibio-fibulary cerclage as described (1). Animals were sacrificed after 2, 4 and 6 weeks of healing. µCT analysis: 26 and 52 weeks old native Achilles tendons of C57BL/6 and Integrin α2−/− mice were investigated after sacrfication and preparation of the tendon (N=6, group). Biomechanics: Achilles’ tendons of 52 weeks old male C57BL/6 and Integrin α2−/− mice were dissected and analyzed as already published (2) under dynamic as well as static measurements (N = 10, group). Histology: Contralateral tendons were investigated in histology and electron microscopy (N = 3, group). Tendon cell culture: Murine Achilles tendons were dissected and the cells were cultured as previously described. The cells were cultivated in αMEM with 10 mM β-glycerol phosphate, 0.2 M L-ascorbate and 10 nM dexamethasone (osteogenic medium) under static condition in normal cell culture plates and under cell stretch condition at a frequency of 0.1 Hz and 10% elongation. The cells were investigated staining for ALP activity and mineralization using alizarin red.

RESULTS SECTION: Integrin α2 deficient tendons showed significantly increased mineralization either in the healing tendon as well as in the aging tendons (Figure 1). During healing after trauma, we could demonstrate enchondral ossification by histological staining for collagen II and X. Similarly, we could detect significantly more cartilage in aging integrin α2 deficient tendon by alician blue staining of histological slices. However, lack of Integrin α2β1 in aging animals led to similar phenotypic features as in tendons of younger animals (2). We observed decreased biomechanical properties as well as increased MMP-2 in integrin α2 deficient animals. Cultivation of isolated tenocytes could show that increased mineralization only occurs under cell stretch condition, whereas in static culture integrin α2 deficient tenocytes have lesser ossification potential.

DISCUSSION: Ectopic mineralization is known to be a leading concomitant symptom for tendinopathy. We could demonstrate here that tendon mineralization appears on a regular basis after trauma and aging on murine tendons. We could as well show that animals lacking integrin α2β1 have an increased tendon mineralization under these tendiopathic conditions. Further on, the weaker biomechanical properties as well as the increased mineralization capabilities under cell stretch conditions of the knock-out led to the conclusion that these effects are driven by mechanical stimulation or stress. Therefore, we conclude that the mechano-dependent, molecular functions of integrin α2β1, i.e. cellular attachment and recognition of the mechanical properties and composition of the ECM, cause the aggravation of the tendiopathic phenotype. We point out to the limitation, that even though the phenotypic effects are strikingly similar to severe tendinopathies, we still have to prove which role the misbalance of matrix proteins play and weather the phenotype we describe is just analogue to typical tendinopathy patients but follows a complete different mechanism. Therefore, we will investigate human patient samples concerning this matter in future.

SIGNIFICANCE/CLINICAL RELEVANCE: Incidence of tendinopathy is increasing due to an aging society and raised functional demands of the elderly. The finding of this study showed, that the biological function of integrin α2β1, which is mechano-stimulated, is be involved in tendinopathy, which may mean that mechanical weakness especially in concert with low integrin α2β1 expression, is a promising venue to predict developing tendinopathies and may be targets for treatment.

REFERENCES:

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Figure 1: Representative µCT images of healing murine Achilles’ tendon after inducing a tenotomy (A) and the corresponding quantification of ossified tissue. B Representative µCT images of Achilles’ tendons of aged male mice and the corresponding quantification.