The Application of Decellularized Tendon Biomaterial and RNA-Interference to Study Integrin-mediated Mechanotransduction in Tenocytes

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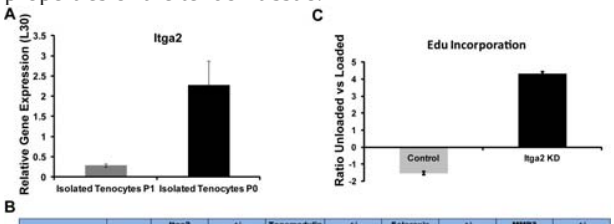
Introduction: Advancements in tendon tissue engineering have been hampered, in part, because of our limited understanding of tendon homeostasis and mechanobiology. Tenocytes play a central role in the health of the tendon by sampling the local environment and responding to mechanical stimulations with changes in their metabolic behavior. Understanding the components involved in recognizing mechanical changes and how they convey the information into a cellular response is hence pivotal. Molecularly, one group of proteins that may play a central role in assessing mechanical stress, communicating with the dynamic environment and responding appropriately to changes in the tendon tissue are integrins. In this study we particularly investigate the role of the collagen I binding integrin alpha2 (Itga2) during mechanotransduction.

Methods: Quantitative as well as normal RT-PCR techniques were applied to identify integrin expression in the bovine digital flexor tendon and to assess changes in gene expression levels throughout our study. Tenocytes were isolated by an enzymatic digestion method and cultured on decellularized bovine tendon tissue. Decellularization was achieved by incubating 30x7x1.5mm tendon sections for several days in a hypertonic buffer, followed by sterilization in 100% acetone for 1hr and several washes in PBS. Decellularization efficiency was assessed by DAPI staining and qRT-PCR for 18S. RNAi was performed to assess the function of the integrin, using a mixture of several small, double stranded RNA oligonucleotides designed against a 600bp region of the bovine Itga2 DNA sequence for 3 days. Subsequently, the tissue was subjected to 5% strain applied under tension at 1Hz in a bioreactor to simulate mechanical loading.

Results: In our expression screen for potential integrins involved in tendon mechanotransduction, we identified the adhesion molecule Itga2 as being expressed in several tendon tissues sampled. Unlike other integrins, we noticed a significant difference between Itga2 expression levels in tenocytes cultured on tissue culture plastic and freshly isolated cells from tendon tissue (Figure 1A), suggesting a mechanism by which tenocytes regulate Itga2 expression based on the substratum on which they are cultured. We therefore decided to use decellularized tendon as a biomaterial to maintain native tenocyte behavior and to address the function of Itga2 in mechanotransduction. Using RNA interference to decrease Itga2 expression and upon cyclic stretching conditions in our bioreactor for 24 hours, we detected changes in expression of various genes including Adamts-5, Collagen I and III, MMP3, Timp1, Scleraxis and Tenomodulin (Figure 1D). We also detected changes in the normal spindle like morphology and enhanced proliferation of tenocytes upon Itga2 knock-down. This finding suggests that Itga2 plays a crucial role in sensing and transducing changes in the mechanical environment, which ultimately affects the biomechanical properties of the tendon tissue.



B

		Itga2	+/-	Tenomodulin	+/-	Scleraxis	+/-	MMP3	+/-
Control KD	Loaded	0.000836	0.000194	0.0106	0.0027	0.00000309	0.00000036	103.14	24.67
Control KD	Unloaded	0.001164	0.000177	0.0023	0.0002	0.00000105	0.00000006	9.47	2.41
Itga2 KD	Loaded	0.000359	0.000024	0.0034	0.0006	0.00000301	0.00000062	47.19	8.69
Itga2 KD	Unloaded	0.000307	0.000070	0.0038	0.0016	0.00000207	0.00000048	10.14	3.15

		Collagen I	+/-	Collagen III	+/-	Timp-1	+/-	Adamts5	+/-
Control KD	Loaded	25816	3610	35.47	3.24	27.91	5.12	16.6	1.6
Control KD	Unloaded	9869	1386	4.77	1.52	4.05	0.66	42.0	4.6
Itga2 KD	Loaded	13478	859	13.53	3.35	4.94	0.66	29.5	1.9
Itga2 KD	Unloaded	11669	1386	6.29	0.30	3.73	0.50	11.0	0.1

Fig.1: A) Itga2 expression in freshly isolated tenocytes (P0) or isolated tenocytes which have been cultured for 7 days on plastic tissue culture dishes (P1). B) Tenocytes were mock transfected (gray shades) or transfected with 1nM esiRNA against Itga2 (pink shades, Itga2 KD) and probed for the indicated gene expression under mechanical loading or unloaded conditions. All tenocytes were derived from the bovine digital flexor tendon. C) 1uM EdU was added to tenocytes seeded on decellularized tendon for 3hrs under loading and non-loading conditions and processed for immunofluorescence. EdU incorporation was assessed by the ratio of EdU positive cells versus total nuclei. Error bars and (+/-) indicate SEM.

Conclusions: Mechanotransduction comprises a complex array of molecular interactions, all which can be modulated based on the mechanical environment encountered. Tendon injuries often occur due to overuse, leading to a degenerative cascade of events in tendinopathy [1]. Here we demonstrate that Itga2 is an important player in sensing and responding to such mechanical forces. Moreover, since Itga2 loss of function revealed changes in several crucial tendon genes its role in tendon homeostasis could be even more substantial when the tendon is mechanically challenged. Therefore, looking at different strain values and various frequencies in the absence of Itga2 may further our molecular understanding of tendon biology in health and disease. Identifying the molecular sensors and understanding their role in sampling the mechanical environment may help to develop better biomaterials for tissue engineering, and efficient treatment options to reduce tissue degeneration and facilitate a faster recovery phase in patients.

References and Acknowledgements:

1.) Arnoczky, S.P. et al., Int J Exp Pathol, 2007. **88**(4): p. 217-26.

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