Abstract

REMOTE ACTIVATION OF ACTIVIN A TYPE II RECEPTOR VIA MAGNETIC NANOPARTICLES FOR TENDON REGENERATION STRATEGIES

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Tendon injuries are a worldwide problem affecting several age groups and stem cell based therapies hold potential for tendon strategies guiding tendon regeneration.

Tendons rely on mechano-sensing mechanisms that regulate homeostasis and influence regeneration. The mechanosensitive receptors available in cell membranes sense the external stimuli and initiate mechanotransduction processes. Activins are members of the TGF-β superfamily which participate in several tendon biological processes. It is envisioned that the activation of the activin receptor, trigger downstream Smad2/3 pathway thus regulating the transcription of tenogenic genes driving stem cell differentiation.

In this work, we propose to target the Activin receptor type IIA (ActRIIA) in human adipose stem cells (hASCs), inducing hASCs commitment towards the tenogenic lineage. Since mechanotransduction can be remotely triggered through magnetic actuation combined with magnetic nanoparticles (MNPs), we stimulated hASCs tagged complexes using a vertical oscillating magnetic bioreactor (MICA Biosystems Ltd). Carboxyl functionalised MNPs (Micromod) were coated with anti-ActRIIA antibody (Abcam) by carbodiimide activation. hASCs were then cultured with MNPs-anti-ActRIIA for 14days with or without magnetic exposure (1Hz, 1h/every other day). hASCs cultured alone in αMEM (negative control) or in αMEM supplemented with ActivinA (R&D systems) (positive control of ActRIIA activation) were used as experimental controls. The tenogenic commitment of hASCs was assessed by real time RT-PCR, immunocytochemistry and quantification of collagen and non-collagenous proteins. Moreover, the phosphorylation of Smad2/3 was also evaluated on hASCs incubated for 2, 10, or 30min under magnetic stimulated (1Hz) and non-stimulated conditions.

The increased gene expression of tendon related markers and higher ECM proteins deposition suggests that remote magnetic activation of ActRIIA promotes effectively hASCs tenogenic commitment. Furthermore, the detection of phospho-Smad2/3 proteins by ELISA (Cell Signaling Technology) was significantly more intense after 10min in hASCs under magnetic stimulation and in comparison to the control groups. These outcomes suggest that ActRIIA is a mechanosensitive receptor that can be remotely activated upon magnetic stimulation.

In conclusion, remotely activation of MNPs tagged hASCs has potential for modulating tenogenic differentiation of stem cells envisioning successful cell therapies for tendon regeneration.

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