Tenomodulin subpopulation of human adipose stem cells as a promising source of tendon progenitor cells

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INTRODUCTION: Cell based strategies envision promising insights for tendon therapies due to the naturally limited cellularity and regeneration of these tissues. Human stem cells from the stromal vascular fraction of adipose tissue (hASCs) have shown their tenogenic potential for tendon strategies[1]. However, the heterogeneous populations within the hASCs pool may hinder their use in specific applications. Tenomodulin (TNMD) has been recognized as an important marker of tendon progenitor cells[2], thus herein we hypothesized that TNMD positive (TNMD+) cells are more prone to differentiate into tendon-like cells. Therefore, a distinct TNMD+ subpopulation was isolated from hASCs by immunomagnetic cell separation[3]. Subsequently, the tenogenic differentiation capacity of TNMD+ cells was evaluated (towards the heterogenous hASCs population obtained from the SVF) in the presence of growth factors (GFs) associated to development, growth and healing of tendon tissue[1]. Cell response to biochemical stimuli was studied analysing cell morphology, tendon-related markers expression, and extracellular matrix (ECM) formation.

METHODS: TNMD+ cells were sorted using Dynabeads® M-450 Epoxy (Invitrogen) coupled with TNMD antibody (Santa Cruz Biotech). Cells isolated without antibody coupling were considered the control population (CTR). Tenogenic differentiation capacity of both TNMD+ and CTR populations was evaluated for up to 28 days in α-medium or with: EGF, bFGF, PDGF-BB or TGF-β1. The time-related effects of GFs supplementation on the expression of tendon-related markers was evaluated by RT-PCR and flow cytometry, while the ability of cells to synthetize a Collagen I and III ECM was analysed by immunocytochemistry.

RESULTS: A TNMD+ subpopulation was successfully obtained by immunomagnetic separation as demonstrated by TNMD deposition in sorted cells in comparison to CTR cells (Fig.1). TNMD+ cells showed enhanced genetic expression of tendon markers, namely SCX, TNMD, and DCN as well as Col I and III over CTR cells for up to 28 days.

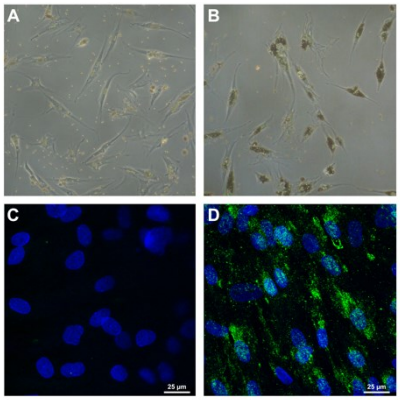

Fig. 1: Micrographs of hASCs: CTR cells (A) and TNMD+ cells (B) 3 days after of immunomagnetic isolation; TNMD immunolocation (green) in CTR (C) and TNMD+ cells (D). DAPI (blue) stains cell nuclei.

SCX and TNMD protein expression by flow cytometry was also increased in TNMD+ cells. Although gene and protein expression were influenced by supplemented GFs with a tendency to increase the expression of tendon markers in TNMD+ cells, no evident differences between supplemented and α-medium were found.

DISCUSSION & CONCLUSIONS: TNMD+ cells show a markedly positive tenogenic response in comparison to CTR cells. This response is supported by the enhanced genetic and protein expression of tendon markers. Thus, the TNMD+ subpopulation is predisposed towards tenogenic lineage and therefore can be used more efficiently as source of tendon progenitor cells.

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