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REVIEW ARTICLE

Current concepts on tenogenic differentiation and clinical applications

Yang Liu [a], Chun-Wai Suen [a], Jin-fang Zhang [a], Gang Li [a,b,c,d,*]

[a] *Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China*
[b] *Stem Cells and Regenerative Medicine Laboratory, Lui Che Woo Institute of Innovative Medicine, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China*
[c] *Key Laboratory for Regenerative Medicine, Ministry of Education, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China*
[d] *The CUHK-ACC Space Medicine Centre on Health Maintenance of Musculoskeletal System, The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, China*

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Summary Tendon is a tissue that transmits force from muscle to bone. Chronic or acute tendon injuries are very common, and are always accompanied by pain and a limited range of motion in patients. In clinical settings, management of tendon injuries still remains a big challenge. Cell therapies, such as the application of stem cells for tenogenic differentiation, were suggested to be an ideal strategy for clinical translation. However, there is still a lack of specific methods for tenogenic differentiation due to the limited understanding of tendon biology currently. This review focuses on the summary of current published strategies for tenogenic differentiation, such as the application of growth factors, mechanical stimulation, biomaterials, coculture, or induced pluripotent stem cells. Current clinical applications of stem cells for treatment of tendon injuries and their limitations have also been discussed in this review.

* Corresponding author. Department of Orthopaedics and Traumatology and Li Ka Shing Institute of Health Sciences, Prince of Wales Hospital, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, New Territories, Hong Kong, China.
E-mail address: gangli@cuhk.edu.hk (G. Li).

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Introduction

Tendon is a tissue that transmits force from muscle to bone. Tendon injures, such as tendinopathy or acute tendon rupture, are a common type of sports injuries. However, current treatments for tendon injures are unsatisfactory and limited to the nonsteroidal anti-inflammatory drug injection, physical therapy, or surgery [1—3]. Tendon tissue engineering has been suggested to be a promising approach for tendon repair. Since bone marrow stem cells (BMSCs) or tendon-derived stem cells (TDSCs) have outstanding self-renewal and multidifferentiation ability, it is a well-recognized strategy to apply them in tendon tissue engineering [4,5]. Although many genes are reported to be involved in tendon development, they also express in a wide range of other tissues, such as muscle, bone, and cartilage. Owing to the limited understanding of specific tendon makers and molecular interactions between transcription factors and signalling pathways, there is still a lack of a specific method for tenogenic differentiation. Currently, various protocols have been reported to be able to induce tenogenic differentiation. This review focuses on the summary of currently published strategies for tenogenic differentiation, such as the application of growth factors, mechanical stimulation, biomaterials, coculture with another cell source, TDSCs, or BMSCs. An advanced understanding of the current strategies on tenogenic differentiation would be beneficial for tendon tissue engineering and its clinical translation in the future.

Tendon biology

Tendon and associated extracellular matrix markers

Tendon formation relies on the combination of the transcription factors, growth factors, and mechanical stimulation during development [6]. In normal tendon, the primary unit of the tendon is the fibre that made up of collagen fibrils with tendon cells residing inside [6]. The dry mass of human tendons is about 30% of the total tendon mass, with water accounting for 70% [7]. From the dry mass of tendon, collagen type I accounts for 65—80%, and elastin takes up about 2% [7,8]. Collagen provides elasticity to the tendon, which is mainly made up of type I collagen (*Col1*) and a small amount of other collagens, such as types III, IV, V, and VI [9]. The extracellular matrix (ECM) functions as the organizer for collagen fibril assembly [5,10,11], and it is composed of proteoglycan, glycoproteins, and other small molecules. Decorin (Dcn) and biglycan (Bgn) are the common small leucine-rich proteoglycans in tendons that help organize the collagen fibre bundles. Targeted knockout of certain proteoglycan can lead to abnormal collagen fibrils in tendons and impair their mechanical properties [12—14]. Other common proteoglycans are fibromodulin and lumican. It was reported that Tenascin-C (Tn-C), a glycoprotein, is regulated by mechanical loading and is upregulated in patients with tendinopathy [15,16]. Moreover, Tn-C also participates in collagen fibre alignment and orientation [7]. Particularly, Tenomodulin (Tnmd) is a type II transmembrane glycoprotein containing a C-terminal antiangiogenic domain, and it is necessary for tenocyte proliferation and tendon maturation [17,18]. The expression of Tnmd is positively regulated by Scleraxis (Scx) [19]. Mice with loss of Tnmd expression showed impaired tenocyte proliferation, reduced tenocyte density, and increased maximal and greater variation of fibril diameters [18].

Transcription factors of tendon

Currently, Scx, Mohawk (Mkx), and early growth response protein 1 (Egr1) have been identified as the transcription factors for tendon development [9,20,21]. Scx, a basic helix—loop—helix transcription factor, is a relatively specific marker of tendon/ligament lineage and has been reported to be induced at the earliest stage during tendon development [22—24]. Mice with Scx knockdown ($Scx^{-/-}$) have severe disruption of force-transmitting tendons, with limited movement of paws and back muscles, and inability to move the tail [20]. It has also been reported that *Scx* could activate *Col1* together with *Nfatc4* (nuclear factor of activated T cells, cytoplasmic 4) [25]. The matrix in the tendon from $Scx^{-/-}$ mutant mice is also disorganized, with intermixing of tenocytes and endotenon cells [20].

Mkx is a membrane of the three amino acid loop extension superclass of a typical homeobox genes expressed in developing tendons [9,26]. Mice with Mkx knockdown ($Mkx^{-/-}$) showed significantly reduced tendon mass and a small collagen fibril diameter [9]. The expression of *Col1A1* is also decreased in $Mkx^{-/-}$ mice, indicating that *Mkx* plays a role in tenogenic differentiation by regulating the production of collagen type I. Moreover, Liu et al [27] also reported that *Mkx* could dramatically activate *Scx* by binding to the tgfb2 promoter, and Mkx showed lower expression in tendinopathy and it is activated during tendon development.

Egr1 is a zinc finger transcription factor, and it was reported to be involved in vertebrate tendon formation [28]. Mice with *Egr1* knockdown ($Egr1^{-/-}$) have weaker mechanical properties, and decreased expression of *Scx*, *Col1A1*, and *Col1A2* was observed in adult tendons [21]. Particularly, it was also mentioned that *Egr1* can promote tenogenic differentiation by targeting transforming growth factor (TGF)-β2. As mentioned before, mechanical stimulation is also necessary for tendon development, especially during the late stage of tenogenic differentiation, to promote the maturation of collagen [29,30]. Activation of *Egr1* has been suggested as a possible mechanism during mechanical stimulation, which promotes the maturation of collagen formation [10,30].

Tendon-derived stem cells

Bi et al [5] first identified and characterized tendon stem cells in tendons from human and mouse, followed by Rui et al [31] in isolating and identifying TDSCs from rat tendon. TDSCs showed multipotent and self-renewal capacities, and they have been suggested as an ideal cell source for tendon tissue engineering. Moreover, it is also found that TDSCs have higher *Tnmd*, *Scx*, *Col1*, *Dcn*, *Bgn* expression; osteogenic differentiation; and chondrogenic differentiation abilities when compared with BMSCs [32].