Experimental Cell Research 341 (2016) 237–242

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

Review Article

Tenogenic differentiation of mesenchymal stem cells and noncoding RNA: From bench to bedside

Ying-fei Lu [a,b], Kai-ming Chan [a,b,c], Gang Li [a,b,c], Jin-fang Zhang [a,b,c,*]

[a] Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong SAR, China
[b] Lui Che Woo Institute of Innovative Medicine, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
[c] Stem Cells and Regenerative Medicine Laboratory, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

ARTICLE INFO

Article history:
Received 21 July 2015
Received in revised form
21 December 2015
Accepted 23 December 2015
Available online 24 December 2015

Keywords:
Tendon
Tendon differentiation
Tendon-derived stem cells
Non-coding RNAs

ABSTRACT

Tendon is a critical unit of musculoskeletal system that connects muscle to bone to control bone movement. More population participate in physical activities, tendon injuries, such as acute tendon rupture and tendinopathy due to overuse, are common causing unbearable pain and disability. However, the process of tendon development and the pathogenesis of tendinopathy are not well defined, limiting the development of clinical therapy for tendon injuries. Studying the tendon differentiation control pathways may help to develop novel therapeutic strategies. This review summarized the novel molecular and cellular events in tendon development and highlighted the clinical application potential of non-coding RNAs and tendon-derived stem cells in gene and cell therapy for tendon injuries, which may bring insights into research and new therapy for tendon disorders.

Contents

1. Introduction

The musculoskeletal system is composed of distinct tissues including bone, muscle, tendon and cartilage. Among them, tendon is a tough band that connects muscle to bone and transmits the force generated by muscle contraction to the bone [1]. To maintain its tension and bear load in a long-period, the tendon has reduced metabolic rate which also slows down its healing potential after injury [2]. Acute tendon rupture and chronic injuries (or tendinopathy) caused by overuse are quite common in athletes and workers [2,3], but the pathogenesis remain poorly understood. Moreover, tendon healing is often accompanied with formation of fibrotically scarring and adhesion, instead of complete regeneration, which may eventually cause partial loss of tendon function.

* Corresponding author at: Room 904, 9/F, Li Ka Shing Institute of Health Institute, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China.
E-mail addresses: gangli@cuhk.edu.hk (G. Li), zhangjf06@cuhk.edu.hk (J.-f. Zhang).

http://dx.doi.org/10.1016/j.yexcr.2015.12.014
0014-4827/

To date, the clinical therapeutic options for tendon injuries were limited to surgical replacement with autografts, allografts, or xenografts [4], with considerable long recovery period. Thus, novel therapeutic strategies are in urgent needed. Studies on the tendon differentiation at molecular level provides insights of the earliest events of tendon development and pathologic changes of tendon.

Tenogenic differentiation involves a series of signaling pathways, transcriptional and epigenetic regulators. Non-coding RNAs (ncRNAs) are a family of functional RNA molecules without being translated into proteins. They serve as important and powerful regulators of various biological activities and play critical roles in a variety of disease progression [5,6]. To date, a few studies have focused on ncRNA functions in tendon development and disorders. In this review, we summarized the molecular events in tendon development and mainly focused on ncRNAs in tendon differentiation and tendinopathy.

2. Molecular basis of tendon development and healing

2.1. Molecular regulation of tendon differentiation during development

Tendon is mainly composed of tenocytes and highly aligned collagen fibers embedded within extracellular matrix (ECM). According to the findings of in vitro studies, the progress of tendon development was demonstrated in Fig. 1. Firstly, embryonic stem cells (ESCs) differentiate into mesenchymal stromal cells (MSCs) [7]; then MSCs further developed into tendon progenitors (or known as tendon-derived stem cells, TDSCs) which are stimulated by the transcription factor Scleraxis (SCX), a key regulator of tenocyte differentiation, and its expression in tenocytes is strongly induced by TGF-beta signaling [8]; the tendon progenitors further differentiate into tendon fibroblasts under control of SCX and Mohawk (MKX), thecritical regulators of tendnogenic differentiation [9,10]. Finally, the tendon fibroblasts mature into tenocytes through secretion and remodeling of surrounding extracellular matrices (ECM) [11]. During the tendon development progress, a number of genes, such as SCX, MKX, and early growth response (EGR) family member EGR1 and EGR2, are responsible for the cell fate determination and regulation of ECM assembly [12–14].

In contrast to bones and cartilage tissues, lack of specific molecular markers during tendon development has limited the mechanism studies in tendon development. SCX was found to continuously express from early somatic cells to mature tendons during mouse embryonic development and was once regarded as a tendon-specific marker [8,9,12,15]. MKX and EGR1 were also reported to mediate tendon differentiation through activating TGF-β pathway [16,17] and regarded as tendnogenic transcriptional factors. Besides these transcription factors mentioned above, genes related to secretion and degradation of tendon matrices, such as collagen type 1 and Tenomodulin (TNMD), were also served as markers to monitor tendon development in the late stage of embryonic development [18].

2.2. Growth factors in tendon development and formation

During tendon development and healing process many growth factors are involved, such as bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), basic fibroblast growth factors (bFGF), transforming growth factor beta (TGF-β), insulin-like factor 1 (IGF-1),platelet-derived growth factor (PDGF) and connective tissue growth factor (CTGF), these factors have been reported to regulate cell differentiation, proliferation, chemotaxis and ECM synthesis [19].

The TGF-β superfamily, including TGF-β, BMPs and GDFs, are the key growth factors for tendon formation. TGF-β signaling has critical roles in vertebrate early tendon development. The TGF-β/SMAD2/3 pathway is identified as the most important pathway in limb tendon development [20] and disruption of TGF-β signaling results in loss of most tendon tissues in mouse embryo [21]. GDFs are also good inducers for tendon formation and regeneration. GDF-5, 6 and 7 (also known as BMP-14, 13 and 12) were shown to modulate tendon matrix synthesis [22–24]. Specially, GDF-5 is found essential in Achilles tendon healing [25]. However, GDFs are also responsible for inducing cartilage and bone formation *in vivo* [26]. More studies on how GDFs regulate cell fate are needed for achieving controlled tendon healing using GDFs.

Other growth factors, such as FGF, IGF-1 and PDGF, are not critical in tendon development, but their expression are increased in tendon healing process, indicating their potential roles in tendon healing. Current studies show that FGF/ERK/MAPK pathway induces tendon differentiation *in vitro* [27], while IGF-1 and PDGF promote cell proliferation, stimulate tenogenic ECM synthesis and enhance tensile strength of tendon in rat models [28–30].

2.3. Mechanical stimuli induced molecular changes in tendon

The mechanical stimuli during muscle movements also play indispensable roles in maintaining tendon functions, including tendon development and repair [31–33]. Studies showed that mechanical loading promotes matrix remodeling in MSCs and activates integrin downstream kinases p38 and ERK1/2 [34,35]. A proteomic analysis of tendon tissue showed that the mechanical force altered expression of a large number of proteins in tendon, including extracellular matrix molecules, intra-cellular signaling molecules, cytoskeleton proteins and inflammatory factors [36]. Among these proteins, collagens I and VI, MMP-14, WNT5A and some inflammatory factors, COX, COX2 and PRDX5 may all contribute to the highly compacted and organized tendon tissue structure [36]. *In vitro* studies revealed that the collagen synthesis was stimulated by mechanical loading, which was probably mediated by growth factors TGF-β, IGF-1 and IL-6 [37]. During tendon healing process in a rat model, mechanical force stimulates tendon growth in late stage by up-regulating a series of tendon-specific genes, such as SCX and tenomodulin [33], while in the

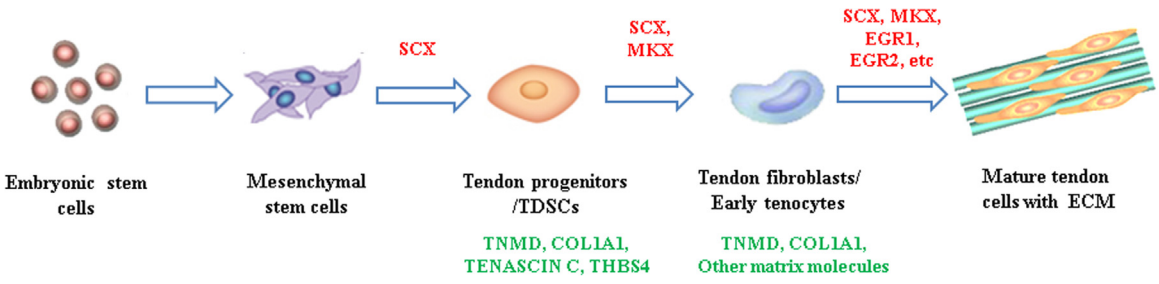

Fig. 1. Hypothetic illustration of tendon development. The transcriptional factors for tendon development are labeled in red, whereas the other molecular markers related to tendon development are labeled in green.