

## A Mini-Review: MicroRNA in Tendon Injuries

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### Abstract

Tendon injury is a difficult clinical problem. Till now, the basic cell biology of tendons is still not fully understood and the management of tendon injury poses a considerable challenge for clinicians. Endogenous microRNAs are short noncoding RNA molecules which can negatively regulate gene expression at the post-transcriptional level. The regulatory role of miRNA has well been studied in many diseases, however the significance of miRNAs in tendon injuries remains elusive. This review summarized the miRNAs related to the pathogenesis of tendinopathy and tendon injuries and highlighted their clinical potential, which may bring new insights into developing a novel advanced strategy toward tendon injury treatment.

**Keywords:** MicroRNA; Tendinopathy; Tendon injuries

### Introduction

Endogenous microRNAs (miRNAs) are short noncoding RNA molecules of 18-24 nucleotides in length. They negatively regulate the target gene expression by binding the 3'-untranslated region (UTR) of their target mRNAs leading to translational repression or mRNA degradation. Many miRNAs have already been identified and they were found to play important roles in multiple cellular processes. It is estimated that more than one-third of the human protein coding genes are regulated by these miRNAs [1]. The clarification that miRNAs participate in the pathogenesis of diseases, especially refractory diseases with unidentified mechanisms, might lead to a novel effective treatment.

Tendon forms an integral part of a musculotendinous unit. They can transmit forces from muscle to rigid bone levers producing joint motion, act as shock absorbers, energy storage sites, and help to maintain posture through their proprioceptive properties. Tendon is consisting of 30% collagen and 2% elastin embedded in an extracellular matrix (ECM) containing 68% water and tenocyte. Generally, the collagen fibers have an orderly parallel arrangement depending on tension direction. Elastin contributes to the flexibility of a tendon. Tenocytes are the main terminally differentiated cells, responsible for synthesis and form of tendon fibers and ground substance in a three-dimensional network [2]. Recent years, scientists identified a population of residing tendon stem/progenitor cells (TSPC) within tendon. These TSPC remain self-renewal, clonogenicity, and three-lineage differentiation capacities. They express tendon-related genes and are able to form tendon and enthesis-like tissues when implanted in vivo [3-5] (Table 1).

Tendon injuries divide into chronic injury, known as tendinopathy, or acute trauma, known as tendon rupture. They are caused by intrinsic or extrinsic factors, either alone or in combination. In acute trauma, extrinsic factors predominate, whilst in chronic cases intrinsic factors also play a role [6-9]. Recently, increasing evidence showed that miRNAs involved in the pathogenesis and experimental treatment of tendon injuries. This mini-review represents a digest of existing evidence on the roles of miRNAs relevant to tendinopathy and tendon injury healing. It is hoped that this article not only unveil the possible significance of miRNAs in tendinopathy but also illuminate the therapeutic potential of related research in tendon injuries treatment.

MicroRNA	Function	References
miR-499	Associated with pathogenesis of tendinopathy	[11]
miR-608	Polymorphic and associated with pathogenesis of tendinopathy	[16]
miR-378, miR-133, miR-206, miR-140, let-7a, let-7e, miR-338, miR-381 and miR-743	Related to mechanical stimuli	[20]
miR-135a	Regulate TSPC senescence by targeting ROCK1	[23]
miR-28 and miR-17-92	Mediate oxidative stress induced-tenocytes apoptosis	[28]
miR-210	Improve tendon injury healing via regulation of angiogenesis	[30]
Engineered TGF- $\beta$ 1 miRNA	Target TGF- $\beta$ and reduce adhesion formation	[34-36]
miR-29b	Prevent tendon adhesion	[37]

**Table 1:** List of miRNAs that may involve in tendon injuries.

### MiRNA is a New Player Participating in the Pathogenesis of Tendinopathy

Tendinopathy is a broad term encompassing painful conditions occurring in and around tendons in response to overuse or ageing, and usually cause pain, stiffness, and loss of strength in the affected area. Histologically, tendinopathy shows disordered haphazard healing with poor healing response, non-inflammatory intratendinous collagen degeneration, fiber disorientation and thinning, hypercellularity, scattered vascular ingrowth, and increased interfibrillar glycosaminoglycans. Many factors have been implicated as mechanisms

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of tendon degeneration, such as hypoxia, ischaemic damage, oxidative stress, hyperthermia, impaired apoptosis, inflammatory mediators, matrix metalloproteinase imbalance and so on [2]. However, the aetiology of tendinopathy has not been fully elucidated and many causes have been theorized.

In order to identify the miRNAs involved in tendinopathy, one approach is to identify differentially expressed miRNAs by comparison of control group and tendinopathy group directly. First, Jelinsky SA et al reported the gene expression profile of tendinopathy, GSE26051, which included 23 control and 18 tendinopathy samples. They identified 983 transcripts with significantly different expression patterns in the diseased tendons through global gene expression profiling, including genes involved in extracellular matrix proteins, MMPs, kinases and chemokines [10]. Further, Cai et al. also used this gene profile to analyze the potential regulatory microRNAs and the target sites of the transcription factors. Based on the molecular signature database, miR-499 was screened and was found to regulate two specific genes, namely CUGBP2 and MYB [11]. CUGBP2 is a RNA-binding protein implicated in the regulation of several post-transcriptional event, involved in pre-mRNA alternative splicing, mRNA translation and stability [12]. MYB, is a transcriptional activator, which plays an important role in the control of stem cell proliferation and differentiation [13]. These information suggest miR-499 may affect tendinopathy through regulating complicated cell processes.

Another approach is to focus on a specific tenogenic gene first, then analyze the expressions of the gene and its target miRNA between control group and tendinopathy group. For example, *COL5A1* encodes the  $\alpha 1$  chain of collagen type V, a quantitatively minor fibrillar collagen that regulates fibrillogenesis [14]. A variant within the 3'-UTR of *COL5A1* is associated with chronic Achilles tendinopathy (AT) and with anterior cruciate ligament injuries in females. Collins M's group reported two major forms of *COL5A1*, named C- and T-alleles, were predominantly identified in the controls and the AT subjects respectively. *COL5A1* mRNA from the tendinopathic phenotype has a higher stability and there is a functional miRNA site for hsa-miR-608 within the *COL5A1* 3'-UTR [15]. Furthermore, they demonstrated polymorphisms within the *COL5A1* 3'-UTR that altered mRNA secondary structure. In addition, the miR-608 gene is also polymorphic and associated with achilles tendinopathy [16].

Moreover, scientists also investigate the miRNAs which are involved in the process of other key factors induced-tendon degeneration. They want to clarify miRNA as a new player participating in its mechanisms. Up to now, there are several representative publications studying the relationship between important pathogenetic factors and miRNAs.

### Mechanical stimuli affects multiple miRNAs expression in tendon

Tendon, as the connective tissue structure, transmit forces from muscle to rigid bone levers producing joint motion. Physiological loading of tendons leads to increases in ECM content and tenocyte density, but the failure of tendons to adapt to physiological loading may cause the development of painful tendinopathy that can severely restrict activities of daily living [17-19]. So it is important to understand how loading affects tendons at the cellular and molecular levels. Mendias CL and coworkers are the first to analyze specific miRNA expression pattern on animal model of tendinopathy induced by overuse. They subjected rats to a 30-min treadmill run and measured gene expression 8 hours later. Compared with the control group without running, there was an increase in the expression of ECM gene, the general cell proliferation

gene, and the tenocyte proliferation and matrix synthesis genes *Scx* and *Tnmd*, which is consistent with the previous studies. More important, they studied the expression of specific miRNA molecules with known roles in the regulation of cell proliferation and ECM synthesis, skeletal muscle growth and adaptation, chondrogenesis and neovascularization, the let-7 cluster, and miRNAs predicted to bind to the 3'-UTR of *Scx* or *Tnmd*. The results showed 12 out of 35 miRNAs evaluated had a change in expression following an acute bout of mechanical loading. In addition, TGF- $\beta$  changed the expression of multiple miRNAs in tendon cells [20]. Although they did not ascertain the role of these changed miRNAs on tenogenesis, they provide a novel insight into the mechanobiology of tendons and indicate that miRNA could play an important role in the adaptation of tendons to growth stimuli.

### MiRNA modulates tendon stem/ progenitor cell senescence

The changes associated with increasing age result in decline in the structure and function of human tendons [19]. Young and healthy tendon tissue consists of abundant extracellular matrix, terminally differentiated tenocytes and resident stem and progenitor cells (TSPC) [21,22]. During aging, various stress factors and mechanical microdamages lead to altered tendon composition and functionality. Kohler J et al report that, in this process, TSPC accumulate several profound changes which altogether result in the exhaustion of their number and functional fitness. Further they found the ROCK kinase pathway (downstream signaling regulates actin stress fiber formation via myosin light chain) might be key players in TSPC aging [22]. MiRNAs are also involved in cellular senescence. Chen L et al further found that miR-135a was a negative regulator of cellular senescence through down-regulating ROCK1 in TSPC [23]. Therefore, the restoration of miR-135a might be a potential therapeutic strategy in tendon aging.

### Oxidative stress induces tenocytes apoptosis through mediating miRNAs expression

Oxidative stress is a key factor causing tissue degeneration [24]. Tenocytes, as the mechano-sensitive musculoskeletal cell, routinely encounter oxidative stress. Evidences show diabetes is associated with increased oxidative damage, accompanied with increased risk of tendon degeneration [25-27]. Poulsen RC et al demonstrated that miR-28, miR-17-92 were involved in oxidative stress combined high glucose-induced tenocyte apoptosis. The results show miR-28 directly inhibits expression of the p53 deacetylase sirtuin 3, allowing accumulation of acetylated p53. The p53 inhibits expression of miR-17-92, a cluster of miRNAs including the Bim (a proapoptotic protein) repressor miR-17. Down-regulation of miR17-92 by p53 allows up-regulation of Bim expression level and increasing Bim-mediated apoptosis [28].

According to the above description, miRNA concepts were mentioned into tendinopathy research in all these studies. However, there is still wild field to identify novel tenogenic miRNAs, and dig deeper into their mechanisms. Especially, those miRNA directly related to tenogenic genes should be considered as the key targets in the subsequent research.

### MiRNA is a Potential Therapeutic Target in the Treatment of Tendon Injuries

Predominantly, scientists study tendon healing through performing on transected animal tendons or ruptured human tendons. It is not clear whether these models, with healing failure response, are relevant to human tendinopathy.

Usually, tendon healing progress are in three overlapping phases.

(1) Inflammatory phase. In the first 24 hour, traumatic tissues collect monocytes and macrophages, the main active cells, and phagocytosis of necrotic materials occurs. Vasoactive and chemotactic factors are released to increase vascular permeability, initiate angiogenesis, stimulate tenocyte proliferation, and recruit more inflammatory cells. Tenocytes gradually migrate to the wound, and initiate to synthesize collagen type III. (2) Proliferative phase. After a few days of tendon injuries, production of type III collagen gets the peak, with lasting for a few weeks. Water content and glycosaminoglycan concentrations still keep at a high level during this stage. (3) Remodeling phase. After approximately six weeks, the healing tissues begin to resize and reshape. First, the repair tissues change from cellular to fibrous. Tenocytes commence to decrease cellularity, while collagen type I synthesizes still at a higher proportion. Meanwhile, tenocytes and collagen fibers form alignedly in the direction of stress. After 10 weeks, fibrous tissues gradually change to scar-like tendon tissue over the course of one year [2].

Thus, it is important to develop specific treatment strategies based on the different phases for improving tendon injury healing. miRNA as an important regulator is well studied in many biological processes. More importantly, one application of miRNA-based therapeutic has already entered Phase 2 clinical trials [29]. For tendon injury healing, there are a few of groundbreaking studies.

One strategy is to accelerate the process of tendon injury healing by miRNA. Usman MA and coworkers' study provided a good example. As mentioned above, angiogenesis vascular supply plays an important role in primary tendon healing, especially during the early healing phase. miR-210 has been reported as a crucial regulator for angiogenesis, which is a key factor for tissue repair. So they local injected synthetic miR-210 into the injured achilles tendons of rats and found it accelerated healing of the tendon. miR-210 induced better regular dense collagen tissue, abundant vessels with up-regulation of expression of VEGF, FGF2 and collagen type I [30].

Another approach is to reduce scar-like tendon tissue by miRNA. Healing of the tendon is frequently accompanied by adhesion formation around the repair sites. Transforming growth factor (TGF)- $\beta$  is responsible for the formation of scars such as adhesions around the healing digital flexor tendons [31-33]. So silence the TGF- $\beta$  expression is considered as a therapeutic strategy for inhibition of the adhesion formation. Tang JB's group designed and engineered miRNAs according to genetic sequences of TGF- $\beta$ 1 and injected the most effectively one mixing with lipofectamine 2000 (a common transfection reagent) on chicken tendon injury model. They found microRNA significantly down-regulated the expression of TGF- $\beta$ 1 *in vitro* and *in vivo*. The application of miRNA did not down-regulated the expression of collagen type I, but down-regulated the collagen type III gene [34]. Furthermore, they improved the microRNA delivery method by producing poly(lactic-co-glycolic acid) (PLGA) nanoparticles/TGF- $\beta$ 1 miRNA plasmid (nanoparticle/ plasmid) complexes. This method had a better transfection efficiency *in vitro* or *in vivo*. More importantly, the grading of adhesions for tendons treated with nanoparticle/plasmid complexes was less severe than that treated with the nanoparticle/mock plasmid complexes. However, the ultimate strength of repaired tendons treated with nanoparticle/plasmid complexes was significantly lower than that of tendons treated with the nanoparticle/mock plasmid complexes [35]. Furthermore, they also try to apply the PLGA nanospheres/TGF- $\beta$ 1 miRNA plasmid complexes. The result showed the expression of transforming growth factor- $\beta$ 1 was significantly down-regulated in healing chicken flexor tendon treated with nanosphere/plasmid.

Histology analysis did not demonstrate any significant inflammation or necrosis in tendons injected with nanosphere/plasmid after surgery [36]. These series of studies point out a possible direction for miRNA application in tendon injury therapy.

In addition, some exogenous compounds can regulate endogenous miRNA expression, which may be considered as the potential drug. Chen Q et al demonstrated that miR-29b mediated chitosan (a natural polymer) induced-prevention of tendon adhesion after surgery by regulating TGF- $\beta$ 1/Smad3 pathway [37]. These results show miRNA pathway can be targeted as the therapeutic mechanism to find new drug in tendon injury healing research.

In conclusion, the on going scientific investigations supply miRNA as a novel clue for pathogenesis and therapeutic method of tendon injuries. In future, more evidence about the role of miRNA in tendon injuries and more knowledge about RNA interference technology *in vivo* will turn miRNA-targeted tendon injury healing treatment from a conceptual chimera into a reality.

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