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Review

Therapeutic potential of GDF-5 for enhancing tendon regenerative healing

Hanyue Li [a], Yini Li [b], Linmei Xiang [c], Shengyu Luo [a], Yan Zhang [d], Sen Li [e, *]

[a] School of Physical Education, Southwest Medical University, PR China
[b] Department of Ultrasound, The Affiliated Hospital of Southwest Medical University, Sichuan, PR China
[c] Department of Dermatology, The Affiliated Hospital of Southwest Medical University, Luzhou, PR China
[d] Luzhou Vocational and Technical College, PR China
[e] Division of Spine Surgery, Department of Orthopedic Surgery, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, PR China

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ABSTRACT

Tendon injury is a common disorder of the musculoskeletal system, with a higher possibility of occurrence in elderly individuals and athletes. After a tendon injury, the tendon suffers from inadequate and slow healing, resulting in the formation of fibrotic scar tissue, ending up with inferior functional properties. Therapeutic strategies involving the application of growth factors have been advocated to promote tendon healing. Growth and differentiation-5 (GDF-5) represents one such factor that has shown promising effect on tendon healing in animal models and in vitro cultures. Although promising, these studies are limited as the molecular mechanisms by which GDF-5 exerts its effect remain incompletely understood. Starting from broadly introducing essential elements of current understanding about GDF-5, the present review aims to define the effect of GDF-5 and its possible mechanisms of action in tendon healing. Nevertheless, we still need more in vivo studies to explore dosage, application time and delivery strategy of GDF-5, so as to pave the way for future clinical translation.

Contents

* Corresponding author. Division of Spine Surgery, Department of Orthopedic Surgery, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, 321 Zhongshan Road, Jiangsu, Nanjing 210000, PR China.
E-mail address: jht187@163.com (S. Li).
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1. Introduction

Tendon injury is a common disorder of musculoskeletal injuries and has an enormous effect on patients' lives, mostly elderly individuals and athletes [1]. With respect to the incidence group, epidemiological investigations show that tendon injury is more common in the elderly population compared to the young [1]. Because of the special anatomical structure of the tendon with avascularity and lack of nerves, the self-repair ability of the tendon is poor. Despite remodeling, the injured tendon is biomechanically and histologically inferior to the intact tendon and never restores the uninjured tendon state [2,3]. Nowadays, treatment modalities for tendon injury fall into two general categories: operative and conservative treatment [4]. Conservative treatments are widely accepted forms of curative treatments for tendon injury since surgical management can easily contribute to infection, nerve injury, and scar formation [5]. However, conservative treatments commonly result in prolonged treatment duration, recurrent injury, and possible loss of function [6]. In fact, these treatment modalities do not fully use the intricate environment of tendon healing to their advantage. Hence, introducing novel strategies to regulate the mechanisms of cells and molecules may lead to more successful treatment strategies for tendon injury.

The healing processes of tendons are expedited by various growth factors that are produced within the injured area. Thus, recently, the focus has been on the biological mechanisms by which tendons heal, and the growth factors involved [7]. Growth factors are involved in the regulation of cell proliferation, migration, differentiation, and ECM production. Therefore, growth factors may be potential therapeutic agents for tendon healing. Growth and differentiation-5 (GDF-5) represents one such factor [8]. GDF-5, also terms as termed as cartilage derived morphogenetic protein-1 (CDMP-1) or bone morphogenetic protein-14 (BMP-14), is a member of the BMP family that belongs to the transforming growth factor-β (TGF-β) superfamily. GDF-5 is best characterized by its cartilage-inducing and bone-inducing potential [9]. Thus, a large number of studies have reported the in-depth mechanisms of GDF-5 in bone and cartilage repair [10,11]. For example, the application of GDF-5 can enhance cartilage repair in cartilage defects of rats by its ability to inhibit inflammation and maintain the balance of anabolism and catabolism of chondrocytes. Also, with respect to bone fracture healing, GDF-5 supplementation shows a significant curative effect by enhancing osteogenic differentiation and anagenesis [12–14]. However, accumulated evidence has confirmed that the GDF-5 ceased to be thought as a chondrogenic and osteogenic factor, as indicated by its name, and is considered a tenogenic factor. The first indication that GDF-5 might hold the potential to promote tendon and ligament repair came from the work of Wolfman et al. [15]. In this study, GDF-5 induces the formation of neotendon and ligament instead of cartilage and bone when it is implanted ectopically into rodents. This study therefore opened the door to consider the possibility that GDF-5 might someday applied therapeutically to enhance tendon and ligament repair in a manner that resembles the use of more traditional BMPs for fracture healing and bone fusion applications [16].

Along the same lines, GDF-5 deficiency contributes to a poor healing process, whereas GDF-5 supplementation exhibits a positive effect on the experimental model of tendon injury [17–19]. These studies further raise the possibility of administration of GDF-5 for the treatment of tendon injury. Despite promising studies in animals, to our knowledge, the underlying mechanisms of GDF-5 on tendon healing have not been explore extensively. Therefore, the purpose of this review is to provide a more precise summary related to the roles and mechanisms of GDF-5 in tendon healing. Other studies not directly involving the effect of GDF-5 in tendon healing will also be discussed when they help to elucidate possible underlying mechanisms for GDF-5 in tendon healing.

1.1. Search strategy

1) Search site: Articles are forming PubMed, a database of papers on biomedical science. 2) Database: MEDLINE. 3) Keywords: GDF-5, BMP-14, CDMP-1, tendon healing, tendon repair, function. 4) Boolean algorithm: ("GDF-5″ OR "BMP-14″ OR "CDMP-1″) OR ("Tendon healing" OR "Tendon repair"). 5) Retrieval timeframe: we searched the selected in journals published from 1983 to 2023). Inclusion and exclusion criteria: Articles were included if the topic is related to GDF-5 or BMP-14 or CDMP-1 or tendon healing, and the article type was a review or experimental paper. The search process was performed as presented in Fig. 1.

2. GDF-5 structure and signaling

GDF-5, also termed as cartilage-derived morphogenetic protein-1 (CDMP-1) or bone morphogenetic protein-14 (BMP-14), is a member of BMP/TGF-β family [20,21]. GDF-5 is synthesized as a large precursor molecule that comprises two main domains: the N-terminal prodomain with a cleavage site and signal sequence and the active C-terminal domain [22]. Subsequently, the precursor molecule is cleaved at a characterized RXXR (Arg, X, X, Arg) site to release the active peptide [23]. This active peptide is highly conserved with seven cysteine residues and contains two domains: the N-terminal region, which forms a tail-like structure within GDF-5 dimers, and the C-terminal region, which is involved in forming homodimers and heterodimers [24].

GDF-5 transduces signals originated from binding to two types of transmembrane serine/threonine kinase types I and II receptors [25]. Among the seven known type I receptors, BMP receptor BMPR-IA and BMPR-IB have been demonstrated to be associated with skeletal patterning [26]. The way in which a ligand–receptor interaction will translate into biological activity such as cell proliferation and migration. Upon GDF-5 binding to types I and II, the receptors confer signaling by activation of Smad 1/5/8 or mitogen-activated protein kinase (MAPK) [27]. In this regard, the phosphorylated Smad 1/5/8 then forms the complex with Smad 4, a common Smad that translocates into the nucleus, where they promote the expression of downstream genes (Fig. 2) [28]. Furthermore, the receptors through which GDF-5 propagates its signaling pathway also activate MAPK signaling pathways such as p38 and extracellular signal-related kinase 1/2. The pattern of receptor interactions is thought to determine whether the Smad pathway or the MAPK pathway is activated. When the ligand binds to type I receptor homodimers, it recruits type II receptors and activates the Smad pathway [29]. However, when the ligand binds to preformed complexes of type I and II receptors, the MAPK pathway gets preferentially activated [30].

3. Overview of GDF-5: implications for therapeutic potential

Over the past decades, GDF-5—reflected by its other names, cartilage-derived morphogenetic protein-1 and bone morphogenetic protein-14—is most widely known for its chondrogenic and osteogenic ability [31]. During embryonal development, GDF-5 is expressed in cartilaginous tissue and exerts a critical role in the formation of joints and long bones [32,33]. Considering that the healing process is in much the same way as the development steps,

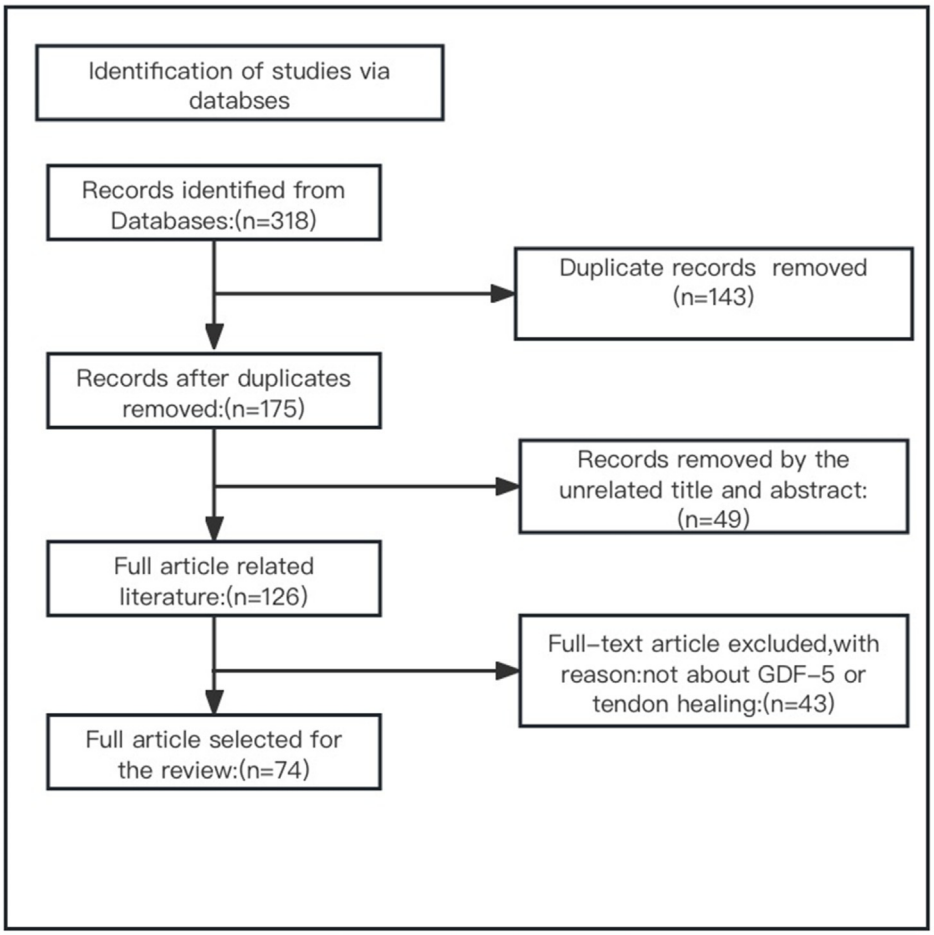

Fig. 1. Article retrieval flow chart with inclusion and exclusion process.

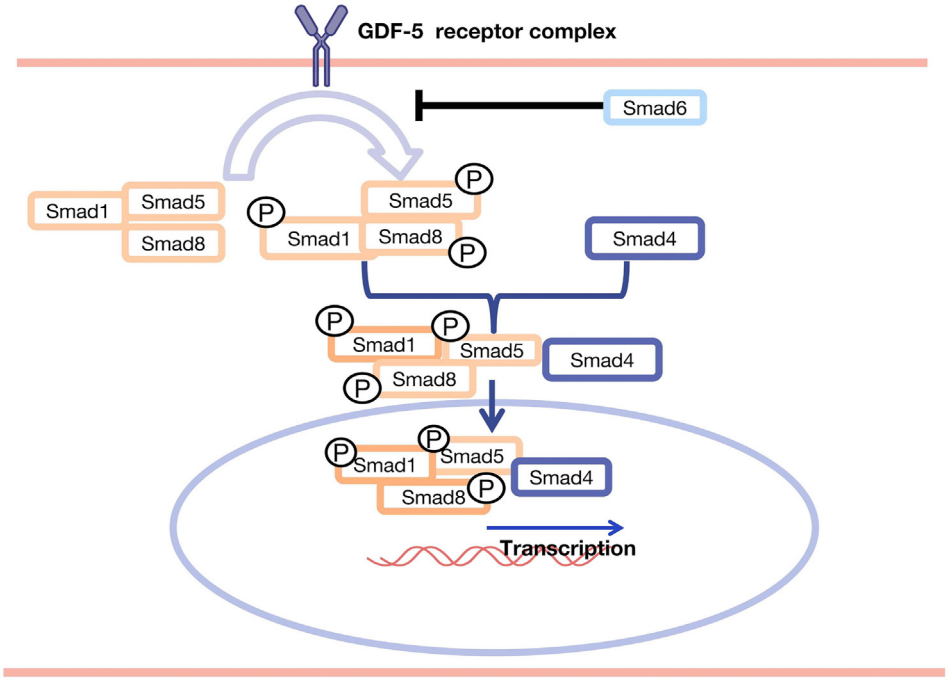

Fig. 2. GDF-5 canonical signaling pathway: After binding to receptor, the receptor complex activates downstream Smad proteins. Smad 1/5/8 are phosphorylated and form a complex with Smad4. Smad 6 functions as endogenous antagonist for Smad 1/5/8 to inhibit excessive signaling transduction.

extensive studies thus have focused on the therapeutic effects of GDF-5 on bone and cartilage healing, wherein the underlying mechanisms of GDF-5 are well established. For example, it is well known that GDF-5 promotes bone repair by stimulating chondrogenic and osteogenic differentiation, promoting angiogenesis, and enhancing bone remodeling after bone fracture [12–14]. Similarly, GDF-5 can also promote cartilage repair by its ability to inhibit inflammation and maintain cartilage homeostasis (as reviewed in Ref. [34]). However, in 1997, a study conducted by Wolfman et al. opened the door to consider the possibility that GDF-5 might be a therapeutic agent for promoting tendon healing, similar to the role of traditional BMPs in fracture healing and bone fusion applications. This study revealed that GDF-5 induces the formation of neotendon and ligament instead of the formation of cartilage and bone when GDF-5 was injected ectopically into rodents [15,35]. However, these findings are not without controversy, as others have found that GDF-5 is able to induce cartilage formation after implantation into muscles [16]. This discrepancy may be explained by several factors, such as differences in recombinant protein production processes and dosage of GDF-5 administration. Along this line, some investigations have revealed the critical role of GDF-5 in the maintenance of tendon tissues. In the first study, researchers analyzed the Achilles tendons of male GDF-5-deficient mice and compared them to the control samples [36]. The results showed that the GDF-5-deficient mice had weaker tendons due to a reduction in collagen. The second study examined tail tendons from GDF-5-mutant mice and found irregular collagen fibrils compared to normal mice. Furthermore, two groups had no differences in collagen and sulfated glycosaminoglycan contents. Accordingly, overlapping expression of other molecules might partially compensate for the loss of GDF-5 in the mutant mice. Despite this possibility, the perturbations caused by GDF-5 deficiency have enhanced the initiation of irreversible events that accelerate the deterioration of tendon structure [37].

Research is ongoing regarding the effect of GDF-5 on tendon healing. One study with GDF-5-mutant rat animal models supports the idea that GDF-5 plays a critical role in tendon healing. In this study, the Achilles tendons were transected and immediately sutured, and a control group was performed on the contralateral limb. Histological, biochemical assessment and ultrastructural measures were characterized. The results showed that the time taken to achieve peak cell density, glycosaminoglycan content, and collagen content was delayed in the GDF-5-deficient Achilles tendon. Interestingly, it was noted that angiogenesis of the injured site was also delayed by one week in mutant mice. This delay is equivalent to the known function of GDF-5 in promoting angiogenesis during bone healing by modulating the rate of vascular invasion and terminal hypertrophic chondrocyte removal [17,18]. However, the mechanisms of GDF-5-induced angiogenesis still remain elusive and thus warrant further investigations. Furthermore, the study revealed that the GDF-5-deficient animals had remarkably increased adipocytes within the injured site, indicating the possibility of abnormal cell differentiation. A mechanical assessment demonstrated that the mutant tendon was still significantly weaker than that of controls at the end of five weeks but had fully recovered by 12 weeks [38]. Still, another study regarding the role of GDF-5 in tendon healing was performed by Nakase et al. In this study, the tendon containing the full-thickness tear margin of the rotator cuff was obtained from patients. The group observed that the expression of CDMP-1 was increased at the site of the cuff tear. Because this study was observational in nature, it did not elucidate whether CDMP-1 promoted torn tendon healing. It does associate well with the above animal investigations hinting that CDMP-1 may play a role in tendon healing [39]. Overall, these findings support the role of GDF-5 in the maintenance and healing of the tendons, which provides a rationale for the application of GDF-5 to promote tendon healing.

4. Tendon scar healing

Injured tendons commonly heal through a scar-mediated manner, which disrupts repair and leads to functional deficits both during tendon healing processes and after completion of the healing processes [40,41]. After tendon trauma, the tendon will undergo three overlapping phases. In the inflammatory phase (a few days), the injury site is infiltrated by erythrocytes and inflammatory cells, particularly macrophages and platelets equipped with growth factors and chemoattractants, which leads to hematoma formation [42]. After several weeks, the proliferative phase commences and lasts for a few weeks. This healing phase is characterized by the prevalence of type III collagen directed by tenocytes. In the remodeling phase, the synthesis of type I collagen dominates, and the ECM leads to better tissue organization. Moreover, the synthetic activity of cells is progressively decreased. The healing tissue appears scar-like, and complete tendon regeneration is never achieved [43]. The resulting scarring tissue lacks the tissue integrity of the innate tissue, and thus, reinjury often occurs [44].

5. GDF-5 in tendon healing: experimental studies

Despite the paucity of reports concerning the role of GDF-5 on tendon healing, some researchers have already evaluated the effect of GDF-5 in tendon healing during GDF-5 administration. Generally, the ability of GDF-5 to promote functional recovery after tendon injury has been reported through direct injection or gene therapy with adeno-associated virus-mediated GDF-5 production. The following section reviews of the literature related to the impact of GDF-5 on tendon healing (Table 1). However, it is worth noting that the dosage and timing of GDF-5 application significantly affect its effectiveness and require careful consideration.

Aspenberg et al. conducted a study to investigate the impact of GDF-5 on tendon healing in the absence of mechanical stimulation. To achieve this, they transected the Achilles tendons of rats at a distance of 5 mm from their insertion and also cut the tibial nerve, leading to unloading and immobilization of the tendons. Next, they injected collagen sponges, either with GDF-5 (1 μg and 10 μg) or without, measuring 1 × 2.5 × 2.5 mm in size. The rats were then subjected to biomechanical and histological examination after two weeks. The results indicated a trend towards better tensile strength in all GDF-5 treated groups, with the 10 μg dosage of GDF-5 having a more significant effect on the tendons' strength. Furthermore, the study found increased cell density and collagen accumulation in the GDF-5 group compared to the group that only received collagen sponges. Notably, none of the GDF-5 treated specimens showed any cartilage/bone growth on the tendons [45]. Also, Rickert et al. conducted a study on the rat Achilles tendon where it was cut transversely and then repaired by suture. The specimens were divided into two groups: a control group that only received sutures and another group that received sutures with GDF-5 (10 μg). The subjects were evaluated through biomechanical and histological examination at 1, 2, 4, and 8 weeks after the tendon operation. The GDF-5-treated group showed remarkably thicker tendon tissue at 1, 2, 4, and 8 weeks, and a significantly increased tensile strength was found at two weeks. This effect may be due to increased cell proliferation and collagen accumulation. However, the histology revealed that the GDF-5 treatment group had an increased accumulation of cartilage-like cells around the injured tendon compared to the control group that did not receive GDF-5. The authors suggested that the chondroinductive ability of GDF-5 could

Table 1
The effect of GDF-5 in tendon healing: experimental studies.

Study	Animal models establish	Dosage	Time post operation	Outcome
[40]	Achilles tendon transected, denervated the calf muscle, and repair	GDF-5: 0, 1, 10 μg	2 weeks	Tensile strength↑
[41]	Achilles tendon transected and repair	GDF-5: 20 μg was distributed onto 20 cm suture (1 μg/cm)	1, 2, 4, weeks	Cell density↑ Tensile strength↑
[42]	Achilles tendon transected and repair	GDF-5: 3×10^{10} adenovirus particles	1, 2, 4, 8, 12 weeks	Max failure load ↑ Cartilage formation↑
[44]	Achilles tendon transected and repair	GDF-5: 0, 0.4, 2 and 10 μg	8 days	Maximum stress↑ Cartilage formation in 10 μg groups↑
[45]	Rotator cuff tendon transected and repair	Recombinant human GDF-5 with suture: 0,24 ng/cm, 55 ng/cm, and 556 ng/cm	3, 6 weeks	Collagen orientation↑ Mechanical strength↑
[43]	Achilles tendon lacerated and repair	GDF-5: recombinant adenoviruses	1, 2, 3 weeks	Cartilage or bone → Tensile strength↑
[19]	Flexor tendon lacerated and repair	GDF-5: 200 μg/ml	3, 6 weeks	Collagen organized ↑ Mechanical strength↑ Cartilage or bone →

be overcome by regulating dosage modification or suture material [46].

Gene therapy with adeno-associated virus-mediated GDF-5 production also has been reported.

Rickert et al. conducted a study to test the feasibility of this delivery strategy for promoting tendon healing. The researchers injected adenovirus particles (3×10^{10}) carrying the GDF-5 gene into the injured Achilles tendon of rats in vivo. In vitro, the results showed that GDF-5 expression peaked at two weeks. In vivo, GDF-5 expression reached its maximum at four weeks, and the GDF-5-treated groups displayed stiffer tendons at eight weeks compared to the group without GDF-5 treatment [47]. Bolt et al. conducted a preclinical animal study where they used adenovirus to infect rats that were continuously expressing GDF-5 after the Achilles tendons were transected and repaired. The control group did not receive any infection after the surgery. The group that received GDF-5 exhibited exuberant healing, more tenocytes, and 70% higher tensile strength with less gapping around the repaired area compared to the control group after two weeks. An important finding was that there was no bone or cartilage formation within tendons at all time points (1 week, 2 weeks, 3 weeks) in the GDF-5 group [48].

In a study by Forslund et al. GDF-5 was also shown to promote tendon healing. After the Achilles tendon of the rats was transected and repaired, acetate buffer containing GDF-5 at different dosages (0, 0.4, 2, and 10 μg) was injected into the injured tendon, and one control group received no treatment. After eight days, the group receiving GDF-5 treatment showed a remarkable dose-dependent increase in stiffness and strength in comparison to the control group. In addition, it was observed that treatment with a low dose of GDF-5 (0.4, 2 μg) did not result in the formation of bone or cartilage in the tendon. On the other hand, specimens treated with a higher dose of GDF-5 (10 μg) showed some formation of cartilage and bone in the tendon. This suggests that lower dosages of GDF-5 may have a promising effect on the healing of tendons [49]. A study was conducted on 48 rats for rotator cuff repair. They were randomly divided into four groups, each comprising 12 rats. The first group was the control group, which received 0 rhGDF-5. The second, third, and fourth groups received 24 ng/cm, 55 ng/cm, and 556 ng/cm of rhGDF-5, respectively. The rhGDF-5 was delivered using sutures coated with a new dip-coat technique. Three weeks after the treatment, the group that received rhGDF-5 showed a lower histological grade, indicating better tendon healing. At six weeks, there was no significant difference in the results between the rhGDF-5 treated group and the other groups. The biomechanical results showed that after three weeks, the rhGDF-5 treated group had a higher stiffness and ultimate tensile load compared to the control group. The 55 ng/cm dosage of rhGDF-5 showed better improvements. However, after 6 weeks, there was no significant difference in the biomechanical strength of the injured tendons. Additionally, all the treatment groups had increased neovascularization compared to the control group [50]. Results from Frank Henn III et al. confirmed the promising effect of GDF-5 on tendon healing. In the study, the flexor tendon of rabbits was cut and repaired. One group of rabbits was treated with a suture coated with GDF-5 (200 μg/mL), while the other group received a suture without GDF-5 (control group). The rabbits in the GDF-5 treatment group showed robust healing, characterized by thicker and hypercellular tendon tissue. The Soslowsky score system was better at 3 weeks and 6 weeks, indicating improved collagen organization compared to the control group. Additionally, the tissue in the GDF-5 treatment group was significantly stiffer at 3 weeks [19].

Above all, these investigations substantiate the potential therapeutic benefits of GDF-5 for improving tendon healing. Although heterotopic bone and cartilage formation are the main problems when delivery of GDF-5 to promote tendon healing, most studies did not observe the formation of bone or cartilage, and some studies indicated that a higher dosage of GDF-5 will result in heterotopic bone and cartilage formation. Hence, further therapeutic studies using GDF-5 to improve tendon healing should demonstrate the dosage that complex the application of GDF-5 as a treatment strategy.

6. Possible mechanisms of GDF-5 for enhancing tendon regenerative healing

Although the above studies provide evidence about the therapeutic potentials of GDF-5 in promoting tendon healing, the underlying mechanisms of GDF-5 in tendon healing remain elusive. A few studies examined the underlying mechanisms in command upon GDF-5 administration (Fig. 3).

The regenerative healing of tendons is the results of successive processes including cell migration, proliferation, that result in reparative tendon formation. However, cell proliferation and migration rate are slow due to the inadequate regenerative capacity of the tendon, which progressively leads to incomplete functional healing of tendon [58]. Results from Keller et al. confirmed the positive effect of GDF-5 on cell proliferation and ECM production. Achilles tendon fibroblasts cultured with GDF-5 (0, 1,10, 100, 1000 ng/ml) for 12 days exhibited higher cell proliferation in a concentration- and time-dependent fashion (3, 6, 9, 12 days) [51].

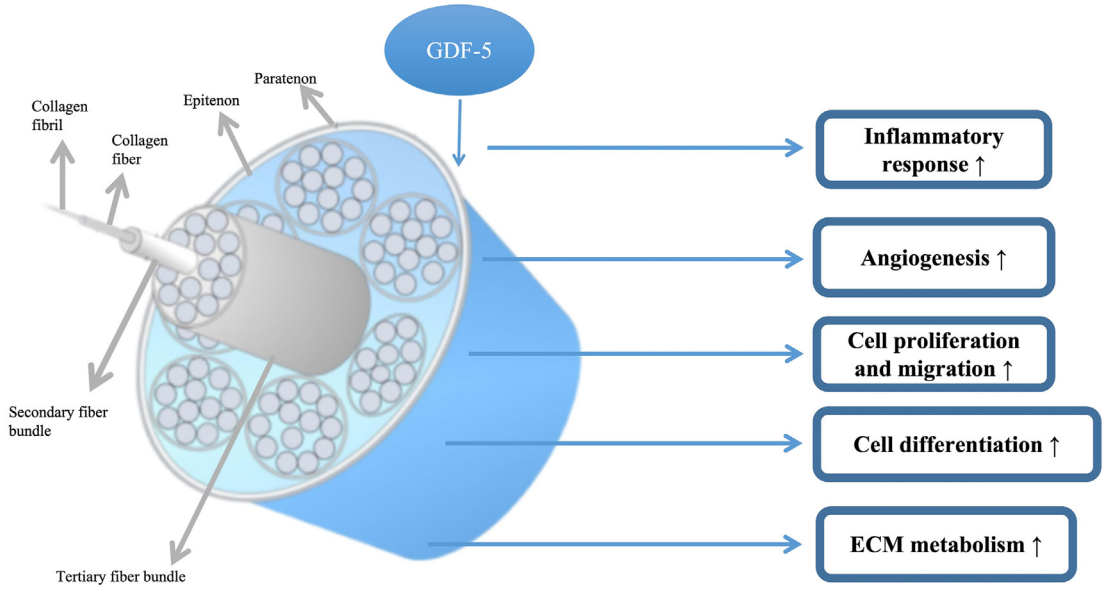

Fig. 3. The possible mechanisms of GDF-5 in tendon healing. ↑, promoted; ↓, suppressed.

On the other hand, it is universally accepted that directional decisions of cell migration are significantly essential for healing process since cellular distribution in the pores of ECM is a key contributory factor to the development of tissue. In rat tendon fibroblasts cultured with GDF-5 have been demonstrated in the following groups at 1, 4, 5, 7 days: (1) 0 μg/ml rhGDF-5; (2) 40 μg/ml rhGDF-5; (3) 200 μg/ml rhGDF-5; (4) 1000 μg/ml rhGDF-5. Cell migration data revealed GDF-5 stimulated cell migration in a dose-dependent chemotactic fashion. Moreover, an important finding is that GDF-5-treatment group also displayed increased collagen synthesis and cell proliferation, but this effect was only observed in above 200 μg/ml rhGDF-5 [52]. Accordingly, we can conjecture that GDF-5 may promote cell migration and proliferation, thereby contribute to a more well-distributed cells in the injured site, lay in the groundwork for the remodeling process, and finally facilitates tendon healing [53].

Additionally, it is widely believed that the hypocellular nature of tendons limits the regenerative ability of tendons, and stem cells can be alternative cells to replenish functional tendon cells at the injured site [54]. Particularly, bone marrow mesenchymal stem cells (BMSCs) are adult stem cells derived from bone marrow with self-replication and differentiation potential cells that can differentiate into various types of cell phenotypes, including tenocytes and fibroblasts, which have been widely applied to empower resident tendon cell populations, with the goal of enhancing tendon regenerative healing [55]. The potential of GDF-5 supplementation to induce BMSCs towards a tenogenic differentiation was confirmed by Wang et al. Experiment with rat BMSCs, treated with different dosage of GDF-5 (0, 10, 20, 50 ng/ml), confirmed that GDF-5 promoted tenogenic differentiation of BMSCs as the expression of SCX, COL I and COL III were higher than that in the control group. In this regard, the COL III expression was not dose-dependent and highest values were reached with 50 ng/ml at 2 days and at 5 days, while the expression of SCX displayed a positive dose-dependent and time-dependent curve in response to GDF-5 [56]. Scx is a specific marker for tendon and ligament, which is responsible for cell proliferation and differentiation and regulating tenomodulin (TNMD) expression [57]. TNMD is another tenocyte marker, and TNMD plays a pivotal role on the cell migration, proliferation, differentiation and inhibits scar tissue formation during tendon healing by encouraging collagen production and tissue remodeling [58]. Treatment of dog BMSCs with 100 ng/ml GDF-5 upregulated the expression of TNMD, COL I and COL III, thereby promoted BMSCs proliferation and tenogenic differentiation [59]. Besides, GDF-5 is more effective for tendon healing if incorporated with BMSCs, more effective therapies for tendon healing. For example, in a study conducted on flexor tendon injured model of dogs, the tendons were randomly divided into four groups: (1) tendon without collagen gel; (2) tendon with BMSC-seeded collagen gel; (3) tendon with GDF-5 collagen gel; (4) tendon with GDF-5 and BMSC collagen gel. Results revealed the highest values of ultimate strength and stiffness reached in GDF-5 and BMSC collagen gel group at 4 weeks compared to other groups, which suggests a more effective healing when BMSCs combine with GDF-5 [59].

Physiological tendon ECM mainly contains water, collagen, proteoglycans. Type I collagen accounts for 95% of ECM, possessing the better mechanical properties, while type III collagen is less abundant, possessing the ability to rapidly form cross-links [60]. Because of this ability of type III collagen, it can rapidly increase during early tendon healing after tendon injury, enabling the stability of injured tendon [61]. Moreover, proteoglycans, such as aggrecan, fibromodulin, decorin and tenascin, serve as key elements of ECM. They are involved in the regulation of collagen production and work with growth factors to control cell proliferation after tendon injury [62]. During proliferative phase, the synthesis of ECM provides structural support for injury site. Specifically speaking, the synthesis of type III collagen and some proteoglycans reaches a peak to rapidly maintain the stability of tendon. Then, type I collagen with the better mechanical properties is produced during remodeling phase, to increase the biomechanical strength of the tendon [63]. Thus, a tendon's extracellular environment is pivotal for its functional recovery. Experiments with fibroblasts extracted from rats Achilles tendon was treated for 4 days with different dosages of GDF-5 (0, 1, 10, 100, 1000 ng/ml). Results shown both type I collagen and type III collagen expression were not dose-dependent and highest values were reached with 100 ng/ml but there was no difference between 1000 ng/ml GDF-5 and control group on the expression of type I collagen [51]. These results may suggest that the 100 ng/ml dosage of GDF-5 has a promising effect

on collagen production, although whether this dosage of GDF-5 would produce a better effect in humans is not clear. In another study on Achilles tendon of mice, 45 mice Achilles tendons were tenotomy and suture repaired, and 10 μg rhGDF-5 or saline was injected into injured site. The mice were assessed histologically. The results showed the expression of ECM genes including procollagen IX, aggrecan, fibromodulin increased in the rhGDF-5 group compared to the saline group. More importantly, GDF-5 may have anti-inflammatory effect on tendon healing, as manifest by decreased the expression of pro-inflammatory genes in this study [64]. Particularly, the proteoglycan aggrecan is mainly present in fibrocartilaginous area of tendon, and it is an important factor for fibroblast proliferation and maintenance the structure of collagen during tendon healing [65]. In this regard, the signaling cascade that causes the upregulation of aggrecan gene maybe closely associated with Smad-mediated transcription activation. Observation of the detailed mechanisms in chondrocyte revealed that GDF-5 activated the Smad pathway, Smad then moved to nucleus to regulate the transcription of ACAN encoding the aggrecan, but this signaling pathway is not completely understood in tendon cells [66]. However, this increased level of aggrecan may be the reason why application of GDF-5 for tendon healing may promote chondroinduction as aggrecan is an important maker of chondrogenic differentiation [67]. In addition to aggrecan, GDF-5 can promote a significant synthesis of proteoglycan tenascin-C in tendon fibroblasts [64]. Tenascin-C is another proteoglycan that involves in maintaining the fibrocartilaginous regions within tendons and has a positive effect on anti-adhesion through reducing cell–matrix adhesion [68]. Given this important anti-adhesive effect of Tenascin-C, it is reasonable to guess that application GDF-5 can decrease the adhesion formation during tendon healing, part of which was mediated by this protein [51,69].

Other ECM components were the ECM remodeling protein, MMPs and TIMPs. During tendon healing, the activity of MMPs and TIMPs plays a vital role in the regulation of ECM turnover and thus determines the composition and aid in regulating tendon function [70]. MMPs are a group of proteinases responsible for the degradation of various ECM components and encouraging tissue remodeling, while TIMPs are a group of proteins involved in inhibiting MMP activity [70]. The MMPs and TIMPs involved in tendon healing are especially MMP-3, MMP-13, and TIMP-2 [71]. More precisely, MMP-3 can trigger the degradation of a wide range of target peptides, while MMP13 is responsible for collagen degradation [72]. TIMP-2 is an inhibitor of several MMPs. The blockage of various MMPs for the treatment of chronic tendinopathy has been demonstrated in the literature with different but positive outcomes [73]. In a rat tendon fibroblast cultured with GDF-5, the expression of MMP-3, MMP-13, and TIMP-2 in tendon fibroblasts is increased at six days, followed by a decreased expression of MMP-13 and TIMP-2 at 12 days [51], these expression changes are consistent with healing tendons. Therefore, these findings may suggest that GDF-5 plays a causative role in the tendon tissue turnover associated with tendon healing. Above all, GDF-5 may promote tendon healing by inhibiting inflammatory response, enhancing angiogenesis, regulating ECM degradation and production, and speeding up cell proliferation, migration, and differentiation. However, much remains to be illuminated concerning the underlying mechanisms of GDF-5.

7. Conclusions and discussion

Thus, while GDF-5 therapy for improving tendon healing lags behind those of bone, cartilage, and joint, it still shows great promise to promote tendon regenerative healing and acquire an optimal result. Increasingly body of studies has revealed how GDF-5 modulates tendon cell behavior by keeping tendon cells in the right way or steering the commitment of stem cells towards differentiated cells to replenish functional tendon cells at the injured site. In this sense, it is important to highlight that the underlying mechanisms of GDF-5 are far from clear; future studies are required to explore, if any, what role and mechanism is played by GDF-5 on tendon healing.

However, with the aim to sufficiently exploit the potential of the GDF-5 delivery for enhancing tendon regenerative healing, we still need to solve these problems: application dosage, delivery strategies, and the timing of GDF-5 application. With respect to application dosage, the difference in GDF-5 delivery dosage is a critical factor limiting the improvement in tendon regenerative healing. For example, in vivo, GDF-5 (0, 24 ng/cm, 55 ng/cm, 556 ng/cm) was delivered by suture into rotator cuff-injured rats. Results revealed that at three weeks, the 24 ng/cm and 556 ng/cm dosage of rhGDF-5 had a more pronounced effect on tendon healing based on a better improvement in collagen organization compared to the other group, while at six weeks, the results of all the rhGDF-5-treatment group are similar compared to others, which indicates the application of GDF-5 at early tendon healing is more promising [50].

On the other hand, the critical issue of application of GDF-5 is its ability to induce bone or cartilage formed in the tendon. In the study by Forslund et al. a low dosage of GDF-5 (0.4, 2 μg) was chosen to avoid the risk of bone or cartilage forming in the tendon, while a higher dosage of GDF-5 (10 μg) resulted in heterotopic bone or cartilage growth [49]. A similar dosage was also observed heterotopic bone or cartilage growth when GDF-5 (10 μg) was administrated into a rat model of Achilles tendon. Although 10 μg of GDF-5 promoted tendon healing, this dosage also resulted in heterotopic bone or cartilage growth after 2 weeks [46]. These results suggest the more dosage of GDF-5 is not always better thus in the future, we should further elucidate the ideal dosage of GDF-5 in the treatment of tendon injury, to fully realize the potential of GDF-5.

Another impediment that should be overcome before translation into clinical practice is delivery strategy. Since the administration of GDF-5 is subjected to rapid clearance, the success of GDF-5-based therapy mainly depends on controlled and consistent delivery devices [7]. As indicated earlier, suture, collagen sponges, and local injections have been used as carriers for delivering GDF-5, and results show these delivery strategies can effectively control and consistent delivery, with the results of improving tendon healing. In fact, learned from other BMPs, BMP-2 and BMP-7 formulations have been approved by the FDA in the United States for clinical settings to improve bone fracture healing, with both using type I collagen as a delivery platform, which may provide cues for paving the application GDF-5 into clinical practice [74]. We hope more well-designed animal investigations about the effect of GDF-5 in tendon healing will allow its full therapeutic potential to be examined in this area.

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Author contributions

HL, YL, LX and SL designed the present manuscript. HL drawn the manuscript. YZ and performed a literature search and selected the studies to be performed. HL revised including the manuscript. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

There are no conflicts of interest in this study.

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