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What We Should Know Before Using Tissue Engineering Techniques to Repair Injured Tendons: A Developmental Biology Perspective

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Tendons connect muscles to bones, and serve as the transmitters of force that allow all the movements of the body. Tenocytes are the basic cellular units of tendons, and produce the collagens that form the hierarchical fiber system of the tendon. Tendon injuries are common, and difficult to repair, particularly in the case of the insertion of tendon into bone. Successful attempts at cell-based repair therapies will require an understanding of the normal development of tendon tissues, including their differentiated regions such as the fibrous mid-section and fibrocartilaginous insertion site. Many genes are known to be involved in the formation of tendon. However, their functional roles in tendon development have not been fully characterized. Tissue engineers have attempted to generate functional tendon tissue *in vitro*. However, a lack of knowledge of normal tendon development has hampered these efforts. Here we review studies focusing on the developmental mechanisms of tendon development, and discuss the potential applications of a molecular understanding of tendon development to the treatment of tendon injuries.

Introduction

The term "tendon" comes from the Latin word *tendere*, meaning to stretch. This is actually counter-intuitive, because although tendon stretching is an important component of proprioception, it is the ability of the tendon to resist tension that is its primary function in transmitting the force of muscle contraction to the skeleton, and thus to generate movement. Tendons generally have a cross-sectional area considerably less than the in-series muscle, and since the force of muscle contraction is transferred to the skeleton directly through the tendon, immense stresses upward of 100 megapascals (MPa),[1] can be placed across tendons during exercise. Tendons are thus highly prone to injury, and unfortunately, their hypocellularity and hypovascularity, compared to other soft tissues, make their natural healing extremely slow and inefficient. Surgical repair of tendons is therefore common.[2] It is estimated that 30 billion dollars are spent on musculoskeletal injuries in the United States each year, and tendon/ligament injuries represent ~45% of these injuries.[3] In addition, surgical repair is often unsuccessful. Approximately 50% of the population by the age of 60 will have suffered a degenerative rotator cuff tear.[4,5] Although small rotator cuff tears have better outcomes, surgical repairs of large tears show failure rates as high as 90% due to muscle contraction, decreased range of joint motion, neurovascular damage, or altered shoulder mechanics.[6,7] Consequently, tendon repairs often require tissue grafts. Allografts are used but can lead to immune rejection.[8,9] Autografts avoid this problem, but can result in considerable donor site morbidity.[10] In addition, it has proved so far impossible to successfully re-create a functional insertion site of the tendon into bone.[11,12] To address these problems, attention has focused recently on the use of tissue engineering to generate replacement tendons. In theory, isolation of stem cell populations from a patient, and their conversion in culture into functional tendon tissue, would obviate both immune rejection and donor site morbidity associated with tendon grafting.

To generate functional and self-renewing tendon tissue, it is necessary to understand the normal processes of tendon development. In particular, we need to understand which stem cell populations in the body are able to form tendon, how they can be directed to do so in culture without simultaneously forming other skeletal tissues, how their growth is controlled, and how normal cell turnover can be re-established and maintained in the tissue-engineered tendon. We also need to understand how cells specified to form tendon become differentiated into either midsubstance or

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insertion site cells, with different histological appearance, molecular components, and mechanical properties of their synthesized proteins. Additionally, we need to understand how the boundaries between these different functional regions of the tendon are established and maintained. Ideally, we would like to develop tissue engineering protocols that use the patient's own stem cells and deliver a series of spatial and temporal signals that mimic the normal developmental pathway of tendon development. In this review, we discuss the current state of knowledge of the normal spatial and temporal developmental processes of tendons, and indicate the areas where research needs to focus.

Structure of Tendon

Tendons have a hierarchical design. Their basic unit is the tenocyte, a fibroblast-like cell that produces collagens, the key elements of tendon structure. The collagen protein forms a triple-helical, rod-shaped molecule that spontaneously associates with other collagen molecules to form a quarter-staggered fibrillar array that establishes the characteristic tendon matrix.[13] Bundles of fibrils form larger primary fiber bundles called fascicles, groups of which associate to form tertiary fiber bundles. These are surrounded by a connective tissue endotenon that contains blood vessels, lymphatics, and nerves.[14] The multiple fiber bundles and endotenon are encompassed by the epitenon, a layer of connective tissue around the outside of the tendon that is continuous with the endotenon that separates individual fiber bundles. On the outside of these tissues is a double-layered sheath of areolar tissue, the paratenon, attached loosely to the outside of the epitenon. The paratenon and epitenon together are sometimes called the peritendon (peritenon or peritendineum) (Fig. 1).[14]

Tendons are composed of multiple molecular constituents. Type I collagen is the major collagen type. There are also minor collagen components, proteoglycans and glycoproteins, including type III collagen, tenascin, cartilage oligomeric matrix protein, decorin, fibronectin, and biglycan.[15–19] The molecular components of tendons and their architectural arrangements have been studied extensively.[20–26] Despite the apparent simplicity of its structure and its comparatively few cell types, mechanistic studies on tendon growth, differentiation, and maintenance are rare, relative to other tissues. One reason for this is that, in the past, there have been few cell type-specific molecular markers of tendon differentiation, which has made it difficult to study developmental mechanism. The discovery of scleraxis (SCX), a tenocyte marker expressed in all tendon progenitor cells, has provided a new opportunity to study tendon development.[27,28] We will discuss this important marker in the next section.

Tendon Formation During Embryonic Development

The vertebrate axial musculoskeletal system originates from somites: dorsally located segmental blocks of mesoderm in the embryo that lie adjacent to the neural tube and notochord. In response to signals from the surrounding tissues, somites differentiate into distinct compartments, which eventually become dermis, muscles, cartilages, and tendons. The development of the dermis, musculature, and skeleton from their somitic compartments (dermatome, myotome, and sclerotome, respectively [Fig. 2A]) is reasonably well

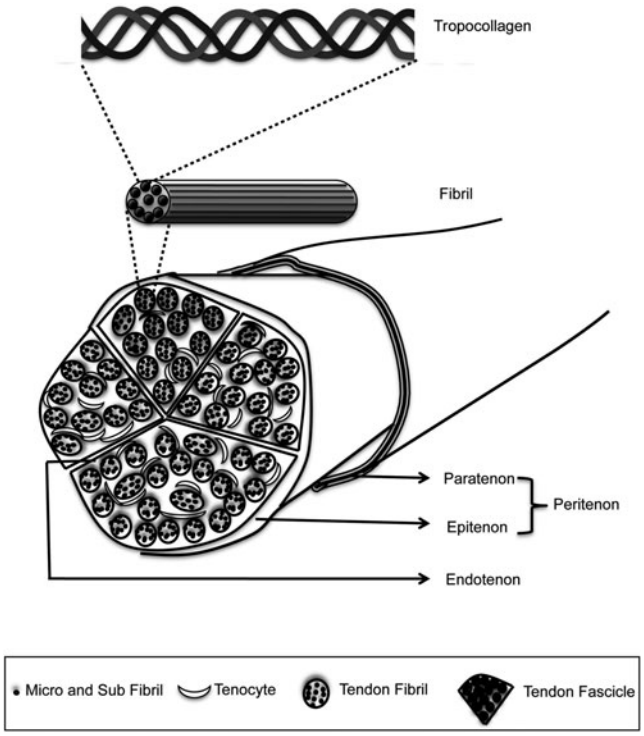

FIG. 1. The hierarchical architecture of tendon. Collagen proteins form a triple helix structure and self-assemble with other collagen molecules to establish the characteristic tendon matrix. Bundles of fibrils form tendon fascicles, the larger primary fiber bundles. Groups of fascicles are bound together by a thin layer of connective tissue named endotenon. Several fiber bundles and endotenon are encompassed by the epitenon. The epitenon is covered by another layer of connective tissue named paratenon. The paratenon and eiptenon are called peritenon.

understood. Until recently, there was no comparable understanding of the origins of the axial tendons that connect axial muscles and skeleton. However, the discovery of the basic-helix-loop-helix transcription factor, SCX, both identified a molecular marker for tendon progenitor cells and allowed more mechanistic studies of axial tendon formation. SCX mRNA is expressed both in fully formed tenocytes and in the progenitor cells of tendons in the embryo.[28] Brent *et al.* demonstrated that SCX-expressing progenitor cells of trunk tendons first appeared between the myotome and sclerotome during somite development. The quail-chick chimera system, in which individual compartments in the chick somite were replaced by the equivalent components of quail somites, was used to identify the origins of these cells.[29] When sclerotome was transplanted from quail to chick, quail cells generated both sclerotome and SCX-expressing cells, but not myotome or dermomyotome. However, when dermomyotome was grafted from quail to chick, quail cells developed into dermomyotome and myotome, but not into SCX-expressing cells. Surgical removal of dermomyotomes before the formation of myotome in chick prevented the expression of *SCX*.[29] The conclusions from this study were that tendon progenitor cells arise in the forming sclerotome, but require signals from the dermomyotome. Expression of molecular markers for cartilage (*Pax1*), or tendon (*Scx*), revealed that

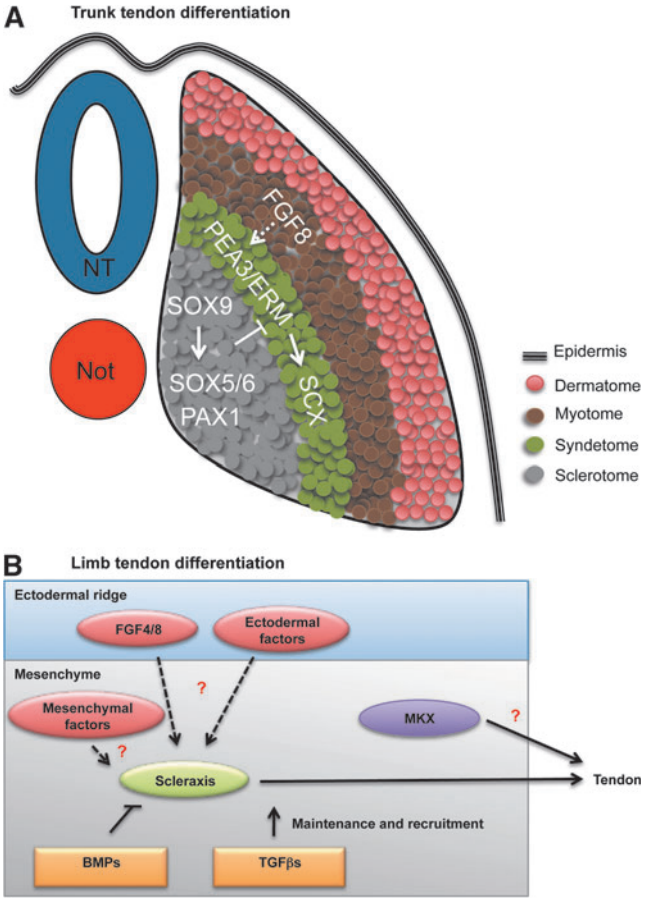

FIG. 2. The current model of tendon differentiation in trunk **(A)** and limb **(B)**. **(A)** FGF8 from the myotome activates expression of SCX via PEA3 and ERM to induce the subjacent sclerotome cells to become tendons. The sclerotomal factors PAX1 and SOX5/6 inhibit the expression of SCX and allow the sclerotome to develop into cartilage. **(B)** The initiation of SCX expression in the limb is still unknown. TGFβ signaling promotes maintenance and recruitment of tendon progenitors and is essential for tendon formation, whereas the homeobox transcription factor Mohawk (MKX) is required for the maturation of tendon. BMP signals have negative influence on tendon development. The roles of other mesenchymal factors on tendon differentiation remain to be characterized. Not, notochord; NT, neural tube; FGF, fibroblast growth factor; SCX, scleraxis; TGFβ, transforming growth factor β; BMP, bone morphogenetic protein.

Scx expression is confined to the dorsolateral regions of the sclerotome, whereas the expression of *Pax1* is more ventromedial. These findings indicated that progenitor cells of tendons arise on the side of the sclerotome adjacent to the myotome. In addition, double *in situ* hybridization with a myogenic marker, *MyoD*, and a tendon marker, *Scx*, revealed that SCX-expressing cells are a distinct population of cells next to the mytome. Thus, *Scx* expression has been used to define a fourth region of somite, the syndetome, which gives rise to tendons connecting the axial muscles to the axial skeleton.[30]

Although the progenitors of muscle, cartilage, and tendon arise in different compartments of the somite, their differentiation is coordinated and interdependent (Fig. 2A). For example, the amount of sclerotome tissue specified to form tendon is controlled by an interaction between somitic muscle and cartilage cell lineages. Removal of the dermomyotome before myotome formation in chicken embryos resulted in the loss of *Scx* expression.[28,29] In addition, in *MyoD* and *Myf5* double-mutant mice, which form no muscle, the expression of *Scx* in the somite was abolished.[30] The muscle precursor region of the somite is therefore essential for the initiation of tendon differentiation. The cartilage precursor cells of the sclerotome seem to play the opposite role in controlling the specification of tendon progenitors. The expression of *Scx* required the downregulation of *Pax1* in the sclerotome (Fig. 2A). Overexpression of *Pax1* in the sclerotome in chicken embryos caused inhibition of *SCX* expression.[29] In *Sox5/Sox6* double-mutant embryos, which develop no cartilage, the expression of *Scx* was slightly upregulated in the dorsolateral sclerotome. Moreover, tendon differentiation markers, such as tenomodulin, were found to be ectopically expressed in the chondroprogenitors of the $Sox5^{-/-}$ /$Sox6^{-/-}$ embryos.[30] It seems that SOX5 and SOX6 inhibit the expression of *Scx* in the sclerotome and thus prevent the chondroprogenitors from adopting a tendon fate activated by signals from the myotome (Fig. 2A). The mechanism by which this repression is mediated is currently not known.

Signals from the myotome must therefore be critical for activation of tendon differentiation in the syndetome. Several fibroblast growth factors (FGFs) are expressed in the myotome, including FGF8 and FGF4.[31] The application of FGF8-soaked beads, or the overexpression of FGF8 by viral infection, caused strong upregulation of *SCX* in the somites.[29,32] Moreover, the inhibition of FGF signaling caused loss of expression of *SCX*. These results indicate that FGF signaling is both necessary and sufficient to induce the expression of *SCX* in the somite.[30] How does the FGF signaling pathway regulate the expression of *SCX* in somite? It has been shown that members of FGF signaling pathway, such as *Fgf8*, *Pea3*, *Erm*, mitogen-activated protein kinase phosphatase 3, and Sprouty, are co-expressed with *Scx* in the somite.[32] Further analysis identified that two Ets transcription factors, *Pea3* and *Erm*, may act downstream of FGF signaling to regulate the transcription of *Scx* directly or indirectly.[32] Evidence also suggests that FGF stimulates the expression of *Scx* via the mitogen-activated protein/extracellular-related kinase signaling pathway.[33] In conclusion, the differentiation of tendon in the somite depends upon a combination of both activating and repressing signals from the other compartments of the somite.

The differentiation of axial tendons occurs adjacent to and in concert with the cartilages and muscles they will connect. However, the differentiation of limb tendons seems to be different. Striated muscles in the limbs arise from muscle progenitor cells that migrate into the limb buds from the somites. However, the cartilages and tendons of the limb arise *in situ*, and unlike the axial tendons, initiation of tendon differentiation in the limb does not seem to require the presence of muscle.[34] Surgical removal of developing muscle in chick did not block the expression of *SCX* in the muscleless wings[28,35]; in the mouse, the SCX-expressing tendon progenitor population appeared in the mesenchyme of the *MyoD/Myf5* double-mutant limbs, which have no muscle.[30] Although section *in situ* hybridization showed a partial overlap between the expression domain of *Scx* and *Pax3*, a

myoblast marker, the expression of *Scx* in the limbs was similar in both wild-type and *Pax3* mutants lacking limb muscle. These studies suggest that the initiation of *Scx* expression in tendon progenitor cells in the limbs does not require signals from myogenic cells. However, in the continued absence of myogenic cells, the expression of *Scx* was not maintained and the morphogenesis of the tendon did not occur normally.[30,34] These results indicate that signals from myogenic cells are not required for the initial establishment of tendon progenitors but are required for their continued differentiation.

If myogenic cells are not responsible for the appearance of SCX-expressing tendon progenitors in the limb, what is its mechanism? Based on the observation that *Scx* is expressed in the subectodermal location of the limb, Schweitzer *et al.* proposed that the ectoderm might play a role (Fig. 2B). Removal of the dorsal limb ectoderm caused the loss of *SCX* expression in the limb mesenchyme, indicating that its expression was induced by ectodermal signals.[28] In keeping with the findings in the trunk tendons, where FGF signals regulate the expression of *SCX*, FGFs would be obvious candidates for the ectoderm-derived signals in the limbs. However, to date, the expression pattern of FGFs in the limb has not been well characterized. *FGF8* is expressed in the apical ectodermal ridge at the time of the initiation of *Scx* expression.[28,36] Nonetheless, the expression of *Fgf8* has not been detected in the proximal region of the limb, making it unlikely that FGF8 is responsible for regulating the expression of *SCX* in the proximal mesenchyme.[28] Another candidate is FGF4, which is expressed in the muscle close to the attachment sites of tendon in chick wings.[35] However, the initiation of early *SCX* expression did not require the presence of muscle, which suggests that FGF4 may not be involved in the initiation of *SCX* expression during the early limb development. Despite that, it may be required for their continued differentiation.

In addition to the FGFs, other potential candidates for regulating tendon development in the limbs include transforming growth factor β (TGFβ) superfamily proteins, since these, as well as their related signaling pathway proteins, are expressed in the tendon during embryonic stages.[37] In the absence of TGFβ signals, as in the TGFβ 2/TGFβ 3 double-mutant, or type II TGFβ receptor null mice, most of the tendons were lost.[38] However, the induction of SCX-expressing tendon progenitors was not affected in these embryos.[38] Bone morphogenetic protein (BMP) family members, including growth and differentiation factor (GDF) isoforms GDF5, 6, and 7, also known as BMP 14, 13, and 12, have been implicated in tendon development and healing. GDF5 is one of the earliest known markers of joint formation.[39,40] Mice deficient in GDF 5, 6, or 7 exhibit tendon ultrastructural, biological, and/or biomechanical abnormalities,[41–43] whereas exogenous delivery of GDF 5, 6, and 7 causes ectopic formation of tendon tissue.[44] However, the involvement of GDFs in the initiation of tendon development in the embryo requires further study.

So, the signals that initiate the expression of SCX in the limb remain unknown. A recent study in *Xenopus* found that *mef2c*, a basic-helix-loop-helix myogenic transcription factor gene, and *scx* were both expressed in the same cells in the embryo, and may cooperate with each other to induce the expression of other tendon genes, such as tenascin C. In addition, a hormone-inducible XMEF2C could induce the expression of Xscleraxis. These results suggest that XMEF2C might act upstream of Xscleraxis and work with Xscleraxis to activate the differentiation of tendon progenitors in *Xenopus*.[45] It will be extremely important to find out whether MEF2C has the same functions in other species.

One of the most interesting aspects of tendon initiation in the limb is the mutual antagonism between BMP and FGF signaling in establishing the size of the SCX-expressing population of cells within the limb bud. Current evidence suggests that in the limb, cartilage and tendon cells arise from a common precursor population whose fate toward tendon or cartilage is regulated by antagonism between BMP and FGF signaling pathways. In chicken embryos, BMP2 stimulates chondrogenesis and inhibits tendon development in the developing limb.[28,35,46] The exogenous inhibitor of BMP signaling, Noggin, promotes tendon differentiation, whereas the inhibition of FGF signaling results in chondrogenesis.[28] It appears, therefore, that the antagonistic relationship between BMP and FGF signaling pathways controls the size of the tendon progenitor population in the limb.

In conclusion, it appears that initiation of tendon differentiation is controlled by different signals in the limb and somite. Although the induction of tendon progenitors does not require the presence of myogenic cells in the limb, their maintenance and further differentiation to tendon do need the participation of muscles. It is evident that FGF signaling is important for the development of tendon but how it does this requires further investigation. The current model of development of tendon in the somite and limb is shown in Figure 2.

Tendon Growth and Differentiation

The growth of tendons depends on the controlled production and turnover of tenocytes, and their differentiation requires the synthesis of the extracellular matrix proteins and proteoglycans characteristic of tendons.[47] SCX has been shown to positively regulate the expression of type I collagen through the tendon-specific element 2 of the procollagen, type1, alpha 1 (*Col1a1*) promoter,[48] which suggests an essential role of SCX in tendon differentiation. However, ablation of *Scx* in mouse embryos does not affect all the tendon tissues. In $Scx^{-/-}$ mice, the force-transmitting tendons were severely disrupted, but ligament and short-range anchoring tendons were not affected.[49] Furthermore, not all type I collagen is lost from the tendons of the *Scx* null mice, suggesting the presence of other factors that regulate the production of type I collagen in tendons. Recent studies revealed that Mohawk (*Mkx*), a homeobox gene, was also expressed in tendon progenitor cells.[50] A null mutation of *Mkx* in mice generated hypoplastic tendons due to the reduction of type I collagen production. Although the tendon mass was decreased in the $Mkx^{-/-}$ mice, the number of tendon cells did not change significantly. In addition, although *Scx* was expressed in *Mkx* null mice, the production of type I collagen was still affected. Thus, the function of *Mkx* seems to be critical in the maturation of tendon.[51] Other homeobox genes are expressed in the developing limb buds.[52] However, the functional roles for these in tendon development remain to be characterized. As mentioned earlier, TGFβ signals play a

critical role in initiating the differentiation of tendons in the embryo. It will be interesting to learn whether TGFβ signals are also involved in the differentiation of tendon at later stages.

In addition to type I collagen, several other proteins and proteoglycans are essential for normal tendon differentiation, including biglycan, decorin, fibromodulin, lumican, and tenomodulin.[20–22,24,53] Although none of these are specific to tendons, tendon differentiation is abnormal in their absence.[54–57] Among these molecules, biglycan and fibromodulin are particularly interesting, because a recent study suggested that they are critical elements for maintaining a niche for tendon progenitor cells.[58] In the absence of biglycan and fibromodulin the organization of tendon fibers was disorganized, and the identity of tendon progenitors, for example, the expression of *Scx* and type I collagen, was lost.[58] These results demonstrate that proteoglycans play an essential role in tendon differentiation. Tenomodulin (*Tnmd*) is also important in tendon differentiation. TNMD is a member of a new family of type II transmembrane glycoproteins. It is expressed in tendons, ligaments, and eyes and is positively regulated by SCX.[59,60] *Tnmd* has been used as a tendon cell-specific marker in both *in vitro* and *in vivo* systems. Targeted mutations of *Tnmd* lead to a decrease in the proliferation of tenocytes and a reduction of tenocyte density. Although the $Tnmd^{-/-}$ mice show increased maximal diameters of collagen fibrils, the deposited amount of extracellular matrix protein is not affected. These findings together suggest that a potential function of TNMD is to ensure the proper formation of the network of collagen.[56] Since the maturation of tendon requires *Mkx* and the proliferation of tenocytes and tendon organization require *Tnmd*, a future direction should be to identify the precise defects caused by the absence of these proteins.

Little is known about the spatial and temporal control of tenocyte proliferation. In the most studied tissues, blood, muscle, and skin, small populations of slow cycling stem cells capable of differentiating into all cell components of the tissue are associated with adjacent cells that form a niche that controls their proliferative behavior. The stem cells divide asymmetrically, so that one daughter cell retains the stem cell property, and the other daughter cell is displaced from the niche and undergoes a series of rapid cell divisions (thus forming a "transit amplifying," or "multiplying progenitor" population) and a restriction in pluripotency to a single cell type. In the developing tendon, such slow-cycling populations of cells have not so far been identified, nor has the spatial position of a potential niche. Culture of tendon cells for long periods has been shown to generate a dividing cell population. These dividing cells express stem cell characteristics such as clonogenicity, multipotency, and self-renewal capacity.[58] However, whether these proliferating cells arose from stem cells, transit amplifying cells, or both is unknown. In addition, although stem cell characteristics were found in the *in vitro* cell culture system, it is unclear whether these distinctions exist in the tendon. It is important to study cell proliferation both spatially and temporally during tendon development. Equally important is to learn if a slow-cycling population of cells is present and to identify them in the tendon. To generate functional and self-renewing tendon tissue by tissue engineering, it will be essential to recreate and localize the stem cell populations in the tendon.

The Enthesis

The formation of the enthesis, the point of insertion of a tendon into bone, is another fascinating and poorly understood component of tendon development. The enthesis does not re-form in adults if damaged, and is not regenerated in a grafted tendon. Since this is an essential functional component of the normal tendon, it will be important to attempt to stimulate its differentiation, both *in vitro* in bio-engineered tendon tissue and *in vivo* in tendon repair. However, current knowledge of the signals and responses that cause differentiation of the enthesis is not sufficient to do this. The fully formed enthesis is generally described as having four parts as the tendon transitions into the bone: tendon, fibrocartilage, mineralized fibrocartilage, and finally bone.[47,61] The composition of the enthesis and its structure has been discussed extensively elsewhere.[62,63] The transition of these four zones of enthesis occurs over a distance of $\sim$1 mm in length. The mechanisms that regulate such a fine series of tissue gradations are not clear. One potential signaling ligand that may be involved is BMP. A recent study has shown that *Bmp4* expression in tenocytes is controlled by SCX, and in the absence of *Bmp4* in the developing forelimb, the formation of bone ridges caused by the pull of tendons is lost.[64] However, since not all bone ridges were lost in the absence of *Bmp4*, other mechanisms must also be involved in enthesis differentiation. Another signaling pathway potentially involved in the formation of the enthesis is the Indian Hedgehog (Ihh) pathway.[63,65] It has been shown that Ihh signaling regulates chondrocyte proliferation and long bone development by cooperating with parathyroid hormone-related protein (PTHrP) at the growth plate.[66] The growth plate is near the end of a long bone and is essential for the development of cartilage and the growth of bone. Interestingly, both *Ihh* and *PTHrP* are also present in entheses, suggesting their potential roles in regulating enthesis development.[65–67] Several genes expressed in the growth plate are also expressed in the enthesis, including collagen type II alpha I (*Col2a1*), collagen X alpha 1(*Col10a1*),[68] and *Sox9*.[69] The co-localization of these genes suggests shared transcriptional regulation in these adjacent structures, and therefore potentially common signaling mechanisms that control their differentiation.

Mechanical cues also seem to play roles in enthesis formation.[63,70] However, little is known about their precise roles. The identification of the signaling pathways that initiate and control the progressive change in structure and function of the tendon at its insertion site is a very high priority. It should also be borne in mind that the tendon initially inserts into the epiphyseal cartilage of the long bone, which only later ossifies as a secondary center of ossification forms. Initiation of enthesis formation is therefore by an initial interaction between tendon and cartilage, not tendon and bone, and it is here that mechanism should be sought.

Tendon Injury and Repair

Tendon injuries are common clinical problems. In the United States, about 45% of the 32.8 million musculoskeletal injuries each year involve tendons and ligaments.[13] Most tendon injuries involve a degenerative component that can take years to develop.[71,72] Tendon degeneration, or tendinosis, can lead to matrix disorganization, mucoid degeneration, and fatty infiltration.[73–76] The hypovascular and

Table 1. *In Vivo* Biological Treatments of Tendon Injury

Treatment	*Type of tendon*	*Deliver methods*	*Results*	*Reference*
bFGF	ACL in dog	Implantation of bFGF pellet	Enhanced the healing process of the injured ACL.	87
bFGF	MCL in rabbit	Carried by fibin gel with recombinant human bFGF	Promoted early formation of repair tissue.	108
bFGF	PT in rat	Injected with increasing doses	Increased the expression of collagen type III but there was no significant difference on ultimate stress and the pyridinoline content between healing tendon and control groups.	86
bFGF	Flexor tendon in dog	Fibrin-heparin-based delivery	Failed to produce improvements in either the mechanical or functional properties of the repair. Increased cellular activity resulted in peritendinous scar formation and diminished range of motion.	109
GDF5	Zone II flexor tendon repairs in a rabbit flexor tendon	Sutures coated with GDF5	All tendons were failed at the repair site But the GDF5 treatment group showed better outcomes on maximum load at early treatment.	110
GDF6	Achilles tendon in rat	Injected GDF6 locally in the defect site	Tendons were 39% stronger than the controls	111
IGF-1	Achilles tendon in rat	Injected LR3-IGF01	Increased the healing rate by reducing inflammation.	82
MSCs from bone marrow	MCL in rat	Injected 10^6 nucleated cells of bone marrow	MSC from bone marrow may serve as a vehicle for therapeutic molecules and to be a source in enhancing healing of ligaments.	112
MSCs from bone marrow	Achilles tendon in rabbit	Implanted with autologous, culture-expanded MSC constructs	Delivered MSC-contracted, organized collagen implants to large tendon defects. Significantly improved the biomechanics, structure, and probably the function of the tendon after injury.	106
MSCs from bone marrow	PT in rabbit	Seeded in collagen-based construct and mechanically stimulated in culture	Matched normal tendon tangent stiffness up to 50% beyond peak *in vivo* forces measured during activities of daily living.	119
IGF-1 and TGF-β1	PT in rabbit	Mixed with fibrin sealant as a delivery vehicle	Significant increase in force at failure, ultimate stress, stiffness, and energy uptake at 2 weeks comparing to the control group.	113
PDGF, PDGF + IGF, PDGF + bFGF	MCL in rat	Directly injected	Increased the healed ligament strength, stiffness and breaking energy in three treatment groups compared to controls.	114
PDGF-BB	MCL in rabbit	Delivered using fibrin sealant	Improved the quality of healing of the MCL.	115
PDGF-BB	Achilles tendon in rats	Delivered using nanoparticles	Increased the healing process.	99
SDF-1	Achilles tendon in rat	Surgically implanted knitted silk-collagen sponge scaffold	The expression of tendon repair gene markers and endogenous SDF-1 were increased. Exhibition of more physiological microstructures with larger diameter collagen fibrils compare to the control group.	116
TGF-β1	ACL in rabbit	Adenoviral vector containing TGF-β1	Induced relatively rapid and continuous proliferation of ACL fibroblasts and high gene expression of collagen type I, collagen type III, and fibronectin mRNA among matrix markers.	117
TGF-β2	MCL in rabbit	Adenoviral vector containing TGF-β2	Increased type I collagen expression and profoundly increased early scar mass.	118
VEGF	Flexor tendon in rabbit	Adenoviral vector containing VEGF165	Induced relatively rapid and continuous proliferation of ACL fibroblasts and high gene expression of collagen type I, collagen type III, and fibronectin mRNA among matrix markers.	117

bFGF, basic fibroblast growth factor; ACL, anterior cruciate ligament; MCL, medial collateral ligament; PT, patellar tendon; GDF, growth and differentiation factor; IGF, insulin-like growth factor; MSC, mesenchymal stem cell; TGFβ, transforming growth factor β; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; SDF-1, stromal cell-derived factor-1.

hypocellular nature of tendons combined with added complications from degeneration over time complicates the treatment choices.[2,75] Although some of the surgical treatments for tendon/ligament injures, such as autografts for anterior cruciate ligaments, show high success rates, the patients often experience chronic pain and other side effects after surgery such as early onset osteoarthritis.[77,78] In particular, investigators have begun to question the recent use of growth factors in clinics to treat tendinopathies and to improve tendon healing. In order to develop more effective treatment options for tendon injuries, we need to learn more about the natural healing process of tendon, and in particular the degree to which it mimics the tendon's normal development.

The origins of the cells responsible for repairing an injured tendon are still the subject of debate. Two healing mechanisms, one extrinsic and the other intrinsic, have been proposed, based on the observations of the healing process of flexor tendons.[79,80] The extrinsic mechanism involves inflammatory cells and fibroblasts migrating in from surrounding tissues, whereas the intrinsic mechanism involves the fibroblast population from the endotenon and epitenon.[80] It seems that both mechanisms may contribute to the process of tendon healing, but most repair is carried out by cells from the epitenon and endotenon. These cells migrate to the lesions and synthesize new matrix.[80,81] However, the molecular mechanism controlling these events, and whether fully differentiated replacement tendon forms at these sites, remains unclear. The use of molecular markers of tenocyte differentiation will help resolve this issue. Various studies have suggested that growth factors participate in the healing process of tendon injuries.[82,83] For example, tendon defects created in mice carrying targeted null mutations in GDF5 showed slower healing than in wild-type mice.[84] Moreover, thicker tendons formed after viral overexpression of *Gdf5* in the tenocytes of rats.[85] These findings indicate that GDF5 may improve the structural outcomes of the regenerated tissue in injured tendons. Further study is needed to support its application in the clinical treatment of tendon diseases. In addition, in the absence of GDF5, the process of

Table 2. Genes Involved in Tendon Development in Mice and Humans

Gene	*Category*	*Phenotypes in gene targeted mice*	*Phenotypes in humans*	*Reference*
Biglycan	Proteologlycan	Disordered collagen fibers.	None reported.	52
Bmp4	Growth factor	Enthesis formation inhibited.	None reported.	41
Collagen, Type I	Collagen	Mutant mice die at E12.5 with vascular defect.	Gene mutations display Ehlers-Danlos syndromes with joint hypermobility, skin hyperlaxity and hyperextensibility; osteogenesis imperfect, bone fragility, blue sclera, and dentinogenesis imperfecta.	16,17
Comp	Glycoprotein	Severe short-limb dwarfism and early-onset osteoarthritis.	Mutations cause multiple epiphyseal dysplasia or pseudoachondroplasia.	19
Decorin	Proteologlycan	Appearance of fragile skin with low tensile strength. The structure of collagen fibrils are irregular.	Associated with Ehlers-Danlos syndromes.	18,26,52
Fibromodulin	Proteologlycan	Abnormal collagen fibrillogenesis in tendons. Thinner collagen fibers.	None reported.	57
Gdf5	Growth factor	Limb shortening and joint dislocation.	Gene mutations cause chondrodysplasia (acromesomelic chondrodysplasia, Hunter-Thompson type).	40,83
Mkx	Transcription factor	Small collagen fibril diameters and a downregulation of type I collagen	None reported.	50,51
Tenomodulin	Glycoprotein	Loss of tenocyte proliferation and reduced tenocyte density	None reported.	56
Tgf2;Tgf3	Growth factor	Double-mutant causes the loss of *Scx* expression and most of the tendon	None reported.	38
Scleraxis	Transcription factor	Null mice display a reduced and disorganized tendon matrix, as well as cellular disorder. The force-transmitting tendons are severely disrupted but ligament and short-range anchoring tendons are not affected	None reported.	49

tendon healing, although slower, still occurs, suggesting the involvement of additional factors in the tendon healing process.[84] FGFs may also play roles in tendon healing after injury, in addition to their role in tendon development. By treating injured rat patellar tendons with FGFs, the healing process was improved due to an increase in cell proliferation and type III collagen expression.[86] Application of FGFs to cruciate ligament injuries in the dog also enhanced the healing process.[87] Moreover, it has been reported that FGF2 expression increased at tendon injury sites in different animal models.[83,88,89] These results suggest that FGF serves as a key factor during wound healing in tendon. TGFβs have also been implicated in tendon wound healing. The mRNA encoding TGFβ1 was found to be increased in the injured tendon and tendon sheath.[90] Its receptors, TGFRI, TGFRII, and TGFRIII, were also upregulated in the injured tendon.[91] The evidence that culturing tendon cells with TGFβ1 protein increased the production of collagen I *in vitro* further indicates the involvement of TGFβ1 in tendon healing.[92] Other growth factors have also been reported to be involved in tendon healing, such as insulin growth factor 1, platelet-derived growth factor-BB (PDGF-BB), and vascular endothelial growth factor.[93–96] Further studies to evaluate the functional roles of these growth factors in tendon healing are necessary. In addition, because the sources of the signaling ligands are not known, nor which cells they act upon, it will be important to identify the cells responding to these cell signals during normal tendon development as well as in tendon repair.

Once we identify signaling ligands that are necessary for normal tendon development, or which improve tendon repair, how do we apply this knowledge in practice? Growth factors could be added directly to treat an injured tendon. Another potential option for introducing growth factors is by gene therapy. Different delivery systems, such as viral or synthetic vectors could be used to introduce genes into the tenocytes in injured tendons, in order to induce or inhibit the expression of target genes.[97] For example, adenovirus-mediated BMP12 expression in the tendon laceration chicken model showed increased type I collagen synthesis as well as tensile strength, indicating improved tendon healing.[98] Use of viral vectors for gene delivery is efficient, but safety must be a major concern in its application to human patients. Nanoparticles have also been used as drug or gene delivery vectors. A recent study using this technique to deliver the *Pdgfb* gene into rats with Achilles tendon injuries showed a significantly faster healing process than in untreated controls.[99] However, it has been reported that some nanoparticles may cause adverse side effects.[100–102] Further study to ensure their safety for medical application is necessary. Although using a transgenic approach for tendon repair is an attractive possibility, one serious concern is how to turn off the function of a transgene after tendon repair. To solve this problem, we need to learn when and where these signals are turned on and off, and how cells respond to them during normal tendon development and repair. In addition, we have to understand if the regulation of cell signaling is different in different regions of tendon. For example, how do cells in the midsubstance respond to the signals during the process of recovery versus cells in the insertion sites? The answers to these questions would be expected to improve the likelihood of using gene therapy for tendon injuries.

One issue that should also be resolved is the origin and differentiation status of the cells that replace injured tendon tissue *in vivo*. Fibroblasts from adjacent connective tissue (the endotenon and epitenon, for example) may be capable of synthesizing collagens, as they do in scar tissue. However, it is also possible that stem/progenitor cells in the connective tissue could initiate *SCX* expression and become tenocytes. Understanding the signals that normally control *SCX* expression in the embryo could dramatically enhance the latter process, or could initiate it if it does not normally occur.

Another alternative is to treat tendon injuries using a stem cell approach. Mesenchymal stem cells (MSCs) derived from adult bone marrow have a significant potential to differentiate into mesenchymal tissues, including tendon and ligament.[103] Although there has been no report showing the presence of MSCs in tendons, it has been shown that a slow-cycling tendon-specific stem cell population might exist in the tendon.[58] In addition, when MSC collagen constructs were inserted into central-third defects of the rabbit patellar tendon, they produced repairs at 12 weeks that matched normal tangent stiffness up to 32% of normal failure force and 50% greater than peak *in vivo* forces recorded during activities of daily living.[104,105] Delivery of cultured MSCs to the injured Achilles tendon of rabbit resulted in the significant improvement of healing with larger cross-sectional area as well as better alignment of collagen fibers.[106] These studies illustrate the potential of using stem cells for treating damaged tendon. However, further study is necessary to understand the biochemical and mechanical signals needed to drive tissue-engineered constructs toward proper tenogenesis.[107]

Conclusions and Future Prospects

Studies of tendon development have demonstrated that tendon is a patterned organ with distinctive sections of the tendon differentiating into different cell types, different cell arrangements, and synthesizing different extracellular matrices. Each section is generated by unknown combinatorial signals acting on the tenocyte progenitor population. Identifying the signals concerned, and the mechanism of their actions during tendon development are both critical for designing more efficient treatments for tendon injuries. For example, we could treat an injured tendon directly with a precise combination of the growth factors to speed up its healing process. In addition, understanding the gene expression patterns during tendon development will provide us diagnostic benchmarks for engineering a correctly differentiating tendon in culture. Tissue engineers also can combine this knowledge with development biology to design better scaffolds or delivery systems that allow tenocytes or the stem cell population from a patient's own body to grow in culture. Several studies have attempted to use biological treatments to repair injured tendons (Table 1).[86,104,106,108–119] However, the efficiency of the restoration from these treatments is unsatisfying. Many potential factors have been implicated in tendon formation, but how they interact and regulate to generate a functional tendon remains to be discovered. Fully understanding normal tendon development will contribute to better outcomes for treating tendon injuries.

Several mouse genetic models for studying tendon growth and differentiation are available now (Table 2). Using a

genetic animal model to explore new aspects of tendon development can provide better understanding of the causes and progression of tendon injuries in humans. Among these opportunities is to use the stem cells from tendons for treating tendon injuries. However, before one can apply stem cell techniques to tendon treatments, the following questions must be addressed. (1) Where are the tendon stem cells? (2) What is their normal niche? And (3) what are the signaling pathways controlling the normal behaviors of the tendon stem cells? By answering these questions, we may be able to re-establish the tendon stem cell population and its niche in a damaged tendon, which will potentiate the chance for long-term maintenance of the repaired or replaced tendon.

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References

1. Ker, R.F., Alexander, R.M., and Bennett M.B. Why are mammalian tendons so thick? J Zool **216,** 309, 1988.
2. Bray, R.C., Rangayyan, R.M., and Frank, C.B. Normal and healing ligament vascularity: a quantitative histological assessment in the adult rabbit medial collateral ligament. J Anat **188(Pt 1),** 87, 1996.
3. Praemer, A., Furner, S., and Rice, D. Musculoskeletal Condition in the United States. Parke Ridge, IL: American Academy of Orthopaedic Surgeons, 1999.
4. Milgrom, C., Schaffler, M., Gilbert, S., and van Holsbeeck, M. Rotator-cuff changes in asymptomatic adults. The effect of age, hand dominance and gender. J Bone Joint Surg Br **77,** 296, 1995.
5. Sher, J.S., Uribe, J.W., Posada, A., Murphy, B.J., and Zlatkin, M.B. Abnormal findings on magnetic resonance images of asymptomatic shoulders. J Bone Joint Surg Am **77,** 10, 1995.
6. Jost, B., Pfirrmann, C.W., Gerber, C., and Switzerland, Z. Clinical outcome after structural failure of rotator cuff repairs. J Bone Joint Surg Am **82,** 304, 2000.
7. Klepps, S., Bishop, J., Lin, J., Cahlon, O., Strauss, A., Hayes, P., *et al.* Prospective evaluation of the effect of rotator cuff integrity on the outcome of open rotator cuff repairs. Am J Sports Med **32,** 1716, 2004.
8. Crossett, L.S., Sinha, R.K., Sechriest, V.F., and Rubash, H.E. Reconstruction of a ruptured patellar tendon with achilles tendon allograft following total knee arthroplasty. J Bone Joint Surg Am **84A,** 1354, 2002.
9. Tadokoro, K., Matsui, N., Yagi, M., Kuroda, R., Kurosaka, M., and Yoshiya, S. Evaluation of hamstring strength and tendon regrowth after harvesting for anterior cruciate ligament reconstruction. Am J Sports Med **32,** 1644, 2004.
10. Chiou, H.M., Chang, M.C., and Lo, W.H. One-stage reconstruction of skin defect and patellar tendon rupture after total knee arthroplasty. A new technique. J Arthroplasty **12,** 575, 1997.
11. Krueger-Franke, M., Siebert, C.H., and Scherzer, S. Surgical treatment of ruptures of the Achilles tendon: a review of long-term results. Br J Sports Med **29,** 121, 1995.
12. Uhthoff, H.K., Trudel, G., and Himori, K. Relevance of pathology and basic research to the surgeon treating rotator cuff disease. J Orthop Sci **8,** 449, 2003.
13. Riley, G. The pathogenesis of tendinopathy. A molecular perspective. Rheumatology (Oxford) **43,** 131, 2004.
14. Levangie, P.K., and Norkin, C.C. Joint Structure and Function: A Comprehensive Analysis, 4th edition. Philadelphia, PA: F. A. Davis Company, 2005.
15. Kjaer, M. Role of extracellular matrix in adaptation of tendon and skeletal muscle to mechanical loading. Physiol Rev **84,** 649, 2004.
16. Lohler, J., Timpl, R., and Jaenisch, R. Embryonic lethal mutation in mouse collagen I gene causes rupture of blood vessels and is associated with erythropoietic and mesenchymal cell death. Cell **38,** 597, 1984.
17. Nuytinck, L., Freund, M., Lagae, L., Pierard, G.E., Hermanns-Le, T., and De Paepe, A. Classical Ehlers-Danlos syndrome caused by a mutation in type I collagen. Am J Hum Genet **66,** 1398, 2000.
18. Reed, C.C., and Iozzo, R.V. The role of decorin in collagen fibrillogenesis and skin homeostasis. Glycoconj J **19,** 249, 2002.
19. Svensson, L., Aszodi, A., Heinegard, D., Hunziker, E.B., Reinholt, F.P., Fassler, R., *et al.* Cartilage oligomeric matrix protein-deficient mice have normal skeletal development. Mol Cell Biol **22,** 4366, 2002.
20. Ansorge, H.L., Meng, X., Zhang, G., Veit, G., Sun, M., Klement, J.F., *et al.* Type XIV collagen regulates fibrillogenesis: premature collagen fibril growth and tissue dysfunction in null mice. J Biol Chem **284,** 8427, 2009.
21. Birk, D.E., and Mayne, R. Localization of collagen types I, III and V during tendon development. Changes in collagen types I and III are correlated with changes in fibril diameter. Eur J Cell Biol **72,** 352, 1997.
22. Birk, D.E., and Trelstad, R.L. Fibroblasts create compartments in the extracellular space where collagen polymerizes into fibrils and fibrils associate into bundles. Ann N Y Acad Sci **460,** 258, 1985.
23. Birk, D.E., and Trelstad, R.L. Extracellular compartments in tendon morphogenesis: collagen fibril, bundle, and macroaggregate formation. J Cell Biol **103,** 231, 1986.
24. Trelstad, R.L., Birk, D.E., and Silver, F.H. Collagen fibrillogenesis in tissues, in a solution and from modeling: a synthesis. J Invest Dermatol **79 Suppl 1,** 109s, 1982.
25. Trelstad, R.L., Birk, D.E., and Silver, F.H. Cellular and collagen fibrillar polarity in developing chick limb tendon. Prog Clin Biol Res **110 Pt A,** 245, 1983.
26. Zhang, G., Ezura, Y., Chervoneva, I., Robinson, P.S., Beason, D.P., Carine, E.T., *et al.* Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. J Cell Biochem **98,** 1436, 2006.
27. Brown, D., Wagner, D., Li, X., Richardson, J.A., and Olson, E.N. Dual role of the basic helix-loop-helix transcription factor scleraxis in mesoderm formation and chondrogenesis during mouse embryogenesis. Development **126,** 4317, 1999.
28. Schweitzer, R., Chyung, J.H., Murtaugh, L.C., Brent, A.E., Rosen, V., Olson, E.N., *et al.* Analysis of the tendon cell fate using Scleraxis, a specific marker for tendons and ligaments. Development **128,** 3855, 2001.
29. Brent, A.E., Schweitzer, R., and Tabin, C.J. A somitic compartment of tendon progenitors. Cell **113,** 235, 2003.
30. Brent, A.E., Braun, T., and Tabin, C.J. Genetic analysis of interactions between the somitic muscle, cartilage and

tendon cell lineages during mouse development. Development **132,** 515, 2005.
31. Kahane, N., Cinnamon, Y., Bachelet, I., and Kalcheim, C. The third wave of myotome colonization by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. Development **128,** 2187, 2001.
32. Brent, A.E., and Tabin, C.J. FGF acts directly on the somitic tendon progenitors through the Ets transcription factors Pea3 and Erm to regulate scleraxis expression. Development **131,** 3885, 2004.
33. Smith, T.G., Sweetman, D., Patterson, M., Keyse, S.M., and Munsterberg, A. Feedback interactions between MKP3 and ERK MAP kinase control scleraxis expression and the specification of rib progenitors in the developing chick somite. Development **132,** 1305, 2005.
34. Kardon, G. Muscle and tendon morphogenesis in the avian hind limb. Development **125,** 4019, 1998.
35. Edom-Vovard, F., Schuler, B., Bonnin, M.A., Teillet, M.A., and Duprez, D. Fgf4 positively regulates scleraxis and tenascin expression in chick limb tendons. Dev Biol **247,** 351, 2002.
36. Edom-Vovard, F., Bonnin, M., and Duprez, D. Fgf8 transcripts are located in tendons during embryonic chick limb development. Mech Dev **108,** 203, 2001.
37. Kuo, C.K., Petersen, B.C., and Tuan, R.S. Spatiotemporal protein distribution of TGF-betas, their receptors, and extracellular matrix molecules during embryonic tendon development. Dev Dyn **237,** 1477, 2008.
38. Pryce, B.A., Watson, S.S., Murchison, N.D., Staverosky, J.A., Dunker, N., and Schweitzer, R. Recruitment and maintenance of tendon progenitors by TGFbeta signaling are essential for tendon formation. Development **136,** 1351, 2009.
39. Settle, S.H., Jr., Rountree, R.B., Sinha, A., Thacker, A., Higgins, K., and Kingsley, D.M. Multiple joint and skeletal patterning defects caused by single and double mutations in the mouse Gdf6 and Gdf5 genes. Dev Biol **254,** 116, 2003.
40. Thomas, J.T., Lin, K., Nandedkar, M., Camargo, M., Cervenka, J., and Luyten, F.P. A human chondrodysplasia due to a mutation in a TGF-beta superfamily member. Nat Genet **12,** 315, 1996.
41. Mikic, B. Multiple effects of GDF-5 deficiency on skeletal tissues: implications for therapeutic bioengineering. Ann Biomed Eng **32,** 466, 2004.
42. Mikic, B., Rossmeier, K., and Bierwert, L. Sexual dimorphism in the effect of GDF-6 deficiency on murine tendon. J Orthop Res **27,** 1603, 2009.
43. Mikic, B., Schalet, B.J., Clark, R.T., Gaschen, V., and Hunziker, E.B. GDF-5 deficiency in mice alters the ultrastructure, mechanical properties and composition of the Achilles tendon. J Orthop Res **19,** 365, 2001.
44. Wolfman, N.M., Hattersley, G., Cox, K., Celeste, A.J., Nelson, R., Yamaji, N., *et al.* Ectopic induction of tendon and ligament in rats by growth and differentiation factors 5, 6, and 7, members of the TGF-beta gene family. J Clin Invest **100,** 321, 1997.
45. della Gaspera, B., Armand, A.S., Sequeira, I., Lecolle, S., Gallien, C.L., Charbonnier, F., *et al.* The Xenopus MEF2 gene family: evidence of a role for XMEF2C in larval tendon development. Dev Biol **328,** 392, 2009.
46. Edom-Vovard, F., and Duprez, D. Signals regulating tendon formation during chick embryonic development. Dev Dyn **229,** 449, 2004.
47. Benjamin, M., and Ralphs, J.R. The cell and developmental biology of tendons and ligaments. Int Rev Cytol **196,** 85, 2000.
48. Lejard, V., Brideau, G., Blais, F., Salingcarnboriboon, R., Wagner, G., Roehrl, M.H., *et al.* Scleraxis and NFATc regulate the expression of the pro-alpha1(I) collagen gene in tendon fibroblasts. J Biol Chem **282,** 17665, 2007.
49. Murchison, N.D., Price, B.A., Conner, D.A., Keene, D.R., Olson, E.N., Tabin, C.J., *et al.* Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons. Development **134,** 2697, 2007.
50. Anderson, D.M., Arredondo, J., Hahn, K., Valente, G., Martin, J.F., Wilson-Rawls, J., *et al.* Mohawk is a novel homeobox gene expressed in the developing mouse embryo. Dev Dyn **235,** 792, 2006.
51. Ito, Y., Toriuchi, N., Yoshitaka, T., Ueno-Kudoh, H., Sato, T., Yokoyama, S., *et al.* The Mohawk homeobox gene is a critical regulator of tendon differentiation. Proc Natl Acad Sci U S A **107,** 10538, 2010.
52. Cohen, D.R., Cheng, C.W., Cheng, S.H., and Hui, C.C. Expression of two novel mouse Iroquois homeobox genes during neurogenesis. Mech Dev **91,** 317, 2000.
53. Elliott, D.H. Structure and function of mammalian tendon. Biol Rev Camb Philos Soc **40,** 392, 1965.
54. Ameye, L., Aria, D., Jepsen, K., Oldberg, A., Xu, T., and Young, M.F. Abnormal collagen fibrils in tendons of biglycan/fibromodulin-deficient mice lead to gait impairment, ectopic ossification, and osteoarthritis. FASEB J **16,** 673, 2002.
55. Danielson, K.G., Baribault, H., Holmes, D.F., Graham, H., Kadler, K.E., and Iozzo, R.V. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol **136,** 729, 1997.
56. Docheva, D., Hunziker, E.B., Fassler, R., and Brandau, O. Tenomodulin is necessary for tenocyte proliferation and tendon maturation. Mol Cell Biol **25,** 699, 2005.
57. Svensson, L., Aszodi, A., Reinholt, F.P., Fassler, R., Heinegard, D., and Oldberg, A. Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. J Biol Chem **274,** 9636, 1999.
58. Thomopoulos, S., Kim, H.M., Rothermich, S.Y., Biederstadt, C., Das, R., and Galatz, L.M. Decreased muscle loading delays maturation of the tendon enthesis during postnatal development. J Orthop Res **25,** 1154, 2007.
59. Oshima, Y., Shukunami, C., Honda, J., Nishida, K., Tashiro, F., Miyazaki, J., *et al.* Expression and localization of tenomodulin, a transmembrane type chondromodulin-I-related angiogenesis inhibitor, in mouse eyes. Invest Ophthalmol Vis Sci **44,** 1814, 2003.
60. Shukunami, C., Takimoto, A., Oro, M., and Hiraki, Y. Scleraxis positively regulates the expression of tenomodulin, a differentiation marker of tenocytes. Dev Biol **298,** 234, 2006.
61. Benjamin, M., Toumi, H., Ralphs, J.R., Bydder, G., Best, T.M., and Milz, S. Where tendons and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or mechanical load. J Anat **208,** 471, 2006.
62. Benjamin, M., and McGonagle, D. Entheses: tendon and ligament attachment sites. Scand J Med Sci Sports **19,** 520, 2009.
63. Thomopoulos, S., Genin, G.M., and Galatz, L.M. The development and morphogenesis of the tendon-to-bone

insertion - what development can teach us about healing. J Musculoskelet Neuronal Interact **10,** 35, 2010.
64. Blitz, E., Viukov, S., Sharir, A., Shwartz, Y., Galloway, J.L., Pryce, B.A., *et al.* Bone ridge patterning during musculoskeletal assembly is mediated through SCX regulation of Bmp4 at the tendon-skeleton junction. Dev Cell **17,** 861, 2009.
65. Koyama, E., Ochiai, T., Rountree, R.B., Kingsley, D.M., Enomoto-Iwamoto, M., Iwamoto, M., *et al.* Synovial joint formation during mouse limb skeletogenesis: roles of Indian hedgehog signaling. Ann N Y Acad Sci 1116, 100, 2007.
66. Vortkamp, A., Lee, K., Lanske, B., Segre, G.V., Kronenberg, H.M., and Tabin, C.J. Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science **273,** 613, 1996.
67. Chen, X., Macica, C., Nasiri, A., Judex, S., and Broadus, A.E. Mechanical regulation of PTHrP expression in entheses. Bone **41,** 752, 2007.
68. Galatz, L., Rothermich, S., VanderPloeg, K., Petersen, B., Sandell, L., and Thomopoulos, S. Development of the supraspinatus tendon-to-bone insertion: localized expression of extracellular matrix and growth factor genes. J Orthop Res **25,** 1621, 2007.
69. Akiyama, H., Kim, J.E., Nakashima, K., Balmes, G., Iwai, N., Deng, J.M., *et al.* Osteo-chondroprogenitor cells are derived from Sox9 expressing precursors. Proc Natl Acad Sci U S A **102,** 14665, 2005.
70. Benhardt, H.A., and Cosgriff-Hernandez, E.M. The role of mechanical loading in ligament tissue engineering. Tissue Eng Part B Rev **15,** 467, 2009.
71. Rees, J.D., Wilson, A.M., and Wolman, R.L. Current concepts in the management of tendon disorders. Rheumatology (Oxford) **45,** 508, 2006.
72. Riley, G. Chronic tendon pathology: molecular basis and therapeutic implications. Expert Rev Mol Med **7,** 1, 2005.
73. Gagey, N., Quillard, J., Gagey, O., Meduri, G., Bittoun, J., and Lassau, J.P. Tendon of the normal supraspinatus muscle: correlations between MR imaging and histology. Surg Radiol Anat **17,** 329, 1995.
74. Khan, K.M., Cook, J.L., Bonar, F., Harcourt, P., and Astrom, M. Histopathology of common tendinopathies. Update and implications for clinical management. Sports Med **27,** 393, 1999.
75. Riley, G. Tendinopathy—from basic science to treatment. Nat Clin Pract Rheumatol **4, 82,** 2008.
76. Spielmann, A.L., Forster, B.B., Kokan, P., Hawkins, R.H., and Janzen, D.L. Shoulder after rotator cuff repair: MR imaging findings in asymptomatic individuals—initial experience. Radiology **213,** 705, 1999.
77. Austin, J.C., Phornphutkul, C., and Wojtys, E.M. Loss of knee extension after anterior cruciate ligament reconstruction: effects of knee position and graft tensioning. J Bone Joint Surg Am **89,** 1565, 2007.
78. Petsche, T.S., and Hutchinson, M.R. Loss of extension after reconstruction of the anterior cruciate ligament. J Am Acad Orthop Surg **7,** 119, 1999.
79. Boyer, M.I. Flexor tendon biology. Hand Clin **21,** 159, 2005.
80. Jones, M.E., Mudera, V., Brown, R.A., Cambrey, A.D., Grobbelaar, A.O., and McGrouther, D.A. The early surface cell response to flexor tendon injury. J Hand Surg Am **28,** 221, 2003.
81. Gelberman, R.H., Manske, P.R., Vande Berg, J.S., Lesker, P.A., and Akeson, W.H. Flexor tendon repair *in vitro*: a comparative histologic study of the rabbit, chicken, dog, and monkey. J Orthop Res **2,** 39, 1984.
82. Kurtz, C.A., Loebig, T.G., Anderson, D.D., DeMeo, P.J., and Campbell, P.G. Insulin-like growth factor I accelerates functional recovery from Achilles tendon injury in a rat model. Am J Sports Med **27,** 363, 1999.
83. Molloy, T., Wang, Y., and Murrell, G. The roles of growth factors in tendon and ligament healing. Sports Med **33,** 381, 2003.
84. Chhabra, A., Tsou, D., Clark, R.T., Gaschen, V., Hunziker, E.B., and Mikic, B. GDF-5 deficiency in mice delays Achilles tendon healing. J Orthop Res **21,** 826, 2003.
85. Rickert, M., Wang, H., Wieloch, P., Lorenz, H., Steck, E., Sabo, D., *et al.* Adenovirus-mediated gene transfer of growth and differentiation factor-5 into tenocytes and the healing rat Achilles tendon. Connect Tissue Res **46,** 175, 2005.
86. Chan, B.P., Fu, S., Qin, L., Lee, K., Rolf, C.G., and Chan, K. Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. Acta Orthop Scand **71,** 513, 2000.
87. Kobayashi, D., Kurosaka, M., Yoshiya, S., and Mizuno, K. Effect of basic fibroblast growth factor on the healing of defects in the canine anterior cruciate ligament. Knee Surg Sports Traumatol Arthrosc **5,** 189, 1997.
88. Kobayashi Mea. Expression of growth factors in the early phase of supraspinatus tendon healing in rabbits. J Shoulder Elbow Surg **15,** 1, 2006.
89. Lee, J., Harwood, F.L., Akeson, W.H., and Amiel, D. Growth factor expression in healing rabbit medial collateral and anterior cruciate ligaments. Iowa Orthop J **18,** 19, 1998.
90. Chang, J., Most, D., Stelnicki, E., Siebert, J.W., Longaker, M.T., Hui, K., *et al.* Gene expression of transforming growth factor beta-1 in rabbit zone II flexor tendon wound healing: evidence for dual mechanisms of repair. Plast Reconstr Surg **100,** 937, 1997.
91. Ngo, M., Pham, H., Longaker, M.T., and Chang, J. Differential expression of transforming growth factor-beta receptors in a rabbit zone II flexor tendon wound healing model. Plast Reconstr Surg **108,** 1260, 2001.
92. Klein, M.B., Yalamanchi, N., Pham, H., Longaker, M.T., and Chang, J. Flexor tendon healing *in vitro*: effects of TGF-beta on tendon cell collagen production. J Hand Surg [Am] **27,** 615, 2002.
93. Alfredson, H., Lorentzon, M., Backman, S., Backman, A., and Lerner, U.H. cDNA-arrays and real-time quantitative PCR techniques in the investigation of chronic Achilles tendinosis. J Orthop Res **21,** 970, 2003.
94. Bidder, M., Towler, D.A., Gelberman, R.H., and Boyer, M.I. Expression of mRNA for vascular endothelial growth factor at the repair site of healing canine flexor tendon. J Orthop Res **18,** 247, 2000.
95. Boyer, M.I., Watson, J.T., Lou, J., Manske, P.R., Gelberman, R.H., and Cai, S.R. Quantitative variation in vascular endothelial growth factor mRNA expression during early flexor tendon healing: an investigation in a canine model. J Orthop Res **19,** 869, 2001.
96. Zhang, F., Liu, H., Stile, F., Lei, M.P., Pang, Y., Oswald, T.M., *et al.* Effect of vascular endothelial growth factor on rat Achilles tendon healing. Plast Reconstr Surg **112,** 1613, 2003.
97. Gerich, T.G., Kang, R., Fu, F.H., Robbins, P.D., and Evans, C.H. Gene transfer to the rabbit patellar tendon: potential

for genetic enhancement of tendon and ligament healing. Gene Ther **3,** 1089, 1996.
98. Lou, J., Tu, Y., Burns, M., Silva, M.J., and Manske, P. BMP-12 gene transfer augmentation of lacerated tendon repair. J Orthop Res **19,** 1199, 2001.
99. Suwalski, A., Dabboue, H., Delalande, A., Bensamoun, S.F., Canon, F., Midoux, P., *et al.* Accelerated Achilles tendon healing by PDGF gene delivery with mesoporous silica nanoparticles. Biomaterials **31,** 5237, 2010.
100. Dhawan, A., and Sharma, V. Toxicity assessment of nanomaterials: methods and challenges. Anal Bioanal Chem **398,** 589, 2010.
101. Pople, P.V., and Singh, K.K. Targeting tacrolimus to deeper layers of skin with improved safety for treatment of atopic dermatitis. Int J Pharm **398,** 165, 2010.
102. Schneider, M., Stracke, F., Hansen, S., and Schaefer, U.F. Nanoparticles and their interactions with the dermal barrier. Dermatoendocrinol **1,** 197, 2009.
103. Liechty, K.W., MacKenzie, T.C., Shaaban, A.F., Radu, A., Moseley, A.M., Deans, R., *et al.* Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after *in utero* transplantation in sheep. Nat Med **6,** 1282, 2000.
104. Juncosa, N., West, J.R., Galloway, M.T., Boivin, G.P., and Butler, D.L. *In vivo* forces used to develop design parameters for tissue engineered implants for rabbit patellar tendon repair. J Biomech **36,** 483, 2003.
105. Juncosa-Melvin, N., Matlin, K.S., Holdcraft, R.W., Nirmalanandhan, V.S., and Butler, D.L. Mechanical stimulation increases collagen type I and collagen type III gene expression of stem cell-collagen sponge constructs for patellar tendon repair. Tissue Eng **13,** 1219, 2007.
106. Young, R.G., Butler, D.L., Weber, W., Caplan, A.I., Gordon, S.L., and Fink, D.J. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. J Orthop Res **16,** 406, 1998.
107. Butler, D.L., Juncosa-Melvin, N., Boivin, G.P., Galloway, M.T., Shearn, J.T., Gooch, C., *et al.* Functional tissue engineering for tendon repair: A multidisciplinary strategy using mesenchymal stem cells, bioscaffolds, and mechanical stimulation. J Orthop Res **26,** 1, 2008.
108. Fukui, N., Katsuragawa, Y., Sakai, H., Oda, H., and Nakamura, K. Effect of local application of basic fibroblast growth factor on ligament healing in rabbits. Rev Rhum Engl Ed **65,** 406, 1998.
109. Thomopoulos, S., Das, R., Sakiyama-Elbert, S., Silva, M.J., Charlton, N., and Gelberman, R.H. bFGF and PDGF-BB for tendon repair: controlled release and biologic activity by tendon fibroblasts *in vitro*. Ann Biomed Eng **38,** 225, 2010.
110. Henn, R.F., 3rd, Kuo, C.E., Kessler, M.W., Razzano, P., Grande, D.P., and Wolfe, S.W. Augmentation of zone II flexor tendon repair using growth differentiation factor 5 in a rabbit model. J Hand Surg Am **35,** 1825, 2010.
111. Forslund, C., and Aspenberg, P. Tendon healing stimulated by injected CDMP-2. Med Sci Sports Exerc **33,** 685, 2001.
112. Watanabe, N., Woo, S.L., Papageorgiou, C., Celechovsky, C., and Takai, S. Fate of donor bone marrow cells in medial collateral ligament after simulated autologous transplantation. Microsc Res Tech **58,** 39, 2002.
113. Lyras, D.N., Kazakos, K., Verettas, D., Chronopoulos, E., Folaranmi, S., and Agrogiannis, G. Effect of combined administration of transforming growth factor-b1 and insulin-like growth factor I on the mechanical properties of a patellar tendon defect model in rabbits. Acta Orthop Belg **76,** 380, 2010.
114. Letson, A.K., and Dahners, L.E. The effect of combinations of growth factors on ligament healing. Clin Orthop Relat Res **308,** 207, 1994.
115. Hildebrand, K.A., Woo, S.L., Smith, D.W., Allen, C.R., Deie, M., Taylor, B.J., *et al.* The effects of platelet-derived growth factor-BB on healing of the rabbit medial collateral ligament. An *in vivo* study. Am J Sports Med **26,** 549, 1998.
116. Shen, W., Chen, X., Chen, J., Yin, Z., Heng, B.C., Chen, W., *et al.* The effect of incorporation of exogenous stromal cell-derived factor-1 alpha within a knitted silk-collagen sponge scaffold on tendon regeneration. Biomaterials **31,** 7239, 2010.
117. Wei, X.L., Lin, L., Hou, Y., Fu, X., Zhang, J.Y., Mao, Z.B., *et al.* Construction of recombinant adenovirus co-expression vector carrying the human transforming growth factor-beta1 and vascular endothelial growth factor genes and its effect on anterior cruciate ligament fibroblasts. Chin Med J (Engl) **121,** 1426, 2008.
118. Spindler, K.P., Dawson, J.M., Stahlman, G.C., Davidson, J.M., and Nanney, L.B. Collagen expression and biomechanical response to human recombinant transforming growth factor beta (rhTGF-beta2) in the healing rabbit MCL. J Orthop Res **20,** 318, 2002.
119. Juncosa-Melvin, N., Shearn, J.T., Boivin, G.P., Gooch, C., Galloway, M.T., West, J.R., *et al.* Effects of mechanical stimulation on the biomechanics and histology of stem cell-collagen sponge constructs for rabbit patellar tendon repair. Tissue Eng **12,** 2291, 2006.

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