Tendon development and diseases

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Key words: Tendon, Scleraxis, Collagen, Tenomodulin, Development

Abstract

Tendon is a uniaxial connective tissue component of the musculoskeletal system. Tendon is involved in force transmission between muscle and bone. Tendon injury is very common and debilitating but tendon repair remains a clinical challenge for orthopedic medicine. In vertebrates, tendon is mainly composed of type I collagen fibrils, displaying a parallel organization along the tendon axis. The tendon-specific spatial organization of type I collagen provides the mechanical properties for tendon function. In contrast to other components of the musculoskeletal system, tendon biology is poorly understood. An important goal in tendon biology is to understand the mechanisms involved in the production and assembly of type I collagen fibrils during development, postnatal formation and healing processes in order to design new therapies for tendon repair. In this review we highlight the current understanding of the molecular and mechanical signals known to be involved in tenogenesis during development, and how development provides insights into tendon healing processes.

Introduction

I - Tendon structure in vertebrates

Tendon proper/tendon midsubstance

Tendon to muscle attachment (myotendinous junction)

Tendon to bone attachment (enthesis)

II - Scleraxis is the main tendon marker during vertebrate development

III - Embryological origins of tendons

Head, limb and trunk tendons in vertebrates

Drosophila tendons

IV - Tendon interactions with other components of the musculoskeletal system

Muscle/tendon interactions

Tendon/bone interactions

V- Intrinsic genes involved in tendon development (other than Scleraxis)

Mohawk (Mkx)

Early growth response (Egr1)

Zn finger transcription Stripe (Drosophila)

VI - Signaling pathways involved in tendon development

Tendon cell specification

Tendon cell differentiation

Signaling pathways involved in Drosophila tendon development

VII – Mechanical forces in tendon development

VIII- Tendon pathologies

Cancer and genetic diseases

Chronic tendon injury/tendinopathy

Acute tendon injury/partial and complete tendon tears

IX - Tendon development as tool for understanding tendon healing

Concluding remarks

Introduction

The musculoskeletal system confers the ability to move. Muscle, tendon and bone are the main components of the musculoskeletal system. Muscle generates forces that are transmitted to bone to allow body motion. Tendon links muscle to bone and is the essential organ of the musculoskeletal system that transmits forces. Tendon is a specialized connective tissue displaying a specific spatial organization of type I collagen fibrils that are organized parallel to the tendon axis. The specific organization of collagen fibrils confers tendon mechanical properties. The molecular and mechanical factors driving collagen production and organization during tendon development, postnatal formation and repair are not fully understood.

Tendon collagen fibrillogenesis consists in the progressive assembly of collagen fibrils that form a functional and mature tendon. Successive and overlapping phases of collagen fibril assembly and growth have been described in tendons[1]. Collagen fibril assembly occurs mostly during fetal stages, while collagen fibril growth and maturation occurs at postnatal stages[1]. The collagen fibril growth and maturation during postnatal stages are accompanied by a dramatic change of tendon mechanical properties. There is a 40,000-fold increase of the elastic modulus between adult tendons versus fetal tendons in chick[2]. Many components of the extracellular matrix (ECM) have been shown to be involved in collagen fibrillogenesis in tendons. Collagens, such as the fibrillar collagens III and V and the non fibrillar FACITs (fibril-associated collagens with interrupted triple helices) collagens XII and XIV, are important for collagen fibril formation, growth, and integrity in tendons (Table 1 and references therein). In addition to fibrillar and FACIT collagens, small leucine-rich proteoglycans (SLRPs) are also involved in type I collagen fibrillogenesis in tendons, mainly by regulating lateral collagen fibril growth[3,4]. Mutations of one SLRP or combination of SLRPs systematically lead to a tendon phenotype in mice (Table 1 and references therein).

The main challenge to decipher the molecular mechanisms underlying tenogenesis is to understand the intrinsic and extrinsic regulators of type I collagen production (transcript and protein levels), collagen fibril assembly and maturation during development. Developmental studies on the musculoskeletal system have focused mainly on muscle, cartilage and bone. The master genes driving the skeletal muscle and cartilage lineages have been identified as the bHLH transcription factors Myf5, MyoD and Mrf4 (muscle) as well as the SOX transcription factor Sox9 (cartilage). The absence of the 3 myogenic regulatory factors, *Myf5*, *MyoD* and *Mrf4* leads to a loss of skeletal muscle in mice[5], while the

overexpression of each myogenic regulatory factor induces myoblast conversion *in vitro* or *in vivo*[6,7]. A loss of *Sox9* activity results in a complete absence of cartilage[8], while overexpression of *Sox9* converts cells to chondrocytes[9]. The master regulator gene(s) of the tendon lineage has (have) not yet been identified. The task is made more difficult because of a lack of specific markers for tendon progenitors and differentiated cells (tenocytes). The main structural and functional component of tendon, type I collagen, is not specific to tendon and is expressed in many other tissues such as bone, skin and cornea. None of the ECM components involved in type I collagen fibrillogenis during tendon formation is specific to tendon; since they are also involved in collagen fibrillogenesis in other tissues[3,4]. Tendons are characterized by the spatial and parallel organization of collagen fibrils. To date, the molecular and cellular mechanisms driving this tendon-specific spatial organization of type I collagen remain completely unknown. It has been shown that fibroblasts, responsible for type I collagen synthesis and organization, cell-autonomously spatially arrange themselves according to their *in vivo* origins[10]. Fibroblasts isolated during fetal stages from tendon, cornea and skin and cultured in the same conditions, adopt a parallel, orthogonal or random organization, respectively[10]. This experiment suggests that fetal tendon fibroblasts intrinsically contain tissue-specific information that drives the parallel organization of type I collagen fibrils. We believe that the identification of genes involved in the early steps of tenogenesis during development will benefit the understanding of the type I collagen fibrillogenesis in tendons, in normal and pathological conditions.

In this review, we describe the current knowledge of tendon development in vertebrates and refer to drosophila tendon development when appropriate to establish parallels between invertebrates and vertebrates. We define the embryological origins of tendon versus the other components of the musculoskeletal system and highlight the intricate development of tendon with that of muscle and cartilage/bone tissues. We list the intrinsic and extrinsic molecular players known to be involved in tendon development and highlight the importance of mechanical forces in tendon development. Finally, we emphasize the parallel between tendon development and tendon healing.

Tendon structure in vertebrates

Tendon is a highly organized hypocellular connective tissue displaying a specific spatial organization of type I collagen fibers (Figure 1). The collagen molecules are synthetized by tendon fibroblasts or tenocytes, which display an elongated shape lying between the collagen

fibers[11]. The cellular composition and collagen organization are not homogenous along the tendon axis and are different at both ends, close to the muscle (myotendinous junction) and the bone (enthesis) interfaces.

Tendon proper/tendon midsubstance

Type I collagen is composed of a triple helix of 2 chains of α1 and one chain of α2 molecules, which are encoded by 2 different genes, *Col1a1* and *Col1a2*. In tendon, type I collagen displays a specific spatial organization that can be visualized at different scales (Figure 1). Collagen molecules assemble together successively forming collagen fibrils, collagen fibers, collagen bundles or fascicles and the tendon unit[12]. Parallel collagen fascicles are separated by the endotenon, a loose connective tissue that also contains fibroblasts as well as blood vessels and nerves[11]. The whole tendon is surrounded by the epitenon and then by a synovial sheath, the paratenon, composed of collagen fibers organized in a perpendicular direction to those of tendon[11,12]. Tendon stem cells have been isolated from mouse, human and rabbit tendons based on colony-forming unit assays[13,14]. However, there is no available marker to allow the visualization of these stem cells *in vivo*.

Tendon and muscle interface (myotendinous junction).

Tendon is attached to muscle via the myotendinous junction. Structurally, the myotendinous junction has been well described. The interface between tendon and muscle cells consists of inderdigitations of the plasma membranes of both tendon and muscle cells, named finger-like processes, which dramatically increase the interface between both cell types[15]. At a molecular level, collagen fibrils produced by tendon cells bind to laminin or integrins present at the level of sarcolemma and produced by muscle cells[16]. The developmental process of the myotendinous junction formation is not well characterized in vertebrates[17]. In contrast, myotendinous junction formation has been well studied in *Drosophila*[18].

Tendon to bone attachment (enthesis)

The region where tendon attaches to bone is called the enthesis. Depending on the attachment sites, fibrous and fibrocartilaginous entheses have been described[19]. Histologically, the fibrocartilaginous enthesis is characterized by different cellular zones, proceeding from tendon to bone: tenocytes, uncalcified fibrocartilage cells, calcified fibrocartilage cells and osteocytes. This cellular arrangement yields a direct connection between soft tissue (tendon) and hard tissue (bone). The part of the bone where the tendon will attach forms an eminence providing a stable anchoring. The development of the interface between tendon and bone has been recently addressed[20]. The maturation of this interface occurs at postnatal stages, leading to mineralization of the enthesis[21].

The different cellular and collagen compositions of the myotendinous junction, tendon proper and enthesis confer the different biomechanical properties of each part of the tendon. Consequently, tendon ruptures can be observed in the tendon midsubstance and at the enthesis but rarely at the myotendinous junction.

Scleraxis is the main tendon marker during vertebrate development

The main structural and functional tendon component, type I collagen, is expressed in many tissues and organs (Figure 2). Consequently, tendon development cannot be studied just by following type I collagen expression. To date, the only early tendon marker in vertebrates is the bHLH transcription factor Scleraxis (Scx)[22,23] (Figures 2,3). Scx has been shown to regulate positively *Col1a1* transcription in mouse tendons[24,25]. However, Scx is not the unique transcription factor driving *Col1a1* transcription in tendons, since in *Scx*-deficient mice *Col1a1* transcription is diminished but not abolished in developing tendons[25]. *Scx* is recognized to be a powerful marker for tendons during chick, mouse and zebrafish development[22,26,27]. *Scx* is also expressed in postnatal tendons[28] but is restricted to epitenon from 4 months postnatally[29] (Figure 3). At early stages, *Scx* is expressed in tendon presumptive regions at the level of branchial arches, somites and limbs[22,26,30]. *Scx* labels tendon progenitor cells and the *Scx*-positive cell population gives rise to tendons[31,32]. However, *Scx* is not the master regulatory gene of the tendon lineage as the myogenic regulatory factors are for the skeletal muscle lineage, since tendons retain their capacity to attach muscle to bone in *Scx* mutant mice[25]. *Scx* mutant mice are viable and mobile[25]. It is possible that *Scx* needs one or several partners to fulfill the function of master gene for tenogenesis. However, in the absence of *Scx* activity, force-transmitting tendons (limb and tail tendons) and intermuscular tendons are severely disrupted, while anchoring tendons (back tendons) are moderately affected[25]. The first tendon defects are observed from E13.5 in mouse limbs, and *Col14a1* and *Tnmd* expression is completely lost in tendons from E16.5 in *Scx* mutant mice[25]. *Tnmd* encodes a type II transmembrane glycoprotein and is considered a highly specific marker of differentiated tenocytes[23,31,33] (Figure 3). *Tmnd* mutant mice display an altered structure of collagen fibrils (shift towards large diameters) in tendons at postnatal stages[34]. *Tnmd* deficient mice also display reduced self-renewal and increased senescence properties of tendon progenitors[35]. In addition to being required for *Tnmd* expression, Scx is also sufficient for *Tnmd* expression[36]. In summary, *Tnmd* is a key marker for differentiated

tenocytes and *Scx* is the unique early tendon marker that provides a powerful tool to study early stages of tendon development.

Embryological origins of tendons

Tendons can be organized into three main groups according to their position in the body, head, trunk and limb tendons (Figure 4). Even if functionally similar, tendons of the different parts of the whole organism have distinct embryological origins, which have been studied mainly using the quail and chick chimera system[37]. Using this technique, it has been shown that vertebrate tendons originate from mesoderm or mesectoderm (neural crest cells). The craniofacial tendons originate from neural crest cells, in mouse, chick and zebrafish[27,30,38]. Axial tendons derive from a somitic compartment, named the syndetome[26]. Limb tendons originate from limb lateral plate[39].

Whatever the tendon group, tendons share the same embryological origins with skeletal tissues such as cartilage and bone, and have origins distinct from those of skeletal muscles. In somites, the syndetome is a subregion of the sclerotome, which gives rise to the axial skeleton, while axial muscles originate from the dermomyotome[26]. In the head, neural crest cells give rise to facial skeleton and tendons, while skeletal muscles originate from head mesoderm[38,40]. In limbs, both skeleton and tendons originate from limb lateral plate, while skeletal muscles derive from somites[41,42]. It should be noted that in the head, tendon progenitors migrate into muscle-containing regions, whereas in limbs, muscle progenitors undergo a migration step towards the limb lateral-plate containing skeleton and tendon progenitors. In contrast to the mesoderm or mesectoderm origins of vertebrate tendons, *Drosophila melanogaster* tendons originate from the ectoderm[43,44]. However, like in vertebrates, drosophila tendons along with the exoskeleton share the same embryological ectoderm origin, which is distinct from that of skeletal muscles derived from mesoderm[43].

Thus in both vertebrates and invertebrates, tendons and skeleton have the same embryological origin, which is different from that of skeletal muscles.

Tendon interactions with other components of the musculoskeletal system

Tendon/muscle interactions

Despite the distinct embryogical origins of the components of the musculoskeletal system, the development of muscle, tendon and cartilage/bone occurs in close spatial and temporal

association. Tendon development requires the presence of muscle, but the modalities of muscle requirement vary with the anatomic locations of tendons (Figure 5). Muscle is required for the initiation of tendon development at the axial level. *Scx* expression is not initiated in the absence of axial muscles. Surgical ablation of dermomyotomes prior to myotome formation leads to an absence of *Scx* expression in chick somites[26]. In E10.5 *Myf5*$^{-/-}$ *; MyoD*$^{-/-}$ double mutant embryos, *Scx* expression is absent in mouse somites[47]. In *myod1-myf5*-deficient zebrafish embryos, *scxa* expression is never initiated in myosepta[27]. In contrast, limb and head tendons initiate their development independently of muscle. In the absence of muscle, *Scx* expression is initiated normally in mouse and zebrafish craniofacial tendons[27,30]. *Scx* expression is also initiated and proceeds normally in muscleless limbs until E12 in *Pax3* mutant mice[22,45] and until E6 in surgically manipulated chick embryos[46]. Similarly with observations in the chick and mouse, *Scxa* is expressed normally in fins of 53-58hpf *myod1-myf5*-deficient zebrafish embryos[27]. The absence of muscle eventually prevents further tendon development and leads to a loss of *Scx* expression in head and limb tendons, in mouse, chick and zebrafish embryos[22,27,30,45]. This demonstrates that muscles are not required for the initiation but are necessary for the maintenance of *Scx* expression in craniofacial and limb tendons (Figure 5).

Muscle is therefore important for the induction of *Scx* expression in axial tendons and for the maintenance of *Scx* expression in cranial and limb tendons, in mouse, chick and zebrafish embryos. This pattern of muscle requirement has been conserved across these vertebrate species. Despite different embryological origins between vertebrates and invertebrates tendon cells (mesoderm versus ectoderm), two phases of tendon formation have been described in fruit fly. In Drosophila, the development of epidermal-derived tendon cells is initiated independently of muscles, but the final differentiation of tendon cells depends on specific interaction with muscles[43,48,49], indicating that drosophila tendon development shares characteristics with that of head and limb vertebrate tendons.

Thus, muscle is required for full tendon formation in vertebrate and invertebrate tendons. We believe that the muscle requirement is related to a requirement for mechanical forces during tendon development.

Tendon/bone interaction

While the role of muscle in tendon development is well demonstrated, the role of cartilage in tendon development is more difficult to address, mainly because tendon and cartilage cells have the same embryological origins. *Sox9a-sox9b*-deficient zebrafish embryos display abnormal craniofacial tendons based on *scxa* and *tnmd* expression[27], suggesting that cartilage

is necessary for the proper organization of tendon cells. However, it is difficult to dissociate tendon and cartilage defects. In somites, cartilage differentiation seems to repress tendon development. It has been observed that *Scx* is upregulated in *Sox5/Sox6* mouse mutant embryos (exhibiting cartilage defects)[47], while overexpression of *Pax1* (known to promote cartilage formation) in sclerotome inhibits *Scx* expression in chick somites[26]. In limbs, cartilage and tendon cell fates also appear to be mutually exclusive. During limb development, the Scx+/Sox9+ progenitors repress *Sox9* (while sparing *Scx*) expression to form the tendon side and downregulate *Scx* (and keep *Sox9*) expression to form the cartilaginous side of the tendon-bone interface[31,32]. However, *Sox9* depletion in *Scx*+ cells does not affect tendon formation other than by altering the bone side of enthesis formation[31,32], suggesting a relative independence of skeleton and tendon formation. However, at the digit levels, it has been reported that tendon blastema formation requires the presence of cartilage[50], indicating differences in tendon development according to proximo-distal position in limbs.

Intrinsic genes involved in tendon development (other than Scleraxis)

To date three transcription factors have been shown to be involved in vertebrate tendon development (Table 1): the bHLH transcription factor Scx[25], the homeobox Mohawk (Mkx)[51,52] and the Zinc finger transcription factor Early growth response 1 (Egr1)[53]. All of them have been shown to regulate *Col1a* gene transcription and type I collagen fibril organization in developing tendons [24,25,51–53]. Each of the three transcription factors Scx, Mkx and Egr1 is alone able to induce tenogenesis in stem cells, based on *Tnmd* expression[54–57]. However, in contrast to *Scx*, *Mkx* and *Egr1* are not specific to tendon, since they display numerous expression sites in addition to developing tendons[53,58,59].

Mohawk (Mkx)

Mkx$^{-/-}$ mutant mice exhibit smaller tendons than wild-type mice and display defects in postnatal growth of tendon collagen fibrils[51,52,60]. The first tendon defects in *Mkx*$^{-/-}$ mice are observed at E16.5 fetal stages[52]. In addition to the reduction of *Col1a1* gene expression, *Mkx*$^{-/-}$ mice display significant reduction in *Tnmd, Fmod,* and *Dcn* gene expression in neonatal tendons[51]. Notably, *Mkx* is expressed in early somites, in progenitor cell populations of skeletal muscle, tendon, cartilage and bone, downstream of the somitic *paraxis* transcription factor[58]. Mkx has been shown to inhibit muscle differentiation in mouse cell culture and to impair muscle development in zebrafish embryos by directly repressing *MyoD* transcription[61–

[63]. This would be consistent with a Mkx role in repressing the muscle lineage and promoting the tendon lineage. However, *Mkx* mutant mice do not display any obvious skeletal muscle defects[60]. *Scx* and *Mkx* expression in developing tendons appears to be normal in *Mkx*$^{-/-}$ and *Scx*$^{-/-}$ mutant mice, respectively, suggesting that *Scx* and *Mkx* act in different genetic cascades during tendon development[51,60].

Early growth response 1 (Egr1)

During fetal development, Egr1 is sufficient for the expression of *Scx, Tnmd* and tendon associated collagens (*Cola1, Col5a1, Col12a1* and *Col14a1*) in chick embryos[53]. *Egr1*$^{-/-}$ mice display defects in collagen fibril organization in tendons at fetal and postnatal stages[53,55]. *Egr1*-deficient tendons show a mechanical weakness and a deficiency in their capacity to heal following injury[55]. In addition to the reduction of *Col1a1* and *Col1a2* gene expression, *Egr1*$^{-/-}$ also displayed significant reductions in the expression of tendon-associated collagens (*Col3a1, Col5a1, Col12a1* and *Col14a1*) and tendon-associated molecules *Tnmd, Fmod* and *Dcn* in fetal limbs and adult tendons[53,55]. *Scx* expression is downregulated, while *Mkx* is not modified in *Egr1*-deficient tendons[53,55].

Stripe (Drosophila)

In Drosophila, the transcription factor *stripe* is the key gene for tendon development[44,48,64]. *Stripe* is the homolog of the vertebrate *Egr* gene family. The *stripe* gene produces two isoforms *stripeA* and *stripeB*. *StripeB* has been shown to be involved in tendon progenitor induction, while *stripeA* is involved at a later muscle-dependent stage of tendon differentiation[48,64,65].

Other transcription factors have been identified as being expressed in developing tendons, either by in situ hybridization experiments[66] or by global transcriptomic or RNA sequencing studies of mouse tendon cells during development[67,68]. Among them, the sine oculis-related homeobox, *Six2* displays a specific expression in chick and mouse autopod tendons[66,68]. However, there is currently no functional data available relating these transcription factors to tendon development.

Although transcription factors have been identified as being involved in tendon development, the intrinsic program driving tenogenesis in vertebrates remains to be fully characterized.

Signaling pathways involved in tendon development

In addition to intrinsic regulators of tenogenesis, the TGF-β and FGF signaling pathways have been shown to be involved in tendon development in mouse and chick embryos[26,46,47,69,70]. Bioinformatics analysis of a transcriptome of tendon cells also highlighted that these two were the main pathways displaying significant regulation during mouse limb development[67].

Tendon cell specification

TGF-β ligand is a potent inducer of *Scx* expression in embryonic mouse limbs, tendon progenitors and mesenchymal stem cells. *Tgfb2* and *Tgfb3* are expressed in early chick and mouse limbs to fulfill a role in *Scx* induction[67,69,71]. In mice, TGF-β2 is sufficient to increase *Scx* expression in E10.5 limbs, tendon progenitors and mesenchymal stem cells[67,69,72]. Moreover, the canonical TGF-β intracellular pathway, SMAD2/3, has been shown to be required for *Scx* expression in E10.5 mouse limbs during the muscle-independent phase of limb tendon formation[67]. Blocking classical TGF-β intracellular pathway using chemical inhibitors also decreases *Scx* expression in zebrafish embryos[27]. However, *Scx* expression appears to be normal in E11.5 limbs of *Tgfb2*$^{-/-}$*;Tgfb3*$^{-/-}$ double mutant mouse embryos[69], suggesting that other TGF-β ligands might be responsible for the initiation of *Scx* expression in mouse limbs. Another TGF-β ligand, myostatin (GDF-8), is a putative candidate to be involved in tendon development, since tendons are small, brittle and hypocellular in *Mstn*$^{-/-}$ mice[73]. Moreover, myostatin treatment of primary culture of mouse tendon fibroblasts increases cell proliferation, in addition to increasing *Scx* and *Tnmd* expression[73].

BMP ligands that signal via the intracellular Smad1/5/8 pathway have the opposite effect from TGF□β□□□□□□□□ and restricts *Scx* expression, while inhibition of BMP signaling using the antagonist Noggin increases *Scx* expression in early chick limbs[22]. The antagonist roles of TGF□β and BMP signaling pathways in tendon cell specification is consistent with their antagonist role in the regulation of fetal muscle progenitors. Myostatin is a potent negative regulator of muscle growth[74], while BMP positively regulates muscle progenitors[75] during embryonic development.

FGF has been shown to be required and sufficient for the initiation of *Scx* expression in somites during axial tendon development. An ectopic source of FGF induces ectopic expression of *Scx* in chick and mouse somites and chick limbs[26,46,47], while inhibition of FGF signaling prevents *Scx* expression[26,47]. *Pea3* (ERK MAPK effector) and *Sprouty2* (ERK MAPK modulator) are both expressed in tendon progenitor regions in chick syndetome and FGF has been shown to act on somitic tendon progenitors via the ERK MAPK intracellular pathway[70,76]. In mouse limbs, the ERK MAPK signaling pathway appears to have a different

effect, since a down-regulation of ERK MAPK was sufficient to increase *Scx* expression in mouse limb explants and in mouse mesenchymal stem cells[67]. Consistent with this result, FGF inhibited *Scx* expression in mouse mesenchymal stem cells[67].

Tendon cell differentiation

In addition to being involved at early stages of tendon induction, the TGF-β and FGF extracellular signals have been shown to be involved in tendon differentiation during the muscle-dependent phase of limb tendon formation[46,67,69]. In the absence of *Tgfb2* and *Tgfb3* function, there is a complete loss of *Scx* expression in head, axial and limb tendons, and subsequently tendons are lost[69]. TGF-β gain-of-function experiments in E12.5 mouse limbs lead to an upregulation of *Scx* and *Tnmd* expression[67,69]. TGF-β gain-of-function experiments in a high-density cell culture system of HH25 chick hindlimbs (micromass) also lead to an up regulation of *Scx* and *Tnmd* expression via the SMAD2/3 intracellular pathway[77]. □□□-interacting □actor (Tgif1)□has been shown to promote the fibrogenic effect of TGF-β□□□□□□□□ SMAD2/3 intracellular pathway□in chick micromass cultures[77]. It has to be noted that the addition of TGF-β ligands in 2D cell culture systems activates *Scx,* but drastically inhibits *Tnmd* expression[55,57,78]. This indicates that TGF-β ligands cannot induce complete tenogenesis in 2D stem cell cultures, in contrast to *ex vivo* experiments, where TGF-β activates *Tnmd* in addition to increasing *Scx* expression in mouse limb explants[67].

FGF has been shown to increase the number of *Scx*-positive cells at the expense of muscle cells in chick limbs during fetal development[46,79]. The expression of the ERK effector *Pea3* and modulator *Spry2* is observed in both muscle and tendon and is increased at the muscle-tendon interface in chick and mouse limbs[80]. However, despite similar expression in fetal chick and mouse tendons of FGF signaling components, FGF appears to have a distinct effect in mouse fetal tendon development compared to that in chick. FGF has been shown to downregulate *Scx* and *Tnmd* expression in mouse tendon cells isolated from E13 mouse embryos at the limb or axial levels[78].

To date, TGF-β□□□□□□□□, BMP/SMAD1/5/8 and FGF/ERK MAPK are the signaling pathways identified as being involved in the regulation of *Scx* expression in vertebrate embryos, although data are still missing to prove that all these pathways play similar roles in *Scx* induction or maintenance in mouse, chick and zebrafish embryos. FGF appears to be crucial for *Scx* induction and maintenance in chick but not in mouse embryos. We also suspect that other signaling pathways are also involved in tendon cell specification or

differentiation. The Wnt pathway is significantly regulated in mouse tendon cells during limb development, according to bioinformatics analysis of a tendon transcriptome[67]. Moreover, Wnt3a has been shown to positively regulate *Six2* expression in autopod tendons in developing chick limbs[66].

In *Drosophila*, signaling pathways have been shown to be involved in the muscle-dependent phase of tendon formation. The ligand Vein produced by muscle cells has been shown to activate the EGFR pathway in the tendon progenitors, leading to the expression of *stripeA*[43,81]. The transmembrane protein Kon-tiki expressed by myotubes target tendon cell through its interaction with Dgrip[82]. All these events lead to a more durable interaction between myotubes and tendon cells through the integrins, notably via the heterodimers αPS1βPS and αPS2βPS integrins[16]. Integrin interactions at the muscle and tendon interface have been shown to maintain the expression of tendon-specific genes such as *stripeA* and *β1-tubulin*[83].

Mechanical forces in tendon development

Mechanical forces are known to be involved in embryonic development by regulating organ formation [84]. Because tendon is a mechanosensitive tissue, mechanical forces are crucial for tendon development. In humans, a diminution of embryo mobility leads to severe abnormalities, including musculoskeletal defects[85]. Mechanical forces control the formation of all components of the musculoskeletal system during embryonic development[86]. Tendons are notably particularly sensitive to the absence of mechanical forces, since they do not form in the absence of muscles[22,45]. The two main pathways known to be involved in tendon development, TGF-β/SMAD2/3 and FGF/ERK MAPK are also involved in mechanotransduction processes[87,88]. It has been shown that mechanical forces regulate *Scx* expression through activation of the TGF-β/SMAD2/3 pathway in adult tenocyte cultures[72]. During development, FGF4 is able to rescue the *Scx* expression in the absence of mechanical movements in chick muscleless limbs[46]. This leads to the hypothesis that TGF-β and FGF signaling pathways are downstream of mechanical forces to regulate tendon development. One possible mechanosensor molecule downstream of mechanical forces and upstream of TGF-β signaling is the transcription factor Egr1. Egr1 is a mechanosensitive gene in the vascular system[89]. Egr1 is involved in tendon development during the muscle-dependent phase in chick and mouse embryos[53] and has been shown to activate *Tgfb2* transcription directly in adult mouse tendons[55]. Another transcription factor, Mkx, involved in tendon

development[51,52] has also been reported to activate *Tgfb2* transcription directly in mouse stem cells[57]. Although there is no reported evidence that *Mkx* is a mechanosensitive gene, we speculate that transcription factors could sense mechanical forces and act upstream of TGF-β signaling during tendon development. Consistent with a mechanosensor role for Egr1, *Egr1* and *Egr2* expression have been reported to be increased within 15 minutes in response to loading in injured rat tendons[90]. The role of Egr1 and Mkx transcription factors as mechanosensors upstream of TGF-β signaling remains to be demonstrated in the context of tendon development. In summary, mechanical forces are important parameters involved in tendon development but the mechanotransduction pathways downstream of forces remain to be characterized.

Tendon pathologies

Tendon is a connective tissue displaying very little cell division[13]. Consequently, there is no cancer in tendon, consistent with the direct correlation between the number of stem cell divisions and variation in cancer risk[91]. Cancers are nevertheless observed in tendon sheaths with the giant-cell tumor of the tendon sheaths (GCTTS). GCTTS is a non malignant condition with an unknown etiology observed mostly but not exclusively in hands[92]. GCTTS is observed at the tendon surface but never arises from tenocytes of the tendon proper and may not arise systematically from tendon sheaths; as it has been suggested to arise from synovial cells[93]. Genetic diseases affecting genes coding for proteins involved in type I collagen fibrillogenesis lead to tendon defects, but also to defects in all connective tissues[94]. Most tendon pathologies involve tendon injuries (Figure 6), which range from chronic to acute. Chronic tendon injury or tendinopathy is characterized by pain and disability. The etiology and pathogenesis of tendinopathy are not well understood, although the main recognized cause of tendinopathy is abnormal mechanical loading[95,96]. Acute tendon injury refers to partial or complete tears as a consequence of trauma[97]. After acute tendon injury, tendons follow the typical wound healing process, including an early inflammatory phase, followed by cell migration, cell proliferation and remodeling phases. However, the healing process is incomplete since healed tendons never regain their original biomechanical properties. The origin of the cells and the molecular mechanisms involved in tendon repair are not well established.

Tendon development as tool for understanding tendon healing

Natural tendon healing is thought to recapitulate tendon developmental processes. Both TGF-β and FGF signaling pathways, identified as being involved in tendon development, have been shown also to be important for tendon healing following injury[97]. TGF-β and FGF ligands are released at the tendon injury sites in animal models[98]. The loss of the canonical intracellular component of TGF-β pathway, *Smad3*, leads to reduced *Col1a1* transcription in healed tendons and to adhesion and scarring defects during tendon healing in *Smad3*$^{-/-}$ mutant mice [99]. Consequently, TGF-β ligands have been studied extensively as therapeutic candidates to promote tendon repair following tendon injury[98]. FGF is also considered as a putative therapeutic target promoting tendon repair. However, the FGF effect on the tendon healing process is not always positive. Local FGF application following tendon injury has been shown to promote cell proliferation in rat[100] and to increase angiogenesis in a canine model[101], but FGF failed to improve mechanical or functional properties of the repaired tendons[100,101]. Interestingly, in a chick tendon injury model, endogenous bFGF expression was down-regulated during the early phase of tendon healing process[102]. In addition, virally-mediated bFGF application enhanced *Scx* gene expression, and improved the biomechanical properties of repaired tendons in chick[103,104]. The beneficial effect of FGF in the tendon healing process in the chick model is consistent with the FGF effect during chick tendon development.

The BMPs have been shown to accelerate tendon-bone junction healing in animal models[105,106]. This effect is consistent with the BMP4 involvement in tendon cells at their bone insertion during deltoid tuberosity development[107].

Concluding remarks

We believe that the understanding of tendon development will provide a basis for the identification of effective treatments of tendon injury. Transcription factors have been identified as promoting tenogenesis using developmental or stem cell models, and have been shown to promote tendon repair in animal models of tendon injury. In addition to transcription factors, signaling pathways have been shown to be involved in tendon development and healing. The relationship between intrinsic and extrinsic regulators of tenogenesis remains to be defined in the context of tendon development and healing and correlated with mechanical forces.

Acknowledgements: We thank Sophie Gournet for illustrations. This work is supported by the FRM, ANR, AFM, INSERM, CNRS and UPMC.

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Figures

Figure 1

Tendon organization

(A) Tendon links muscle to bone and is attached at one end to muscle by the myotendinous junction and at the other end to bone by the enthesis. Tendon is mainly composed of type collagen and of very few cells. Type I collagen displays a specific spatial organization parallel to the tendon axis. Tendon is formed of collagen fascicles, which are composed of collagen fibers, which are formed of collagen fibrils. The endotenon separates collagen fascicles. Tendon is surrounded by the tendon sheaths named the peritenon, which comprises paratenon and epitenon. (B,C) Collagen fibres and fibrils can be visualized at different scales with electron microscopy. Electron microscopy of transverse sections of a mouse Achilles tendon showing collagen fibrils (B,C).

Figure 2

Expression of *Col1a1* and *Scx* in chick limbs

(A-D) Adjacent and transverse forelimb sections of Embryonic Day 10 (E10) chick embryos were hybridized with Col1a1 (A,B) and Scx (C,D) probes (blue) and then immunostained with the MF20 antibody, which recognizes myosins in skeletal muscles (brown). *Col1a1* is expressed in tendons but also around cartilage elements, in feather buds and connective tissues (A). *Scx* is expressed in tendons (C). (B,D) are higher magnifications of two dorsal muscles of forelimbs. *Col1a1* is expressed in tendons and muscle connective tissue (B), while Scx is expressed only in tendons (D). u, ulna; r, radius.

Figure 3

Expression of *Scx* and *Tnmd* in chick limbs and schematic representation of *Scx* expression in developmental, postnatal and adult tendons. (A-D) In situ hybridization to adjacent and transverse forelimb sections of Embryonic Day 9 (E9) chick embryos with Scx (A,C) and Tnmd (B,D) probes. *Scx* and *Tnmd* are expressed in tendons. (E) *Scx*-positive cells are schematized in green. During development, *Scx* expression is expressed in all tendon cells. During tendon maturation at postnatal stages, *Scx* is expressed in the tendon proper, endotendon and external sheaths including epitenon and paratenon, but is restricted to the epitenon by the 4^{th} postnatal month.

Figure 4

Distinct embryological origins of vertebrate tendons. Tendons can be divided into head, axial and limb tendons. Head tendons originate from neural crest cells (orange). Axial tendons originate from somites (purple). Limb tendons originate from limb lateral plate (green).

Figure 5

Muscle-dependency for head, limb and axial tendon development. Muscle and tendon are schematized in red and green, respectively. In the head (A) and limbs (B), tendons initiate their development independently of muscle, but further tendon development requires the presence of muscle. In contrast, the initiation of axial tendon development requires the presence of muscle (C).

Figure 6

Schematic representation of tendon pathologies. (A) Normal tendons. (B) Tendons in genetic diseases affecting collagen fibrillogenesis. (C) Chronic tendon injury or tendinopathy. (D) Acute tendon injury.

Table 1

List of molecules involved in tendon development.

Tendon phenotypes reported in mice during development, postnatal or adult stages. Studies reporting a tendon phenotype performed in chick, zebrafish or *Drosophila* are also reported.