



At the Crux of Joint Crosstalk: TGF β Signaling in the Synovial Joint

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Abstract

Purpose of Review The effect of the transforming growth factor beta (TGF β) signaling pathway on joint homeostasis is tissue-specific, non-linear, and context-dependent, representing a unique complexity in targeting TGF β signaling in joint disease. Here we discuss the variety of mechanisms that TGF β signaling employs in the synovial joint to maintain healthy joint crosstalk and the ways in which aberrant TGF β signaling can result in joint degeneration.

Recent Findings Osteoarthritis (OA) epitomizes a condition of disordered joint crosstalk in which multiple joint tissues degenerate leading to overall joint deterioration. Synovial joint tissues, such as subchondral bone, articular cartilage, and synovium, as well as mesenchymal stem cells, each demonstrate aberrant TGF β signaling during joint disease, whether by excessive or suppressed signaling, imbalance of canonical and non-canonical signaling, a perturbed mechanical microenvironment, or a distorted response to TGF β signaling during aging.

Summary The synovial joint relies upon a sophisticated alliance among each joint tissue to maintain joint homeostasis. The TGF β signaling pathway is a key regulator of the health of individual joint tissues, and the subsequent interaction among these different joint tissues, also known as joint crosstalk. Dissecting the sophisticated function of TGF β signaling in the synovial joint is key to therapeutically interrogating the pathway to optimize overall joint health.

Keywords TGF beta · Joint crosstalk · Osteoarthritis · Subchondral bone · Articular cartilage

Introduction

The synovial joint consists of multiple tissues, including articular cartilage, subchondral bone, meniscus, and synovium, that interact biologically and mechanically to maintain joint health [1]. During joint degeneration, the disruption of one tissue can lead to overall deterioration by shifting biomechanical loads, increasing inflammation, altering paracrine factors, or inducing aberrant cell-intrinsic signaling. These interactions among joint tissues are referred to as joint crosstalk. Though joint crosstalk is critical for joint health, it can also exacerbate disease. Unravelling the mechanisms and causal relationships among these tissues

has been challenging and has limited the development of therapies that modify osteoarthritis (OA) progression.

The transforming growth factor beta (TGF β) signaling pathway plays an intricate role in maintaining healthy joint crosstalk by carefully regulating homeostasis of each tissue. The delicate balance of TGF β signaling throughout the joint is non-linear and relies upon control of effector selection, mechanical cues, and spatial localization. Here, we discuss the multifaceted function of TGF β signaling in joint homeostasis and disease.

Overview of the TGF β Signaling Pathway

The TGF β signaling pathway controls cell behavior through hierarchical, context-dependent, and coordinated regulation [2–4]. The expression pattern of the three TGF β ligand isoforms, TGF β 1, β 2, and β 3, is spatially and temporally regulated throughout the lifespan. Each joint tissue expresses ligands capable of activating the TGF β signaling pathway, as well as the receptors and effectors needed to respond. The expression pattern and relative activity of each component

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are tightly regulated, which contributes to the diverse effects of TGF β in the joint.

The TGF β ligands are secreted as inactive ligands, consisting of the latency-associated peptide and the mature peptide. This complex is then secured to the extracellular matrix (ECM) through latent TGF β binding proteins (LTBP) and other proteins, such as biglycan, fibrillin, and thrombospondin, many of which have also been implicated in joint homeostasis or disease [5]. Not only do these mechanisms function to locally sequester TGF β in the ECM, but they also add multiple layers of regulation to TGF β activation, to be discussed later. Briefly, latent TGF β is activated through a variety of mechanisms, including those that are sensitive to the mechanical or chemical microenvironment [2]. This ECM-dependent control of TGF β activity is especially important in the joint. First, tissues with abundant ECM, such as hyaline cartilage and bone, are reservoirs for latent TGF β . Second, ECM changes that typify joint disease can indirectly disrupt the tight local control of TGF β sequestration and activity.

Once latent TGF β ligand becomes activated, it binds to two type II serine/threonine kinase receptors (T β RII), which then recruit and phosphorylate two type I serine/threonine kinase receptors (T β RI) to form a heterotetrameric transmembrane receptor complex [6]. The type I receptors, also termed activin receptor-like kinases (ALKs), calibrate ligand binding affinity and effector selection. Upon ligand binding, the receptors are phosphorylated, which permits effectors to be recruited and activated, including the canonical SMAD2/3 protein and multiple non-canonical effectors, such as SMAD1/5/8, Erk, JNK, Akt, and p38 [5]. Canonically, SMAD2/3-dependent TGF β signaling is mediated by the ALK5 type I receptor, and SMAD1/5/8-dependent TGF β signaling is mediated by the ALK1 type I receptor, illustrating one mechanism of effector selection in TGF β signaling [5]. Once activated, phosphorylated SMAD3 (pSMAD3) forms a complex with the co-Smad SMAD4 and translocates to the nucleus where it binds directly to Smad-binding elements in the promoter, or interacts indirectly with other transcription factors, to alter gene expression [6].

TGF β signaling exhibits exquisite internal control through negative feedback by a variety of mechanisms. Inhibitory Smads *SMAD6* and *SMAD7* are induced by TGF β , activin, and BMP signaling and regulate signaling by competitively interfering with the receptor-Smad complex to prevent Smad activation [7]. Additionally, *SMAD6* and *SMAD7* inhibit TGF β signaling by recruiting Smurf E3 ubiquitin ligases, SMURF1 and SMURF2, which ubiquitinate the TGF β receptors and Smads, tagging them for degradation [7]. Multiple other mechanisms of transcriptional and post-transcriptional control, including targeting components of the TGF β pathway through microRNAs (miRNAs), sumoylation, and regulated localization, also tune the functional activity of

this pathway [5, 7]. Another level of complexity in effector regulation is that, in certain contexts, non-canonical effectors can themselves regulate the activity of SMAD2/3, thus impacting downstream signaling and gene expression [2].

Here, we focus on the function of TGF β in skeletal cells and tissues in joint health and disease. Spatial and temporal control of TGF β signaling is exerted at multiple levels of the pathway and is context dependent. Disrupting this balance can lead to joint degeneration.

Overview of Joint Health and Disease

While there are a variety of joint diseases, this review will focus primarily on the impact of TGF β signaling in OA. OA is a chronic and debilitating joint disease that diminishes mobility and causes severe pain. OA affects 30 million Americans [8] and is a leading cause of disability globally [9]. There is no currently available pharmacologic agent that can delay or prevent the development of OA, and end-stage OA often deteriorates to the point that a joint replacement is necessary. Given the dearth of treatment options for those suffering from OA, there is a pressing need to understand the cellular mechanisms that contribute to joint homeostasis. As mentioned previously, hierarchical regulation of the TGF β signaling pathway indicates that differential function in various levels of the pathway could result in dysregulated TGF β signaling (Fig. 1). Importantly, either diminished or excessive TGF β signaling can perturb joint health.

One genetic syndrome vividly demonstrates the importance of the TGF β pathway on joint health. Aneurysms osteoarthritis syndrome (AOS) is a familial syndromic form of aortic aneurysm and early-onset OA that is the result of loss-of-function mutations of *SMAD3*. For example, one familial form involves a truncating mutation that removes the MH2 domain, while another familial form involves missense mutations that result in conformational changes of the SMAD3 protein, disrupting protein trimerization [10, 11]. Other musculoskeletal anomalies can also be present in patients with AOS, including intervertebral disc degeneration, osteochondritis dissecans, and meniscal anomalies [11]. Most mutations that lead to AOS are located in the MH2 domain, which is important for SMAD association with TGF β receptors, SMAD3/SMAD4 oligomerization, and SMAD-dependent transcriptional control [11]. Despite the loss-of-function mutation in *SMAD3*, patients with AOS paradoxically demonstrate increased total levels of SMAD3 protein, excessive activation and nuclear localization of SMAD2 and SMAD3, and higher levels of TGF β 1 in the aortic wall [10]. Taken together, these observations suggest a role for enhanced TGF β signaling in AOS. While the level of TGF β and downstream effectors was not assessed in musculoskeletal tissues, it is possible that early-onset OA

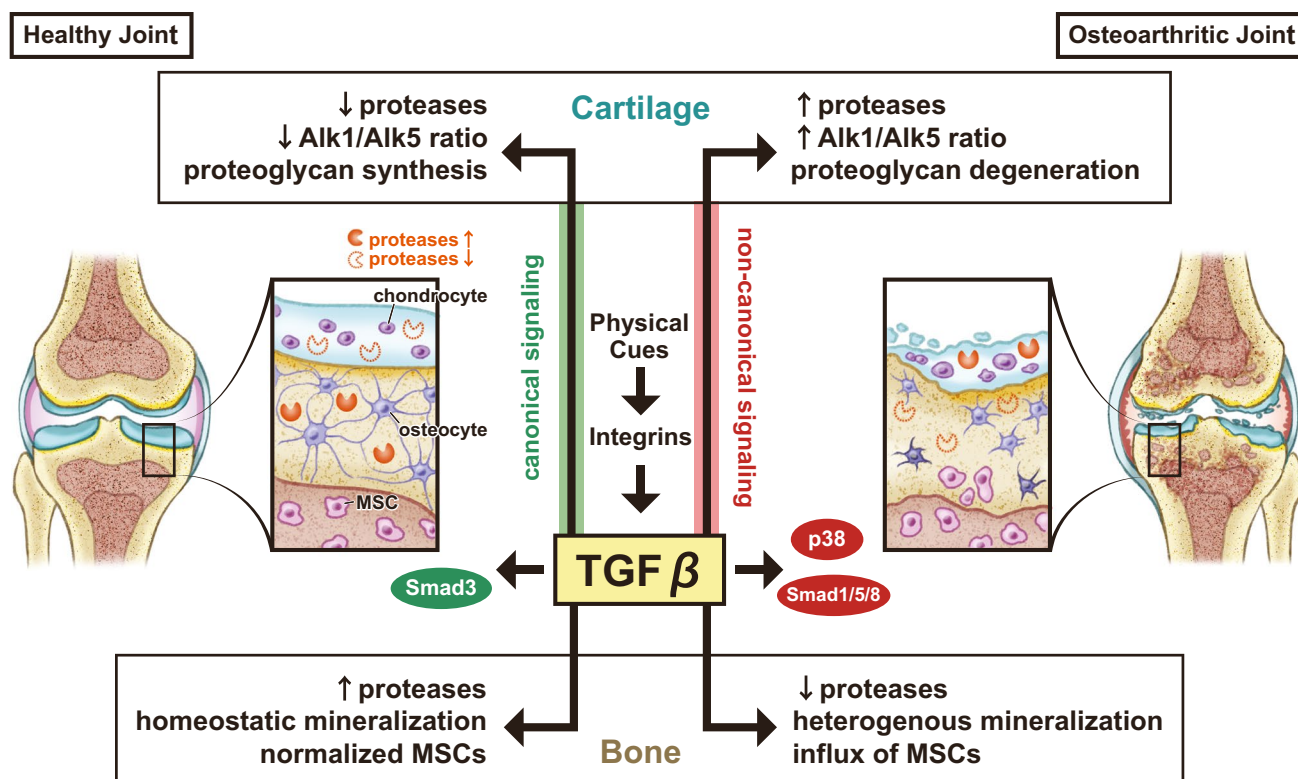


Fig. 1 Synovial joint homeostasis relies upon intricate control of TGF β signaling across multiple joint tissues. Disruption of the complex hierarchical regulation of TGF β signaling, whether through physical cues, receptor recruitment, or effector selection, impairs synovial joint health and results in joint degeneration in a tissue-

specific manner. Proteases, such as MMP13, have differential TGF β -dependent regulation within each tissue in joint disease, such that elevated proteases in subchondral bone improve bone quality and joint health, yet elevated proteases in cartilage leads to cartilage degeneration

in these patients coincides with excessive TGF β signaling in the joint.

Dysregulated TGF β signaling has been found in various synovial joint tissues in OA. Bone from patients with OA exhibits increased expression of TGF β 1 ligand and *TGFBI* and *SMAD3* gene expression, even at sites distant to joint degeneration [12–14]. Other changes observed in OA bone include altered collagen I production, *RUNX2* expression, osteocalcin, and WNT signaling targets, each of which plays a role in osteoblast differentiation [12, 14, 15]. In human cartilage, *SMAD3* mRNA expression is higher in OA cartilage than in non-arthritic control tissue [16], providing evidence that in some cases, the TGF β /SMAD3 pathway may be overactive in OA cartilage leading to degeneration. In synovium and synovial fluid from patients with OA, elevated levels of TGF β 1 ligand are a strong predictor of OA progression [17].

Genome-Wide Association Studies (GWAS) provide compelling evidence of the multi-level genetic role of TGF β signaling in joint disease. Single nucleotide polymorphisms (SNPs) in introns or exons of the *SMAD3* gene have been associated with increased risk of OA [18–21]. More specifically, a missense mutation of an exon of the *SMAD3* gene correlates with increased serum levels of

MMP2 and MMP9, enzymes that play a role in articular cartilage degeneration [18]. Genetic variation in intronic SNPs of *SMAD3* also increases risk of hip OA and knee OA, although the specific function of the SNPs is unclear given their intronic location in the *SMAD3* gene [19–21]. In concert with the increased risk of OA with *SMAD3* mutations, *SMAD3* may have a pleiotropic effect on bone mineral density [20]. At the level of the ligand, a SNP within the *LTBP3* gene, encoding a latent TGF β binding protein that can regulate the availability and activity of TGF β ligand, was associated with increased risk and clinical severity of hip OA [22]. Noncoding mutations in *GDF5*, a member of the TGF β super family, or p38 MAP kinase-mediated signaling, a “non-canonical” effector of the TGF β pathway, are causal variants in OA development [23••]. A single variant within the *TGFBI* gene has been postulated as causal for OA development, a finding that coincides with significant genome-wide enrichment for other genes implicated in TGF β -signaling, such as *LTBP1*, *LTBP3*, *SMAD3*, and *RUNX2* [24••]. Evidence of a genetic basis for TGF β mutations in OA development motivates the mechanistic study of TGF β in joint health to identify appropriate therapeutic targets.

Mechanoregulation of TGF β Signaling

At the cellular level, changes in the mechanical micro-environment can alter the effect of TGF β signaling at different hierarchical levels of the signaling pathway in each joint tissue (Table 1) [25]. Cellular forces resulting from changes in ECM stiffness or cytoskeletal tension can directly activate the TGF β ligand, rendering it available for downstream signaling [26]. Colocalization of TGF β receptors T β RI and T β RII to form heteromeric complexes capable of activating downstream signaling is sensitive to cytoskeletal tension in chondrocytes and fluid flow shear stress in osteocytes [27, 28]. Similarly, cytoskeletal tension primes the activity of the chondrogenic transcription factor SOX9 in response to TGF β or mechanical stimulation [29]. A discrete cell substrate stiffness that is consistent with the physiologic stiffness of healthy articular cartilage stimulates maximal TGF β -dependent SMAD3 phosphorylation, nuclear localization, and transactivation of chondrogenic genes; substrates that are more or less stiff than healthy articular cartilage diminish the ability of TGF β signaling to induce chondrogenesis [30].

Joint loading presents a tissue-level mechanical cue that regulates the function of TGF β ligand to modulate metabolic activity of chondrocytes. Both the superficial zone chondrocytes and the synoviocytes secrete latent TGF β into the synovial fluid, which becomes activated with joint motion, and accumulates in the superficial zone of articular cartilage [31]. Mechanical shearing within the joint activates latent TGF β 1 in the synovial fluid, rendering it available to stimulate TGF β signaling in chondrocytes [32]. Though active TGF β accumulates in the superficial zone, it is unable to penetrate deeper into middle and deep zones of cartilage [31]. In this way, superficial

chondrocytes may receive adequate active TGF β from synovial fluid to maintain homeostatic TGF β signaling. In fact, physiologic dynamic compression of devitalized cartilage explants does not directly activate ECM-bound latent TGF β in the deep zone of articular cartilage [33]. Therefore, activation of local TGF β stores within the deep zone requires chondrocyte-dependent mechanisms. Taken together, articular cartilage relies upon synovial and cartilage-derived TGF β , activated by mechanical and chondrocyte-dependent mechanisms, to sustain TGF β signaling across the different articular cartilage zones.

Both physiologic and excessive loading of articular cartilage results in rapid induction of the canonical TGF β signaling pathway [34]. In response to dynamic compression of either physiologic or excessive force, articular cartilage explants increase the mRNA levels for established TGF β -inducible genes *Serpine1* and *Smad7*, and production of *Tgfb1* mRNA through an ALK5-dependent mechanism, demonstrating that TGF β signaling and TGF β production respond to cartilage loading [34]. The loss of compressive loading of cartilage explants results in a rapid decrease in TGF β signaling, as shown by the reduction of pSMAD2 in chondrocytes and decreased expression of TGF β -responsive genes *Serpine1*, *Smad7*, and *Alk5*, that is restored upon re-loading [35]. As a consequence of the unloading-dependent loss of TGF β signaling, increased expression of *Col10a1* mRNA, a marker of chondrocyte hypertrophy, is observed, suggesting a TGF β -dependent mechanism that couples joint loading to cartilage homeostasis [35].

In response to load in either cartilage or bone, relative *Alk5* expression increases, but the consequences of this change for joint homeostasis depend upon the cell compartment. In cartilage, loading reduces *Alk1* expression and increases *Alk5* expression to support canonical TGF β signaling, unveiling one mechanism by which the balance between

Table 1 Mechanoregulation of TGF β signaling in the synovial joint

	Reference
Bone	
Colocalization of T β RI and T β RII in osteocytes is sensitive to fluid shear stress	[28]
Loss of osteocytic T β RII impedes subchondral bone response to changes in load	[43•]
Cartilage	
Colocalization of T β RI and T β RII in chondrocytes is sensitive to cytoskeletal tension	[27]
Chondrogenic activation of SOX9 in response to TGF β is sensitive to cytoskeletal tension	[29]
ECM stiffness affects chondrogenic potential of TGF β signaling	[30]
Joint motion activates latent TGF β in superficial zone of articular cartilage	[31]
Cartilage loading reduces <i>Alk1</i> and increases <i>Alk5</i> mRNA expression	[34, 35]
Aged cartilage has reduced ability to induce canonical TGF β signaling with load	[38]
Synovium	
Mechanical shearing activates synovial fluid latent TGF β 1	[32]
Mesenchymal stem cells	
Changes in load increases ALK5 in subchondral bone MSCs without increasing ALK1	[39]

canonical and non-canonical TGF β signaling in cartilage is preserved [34, 35]. In aging cartilage, the relative ratio of ALK1/ALK5 receptors is increased, thus favoring a non-canonical TGF β signaling pathway [36, 37]. In response to either physiologic or excessive mechanical load, aged cartilage has a reduced ability to induce canonical TGF β signaling, with reduced overall levels of phosphorylated SMAD2 and diminished nuclear localization of phosphorylated SMAD2 [38]. Thus, the ability of loading to sustain chondrocyte homeostasis may decline with age, and other avenues to promote cartilage health may become relevant, while canonical TGF β signaling becomes less favored. Interestingly, perturbed joint loading due to transection of the anterior cruciate ligament (ACL) increases ALK5 in subchondral bone mesenchymal stem cells (MSCs) without increasing ALK1 [39]. The extent to which the MSC response to ACL injury reflects cellular exposure to excessive or diminished loads, or a cell type-specific response to altered joint loading, remains to be determined.

Subchondral bone, likewise, is dynamically loaded during joint motion and provides mechanical support to the overlying cartilage. The load-induced response of bone is largely mediated by osteocytes, the primary mechanosensors of bone [40]. Osteocytic TGF β signaling is required for bone mechanosensation, as well as the control of bone quality [41, 42]. We found that increased loading on the

articular cartilage and underlying subchondral bone, as a result of meniscal injury in mice, represses the expression of T β RII in subchondral bone plate osteocytes [43•]. Likewise, subchondral bone from mice with a genetic deficiency in osteocytic T β RII fails to respond to injury-induced load. Therefore, TGF β signaling is both regulated by and required for osteocytic mechanosensitivity [43•]. In the same model of osteocytic TGF β deficiency, articular cartilage deteriorates, suggesting that the mechanosensitive function of the subchondral bone plate requires osteocytic TGF β signaling in order to maintain the health of cartilage [43•].

Tissue-Specific Function of TGF β Signaling in Joint Disease

TGF β plays a tissue-specific role in joint homeostasis and joint disease, contributing to the sophisticated function of TGF β in the joint (Table 2). For example, inhibiting TGF β within the joint can prevent osteophyte formation but simultaneously exacerbate cartilage degeneration [44]. Tissue-level evidence in clinical specimens offers insight into the role of TGF β in joint disease, and transgenic mouse models of joint disease with a tissue-specific ablation of key receptors or effectors within the TGF β signaling pathway provide

Table 2 Tissue-specific differences of TGF β signaling leading to OA

		Reference
Bone		
Excessive:	Increased <i>TGFβ1</i> mRNA, TGF β 1 ligand, and <i>SMAD3</i> mRNA in human OA bone	[12–14]
	Increased <i>TGFβ1</i> mRNA, TGF β 1 ligand, and <i>SMAD3</i> mRNA in primary OA osteoblasts	[45–49]
	Camurati-Engelmann disease with excessive levels of TGF β 1 secreted by osteoblasts develop OA	[39]
Repressed:	Loss of osteocytic T β RII leads to cartilage degeneration	[43•]
Cartilage		
Excessive:	Increased TGF β 1 and β 2 ligands in human OA cartilage	[58]
	<i>SMAD3</i> mRNA higher in human OA cartilage	[16]
Repressed:	Loss of chondrocyte-intrinsic SMAD3, T β RII, or ALK5 in mice induces OA	[71–73]
	Downregulation of TGF β ligand, reduced TGF β -induced proteoglycan synthesis, and decreased T β RI and T β RII in aging cartilage	[85, 91]
Altered canonical/ non-canonical TGF β signaling:	Increased ALK1/ALK5 receptor ratio increases MMP13 in OA cartilage	[36]
	T β RI competitively antagonizes BMP signaling	[67•]
	SMAD1/5/8 signaling promotes hypertrophic chondrocyte differentiation during chondrogenesis	[74]
	Increased ALK1/ALK5 receptor ratio in aging murine and bovine cartilage	[36, 37]
Synovium		
Excessive:	Increased TGF β ligand in synovial fluid induces OA	[17, 64–66]
Repressed:	Reduced TGF β signaling due to dominant-negative mutation of T β RII exhibit synovial hyperplasia	[68]
	Synovial fluid TGF β ligand antagonizes degenerative effects of IL-1 on cartilage	[87, 88]
Mesenchymal stem cells		
Excessive:	Excessive TGF β signaling in MSCs after injury results in OA	[39, 89]
Repressed:	Higher concentrations of T β RI inhibitor induce proteoglycan loss in cartilage	[39]

powerful tools for isolating the tissue- or cell-type-specific functions of TGF β in joint disease.

Bone-Intrinsic Role of TGF β in Joint Health and Disease

The association of altered bone mass and mineralization with OA progression [1], coupled with differences in expression of genes critical for TGF β signaling in OA bone [12–14], has led to an interest in understanding the role of TGF β in bone during joint degeneration. Disrupted TGF β signaling may participate in several possible mechanisms by which bone may contribute to cartilage degeneration in joint disease. These mechanisms include altering joint mechanics, disrupting nutrient and vasculature exposure through the osteocytic lacunocanalicular network, or changing levels of paracrine factors that disrupt cartilage health. All three bone-cell types — osteoblasts, osteoclasts, and osteocytes — are distinctly regulated by TGF β signaling in joint health and disease.

Studies of TGF β signaling in whole-bone samples from OA human joints have found increased TGF β ligand production and *TGF β 1* and *SMAD3* gene expression, relative to non-arthritic joints, even in bone specimens taken from sites distant from the OA joint [12–14]. Microarray gene expression of bone from OA patients demonstrates significant increases in expression of *SMAD3* [12]. These disruptions in TGF β signaling occur in the context of altered WNT/ β -catenin signaling [12, 14]. Taken together, the differential gene expression patterns in OA bone are consistent with perturbed osteoblast differentiation, altered bone formation, and heterogeneous mineralization. Given the mixture of bone-cell types within whole bone, it is necessary to further delineate the specific contributions of each cell type to TGF β signaling in joint homeostasis to better characterize the bone-intrinsic role.

The evaluation of primary osteoblasts isolated from human OA specimens supports an osteoblast-intrinsic role of TGF β in OA. Similar to findings in whole bone extracts, primary osteoblasts obtained from OA bone express increased levels of *TGF β 1* mRNA, TGF β 1 ligand, and *SMAD3* mRNA [45–49]. OA osteoblasts exhibit a TGF β -dependent shift from the normal ratio of *COL1A1*/*COL1A2* mRNA of approximately 2:1 to levels 2–threefold higher than that in normal osteoblasts, which blunts the ability of osteoblasts to generate a mineralized ECM in vitro [15, 47]. When TGF β 1 is inhibited, OA osteoblasts correct the abnormal *COL1A1*/*COL1A2* ratio at the mRNA level and mineralize normally [47]. In murine osteoblasts, *SMAD3* represses the function of *RUNX2* [50], a key regulator of osteoblast differentiation and mineralization. Increased *SMAD3* in human OA osteoblasts is associated with the calcium:phosphorous ratio in

bone mineral [45], likely through its regulation of *RUNX2*-dependent mineralization.

TGF β signaling in OA osteoblasts exhibits an epistatic relationship with the Wnt signaling pathway, an important regulatory pathway in bone formation [45, 48, 49]. TGF β antagonizes the Wnt signaling pathway in OA osteoblasts by increasing the mRNA expression of Dickkopf-2 (*DKK2*), which diminishes osteoblastic mineralization in response to BMP-2 [48]. Increased hepatocyte growth factor (*HGF*) mRNA and HGF protein in OA osteoblasts increase *TGF β 1* expression, which subsequently inhibits the Wnt signaling pathway and blunts BMP-2-dependent mineralization [49]. The loss of HGF, therefore, favors BMP-2 signaling through *SMAD1/5/8* and stimulates the canonical Wnt signaling pathway [49].

Camurati-Engelmann disease (CED) is a disease of poor bone quality due to excessive levels of TGF β 1. A mouse model of CED, in which the *Col1A1* promoter drives expression of *Tgfb1* with the point mutation H222D, results in higher levels of active TGF β 1 secreted by osteoblasts [39]. Coupled with the disrupted subchondral bone, these mice develop thin articular cartilage with relative hypocellularity, thick calcified cartilage, and excessive subchondral bone vascularity [39]. The subchondral bone in these mice exhibits increased levels of nestin-positive MSCs and osterix-positive osteoprogenitors, suggesting that excessive TGF β signaling alters the joint environment to increase recruitment of MSCs. This, in turn, increases the number of available osteoprogenitors [39]. This MSC-dependent mechanism illustrates how excessive TGF β secreted by subchondral bone osteoblasts can exacerbate OA.

On the other hand, inadequate subchondral bone TGF β signaling also has negative consequences for joint homeostasis. TGF β signaling in osteocytes is essential for function of these cells, regulating their ability to maintain the lacunocanalicular network through osteocytic perilacunar/canalicular remodeling (PLR) [41, 42]. In addition to their role in maintaining bone quality, osteocytes have recently emerged as cellular contributors to OA [43•, 51•]. More specifically, osteocytic TGF β signaling is required for the response of subchondral bone to joint injury [43•]. Without this key signaling pathway, subchondral bone homeostasis is disturbed, resulting in arthritic cartilage degeneration [43•].

As mediators of bone homeostasis, osteoclasts are inevitably involved in joint health. The altered mass and mineralization in OA subchondral bone suggest an imbalance in bone remodeling by osteoblasts and osteoclasts, for which TGF β is a critical coupling factor [52]. Osteoclasts can play a direct or indirect role on the effect of TGF β within the joint. Directly, osteoclasts require TGF β for osteoclastogenesis. Osteoclasts also locally control the level of active TGF β by liberating and activating latent TGF β from the bone matrix [53–55]. In an ACLT model of OA, osteoclast

suppression with alendronate reduces local TGF β activation, with a corresponding reduction in matrix metalloproteinase 13 (MMP13) in articular cartilage and osteophyte formation [56]. Indirectly, osteoclasts are sensitive to aberrant TGF β signaling within the joint because it can alter *Rankl* expression by osteocytes and interfere with osteoblast-osteoclast coupling during bone remodeling [41, 57]. Therefore, the activity of osteoclasts can impact the level of active TGF β signaling, and vice versa, with broader impacts throughout the joint.

Cartilage-Specific Role of TGF β in Joint Health and Disease

Human osteoarthritic cartilage shows a complex disruption of homeostatic TGF β signaling at various levels involving ligand, receptor, effector, and negative regulators, underscoring the importance of studying the hierarchy of altered TGF β signaling in OA. There are at least three ways that altered TGF β signaling in human cartilage is implicated in OA. First, excessive activity of the pathway is observed in human OA cartilage: the expression of genes encoding TGF β 1 and β 2 ligands is positively correlated with cartilage degeneration [58]. Likewise, *SMAD3* mRNA levels are significantly higher in OA cartilage, in a manner that is unexplained by DNA methylation in the *SMAD3* promoter region [16]. Second, there is a shift from canonical to non-canonical signaling: the mRNA expression of *ALK1* in human OA cartilage is correlated with chondrocytic *MMP13* mRNA expression, whereas *ALK5* mRNA correlates with aggrecan and type II collagen mRNA expression. Since *ALK1* preferentially activates *SMAD1/5/8*, and *ALK5* preferentially activates *SMAD2/3*, this suggests a role for TGF β receptor and effector selection in expression of genes that are protective or deleterious for cartilage [36]. Third, there is aberrant negative regulation of the signaling pathway: in human OA cartilage, higher levels of *SMURF2*, a multi-level negative regulator of TGF β signaling, can be observed compared to healthy cartilage [59]. *SMURF2* ubiquitinates *SMADs* and the *T β RI* receptor to promote degradation and reduce TGF β signaling. Therefore, high levels of *SMURF2* indicate a general reduction in TGF β signaling.

In addition to direct regulation of the signaling pathway, TGF β signaling can regulate, and be regulated by, microRNAs (miRNAs) to affect downstream signaling and OA gene expression. The expression of miR-140, for instance, is repressed by TGF β signaling and significantly reduced in human OA cartilage [60–62], whereas miR-455, which promotes TGF β /*SMAD3* signaling, is also repressed in OA cartilage [63]. Overall, these observations demonstrate an association of cartilage degeneration with aberrant TGF β signaling, whether by suppressed or excessive activity, a shift from canonical to non-canonical signaling, or altered

negative regulation. While these associations in human cartilage from end-stage OA show clinical relevance and motivate further study of TGF β signaling in chondrocytes, other approaches are needed to establish causal mechanisms that operate earlier in the disease process.

Findings in animal models reinforce the importance of an optimal level and type of TGF β signaling in the joint. Increased TGF β in the synovial fluid, whether by intra-articular administration of TGF β or overexpression of TGF β by the synovium, induces cartilage degeneration and osteophyte formation [64–66]. Elevated TGF β signaling activates a positive feedback loop that further increases expression of *Tgfb1*, *b2*, and *b3* mRNAs in the synovium and cartilage, relative to vehicle-injected controls [64–66]. Conversely, inhibition of endogenous TGF β by systemic injection of scavenging soluble *T β RII* prevents osteophyte formation but, likewise, induces proteoglycan degeneration in cartilage [44]. At the chondrocyte-level, although the expression of both receptors declines with age, the number of cells positive for *ALK5* in OA cartilage decreases more rapidly than those positive for *ALK1*. This consequently increases the *ALK1/ALK5* ratio and shifts the function from *ALK5*-dependent collagen II and aggrecan expression and, instead, favoring *SMAD1/5/8* signaling and *MMP13* production [36]. In cartilage development at the growth plate, *T β RI* competitively antagonizes *BMP* signaling mediated by *ALK1*, thus shifting the balance of TGF β signaling [67•]. Together, these results highlight the importance of an optimal quantity and quality of TGF β signaling, since excessive or inhibited TGF β activity, or a shift in the balance of canonical and non-canonical TGF β signaling, can exacerbate cartilage degeneration.

Transgenic mouse models with disrupted TGF β signaling in multiple tissues have likewise demonstrated the requirement of TGF β signaling in cartilage health. Mice with a dominant-negative mutation of the gene encoding *T β RII* in the articular cartilage, synovium, periosteum, and perichondrium exhibit a severe OA phenotype with substantial cartilage loss and osteophyte formation [68]. This mutation suppresses the responsiveness to TGF β ligand, thus promoting terminal differentiation of chondrocytes, reducing proteoglycan synthesis, and increasing type X collagen [68]. Mice with a global loss of canonical TGF β effector *SMAD3* similarly develop severe OA [69, 70]. Taken together, these two mouse models illustrate the chondroprotective role of TGF β in synovial joint tissues, motivating mechanistic study of the tissue-specific effects of TGF β in articular cartilage.

Because OA involves the coordinated disruption of multiple joint tissues, mouse models employing chondrocyte-intrinsic mutations in the TGF β signaling pathway can diminish the apparently confounding effects of TGF β signaling in joint crosstalk. Toggling this pathway experimentally by targeting the receptors, effectors, and negative regulators in chondrocyte-intrinsic models, for example,

by driving transgenic expression of Cre recombinase or other genes using a *Col2a1* promoter, isolates the relative chondroprotective nature of TGF β . As mentioned previously, SMURF2 levels are elevated in human OA cartilage, disrupting homeostatic TGF β signaling [59]. Mice with a chondrocyte-intrinsic overexpression of SMURF2 develop spontaneous OA with loss of articular cartilage, increased subchondral bone sclerosis, and increased mRNA expression of type X collagen and elevated levels of MMP13 protein in the articular cartilage [59], demonstrating the chondrocyte-intrinsic requirement of balanced TGF β signaling. Likewise, mice with a chondrocyte-intrinsic loss of *Smad3* develop OA and demonstrate decreased expression of genes encoding aggrecan and collagen II associated with increased MMP13 protein levels and enzyme activity in the articular cartilage, thus indicating that chondrocyte-intrinsic SMAD3 is essential for maintenance of healthy cartilage [71]. At the receptor level, chondrocyte-specific ablation of the gene encoding either T β RII [72] or ALK5 [73] promotes joint degeneration by inducing genes implicated in cartilage destruction. More specifically, the deletion of *Tgfr2* in chondrocytes exacerbates OA and induces *Runx2*, *Mmp13*, and *Adamts5* gene expression in articular cartilage [72]. Interestingly, the deleterious effects of chondrocytic *Tgfr2* ablation were mediated by activities of proteinases encoded by *Mmp13* and *Adamts5*, since deletion of either gene or treatment with an MMP13 inhibitor ameliorated the OA phenotype in this model [72]. Likewise, in chondrocyte culture, siRNA-mediated inhibition of T β RII upregulated *Mmp13* and *Col10a1* gene expression through a RUNX2-dependent mechanism [72]. Furthermore, chondrocyte-intrinsic deletion of *Alk5* resulted in a spontaneous OA phenotype with articular cartilage degradation, synovial hyperplasia, osteophyte formation, and subchondral bone sclerosis, with reduced SMAD3 phosphorylation and increased *Mmp13*, *Adamts5*, and *Col10a1* gene expression and elevated protein levels of MMP13 and ADAMTS5 [73].

Some of the effects of TGF β signaling in cartilage are explained by its ability to control chondrocyte differentiation. Prior to chondrogenesis, MSCs require both ALK5-dependent SMAD2/3 activity and ALK1-dependent SMAD1/5/8 activity, in concert with SMAD4, to induce *COL2A1* gene expression and subsequently produce increased protein levels of collagen II [74–76]. As chondrogenesis proceeds, the function of SMAD1/5/8 shifts to promote terminal hypertrophic chondrocyte differentiation, such that it inhibits collagen II production and increases MMP13, collagen X, and alkaline phosphatase [74]. On the other hand, SMAD3 acts to expand the pool of chondrocytes by stimulating their differentiation and preventing their hypertrophy [77–79]. In MSCs, TGF β increases the transcriptional activity of SOX9 and the gene expression of *COL2A1* [78]. Through SMAD2/3-dependent signaling, TGF β inhibits chondrocyte

hypertrophy [77, 78] and decreases expression of the genes encoding collagen X and other terminal differentiation markers [79]. TGF β tightly regulates its own signaling in chondrocytes, both by positive feedback that increases the expression of TGF β receptors and SMAD3 [80] and by negative feedback that can reduce mRNA stability of TGF β receptors, decrease SMAD3, and increase SMAD7 [80]. Decreased levels of TGF β signaling shift chondrocytes from expression of chondroprotective genes such as *SOX9* and *COL2A1* to hypertrophic genes such as *COL10A1* [80]. In addition to the availability of TGF β ligand and the relative balance of SMAD2/3 signaling, the chondrogenic response to TGF β depends on mechanical cues from the ECM [30], as mentioned previously.

Among the constituents of the TGF β pathway that control chondrocyte homeostasis, the most well studied is SMAD3, motivated by the association of human OA and *SMAD3* variants identified in GWAS [19–21]. In chondrocytes, SMAD3-dependent TGF β signaling promotes cartilage health by repressing RUNX2-inducible *Mmp13* and *Col10* gene expression and tempering BMP signaling [71, 72, 81], each through mechanisms that diminish non-canonical TGF β signaling. Chondrocytes exhibit a time-dependent response to exogenous TGF β , with an early repression of *Mmp13* mRNA and MMP13 protein requiring SMAD3 and a later induction of *Mmp13* mRNA and MMP13 protein requiring p38 [71]. Importantly, in the absence of SMAD3, non-canonical TGF β signaling through p38 is favored, inducing the cartilage-destructive enzyme MMP13 [71]. Primary chondrocytes from mice lacking SMAD3 show enhanced BMP-related gene expression by increasing *Smad1*, *Smad5*, *BMP2*, and *BMP6* mRNA levels, resulting in increased phosphorylated SMAD1/5/8 and *Col10* mRNA expression and accelerating chondrocyte maturation [81]. This aberrant signaling can be blocked by overexpressing *Smad2* and *Smad3* or inhibiting BMP signaling [81]. TGF β likewise counteracts the effects of MMPs and protects the local cartilage ECM by inducing expression of tissue inhibitor of metalloproteinases-3 (TIMP-3) through SMAD2/3-dependent and ERK1/2-dependent mechanisms [82, 83], illustrating the multiple avenues by which SMAD3 functions to promote chondrocyte homeostasis.

TGF β and Synovial Inflammation

As in cartilage, a balanced level of TGF β signaling in the synovium and synovial fluid is required for healthy joint homeostasis, with insufficient or excessive TGF β levels compromising joint health. The ability of TGF β to induce a suppressive or inflammatory immune response within the synovium is context-dependent [84]. The synovium is a source of TGF β ligand within the joint [32, 85], and the level of TGF β in synovial fluid correlates positively with

OA severity [17]. TGF β is also a key mediator of synovial hyperplasia in rheumatoid arthritis [86]. Either excessive or suppressed TGF β in the synovium or the synovial fluid can result in synovial hyperplasia. For instance, mice with reduced TGF β signaling throughout the joint due to a dominant-negative mutation of the gene encoding T β RII in multiple joint tissues exhibit synovial hyperplasia [68]. Excessive levels of TGF β within the joint, as a result of intra-articular injection of exogenous TGF β , likewise promote synovial hyperplasia [64]. On the other hand, synovial inflammation with joint degeneration involves production of inflammatory cytokines, such as interleukin (IL)-1. TGF β antagonizes the degenerative effects of IL-1 on cartilage [87, 88]. Together, TGF β and the synovium can participate in bidirectional feedback, where synovial production of TGF β can have broader effects on other joint tissues, and TGF β signaling in other joint tissues can affect the synovium.

Mesenchymal Stem Cells and TGF β in Joint Health and Disease

In addition to the role of subchondral bone osteoblasts, osteoclasts, and osteocytes in synovial joint homeostasis, there is evidence of an influx of bone marrow MSCs into the subchondral bone during OA progression, which induce angiogenesis and differentiate into osteoblasts to further perturb the subchondral bone environment [39]. Joint injury from ACL transection increases the level of nestin-positive MSCs in the subchondral bone marrow and osteoprogenitor clusters in the bone marrow [39, 89]. Systemic administration of a T β RI inhibitor can reduce the number of MSCs and osteoprogenitors, normalize the subchondral bone, and attenuate OA after injury [39]. The benefits of the T β RI inhibitor on joint health are tissue-dependent; higher concentrations of the T β RI inhibitor not only mitigate injury-induced changes in subchondral bone structure but also induce proteoglycan loss in cartilage, underscoring the tight control of TGF β that is necessary for joint health [39]. The relative benefits of inhibiting TGF β signaling within the subchondral compartment are mediated by nestin-positive MSCs. A nestin-positive MSC-specific ablation of the gene encoding T β RII recapitulates findings with the pharmacologic T β RI inhibitor, with protection of subchondral bone microarchitecture and cartilage proteoglycan levels after ACL transection [39].

With increased levels of TGF β in circulation after injury, targeting the TGF β signaling pathway at the level of the ligand, rather than receptor, could likewise normalize the aberrant environment after injury. Local treatment to the subchondral bone or systemic treatment with an inhibitor of TGF β 1, β 2, and β 3 reduces the number and mobilization of nestin-positive MSCs in the subchondral bone and diminishes angiogenesis after ACL transection [39, 89]. This effect is dose-dependent, such that lower and higher

concentrations lead to proteoglycan loss [89]. As discussed below, when considering the benefits of inhibiting excess TGF β signaling post-injury, care must be taken not to compromise the essential role of this growth factor in other joint cell types where it also supports joint homeostasis.

TGF β in Aging and OA

Aging can have broad effects on TGF β signaling in multiple joint tissues. Because the prevalence of OA increases with age, considering the shift in function of TGF β with age could identify a distinct age-related pathophysiology of cartilage degeneration. Furthermore, studying spontaneous age-related OA allows for the evaluation of joint degeneration in the absence of the substantial mechanical and inflammatory changes that occur with joint injury in post-traumatic OA models.

Among the many roles of TGF β in the joint is its interaction with IL-1, a pro-inflammatory cartilage destructive cytokine that is upregulated with age and following injury [90]. TGF β and IL-1 interact, such that TGF β can protect against the deleterious effects of IL-1 on proteoglycan synthesis [87, 88]. With age, however, IL-1-induced cartilage degeneration outpaces the protective effects of TGF β [87, 88, 91], mediated in part by nitric oxide production [88]. While TGF β blocks IL-1-induced nitric oxide production in young mice, TGF β is unable to induce the same response in old mice, likely due to decreased expression of TGF β receptors [88]. Interestingly, while TGF β shows protective effects on cartilage synthesis in the presence of IL-1, it exacerbates the inflammatory response, generating a severe synovitis, underscoring the importance of delineating the tissue-specific effects of TGF β [87]. A similar role for IL-1 and TGF β crosstalk has been shown in equine cartilage, where TGF β -induced proteoglycan synthesis is diminished by the addition of IL-1 [92].

Aging cartilage exhibits decreased levels of TGF β 1, β 2, and β 3 ligands, diminished TGF β -induced proteoglycan synthesis, and reduced the number of cells positive for T β RI and T β RII protein [85, 91]. This age-related suppression of TGF β ligands and receptors results in decreased phosphorylated SMAD2 without a reduction in overall SMAD2 expression, suggesting reduced active canonical TGF β signaling [91]. Furthermore, a mouse model of spontaneous OA demonstrated lower levels of TGF β 3 ligand and phosphorylated SMAD2 over the course of OA progression with a complete loss by 1 year of age, coinciding with an increase in protein levels of BMP-2 with age [85].

Across species, articular cartilage exhibits a shift from canonical to non-canonical TGF β signaling during aging [36, 37, 93]. In aging murine cartilage, increased ALK1/ALK5 ratio favors TGF β -induced SMAD1/5/8 signaling and

MMP13 production [36]. A similar increase in the ALK1/ALK5 ratio is observed in aged bovine cartilage [37], and aged chondrocytes from guinea pigs demonstrate a progressive shift of SMAD2/3 signaling to SMAD1/5/8 signaling [93], illustrating the age-related shift in TGF β function across multiple species. Aged bovine cartilage demonstrates reduced activation of SMAD2/3 signaling and nuclear localization in response to either stimulation with TGF β or mechanical activation [38]. These changes with age precede gross degeneration of cartilage and, therefore, may be early signs of OA [38]. Together, these findings emphasize the importance of the relative balance of canonical and non-canonical TGF β signaling in cartilage health.

Interactions of TGF β and Other Joint Tissues

Aging also impacts the TGF β -induced collagen production in ligament fibroblasts. With aging, the ability of medial collateral ligament (MCL)-derived fibroblasts to synthesize collagen in response to TGF β is diminished relative to young controls [94]. While overall collagen synthesis decreases at all doses in MCL-derived fibroblasts from older rabbits, the sensitization to TGF β is higher in aged animals, such that relative to controls lacking TGF β , collagen synthesis increases with increasing doses of TGF β [94]. These findings suggest that the diminished mechanical integrity and prolonged healing with injury of aged ligaments may be due to a weakened response to TGF β . Coupled with the increased sensitization to TGF β , treatment with TGF β could, thus, improve the mechanical stability of the ligament.

One area of interest in the intersection of TGF β and joint homeostasis is the extent of crosstalk with the nervous system. Nerve growth factor (NGF) is a key driver of the musculoskeletal pain response. Despite the lack of apparent sequence similarities, TGF β and NGF demonstrate similarities in topological structure that place them in a common growth factor superfamily [5]. Anti-NGF therapies in OA reduce knee OA pain [95] but increase the incidence of rapidly progressive OA [96]. Therefore, understanding the extent to which TGF β is involved in joint pain could provide further context for the complexity of targeting NGF.

Targeting TGF β for Treatment of Joint Disease

Careful consideration must be taken when developing TGF β -targeting treatments for OA due to the complex role of TGF β in joint health and disease. Treatment strategies need exquisite control of multiple factors, including optimal dose, tissue-specific effects, selection of downstream TGF β receptors and effectors, and local mechanical cues.

For instance, injury can induce excessive levels of TGF β ligand within the joint [39], suggesting that returning TGF β to pre-injury levels would encourage joint health. However, injury also represses osteocytic T β RII in the subchondral bone, and reduced levels of TGF β ligand may further reduce downstream osteocytic TGF β signaling [43•]. Furthermore, in aging cartilage, the relative balance of canonical and non-canonical TGF β signaling is disrupted [36, 37, 93], complicating the efforts of targeting this pathway by enhancing or inhibiting TGF β .

Therapeutics have been developed that target different levels of the TGF β signaling pathway in the clinical setting, primarily in the context of cancer or fibrotic disease [97, 98]. Recently, human chondrocytes virally transduced with a gene containing TGF β 1 have been employed in the setting of patients with OA in a phase II clinical trial. Patients with knee OA received an intra-articular injection of either placebo or transduced allogenic chondrocytes expressing TGF β 1, which significantly improved clinical pain scores [99, 100]. Further work is needed to fully uncover the relative benefit of delivering TGF β 1-producing chondrocytes, designed to be a cell-mediated cytokine gene therapy. Another critical consideration is the likelihood that OA originates through several distinct mechanisms, each of which may respond differently to changes in the level or type of TGF β signaling. Overall, the complex nature of the TGF β signaling pathway suggests that therapeutically regulating TGF β would require a precise understanding of the underlying etiology of OA and the specific disruption in TGF β to successfully use it as a therapeutic target of OA.

Conclusion

In conclusion, TGF β signaling plays a sophisticated function in maintaining healthy joint crosstalk that is non-linear and depends upon effector selection, physical and mechanical cues, and tissue-specific function to support joint health. Synovial joints facilitate smooth motion and load transfer through integrated function of multiple tissues, including articular cartilage, bone, ligaments, tendons, synovium, and menisci. Under normal conditions, the biological and mechanical activities of these tissues are exquisitely coordinated, yet in the setting of aberrant TGF β signaling, one or more of these joint tissues can deteriorate leading to overall joint destruction. Regulation of the TGF β signaling pathway can occur at different levels, and disrupting the homeostatic TGF β signaling at any level of the pathway can have broad effects through crosstalk among multiple joint tissues.

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Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards.

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