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TGF β Signaling in Cartilage Development and Maintenance

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Abstract

Members of the transforming growth factor beta (TGF β) superfamily of secreted factors play essential roles in nearly every aspect of cartilage formation and maintenance. However, the mechanisms by which TGF β s transduce their effects in cartilage *in vivo* remain poorly understood. Mutations in several TGF β family members, their receptors, extracellular modulators, and intracellular transducers have been described, and these usually impact the development of the cartilaginous skeleton. Furthermore, genome-wide association studies have linked components of the (TGF β) superfamily to susceptibility to osteoarthritis. This review focuses on recent discoveries from genetic studies in the mouse regarding the regulation of TGF β signaling in developing growth plate and articular cartilage, as well as the different modes of crosstalk between canonical and noncanonical TGF β signaling. These new insights into TGF β signaling in cartilage may open new prospects for therapies that maintain healthy articular cartilage.

Keywords

TGF β signaling; cartilage; development; maintenance

Introduction

Members of the transforming growth factor beta (TGF β) superfamily of secreted factors play essential roles in nearly every aspect of development, from the generation of germ cells, through gastrulation and organ formation, and into postnatal life. Mutations in several TGF β family members, their receptors, extracellular modulators, and intracellular transducers have been described, and these commonly impact the development of the cartilaginous skeleton. Furthermore, genome-wide association studies have linked components of the (TGF β) superfamily to susceptibility to osteoarthritis. This review focuses on recent discoveries from genetic studies in the mouse regarding the regulation of TGF β signaling in developing

growth plate and articular cartilage, as well as the different modes of crosstalk between canonical and noncanonical TGF β signaling. These new insights into TGF β signaling in cartilage may open new prospects for therapies that maintain healthy articular cartilage.

ENDOCHONDRAL BONE FORMATION

The skeleton is composed primarily of cartilage and bone. Throughout the axial and appendicular skeleton, with the exception of the skull, the skeleton is formed from a hyaline cartilage template. During development, cells from three distinct lineages (sclerotome, paraxial mesoderm, and neural crest) undergo chondrogenesis through a similar sequence of events to form the cartilage of the embryonic skeleton (Long and Ornitz, 2013; Pitsillides and Beier, 2011) (Fig. 1). The first overt sign of chondrogenesis is aggregation of mesenchymal chondroprogenitor cells into condensations. This process is mediated by elevated expression of various cell adhesion molecules, such as neural cadherin (N-cadherin) and neural cell adhesion molecule (NCAM). These molecules mediate crucial cell–cell interactions and are critical for maintenance of the expression of Sox9, the transcription factor currently known to act earliest in the chondrogenic program (Akiyama and Lefebvre, 2011).

GROWTH PLATE CARTILAGE FORMATION

Cells at the core of the condensations differentiate into chondrocytes. This involves a change in morphology from fibroblast-like to more spherical, along with a significant increase in synthesis of specific extracellular matrix (ECM) molecules. The Sox9 mediated transcriptional program continues, with the collaboration of the structurally related transcription factors Sox5 and 6, driving the expression of collagen types II, IX, and XI, and the major proteoglycan of cartilage, aggrecan (Karsenty et al., 2009; Kronenberg, 2003; Long and Ornitz, 2013). Cells at the periphery of the condensations retain a fibroblastic morphology, and continue to express type I collagen, giving rise to the structure known as the perichondrium (for review see, Karsenty and Kronenberg, 2009).

Chondrocytes in the cores of the condensations initially undergo rapid proliferation that leads to linear growth of the developing skeletal element. Subsequently, chondrocytes in the centers of the elements exit the cell cycle and execute a well-coordinated program of maturation. This ordered process of proliferation and differentiation leads to the formation of stratified zones of cells at different stages of the cell cycle, with continued expression of Sox9 throughout the resting and proliferating chondrocytes. From the ends of the element to the center, these zones include a layer of relatively quiescent cells (resting zone) that exhibit a round cell morphology, a zone of rapidly proliferating cells that have a more flattened morphology and form stacks (columnar or proliferative zone), a zone of postmitotic cells that begin to enlarge and are characterized by the expression of *Indian Hedgehog* (*Ihh*) and decreased expression of *Sox9* (prehypertrophic zone), and a zone of terminal enlarged chondrocytes (hypertrophic zone). Most of these undergo cell death, leaving an ECM that is replaced by bone-forming osteoblasts (Shapiro et al., 2005). Hypertrophic chondrocytes produce a unique mineralized ECM containing type X collagen. These cells also produce matrix metalloproteinase 13 (MMP-13), which modifies the ECM to facilitate vascular

invasion. The invading vasculature permits the entry of osteoprogenitors, which differentiate into osteoblasts. These cells build the bone matrix and subsequently replace the cartilage.

ARTICULAR CARTILAGE FORMATION AND MAINTENANCE

Unlike growth plate cartilage, which is eventually replaced by bone in most species, articular cartilages are permanent structures. Articular cartilage is formed during embryonic stages at sites of joint formation, but is not replaced by bone and instead remains and develops during postnatal stages of growth (Chan et al., 2012). Articular cartilage is distinct from growth plate cartilage in terms of ECM content, cellular organization, and mechanical properties (Iwamoto et al., 2013). Briefly, there are fewer cells in this structure, and they are embedded as solitary cells within a distinct ECM that contains more collagen crosslinks than in growth plate cartilage. Mature articular cartilage has a zonal organization that is divided into a superficial layer, a mid layer, the deep layer, and the calcified layer, in order from the surface of articular cartilage toward the bone (Las Heras et al., 2012; Poole, 2003).

Within this cartilage, through mechanisms that are not well understood, the nonhypertrophic Sox trio (Sox9/5/6) program is maintained and chondrocyte differentiation is blocked, resulting in a permanent cartilage residing at the end of the long bones. However, during osteoarthritis, articular chondrocytes lose their inactive phenotype, and undertake hypertrophic chondrocyte terminal differentiation, thus expressing markers ColX and MMP-13.

The different morphologies and functions of cartilage across the lifespan are supported by the differences in proliferation and differentiation of chondrocytes, tightly controlled by many cytokines and their intracellular signaling pathways. These important cytokines include the transforming growth factor-beta (TGF β) superfamily, Wnts, Hedgehog, Notch, and FGFs (Gao et al., 2013; Long and Ornitz, 2013; Pan et al., 2008).

OVERVIEW OF BMP/TGF β SIGNALING

The TGF β superfamily consists of two subfamilies. The TGF β subfamily includes TGF β s (1, 2, and 3), Activin (A and B), Nodals, myostatin (GDF-8), and Mullerian inhibiting substance. The bone morphogenetic protein (BMP) subfamily consists of BMPs 2, 4–10, and the growth and differentiation factors (GDFs) (Gordon and Blobe, 2008; Guo and Wang, 2009; Hinck, 2012). Ligands are usually assigned to either of these subfamilies based on the utilization of the downstream signaling mediators known as the receptor-regulated Smad proteins (R-Smads). Members of the TGF β subfamily usually transduce signals through R-Smads 2 and 3, while members of the BMP subfamily transduce signals through R-Smads 1, 5, and 8 (Burks and Cohn, 2011; Weiss and Attisano, 2013). Smad4 is a cofactor that forms a complex with the activated R-Smads from both groups; it is thought to be essential for canonical signaling. The third group includes the inhibitory Smads 6 and 7 (I-Smads), which act as inhibitors on the BMP and TGF β signaling cascade by various mechanisms (Song et al., 2009).

TGF β ligands initiate signaling cascades across the cell membrane by binding and assembling a receptor complex on the cell surface (Fig. 2). These complexes are assembled

from serine/threonine kinase types I and II receptors (Hinck, 2012; Massague, 2012). Upon ligand binding, the type II receptor is activated and transphosphorylates the type I receptor (Song et al., 2009). The type I receptors are termed ALKs (activin receptor-like kinases), of which seven have been discovered (Hinck, 2012; ten Dijke et al., 1994; van der Kraan et al., 2009). ALK 1, 2, 3, and 6 bind BMPs and signal via the R-Smads1/5/8; ALK 4, 5, and 7 bind activins and TGF β s and signal through R-Smads2/3 (Hinck, 2012; Massague, 2012b; Weiss and Attisano, 2013). ALK5 is the canonical type I receptor for TGF β s and activates Smads2/3. However, TGF β can also bind to ALK1 and ALK2 in some cell types to activate R-Smads 1,5,8, thus activating the BMP pathway (van der Kraan et al., 2009). There are five type II receptors, including T β RII, ActRII, ActRIIb, BMPRII, and MISRII (Hinck, 2012; Weiss and Attisano, 2013).

Upon receptor activation, TGF β /BMPs can signal through canonical and noncanonical pathways (Qiao et al., 2005; ten Dijke et al., 2002; Yoon et al., 2004). In the canonical pathway, activated type I receptors trigger phosphorylation of specific R-Smads, which then complex with Smad4, and translocate into the nucleus to direct transcriptional responses in combination with other gene specific transcription factors (Song et al., 2009). In addition, TGF β /BMPs signal through a variety of noncanonical, Smad-independent avenues, utilizing MAP kinases, TAK1, RhoA, and mTOR pathways (Moustakas and Heldin, 2005; Mu et al., 2012; Yamaguchi et al., 1995; Yonekura et al., 1999; Zhang, 2009).

TGF β Signaling in Cartilage Development

TGF β s play critical roles in regulating chondrocyte differentiation from early to terminal stages, including condensation, proliferation, terminal differentiation, and maintenance of articular chondrocytes (Li et al., 2005; Serra and Chang, 2003; Serra et al., 1997; van der Kraan et al., 2009; Yang et al., 2001). All three TGF β isoforms are expressed in mesenchymal condensations. TGF β 3 is highly expressed in ribs and vertebral cartilage, whereas TGF β 1 and TGF β 2 expression is barely detected (Pelton et al., 1991). Levels of expression of all of these ligands are reduced at later stages of development in cartilage (Pelton et al., 1990, 1991). In the perichondrium, TGF β 3 is expressed at higher levels than other TGF β s (Pelton et al., 1990, 1991). In appendicular growth plates, TGF β 1 and TGF β 3 are expressed mainly in the proliferative and hypertrophic zones, whereas TGF β 2 is expressed in all zones, but at its highest levels in the hypertrophic zone (Horner et al., 1998; Millan et al., 1991; Sandberg et al., 1988; Thorp et al., 1992).

TGF β S IN PRECHONDROCYTE CONDENSATION

There is a considerable amount of in vitro evidence to indicate that TGF β signaling pathways promote mesenchymal condensation. In vitro data demonstrate that TGF β 1 induces mesenchymal cell condensation via up-regulation of N-cadherin and fibronectin (FN) (Song et al., 2007; Tuli et al., 2003). TGF β 1 treatment initiates chondrogenesis of mesenchymal progenitor cells (Tuli et al., 2003). TGF β 2 and TGF β 3 are even more effective, causing a twofold greater accumulation of glycosaminoglycan (Barry et al., 2001). However, knockouts for individual TGF β ligands do not exhibit phenotypes that support an essential role for TGF β s in condensation in vivo. *Tgfb1* null mice that survive to birth do not exhibit any skeletal defects, but die from diffuse inflammation (Shull et al., 1992). However,

at least 50% of *Tgfb1*^{-/-} embryos die prior to the onset of skeletal development (Dunker et al., 2001), raising the possibility that this ligand could play a role in early skeletal patterning on specific genetic backgrounds. This possibility can now be addressed because a conditional allele for TGFβ1 has been generated (Azhar et al., 2009).

If TGFβs play an essential role in condensation in vivo, this should be seen in mice deficient for the TGFβ receptor ALK5, as this is the primary receptor that transduces TGFβ signals. *Alk5*^{-/-} mice die at midgestation, exhibiting severe defects in vascular development in the yolk sac and placenta that preclude an analysis of skeletal formation (Larsson et al., 2001). Conditional ablation of *Alk5* using *Dermo1-Cre*, which targets skeletal progenitor cells prior to condensation, results in cartilage malformation and short limbs (Matsunobu et al., 2009). In these mice, chondrocytes proliferate and differentiate, but ectopic cartilaginous tissues protrude into the perichondrium, a phenotype related to the abnormally thin perichondrial layer. These and other studies in which the impact of TGFβ signaling has been investigated at early stages of chondrogenesis in the mouse are discussed in greater detail below, but in vivo genetic data demonstrating an essential role for TGFβ signaling in condensation stages are lacking.

TGFβ SIGNALING IN JOINT FORMATION

Several studies have revealed an essential role for TGFβ signaling through the type II receptor TβRII in appendicular and axial joint formation. Constitutive deletion of *Tgfb2* causes defects in yolk sac hematopoiesis and vasculogenesis, resulting in embryonic lethality around 10.5 days of gestation (Oshima et al., 1996). In *Tgfb2;Prx1-Cre* mice, where the *Prx-1* limb enhancer drives Cre recombinase expression in limb mesenchyme beginning at E9.5 and prior to condensation, loss of TβRII signaling resulted in the absence of interphalangeal joints (Spagnoli et al., 2007). The chemokine MCP-5 was recently identified as a key target for TβRII in the joint; TGFβ signaling was shown to be required to down-regulate MCP5 expression in joint interzone cells, to prevent the acquisition of a chondrogenic fate (Longobardi et al., 2012).

There are some similarities in defects in *Alk5;Dermo1-Cre* and *Tgfb2;Prx1-Cre* conditional knockout mice. Both *Dermo1-Cre* and *Prx1-Cre* are expressed in mesenchymal progenitors, and in each case, the mice develop short-limbed dwarfism, abnormal sternums, and defects in joint formation (Seo and Serra, 2007; Spagnoli et al., 2007), suggesting that TGFβRII and ALK5 work together, likely in a complex, in mesenchymal progenitor cells. However, there is a distinction as to where joint fusion defects arise in these two mutants. *Tgfb2;Prx1-Cre* mice develop fused phalangeal joints, while *Alk5;Dermo1-Cre* mice showed normal phalangeal joints, but partially fused knee joints (Matsunobu et al., 2009). In addition, there was a decrease in chondrocyte proliferation and a delay of late hypertrophic differentiation in *Tgfb2;Prx1-Cre* mice that was not seen in *Alk5;Dermo1-Cre* growth plates (Matsunobu et al., 2009). The phenotypic differences in these two mouse models may result from the differences in *Prx1-Cre* and *Dermo1-Cre* expression patterns. *Prx1-Cre* is expressed earlier than *Dermo1-Cre* in limb mesenchymal progenitors (Rodda and McMahon, 2006). It is also possible that the expression levels of *Dermo1-Cre* and *Prx1-Cre* differ in mesenchyme progenitors, perichondrial cells, and chondrocytes. Finally, as discussed below, the

differences between *Alk5* and *Tgfb2* mutant mice may reflect the fact that these two receptors do not have to work together in all tissues to transduce TGF β signals.

TGF β SIGNALING IN THE GROWTH PLATE

As discussed above, mice lacking TGF β 1 do not exhibit an obvious growth plate phenotype (Kaartinen and Heisterkamp, 1995; Shull, 1992). Similarly, while loss of TGF β 3 leads to perinatal lethality, defects in chondrogenesis are not observed (Kaartinen and Heisterkamp, 1995; Proetzel, 1995). However, mice lacking TGF β 2 present with generalized chondrodysplasia that appears to have an onset at late gestation stages (Sanford, 1997). Furthermore, it has been shown that loss of TGF β 2 (but not loss of TGF β 3) prevents the ability of exogenous Shh (sonic hedgehog) to block hypertrophic differentiation in ex vivo metatarsal cultures (Alvarez, 2002). In summary, of the three TGF β ligands, the evidence suggests that TGF β 2 may be the predominant one impacting chondrogenesis in vivo.

The evidence for a role for TGF β signaling in the growth plate is clearer at the level of the receptors, although these roles are still not well understood. The majority of loss-of-function studies have been conducted using targeted deletions or overexpression of dominant-negative forms of *Tgfb2* (the gene encoding T β RII). *Tgfb2;Prx1-Cre* growth plates display elevated Indian hedgehog (Ihh) expression, along with a delay in the onset of hypertrophy and decrease in levels of *Col10a1* expression in hypertrophic cells (Spagnoli et al., 2007). This result is more severe than but consistent with the phenotype resulting from blockade of TGF β signaling by the overexpression of a dominant negative form of T β RII (dnT β RII) in condensing mesenchymal cells and chondrocytes; this resulted in absence of hypertrophic chondrocytes at 14.5 dpc, suggesting impaired hypertrophic chondrocyte differentiation (Hiramatsu et al., 2011).

Interestingly, in contrast to the above results where deletion or transgenic overexpression of T β RII occurs prior to the onset of formation of differentiated (*Col2a1*-expressing) chondrocytes, conditional deletion of the T β RII in *Col2a1*-expressing chondrocytes did not lead to obvious defects in appendicular elements (Baffi et al., 2004). These findings strongly suggest that T β RII transduces TGF β signaling at pre-chondrogenic stages, but may not have as substantial a role at later stages. Consistent with this speculation, defects in *Tgfb2;Col2a1-Cre* mice were restricted to axial elements, where defective segmentation and formation of intervertebral discs was found (Baffi et al., 2004). In axial elements, *Col2a1* is activated in somites, considerably earlier than the onset of *Col2a1* expression in chondrocytes within appendicular elements.

Given the lack of severe cartilage phenotypes in appendicular elements when *Tgfb2* is ablated in differentiated chondrocytes, the question arises as to whether TGF β signaling plays a substantial role in growth plate chondrocytes in vivo. This would be best addressed by studies in which the type I receptor ALK5 is ablated. ALK5 clearly has an important role in the formation of the perichondrium, as revealed in the *Alk5;Dermo1-Cre* mice discussed above (Matsunobu et al., 2009). However, the role, if any, of ALK5 in growth plate chondrocytes has not yet been investigated in vivo.

Investigating the role of TGF β signaling at the level of ALK5 gains importance because it was shown recently that T β RII may be dispensable for TGF β signaling in some settings; TGF β ligands are able to elicit signals in T β RII (*Tgfr2*) mutant mice (Iwata et al., 2012). Loss of *Tgfr2* in cranial neural crest cells results in elevated expression of TGF β 2 and the type III TGF β receptor (T β RIII; also known as betaglycan). T β RIII can keep TGF β at the cell surface and can promote signaling by presenting ligand to the T β RI/T β RII complex (Shi and Massague, 2003). However, Iwata et al. (2012) demonstrated the existence of an ALK5/T β RIII-mediated, T β RII-independent signaling pathway that was essential for proliferation in the palatal mesenchyme. In palatal mesenchyme, T β RI/T β RII guides TGF β signaling through Smad2 and Smad3, while T β RI/T β RIII transduces a signal through the noncanonical TAK1/p38 pathway (Bernabeu et al., 2009; Iwata et al., 2012). Of interest, T β RIII has high affinity for TGF β 2, but not for TGF β 1 or TGF β 3. Thus, utilization of this T β RI/T β RIII pathway may explain why skeletal phenotypes are observed in TGF β 2-deficient mice, but not in mice lacking TGF β 1 or TGF β 3, and why loss of T β RII does not exhibit a strong phenotype in growth plate chondrocytes. Finally, it has been reported that ALK5 can form a complex with other type II receptors, such as ACTRII (Andersson et al., 2006; Rebbapragada et al., 2003; Tsuchida et al., 2008; Wu et al., 2003). Whether TGF β relays signals through ALK5/T β RIII or ALK5/ACTRII complexes during chondrogenesis warrants further investigation.

TGF β SIGNALING IN POSTNATAL/ARTICULAR CARTILAGE

In postnatal cartilage homeostasis, TGF β s act as inhibitors of terminal hypertrophic differentiation in chondrocytes. TGF β 1 arrests differentiation at an early stage of hypertrophy in bovine synovial explants (Shintani et al., 2013), and TGF β 3 inhibits terminal differentiation of chondrocytes from cultured mesenchymal stem cells (Mueller et al., 2010; Mueller and Tuan, 2008). In micromass culture using mouse limb bud cells, TGF β treatment delayed chondrocyte maturation and hypertrophy, and in accordance, inhibited expression of type X collagen, VEGF, MMP13, and osteocalcin (Zhang et al., 2004). TGF β 1 prevents the terminal differentiation of epiphyseal chondrocytes into hypertrophic cells (Ballock et al., 1993). These data along with the previously discussed data showing that T β RII is required for hypertrophy at early stages, indicate that TGF β promotes the initial stages of chondrocyte differentiation, but represses terminal hypertrophic differentiation.

T β RII has profound roles in maintaining cartilage integrity. T β RII inhibits terminal differentiation and hypertrophy in articular chondrocytes. Deletion of *Tgfr2* in early postnatal chondrocytes, achieved using tamoxifen-inducible; *Tgfr2*; *Col2-CreER* mice, results in up-regulation of Runx2, Mmp13, and Adamts5 expression in articular cartilage tissue, leading to progressive development of an osteoarthritis (OA)-like phenotype (Shen et al., 2013). One caveat of these studies is that the ablation was carried out in 2-week old mice. No significant changes were reported in growth plate chondrocytes, strongly suggesting that the effects seen on formation of articular cartilage were direct. However, studies in which TGF β signaling is ablated in adult articular cartilage will be needed to investigate the impact of these pathways on maintenance of articular cartilage.

Whether and how ALK5 acts in postnatal articular cartilage development and maintenance are not well understood and should be further investigated. ALK5 is expressed in murine and human cartilage, but its expression level decreases in normal aging and OA cartilage (Blaney Davidson et al., 2009). In vitro knockdown of ALK5 in articular chondrocytes leads to elevated expression of MMP13, a marker of terminally differentiated chondrocytes and a major cartilage-degrading enzyme in OA (Billinghurst et al., 1997; Blaney Davidson et al., 2009). These results strongly suggest that ALK5 plays an important role in inhibiting chondrocyte terminal differentiation in articular cartilage, but this remains to be confirmed in vivo.

It has been shown that TGF β can activate canonical BMP pathways through engagement of ALK1 (Goumans et al., 2002), and that this pathway leads to activation of Smads1/5/8 in articular cartilage (van der Kraan et al., 2009). Moreover, the ALK1/ALK5 ratio is elevated in aged and OA articular chondrocytes as a consequence of an age-related decline in ALK5 expression (Blaney Davidson et al., 2009; van der Kraan et al., 2012). The increased ALK1/ALK5 ratio is correlated with a shift from Smad2/3 to Smad1/5/8 signaling during aging and OA in murine cartilage (Blaney Davidson et al., 2009; van der Kraan et al., 2012). Moreover, there is a significant correlation between ALK1 and MMP13 mRNA expression in the cartilage of human OA knee joints (Blaney Davidson et al., 2009). The effects of ALK1/Smad1/5/8 signaling on expression of many genes are opposite to those of ALK5/Smad2/3 in cartilage; thus, the outcome of TGF β treatment will differ depending on the constellation of receptors in cartilage. The above discussion focuses on the role of TGF β in articular chondrocytes. It is important to bear in mind that TGF β signaling through Smad2/3 and Smad1/5 affects multiple joint tissues, including ligament, meniscus, subchondral bone, and synovium, and signaling through either pathway (Smad2/3) versus (Smad1/5/8) can have both protective and harmful effects (Plaas et al., 2011). Evaluation of the physiological significance of these effects with respect to joint health and OA will require additional in vivo studies.

Smad-Dependent TGF β Signaling Functions in Cartilage

Smad2 and Smad3 are expressed throughout the growth plate. Smad2 is preferentially expressed in proliferative and prehypertrophic chondrocytes, whereas Smad3 is expressed at higher levels in prehypertrophic and hypertrophic chondrocytes. Smad4 is expressed in all zones (Billiar et al., 2004; Sakou et al., 1999). Smad2 and Smad3 have distinct roles in mediating TGF β signaling. Smad3 binds DNA directly, whereas Smad2 regulates gene expression by interacting with Smad3 or other transcriptional factors (Massague et al., 2005). Smad2 is essential at early stages of embryonic development, while Smad3 may play a more important role in adult life (Song et al., 2009). *Smad2*^{-/-} mice die at embryonic day 7.5–12.5 (Heyer et al., 1999; Nomura and Li, 1998; Waldrip et al., 1998; Weinstein et al., 1998). *Smad3*^{-/-} mice are viable, but develop metastatic colorectal cancer, defects in the immune system, and present with OA-like symptoms (Datto et al., 1999; Li et al., 2006; Yang et al., 1999, 2001; Zhu et al., 1998). *Smad3*^{-/-} mice develop degenerative joint disease resembling human OA, as characterized by increased chondrocyte hypertrophy and the presence of type X collagen-positive cells in articular cartilage, progressive loss of the joint

surface, formation of osteophytes, and decreased production of proteoglycans in synovial joints (Yang et al., 2001).

As is the case for *Tgfb2* conditional knockout mice (*Tgfb2;Col2-cre*) (Baffi et al., 2004), *Smad3* conditional knockout mice (*Smad3; Col2-Cre*) do not exhibit profound cartilage defects at prenatal stages (Chen et al., 2012). Depletion of *Smad3* in chondrocytes causes progressive articular cartilage degeneration, associated with increased expression of MMP13 and deficiency in key cartilage matrix constituents type II collagen and aggrecan (Chen et al., 2012).

The *in vivo* function of Smad2 in cartilage is still not clear. Overexpression of either Smad2 or Smad3 can block the spontaneous maturation observed in *Smad3*-deficient chondrocytes (Li et al., 2006). These data indicate that in spite of the fact that Smad2 and Smad3 regulate different sets of genes, Smad2 may partially compensate for Smad3 in preventing chondrocyte terminal differentiation. The extent to which TGF β signaling utilizes Smad-dependent versus Smad-independent signaling in aspects of cartilage development and maintenance *in vivo* is as yet unclear, because direct comparisons of phenotypes using the same Cre drivers to ablate Smads versus TGF β receptors have not been reported.

Noncanonical TGF β Signaling in Cartilage

There exist numerous Smad-independent noncanonical pathways for transduction of TGF β signals, including various MAPK, Rho-like GTPase, and phosphatidylinositol-3-kinase (PI3K)/AKT pathways (Moustakas and Heldin, 2005; Mu et al., 2012; Yeganeh et al., 2013; Zhang, 2009). The extent to which TGF β s mediate their effects through these pathways in cartilage *in vivo* is unknown, but there is solid evidence that these pathways are important for chondrogenesis.

TAK1 AND p38 IN CARTILAGE

The most extensively studied noncanonical pathways are those mediated by TGF β activating kinase 1 (TAK1), a member of the MAPKKK family. TAK1 is activated by type I BMP and TGF β receptors, and subsequently activates several MAP kinases (MAPKs), including p38, JNK, and ERK. Many reports have shown that MAPK activation converges with Smad signaling downstream of TGF β to regulate cell apoptosis and epithelial–mesenchymal transition (EMT) (Holm et al., 2011; Lamouille and Derynck, 2007; Massague, 2012b; Mu et al., 2012; Wu and Hill, 2009). For example, the TAK1-JNK/p38 cascade functions in conjunction with the Smad-dependent pathway to regulate TGF β -induced apoptosis. Moreover, siRNA knockdown of TRAF6, an upstream activator of TAK1, or treatment of cells with a chemical inhibitor of p38, efficiently blocked TGF β -mediated apoptosis (Sorrentino et al., 2008; Yamashita et al., 2008; Yu et al., 2002). TGF β promotes tumor growth by inducing EMT through a combination of Smad-dependent and Smad-independent effects mediated by p38 (Lee et al., 2006; Massague, 2012a; Thiery, 2003). Cooperation of Smad4 and p38 mediated signaling pathways is also required for normal tooth and palate formation (Xu et al., 2008). It is highly likely that TGF β mediates its effects in chondrocytes through both canonical and noncanonical pathways.

In growth plate chondrocytes, TAK1 is critical for stimulating chondrocyte proliferation and differentiation. However, in developing cartilage, BMPs, rather than TGF β , appear to be the major activators of TAK1. Whether this is the case in articular cartilage is unknown. Conditional deletion of TAK1 in cartilage (*Tak1;Col2Cre*) results in chondrodysplasia characterized by neonatal-onset runting, delayed formation of secondary ossification centers, and defects in formation of the elbow and tarsal joints (Greenblatt et al., 2010). Data from another research group showed that deletion of *Tak1* in chondrocytes resulted in multiple developmental cartilage defects, including decreased chondrocyte proliferation and survival, delayed onset of hypertrophy, reduced MMP13 expression, and a failure to maintain interzone cells of the elbow joint (Gunnell et al., 2010). These defects resemble those seen in mice deficient for BMP receptors or ligands more than they do the phenotypes of mice deficient for components of TGF β pathways. In accordance, chondrocytes from these mice show evidence of defective BMP signaling in vivo and in vitro. Somewhat unexpectedly, deletion of TAK1 seems to affect not only activation of the p38 MAPK signaling cascade, but also activation of canonical BMP Smad1/5/8. Deletion of *Tak1* in limb mesenchyme (*Tak1;Prx1Cre*) resulted in widespread joint fusions, likely owing to the commitment of joint inter-zone cells to the chondrocyte lineage (Gunnell et al., 2010). Since TAK1 activates both p38/JNK/ERK MAPK and Smad1/5/8 pathways, the defects seen in *Tak1;Col2Cre* mice likely reflect reduced signaling through both canonical and noncanonical BMP pathways.

Although the above studies indicate that BMPs are major mediators of TAK1 pathways, TGF β signaling also depends on TAK1 in several aspects of chondrogenesis. The most extensive data comes from studies of p38, a downstream effector of TAK1 signaling. Studies with genetically modified mice show that p38 pathways have various functions in cartilage, including inhibiting chondrocyte proliferation and differentiation, and maintaining cartilage integrity. p38 has 4 isoforms, α , β , γ , and δ , but only α , β , and γ are detectable in mouse cartilage (Li et al., 2010). Transgenic overexpression in chondrocytes of activated MKK6, a downstream mediator of TAK1 and an upstream activator of p38, resulted in dwarfism, inhibition of chondrocyte proliferation and differentiation, and a delay in primary and secondary ossification (Zhang et al., 2006). Inhibition of p38 in transgenic mice by cartilage-specific expression of a dominant-negative p38 (*Col2a1-p38-DN*) resulted in severely deficient endochondral bone formation and reduced limb length (Namdari et al., 2008). p38-DN heterozygotes developed osteoarthritis-like symptoms, indicating that chronic p38 deficiency is harmful to articular cartilage (Namdari et al., 2008). Whether this is due to early chondrogenesis defects that affect joint shape, or to a role for p38 in adult articular cartilage, is an important unknown.

p38 also has catabolic functions in cartilage. Increased p38 phosphorylation was found in human OA cartilage (Fan et al., 2007). *Col10a1* is expressed in all hypertrophic chondrocytes, but *MMP13* expression is restricted to the most terminally differentiated hypertrophic chondrocytes (Inada et al., 2004; MacLean et al., 2003). p38 inhibits *Col10a1* expression (Li et al., 2010), but activates *MMP13* expression (Chen et al., 2012). These results suggest that p38 prevents onset of chondrocyte hypertrophy, but stimulates their terminal differentiation, an effect also seen for TGF β , as discussed above.

CROSSTALK BETWEEN SMAD3 AND p38 IN CARTILAGE

TGF β can activate MAPK pathways through Smad-independent and Smad-dependent pathways. For example, a mutant T β RI receptor defective in Smad binding and activation, but retaining an intact kinase activity, is able to mediate TGF β -induced activation of JNK and p38 through TAK1 (Itoh et al., 2003; Yu et al., 2002), indicating that Smads can be dispensable for TGF β activation of JNK and p38. However, several studies have demonstrated that Smad2/3 and p38 act in concert to regulate aspects of chondrogenesis. For example, in vitro assays using chondrocytic ATDC5 cells showed that although TGF β -dependent activation of p38 and ERK1/2 does not influence activation of R-Smads by TGF β , inhibition of p38 or ERK1/2 inhibited TGF β -induced transcriptional activity of both Smad2 and Smad4 (Watanabe et al., 2001). Hence, these studies suggest that TGF β -induced activation of p38 or ERK1/2 is essential for transcriptional activation of Smad2 and Smad4, and for maximal activation of specific Smad-dependent transcriptional responses in ATDC5 cells (Watanabe et al., 2001).

Smad3 has been shown to modulate p38 activity. For example, Smad3 regulates p38 phosphorylation in chondrocytes. Assays using primary chondrocytes from *Smad3*^{-/-} mice showed that loss of Smad3 promotes inactivation of p38, most likely by disrupting a pSmad3-p38 complex, thereby abrogating signaling through a TAK1/p38/ATF-2 pathway. Over-expression of ATF-2 or treatment with the p38 activator anisomycin inhibited expression of type X collagen, suggesting that Smad3 and p38 cooperate to repress the onset of hypertrophy (Li et al., 2010). p38 can also act independently of Smad3 to promote induction of MMP13 in growth plate chondrocytes (Chen et al., 2012). Smad3-mediated TGF β signals transiently repress MMP13 expression. However, after 24 hr of TGF β treatment, there is an increase of MMP13 expression; this induction is mediated by p38, but not Smad3 (Chen et al., 2012). Hence, a switch from Smad3-mediated signals to p38-mediated signals changes the outcome of TGF β treatment from repression to activation of MMP13 expression. Whether this switch to a Smad3-independent effect correlates with a change in receptor utilization towards ALK1 is an interesting possibility.

In contrast to the above studies demonstrating cooperativity between Smad and p38 pathways, MAPK pathways negative regulate Smad signaling through phosphorylation of Smad linker sites (Fuentelba et al., 2007; Gao et al., 2009). The BMP and TGF β type I receptors phosphorylate R-Smads at the C-terminal SXS site to initiate signal propagation. Subsequently, the R-Smad linker region is phosphorylated by MAPK, leading to a primed substrate for glycogen synthase kinase 3 (GSK3). GSK3 creates binding sites for the E3 ubiquitin protein ligases SMURF1 (SMAD-specific E3 ubiquitin protein ligase 1) or NEDD4L (neural precursor cell expressed developmentally downregulated protein 4-like), which target SMAD proteins for polyubiquitination and proteasome-mediated degradation (Alarcon et al., 2009; Fuentelba et al., 2007; Gao et al., 2009). Besides MAPK pathways, PI3K/Akt pathway can also antagonize Smad-mediated effects. Akt can directly interact with Smad3 and inhibit Smad3-phosphorylation, nuclear localization, and Smad3-mediated transcription (Conery et al., 2004; Remy et al., 2004). The extent to which noncanonical pathways interact with Smad pathways in cartilage development remains poorly understood.

JNK, ERK, PI3K, AND RHO GTPASE PATHWAYS IN CARTILAGE

When compared with p38, there is less information on the functions of JNK and ERK pathways in cartilage. JNK1 and JNK2 are expressed in chondrocytes, but deletion of either isoform has not been associated with a skeletal phenotype (Beier and Loeser, 2010), suggesting that JNK1 and JNK2 may have overlapping functions in cartilage. High levels of activated JNK are seen in human OA cartilage (Clancy et al., 2001; Fan et al., 2007). In vitro experiments showed that inhibiting JNK blocks MMP13 expression in human chondrocytes (Im et al., 2007; Loeser et al., 2003), implicating JNK in the progression of OA.

Chondrocytes also express both ERK1 and ERK2. ERK1-null mice have no obvious skeletal or growth abnormalities (Pages et al., 1999), and ERK2 null mice die very early in embryogenesis as a result of defective trophoblast development (Saba-EI-Leil et al., 2003). Constitutive activation of MEK1 in chondrocytes inhibits hypertrophic differentiation of growth plate chondrocytes, and negatively regulates bone growth without inhibiting chondrocyte proliferation, resulting in achondroplasia-like dwarfism (Murakami et al., 2004). Similar to p38 and JNK, ERK plays a role in stimulating MMP13 expression in human chondrocytes, and inhibiting ERK prevents MMP13 expression (Forsyth et al., 2002; Loeser et al., 2003). In vitro assay results showed that all three major MAP kinases (ERK1/2, p38a, and JNK1/2) must be activated at the same time to induce MMP expression, while inhibition of any one of the three is sufficient to inhibit MMP13 expression (Forsyth et al., 2002; Loeser et al., 2003).

The PI3K pathway has various stage-specific functions in cartilage development. Transgenic overexpression of an activated form of Akt, a downstream target of PI3K, in cartilage increased chondrocyte proliferation in the resting zone, and delayed hypertrophic differentiation in the growth plate, but promoted hypertrophic differentiation in craniobasal cartilaginous elements and vertebrae (Rokutanda et al., 2009). The differential effects on hypertrophy were shown to be a result of engagement of different pathways downstream of Akt. Organ culture experiments showed that Akt relays signals through mTOR, FoxO, and GSK3 pathways. The Akt–mTOR pathway was responsible for promoting chondrocyte proliferation, maturation, and cartilage matrix production. The Akt–FoxO pathway enhanced chondrocyte proliferation, but inhibited chondrocyte maturation and cartilage matrix production, while the Akt–GSK3 pathway negatively regulated three of the cellular processes in limb skeletons but not in vertebrae, as a result of less GSK3 expression in vertebrae (Rokutanda et al., 2009).

Cartilage-specific inactivation of PTEN, the main phosphatase counteracting PI3K activity, leads to profound defects in skeletal development in mice, including skeletal overgrowth, disorganization of growth plates with increasing resting cell proliferation, and fusion of the primary and secondary ossification centers (Ford-Hutchinson et al., 2007; Hsieh et al., 2009; Yang et al., 2008). As is the case for Akt, the function of PTEN varies depending on the time and location of skeletal development. The loss of PTEN delays chondrocyte hypertrophy in embryonic growth plates (Yang et al., 2008), whereas it accelerates hypertrophic chondrocyte maturation in adult mice (Ford-Hutchinson et al., 2007). Among the Akt isoforms (Akt1, Akt2, and Akt3), Akt1 is the most highly expressed in

chondrocytes. *Akt1*^{-/-} mice are small and have normal proliferative and hypertrophic zones, but exhibit decreased calcification in the growth plate. *Akt1*^{-/-} mice formed fewer osteophytes in medial collateral ligament transection induced OA (Fukai et al., 2010).

The in vivo functions of Rho GTPases in chondrogenesis are not clear because in vivo models are lacking (e.g., conditional knockout mice). In vitro data indicate that RhoA, one of the main prototypes of Rho GTPase families, inhibits early chondrogenesis and hypertrophic chondrocyte differentiation by repressing Sox9 expression (Woods et al., 2005; Woods and Beier, 2006; Kumar and Lassar, 2009; reviewed in Beier and Loeser, 2010).

In summary, p38, JNK, ERK, PI-3 Kinase, and Rho GTPases pathways have different functions in regulating chondrocyte proliferation and differentiation according to the development stage and type of skeletal element (Table 1). p38, ERK, and PI3K pathways both promote and inhibit chondrocyte terminal differentiation. As a result, attempts at pharmacological intervention for OA using these pathways as targets must take these temporal and spatial differences in function into consideration. The extent to which these pathways are regulated by TGF β in vivo is unknown, as is the extent to which TGF β transduces its signals through these pathways in cartilage.

CROSSTALK BETWEEN TGF- β SIGNALING AND BMP SIGNALING

BMP pathways control nearly every aspect of chondrogenesis (Song et al., 2009; Yoon and Lyons, 2004). Thus, understanding how BMP and TGF β pathways intersect is fundamental to understand the mechanisms controlling cartilage formation and maintenance. TGF β enhances BMP2-induced chondrogenesis in bovine synovial explants, improves the hyaline-like properties of neocartilage, and arrests differentiation at an early stage of hypertrophy (Shintani et al., 2013). In undifferentiated ATDC5 cells, which represent a proliferative stage, TGF β enhanced BMP signaling, while BMP2 significantly reduced levels of TGF β signaling (Keller et al., 2011). These results suggest that TGF β promotes BMP signaling during early chondrogenesis and cell proliferation.

On the other hand, in vivo data demonstrated that Smad3 can repress Smad1/5/8 activation to prevent chondrocyte hypertrophy (Li et al., 2006). In accordance, there is an increase in the level of pSmad1/5/8 activity with the loss of Smad3. *Smad3*^{-/-} chondrocytes were more responsive to BMP2, exhibiting increased type X collagen expression, pSmad1/5/8 levels, and BMP-responsive luciferase reporter activity (Li et al., 2006). In vitro assays using MDA-MB-231 breast cancer cell lines showed that TGF β inhibits BMP responses by inducing the formation of pSmad3-pSmad1/5 complexes, which bind to BMP-responsive elements and mediate TGF β -induced transcriptional repression (Gronroos et al., 2012). In ATDC5 cells, TGF β suppresses BMP signaling and chondrocyte hypertrophy via SnoN, a transcriptional corepressor (Kawamura et al., 2012). SnoN is induced by TGF β signaling in maturing chondrocytes and suppresses the BMP-Smad signaling pathway to inhibit hypertrophic maturation of chondrocytes (Kawamura et al., 2012).

Summary and Perspectives

There has been considerable progress toward understanding the physiological functions of TGF β signaling network components and downstream pathways during cartilage development and maintenance. However, many questions remain regarding the relative importance of various pathways downstream of TGF β , the role of TGF β as opposed to other growth factors in activating these pathways, and the mechanisms by which TGF β -regulated canonical and noncanonical pathways intersect. It is clear that the composition of TGF β receptor complexes and crosstalk between Smad and non-Smad signaling pathways determines the final cellular response. Not every signaling component, however, is well understood with regard to *in vivo* functions at different developmental stages. For example, the functions of ALK5, ALK1, Smad2, and Smad4 in mediating TGF β actions in cartilage development and maintenance are still not clear. Given that TGF β can transduce both canonical TGF β (Smad2/3) and BMP (Smad1/5/8) signals that have fundamentally different and usually opposing effects in cartilage, understanding the extent to which TGF β utilizes BMP pathways *in vivo* is also an important goal.

References

- Akiyama H, Lefebvre V. Unraveling the transcriptional regulatory machinery in chondrogenesis. *J Bone Miner Metab.* 2011; 29:390–395. [PubMed: 21594584]
- Alarcon C, Zaromytidou AI, Xi Q, et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell.* 2009; 139:757–769. [PubMed: 19914168]
- Alvarez J, Sohn P, Zeng X, et al. TGF β 2 mediates the effects of Hedgehog on hypertrophic differentiation and PTHrP expression. *Development.* 2002; 129:1913–1924. [PubMed: 11934857]
- Azhar M, Yin M, Bommireddy R, et al. Generation of mice with a conditional allele for transforming growth factor beta 1 gene. *Genesis.* 2009; 47:423–431. [PubMed: 19415629]
- Baffi MO, Slattery E, Sohn P, et al. Conditional deletion of the TGF-beta type II receptor in Col2a expressing cells results in defects in the axial skeleton without alterations in chondrocyte differentiation or embryonic development of long bones. *Dev Biol.* 2004; 276:124–142. [PubMed: 15531369]
- Ballock RT, Heydemann A, Wakefield LM, et al. TGF-beta 1 prevents hypertrophy of epiphyseal chondrocytes: Regulation of gene expression for cartilage matrix proteins and metalloproteases. *Dev Biol.* 1993; 158:414–429. [PubMed: 8344460]
- Barry F, Boynton RE, Liu B, et al. Chondrogenic differentiation of mesenchymal stem cells from bone marrow: differentiation-dependent gene expression of matrix components. *Exp Cell Res.* 2001; 268:189–200. [PubMed: 11478845]
- Beier F, Loeser RF. Biology and pathology of Rho GTPase, PI-3 kinase-Akt, and MAP kinase signaling pathways in chondrocytes. *J Cell Biochem.* 2010; 110:573–580. [PubMed: 20512918]
- Benazet JD, Pignatti E, Nugent A, et al. Smad4 is required to induce digit ray primordia and to initiate the aggregation and differentiation of chondrogenic progenitors in mouse limb buds. *Development.* 2012; 139:4250–4260. [PubMed: 23034633]
- Bernabeu C, Lopez-Novoa JM, Quintanilla M. The emerging role of TGF-beta superfamily coreceptors in cancer. *Biochim Biophys Acta.* 2009; 1792:954–973. [PubMed: 19607914]
- Billiar RB, St Clair JB, Zachos NC, et al. Localization and developmental expression of the activin signal transduction proteins Smads 2, 3, and 4 in the baboon fetal ovary. *Biol Reprod.* 2004; 70:586–592. [PubMed: 14585818]
- Billingham RC, Dahlberg L, Ionescu M, et al. Enhanced cleavage of type II collagen by collagenases in osteoarthritic articular cartilage. *J Clin Investig.* 1997; 99:1534–1545. [PubMed: 9119997]

- Blaney Davidson EN, Remst DF, et al. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol.* 2009; 182:7937–7945. [PubMed: 19494318]
- Burks TN, Cohn RD. Role of TGF-beta signaling in inherited and acquired myopathies. *Skelet Muscle.* 2011; 1:19. [PubMed: 21798096]
- Chen CG, Thuillier D, Chin EN, et al. Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis. *Arthritis Rheum.* 2012; 64:3278–3289. [PubMed: 22674505]
- Chu GC, Dunn NR, Anderson DC, et al. Differential requirements for Smad4 in TGF beta-dependent patterning of the early mouse embryo. *Development.* 2004; 131:3501–3512. [PubMed: 15215210]
- Clancy R, Rediske J, Koehne C, et al. Activation of stress-activated protein kinase in osteoarthritic cartilage: Evidence for nitric oxide dependence. *Osteoarthritis Cartilage.* 2001; 9:294–299. [PubMed: 11399092]
- Conery AR, Cao Y, Thompson EA, et al. Akt interacts directly with Smad3 to regulate the sensitivity to TGF-beta induced apoptosis. *Nat Cell Biol.* 2004; 6:366–372. [PubMed: 15104092]
- Datto MB, Frederick JP, Pan L, et al. Targeted disruption of Smad3 reveals an essential role in transforming growth factor beta-mediated signal transduction. *Mol Cell Biol.* 1999; 19:2495–2504. [PubMed: 10082515]
- Dunker N, Schuster N, Kriegelstein K. TGF-beta modulates programmed cell death in the retina of the developing chick embryo. *Development.* 2001; 128:1933–1942. [PubMed: 11493517]
- Estrada KD, Wang WG, Retting KN, et al. Smad7 regulates terminal maturation of chondrocytes in the growth plate. *Dev Biol.* 2013; 382:375–384. [PubMed: 23994637]
- Fan Z, Soder S, Oehler S, et al. Activation of interleukin-1 signaling cascades in normal and osteoarthritic articular cartilage. *Am J Pathol.* 2007; 171:938–946. [PubMed: 17640966]
- Ford-Hutchinson AF, Ali Z, Lines SE, et al. Inactivation of Pten in osteochondroprogenitor cells leads to epiphyseal growth plate abnormalities and skeletal overgrowth. *J Bone Miner Res.* 2007; 22:1245–1259. [PubMed: 17456009]
- Forsyth CB, Pulai J, Loeser RF. Fibronectin fragments and blocking antibodies to alpha2beta1 and alpha5beta1 integrins stimulate mitogen-activated protein kinase signaling and increase collagenase 3 (matrix metalloproteinase 13) production by human articular chondrocytes. *Arthritis Rheum.* 2002; 46:2368–2376. [PubMed: 12355484]
- Fuentealba LC, Eivers E, Ikeda A, et al. Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal. *Cell.* 2007; 131:980–993. [PubMed: 18045539]
- Fukai A, Kawamura N, Saito T, et al. Akt1 in murine chondrocytes controls cartilage calcification during endochondral ossification under physiologic and pathologic conditions. *Arthritis Rheum.* 2010; 62:826–836. [PubMed: 20187155]
- Gao L, Sheu TJ, Dong Y, et al. TAK1 regulates Sox9 expression in chondrocytes and is essential for postnatal development of the growth plate and articular cartilages. *J Cell Sci.* 2013; 126:5704–5713. [PubMed: 24144697]
- Gao S, Alarcon C, Sapkota G, et al. Ubiquitin ligase Nedd4L targets activated Smad2/3 to limit TGF-beta signaling. *Mol Cell.* 2009; 36:457–468. [PubMed: 19917253]
- Gordon KJ, Blobel GC. Role of transforming growth factor-beta superfamily signaling pathways in human disease. *Biochim Biophys Acta.* 2008; 1782:197–228. [PubMed: 18313409]
- Goumans MJ, Valdimarsdottir G, Itoh S, et al. Balancing the activation state of the endothelium via two distinct TGF-beta type I receptors. *EMBO J.* 2002; 21:1743–1753. [PubMed: 11927558]
- Greenblatt MB, Shim JH, Glimcher LH. TAK1 mediates BMP signaling in cartilage. *Ann NY Acad Sci.* 2010; 1192:385–390. [PubMed: 20392264]
- Gronroos E, Kingston IJ, Ramachandran A, et al. Transforming growth factor beta inhibits bone morphogenetic protein-induced transcription through novel phosphorylated Smad1/5–Smad3 complexes. *Mol Cell Biol.* 2012; 32:2904–2916. [PubMed: 22615489]
- Gunnell LM, Jonason JH, Loiselle AE, et al. TAK1 regulates cartilage and joint development via the MAPK and BMP signaling pathways. *J Bone Miner Res.* 2010; 25:1784–1797. [PubMed: 20213696]

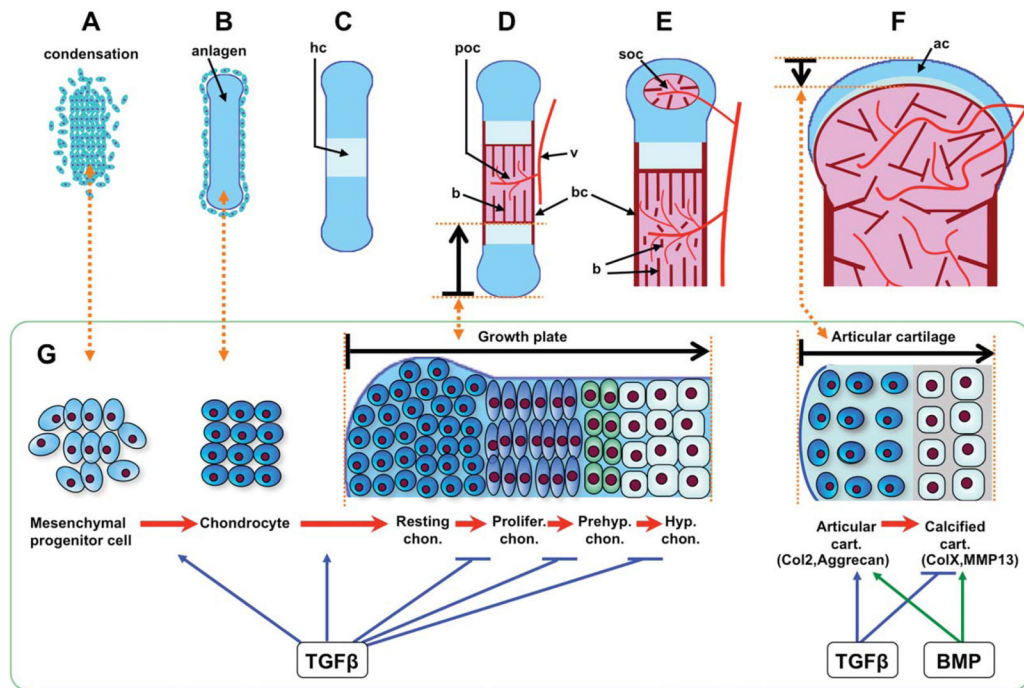
- Guo X, Wang XF. Signaling cross-talk between TGF-beta/BMP and other pathways. *Cell Res.* 2009; 19:71–88. [PubMed: 19002158]
- Heyer J, Escalante-Alcalde D, Lia M, et al. Postgastrulation Smad2-deficient embryos show defects in embryo turning and anterior morphogenesis. *Proc Natl Acad Sci USA.* 1999; 96:12595–12600. [PubMed: 10535967]
- Hinck AP. Structural studies of the TGF-betas and their receptors: Insights into evolution of the TGF-beta superfamily. *FEBS Lett.* 2012; 586:1860–1870. [PubMed: 22651914]
- Hiramatsu K, Iwai T, Yoshikawa H, et al. Expression of dominant negative TGF-beta receptors inhibits cartilage formation in conditional transgenic mice. *J Bone Miner Metab.* 2011; 29:493–500. [PubMed: 21213115]
- Holm TM, Habashi JP, Doyle JJ, et al. Noncanonical TGFbeta signaling contributes to aortic aneurysm progression in Marfan syndrome mice. *Science.* 2011; 332:358–361. [PubMed: 21493862]
- Horner A, Kemp P, Summers C, et al. Expression and distribution of transforming growth factor-beta isoforms and their signaling receptors in growing human bone. *Bone.* 1998; 23:95–102. [PubMed: 9701467]
- Hsieh SC, Chen NT, Lo SH. Conditional loss of PTEN leads to skeletal abnormalities and lipoma formation. *Mol Carcinogen.* 2009; 48:545–552.
- Im HJ, Muddasani P, Natarajan V, et al. Basic fibroblast growth factor stimulates matrix metalloproteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase Cdelta pathways in human adult articular chondrocytes. *J Biol Chemistry.* 2007; 282:11110–11121.
- Inada M, Wang Y, Byrne MH, et al. Critical roles for collagenase-3 (Mmp13) in development of growth plate cartilage and in endochondral ossification. *Proc Natl Acad Sci USA.* 2004; 101:17192–17197. [PubMed: 15563592]
- Itoh S, Thorikay M, Kowanz M, et al. Elucidation of Smad requirement in transforming growth factor-beta type I receptor-induced responses. *J Biol Chem.* 2003; 278:3751–3761. [PubMed: 12446693]
- Iwamoto M, Ohta Y, Larmour C, et al. Toward regeneration of articular cartilage. *Birth Defects Res.* 2013; 99:192–202.
- Iwata J, Hacia JG, Suzuki A, et al. Modulation of noncanonical TGF-beta signaling prevents cleft palate in Tgfb2 mutant mice. *J Clin Investig.* 2012; 122:873–885. [PubMed: 22326956]
- Kaartinen V, Voncken JW, Shuler CF, et al. Abnormal lung development and cleft palate in mice lacking TGF-b3 indicates defects of epithelial–mesenchymal interaction. *Nat Genet.* 1995; 11:415–421. [PubMed: 7493022]
- Karsenty G, Kronenberg HM, Settembre C. Genetic control of bone formation. *Ann Rev Cell Dev Biol.* 2009; 25:629–648. [PubMed: 19575648]
- Kawamura I, Maeda S, Imamura K, et al. SnoN suppresses maturation of chondrocytes by mediating signal cross-talk between transforming growth factor-beta and bone morphogenetic protein pathways. *J Biol Chem.* 2012; 287:29101–29113. [PubMed: 22767605]
- Keller B, Yang T, Chen Y, et al. Interaction of TGFbeta and BMP signaling pathways during chondrogenesis. *PloS one.* 2011; 6:e16421. [PubMed: 21297990]
- Kronenberg HM. Developmental regulation of the growth plate. *Nature.* 2003; 423:332–336. [PubMed: 12748651]
- Kulkarni AB, Karlsson S. Transforming growth factor-beta 1 knockout mice. A mutation in one cytokine gene causes a dramatic inflammatory disease. *Am J Pathol.* 1993; 143:3–9. [PubMed: 8317552]
- Kumar D, Lassar AB. The transcriptional activity of Sox9 in chondrocytes is regulated by RhoA signaling and actin polymerization. *Mol Cell Biol.* 2009; 29:4262–4273. [PubMed: 19470758]
- Lamouille S, Derynck R. Cell size and invasion in TGF-beta-induced epithelial to mesenchymal transition is regulated by activation of the mTOR pathway. *J Cell Biol.* 2007; 178:437–451. [PubMed: 17646396]
- Larsson J, Goumans MJ, Sjostrand LJ, et al. Abnormal angiogenesis but intact hematopoietic potential in TGF-beta type I receptor-deficient mice. *EMBO J.* 2001; 20:1663–1673. [PubMed: 11285230]

- Las Heras F, Gahunia HK, Pritzker KP. Articular cartilage development: a molecular perspective. *Orthop Clin N Am*. 2012; 43:155–171.
- Lee JM, Dedhar S, Kalluri R, et al. The epithelial–mesenchymal transition: New insights in signaling, development, and disease. *J Cell Biol*. 2006; 172:973–981. [PubMed: 16567498]
- Li TF, Darowish M, Zuscik MJ, et al. Smad3-deficient chondrocytes have enhanced BMP signaling and accelerated differentiation. *J Bone Miner Metab*. 2006; 21:4–16.
- Li TF, Gao L, Sheu TJ, et al. Aberrant hypertrophy in Smad3-deficient murine chondrocytes is rescued by restoring transforming growth factor beta-activated kinase 1/activating transcription factor 2 signaling: A potential clinical implication for osteoarthritis. *Arthritis Rheum*. 2010; 62:2359–2369. [PubMed: 20506210]
- Li TF, O’Keefe RJ, Chen D. TGF-beta signaling in chondrocytes. *Front Biosci*. 2005; 10:681–688. [PubMed: 15569609]
- Loeser RF, Forsyth CB, Samarel AM, et al. Fibronectin fragment activation of proline-rich tyrosine kinase PYK2 mediates integrin signals regulating collagenase-3 expression by human chondrocytes through a protein kinase C-dependent pathway. *J Biol Chem*. 2003; 278:24577–24585. [PubMed: 12730223]
- Long F, Ornitz DM. Development of the endochondral skeleton. *Cold Spring Harbor Perspect Biol*. 2013; 5:a008334.
- Longobardi L, Li T, Myers TJ, et al. TGF-beta type II receptor/MCP-5 axis: At the crossroad between joint and growth plate development. *Dev Cell*. 2012; 23:71–81. [PubMed: 22814601]
- MacLean HE, Kim JI, Glimcher MJ, et al. Absence of transcription factor c-maf causes abnormal terminal differentiation of hypertrophic chondrocytes during endochondral bone development. *Dev Biol*. 2003; 262:51–63. [PubMed: 14512017]
- Massague J. TGF-beta signaling in development and disease. *FEBS Lett*. 2012a; 586:1833. [PubMed: 22651913]
- Massague J. TGFbeta signalling in context. *Nat Rev Mol Cell Biol*. 2012b; 13:616–630. [PubMed: 22992590]
- Massague J, Seoane J, Wotton D. Smad transcription factors. *Genes Dev*. 2005; 19:2783–2810. [PubMed: 16322555]
- Matsunobu T, Torigoe K, Ishikawa M, et al. Critical roles of the TGF-beta type I receptor ALK5 in perichondrial formation and function, cartilage integrity, and osteoblast differentiation during growth plate development. *Dev Biol*. 2009; 332:325–338. [PubMed: 19501582]
- Millan FA, Denhez F, Kondaiah P, et al. Embryonic gene expression patterns of TGF beta 1, beta 2 and beta 3 suggest different developmental functions in vivo. *Development*. 1991; 111:131–143. [PubMed: 1707784]
- Moustakas A, Heldin CH. Non-Smad TGF-beta signals. *J Cell Sci*. 2005; 118:3573–3584. [PubMed: 16105881]
- Mu Y, Gudey SK, Landstrom M. Non-Smad signaling pathways. *Cell Tissue Res*. 2012; 347:11–20. [PubMed: 21701805]
- Mueller MB, Fischer M, Zellner J, et al. Hypertrophy in mesenchymal stem cell chondrogenesis: effect of TGF-beta isoforms and chondrogenic conditioning. *Cells Tissues Organs*. 2010; 192:158–166. [PubMed: 20407224]
- Mueller MB, Tuan RS. Functional characterization of hypertrophy in chondrogenesis of human mesenchymal stem cells. *Arthritis Rheum*. 2008; 58:1377–1388. [PubMed: 18438858]
- Murakami S, Balmes G, McKinney S, et al. Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. *Genes Dev*. 2004; 18:290–305. [PubMed: 14871928]
- Namdari S, Wei L, Moore D, et al. Reduced limb length and worsened osteoarthritis in adult mice after genetic inhibition of p38 MAP kinase activity in cartilage. *Arthritis Rheum*. 2008; 58:3520–3529. [PubMed: 18975318]
- Nomura M, Li E. Smad2 role in mesoderm formation, left–right patterning and craniofacial development. *Nature*. 1998; 393:786–790. [PubMed: 9655392]
- Oshima M, Oshima H, Taketo MM. TGF-beta receptor type II deficiency results in defects of yolk sac hematopoiesis and vasculogenesis. *Dev Biol*. 1996; 179:KC 297–302.

- Pages G, Guerin S, Grall D, et al. Defective thymocyte maturation in p44 MAP kinase (Erk 1) knockout mice. *Science*. 1999; 286:1374–1377. [PubMed: 10558995]
- Pan Q, Yu Y, Chen Q, et al. Sox9, a key transcription factor of bone morphogenetic protein-2-induced chondrogenesis, is activated through BMP pathway and a CCAAT box in the proximal promoter. *J Cell Physiol*. 2008; 217:228–241. [PubMed: 18506848]
- Pelton RW, Dickinson ME, Moses HL, et al. In situ hybridization analysis of TGF beta 3 RNA expression during mouse development: comparative studies with TGF beta 1 and beta 2. *Development*. 1990; 110:609–620. [PubMed: 1723948]
- Pelton RW, Saxena B, Jones M, et al. Immunohistochemical localization of TGF beta 1, TGF beta 2, and TGF beta 3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development. *J Cell Biol*. 1991; 115:1091–1105. [PubMed: 1955457]
- Pitsillides AA, Beier F. Cartilage biology in osteoarthritis—Lessons from developmental biology. *Nature reviews Rheumatology*. 2011; 7:654–663.
- Plaas A, Velasco J, Gorski DJ, et al. The relationship between fibrogenic TGFbeta1 signaling in the joint and cartilage degradation in post-injury osteoarthritis. *Osteoarthritis and cartilage/OARS. Osteoarthritis Res Soc*. 2011; 19:1081–1090.
- Poole AR. Biochemical/immunochemical biomarkers of osteoarthritis: Utility for prediction of incident or progressive osteoarthritis. *Rheum Dis Clin N Am*. 2003; 29:803–818.
- Proetzel G, Pawlowski SA, Wiles MV, et al. Transforming growth factor beta 3 is required for secondary palate fusion. *Nature*. 1995; 11:409–414.
- Remy I, Montmarquette A, Michnick SW. PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. *Nat Cell Biol*. 2004; 6:358–365. [PubMed: 15048128]
- Rodda SJ, McMahon AP. Distinct roles for Hedgehog and canonical Wnt signaling in specification, differentiation and maintenance of osteoblast progenitors. *Development*. 2006; 133:3231–3244. [PubMed: 16854976]
- Rokutanda S, Fujita T, Kanatani N, et al. Akt regulates skeletal development through GSK3, mTOR, and FoxOs. *Dev Biol*. 2009; 328:78–93. [PubMed: 19389373]
- Saba-El-Leil MK, Vella FD, Vernay B, et al. An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development. *EMBO Rep*. 2003; 4:964–968. [PubMed: 14502223]
- Sakou T, Onishi T, Yamamoto T, et al. Localization of Smads, the TGF-beta family intracellular signaling components during endochondral ossification. *J Bone Miner Metab*. 1999; 14:1145–1152.
- Sandberg M, Autio-Harminen H, Vuorio E. Localization of the expression of types I, III, and IV collagen, TGF-beta 1 and c-fos genes in developing human calvarial bones. *Dev Biol*. 1988; 130:324–334. [PubMed: 3053296]
- Sanford LP, Ormsby I, Gittenberger-de Groot AC, et al. TGF β 2 knockout mice have multiple developmental defects that are nonoverlapping with other TGF β knockout phenotypes. *Development*. 1997; 124:2659–2670. [PubMed: 9217007]
- Seo HS, Serra R. Deletion of Tgfbr2 in Prx1-cre expressing mesenchyme results in defects in development of the long bones and joints. *Dev Biol*. 2007; 310:304–316. [PubMed: 17822689]
- Serra R, Chang C. TGF-beta signaling in human skeletal and patterning disorders. *Birth Defects Res Part C*. 2003; 69:333–351.
- Serra R, Johnson M, Filvaroff EH, et al. Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol*. 1997; 139:541–552. [PubMed: 9334355]
- Shapiro IM, Adams CS, Freeman T, et al. Fate of the hypertrophic chondrocyte: Microenvironmental perspectives on apoptosis and survival in the epiphyseal growth plate. *Birth Defects Res Part C*. 2005; 75:330–339.
- Shen J, Li J, Wang B, et al. Deletion of the Type II TGF-beta receptor gene in articular chondrocytes leads to a progressive OA-like phenotype in mice. *Arthritis Rheum*. 2013; 65:3107–3119. [PubMed: 23982761]
- Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell*. 2003; 113:685–700. [PubMed: 12809600]

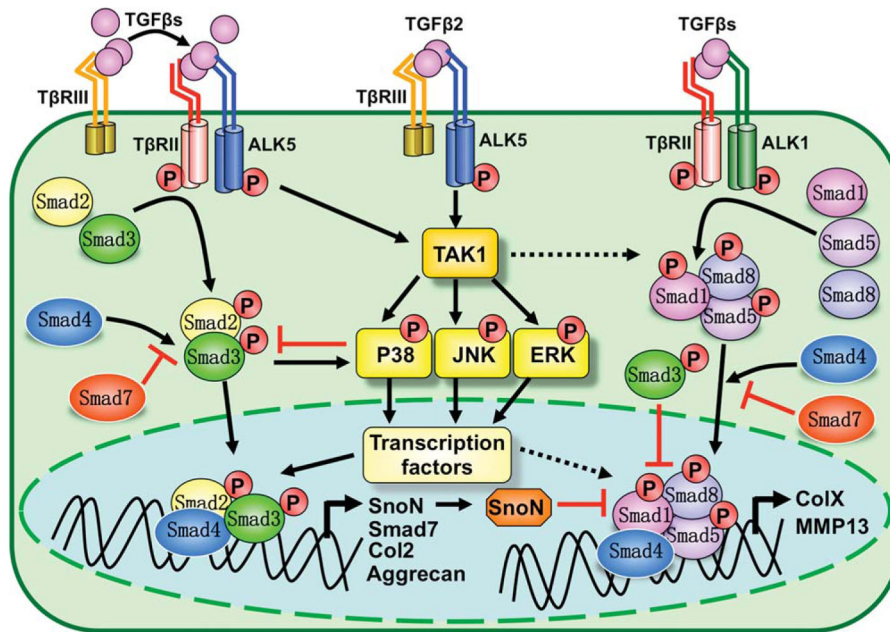
- Shintani N, Siebenrock KA, Hunziker EB. TGF- β 1 enhances the BMP-2-induced chondrogenesis of bovine synovial explants and arrests downstream differentiation at an early stage of hypertrophy. *PLoS one*. 2013; 8:e53086. [PubMed: 23301025]
- Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359:693–699. [PubMed: 1436033]
- Shull MM, Ormsby I, Kier AB, et al. Targeted disruption of the mouse transforming growth factor- β 1 gene results in multifocal inflammatory disease. *Nature*. 1992; 359:693–699. [PubMed: 1436033]
- Song B, Estrada KD, Lyons KM. Smad signaling in skeletal development and regeneration. *Cytokine Growth Factor Rev*. 2009; 20:379–388. [PubMed: 19926329]
- Song JJ, Aswad R, Kanaan RA, et al. Connective tissue growth factor (CTGF) acts as a downstream mediator of TGF- β 1 to induce mesenchymal cell condensation. *J Cell Physiol*. 2007; 210:398–410. [PubMed: 17111364]
- Sorrentino A, Thakur N, Grimsby S, et al. The type I TGF- β receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nat Cell Biol*. 2008; 10:1199–1207. [PubMed: 18758450]
- Spagnoli A, O’Rear L, Chandler RL, et al. TGF- β signaling is essential for joint morphogenesis. *J Cell Biol*. 2007; 177:1105–1117. [PubMed: 17576802]
- Ten Dijke P, Yamashita H, Ichijo H, et al. Characterization of type I receptors for transforming growth factor- β and activin. *Science*. 1994; 264:101–104. [PubMed: 8140412]
- Thiery JP. Epithelial–mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol*. 2003; 15:740–746. [PubMed: 14644200]
- Thorp BH, Anderson I, Jakowlew SB. Transforming growth factor- β 1, - β 2 and - β 3 in cartilage and bone cells during endochondral ossification in the chick. *Development*. 1992; 114:907–911. [PubMed: 1618152]
- Tuli R, Tuli S, Nandi S, et al. Transforming growth factor- β -mediated chondrogenesis of human mesenchymal progenitor cells involves N-cadherin and mitogen-activated protein kinase and Wnt signaling cross-talk. *J Biol Chem*. 2003; 278:41227–41236. [PubMed: 12893825]
- van der Kraan PM, Blaney Davidson EN, Blom A, et al. TGF- β signaling in chondrocyte terminal differentiation and osteoarthritis: Modulation and integration of signaling pathways through receptor-Smads. *Osteoarthritis Cartilage*. 2009; 17:1539–1545. [PubMed: 19583961]
- van der Kraan PM, Goumans MJ, Blaney Davidson E, et al. Age-dependent alteration of TGF- β signalling in osteoarthritis. *Cell Tissue Res*. 2012; 347:257–265. [PubMed: 21638205]
- Waldrip WR, Bikoff EK, Hoodless PA, et al. Smad2 signaling in extraembryonic tissues determines anterior–posterior polarity of the early mouse embryo. *Cell*. 1998; 92:797–808. [PubMed: 9529255]
- Watanabe H, de Caestecker MP, Yamada Y. Transcriptional cross-talk between Smad, ERK1/2, and p38 mitogen-activated protein kinase pathways regulates transforming growth factor- β -induced aggrecan gene expression in chondrogenic ATDC5 cells. *J Biol Chem*. 2001; 276:14466–14473. [PubMed: 11278290]
- Weinstein M, Yang X, Li C, et al. Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc Natl Acad Sci USA*. 1998; 95:9378–9383. [PubMed: 9689088]
- Weiss A, Attisano L. The TGF β superfamily signaling pathway. *Dev Biol*. 2013; 2:47–63.
- Woods A, Beier F. RhoA/ROCK signaling regulates chondrogenesis in a context-dependent manner. *J Biol Chem*. 2006; 281:13134–13140. [PubMed: 16565087]
- Woods A, Wang G, Beier F. RhoA/ROCK signaling regulates Sox9 expression and actin organization during chondrogenesis. *J Biol Chem*. 2005; 280:11626–11634. [PubMed: 15665004]
- Wu MY, Hill CS. Tgf- β superfamily signaling in embryonic development and homeostasis. *Dev Cell*. 2009; 16:329–343. [PubMed: 19289080]
- Xu X, Han J, Ito Y, et al. Ectodermal Smad4 and p38 MAPK are functionally redundant in mediating TGF- β /BMP signaling during tooth and palate development. *Dev Cell*. 2008; 15:322–329. [PubMed: 18694570]

- Yamaguchi K, Shirakabe K, Shibuya H, et al. Identification of a member of the MAPKKK family as a potential mediator of TGF-beta signal transduction. *Science*. 1995; 270:2008–2011. [PubMed: 8533096]
- Yamashita M, Fatyol K, Jin C, et al. TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Mol Cell*. 2008; 31:918–924. [PubMed: 18922473]
- Yang G, Sun Q, Teng Y, et al. PTEN deficiency causes dyschondroplasia in mice by enhanced hypoxia-inducible factor 1alpha signaling and endoplasmic reticulum stress. *Development*. 2008; 135:3587–3597. [PubMed: 18832389]
- Yang X, Chen L, Xu X, et al. TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol*. 2001; 153:35–46. [PubMed: 11285272]
- Yang X, Letterio JJ, Lechleider RJ, et al. Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-beta. *EMBO J*. 1999; 18:1280–1291. [PubMed: 10064594]
- Yeganeh B, Mukherjee S, Moir LM, et al. Novel non-canonical TGF-beta signaling networks: emerging roles in airway smooth muscle phenotype and function. *Pulm Pharmacol Therapeut*. 2013; 26:50–63.
- Yonekura A, Osaki M, Hirota Y, et al. Transforming growth factor-beta stimulates articular chondrocyte cell growth through p44/42 MAP kinase (ERK) activation. *Endocrine J*. 1999; 46:545–553. [PubMed: 10580747]
- Yoon BS, Lyons KM. Multiple functions of BMPs in chondrogenesis. *J Cell Biochem*. 2004; 93:93–103. [PubMed: 15352166]
- Yu L, Hebert MC, Zhang YE. TGF-beta receptor-activated p38 MAP kinase mediates Smad-independent TGF-beta responses. *EMBO J*. 2002; 21:3749–3759. [PubMed: 12110587]
- Zhang JH, Tan XH, Li WL, et al. Smad4 is required for the normal organization of the cartilage growth plate. *Dev Biol*. 2005; 284:311–322. [PubMed: 16023633]
- Zhang R, Murakami S, Coustry F, et al. Constitutive activation of MKK6 in chondrocytes of transgenic mice inhibits proliferation and delays endochondral bone formation. *Proc Natl Acad Sci USA*. 2006; 103:365–370. [PubMed: 16387856]
- Zhang X, Ziran N, Goater JJ, et al. Primary murine limb bud mesenchymal cells in long-term culture complete chondrocyte differentiation: TGF-beta delays hypertrophy and PGE2 inhibits terminal differentiation. *Bone*. 2004; 34:809–817. [PubMed: 15121012]
- Zhang YE. Non-Smad pathways in TGF-beta signaling. *Cell Res*. 2009; 19:128–139. [PubMed: 19114990]
- Zhu Y, Richardson JA, Parada LF, et al. Smad3 mutant mice develop metastatic colorectal cancer. *Cell*. 1998; 94:703–714. [PubMed: 9753318]

**FIGURE 1.**

Process of endochondral bone formation and TGFβ's role during cartilage development. (A) Mesenchymal progenitor cells condense. (B) Cells of condensations become chondrocytes that synthesize a collagen II and aggrecan-rich extracellular matrix (ECM), constructing a cartilage template (anlagen) for endochondral ossification. (C) Chondrocytes at the center of condensation stop proliferating and become hypertrophic chondrocytes that synthesize a collagen X-rich ECM, the hypertrophic cartilage (hc) is calcified and provides the niche for vascular invasion. (D) Hypertrophic chondrocytes undergo apoptosis and attract blood vessels (v) that bring in precursors of osteoblasts, which secrete collagen I into ECM, and build the calcified bone (b) in the primary ossification center (poc). Perichondrial cells adjacent to hypertrophic chondrocytes also become osteoblasts, forming bone collar (bc). Chondrocytes continue to proliferate and differentiate in the growth plate that drives the bone elongation. Chondrocytes differentiate into four different cell subtypes, including resting (Rc), proliferative (Plc), prehypertrophic (Phc), and hypertrophic chondrocyte (Hc). (E) At the end of the bone, the secondary ossification center (soc) forms through cycles of chondrocyte hypertrophy, vascular invasion, and osteoblast activity. The soc separates the epiphyseal cartilage from the growth plate cartilage. (F) Epiphyseal cartilage becomes articular cartilage (ac) at the end of bone. In humans the primary and secondary ossification centers fuse after puberty, whereas in adult mice a narrow region of growth plate cartilage remains. (G) During cartilage development, TGFβ signaling promotes mesenchymal cell condensation and differentiation into chondrocytes, and maintains resting chondrocytes in a quiescent stage. It prevents chondrocytes from further differentiating into proliferative cells, and inhibits prehypertrophic and hypertrophic differentiation. In articular cartilage, TGFβ signaling cooperates with BMP signaling to stimulate anabolic function of chondrocytes, for example, enhancing collagen II (Col2) and aggrecan expression. On the contrary, TGFβ

signaling antagonizes BMP signaling to prevent calcification and catabolic gene expression, such as collagen X (ColX) and matrix metalloproteinase 13 (MMP13).

**FIGURE 2.**

Canonical and noncanonical TGF β signaling pathways in cartilage formation and maintenance. Canonical Smad-dependent TGF β signaling is initiated by TGF β ligand binding to receptors and assembly of heteromeric complexes of type II (T β RII) and type I (ALK5) receptors. Type III (T β RIII) receptor stabilizes TGF β s, particularly TGF β 2, at the cell membrane, and facilitates presentation of TGF β s to T β RII and ALK5, which are activated and then transduce signals through Smad2 and 3. Activated Smads form a complex with Smad4 and then translocate into the nucleus, where they interact with other transcription factors to regulate target gene expression. Activated ALK5 can relay signaling to noncanonical TAK1-mediated pathways, which are also the primary pathways activated by T β RIII/ALK5 complexes that do not contain T β RII. TAK1 pathways act either by converging with or repressing Smad2/3, depending on the cell context. TGF β s can also activate Smad1/5/8 (BMP) pathways through ALK1 receptor. The balance of signaling through Smad2/3 versus Smad1/5/8 thus depends on ratios of ALK1 and ALK5 expression. Smad2/3-mediated TGF β signaling generally counteracts Smad1/5/8 (BMP) pathways through inhibiting transcriptional activity by forming mixed Smad3/Smad1/5 complexes, or through SnoN, and blocking Smad1/5/8 interaction with Smad4. TAK1 can activate and cooperate with Smad1/5/8 to regulate cartilage development, but whether and how TAK1 mediated TGF β signaling interacts with Smad1/5/8 is still not clear.

TABLE 1

Genetic Modified Mouse Models on TGF β Signaling on Cartilage Formation and Maintaining

Gene	Models	Defects	References
Tgf β 1	-/-	No defect in cartilage formation, 50% embryos die early	Shull et al. (1992), Dunker et al. (2001)
Tgf β 2	-/-	Generalized chondrodysplasia	Sanford et al. (1997)
Tgf β 3	-/-	No defects in chondrogenesis, perinatal lethality	Proetzel et al. (1995), Kaartinen et al. (1995)
Alk5	Flox; Dermo1-Cre	Fused knee joints, short limbs and ectopic cartilaginous protrusions	Matsunobu et al. (2009)
Tgfb2	Flox; Prx1-Cre	Fused phalangeal joints, decreased chon. proliferation, delayed hypertrophic differentiation	Longobardi et al. (2012)
	Flox; Wnt1-Cre	Craniofacial deformities	Iwata et al. (2012)
	Flox; Col2a1-Cre	No defect in appendicular elements, defective segmentation and formation of intervertebral discs	Baffi et al. (2004)
	Flox; Col2-CreER	osteoarthritis(OA)-like phenotype	Shen et al. (2013)
DN-Tgfb2 ^a	Col11a2-promoter/enhancer; Flox; Prx1-Cre	Hypoplasia, absence of hypertrophic chondrocytes at 14.5 dpc	Hiramatsu et al. (2011)
Smad3	-/-	Increased chondrocyte hypertrophy, osteoarthritis-like symptoms	Yang et al. (2001)
	Flox; Col2a1-Cre	No cartilage defects at prenatal stages, articular cartilage degeneration	Chen et al. (2012)
Smad4	-/-	Early embryonic lethal	Chu et al. (2004)
	Flox; Col2a1-Cre	Dwarfism, disorganized growth plate, delay in chondrocyte maturation	Zhang et al. (2005)
	Flox; Prx1-Cre	Halted limb bud development and carpal fusions	Benazet et al. (2012)
Smad7	-/-	Early postnatal lethality, retained proliferative chondrocytes, hypocellular cores, anterior/posterior transformation.	Estrada et al. (2013)
Tak1	Flox; Prx1-Cre	Survive to the weaning stag, decreased chondrocyte proliferation, delays in both the onset and progression of chondrocyte maturation, joint fusions,	Gunnell et al. (2010)
	Flox; Col2-Cre	Die before birth, decreased chondrocyte proliferation and survival, delayed onset of hypertrophy, elbow abnormalities	Gunnell et al. (2010)
	Flox; Col2a1-Cre	Survive postnatally for 2–3 weeks, neonatal-onset runting, decreased chondrocyte proliferation, delayed formation of secondary ossification centers, and defects in formation of the elbow and tarsal joints	Greenblatt et al. (2010)
	Flox; Col2-CreER	Display severe growth retardation and OA-like phenotype	Gao et al. (2013)
CA-MKK6 ^a	Col2a1 promoter	Dwarfism, inhibition of both chondrocyte proliferation and differentiation, and a delay in primary and secondary ossification	Zhang et al. (2006)
DN-P38 ^a	Col2 promoter	Dwarfism, OA-like phenotype	Namdari et al. (2008)
CA-MEK1 ^a	Col2a1 promoter	Dwarfism, inhibits hypertrophic differentiation, no defect of chondrocyte proliferation	Murakami et al. (2004)
Akt1	-/-	Dwarfism, exhibit decreased calcification in the growth plate with fewer osteophyte formation in OA	Fukai et al. (2010)

Gene	Models	Defects	References
Pten	Flox; Col2a1-Cre	Dyschondroplasia, defects in chondrocyte proliferation and maturation, and exhibit aberrant neoplastic cores	Yang et al. (2008)

^aCA: Constitutive active, DN: Dominant negative.