



HAL
open science

Regulation and Role of TGF β Signaling Pathway in Aging and Osteoarthritis Joints

Catherine Baugé, Nicolas Girard, Eva Lhuissier, Celine Bazille, Karim Boumediene

► **To cite this version:**

Catherine Baugé, Nicolas Girard, Eva Lhuissier, Celine Bazille, Karim Boumediene. Regulation and Role of TGF β Signaling Pathway in Aging and Osteoarthritis Joints. *Aging and disease*, 2014, 5 (6), pp.394-405. 10.14336/AD.2014.0500394 . hal-01147762

HAL Id: hal-01147762

<https://hal.science/hal-01147762>

Submitted on 1 May 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Review Article

Regulation and Role of TGF β Signaling Pathway in Aging and Osteoarthritis Joints

Catherine Baugé^{1,2,*}, Nicolas Girard^{1,2}, Eva Lhuissier^{1,2}, Celine Bazille^{1,2,3},
Karim Boumediene^{1,2}.

¹ Normandie Univ, France

² UNICAEN, EA4652 MILPAT, Caen, France

³ Service d'Anatomie Pathologique, CHU, Caen, France

[Received October 16, 2013; Revised December 4, 2013; Accepted December 4, 2013]

ABSTRACT: Transforming growth factor beta (TGF β) is a major signalling pathway in joints. This superfamily is involved in numerous cellular processes in cartilage. Usually, they are considered to favor chondrocyte differentiation and cartilage repair. However, other studies show also deleterious effects of TGF β which may induce hypertrophy. This may be explained at least in part by alteration of TGF β signaling pathways in aging chondrocytes. This review focuses on the functions of TGF β in joints and the regulation of its signaling mediators (receptors, Smads) during aging and osteoarthritis.

Key words: osteoarthritis, TGFbeta, chondrocytes, aging

Osteoarthritis, an aging disease

Osteoarthritis (OA) is the most common form of arthritis with over 151 million sufferers worldwide [1]. It is one of the most common diagnoses in general practice [2]. OA can occur in any joint but is more common in the joints of the hand, knee, and hip [3]. It is mainly characterized by the progressive degradation of cartilage [1]. However, OA is not only a disease of cartilage; it affects all the tissues of the joint, including synovium, subchondral bone, capsule, ligaments, periarticular muscles and the sensory nerves whose termini lie within these tissues. Furthermore, OA may be resulted from abnormalities in any of the above tissues. OA leads to pain, functional impairment and limited movements, which in turn contribute to reduced social interactions and may lead to depression [3].

The major tissue affected by OA is articular cartilage. This latter is located on the surfaces of joints involved in mechanical movement [4]. This layer of hyaline cartilage protects bone to continual compression and friction and acts as a lubricant to facilitate movement [5]. Cartilage is

composed primarily of water, collagen, proteoglycans, and chondrocytes (the only resident cells) [4]. Chondrocytes respond to changes induced by joint loading, cytokines, growth factors, and the presence of fragmented matrix molecules in the extracellular matrix of cartilage [1], and are responsible for maintaining a balanced cartilage turnover [6].

Healthy articular cartilage is a stable tissue that functions for decades to keep normal joint movement possible. In contrast, osteophytic cartilage is temporary and subjects to endochondral ossification. This is thought to occur in OA cartilage at least in a part of the patients, and to induce changes in gene expression of chondrocytes [7]. Compared to intact human articular cartilage, osteophytic chondrocytes express more genes involved in endochondral ossification, such as BMPs or Runx2, and also enzymes mediating tissue remodeling, like MMP9 and MMP13. In contrast, expression of inhibitors for the BMP-signaling pathways is decreased. This blocking of BMP signaling in healthy articular cartilage may inhibit chondrocyte terminal differentiation, and the loss of this blockage and elevated BMP signaling might release the

*Correspondence should be addressed to: Catherine Baugé EA4652, UFR de médecine, Université de Caen, CS14032 Caen cedex 5, France. Email: catherine.bauge@unicaen.fr

brake on chondrocyte endochondral ossification and matrix breakdown, and participate to osteophyte formation.

OA is one of the most common sources of pain and disability in the elderly [8,9] and age is considered as the single greatest risk factor [10,11]. Indeed, OA development is highly age-related. For instance, the prevalence of radiographic knee OA, the most common location, increased with each decade of life from 33% among those aged 60–70 to 43.7% among those over 80 years of age [12]. The prevalence of primary hip OA also increases with age from 0.7% in the 40–44 age group to 14% in the 85+ age group [13].

There is mounting evidences that the changes occurring in the articular cartilage during the development of OA are the result of an age-related loss in normal homeostasis. The chondrocyte is the one cell type present in articular cartilage, and therefore is responsible for both synthesis and breakdown of the cartilaginous extracellular matrix [14]. Signals generated by cytokines, growth factors, and cartilage matrix regulate chondrocyte metabolic activity. In OA cartilage, it appears that the inflammatory and catabolic signals are in excess relative to anabolic factors. This imbalance promotes increased production of matrix degrading enzymes by chondrocytes, including matrix metalloproteinases, aggrecanases and other proteases that degrade the cartilage matrix. These changes that can also occur in aging chondrocyte, appear to contribute to the loss in homeostasis, and in particular in the loss of TGFβ signaling responsiveness and will be discussed next.

TGFβ signaling pathways

The transforming growth factor-β (TGFβ) superfamily is comprised of almost forty ligands responsible for numerous cellular processes including early embryonic development, tissue patterning and homeostasis, bone formation, wound healing and fibrosis [15,16]. In cartilage, the main representatives of this superfamily are TGFβ and BMP. Both of them are crucial for normal joint development and homeostasis and have been implicated in the pathogenesis of OA.

Members of the TGFβ superfamily are synthesized as large precursor molecules that are proteolytically processed in the Golgi apparatus by the convertase family of endonucleases. They are secreted from cells as a dimeric small latent complex (SLC) comprising non-covalently associated latency-associated propeptide (LAP) and active TGFβ and/or as a large LLC comprising SLC bound covalently to a latent TGFβ-binding protein (LTBP) [17,18]. Physiological activation mechanisms leading to receptor signaling are incompletely understood. They may involve LTBP-1-mediated proteolytic release,

thrombospondin-1 (TSP-1) competition with SLC, integrin presentation, pH changes, and reactive oxygen species [17–20].

Once activated, the TGFβ superfamily (including bone morphogenetic proteins (BMPs)) signals via heteromeric complexes of type I (ALK) and type II receptors, recruiting downstream R-Smad proteins (Receptor-regulated Smad: Smad1, Smad2, Smad3, Smad5, and Smad8) and co-Smads (Smad4, serves as a common partner for all R-Smad) before translocating to the nucleus and act as a transcription factor on target genes [21]. TGFβ can also exert its biological effects *via* non-Smad pathways [22]. In noncanonical pathways, TGFβs may transduce their signals via MAP kinases through TGFβ-activating kinase (TAK1). They can utilize a multitude of intracellular signaling pathways including extracellular regulated kinase (ERK) [23], p38 kinase [21], c-Jun N-terminal kinase (JNK) [24], phosphatidylinositol-3-kinase (PI3K)/AKT [25], or Rho-like GTPase [26] signaling pathways, to regulate cell function in coordination with the Smad pathway.

Seven type I receptors (ALK) and five type II receptors exist. They are all single-pass transmembrane receptors, which contain intracellular serine/threonine kinase domains. ALK is unable to directly bind its ligand, but forms a high-affinity heteromeric receptor complex with TβRII in its presence. Upon assembly, the intracellular domain of ALK is phosphorylated by TβRII on a conserved GS domain, leading the activation of its kinase activity and the phosphorylation of R-Smads [27,28]. The recruitment of R-Smads to the cytoplasmic domains of the ALK/TβRII complex, is facilitated by the Smad anchor for receptor activation (SARA) [29]. Upon activated, R-Smads modify their conformation, thereby facilitating their heteromerization with Smad4 which allows translocation to the nucleus, where it acts to regulate the transcription of various target genes [28].

Typically, TGFβ signals via ALK5 which phosphorylates Smad2/3, while BMP typically signals through ALK1, 2, 3, and 6 phosphorylating Smad1/5/8. In chondrocyte differentiation, Smad2/3 is required for early chondrogenesis and to block terminal differentiation while Smad1/5/8 is necessary for terminal differentiation [30–32]. All these R-Smads play critical roles as transcriptional regulators in osteoblastogenesis and chondrogenesis.

Downregulation of TGFβ signaling is mediated extracellularly by ligand antagonists, and intracellularly by attenuation of R-Smad activity, in part by inhibitory Smads (I-Smad) 6 and 7. I-Smads recruit E3 ubiquitin ligases to type I receptors, leading to their degradation [33,34]. In addition, I-Smads can interfere with R-Smad phosphorylation [35]. While Smad6 specifically inhibits

the BMP pathway, Smad7 can inhibit both BMP and TGFβ pathways [36].

Role of TGFβ superfamily in cartilage: angel or devil?

TGFβ superfamily plays numerous roles in cartilage. Some are benefit, whereas other may favor OA process.

TGFβ sub-family (TGFβ1, 2 and 3)

TGFβ1, 2, and 3 are expressed by perichondrial cells and hypertrophic chondrocytes. The expressions of TGFβ1 and 2 are maintained in adult articular cartilage, suggesting a role for the TGFβ pathway in the maintenance of permanent cartilage [37,38]. Concerning receptors, TGFβ receptor type 1 (ALK5) and TGF beta receptor II (TGFβr2 or TβRII) are expressed in perichondrial cells as well as proliferating and hypertrophic chondrocytes within the growth plate.

The mechanical shearing of synovial fluid induced during joint motion, rapidly activates latent TGFβ, which is secreted from both synovium and articular cartilage. The active TGFβ peptide accumulates in the superficial zone of articular cartilage, due to the presence of an overwhelming concentration of unspecific TGFβ binding sites in the extracellular matrix. This leads to high level of active TGFβ in the superficial zone of cartilage. However, the active peptide is unable to penetrate deeper into the middle and deep zones of cartilage [39].

The role of TGFβ in joints is controversial [40]. While TGFβ may favor osteoarthritis [41], it also favors chondrogenesis. It promotes the differentiation of embryonic chick limb cartilage [42] and up-regulates a number of molecules associated with prechondrogenic condensation [43–45]. Besides, primary chondrogenesis derived from mesenchymal stem cells (MSCs) needs TGFβ signals [46–48]. TβRII plays a role major in this process. Its expression is induced during chondrogenesis [49,50], and its downregulation induced by retinoid acid treatment inhibits chondrogenesis of mouse embryonic palate mesenchymal cells [51]. Furthermore, TGFβs have been shown to inhibit chondrocyte hypertrophy. This action was supported by phenotypes of mice overexpressing a dominant-negative form of TGFβr2 [43] and *Smad3*^{-/-} mice [31]; these mutant mice showed severe progressive osteoarthritis, in which the hypertrophic zone was enlarged and the proliferating zone was reduced in postnatal articular and growth plate chondrocytes.

In addition, TGFβ1 stimulates chondrocyte synthetic activity and decreases the catabolic activity of IL-1 [52–57]. TGFβ increases the synthesis of protease inhibitors such as tissue inhibitor of metalloproteinase (TIMP) and decreases the production of several MMP. It counteracts

NO production induced by IL1 [58]. In addition, TGFβ is able to increase the production of essential cartilage matrix molecules such as aggrecan and type II collagen [54,59], and prevent loss of proteoglycan in articular cartilage during experimental OA [57,60–62]. TGFβ also functions as anti-arthritis [63,64] and is able to block inflammation *in vivo* [65]. TGFβ also stimulated extracellular matrix (ECM) synthesis and has been evaluated *in vitro* in rabbit models of acute cartilage injury [66–68].

However, other studies show a negative effect of TGFβ on cartilage. It induces the synthesis of MMP-13 (collagenase-3) in a subpopulation of human articular chondrocytes [69] or MMP-9 in normal equine chondrocytes [70]. In synovial lining cells, TGFβ has also been shown to increase the synthesis of aggrecanases (ADAMTS4/5), MMP-1 as well as the expression of pro-inflammatory cytokines [71]. Enhancement of these genes could result in accelerated breakdown of cartilage [72]. Consequently, TGFβ could contribute to the progression of inflammation and joint destruction in RA [73,74]. Moreover, repeated local administration of TGFβ resulted in OA-like changes in articular cartilage [41].

This differential effect of TGFβ responses may be explained by the modulation of canonical Smad signaling pathways by TGFβ itself. Indeed, our recent research works showed that TGFβ1 exerts a biphasic effect on chondrocytes, at least *in vitro* [75]. A short TGFβ1 administration induces Sox9 expression, followed by induction of collagen type II expression. This effect was transient, but a second peak of collagen II expression appears later. These data suggest that at least two different mechanisms are responsible for cell response to TGFβ. A short TGFβ administration may activate the Smad2/3 pathway (upregulation of TβRI, TβRII and Smad3, and phosphorylation of Smad2/3), leading to an increase of Sox9, which, in turn, may induce collagen type II expression. This is supported by the upregulation of ALK5, and Smad3 observed after a short administration of TGFβ1, which is correlated to phosphorylation of Smad2/3. At contrary, continuous TGFβ exposure leads to a negative feedback loop, characterized by a reduction of ALK5, TβRII and Smad3 expression and simultaneous induction of the inhibitory Smad7. This leads to the blockage of Smad2/3-mediated TGFβ signalling and reduction of Sox9. This late response is also associated with increased atypical collagen expression (COL1A1 and COL10A1) and reduction of aggrecan expression. These data suggest that a non canonical pathway could be involved in this late response to TGFβ. Several pathways may be implied. In particular, the reduction of ALK5 expression may change the ratio between ALK5 and ALK1, another type I TGFβ receptor recently identified in chondrocytes, favoring TGFβ signalling via the

Smad1/5/8 route and, subsequently, chondrocyte terminal differentiation [76,77].

Bone morphogenetic proteins (BMPs)

BMPs have multiple important roles during skeletal formation [16,78]. They are expressed by chondrocytes. The perichondrium expresses *Bmp2*, 3, 4 and 7 [79–82], the hypertrophic chondrocytes expressed *Bmp2* and *Bmp6* [83], and the proliferating chondrocytes expressed *Bmp7* [84].

In humans, there are three type I receptors (BMPRIA, BMPRII and ACVRI) and three type II receptors (BMPRII, ActRIIA and ActRIIB) that bind to BMP ligands to signal. BMP receptor type 1A (*Bmpr1a*), also called as ALK3, is highly expressed in perichondrial cells, proliferating chondrocytes, and hypertrophic chondrocytes; BMP receptor type 1B (*Bmpr1b*, ALK6) is expressed throughout the growth plate and in the perichondrium; and activin A receptor type 1 (*Acvr1*, ALK2) is expressed in resting and proliferating chondrocytes [80,85–87]. BMP receptor type II (*Bmpr2*) is expressed throughout the growth plate. The specificity of signaling is primarily determined by type I receptors [88]; however, the specificity of ligand binding is altered by the combination of type I and II receptors [89]. It has been reported that BMPRIA is a potent receptor of BMP2 and BMP4 [90,91], and ACVR1 is a receptor of BMP7 [92]. The majority of BMP signaling in cartilage development occurs via the canonical pathway through R-Smads 1/5/8. It play a critical role in skeletal development, bone formation and stem cell differentiation [93,94]. Thus, mice lacking R-Smads1/5/8 present severe chondrodysplasia [95].

BMPs derive their name from their potent ability to induce ectopic bone formation when subcutaneously implanted in rodents [96]. Then, numerous studies reported that BMPs stimulate osteoblast differentiation. However, the effects of BMP signaling on chondrocyte are still debated. Both *in vitro* and *in vivo* evidence suggest that BMP signaling promotes or inhibits the hypertrophic differentiation [96–99]. In the earliest stage of chondrogenesis, BMP signaling promotes mesenchymal cells to differentiate into chondrocytes and stimulates chondrocyte proliferation by inducing *Sox9* expression [30,100,101]. BMP signaling also promotes chondrocyte hypertrophy and is required for endochondral bone formation [85,95,98,102].

In vitro, BMP-2 is able to maintain or restore the differentiated phenotype of adult chondrocytes [103,104]. However, in cultures of embryonic chondrocytes, BMP-2 induced chondrogenesis can continue to hypertrophy [105], even to osteoblast differentiation characterized by osteocalcin expression [102]. In cultures

of human mesenchymal stem cells, BMP-2 and BMP-9 increase the synthesis of cartilage-specific proteins [106]. Comparing the ability of BMP-2, BMP-4 and BMP-6 to promote the differentiation of mesenchymal stem cells from bone marrow toward chondrocyte showed that BMP-2 appears to be the most effective [107]. However, under BMP-2, mesenchymal stem cells can possibly continue their differentiation to hypertrophy and osteogenesis [108].

BMP-14, also known as cartilage-derived morphogenetic protein (CDMP-1) or GDF-5 (growth differentiation factor-5) plays also a major role in cartilage. Variations in its gene in humans have been associated with the development of osteoarthritis [109]. BMP-14 shows also some capacity to stimulate cartilage matrix synthesis. It induces the differentiation of mesenchymal stem cells into chondrocytes and promoted increased accumulation of GAG and type II collagen during pellet culture [110]. Chubinskaya *et al.* reported that addition of GDF-5 resulted in an increase in proteoglycan accumulation in adult human articular chondrocytes cultured in alginate beads for 9 days, compared with controls without growth factors [111].

Deregulation of TGF β signalling in old and OA joint (Figure 1)

Because OA is rare in young adults and even serious joint injuries usually don't manifest as OA until years later [112], it appears that young joint tissues can compensate, to some degree, to abnormal mechanical stress. But with aging, the ability to compensate to stress declines. Older adults who experience a joint injury develop OA much more rapidly than younger adults with a similar injury [113]. If the basic cellular mechanisms that maintain tissue homeostasis decline with aging, then the response to stress or joint injury will not be adequate and joint tissue destruction and OA will be the result. A mechanism possible may be a deregulation of TGF β signaling with age leading to the decline of anabolic activity of chondrocytes. In particular, several studies suggest that modifications of chondrocyte phenotype during aging result from alteration of the TGF β signalling, decline which may be at the root of OA development.

This notion is supported by studies demonstrating an age-associated decrease in proteoglycan synthesis in equine cartilage in response to TGF β I [114]. Similar decreases in TGF β responsiveness have been seen in human immature and mature cartilage explants [115]. Furthermore, 3D culture of human chondrocytes from old donors (over 40) did not show any increase in proteoglycan content following TGF β I treatment, contrasting with observations in chondrocyte cultures from young donors [116].

Blaney Davidson showed that levels of TGFβ2 and TGFβ3 (but not TGFβ1) decrease with age as does the level of TGF-β receptors I (ALK5) and II [117]. The decline of ALK5 and TβRII lead an alteration in Smad recruitment, as confirmed by the loss of phosphorylated-Smad2 in old murine chondrocytes [117], leading to illegitimate entry of chondrocytes into hypertrophy and disruption of normal cartilage homeostasis [118]. This age-induced downregulation of TβRII has also been

reported from cultures of human chondrocytes [50], and has been associated to a loss of Smad2/3 phosphorylation and an increase of collagen type X expression, MMP13 and Adamts5 [75,119]. The role of TβRII in hypertrophy is corroborated by in vivo data which show that TβRII-deficient mice have a reduced proliferation of chondrocytes and an accelerated early hypertrophic differentiation [120].

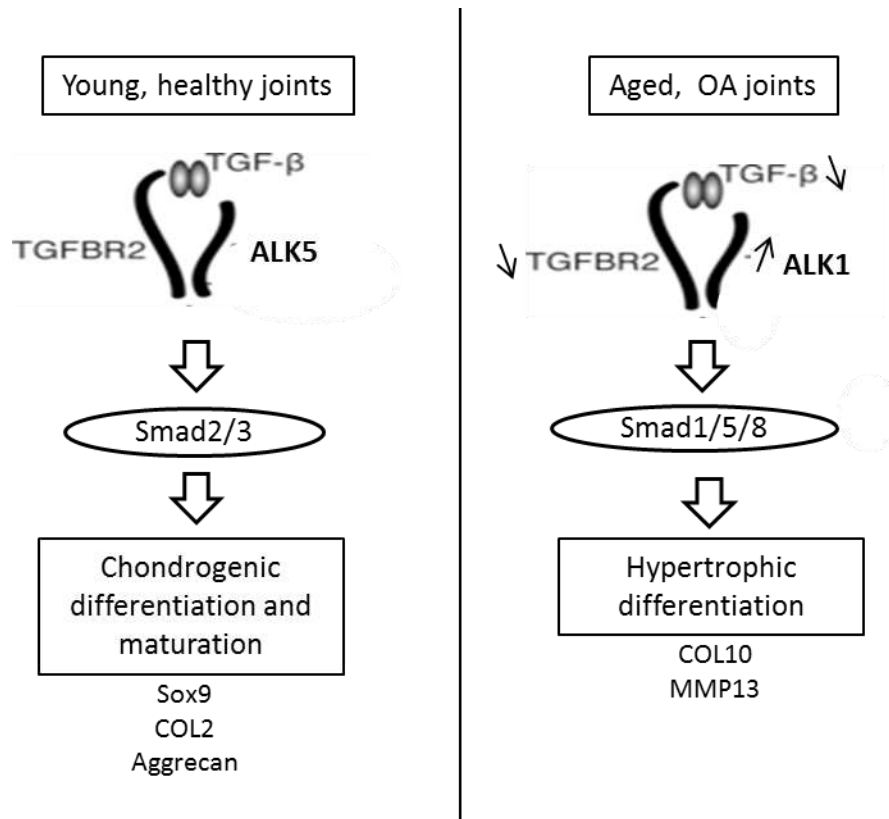


Figure 1. Role of TGF-beta in healthy and OA cartilage. TGFβ signals through TβRII and ALK5 in young and healthy cartilage eliciting chondrogenic factors. In aged or OA cartilage, TβRII and ALK5 breakdown occurs while ALK1 expression is enhanced. Therefore, TGFβ signalling shifts from Smad2/3 to Smad 1/5/8 leading to COL10 and MMP13 expression.

Besides, age also reduces ALK1 expression, but the extent of this decrease is not as great as that in ALK5, suggesting a shift from Smad2/3 signaling via ALK5 to Smad1/5/8 via ALK1 in aging chondrocytes [76,121]. The reduction of ALK5/ALK1 ratio could shift chondrocyte differentiation towards a more hypertrophic

phenotype expressing markers characteristic of OA. Indeed, ALK1 overexpression and ALK5 inhibition can induce MMP-13, while ALK5 expression induces AGC1 expression. Subsequently, all these deregulations of TGFβ receptor expression lead to the decline of anabolic response and favors hypertrophy of chondrocytes.

Surprisingly, it has been observed that aging is associated to an increase of Smad3 expression [50,117]. This Smad3 upregulation may be a consequence of the loss of TGF β signaling due to the decline of receptor expression. Since Smad3 acts as one important TGF β signaling pathway member to develop and/or maintain the phenotype of chondrocytes [31,122] and to stimulate chondrogenesis [123], it is possible that the increased Smad3 expression observed during aging could be a compensatory mechanism to promote cartilaginous phenotype. Moreover, it can be also due to a direct regulation of Smad3 gene expression by TGF β . Given that TGF β reduces Smad3 expression [75,124], the reduction of TGF β signaling may upregulate Smad3.

The deregulation of TGF β signaling is also found in OA cartilage. It is now admitted that OA chondrocytes lose their capacity to respond to TGF β . This decrease of TGF β responsiveness is correlated to a decrease of T β R2 expression in OA cartilage [125]. This downregulation of T β R2 cannot be only imputed to aging, since it is also found in experimental induced-OA cartilage in young rabbit [126]. At least, another mechanism may explain this downregulation, namely the increase of IL1 level in OA joint. Indeed, we have now well-established that this proinflammatory cytokine reduces T β R2 gene transcription [127] and increases receptor degradation [128] making cells insensitive to TGF β [53]. Furthermore, OA development is accompanied by a decrease of ALK5 [125,126]. These deregulations of TGF β receptors may be one of OA roots.

The response to BMP in aging is less well reported. However, rabbit intervertebral disc cells show reduced proteoglycan synthesis in response to BMP2 in old compared to young animals [129].

Potential of TGF β in the development of novel therapeutic strategies to treat cartilage defects and OA

TGF β family members, mainly TGF β 1, TGF β 3 or BMP2, are often used for the development of cartilage engineering strategy. These growth factors can be introduced by different ways: direct addition to the culture medium, overexpression in genetically engineering cells [130], construction of polymeric systems that provide for the controlled release of growth factors [131], direct incorporation of plasmid DNA encoding growth factors into scaffolds [132,133], and embedding cationic polymeric gene delivery systems that encode growth factors into scaffolds for sustained release of pDNA [134,135].

TGF β 1 is an important growth factor in tissue engineering for cartilage repair. It has been shown to promote chondrocyte proliferation and differentiation, both of which are important features of effective cartilage

regeneration [132,136,137]. TGF β is also known to be a potent inducer of stem cells chondrogenic differentiation [138–140] and favor the differentiation of MSCs to form ectopic cartilage *in vivo* [141]. Supplementation with TGF β 1 could initiate and promote chondrogenesis of synovium-derived stem cell (SDSCs), but TGF β 1 alone was insufficient to fully differentiate SDSCs into chondrocytes. However, it is reported that TGF β inhibits early chondrogenic induction of human ESCs but is required at the later stages of the differentiation, and TGF β can sustain an undifferentiated population of ESCs within the differentiation culture, suggesting that caution should be exercised to avoid possible teratoma formation *in vivo* when using TGF β as a chondrogenic inducer of ESCs [142]. In addition, a high dose of TGF β 1 *via* intra-articular injection is known to induce chemotaxis and activation of inflammatory cells, resulting in characteristic cartilage defects such as fibrosis and osteophyte formation [135,143,144]. Therefore, it is evident that TGF β 1 should be administered in a controlled manner to minimize adverse effects.

Another TGF β superfamily member often used for the development of cartilage engineering strategy is BMP2. Since BMP-2 was more potent than TGF β 1 in inducing not only the expression of the gene for type-II collagen but also the post-translational production and secretion of the protein itself, it would appear to be the more promising candidate of the two for the generation of a hyaline type of cartilage at least from synovial explants [145]. However, BMP-2 alone was unable to effect the complete differentiation of synovial explants into a typically hyaline type of articular cartilage throughout the entire tissue volume, and the synovial cells underwent full downstream differentiation into the terminal hypertrophic state, leading to calcification of the extracellular matrix.

All these experiments showed that a limit of this strategy is the development of adverse effects, mainly the development of a hypertrophic cartilage characterized by type X collagen and Runx2 expression, or a fibroblastic cartilage with a high expression of type I collagen. Find a way to reduce these adverse effects is subsequently essential to the development of an efficient strategy of tissue engineering for cartilage repair. Some researchers propose to co-treat cell with TGF β and BMP2. Pretreatment with TGF β could prevent fully differentiation of MSCs encapsulated in alginate beads into osteoblasts [146]. Although BMP-2 induces osteogenic and chondrogenic phenotypes in alginate-encapsulated adipose-derived stem cells, TGF β 1 can inhibit BMP-2-induced differentiation of the osteogenic lineage, and combined growth factor treatment shows a synergistic effect on the expression of cartilage-specific genes and elevated release of cartilage-specific ECM proteins [147]. Another way to reduce efficiently the

adverse effects of TGFβ or BMP2 addition might modulate TGFβ receptor expression. Indeed, we found that TβRII expression level is intimately linked to differentiation status of chondrocytes, and that TβRII ectopic expression permits to restore TGFβ1 responsiveness as well as to increase the expression of some specific cartilage matrix components in chondrocytes [50]. Subsequently, the reintroduction of TβRII in chondrocytes may improve the current strategies of cartilage engineering. Indeed some studies show a benefic effect of TGFβ transgenesis for inducing chondrocyte phenotype. However, since TGFβ at long term, has a negative effect due to the loss of TβRII [75], it may be interesting to co-transfect TβRII in order to maintain benefic effect of TGFβ1 [50]. Similarly, it may be pertinent to reintroduce ALK5 in OA chondrocytes before reimplantation in patients.

Acknowledgements

Research of the authors was supported by European Community in the context of Genostem integrated project (6th framework program), Conseil regional de Basse-Normandie, and Société Française de Rhumatologie (SFR). NG is a recipient of a fellowship from Conseil regional de Basse-Normandie.

References

- [1] Wieland HA, Michaelis M, Kirschbaum BJ, Rudolph KA (2005). Osteoarthritis - an untreatable disease? *Nat Rev Drug Discov*, 4:331–44.
- [2] Guccione AA, Felson DT, Anderson JJ, Anthony JM, Zhang Y, Wilson PW, et al. (1994). The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. *Am J Public Health*, 84:351–8.
- [3] Bijlsma JW, Berenbaum F, Lafeber FP (2011). Osteoarthritis: an update with relevance for clinical practice. *The Lancet*, 377:2115–26.
- [4] Pearle AD, Warren RF, Rodeo SA (2005). Basic Science of Articular Cartilage and Osteoarthritis. *Clin Sports Med*, 24:1–12.
- [5] Sun HB (2010). Mechanical loading, cartilage degradation, and arthritis. *Ann N Y Acad Sci*, 1211:37–50.
- [6] Goldring MB, Marcu KB (2009). Cartilage homeostasis in health and rheumatic diseases. *Arthritis Res Ther*, 11:224.
- [7] Gelse K, Ekici AB, Cipa F, Swoboda B, Carl HD, Olk A, et al. (2012) Molecular differentiation between osteophytic and articular cartilage – clues for a transient and permanent chondrocyte phenotype. *Osteoarthritis Cartilage*, 20:162–71.
- [8] Arden N, Nevitt MC (2006). Osteoarthritis: Epidemiology. *Best Pract Res Clin Rheumatol*, 20:3–25.
- [9] Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, et al. (2008) Estimates of the Prevalence of Arthritis and Other Rheumatic Conditions in the United States, Part II. *Arthritis Rheum*, 58:26–35.
- [10] Wu Z, Schimmele CM, Chappell NL (2012). Aging and Late-Life Depression. *J Aging Health*, 24:3–28.
- [11] Anderson AS, Loeser RF. (2010) Why is Osteoarthritis an Age-Related Disease? *Best Pract Res Clin Rheumatol*, 24:15.
- [12] Felson DT, Naimark A, Anderson J, Kazis L, Castelli W, Meenan RF (1987). The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis Rheum*, 30:914–8.
- [13] Dagenais S, Garbedian S, Wai EK (2009). Systematic Review of the Prevalence of Radiographic Primary Hip Osteoarthritis. *Clin Orthop*, 467:623–37.
- [14] Goldring MB, Goldring SR (2007). Osteoarthritis. *J Cell Physiol*, 213:626–34.
- [15] Attisano L, Cárcamo J, Ventura F, Weis FM, Massagué J, Wrana JL (1993). Identification of human activin and TGF beta type I receptors that form heteromeric kinase complexes with type II receptors. *Cell*, 75:671–80.
- [16] Hogan BL (1996). Bone morphogenetic proteins in development. *Curr Opin Genet Dev*, 6:432–8.
- [17] Annes JP, Munger JS, Rifkin DB (2003). Making sense of latent TGFβ activation. *J Cell Sci*, 116:217–24.
- [18] Hyytiäinen M, Penttinen C, Keski-Oja J (2004). Latent TGF-beta binding proteins: extracellular matrix association and roles in TGF-beta activation. *Crit Rev Clin Lab Sci*, 41:233–64.
- [19] Fontana L, Chen Y, Prijatelj P, Sakai T, Fässler R, Sakai LY, et al. (2005) Fibronectin is required for integrin αvβ6-mediated activation of latent TGF-β complexes containing LTBP-1. *FASEB J*, 19:1798–808.
- [20] Gomez-Duran A, Mulero-Navarro S, Chang X, Fernandez-Salguero PM (2006). LTBP-1 blockade in dioxin receptor-null mouse embryo fibroblasts decreases TGF-β activity: Role of extracellular proteases plasmin and elastase. *J Cell Biochem*, 97:380–92.
- [21] Nakao A, Imamura T, Souchelnytskyi S, Kawabata M, Ishisaki A, Oeda E, et al. (1997). TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4. *EMBO J*, 16:5353–62.
- [22] Moustakas A, Heldin C-H (2005). Non-Smad TGF-β signals. *J Cell Sci*, 118:3573–84.
- [23] Hartsough MT, Mulder KM. (1995). Transforming growth factor beta activation of p44mapk in proliferating cultures of epithelial cells. *J Biol Chem*, 270:7117–24.
- [24] Engel ME, McDonnell MA, Law BK, Moses HL (1999). Interdependent SMAD and JNK signaling in transforming growth factor-beta-mediated transcription. *J Biol Chem*, 274:37413–20.
- [25] Wilkes MC, Mitchell H, Penheiter SG, Doré JJ, Suzuki K, Edens M, et al (2005). Transforming growth factor-beta activation of phosphatidylinositol 3-kinase is independent of Smad2 and Smad3 and regulates fibroblast responses via p21-activated kinase-2. *Cancer Res*, 65:10431–40.
- [26] Bhowmick NA, Ghiassi M, Bakin A, Aakre M, Lundquist CA, Engel ME, et al. (2001) Transforming

- growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Mol Biol Cell*, 12:27–36.
- [27] Wrana JL, Attisano L, Wieser R, Ventura F, Massagué J (1994). Mechanism of activation of the TGF-beta receptor. *Nature*, 370:341–7.
- [28] Attisano L, Wrana JL. Signal transduction by the TGF-beta superfamily (2002). *Science*, 296:1646–7.
- [29] Tsukazaki T, Chiang TA, Davison AF, Attisano L, Wrana JL (1998). SARA, a FYVE domain protein that recruits Smad2 to the TGFbeta receptor. *Cell*, 95:779–91.
- [30] Denker AE, Nicoll SB, Tuan RS (1995). Formation of cartilage-like spheroids by micromass cultures of murine C3H10T1/2 cells upon treatment with transforming growth factor-beta 1. *Differ Res Biol Divers*, 59:25–34.
- [31] Yang X, Chen L, Xu X, Li C, Huang C, Deng CX (2001). TGF-beta/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J Cell Biol*, 153:35–46.
- [32] Yoon DM, Fisher JP (2006). Chondrocyte signaling and artificial matrices for articular cartilage engineering. *Adv Exp Med Biol*, 585:67–86.
- [33] Inoue Y, Imamura T (2008). Regulation of TGF-beta family signaling by E3 ubiquitin ligases. *Cancer Sci*, 99:2107–12.
- [34] Murakami G, Watabe T, Takaoka K, Miyazono K, Imamura T (2003). Cooperative inhibition of bone morphogenetic protein signaling by Smurf1 and inhibitory Smads. *Mol Biol Cell*, 14:2809–17.
- [35] Nakao A, Afrakhte M, Morán A, Nakayama T, Christian JL, Heuchel R, et al. (1997) Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature*, 389:631–5.
- [36] Massagué J, Seoane J, Wotton D (2005). Smad transcription factors. *Genes Dev*, 19:2783–810.
- [37] Morales TI, Joyce ME, Sobel ME, Danielpour D, Roberts AB (1991). Transforming growth factor-beta in calf articular cartilage organ cultures: synthesis and distribution. *Arch Biochem Biophys*, 288:397–405.
- [38] Ellingsworth LR, Brennan JE, Fok K, Rosen DM, Bentz H, Piez KA, et al. (1986) Antibodies to the N-terminal portion of cartilage-inducing factor A and transforming growth factor beta. Immunohistochemical localization and association with differentiating cells. *J Biol Chem*, 261:12362–7.
- [39] Albro MB, Nims RJ, Cigan AD, Yeroushalmi KJ, Alliston T, Hung CT, et al. (2013) Accumulation of exogenous activated TGF- β in the superficial zone of articular cartilage. *Biophys J*, 104:1794–804.
- [40] Pujol JP (1999). TGF-beta and osteoarthritis: in vivo veritas? *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 7:439–40.
- [41] Van Beuningen HM, Glansbeek HL, van der Kraan PM, van den Berg WB (2000). Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 8:25–33.
- [42] Kulyk WM, Rodgers BJ, Greer K, Kosher RA (1989). Promotion of embryonic chick limb cartilage differentiation by transforming growth factor-beta. *Dev Biol*, 135:424–30.
- [43] Serra R, Johnson M, Filvaroff EH, LaBorde J, Sheehan DM, Derynck R, et al. (1997). Expression of a truncated, kinase-defective TGF-beta type II receptor in mouse skeletal tissue promotes terminal chondrocyte differentiation and osteoarthritis. *J Cell Biol*, 139:541–52.
- [44] Chimal-Monroy J, D'áz de León L (1999). Expression of N-cadherin, N-CAM, fibronectin and tenascin is stimulated by TGF-beta1, beta2, beta3 and beta5 during the formation of precartilaginous condensations. *Int J Dev Biol*, 43:59–67.
- [45] Grimaud E, Heymann D, Ráđini F (2002). Recent advances in TGF-beta effects on chondrocyte metabolism. Potential therapeutic roles of TGF-beta in cartilage disorders. *Cytokine Growth Factor Rev*, 13:241–57.
- [46] Reddi AH (1994). Symbiosis of biotechnology and biomaterials: applications in tissue engineering of bone and cartilage. *J Cell Biochem*, 56:192–5.
- [47] Andriamanalijaona R, Duval E, Raoudi M, Lecourt S, Vilquin JT, Marolleau JP, et al. (2008). Differentiation potential of human muscle-derived cells towards chondrogenic phenotype in alginate beads culture. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 16:1509–18.
- [48] Longobardi L, O'Rear L, Aakula S, Johnstone B, Shimer K, Chytil A, et al. (2006). Effect of IGF-I in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 21:626–36.
- [49] Mizuta H, Sanyal A, Fukumoto T, Fitzsimmons JS, Matsui N, Bolander ME, et al. (2002). The spatiotemporal expression of TGF-beta1 and its receptors during periosteal chondrogenesis in vitro. *J Orthop Res Off Publ Orthop Res Soc*, 20:562–74.
- [50] Baugé C, Duval E, Ollitrault D, Girard N, Leclercq S, Galéra P, et al. (2013) Type II TGF β receptor modulates chondrocyte phenotype. *Age Dordr Neth*, 35:1105–16.
- [51] Yu Z, Xing Y (2006). All-trans retinoic acid inhibited chondrogenesis of mouse embryonic palate mesenchymal cells by down-regulation of TGF-beta/Smad signaling. *Biochem Biophys Res Commun*, 340:929–34.
- [52] Lotz M, Rosen F, McCabe G, Quach J, Blanco F, Dudler J, et al. (1995). Interleukin 1 beta suppresses transforming growth factor-induced inorganic pyrophosphate (PPi) production and expression of the PPi-generating enzyme PC-1 in human chondrocytes. *Proc Natl Acad Sci U S A*, 92:10364–8.
- [53] Baugé C, Legendre F, Leclercq S, Elissalde JM, Pujol JP, Galéra P, et al. (2007). Interleukin-1 β impairment of transforming growth factor β 1 signaling by down-regulation of transforming growth factor β receptor type II and up-regulation of Smad7 in human articular chondrocytes. *Arthritis Rheum*, 56:3020–32.
- [54] Ráđini F, Mauviel A, Pronost S, Loyau G, Pujol JP (1993). Transforming growth factor beta exerts opposite effects from interleukin-1 beta on cultured rabbit

- articular chondrocytes through reduction of interleukin-1 receptor expression. *Arthritis Rheum*, 36:44–50.
- [55] Pronost S, Segond N, Macro M, Rđini F, Penfornis H, Jullienne A, et al. (1995). Modulation of interleukin-1 receptor expression by transforming growth factor-beta in cultured rabbit articular chondrocytes: analysis by reverse transcription-polymerase chain reaction. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 3:147–55.
- [56] Pujol JP, Galera P, Redini F, Mauviel A, Loyau G (1991). Role of cytokines in osteoarthritis: comparative effects of interleukin 1 and transforming growth factor-beta on cultured rabbit articular chondrocytes. *J Rheumatol Suppl*, 27:76–9.
- [57] Demoor-Fossard M, Boittin M, Redini F, Pujol JP (1999). Differential effects of interleukin-1 and transforming growth factor beta on the synthesis of small proteoglycans by rabbit articular chondrocytes cultured in alginate beads as compared to monolayers. *Mol Cell Biochem*, 199:69–80.
- [58] Amin AR, Abramson SB (1998). The role of nitric oxide in articular cartilage breakdown in osteoarthritis. *Curr Opin Rheumatol*, 10:263–8.
- [59] Yaeger PC, Masi TL, de Ortiz JL, Binette F, Tubo R, McPherson JM (1997). Synergistic action of transforming growth factor-beta and insulin-like growth factor-I induces expression of type II collagen and aggrecan genes in adult human articular chondrocytes. *Exp Cell Res*, 237:318–25.
- [60] Daireaux M, Redini F, Loyau G, Pujol JP (1990). Effects of associated cytokines (IL-1, TNF-alpha, IFN-gamma and TGF-beta) on collagen and glycosaminoglycan production by cultured human synovial cells. *Int J Tissue React*, 12:21–31.
- [61] Gal éra P, Vivien D, Pronost S, Bonaventure J, Rđini F, Loyau G, et al. (1992) Transforming growth factor-beta 1 (TGF-beta 1) up-regulation of collagen type II in primary cultures of rabbit articular chondrocytes (RAC) involves increased mRNA levels without affecting mRNA stability and procollagen processing. *J Cell Physiol*, 153:596–606.
- [62] Redini F, Galera P, Mauviel A, Loyau G, Pujol JP (1998). Transforming growth factor beta stimulates collagen and glycosaminoglycan biosynthesis in cultured rabbit articular chondrocytes. *FEBS Lett*, 234:172–6.
- [63] Brandes ME, Allen JB, Ogawa Y, Wahl SM (1991). Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals. *J Clin Invest*, 87:1108–13.
- [64] Thorbecke GJ, Shah R, Leu CH, Kuruvilla AP, Hardison AM, Palladino MA (1992). Involvement of endogenous tumor necrosis factor alpha and transforming growth factor beta during induction of collagen type II arthritis in mice. *Proc Natl Acad Sci U S A*, 89:7375–9.
- [65] Cho M-L, Min S-Y, Chang S-H, Kim K-W, Heo S-B, Lee S-H, et al. (2006). Transforming growth factor beta 1 (TGF-beta1) down-regulates TNFalpha-induced RANTES production in rheumatoid synovial fibroblasts through NF-kappaB-mediated transcriptional repression. *Immunol Lett*, 105:159–66.
- [66] Ellman MB, An HS, Muddasani P, Im H-J (2008). Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. *Gene*, 420:82–9.
- [67] Kurth T, Hedbom E, Shintani N, Sugimoto M, Chen FH, Haspl M, et al. (2007). Chondrogenic potential of human synovial mesenchymal stem cells in alginate. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 15:1178–89.
- [68] Lee CH, Cook JL, Mendelson A, Moiola EK, Yao H, Mao JJ (2010). Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. *Lancet*, 376:440–8.
- [69] Shlopov BV, Smith GN Jr, Cole AA, Hasty KA (1999). Differential patterns of response to doxycycline and transforming growth factor beta1 in the down-regulation of collagenases in osteoarthritic and normal human chondrocytes. *Arthritis Rheum*, 42:719–27.
- [70] Thompson CC, Clegg PD, Carter SD (2001). Differential regulation of gelatinases by transforming growth factor beta-1 in normal equine chondrocytes. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 9:325–31.
- [71] Cheon H, Yu S-J, Yoo DH, Chae IJ, Song GG, Sohn J (2002). Increased expression of pro-inflammatory cytokines and metalloproteinase-1 by TGF-beta1 in synovial fibroblasts from rheumatoid arthritis and normal individuals. *Clin Exp Immunol*, 127:547–52.
- [72] Yamanishi Y, Boyle DL, Clark M, Maki RA, Tortorella MD, Arner EC, et al. (2002). Expression and regulation of aggrecanase in arthritis: the role of TGF-beta. *J Immunol Baltim Md*, 168:1405–12.
- [73] Allen JB, Manthey CL, Hand AR, Ohura K, Ellingsworth L, Wahl SM (1990). Rapid onset synovial inflammation and hyperplasia induced by transforming growth factor beta. *J Exp Med*, 171:231–47.
- [74] Fava RA, Olsen NJ, Postlethwaite AE, Broadley KN, Davidson JM, Nanney LB, et al. (1991) Transforming growth factor beta 1 (TGF-beta 1) induced neutrophil recruitment to synovial tissues: implications for TGF-beta-driven synovial inflammation and hyperplasia. *J Exp Med*, 173:1121–32.
- [75] Baug éC, Cauvard O, Leclercq S, Gal éra P, Boum édiene K (2011). Modulation of transforming growth factor beta signalling pathway genes by transforming growth factor beta in human osteoarthritic chondrocytes: involvement of Sp1 in both early and late response cells to transforming growth factor beta. *Arthritis Res Ther*, 13:R23.
- [76] Blaney Davidson EN, Remst DFG, Vitters EL, van Beuningen HM, Blom AB, Goumans M-J, et al. (2009). Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol Baltim Md*, 182:7937–45.
- [77] Hellingman CA, Blaney Davidson E, Koevoet W, Vitters EL, van den Berg WB, van Osch G, et al. (2011). Smad signaling determines chondrogenic differentiation of bone-marrow derived mesenchymal stem cells: Inhibition of Smad 1/5/8P prevents terminal differentiation and calcification. *Tissue Eng Part A*, 17:1157–67.

- [78] Zhao G-Q (2003). Consequences of knocking out BMP signaling in the mouse. *Genes New York N*, 35:43–56.
- [79] Pathi S, Rutenberg JB, Johnson RL, Vortkamp A (1999). Interaction of Ihh and BMP/Noggin signaling during cartilage differentiation. *Dev Biol*, 209:239–53.
- [80] Zou H, Wieser R, Massagué J, Niswander L (1997). Distinct roles of type I bone morphogenetic protein receptors in the formation and differentiation of cartilage. *Genes Dev*, 11:2191–203.
- [81] Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, et al. (2001). Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet*, 27:84–8.
- [82] Haaijman A, Burger EH, Goei SW, Nelles L, ten Dijke P, Huylebroeck D, et al. (2000) Correlation between ALK-6 (BMP-IB) distribution and responsiveness to osteogenic protein-1 (BMP-7) in embryonic mouse bone rudiments. *Growth Factors Chur Switz*, 17:177–92.
- [83] Lyons KM, Pelton RW, Hogan BL (1990). Organogenesis and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenetic protein-2A (BMP-2A). *Dev Camb Engl*, 109:833–44.
- [84] Lyons KM, Hogan BL, Robertson EJ (1995). Colocalization of BMP 7 and BMP 2 RNAs suggests that these factors cooperatively mediate tissue interactions during murine development. *Mech Dev*, 50:71–83.
- [85] Minina E, Wenzel HM, Kreschel C, Karp S, Gaffield W, McMahon AP, et al (2001). BMP and Ihh/PTHrP signaling interact to coordinate chondrocyte proliferation and differentiation. *Dev Camb Engl*, 128:4523–34.
- [86] Sakou T, Onishi T, Yamamoto T, Nagamine T, Sampath T K, Ten Dijke P (1999). Localization of Smads, the TGF-beta family intracellular signaling components during endochondral ossification. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 14:1145–52.
- [87] Yi SE, LaPolt PS, Yoon BS, Chen JY, Lu JK, Lyons KM (2001). The type I BMP receptor Bmpr1B is essential for female reproductive function. *Proc Natl Acad Sci U S A*, 98:7994–9.
- [88] Cárcamo J, Weis FM, Ventura F, Wieser R, Wrana JL, Attisano L, et al. (1994). Type I receptors specify growth-inhibitory and transcriptional responses to transforming growth factor beta and activin. *Mol Cell Biol*, 14:3810–21.
- [89] Massagué J (1996). TGFbeta signaling: receptors, transducers, and Mad proteins. *Cell*, 85:947–50.
- [90] Keller S, Nickel J, Zhang J-L, Sebald W, Mueller TD (2004). Molecular recognition of BMP-2 and BMP receptor IA. *Nat Struct Mol Biol*, 11:481–8.
- [91] Hatta T, Konishi H, Katoh E, Natsume T, Ueno N, Kobayashi Y, et al. (2000). Identification of the ligand-binding site of the BMP type IA receptor for BMP-4. *Biopolymers*, 55:399–406.
- [92] Macías-Silva M, Hoodless PA, Tang SJ, Buchwald M, Wrana JL (1998). Specific activation of Smad1 signaling pathways by the BMP7 type I receptor, ALK2. *J Biol Chem*, 273:25628–36.
- [93] Kang Q, Sun MH, Cheng H, Peng Y, Montag AG, Deyrup AT, et al. (2004). Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. *Gene Ther*, 11:1312–20.
- [94] Luther G, Wagner ER, Zhu G, Kang Q, Luo Q, Lamplot J, et al. (2011). BMP-9 induced osteogenic differentiation of mesenchymal stem cells: molecular mechanism and therapeutic potential. *Curr Gene Ther*, 11:229–40.
- [95] Retting KN, Song B, Yoon BS, Lyons KM (2009). BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. *Dev Camb Engl*, 136:1093–104.
- [96] Yoon BS, Pogue R, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, et al. (2006). BMPs regulate multiple aspects of growth-plate chondrogenesis through opposing actions on FGF pathways. *Dev Camb Engl*, 133:4667–78.
- [97] Grimsrud CD, Romano PR, D'Souza M, Puzas JE, Reynolds PR, Rosier RN, et al. (1999). BMP-6 is an autocrine stimulator of chondrocyte differentiation. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 14:475–82.
- [98] Kobayashi T, Lyons KM, McMahon AP, Kronenberg HM (2005). BMP signaling stimulates cellular differentiation at multiple steps during cartilage development. *Proc Natl Acad Sci U S A*, 102:18023–7.
- [99] Bandyopadhyay A, Tsuji K, Cox K, Harfe BD, Rosen V, Tabin CJ (2006). Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis. *PLoS Genet*, 2:e216.
- [100] Zehentner BK, Dony C, Burtscher H (1999). The transcription factor Sox9 is involved in BMP-2 signaling. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 14:1734–41.
- [101] Yoon BS, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, Lyons KM (2005). *Bmpr1a* and *Bmpr1b* have overlapping functions and are essential for chondrogenesis in vivo. *Proc Natl Acad Sci U S A*, 102:5062–7.
- [102] Valcourt U, Gouttenoire J, Moustakas A, Herbage D, Mallein-Gerin F (2002). Functions of transforming growth factor-beta family type I receptors and Smad proteins in the hypertrophic maturation and osteoblastic differentiation of chondrocytes. *J Biol Chem*, 277:33545–58.
- [103] Kim D-J, Moon S-H, Kim H, Kwon U-H, Park M-S, Han K-J, et al. (2003). Bone morphogenetic protein-2 facilitates expression of chondrogenic, not osteogenic, phenotype of human intervertebral disc cells. *Spine*, 28:2679–84.
- [104] Gründer T, Gaissmaier C, Fritz J, Stoop R, Hortschansky P, Mollenhauer J, et al. (2004). Bone morphogenetic protein (BMP)-2 enhances the expression of type II collagen and aggrecan in chondrocytes embedded in alginate beads. *Osteoarthritis Cartilage* OARS *Osteoarthritis Res Soc*, 12:559–67.
- [105] Kramer J, Hegert C, Guan K, Wobus AM, Müller PK, Rohwedel J (2000). Embryonic stem cell-derived chondrogenic differentiation in vitro: activation by BMP-2 and BMP-4. *Mech Dev*, 92:193–205.

- [106] Majumdar MK, Wang E, Morris EA (2001). BMP-2 and BMP-9 promotes chondrogenic differentiation of human multipotential mesenchymal cells and overcomes the inhibitory effect of IL-1. *J Cell Physiol*, 189:275–84.
- [107] Sekiya I, Larson BL, Vuoristo JT, Reger RL, Prockop DJ (2005). Comparison of effect of BMP-2, -4, and -6 on in vitro cartilage formation of human adult stem cells from bone marrow stroma. *Cell Tissue Res*, 320:269–76.
- [108] Nochi H, Sung JH, Lou J, Adkisson HD, Maloney WJ, Hruska KA (2004). Adenovirus mediated BMP-13 gene transfer induces chondrogenic differentiation of murine mesenchymal progenitor cells. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 19:111–22.
- [109] Vaes RBA, Rivadeneira F, Kerkhof JM, Hofman A, Pols HAP, Uitterlinden AG, et al. (2009). Genetic variation in the GDF5 region is associated with osteoarthritis, height, hip axis length and fracture risk: the Rotterdam study. *Ann Rheum Dis*, 68:1754–60.
- [110] Bai X, Xiao Z, Pan Y, Hu J, Pohl J, Wen J, et al. (2004). Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. *Biochem Biophys Res Commun*, 325:453–60.
- [111] Chubinskaya S, Segalite D, Pikovsky D, Hakimiyan AA, Rueger DC (2008). Effects induced by BMPS in cultures of human articular chondrocytes: comparative studies. *Growth Factors Chur Switz*, 26:275–83.
- [112] Gelber AC, Hochberg MC, Mead LA, Wang NY, Wigley FM, Klag MJ (2000). Joint injury in young adults and risk for subsequent knee and hip osteoarthritis. *Ann Intern Med*, 133:321–8.
- [113] Roos H, Adalberth T, Dahlberg L, Lohmander LS (1995). Osteoarthritis of the knee after injury to the anterior cruciate ligament or meniscus: the influence of time and age. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 3:261–7.
- [114] Iqbal J, Dudhia J, Bird JL, Bayliss MT (2000). Age-related effects of TGF-beta on proteoglycan synthesis in equine articular cartilage. *Biochem Biophys Res Commun*, 274:467–71.
- [115] Hickery MS, Bayliss MT, Dudhia J, Lewthwaite JC, Edwards JCW, Pitsillides AA (2003). Age-related changes in the response of human articular cartilage to IL-1alpha and transforming growth factor-beta (TGF-beta): chondrocytes exhibit a diminished sensitivity to TGF-beta. *J Biol Chem*, 278:53063–71.
- [116] Barbero A, Grogan S, Schäfer D, Heberer M, Mainil-Varlet P, Martin I (2004). Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 12:476–84.
- [117] Blaney Davidson E, Scharstuhl A, Vitters E, van der Kraan P, van den Berg W (2005). Reduced transforming growth factor-beta signaling in cartilage of old mice: role in impaired repair capacity. *Arthritis Res Ther*, 7:R1338–R1347.
- [118] Van der Kraan PM, van den Berg WB (2012). Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 20:223–32.
- [119] Shen J, Li J, Wang B, Jin H, Wang M, Zhang Y, et al. (2013). Deletion of the Type II TGF- β receptor gene in articular chondrocytes leads to a progressive OA-like phenotype in mice. *Arthritis Rheum*, 65:3107–19.
- [120] Seo H-S, Serra R (2007). Deletion of Tgfr2 in Prx1-cre expressing mesenchyme results in defects in development of the long bones and joints. *Dev Biol*, 310:304–16.
- [121] Finnson KW, Parker WL, ten Dijke P, Thorikay M, Philip A (2008). ALK1 opposes ALK5/Smad3 signaling and expression of extracellular matrix components in human chondrocytes. *J Bone Miner Res Off J Am Soc Bone Miner Res*, 23:896–906.
- [122] Van de Laar IMBH, Oldenburg RA, Pals G, Roos-Hesslink JW, de Graaf BM, Verhagen JMA, et al. (2011). Mutations in SMAD3 cause a syndromic form of aortic aneurysms and dissections with early-onset osteoarthritis. *Nat Genet*, 43:121–6.
- [123] Furumatsu T, Tsuda M, Taniguchi N, Tajima Y, Asahara H (2005). Smad3 induces chondrogenesis through the activation of SOX9 via CREB-binding protein/p300 recruitment. *J Biol Chem*, 280:8343–50.
- [124] Mori Y, Chen SJ, Varga J (2000). Modulation of endogenous Smad expression in normal skin fibroblasts by transforming growth factor-beta. *Exp Cell Res*, 258:374–83.
- [125] Verdier M-P, Seit \acute{e} S, Guntzer K, Pujol J-P, Boum \acute{e} di \acute{e} ne K (2005). Immunohistochemical analysis of transforming growth factor beta isoforms and their receptors in human cartilage from normal and osteoarthritic femoral heads. *Rheumatol Int*, 25:118–24.
- [126] Boumediene K, Conrozier T, Mathieu P, Richard M, Marcelli C, Vignon E, et al. (1998). Decrease of cartilage transforming growth factor-beta receptor II expression in the rabbit experimental osteoarthritis--potential role in cartilage breakdown. *Osteoarthr Cartil OARS Osteoarthr Res Soc*, 6:146–9.
- [127] Baug \acute{e} C, Beauchef G, Leclercq S, Kim SJ, Pujol JP, Gal \acute{e} ra P, et al. (2008). NF κ B mediates IL-1 β -induced down-regulation of T β RII through the modulation of Sp3 expression. *J Cell Mol Med*, 12:1754–66.
- [128] Baug \acute{e} C, Girard N, Leclercq S, Gal \acute{e} ra P, Boum \acute{e} diene K (2012). Regulatory mechanism of transforming growth factor beta receptor type II degradation by interleukin-1 in primary chondrocytes. *Biochim Biophys Acta*, 1823:983–6.
- [129] Fei Q-M, Jiang X-X, Chen T-Y, Li J, Murakami H, Tsai K-J, et al. (2006). Changes with age and the effect of recombinant human BMP-2 on proteoglycan and collagen gene expression in rabbit anulus fibrosus cells. *Acta Biochim Biophys Sin*, 38:773–9.
- [130] Guo C-A, Liu X-G, Huo J-Z, Jiang C, Wen X-J, Chen Z-R (2007). Novel gene-modified-tissue engineering of cartilage using stable transforming growth factor-beta1-transfected mesenchymal stem cells grown on chitosan scaffolds. *J Biosci Bioeng*, 103:547–56.
- [131] Lee JE, Kim KE, Kwon IC, Ahn HJ, Lee S-H, Cho H, et al. (2004). Effects of the controlled-released TGF-beta 1 from chitosan microspheres on chondrocytes cultured in

- a collagen/chitosan/glycosaminoglycan scaffold. *Biomaterials*, 25:4163–73.
- [132] Guo T, Zhao J, Chang J, Ding Z, Hong H, Chen J, et al. (2006). Porous chitosan-gelatin scaffold containing plasmid DNA encoding transforming growth factor-beta1 for chondrocytes proliferation. *Biomaterials*, 27:1095–103.
- [133] Diao H, Wang J, Shen C, Xia S, Guo T, Dong L, et al. (2009). Improved cartilage regeneration utilizing mesenchymal stem cells in TGF-beta1 gene-activated scaffolds. *Tissue Eng Part A*, 15:2687–98.
- [134] Peng L, Cheng X, Zhuo R, Lan J, Wang Y, Shi B, et al. (2009). Novel gene-activated matrix with embedded chitosan/plasmid DNA nanoparticles encoding PDGF for periodontal tissue engineering. *J Biomed Mater Res A*, 90:564–76.
- [135] Lu H, Lv L, Dai Y, Wu G, Zhao H, Zhang F (2013). Porous Chitosan Scaffolds with Embedded Hyaluronic Acid/Chitosan/Plasmid-DNA Nanoparticles Encoding TGF-β1 Induce DNA Controlled Release, Transfected Chondrocytes, and Promoted Cell Proliferation. *PLoS One* [Internet], Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3720934/>
- [136] Chung C, Burdick JA (2008). Engineering cartilage tissue. *Adv Drug Deliv Rev*, 60:243–62.
- [137] Park H, Temenoff JS, Holland TA, Tabata Y, Mikos AG (2005). Delivery of TGF-beta1 and chondrocytes via injectable, biodegradable hydrogels for cartilage tissue engineering applications. *Biomaterials*, 26:7095–103.
- [138] Kim H-J, Kim Y-J, Im G-I (2009). Is continuous treatment with transforming growth factor-beta necessary to induce chondrogenic differentiation in mesenchymal stem cells? *Cells Tissues Organs*, 190:1–10.
- [139] Kawamura K, Chu CR, Sobajima S, Robbins PD, Fu FH, Izzo NJ, et al. (2005). Adenoviral-mediated transfer of TGF-beta1 but not IGF-1 induces chondrogenic differentiation of human mesenchymal stem cells in pellet cultures. *Exp Hematol*, 33:865–72.
- [140] Solorio LD, Fu AS, Hernández-Irizarry R, Alsberg E (2010). Chondrogenic differentiation of human mesenchymal stem cell aggregates via controlled release of TGF-beta1 from incorporated polymer microspheres. *J Biomed Mater Res A*, 92:1139–44.
- [141] Fan H, Hu Y, Qin L, Li X, Wu H, Lv R (2006). Porous gelatin-chondroitin-hyaluronate tri-copolymer scaffold containing microspheres loaded with TGF-beta1 induces differentiation of mesenchymal stem cells in vivo for enhancing cartilage repair. *J Biomed Mater Res A*, 77:785–94.
- [142] Yang Z, Sui L, Toh WS, Lee EH, Cao T (2009). Stage-dependent effect of TGF-beta1 on chondrogenic differentiation of human embryonic stem cells. *Stem Cells Dev*, 18:929–40.
- [143] Elder SH, Nettles DL, Bumgardner JD (2004). Synthesis and characterization of chitosan scaffolds for cartilage-tissue engineering. *Methods Mol Biol Clifton NJ*, 238:41–8.
- [144] Shi D, Cai D, Zhou C, Rong L, Wang K, Xu Y (2005). Development and potential of a biomimetic chitosan/type II collagen scaffold for cartilage tissue engineering. *Chin Med J (Engl)*, 118:1436–43.
- [145] Shintani N, Siebenrock KA, Hunziker EB (2013). TGF-β1 enhances the BMP-2-induced chondrogenesis of bovine synovial explants and arrests downstream differentiation at an early stage of hypertrophy. *PLoS One*, 8:e53086.
- [146] Mehlhorn AT, Schmal H, Kaiser S, Lepski G, Finkenzeller G, Stark GB, et al. (2006). Mesenchymal Stem Cells Maintain TGF-β-Mediated Chondrogenic Phenotype in Alginate Bead Culture. *Tissue Eng*, 12:1393–403.
- [147] Mehlhorn AT, Niemeyer P, Kaschte K, Muller L, Finkenzeller G, Hartl D, et al. (2007). Differential effects of BMP-2 and TGF-beta1 on chondrogenic differentiation of adipose derived stem cells. *Cell Prolif*, 40:809–23.