Rheumatology : Current Research
ISSN: 2161-1149

Gelse et al., Rheumatology 2012, S3
DOI: 10.4172/2161-1149.S3-002

Review Article Open Access

Endochondral Ossification in Cartilage Repair Tissue Hampers Bone Marrow Stimulating Techniques

Gelse K[1]*, Beyer C[2], Welsch G[1] and Blanke M[1]

[1]University Hospital Erlangen, Department of Orthopaedic Trauma Surgery, Krankenhausstr. 12, 91054 Erlangen, Germany
[2]University Hospital Erlangen, Department of Internal Medicine III and Institute of Clinical Immunology, Krankenhausstr. 12, 91054 Erlangen, Germany

Abstract

Bone marrow-stimulating techniques are frequently applied to induce cartilage repair. Apart from insufficient chondrogenesis of the ingrowing bone marrow stem cells (BMSCs), these techniques are hampered by excessive ossification with formation of intralesional osteophytes, in which the ingrowing BMSCs tend to undergo the inherent programme of endochondral ossification. Within this programme, the chondrocyte phenotype only represents a transient state that is followed by terminal chondrocyte differentiation and replacement of the cartilaginous tissue by osseous tissue. The transcription factor Runx2 is considered the driving force for endochondral ossification, which integrates signals from growth factors that are released from the bone marrow, including bone morphogenetic proteins (BMPs), fibroblast growth factor-2 and members of the Wnt-family among others.

Anti-hypertrophic factors such as PTHrP or anti-angiogenic proteins including Chondromodulin-I or Thrombospondin-1 can inhibit the endochondral ossification. In addition, antagonists of BMP- and Wnt-signalling can stabilize the non-hypertrophic chondrocyte phenotype. The generation of stable cartilage tissue, however, does not only depend on extracellular factors but also on the fate of the originating cell population. Regardless of the spectrum of specific stimuli, BMSCs are prone to finally become osteocytes rather than chondrocytes. Since there is increasing evidence for epigenetic regulation including DNA methylation and histone modification for cartilage-relevant genes, future studies will have to explore the role of genomic imprinting of adult BMSCs. However, as long as tools that stabilize a chondrocyte-specific phenotype of adult BMSCs are not available in clinical routine, the transplantation of differentiated chondrocytes may remain the method of choice for cartilage repair.

Basic Problems of Cartilage Defects Treated by Bone Marrow-Stimulating Techniques

Articular cartilage has only limited capacities for spontaneous healing in case of injury or degeneration, since the adjacent chondrocytes are largely nonmotile and remain entrapped within the surrounding matrix and, in the nonvascularized tissue, mesenchymal stem cells (MSC) have limited access to cartilage lesions. Thus, the introduction of a new cell population seems necessary to generate cartilage repair tissue. Bone marrow-stimulating techniques, such as microfracturing (MFX) or abrasion of the subchondral bone plate within the defects areas, are simple, minimally-invasive and cost-effective cartilage repair approaches that are frequently applied in clinical settings [1,2]. These techniques allow stem or progenitor cells from the bone marrow to enter the cartilage lesions. Captured within a blood clot, these bone marrow-derived stem cells (BMSCs) proliferate and produce a repair tissue that may completely fill up the defect and contribute to relieve in symptoms of the patient [1-3]. However, the forming repair tissue lacks the biomechanical properties of hyaline articular cartilage and often fails in the long run, which results in deterioration of function and recurrence of clinical symptoms [2]. The inferior quality of the repair tissue results from improper cellular differentiation of the BMSCs, which is confronted with two basic problems:

a. In upper zones of the repair tissue, the chondrogenic differentiation appears to be incomplete. Thus, the ingrowing progenitor cells fail to fully differentiate into chondrocytes, which leads to the formation of fibrous or fibrocartilaginous tissue characterized by inferior biomechanical stiffness (Figure 1a) [4-8].

b. In the deeper zones of the repair tissue, the ingrowing progenitor cells tend to pass through the cascade of chondrogenic differentiation beyond the status of the mature chondrocyte and undergo terminal differentiation. The resulting chondrocyte hypertrophy is typically followed by endochondral ossification (Figure 1b). The forming osseous tissue often exceeds the original level of the subchondral bone plate in terms of intralesional osteophytes (Figure 1 and 2) [8-10].

While incomplete chondrogenesis and the formation of fibrocartilage is a well-recognized problem, the formation of intralesional osteophytes has just recently awakened more interest. Several studies have shown that excessive bone formation occurs in up to 70% of all lesions treated by MFX [2,3,9-13]. In experimental studies on minipigs, the volume of excessive osseous tissue accounted for more than 20% in relation to the total volume of the repair tissue [8,9]. Intralesional osteophytes affect the biomechanical properties of the repair tissue by increasing the overall stiffness [4], which might interfere with the durability of the overlying thinned cartilaginous layer. Our review will discuss the mechanisms of the undesirable endochondral ossification within cartilage repair tissue.

Endochondral Ossification – the Endpoint of the Chondrogenic Differentiation Cascade

Ingrowing BMSCs, the cellular key players of bone marrow-stimulating techniques, are multipotent progenitor cells. Upon

***Corresponding author:** Gelse K, Kolja University Hospital Erlangen, Department of Orthopaedic Trauma Surgery, Krankenhausstr. 12, 91054 Erlangen, Germany, Tel: 0049-9131-8542121; Fax: 0049-9131-8533300; E-mail: kolja.gelse@web.de

Received December 02, 2011; **Accepted** January 26, 2012; **Published** February 03, 2012

Citation: Gelse K, Beyer C, Welsch G, Blanke M (2012) Endochondral Ossification in Cartilage Repair Tissue Hampers Bone Marrow Stimulating Techniques. Rheumatology S3:002. doi:10.4172/2161-1149.S3-002

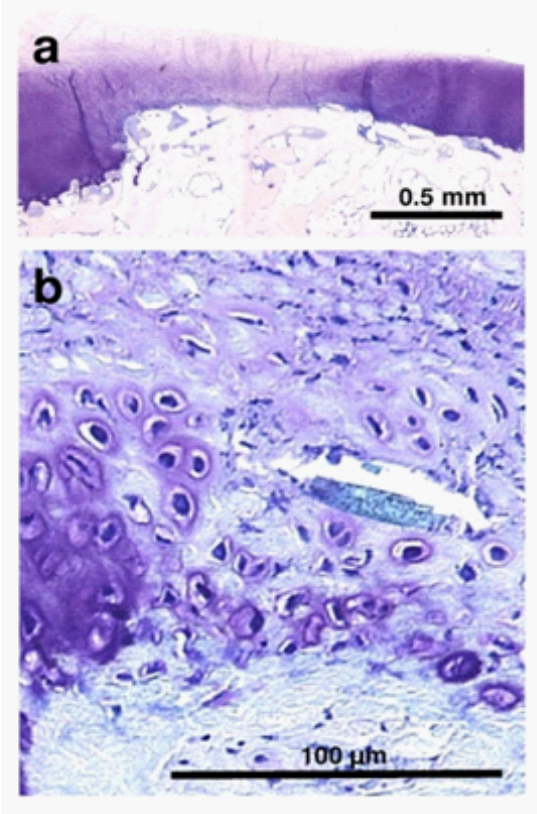

Figure 1: Chondrocyte hypertrophy and excessive bone formation following MFX in an experimental model.

(**a**) Histological section of cartilage repair tissue within the knee joint of a minipig model 6 months after treatment by MFX. The repair tissue is characterized by excessive outgrowth of the subchondral bone tissue and overlying fibrocartilaginous tissue. (**b**) High magnification of the repair tissue in a minipig model 6 weeks following MFX. Repair cells of deeper zones differentiate into hypertrophic chondrocytes prior to be replaced by endochondral ossification (Toluidin blue staining).

stimulation, BMSCs can be directed towards the chondrogenic lineage [14]. Nevertheless, their differentiation potential is not confined to chondrogenesis since they also have the capability to differentiate into other mesenchymal cells, including osteoblasts, fibroblasts, tenocytes, adipocytes or myoblasts [15,16]. Thus, the differentiation towards the chondrogenic lineage does not occur spontaneously but depends on the stimulation by specific factors or a combination of those, e.g. dexamethasone and Transforming Growth Factor-β (TGFβ) [14]. Beyond that, the adopted chondrocytic phenotype is not only characterized by upregulation of cartilage-specific genes such as collagen type II and IX as well as aggrecan, but also by markers for chondrocyte hypertrophy, such as collagen type X, alkaline phosphatase and matrix metalloproteinase-13 (MMP13) [14,17,18]. Thus, the induced chondrocyte phenotype is not stable but is prone to further undergo terminal differentiation, which is followed by endochondral ossification. In this context, a recent study showed that BMSC-derived cell pellets failed to form stable cartilage tissue, but rather underwent endochondral ossification and formed osseous tissue when implanted into subcutaneous pouches of SCID mice [18]. Interestingly, the predisposition for osteogenesis and matrix calcification following ectopic transplantation into SCID mice is not confined to BMSCs, but has also been described for MSCs from adipose tissue or the synovial membrane [19,20].

Apart from soluble mediators, the micro-environment which includes other cells, blood vessels, or matrix components, may play an important role for cellular differentiation of BMSCs. For example, a dense capillary network providing high oxygen levels may interfere with the maintenance of the chondrocyte phenotype following ectopic transplantation into subcutaneous tissue pouches. By contrast, low oxygen levels, contact to a synovial environment and the impact of certain biomechanical forces may favour chondrogenic differentiation [21,22].

Basically, adult MSCs recapitulate differentiation processes that are analogous to those within the embryonal limb bud or the growth plate of the growing skeleton, or to those within fracture callus or growing osteophytes of the adult organism [23-26]. Chondrogenesis and the subsequent endochondral ossification is a spatio-temporal process. As seen in the growth plate, the cellular differentiation stages simultaneously occur within the repair tissue. Thus, repair cartilage tissue induced by bone marrow-stimulating techniques is typically characterized by a stratified pattern.

In superficial and middle zones, the chondrogenic differentiation usually remains incomplete resulting in the formation of fibrocartilage that is typically positive for collagen type I but negative for collagen type II. Underneath, a zone of varying extent may contain differentiated chondrocytes that produce a hyaline-like matrix positive for collagen type II. In deeper zones, the cellular differentiation process typically progresses towards the terminal state in which the cells undergo hypertrophy characterized by expression of collagen type X [8,27]. Similar to the growth plate, these cells are putatively prone to undergo apoptosis and to be replaced by endochondral ossification [28]. Development of bone trabeculae within the deepest repair tissue zones results in formation of bone marrow spaces and the invasion of blood vessels [8,9].

Bone marrow-stimulating techniques significantly affect the integrity of the subchondral bone plate depending on the applied method [29]. In contrast to healthy articular cartilage, the repair tissue typically lacks a definite tide-mark and a plane subchondral bone plate. By contrast, the repair tissue may show signs of bone resorption and cyst formation, or even more often, excessive endochondral ossification leading to bone overgrowth above the projected tide-mark (Figure 1 and Figure 2) [2,3,10-13,29]. Such outgrowths alter the biomechanical properties of the joint surface [4] and interfere with the integritiy of the rather thin and soft overlying fibrocartilaginous repair tissue, a process that may result in cartilage degeneration.

In view of the long-term outcome of bone marrow-stimulating techniques, it is important to mention that there is a point of no return once the irreversible endochondral differentiation programme has started and that excessive bone formation will not be remodelled into cartilage tissue again.

Mediators of Terminal Differentiation and Endochondral Ossification

Excessive endochondral ossification of cartilage repair tissue upon bone marrow-stimulating techniques is a process that basically occurs independently from application of exogenous growth factors. By contrast, terminal differentiation of invading BMSCs is driven by endogenous cell programs and is supported by factors that are incidentally released from bone marrow by penetration of the subchondral bone plate. Indeed, the bone marrow stores and releases a multitude of growth factors including BMPs, FGFs, VEGF, TGFβ, IGF-1 or PDGF (Figure 3) [30-32].

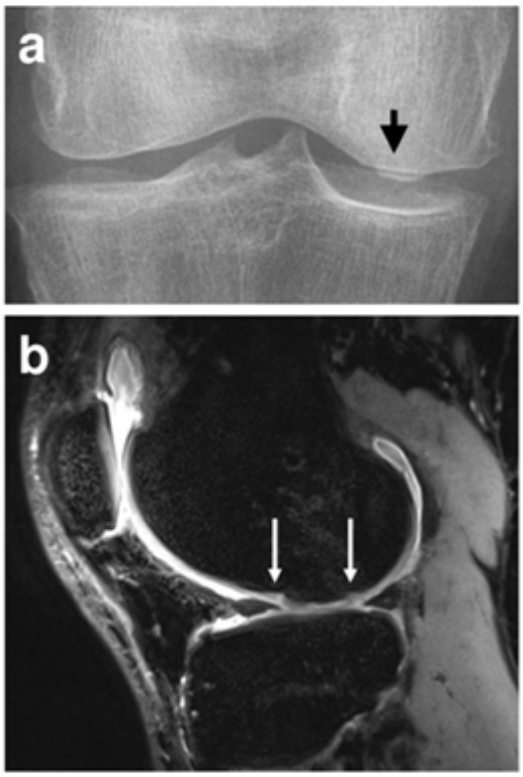

Figure 2: Clinical cases of intralesional osteophyte formation following MFX.

(**a**) Radiograph of a knee joint 3 years following MFX of the medial femoral condyle The arrow indicates the formation of an intralesional osteophyte (50-year-old patient). (**b**) MRI image (PD fat sat sequence) 24 months after MFX of the lateral femoral condyle (32-year-old patient). Excessive bone formation is evident within the former defect area (arrows delineate the defect borders).

In particular, members of the TGFβ-superfamily, including TGFβ, BMP-2, -4 and -7 and GDF-5 (CDMP-1) are potent chondroinductive growth factors [14,24,33-35]. For cartilage repair, these proteins have been considered promising tools to promote chondrogenic differentiation of MSCs. The exogenous application of these factors in experimental settings could significantly improve the quality of cartilage repair tissue that was characterized by a hyaline matrix instead of fibrocartilaginous tissue [8,36-39].

Beyond their chondroinductive properties, however, in particular BMPs were originally considered bone-inducing factors [40]. Thus, they also strongly promote chondrocyte hypertrophy and endochondral ossification [8,24,41-43]. Intraarticular administration or overexpression of BMP-2 or TGFβ, which resulted in the formation of significant mature osteophytes [44-46], highlighted the osteoinductive capability of these mediators even within the synovial joint. We have recently examined the repair of cartilage lesions within the knee joints of minipigs that were treated by MFX combined with additional application of matrix-bound recombinant BMP-7(also known as osteogenic protein-1 (OP-1)). Six weeks after MFX, we observed that the additional application of BMP-7 (OP-1) significantly promoted chondrogenic differentiation of the ingrowing cells, but also supported their terminal differentiation and increased the amount of hypertrophic chondrocytes surrounded by a collagen type X-positive matrix. At week 26, the transiently formed cartilage tissue with hypertrophic cells was replaced by excessive bone tissue leading to the formation of impressive intralesional osteophytes [8]. Thus, our findings demonstrated both the chondrogenic and osteogenic effects of BMPs. The concentrations of endogenous BMPs released from the bone marrow may be lower than those applied experimentally, but a prolonged release from ample endogenous storages might have similar effects leading to the formation of intralesional osteophytes as seen in many clinical cases. Two other important members of the TGFβ-superfamily, TGFβ and GDF5 (CDMP-1), also exert chondroinductive effects but have a less prominent impact on promoting chondrocyte hypertrophy [47-50], which explains the favorable effect of both factors observed in a number of experimental cartilage repair studies [51-54].

VEGF, a potent pro-angiogenic factor that exerts a crucial role in endochondral ossification, is also released from the bone marrow [31,32]. VEGF appears to promote the invasion of blood vessels, which is required for the replacement of cartilage by bone [55]. Indeed, in MFX-induced cartilage repair tissue, we could identify ingrowing vascular structures within the osseous tissue of intralesional osteophytes [8,9]. In addition to its pro-angiogenic effects, VEGF and its receptors are also expressed by both articular chondrocytes and growth plate chondrocytes [56,57]. In cartilage, VEGF acts as a mitogen and also propagates the terminal differentiation of chondrocytes. It inhibits the expression of cartilage-specific genes like collagen type II and aggrecan [58] and increases the secretion of matrix-degrading MMPs, in particular MMP13 [59]. In this respect, therapeutic approaches focus on the inhibition of VEGF to protect and stabilize the chondrocyte phenotype. Indeed, the formation of cartilage repair tissue within osteochondral defects could be improved by intravenous administration of monoclonal antibodies against VEGF with formation of cartilaginous repair tissue that resisted endochondral

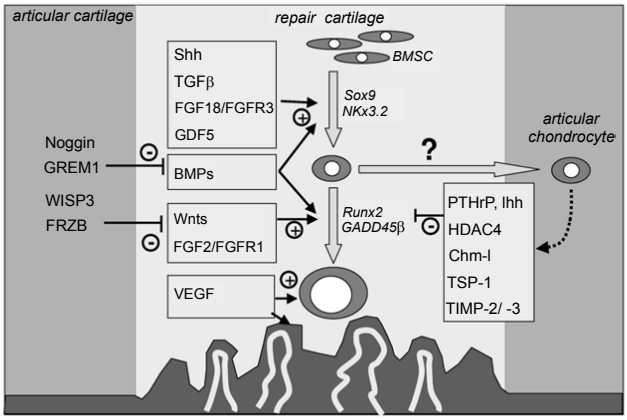

Figure 3: Extracellular determinants and cellular processes of cartilage repair tissue induced by bone marrow-stimulating techniques.

Penetration of the subchondral bone plate allows both the ingrowth of BMSC and the release of growth factors. BMSCs proliferate and contribute to filling of the defect. BMSCs undergo chondrogenic differentiation, which is promoted by factors including Shh, TGFß, FGF18 or GDF5 and mediated by the transcription factors Sox9 and Nkx3.2. Particularly in deeper zones, the cells tend to undergo further terminal differentiation, a process that is mediated by the transcription factors Runx2 and GADD45ß and supported by growth factors including BMPs, Wnts, FGF2 and VEGF. Hypertrophic differentiation is inhibited by factors that are predominantly released by articular chondrocytes including PTHrP, HDAC4, Chm-I, TSP-1 or TIMP-2/-3. Furthermore, articular cartilage produces GREM1, WISP3 and FRZB, which inhibit BMP and Wnt-signalling. To date it is not known if chondrocytic-differentiated BMSCs may adopt a permanent articular chondrocyte phenotype.

ossification [60]. In line with these findings, transplantation of MSCs overexpressing sFlt-1, the soluble splice variant of VEGF receptor-1 which reduces free circulating levels of VEGF, prevented bone invasion into BMP-4-induced cartilage repair tissue [60].

Fibroblast Growth Factors (FGFs) are produced by fibroblasts within the bone marrow and released upon penetration of the subchondral bone plate [30]. The network effect of FGFs on chondrogenesis is complex and includes signals from 22 different ligands and three different receptors [61]. In the growth plate, FGFR3 prevents chondrocyte hypertrophy and stabilizes the chondrocyte phenotype. A number of FGFs are capable of activating FGFR3, but expression analyses suggest that FGF9 and FGF18 are the preferred ligands of this receptor [62]. By contrast, FGFR1 is upregulated in the prehypertrophic and hypertrophic chondrocytes of the growth plate and its preferred ligand FGF2 (bFGF) promotes terminal chondrocyte differentiation and endochondral ossification [61-63]. Similar findings were shown for MSCs, in which FGFR1 and its ligand FGF2 promoted their differentiation into a hypertrophyic chondrocyte phenotype [64]. Since FGF2 is released from fibroblasts of the bone marrow, it may be an important factor to stimulate chondrocyte hypertrophy and excessive bone formation following opening of the bone marrow space by MFX [30].

Within the growth plate, Wnt/β-catenin signalling promotes chondrocyte hypertrophy and induces osteoblast differentiation with subsequent endochondral ossification [65,66]. Within the cell, both BMP- and Wnt/β-catenin signalling result in increased activity of the runt-domain transcription factor 2 (Runx2), which is the key transcription factor for osteoblast differentiation. In this context, Runx2 activity leads to the expression of proteins typical of hypertrophic cartilage, including Col10a1, alkaline phosphatase (ALP), osteocalcin (BGLAP) and MMP13 [24,67-69]. Nevertheless, the transcriptional activation of these genes may also depend on factors other than Runx2. In this respect, GADD45β is considered an important co-factor that acts in synergy with Runx2 to induce transcription of Col10a1 and MMP13 [70]. Interestingly, GADD45β itself was identified to be an early response gene of BMP/Smad-signalling [70].

Factors That Stabilize the Chondrocyte Phenotype and Inhibit Hypertrophy

Clinical experience shows that the osseous fusion of arthrodeses is interfered by interposed articular cartilage [71], since articular cartilage by itself exerts strong anti-angiogenic and anti-osteogenic effects. In this context, we could demonstrate that the transplantation of differentiated chondrocytes into microfractured lesions effectively inhibited excessive endochondral ossification within the repair tissue [9]. The cartilage matrix is rich in proteins with anti-angiogenic properties, including Chondromodulin-I (Chm-I), Thrombospondin-1 and -2 (TSP-1, -2) and Tissue Inhibitors of Metalloproteinases-2 and –3 (TIMP-2, -3) (Figure 3) [72]. Recently, we have demonstrated in a minipig model that the application of recombinant TSP-1 could significantly prevent chondrocyte hypertrophy and excessive ossification within cartilage repair tissue induced by MFX and stimulated with BMP-7/OP-1 [8]. In a similar model, the overexpression of Chm-I within MFX-treated lesions could also prevent terminal chondrocyte differentiation and the formation of intralesional osteophytes (Figure 4) [27].

Chm-I and TSP-1 may not only have anti-angiogenic effects on endothelial cells but also direct anti-hypertrophic activity on the repair cells. This dual action seems obvious, since the occurrence of hypertrophic chondrocytes significantly precedes the ingrowth

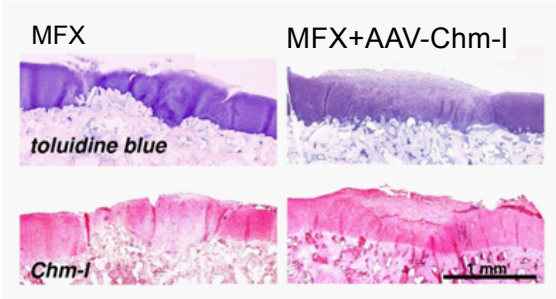

Figure 4: Stabilizing effect of Chondromodulin-I on the chondrocyte phenotype.

Histological sections of cartilage repair tissue within the knee joint of a minipig model 6 months after treatment by microfracturing alone (MFX) or combined with overexpression of Chondromodulin-I (Chm-I) (MFX + AAVChm-I), which was performed by Adenovirus-Associated-Virus (AAV)-mediated gene transfer as described previously [27]. Repair cartilage induced by MFX was characterized by only weak immunostaining for Chm-I, but was typically associated with outgrowths of the subchondral bone plate. By contrast, efficient AAV-mediated overexpression of Chm-I was confirmed by strong immunostaining for Chm-I within the repair matrix treated by MFX + AAVChm-I, which stabilized the chondrocyte phenotype and inhibited the formation of intralesional osteophytes.

of vessels [8,27]. The direct anti-hypertrophic effect of TSP-1 on chondrocytes may be at least in part mediated by inhibiting the expression of GADD45β, a crucial transcriptional enhancer for the expression of Col10a1 and MMP13 [8,70]. The anti-angiogenic and chondrocyte-stabilizing effects of Chm-I have not been fully identified yet but may involve modulation of the cell cycle and cell proliferation by inducing the cell cycle inhibitor $p21^{cip1/waf1}$ [27,73]. Cell cycle arrest or cellular quiescence with up-regulation of $p21^{cip1/waf1}$ is a characteristic feature of post-mitotic articular chondrocytes and stabilizes the typical chondrocyte phenotype [74,75].

Other secreted factors that act in a paracrine manner may also mediate the anti-hypertrophic effect of transplanted chondrocytes within MFX-treated lesions. Among those, PTHrP may be the most relevant candidate. This peptide is involved in a negative feedback-loop described for the growth plate [76]: The expression of PTHrP in the periarticular perichondrium is induced by Indian hedgehog (Ihh) which is released from hypertrophic chondrocytes. PTHrP, in turn, acts in a gradient to prevent or delay premature hypertrophic differentiation of chondrocytes of the proliferating zone [76]. In the adult, we detected a significantly higher expression of PTHrP in articular chondrocytes compared with transiently differentiated chondrocyte-like cells within the cartilaginous cap of osteophytes [77]. In other cell culture experiments, PTHrP was secreted by articular chondrocytes and suppressed the hypertrophic differentiation of co-cultured MSCs in a paracrine manner [78]. The biological action of PTHrP is tightly coupled with the action of the transcription factor Sox9, the key element in inducing and stabilizing the chondrocyte phenotype. While Sox9 was shown to transactivate PTHrP gene expression [79], PTHrP itself phosphorylates Sox9 and thereby increases its transcriptional activity [80]. Thus both factors act in concert to inhibit chondrocyte hypertrophy and matrix calcification.

Osteophytes may represent a model for secondary cartilage formation and they share great similarities with MFX-induced cartilage repair tissue. Both tissues arise from mesenchymal progenitor cells that transiently differentiate into a chondrocyte-like phenotype. Particularly within the deeper zones of both tissues, the cells tend to undergo hypertrophy, which is followed by endochondral ossification

[25]. Because of these analogies, the development of osteophytes may be a useful model to study endochondral ossification in MFX-induced cartilage repair. In this context, we have recently performed a genome-wide microarray analysis comparing the gene expression of permanent articular cartilage with that of the cartilaginous cap of osteophytes of adult joints. In this comparative analysis, we identified GREM1 as one of the most differentially expressed genes with a more than 20-fold upregulation in articular chondrocytes compared with osteophytic chondrocytes [77]. GREM1, a functional BMP-antagonist, binds and blocks the action of BMP-2, -4 and-7 [81]. In early skeletal development, GREM1 is involved in a self-regulatory feedback system that maintains the chondrocyte phenotype during limb formation [82]. GREM1 also plays a central role in epiphyseal development with a significantly higher expression in the region of the epiphyses compared to the hypertrophic physeal zone of developing bones of 7-day-old mice [83]. GREM1 is supposed to maintain the chondrocyte phenotype within the epiphyses, while the action of BMPs propagates endochondral ossification in the physes. Accordingly, a recent study based on an expression analysis of epiphyseal and growth plate cartilage of new-born rats revealed the presence of a gradient in BMP-activity that increases from the epiphyses towards the hypertrophic zone. Thus, epiphyseal cartilage, the origin of the developing articular cartilage, exhibited lower expression of BMPs-2, -4, -6 and -7 and higher expression of the BMP antagonists GREM1 and Noggin when compared with the hypertrophic zone [84]. This BMP-gradient of the growth plate may also be transferred to the adult articular cartilage, in which low levels of BMPs [85] help to maintain the cells in a quiescent, non-hypertrophic state.

Our recent genome-wide expression analysis revealed that two further inhibitors of growth factor signaling, WNT1 inducible signaling pathway protein-3 (WISP3) and Frizzled-related Protein (FRZB), were upregulated in stable permanent articular chondrocytes compared to transient osteophytic chondrocytes [77]. WISP3 is an inhibitor of both BMP- and Wnt-signaling and has stabilizing effects on the chondrocyte phenotype by inhibiting cellular maturation and hypertrophy [86-88]. FRZB predominantly antagonizes the signaling of Wnt ligands. In the developing skeleton, FRZB is prominently expressed within the epiphyses. Since Wnt-signaling promotes osteoblast differentiation and endochondral ossification, FRZB delays transformation to hypertrophy and inhibits trabecular bone development [83,89]. The lack of FRZB in FRZB(-/-) mice displayed increased periosteal appositional new bone formation and osteoarthritis-like changes [90]. Furthermore, variants of the FRZB were associated with hip osteoarthritis in humans associated with increased osteophyte formation [91,92].

Taken together, there is increasing evidence that the generation and the maintenance of a stable, non-hypertrophic chondrocyte phenotype does not only depend on mitogenic and anabolic factors but also on the modulating function of antagonistic and inhibitory factors.

Bmscs Differ from Articular Chondrocytes with Respect to Their Cellular Fate

As discussed above, BMSCs adopt a transient rather than permanent chondrocyte phenotype and tend to undergo terminal differentiation, which is followed by endochondral ossification. In this context, the induction and, even more important, the maintenance of a chondrocytic phenotype does not occur spontaneously, but incessantly depends on the influence of certain exogenous factors. For example, we could recently demonstrate that BMSCs, indeed, adopted a non-hypertrophic chondrocytic phenotype by applying the prochondrogenic stimulus BMP-7/OP-1 and simultaneously the anti-angiogenic and anti-hypertrophic factor TSP-1 [8]. However, the follow-up period of this study was limited to 6 months and it may be speculated that, as soon as the influence of the exogenously applied therapeutic proteins declines, a fibroblastic dedifferentiation or terminal differentiation of the cells will occur. Such secondary dedifferentiation was obvious in another study, in which BMP-2 gene transfer only transiently induced a chondrocyte-like phenotype of mesenchymal repair cells and in course of a declining BMP-2 stimulus, the cells reverted back to a fibroblastic phenotype [37]. Thus, ever ongoing stimuli seem mandatory to maintain chondrocytic differentiation of BMSCs.

Since the physiological role of BMSCs in the adult is to serve for fracture healing, it appears obvious that the restorative response does not stop with cartilage formation but further progresses to bone formation. Of note, MSCs from other origins, such as the synovial membrane, adipose tissue, muscle, periosteum or perichondrium, also show chondrogenic potential, but permanently stable chondrogenic differentiation could not be demonstrated for any of these cell populations so far [18-20,93,94].

The use of differentiated articular chondrocytes for cartilage repair approaches might help to overcome this obstacle. Articular chondrocytes are known to spontaneously produce a cocktail of anti-hypertrophic factors, including PTHrP, TSP-1 and Chm-I [8,9,27,78]. In co-culture experiments, articular chondrocytes effectively inhibited hypertrophic differentiation of MSCs [78,95,96]. Furthermore, the simultaneous transplantation of differentiated chondrocytes into microfractured lesions could prevent excessive endochondral ossification [9]. Even the application of undigested cartilage tissue fragments into cartilage lesions reduced the formation of intralesional osteophytes [10]. Thus, the presence of differentiated chondrocytes or cartilage tissue within repair tissue seems to be beneficial.

Beyond that, there is increasing evidence that articular cartilage itself contains a progenitor cell population, which is located particularly within the superficial zones and which may be capable to restore smaller superficial lesions [97-102]. Although this endogenous repair capacity is commonly not sufficient to heal clinically symptomatic larger cartilage defects, this predetermined cell population may represent a favourable source for cartilage repair [96-99]. This cell population may clinically be utilized by the transplantation of allogeneic juvenile minced epiphyseal cartilage fragments [103], which may evolve into a promising alternative to autologous chondrocyte transplantation.

Based on the current knowledge, one might speculate that genetic imprinting during early joint formation may differentiate between the determination of a permanent articular chondrocyte originating from cap of the epiphyses and the determination of a transient growth plate chondrocytes originating from the resting zone. Epigenetic mechanisms include gene activation by histone acetylation and gene silencing by methylation of CpG-rich DNA regions. Recently, epigenetic regulation of the COL10a1 gene in chondrocytes has been demonstrated: While hypermethylation of the COL10a1 gene in articular chondrocytes results in silencing of this hypertrophic marker, demethylation in MSC is associated with induction of Col10a1 [104]. By contrast, the COL2a1 gene is sparsely methylated in chondrocytes [105]. Histone acetylation facilitates transcription by modulating the chromatin structure. In this respect, histone deacetylases (HDAC) exert a transcriptional repression. HDAC4, which is expressed in prehypertrophic chondrocytes, may have a central role in skeletal development by inhibiting the expression of Runx2 and thus regulating chondrocyte hypertrophy and endochondral ossification [106].

Outlook

Future therapeutic approaches, which have to focus on the stabilization of the chondrocytic phenotype and inhibition of chondrocyte hypertrophy, will have to consider the following challenges: 1. the identification of the appropriate repair cell population under consideration of genetic imprinting mechanisms that may predetermine a chondrogenic fate of progenitor cells. 2 the interplay of different signalling molecules, that will not only include anabolic growth factors, but also antagonists and inhibitory factors. Until these aspects have not completely been identified, the transplantation of differentiated chondrocytes may remain the method of choice to induce hyaline-like repair cartilage and to avoid excessive bone formation.

Acknowledgements

This work was supported by the ELAN Fonds of the University Hospital Erlangen and the German Research Foundation (DFG) Grant GE 1975/2-1.

References

1. Steadman JR, Briggs KK, Rodrigo JJ, Kocher MS, Gill TJ, et al. (2003) Outcomes of microfracture for traumatic chondral defects of the knee: average 11-year follow-up. Arthroscopy 19: 477-484.
2. Kreuz PC, Steinwachs MR, Erggelet C, Krause SJ, Konrad G, et al. (2006) Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthritis Cartilage 14: 1119-1125.
3. Mithoefer K, Williams RJ 3rd, Warren RF, Potter HG, Spock CR, et al. (2005) The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. J Bone Joint Surg Am 87: 1911-1920.
4. Gelse K, Olk A, Eichhorn S, Swoboda B, Schoene M, et al. (2010) Quantitative ultrasound biomicroscopy for the analysis of healthy and repair cartilage tissue. Eur Cell Mater 19: 58-71.
5. Franke O, Durst K, Maier V, Göken M, Birkholz T, et al. (2007) Mechanical properties of hyaline and repair cartilage studied by nanoindentation. Acta Biomater 3: 873-881.
6. Shapiro F, Koide S, Glimcher MJ (1993) Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. J Bone Joint Surg Am 75: 532-553.
7. Saris DB, Vanlauwe J, Victor J, Haspl M, Bohnsack M, et al. (2008) Characterized chondrocyte implantation results in better structural repair when treating symptomatic cartilage defects of the knee in a randomized controlled trial versus microfracture. Am J Sports Med 36: 235-246.
8. Gelse K, Klinger P, Koch M, Surmann-Schmitt C, von der Mark K, et al. (2011) Thrombospondin-1 prevents excessive ossification in cartilage repair tissue induced by osteogenic protein-1. Tissue Eng Part A 17: 2101-2112.
9. Blanke M, Carl HD, Klinger P, Swoboda B, Hennig F, et al. (2009) Transplanted chondrocytes inhibit endochondral ossification within cartilage repair tissue. Calcif Tissue Int 85: 421-433.
10. Cole BJ, Farr J, Winalski CS, Hosea T, Richmond J, et al. (2011) Outcomes after a single-stage procedure for cell-based cartilage repair: a prospective clinical safety trial with 2-year follow-up. Am J Sports Med 39: 1170-1179.
11. Brown WE, Potter HG, Marx RG, Wickiewicz TL, Warren RF (2004) Magnetic resonance imaging appearance of cartilage repair in the knee. Clin Orthop Relat Res 214-223.
12. Henderson IJ, La Valette DP (2005) Subchondral bone overgrowth in the presence of full-thickness cartilage defects in the knee. Knee 12: 435-440.
13. Gomoll AH, Madry H, Knutsen G, van Dijk N, Seil R, et al. (2010) The subchondral bone in articular cartilage repair: current problems in the surgical management. Knee Surg Sports Traumatol Arthrosc 18: 434-447.
14. Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 238: 265-272.
15. Prockop DJ (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276: 71-74.
16. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147.
17. Winter A, Breit S, Parsch D, Benz K, Steck E, et al. (2003) Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue-derived stromal cells. Arthritis Rheum 48: 418-429.
18. Pelttari K, Winter A, Steck E, Goetzke K, Hennig T, et al. (2006) Premature induction of hypertrophy during in vitro chondrogenesis of human mesenchymal stem cells correlates with calcification and vascular invasion after ectopic transplantation in SCID mice. Arthritis Rheum 54: 3254-3266.
19. De Bari C, Dell'Accio F, Luyten FP (2004) Failure of in vitro-differentiated mesenchymal stem cells from the synovial membrane to form ectopic stable cartilage in vivo. Arthritis Rheum 50: 142-150.
20. Hennig T, Lorenz H, Thiel A, Goetzke K, Dickhut A, et al. (2007) Reduced chondrogenic potential of adipose tissue derived stromal cells correlates with an altered TGFbeta receptor and BMP profile and is overcome by BMP-6. J Cell Physiol 211: 682-691.
21. Schätti O, Grad S, Goldhahn J, Salzmann G, Li Z, et al. (2011) A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. Eur Cell Mater 22: 214-225.
22. Robins JC, Akeno N, Mukherjee A, Dalal RR, Aronow BJ, et al. (2005) Hypoxia induces chondrocyte-specific gene expression in mesenchymal cells in association with transcriptional activation of Sox9. Bone 37: 313-322.
23. Scotti C, Tonnarelli B, Papadimitropoulos A, Scherberich A, Schaeren S, et al. (2010) Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering. Proc Natl Acad Sci U S A 107: 7251-7256.
24. Goldring MB, Tsuchimochi K, Ijiri K (2006) The control of chondrogenesis. J Cell Biochem 97: 33-44.
25. Gelse K, Söder S, Eger W, Diemtar T, Aigner T (2003) Osteophyte development--molecular characterization of differentiation stages. Osteoarthritis Cartilage 11: 141-148.
26. Wuelling M, Vortkamp A (2010) Transcriptional networks controlling chondrocyte proliferation and differentiation during endochondral ossification. Pediatr Nephrol 25: 625-631.
27. Klinger P, Surmann-Schmitt C, Brem M, Swoboda B, Distler JH, et al. (2011) Chondromodulin 1 stabilizes the chondrocyte phenotype and inhibits endochondral ossification of porcine cartilage repair tissue. Arthritis Rheum 63: 2721-2731.
28. Shapiro IM, Adams CS, Freeman T, Srinivas V (2005) Fate of the hypertrophic chondrocyte: microenvironmental perspectives on apoptosis and survival in the epiphyseal growth plate. Birth Defects Res C Embryo Today 75: 330-339.
29. Chen H, Chevrier A, Hoemann CD, Sun J, Ouyang W, et al. (2011) Characterization of subchondral bone repair for marrow-stimulated chondral defects and its relationship to articular cartilage resurfacing. Am J Sports Med 39: 1731-1740.
30. Soltan M, Smiler D, Choi JH (2009) Bone marrow: orchestrated cells, cytokines, and growth factors for bone regeneration. Implant Dent 18: 132-141.
31. Giannoudis PV, Pountos I, Morley J, Perry S, Tarkin HI, et al. (2008) Growth factor release following femoral nailing. Bone 42: 751-757.
32. Schmidmaier G, Herrmann S, Green J, Weber T, Scharfenberger A, et al. (2006) Quantitative assessment of growth factors in reaming aspirate, iliac crest, and platelet preparation. Bone 39: 1156-1163.
33. Gelse K, von der Mark K, Aigner T, Park J, Schneider H (2003) Articular cartilage repair by gene therapy using growth factor-producing mesenchymal cells. Arthritis Rheum 48: 430-441.
34. Huang AH, Motlekar NA, Stein A, Diamond SL, Shore EM, et al. (2008) High-throughput screening for modulators of mesenchymal stem cell chondrogenesis. Ann Biomed Eng 36: 1909-1921.
35. Kan A, Ikeda T, Saito T, Yano F, Fukai A, et al. (2009) Screening of chondrogenic factors with a real-time fluorescence-monitoring cell line ATDC5-C2ER: identification of sorting nexin 19 as a novel factor. Arthritis Rheum 60: 3314-3323.
36. Kuo AC, Rodrigo JJ, Reddi AH, Curtiss S, Grotkopp E, et al. (2006) Microfracture and bone morphogenetic protein 7 (BMP-7) synergistically stimulate articular cartilage repair. Osteoarthritis Cartilage 14: 1126-1135.

37. Gelse K, Mühle C, Franke O, Park J, Jehle M, et al. (2008) Cell-based resurfacing of large cartilage defects: long-term evaluation of grafts from autologous transgene-activated periosteal cells in a porcine model of osteoarthritis. Arthritis Rheum 58: 475-488.

38. Sellers RS, Zhang R, Glasson SS, Kim HD, Peluso D, et al. (2000) Repair of articular cartilage defects one year after treatment with recombinant human bone morphogenetic protein-2 (rhBMP-2). J Bone Joint Surg Am 82: 151-160.

39. Yang HS, La WG, Bhang SH, Kim HJ, Im GI, et al. (2011) Hyaline cartilage regeneration by combined therapy of microfracture and long-term bone morphogenetic protein-2 delivery. Tissue Eng Part A 17: 1809-1818.

40. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, et al. (1988) Novel regulators of bone formation: molecular clones and activities. Science 242: 1528-1534.

41. Volk SW, Luvalle P, Leask T, Leboy PS (1998) A BMP responsive transcriptional region in the chicken type X collagen gene. J Bone Miner Res 13: 1521-1529.

42. Steinert AF, Proffen B, Kunz M, Hendrich C, Ghivizzani SC, et al. (2009) Hypertrophy is induced during the in vitro chondrogenic differentiation of human mesenchymal stem cells by bone morphogenetic protein-2 and bone morphogenetic protein-4 gene transfer. Arthritis Res Ther 11: R148.

43. Tou L, Quibria N, Alexander JM (2003) Transcriptional regulation of the human Runx2/Cbfa1 gene promoter by bone morphogenetic protein-7. Mol Cell Endocrinol 205: 121-129.

44. Gelse K, Jiang QJ, Aigner T, Ritter T, Wagner K, et al. (2001) Fibroblast-mediated delivery of growth factor complementary DNA into mouse joints induces chondrogenesis but avoids the disadvantages of direct viral gene transfer. Arthritis Rheum 44: 1943-1953.

45. van Beuningen HM, van der Kraan PM, Arntz OJ, van den Berg WB (1994) Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint. Lab Invest 71: 279-290.

46. Blaney Davidson EN, Vitters EL, van Beuningen HM, van de Loo FA, van den Berg WB, et al. (2007) Resemblance of osteophytes in experimental osteoarthritis to transforming growth factor beta-induced osteophytes: limited role of bone morphogenetic protein in early osteoarthritic osteophyte formation. Arthritis Rheum 56: 4065-4073.

47. Storm EE, Kingsley DM (1999) GDF5 coordinates bone and joint formation during digit development. Dev Biol 209: 11-27.

48. Zhang X, Ziran N, Goater JJ, Schwarz EM, Puzas JE, et al. (2004) Primary murine limb bud mesenchymal cells in long-term culture complete chondrocyte differentiation: TGF-beta delays hypertrophy and PGE2 inhibits terminal differentiation. Bone 34: 809-817.

49. Mueller MB, Fischer M, Zellner J, Berner A, Dienstknecht T, et al. (2010) Hypertrophy in mesenchymal stem cell chondrogenesis: effect of TGF-beta isoforms and chondrogenic conditioning. Cells Tissues Organs 192: 158-166.

50. Coleman CM, Tuan RS (2003) Growth/differentiation factor 5 enhances chondrocyte maturation. Dev Dyn 228: 208-216.

51. Pagnotto MR, Wang Z, Karpie JC, Ferretti M, Xiao X, et al. (2007) Adeno-associated viral gene transfer of transforming growth factor-beta1 to human mesenchymal stem cells improves cartilage repair. Gene Ther 14: 804-813.

52. Chen J, Chen H, Li P, Diao H, Zhu S, et al. (2011) Simultaneous regeneration of articular cartilage and subchondral bone in vivo using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. Biomaterials 32: 4793-4805.

53. Guo X, Zheng Q, Yang S, Shao Z, Yuan Q, et al. (2006) Repair of full-thickness articular cartilage defects by cultured mesenchymal stem cells transfected with the transforming growth factor beta1 gene. Biomed Mater 1: 206-215.

54. Katayama R, Wakitani S, Tsumaki N, Morita Y, Matsushita I, et al. (2004) Repair of articular cartilage defects in rabbits using CDMP1 gene-transfected autologous mesenchymal cells derived from bone marrow. Rheumatology (Oxford) 43: 980-985.

55. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, et al. (1999) VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 5: 623-628.

56. Pfander D, Körtje D, Zimmermann R, Weseloh G, Kirsch T, et al. (2001) Vascular endothelial growth factor in articular cartilage of healthy and osteoarthritic human knee joints. Ann Rheum Dis 60: 1070-1073.

57. Bluteau G, Julien M, Magne D, Mallein-Gerin F, Weiss P, et al. (2007) VEGF and VEGF receptors are differentially expressed in chondrocytes. Bone 40: 568-576.

58. Chen XY, Hao YR, Wang Z, Zhou JL, Jia QX, et al. (2011) The effect of vascular endothelial growth factor on aggrecan and type II collagen expression in rat articular chondrocytes. Rheumatol Int.

59. Pufe T, Harde V, Petersen W, Goldring MB, Tillmann B, et al. (2004) Vascular endothelial growth factor (VEGF) induces matrix metalloproteinase expression in immortalized chondrocytes. J Pathol 202: 367-374.

60. Nagai T, Sato M, Kutsuna T, Kokubo M, Ebihara G, et al. (2010) Intravenous administration of anti-vascular endothelial growth factor humanized monoclonal antibody bevacizumab improves articular cartilage repair. Arthritis Res Ther 12: R178.

61. Ellman MB, An HS, Muddasani P, Im HJ (2008) Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene 420: 82-89.

62. Ornitz DM (2005) FGF signaling in the developing endochondral skeleton. Cytokine Growth Factor Rev 16: 205-213.

63. Minina E, Kreschel C, Naski MC, Ornitz DM, Vortkamp A (2002) Interaction of FGF, Ihh/Pthlh, and BMP signaling integrates chondrocyte proliferation and hypertrophic differentiation. Dev Cell 3: 439-449.

64. Hellingman CA, Koevoet W, Kops N, Farrell E, Jahr H, et al. (2010) Fibroblast growth factor receptors in in vitro and in vivo chondrogenesis: relating tissue engineering using adult mesenchymal stem cells to embryonic development. Tissue Eng Part A 16: 545-556.

65. Day TF, Guo X, Garrett-Beal L, Yang Y(2005) Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 8: 739-750.

66. Tamamura Y, Otani T, Kanatani N, Koyama E, Kitagaki J, et al. (2005) Developmental regulation of Wnt/beta-catenin signals is required for growth plate assembly, cartilage integrity, and endochondral ossification. J Biol Chem 280: 19185-19195.

67. Dong YF, Soung do Y, Schwarz EM, O'Keefe RJ, Drissi H (2006) Wnt induction of chondrocyte hypertrophy through the Runx2 transcription factor. J Cell Physiol 208: 77-86.

68. Komori T (2010) Regulation of bone development and extracellular matrix protein genes by RUNX2. Cell Tissue Res 339: 189-195.

69. Mackie EJ, Ahmed YA, Tatarczuch L, Chen KS, Mirams M (2008) Endochondral ossification: how cartilage is converted into bone in the developing skeleton. Int J Biochem Cell Biol 40: 46-62.

70. Ijiri K, Zerbini LF, Peng H, Correa RG, Lu B, et al. (2005) A novel role for GADD45beta as a mediator of MMP-13 gene expression during chondrocyte terminal differentiation. J Biol Chem 280: 38544-38555.

71. Johnson JT, Schuberth JM, Thornton SD, Christensen JC (2009) Joint curettage arthrodesis technique in the foot: a histological analysis. J Foot Ankle Surg 48: 558-564.

72. Shukunami C, Oshima Y, Hiraki Y (2005) Chondromodulin-I and tenomodulin: a new class of tissue-specific angiogenesis inhibitors found in hypovascular connective tissues. Biochem Biophys Res Commun 333: 299-307.

73. Mera H, Kawashima H, Yoshizawa T, Ishibashi O, Ali MM, et al. (2009) Chondromodulin-1 directly suppresses growth of human cancer cells. BMC Cancer 9: 166.

74. Negishi Y, Ui N, Nakajima M, Kawashima K, Maruyama K, et al. (2001) p21Cip-1/SDI-1/WAF-1 gene is involved in chondrogenic differentiation of ATDC5 cells in vitro. J Biol Chem 276: 33249-33256.

75. Stewart MC, Farnum CE, MacLeod JN (1997) Expression of p21CIP1/WAF1 in chondrocytes. Calcif Tissue Int 61: 199-204.

76. Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, et al. (1996) Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273: 613-622.

77. Gelse K, Ekici AB, Cipa F, Swoboda B, Carl HD, et al. (2012) Molecular differentiation between osteophytic and articular cartilage - clues for a transient and permanent chondrocyte phenotype. Osteoarthritis Cartilage 20: 162-171.

78. Fischer J, Dickhut A, Rickert M, Richter W (2010) Human articular chondrocytes

secrete parathyroid hormone-related protein and inhibit hypertrophy of mesenchymal stem cells in coculture during chondrogenesis. Arthritis Rheum 62: 2696-2706.

79. Amano K, Hata K, Sugita A, Takigawa Y, Ono K, et al. (2009) Sox9 family members negatively regulate maturation and calcification of chondrocytes through up-regulation of parathyroid hormone-related protein. Mol Biol Cell 20: 4541-4551.

80. Huang W, Chung UI, Kronenberg HM, de Crombrugghe B (2001) The chondrogenic transcription factor Sox9 is a target of signaling by the parathyroid hormone-related peptide in the growth plate of endochondral bones. Proc Natl Acad Sci U S A 98: 160-165.

81. Merino R, Rodriguez-Leon J, Macias D, Gañan Y, Economides AN, et al. (1999) The BMP antagonist Gremlin regulates outgrowth, chondrogenesis and programmed cell death in the developing limb. Development 126: 5515-5522.

82. Benazet JD, Bischofberger M, Tiecke E, Goncalves A, Martin JF, et al. (2009) A self-regulatory system of interlinked signaling feedback loops controls mouse limb patterning. Science 323: 1050-1053.

83. Shapiro F, Flynn E, Calicchio ML (2009) Molecular differentiation in epiphyseal and physeal cartilage. Prominent role for gremlin in maintaining hypertrophic chondrocytes in epiphyseal cartilage. Biochem Biophys Res Commun 390: 570-576.

84. Nilsson O, Parker EA, Hegde A, Chau M, Barnes KM, et al. (2007) Gradients in bone morphogenetic protein-related gene expression across the growth plate. J Endocrinol 193: 75-84.

85. Anderson HC, Hodges PT, Aguilera XM, Missana L, Moylan PE (2000) Bone morphogenetic protein (BMP) localization in developing human and rat growth plate, metaphysis, epiphysis, and articular cartilage. J Histochem Cytochem 48: 1493-1502.

86. Yang Y, Liao E (2007) Mutant WISP3 triggers the phenotype shift of articular chondrocytes by promoting sensitivity to IGF-1 hypothesis of spondyloepiphyseal dysplasia tarda with progressive arthropathy (SEDT-PA). Med Hypotheses 68: 1406-1410.

87. Nakamura Y, Weidinger G, Liang JO, Aquilina-Beck A, Tamai K, et al. (2007) The CCN family member Wisp3, mutant in progressive pseudorheumatoid dysplasia, modulates BMP and Wnt signaling. J Clin Invest 117: 3075-3086.

88. Cui RR, Huang J, Yi L, Xie H, Zhou HD, et al. (2007) WISP3 suppresses insulin-like growth factor signaling in human chondrocytes. Mol Cell Endocrinol 279: 1-8.

89. Bodine PV, Zhao W, Kharode YP, Bex FJ, Lambert AJ, et al. (2004) The Wnt antagonist secreted frizzled-related protein-1 is a negative regulator of trabecular bone formation in adult mice. Mol Endocrinol 18: 1222-1237.

90. Lories RJ, Peeters J, Bakker A, Tylzanowski P, Derese I, et al. (2007) Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. Arthritis Rheum 56: 4095-4103.

91. Lane NE, Lian K, Nevitt MC, Zmuda JM, Lui L, et al. (2006) Frizzled-related protein variants are risk factors for hip osteoarthritis. Arthritis Rheum 54: 1246-1254.

92. Evangelou E, Chapman K, Meulenbelt I, Karassa FB, Loughlin J, et al. (2009) Large-scale analysis of association between GDF5 and FRZB variants and osteoarthritis of the hip, knee, and hand. Arthritis Rheum 60: 1710-1721.

93. Peng H, Huard J (2004) Muscle-derived stem cells for musculoskeletal tissue regeneration and repair. Transpl Immunol 12: 311-319.

94. Park J, Gelse K, Frank S, von der Mark K, Aigner T, et al. (2006) Transgene-activated mesenchymal cells for articular cartilage repair: a comparison of primary bone marrow-, perichondrium/periosteum- and fat-derived cells. J Gene Med 8: 112-125.

95. Bian L, Zhai DY, Mauck RL, Burdick JA (2011) Coculture of human mesenchymal stem cells and articular chondrocytes reduces hypertrophy and enhances functional properties of engineered cartilage. Tissue Eng Part A 17: 1137-1145.

96. Cooke ME, Allon AA, Cheng T, Kuo AC, Kim HT, et al. (2011) Structured three-dimensional co-culture of mesenchymal stem cells with chondrocytes promotes chondrogenic differentiation without hypertrophy. Osteoarthritis Cartilage 19: 1210-1218.

97. McCarthy HE, Bara JJ, Brakspear K, Singhrao SK, Archer CW (2011) The comparison of equine articular cartilage progenitor cells and bone marrow-derived stromal cells as potential cell sources for cartilage repair in the horse. Vet J .

98. Williams R, Khan IM, Richardson K, Nelson L, McCarthy HE, et al. (2010) Identification and clonal characterisation of a progenitor cell sub-population in normal human articular cartilage. PLoS One 5: e13246.

99. Chang HX, Yang L, Li Z, Chen G, Dai G (2011) Age-related biological characterization of mesenchymal progenitor cells in human articular cartilage. Orthopedics 34: e382-388.

100. Alsalameh S, Amin R, Gemba T, Lotz M (2004) Identification of mesenchymal progenitor cells in normal and osteoarthritic human articular cartilage. Arthritis Rheum 50: 1522-1532.

101. Nakamura N, Horibe S, Toritsuka Y, Mitsuoka T, Natsu-ume T, et al. (2008) The location-specific healing response of damaged articular cartilage after ACL reconstruction: short-term follow-up. Knee Surg Sports Traumatol Arthrosc 16: 843-848.

102. Dowthwaite GP, Bishop JC, Redman SN, Khan IM, Rooney P, et al. (2004) The surface of articular cartilage contains a progenitor cell population. J Cell Sci 117: 889-897.

103. McCormick F, Yanke A, Provencher MT, Cole BJ (2008) Minced articular cartilage--basic science, surgical technique, and clinical application. Sports Med Arthrosc 16: 217-220.

104. Zimmermann P, Boeuf S, Dickhut A, Boehmer S, Olek S, et al. (2008) Correlation of COL10A1 induction during chondrogenesis of mesenchymal stem cells with demethylation of two CpG sites in the COL10A1 promoter. Arthritis Rheum 58: 2743-2753.

105. Fernández MP, Young MF, Sobel ME (1985) Methylation of type II and type I collagen genes in differentiated and dedifferentiated chondrocytes. J Biol Chem 260: 2374-2378.

106. Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, et al. (2004) Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. Cell 119: 555-566.