Abstract

Introduction

Osteoarthritis (OA) in no longer considered as a disease of articular cartilage only. In more recent times, researchers opine that OA could be a disease of joint as well. Clinical and basic studies now question this notion. Indeed, current evidence indicates OA may be a disease involving abnormal bone tissue modelling or remodelling caused by alterations in mesenchymal stem cell (MSC) recruitment and differentiation. Biological factors produced by OA osteoblasts have been shown to affect chondrocyte function, causing alterations in MSC recruitment/differentiation. One such factor may be transforming growth factor-β1 (TFG-β1). The aim of this review was to discuss the rationale for a new paradigm shift involving TFG-β1 in OA.

Conclusion

Recent studies have contributed to a shift in researchers’ understanding of OA and the causative events that lead to it.

Introduction

Osteoarthritis (OA) is the most common form of arthritis. OA is characterized by progressive articular cartilage loss, appositional new bone formation with sclerosis and abnormal vascularization of subchondral trabeculae and plate, and formation of osteophytes1–3. Synovitis is often observed and is considered secondary to changes in hard tissues within the joint. The widely held belief that OA is a disease of articular cartilage, with cartilage erosion being the main identifying feature, has been a subject for debate, particularly in recent years, and several investigations have led to the postulation that bone changes may account for subsequent joint deterioration and development of OA. OA risk factors in humans include age, gender, genetic predisposition, mechanical stress and/or joint trauma, and obesity. Bone mass in OA patients is better preserved than in healthy individuals, independently of body weight. Moreover, increase in bone mass is noted for both the usually affected sites, such as the knee and the hip, as well as non-synovial sites, such as the lumbar spine. Genetic studies indicate a recessively inherited gene and a multifactorial component. Hence, the ‘gene’ is more likely to express a factor involved in a metabolic pathway than a structural skeletal protein. Studies examining a metabolic link between obesity and OA have reported conflicting findings. Some studies were able to show a significant association between hypertension, uric acid, cholesterol and OA, whereas others failed to substantiate these relationships7,8. Therefore, a unifying hypothesis taking into account all features of pathology could be that OA is a metabolic disease in which systemic and/or local factors induce changes in skeletal tissues by modifying the formation and biosynthetic activity of cells derived from mesenchymal stem cell (MSC). As tissue homeostasis is disturbed and joint integrity impaired, normal wear and tear could then lead to cartilage damage, the hallmark of OA. The loss of cartilage may then be a result of mechanical forces, but this would not cause the disease itself. Furthermore, OA process may modify the formation and biosynthetic activity of MSC9. In as much as adipocytes share a common MSC precursor with osteoblasts (Ob), chondrocytes, tenocytes and myoblasts, all cells affected by OA, a link between lipid metabolism and connective tissues is probable. Such a link may be related to leptin, a known factor involved in body weight regulation and obesity, and in lipid metabolism. Leptin causes the local differentiation of mesenchymal stromal cells into Ob within the bone marrow, while impeding the maturation of adipocytes10. Indeed, Ob increases in proportion to an increase in adipocytes and chondrocytes and is blunted by using bone marrow stromal cells from OA patients11, therefore implying a key role for leptin in this process. The activity of the Wnt/β-catenin signalling pathway is also crucial for the recruitment and differentiation of MSC into osteoblasts and adipocytes. Indeed, β-catenin levels must vary within a narrow limit at specific time points during MSC, pre-Ob and Ob development. This critical review discusses OA and the rationale for a new paradigm shift involving transforming growth factor-β1 (TFG-β1).

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by relevant ethics committees related to the institutions.
in which they were performed. All human subjects in these referenced studies gave informed consent to participate in these studies.

What are the causes of abnormal subchondral bone remodelling in OA?

Abnormal indices of bone remodelling are observed in OA patients, and in animal models with spontaneous OA. Indeed, increased subchondral bone activity in OA, as judged by enhanced uptake of technetium labelled diphosphonate, predicts cartilage loss, and cartilage lesions do not progress without significant subchondral activity. Alterations of the bony bed precede cartilage changes in the Macaca fascicularis primate model of OA, and in the Dunkin–Hartley guinea pig model. This is associated with intensified remodelling of the subchondral bone and increased bone stiffness, which results in the bone no longer being an effective shock absorber. In addition, bone is not the only dense tissue in joints. Indeed, the calcified cartilage tissue is also dense and even more so in OA calcified tissue, hence probably not providing a tissue of intermediate stiffness under these conditions. However, subchondral bone stiffness in OA may be part of a more generalized bone alteration leading to an apparent increased bone mineral density (BMD) or bone volume as observed in most obese individuals. An extremely hypermineralized articular calcified cartilage and subchondral bone tissue of femoral heads can be observed by quantitative backscattered electron imaging in OA patients. Such a localized increase in BMD was confirmed using Fourier transform infrared spectroscopy of OA knee joints, suggesting that this is related to a remineralization of the subchondral bone tissue. In addition, BMD of the subchondral trabecular bone correlates with progressive joint space narrowing in knee OA and with osteophytes. Increased bone density and osteoid volume are often more severe than cartilage changes in animal models of spontaneous OA. However, an apparent increase in BMD in OA may be due to an increase in material density and need not result from an increase in mineral density caused by increased osteoid collagen matrix that is under-mineralized. Such an increase of osteoid volume in OA bone tissue is also an indication of abnormal mineralization. OA bone’s hardness is 7% lower than that of osteoporotic bone, indicating that OA bone has a reduced elastic modulus. These results support the view of a more generalized bone metabolic disease in OA and that alteration of subchondral bone integrity is a key event in OA.

Evidence for a role of abnormal bone cell metabolism in OA

A high body mass index (BMI) as observed in OA patients is linked with a higher bone mass, and increased BMD suggests new bone synthesis exceeds degradation in OA. Indeed, elevated osteocalcin serum levels in women with hand OA and in cortical bone explants, and the observation that insulin-like growth factors (IGF-I and II) and TGF-β levels are higher in samples of iliac crest bone of patients with OA, hence at a site distant from weight-bearing joints, suggest an increased bone anabolism in OA. Moreover, an imbalance between collagen and non-collagen protein synthesis like osteocalcin can lead to an increase in bone volume without a concomitant increase in BMD. Abnormal collagen content may also lead to abnormal mineralization as only native collagen type I fibrils can mineralize. Intriguingly, collagen type I is elevated in trabecular bone of femoral heads of OA patients, and leptin can directly stimulate the synthesis of the a1 chain of collagen type I in in vitro Ohs. However, collagen type I is composed of an heterotrimer of a1 and a2 chains at an average ratio of 2.4:1 in normal bone, yet this varied between 4:1 and 17:1 in OA bone tissue. In vivo, the increase in bone collagen matrix could also be linked to altered MSC differentiation into Obs, since in OA patients Obs maturation from bone marrow stromal cells is enhanced while that of adipocytes and chondrocytes is blunted.

Role of MSCs in OA

The potential role of bone tissue in OA initiation or progression may be due to its capacity to serve as a reservoir for MSCs and to provide nutrition of the hyaline cartilage. The role of MSC in the appearance and/or progression of OA is a key issue that received recent attention. Based on the increasing data on abnormal behaviour and phenotypic features of osteoblasts, chondrocytes, myoblasts and tenocytes in OA joints, it is possible to conclude that MSC development and differentiation are likely to be altered in affected individuals. Recent evidence indicates that MSC numbers, proliferation rate, population-doubling time and the capacity to differentiate into different lineage cells may be altered in OA, yet no mechanism(s) responsible for this situation have been identified. The response to cytokines and growth factors by MSC in OA individuals may also be altered. These results indicate that the differentiation of MSC into target cells could be altered in vivo, it could lead to abnormal tissue homeostasis. It also suggests that cells not presently residing in the affected tissue may profoundly affect behaviour and homeostasis of this tissue. Moreover, because the chondrogenic and adipogenic capacity of OA MSC is impaired, OA MSC either remains undifferentiated or differentiated into limited lineage cell, such as the osteogenic line. This could explain why all joint tissues except bone are impaired in OA individuals. Indeed, although muscle strength is reduced beyond normal age-related loss, possibly due to

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muscle cell dysfunction, alterations in the differentiation capacity of MSC to form myocytes may also be altered in these individuals, which was never demonstrated however. The alteration in OA MSC may also affect the important immunoregulatory role played by MSC. This regulatory role is modulated by specific signalling molecules such as TGF-β1 and hepatocyte growth factor (HGF).

Hence, it is important to remember the key role played by cytokines and growth factors in osteophyte formation, the elevation of TGF-β1 and HGF levels in OA cartilage, and elevated messenger RNA (mRNA) and protein levels of both TGF-β1 and HGF in OA osteoblasts. MSC isolated from adipose tissue secrete HGF that can decrease TGF-β1 production in co-cultures with chondrocytes, indicating another potential crosstalk between different joint tissues. Osteophyte formation may be considered a repair response to stabilize the damaged joints, and it requires the local recruitment of specific MSC. Under normal conditions, local TGF-β1 levels are kept at a normal level in response to shear stress in synovial membranes, whereas OA could be viewed as an exaggerated response to shear stress or a continued response to leading to abnormally high levels of TGF-β1.

This could alter the Wnt signalling pathway triggered in MSC and lead to abnormal recruitment of pre-Ob and Ob at the expense of chondrocytes and adipocytes.

**Role of the Wnt signalling pathway in MSC lineage commitment**

Wnt signalling plays a crucial role in MSC self-renewal in adult tissues. MSCs serve as a reservoir for tissue renewal following trauma, disease and aging. Via its interaction with frizzled (Fz) and LRPS/6 co-receptors, Wnts inactivate the axin–GSK3β complex. This latter complex phosphorylates and drives the degradation of β-catenin, whereas Wnts inhibit this activity. Non-phosphorylated β-catenin then translocates into the nucleus to form a complex with T-cell factor (TCF)/lymphoid enhancer-binding factor (LEF) transcription factors to activate Wnt target genes. Some Wnts do not activate this canonical pathway but act via other effectors, including JNK, Rho GTPase or Ca2+/PKC. Wnt3a and Wnt7b are among the most potent Wnt agonists in bone tissue. Five families of extracellular Wnt antagonists have been identified: secreted frizzled-related proteins (sFRP), Wnt inhibitory factor 1 (Wif1), Cerberus, Wise and Dickkopfs (DKK). In addition, Sclerostin (SOST) a glycoprotein secreted mostly by mature osteoblasts/osteocytes and belonging to the cysteine-knot protein of the DAN family, also inhibits Wnt signalling. The MSCs isolated from adult bone marrow are multi-potent and give rise to tissues, including bone, cartilage, muscle and adipose, and a number of critical transcription factors are involved in this commitment of MSC-derived lineages. Genetic studies have identified that Wnt/β-catenin activity is essential for normal osteogenesis. In mice and humans, targeted overexpression of Wnts or deficiency of Wnt antagonists is associated with increased bone formation. Although some in vitro studies showed that Wnts stimulate the differentiation of murine (m)MSC toward the osteoblastic lineage, both stimulatory or inhibitory effects have been reported for human (h) MSC, possibly linked with culture conditions. However, recent studies strongly suggest that under conditions permissive for in vitro binary lineage differentiation of adipocytes and osteoblasts, reminiscent of the in vivo situation, differences in sensitivity for Wnts alter this equilibrium and shift the commitment of normal hMSC from adipocytes towards osteoblasts. Moreover, under normal conditions the local balance of Wnts and Wnt antagonists, and the resulting Wnt/β-catenin signalling, in mMSC triggers adipogenesis and osteogenesis where Wnts at low concentrations potently block adipogenesis while stimulating the recruitment of osteoprogenitors. As osteoprogenitors then progress though their differentiation towards Ob, they progressively express more Wnts and Wnt antagonists that locally control this balance to further promote either adipogenesis or osteogenesis of neighbouring cells. This local triggering of Wnt/β-catenin signalling then increases the expression of other Wnts by differentiating mMSC into osteoblasts, namely, Wnt 7b, Wnt10b, DKKs and sFRPs. This suggests that the balance of these Wnts and Wnt antagonists could be altered in these individuals, which is the expression of abnormal phenotype markers and mineralization.

**Role of TGF-β1 in cartilage and bone tissue: involvement in OA pathogenesis**

TGF-β1 plays a dual role in skeletal tissues. Although it is an essential anabolic growth factor for cartilage growth and as such used in vitro for cartilage bioengineering, it can also cause OA-like features upon prolonged exposure. TGF-β1 also drives osteophyte formation. Early observations of OA bone tissue samples reported increased levels of TGF-β1 and IGF-1 even in non-weight-bearing joints, implying that OA could be a bone disease. Moreover, TGF-β1 levels remain elevated in isolated bone explants and in vitro osteoblasts of OA patients. A recent study indicated that targeted overexpression of TGF-β1 in mouse osteoblasts, but not in other joint tissues, lead to OA-like features, whereas inhibition of TGF-β1 activity in subchondral bone prevented the degeneration of cartilage in mice. Most important, TGF-β1 overexpression in mouse
osteoblasts leads to the alteration of Wnt signalling targets and abnormal release of secreted factors involved in MSC recruitment. This observation is reminiscent of those previously made using targeted overexpression of TGF/bone morphogenetic protein (BMP) inhibitors that reduced osteophyte formation and disease progression in an induced OA mouse model. This would concur with previous observations of alterations of TGF-β1 production in human OA bone tissue and osteoblasts. Our recent observation that the activity of Wnt/β-catenin signalling is reduced in human OA Ob compared to normal due to an elevated expression of the Wnt antagonist Dickkopf-2 (DKK2) is therefore timely. Moreover, TGF-β1 drives DKK2 expression in human OA Ob (44), and they both inhibit Ob mineralization as it occurs in human OA bone tissue.

As the aforesaid deleterious effects of TGF-β1 are driven via the ALK5 receptor pathway, which triggers SMAD2/3, it was suggested that direct intervention on this signalling route could be beneficial for OA. This can now be reconciled with the anabolic role of TGF-β1 in cartilage. In chondrocytes, TGF-β1 induces tissue inhibitors of metalloproteinases (TIMP), counteracts the inflammatory role played by IL-1, and can prevent hypertrophy, a chondrocyte phenotype found in OA, via the SMAD2/3 pathway. Altered SMAD3 expression may be possible in OA as single-nucleotide polymorphisms (SNPs) have been observed in populations of Northern Europe and China affected by OA, whereas abnormal SMAD3 expression and abnormal relationship with bone mineralization have been observed in OA versus normal bone tissue and osteoblasts. For the European study, genetic variation in the Smad3 gene has been associated with knee and hip OA A (66). Unfortunately, this study does not identify whether these mutations could induce a gain or loss of function. Therefore, targeting the anaplastic lymphoma kinase (ALK5) pathway for treating OA may still be complicated. Of note, recent studies have indicated that reduced SMAD2/3 activity could be detrimental to cartilage and prolonged exposure to TGF-β1 can drive SMAD1/5/8 after interacting with ALK1. This triggers MMP-13, which is a potent driving force for the loss of articular cartilage. This shift in ALK5 towards ALK1 activity is observed not only in aging but also in OA pathogenesis, implying that ALK1 would be a more interesting target for therapeutic intervention regarding cartilage, whereas this route does not seem to play a key role in bone. Moreover, as abnormal TGF-β1 levels alter not only cartilage but also MSC recruitment and differentiation, it would be important to more closely understand factor(s) involved in this paracrine signalling. Although the authors suggested Wnts or Wnt targets could be involved, more input is definitely needed to determine which factor(s) are involved. Among potential hypotheses, we cannot overlook that obesity is a high-risk factor for developing OA, and leptin has been pointed out as a key factor in obesity. Leptin, the product of the obese (ob) gene, is a 16-kDa protein produced by white adipocytes and other cells such as skeletal muscles and osteoblasts. Leptin favours the local differentiation of MSC into Ob within the bone marrow while impeding the maturation of adipocytes, an effect reminiscent of the observations made with bone-marrow stromal cells from OA patients and implying a key role for leptin in this process. Last, leptin has been shown to promote TGF-β1 expression in joint tissues of rats, and the role of TGF-β1 in OA is now considered a key event in the pathogenesis of this disease, although it is still not known what would trigger TGF-β1 levels locally.

Conclusion

Recent studies have contributed to a shift in our understanding of OA and the causative events that may lead to it. We moved from a general consensus on cartilage disease towards a bona fide bone disease, to a joint disease involving all joints, and now we are progressing to understand that local signals emanating from osteoblasts (possibly chondrocytes, though this has not been tested) trigger abnormal recruitment of MSCs that alter tissue integrity and signal homeostasis. Such a local factor could be TGF-β1, and abnormal active TGF-β1 levels could be achieved via altered metabolic signals such as leptin and shear stress in joint tissues.

References


Critical review


49. Veeman MT, Axelrod JD, Moon RT. A second canon. Functions and mechanisms of canonical WNT signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005 May;8(5):739–50.


55. Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005 May;8(5):739–50.


