The Role of Leptin in Osteoarthritis and Cartilage Metabolism

a report by
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Leptin is a 16kDa non-glycosylated peptide hormone encoded by the obese gene (ob), the murine homologue of the human gene LEP.1 It is mainly produced by adipocytes, and circulating leptin levels have been directly correlated with white adipose tissue mass. Leptin influences bodyweight homeostasis through its effects on food intake and energy expenditure by negative feedback at the hypothalamic nuclei, while it is also produced by non-adipocytic cells and targets extrahypothalamic tissues.2–4 It is a pleiotropic hormone implicated as a regulatory molecule in various physiological processes such as lipid metabolism, haemopoiesis, immune system modulation, angiogenesis, ovarian function, reproduction and inflammation.5–12 Leptin exerts its biological actions by binding to its receptors. These are encoded by the gene diabetes (db) and belong to the class I cytokine receptor superfamily. In humans, there exist at least five isoforms of the leptin receptor, generated by messenger RNA (mRNA) alternative splicing, which are membrane-spanning glycoproteins with cytoplasmic domains of ranging length.13,14 The long isoform of leptin receptor (Ob-Rb) contains the intracellular motifs required for the activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signal transduction pathway. Recently, it has also been suggested that, besides its signalling in the JAK/STAT pathway, Ob-Rb is capable of signal transduction by using alternative signalling pathways that link tyrosine phosphorylation events to renin–angiotensin system (RAS) activation, probably representing a critical step of cell proliferation and differentiation.3,14,15 Alternative splicing of db gives rise to six receptor isoforms: the soluble form Ob-Re, which lacks a cytoplasmic domain; four forms with short cytoplasmic domains (Ob-Ra, Ob-Rc, Ob-Rd and Ob-Rf); and the long form Ob-Rb, which is found in almost all tissues and appears to be the only form capable of transducing the leptin signal (see Figure 1).

Increasing evidence suggests that leptin is a novel pro-inflammatory adipocyte-derived factor that operates in the cytokine network by linking immune and inflammatory processes to the neuroendocrine system.16 A pro-inflammatory role of leptin through its receptor Ob-Rb has been suggested in inflammatory conditions such as multiple sclerosis, rheumatoid arthritis, non-alcoholic steatohepatitis, autoimmune encephalomyelitis and other diseases, probably by the induction of pro-inflammatory factors such as tumour necrosis factor (TNF), interferon (IFN)-γ, interleukin (IL)-2, cyclo-oxygenase (COX)-2 and nitric oxide production.17,18 It has been shown that leptin may display pro- or anti-inflammatory effects in the joints depending on the immune response.19,20 Leptin, as mentioned above, is more than an obesity molecule. Indeed, it is also a major regulator of bone mass, and the role of leptin in bone homeostasis was recently reviewed. However, it was not until late 2001 that the first report on the effect of leptin on chondrocytes was published by Figenschau et al.21 This paper led to a renewed interest in the potential roles of leptin in this cell type, and other data soon appeared.

The potential role of leptin in OA has been supported by the relationship between obesity and increased risk of OA development.22 This positive association has been observed not only for weight-bearing joints, such as knees, but also for non-weight-bearing joints, such as those in the hands.23–26 In addition, the functional isoform of leptin’s receptor (Ob-Rb) has been detected in cultured human adult articular chondrocytes, and leptin treatment of isolated cells in vitro has been shown to stimulate matrix synthesis.27 It has been suggested that leptin has a local effect on OA cartilage, as its levels in the synovial fluid (SF) have been found to be higher than those in the serum.28 Further evidence for the involvement of leptin in OA comes from our observation that leptin was found in the SF of OA patients and that its concentration was correlated with BMI (see Figure 2).29

The role of leptin in OA confirmed the hypothesis that OA is a metabolic disease in which systemic and local factors significantly contribute to the prominent dysregulation present in the disease. The first relevant observation of our research team is that there is differential expression of leptin and its functional receptor in severely damaged cartilage and in minimally affected cartilage; expression is higher in the former.29 This type of experimental approach avoids external interferences of other variables such as age, genetic background, differences in the pharmacological treatments and artifacts due to specimen collection. All comparisons were carried out between cartilage samples from the same individual. Although we acknowledged that minimally affected OA cartilage cannot be considered as normal cartilage, we could assume that mildly damaged OA cartilage adjacent to OA lesions represents a pre-challenged tissue that has all the potential to perpetuate the degenerative process and can be considered easily as a mirror of what is happening along the development of the disease. The increased expression of leptin in markedly damaged
cartilage, together with elevated leptin levels in SF (which could be also enhanced by circulating levels through the increase of vascular permeability in inflamed joints) suggested that leptin may trigger cartilage destruction, particularly when associated with some local factors. Actually, leptin synergises with IL-1 (see Figure 3), whose expression has been found to be markedly influenced by leptin itself in chondrocytes to increase nitric oxide production. Nitric oxide is known to interfere with chondrocyte function, resulting in loss of cartilage matrix, inhibition of type II collagen synthesis and activation of metalloproteinases. Regarding this last issue, we reported a leptin dose-dependent increase of MMP9 and MMP13, two of the major cartilage matrix-degrading metalloproteinases that mediate the destructive process in OA, indicating a catabolic role of leptin in cartilage metabolism.

After confirming the expression of leptin and its receptors in cartilage, we investigated the effect of leptin on cell proliferation and apoptosis in normal and osteoarthritic cultured chondrocytes. A stimulatory effect of leptin on chondrocyte proliferation was demonstrated in short-term cultures of normal chondrocytes incubated with the addition of serum. We showed that leptin enhanced cell proliferation in short-term cultures of normal chondrocytes; however, in long-term cultures this stimulatory effect was lost. When chondrocytes were treated with increasing leptin concentrations, their cell proliferation was decreased, while no increase was observed in their apoptosis rate compared with the untreated cells, indicating a long-term detrimental effect of leptin on cell viability and not on apoptosis. It has been suggested that the leptin–receptor axis is important for cell proliferation in many tissues.

OA can be considered as a metabolic disease in which all the components of the joint, including bone, muscles, synovia and cartilage, are generally affected by adipokine dysregulation or hyperactivity. The effects of leptin discussed above may participate in the pathogenesis of OA, together with mechanical overload due to the activation of mechanoreceptors at the chondrocyte surface as well as other specific conditions such as obesity and its associated complications of vascular inflammation, type 2 diabetes and severe alteration of lipids and glucose metabolism. However, in light of the recent published literature, it is now evident that leptin is involved in OA development and that it may be the missing link between biomechanical and metabolic factors involved in OA.

Our observation that leptin and its receptors at the mRNA and protein levels are expressed in OA articular chondrocytes indicates that this adipokine/ cytokine can indeed affect cartilage metabolism directly. The observed intra-joint difference of leptin and its functional receptor expression between severely and mildly affected osteoarthritic cartilage depending on lesion severity related leptin to the grade of cartilage destruction and, consequently, to OA progression. Furthermore, it can be suggested that in patients with osteoarthritis there is a unique microenvironment in the cartilage characterised by enhanced locally produced leptin levels, which induce IL-1β and MMP-9 and MMP-13 production by chondrocytes, reinforcing the scenario that leptin acts as a pro-inflammatory cytokine with a catabolic role on cartilage metabolism. Figure 4 shows a schematic representation of key physiopathological actions and targets of leptin at the bone and cartilage level.

These data also might have important implications for novel therapeutic strategies regarding osteoarthritis. We recently blocked leptin’s expression in chondrocytes using the new technology of small interfering RNA (siRNA) transferred in liposomes, and observed that leptin was about 65% downregulated 24 hours after transfection and was completely shut off 48 hours after siRNA transfection. Additionally, MMP-13 expression was significantly reduced, and continued to decrease even 96 hours after treatment.

The above experiments confirmed the specificity of siRNA treatment by liposomes and the effectiveness of leptin downregulation, pointing towards a possible therapeutic potential using siRNA treatment against leptin for OA treatment. However, the current understanding of the actions of leptin and other newly recognised adipokines is still too