Expression of adipokines in osteoarthritis osteophytes and their effect on osteoblasts

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Abstract

Objective

Osteophyte formation in osteoarthritis (OA) is mediated by increased osteoblast activity, which is -in turn- regulated by the Wnt signaling pathway. Obesity is regarded a risk factor in OA, yet little is known about the interaction between adipose tissue-derived factors, the adipokines, and bone formation, although adipokines are associated with the pathogenesis of OA. Therefore, the effect of adipokines on bone and cartilage forming cells and osteophyte development was analyzed.

Methods

Human OA osteophytes were histologically characterized and adipokine expression was evaluated by immunohistochemistry. Osteoblasts and chondrocytes were isolated from OA tissue and stimulated with adiponectin, resistin, or visfatin. Cytokine and osteoblast/chondrocyte markers were quantified and activation of Wnt and p38 MAPK signaling was analyzed.

Results

Adiponectin, resistin, and visfatin were expressed in OA osteophytes by various articular cell types. Stimulation of OA osteoblasts with adiponectin and of OA chondrocytes with visfatin led to an increased release of proinflammatory mediators but not to osteoblast differentiation or activation. Additionally, visfatin increased matrix degrading factors in chondrocytes. Wnt signaling was not altered by adipokines, but adiponectin induced p38 MAPK signaling in osteoblasts.

Conclusion

Adipokines are present in OA osteophytes, and adiponectin and visfatin increase the release of proinflammatory mediators by osteoblasts and chondrocytes. The effects of adiponectin were mediated by p38 MAPK but not Wnt signaling in osteoblasts. Therefore, the results support the idea that adipokines do not directly influence osteophyte development but the proinflammatory conditions in OA.

1. Introduction

Osteoarthritis (OA) is one of the most common forms of arthritis [1]. Factors contributing to OA are aging, obesity, trauma, and inflammation leading to functional impairment [1]. OA is characterized by cartilage degradation [1] but also involves moderate to severe synovial inflammation, remodeling of subchondral bone and osteophyte development [2]. Osteophytes are fibrocartilage-capped bony outgrowths at the joint borders [3], very common in OA and more prominent than e.g. in rheumatoid arthritis (RA). Osteophytes usually develop on the outside of the cortical bone and originate from periosteum [4]. It is unclear whether osteophyte formation is a functional adaptation or a pathological phenomenon. Osteophytes may arise as adaptation to joint instability as repair mechanism or may develop due to remodeling processes leading to chondrogenesis of progenitor cells from the periosteum [5]. Interestingly, osteophyte development can be triggered by various factors, e.g. mechanical forces, growth factors and cytokines stimulating the proliferation of mesenchymal stem cells in the periosteum [6]. Inside the developing osteophyte, cells undergo chondrogenesis and mature hypertrophic chondrocytes are subsequently replaced with osteoblasts mediating bone formation [6]. An increased osteoblast function has been assumed, most likely due to changes in Wnt signaling, a central pathway of bone remodeling. It controls the differentiation of mesenchymal progenitor cells into chondrocytes or osteoblasts [7]. In mature osteoblasts, Wnt signaling controls the expression of osteoprotegerin (OPG), which influences osteoclast differentiation and activity and, therefore, controlling bone resorption [8].

In inflammatory rheumatic diseases, especially in RA and OA, adipokines such as adiponectin, resistin, or visfatin are involved in disease pathophysiology [9,10]. Adipokines are bioactive substances formed mainly by cells of the adipose tissue [11,12]. Adiponectin has several metabolic effects, e.g. a positive effect on insulin resistance or liver function. However, adiponectin also has an immunomodulatory potential, e.g. it inhibits macrophage function [13], or induces TNF-α (tumor necrosis factor α) in monocytes. In addition, adiponectin reduces the expression of adhesion molecules on inflammatory cells [14]. In RA, adiponectin is present in high levels in the synovial fluid compared to OA patients [15]. In synovium, adiponectin is localized particularly in the RA and OA synovial lining layer and around vessels. Stimulation of RA but also OA synovial fibroblasts with adiponectin results in increased interleukin (IL) - 6 and pro-matrix-metalloproteinase (MMP) - 1 synthesis, factors contributing to inflammation and matrix degradation [16]. Resistin is mainly produced by monocytes and macrophages in adult humans [17]. The serum concentration of resistin correlates with inflammatory markers. Compared to OA patients, resistin is increased in serum and synovium of RA patients. However, it is present in the synovial lining and sublining layer in RA as well as OA tissues, e.g. in fibroblasts, macrophages, and B-cells [15,18]. Stimulation of macrophages with resistin induced proinflammatory cytokines, e.g. IL-6, IL-12, or TNF-α. In mouse models, arthritis could be induced by local injection of resistin into joints suggesting a pro-inflammatory potential in arthritis [18]. Visfatin is present in synovial fluid and serum of RA and OA patients [15,19], although levels in RA are higher than in OA patients. Besides adipocytes, visfatin is expressed in cells of the synovial lining layer, lymphocyte aggregates and endothelial cells. Visfatin has pro-inflammatory potential, it activates human leukocytes, induces co-stimulatory molecules on the cell surface as well as pro-inflammatory cytokines in monocytes [15]. Visfatin can be induced in synovial fibroblasts e.g. by IL-6 [20]. In addition, visfatin seems to be able to protect fibroblasts and neutrophils from apoptosis [21]. Visfatin levels correlate with degree of inflammation and disease activity as could be shown for RA patients [22]. Visfatin seems to have an additional impact on OA [9,10], due of its increased production by chondrocytes in OA cartilage [23]. Visfatin was shown to induce MMP3 and 13 as well as the expression of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) 4 and 5 in chondrocytes [9,10]. These data suggest that visfatin affects inflammation and the survival of cells responsible for mediating inflammation and destruction in arthritic diseases [9,10,24].

In chronic-inflammatory rheumatic diseases such as RA, several inhibitors of the Wnt signaling pathway, e.g. DKK1 or SFRPs, are increased at inflammation-mediated sites of erosion [25]. In the collagen-induced arthritis (CIA) mouse model, neutralizing anti-DKK1 antibodies on the one hand reduce joint erosion but on the other hand increase the formation of osteophytes, which are rather untypical in CIA [26]. In spondyloarthropathies, DKK1 also appears to play an important role in the pathological formation of new bone [27,28]. The inflammatory stimuli TNF-α and LPS induced WNT5A in bone marrow stromal cells, which in turn regulated cytokine and chemokine production [29]. Besides, adiponectin and other adipokines (WISP-2, SFRP5) were shown to be involved in Wnt signaling [30–32]. This led us to our hypothesis that there may also be a link between adipokines and Wnt signaling in osteophyte formation.

In our study, we investigated the effects of the adipokines adiponectin, resistin, and visfatin in the context of OA osteophyte development because their immunomodulatory properties are well described and they are three of the most prevalent adipokines in the research focus of rheumatic diseases.