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Review Article

The role of vascular endothelial growth factor in osteoarthritis: a review

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Abstract: Osteoarthritis (OA) is the most prevalent form of joint disease, with a high prevalence in the elderly. Its primary clinical symptoms are joint pain and restricted movement, with main pathologies of synovitis, cartilage degeneration, subchondral bone sclerosis, and osteophyte formation. Vascular endothelial growth factor (VEGF) is expressed in articular cartilage and increases in expression levels have been associated with progression of OA. Moreover, VEGF has been found to play an important role in the pathogenesis of OA of synovitis, cartilage degeneration, subchondral bone sclerosis, and osteophyte formation. The aim of this article was to examine the role of VEGF in OA and related studies.

Keywords: Vascular endothelial growth factor, osteoarthritis, articular cartilage, subchondral bone

Introduction

Osteoarthritis (OA) is a joint disease accompanied by the degeneration of articular cartilage. It is more likely to occur in middle-aged and elderly populations [1]. It is caused by the interaction of many factors, such as mechanical stress, biochemistry, and heredity. Clinical manifestations of OA are mainly joint pain and movement limitation, involving many joints in the whole body but more common in knee-weight joints [2]. Pathological features include synovitis, cartilage degeneration, subchondral bone sclerosis, and osteophyte formation [3]. Diagnosis of OA mainly depends on clinical symptoms, signs, and imaging data. Early treatment of OA consists of medication of analgesia and cartilage protection. In late-stage OA, arthroscopic surgery and arthroplasty are recommended.

Pesesse et al. [4] showed that progression of OA was related to angiogenesis and that vascular endothelial growth factor (VEGF) was one of the most important factors inducing angiogenesis. Hamilton et al. [5] demonstrated that VEGF plays an important role in the occurrence and development of OA. The role of VEGF in OA is reviewed in this study.

Basic biological characteristics and functions of VEGF

In the 1970s, VEGF was found to be an unknown factor that could increase vascular permeability and promote blood vessel formation. It was then called vascular permeability factor. In 1989, Ferrara N et al. [6] isolated and purified vascular endothelial growth factor from bovine pituitary follicular stellate cells. It was successfully identified and sequenced by American scientists. It was proven that vascular permeability factor and vascular endothelial growth factor were the same protein encoded by the same gene. Later, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor were found to be five other members of the VEGF family [7]. Various members of the VEGF family are located on a different chromosome. Located on chromosome 6p21.3, the human VEGF gene is 28kb in length and contains 8 exons and 7 introns. The gene of encoding VEGF is about 14

kb in length and is a disulfide-linked molecule with a relative molecular mass of 35,000-44,000 source dimer glycoprotein. The VEGF that is usually referred to is VEGF-A, which contains five subtypes (VEGF-Al21, VEGF-Al45, VEGF-Al65, VEGF-Al89, and VEGF-A206). The difference lies mainly in the different abilities of combination with heparin. VEGF-Al21 and VEGF-Al65 are soluble secretory proteins, which are more common *in vivo* [8, 9]. VEGF has 4 receptors: Fms-like tyrosine kinase-1 (Flt-1/VEGFR-1), kinase domain receptor (KDR/FLK-1/VEGFR-2), Fms-like tyrosine kinase-4 (Flt-4/VEGFR-3), and Neuropilin (NRP) [10]. Both VEGFR-1 and VEGFR-2 have extracellular immunoglobulin-like domains, single transmembrane regions, and intracellular tyrosine kinase-like structures. However, the former has a higher ability to bind VEGF than the latter. The function of various receptors is slightly different. VEGFR-1 contributes to the migration and formation of vascular endothelial cells. VEGFR-2 can increase microvascular permeability, endothelial cell division, and proliferation. The function of VEGFR-3 is mainly to cause proliferation of lymphatic endothelial cells. Chondrocytes express these three receptors only in OA, while normal chondrocytes do not [11]. VEGF is a potent inducer of vascular endothelial cells, which can induce proliferation of vascular endothelial cells, increase capillary permeability, and cause extravasation of fibrinogen, collagenase, and protease in blood vessels. They provide environmental conditions for the migration and angiogenesis of vascular endothelial cells [12]. Jansen et al. [13] experimentally induced OA in New Zealand rabbits, demonstrating that mRNA and protein of VEGF were detected in early osteoarthritic cartilage in the experimental group, whereas mRNA and protein of VEGF remained negative in the control group. It was suggested that VEGF participated in early OA changes and regulating VEGF production may be a new method for treating OA in the future. Lingaraj et al. [14] experimentally studied expression of VEGF in OA during growth, maturation, and degeneration of articular cartilage, finding that the temporal and spatial distribution of VEGF was expressed during the growth of articular cartilage, rested during maturation, and re-expressed in OA. Saetan et al. [15] showed that VEGF levels in plasma and synovial fluid were positively correlated with severity of knee OA. Therefore, VEGF may helpful in monitoring the severity of OA and may play an important role in the development and progression of knee OA.

The role of VEGF in angiogenesis

Normal articular cartilage does not contain blood vessels because angiogenic inhibitory factors (such as thrombospondin-1 (TSP-1), chondromodulin-1 (CHM-1), Troponin-1 (TN-1), and proteoglycans) and angiogenic growth factors (such as VEGF, basic fibroblast growth factor, and insulin-like growth factor-1) are in a state of dynamic equilibrium [16, 17]. TN-1 is a contractile protein found in human cartilage experiments that could contract skeletal muscle and myocardium, inhibiting the proliferation of capillary endothelial cells [18]. Kern et al. [19] found that TN-1 had an anti-angiogenic effect and could inhibit VEGF production and endothelial cell division. Its effect was related to its protein Glu94-Leu123 peptide. In normal cartilage, TSP-1 is present mainly in the middle and upper deep zone. In mild and moderate osteoarthritic cartilage, an increased number of TSP-1 expressing chondrocytes are seen. In severe osteoarthritic cartilage, a decrease in the number of TSP-1 synthesizing chondrocytes have been observed [20]. Jou et al. [21] intra-articularly injected adenoviral vector coding for TSP-1 into a rat model of collagen-induced arthritis, aiming to study the role of TSP-1 in OA. The decrease in the number of VEGF in the synovium was found to significantly improve the clinical course of arthritis, suggesting that TSP-1 could inhibit the formation of VEGF and alleviate the development of arthritis. Shukunami et al. [22] demonstrated that CHM-1 could antagonize the production of VEGF and inhibit endothelial cell proliferation. Hayami et al. [23] found that expression of CHM-1 in the superficial region of articular cartilage decreased in the early stages of OA. In advanced OA, expression of CHM-1 in all regions of articular cartilage was reduced and the number of VEGF-expressing cells was increased. High expression of CHM-1 was detected in articular cartilage of growing and normal adult joints, demonstrating its inhibitory effects on angiogenesis of articular cartilage. In OA cartilage, expression of CHM-I was decreased and expression of VEGF and other pro-angiogenic factors were relatively increased. Smith et al. [24] found that proteoglycans were strong inhibitors

of chondrogenesis and could prevent their binding to tyrosine kinase receptors by binding to angiogenic factors, such as VEGF, thereby blocking their proliferation in the extracellular matrix. Loss of proteoglycans in the extracellular matrix has been associated with blood vessels invading cartilage. Mechanical stress, inflammatory factors, and hypoxia induced chondrocytes to secrete VEGF and increased VEGF contributed to the formation of vascular arthritis [25]. Cartilage promoted expression of hypoxia-inducible factor (HIF)-2α in a hypoxic environment. With the development of OA, VEGF and HIF-2α were upregulated in cartilage and synovial cells. HIF-2α may upregulate expression of VEGF in cartilage and synovial cells, thus making OA worse [26].

The role of VEGF in cartilage degeneration

The structure of articular cartilage can be divided into four layers from top to bottom: fibrous layer, proliferative layer, hypertrophy layer, and calcified cartilage layer. After histological staining, a wavy line which was called the tidal line could be found between the proliferative and hypertrophy layer [27]. Cartilage is composed of chondrocytes and extracellular matrix, while the extracellular matrix contains mainly proteoglycans and collagen. The extracellular matrix has physical functions, such as support, compression resistance, protection, and water retention. It is an important medium for chondrocytes to conduct information transmission and obtain nutrition. The balance of the extracellular matrix to maintain dynamic metabolism could make the physiological function of chondrocytes normal. Cartilage degeneration is when extracellular matrix of cartilage degrades more than synthesizes, which is also an important pathological feature of OA. Chen et al. [28] studied the effects of VEGF on expression of proteoglycans and type II collagen *in vitro*, finding that VEGF may cause articular cartilage regression by inhibiting the synthesis and expression of proteoglycans and type II collagen. Matrix metalloproteinases (MMPs) and disintegrin-like metalloproteinases containing platelet-binding protein motifs (ADAMTs) are degradative enzymes of the extracellular matrix of cartilage. VEGF plays an important role in the early development of OA due to its ability to increase expression of MMPs and reduce expression of its inhibitors (tissue inhibitors of metalloproteinases (TIMPs)). Pufe et al. [29] also showed that VEGF induced cell proliferation, stimulated MMPs production, and inhibited TIMPs expression in human chondrocytes. Chondrocyte proliferation and matrix remodeling are two processes of cartilage growth and degeneration, respectively. VEGF increased the secretion of MMP-1, particularly MMP-13, which could be effectively reduced by an inhibitor of VEGFR-2 kinase activity. VEGF reduced expression of TIMP-1, particularly TIMP-2. Under hypoxic conditions, the reduction in TIMPs levels in cartilage was even greater. These findings suggest that VEGF plays an important role in the destructive process of OA [30].

The role of VEGF in subchondral bone sclerosis

Studies have shown that subchondral bone plays an important role in the pathology of OA [31]. In early OA, subchondral bone manifests as accelerating bone remodeling and bone loss. In late OA, it manifests as slowing and hardening of bone remodeling. The change in subchondral bone is an adaptive compensatory change in biochemical signaling and mechanical mechanics of OA. This altered signal transmission occurs primarily through cellular regulation [32]. The dynamic balance between osteoclasts and osteoblasts influences bone resorption and bone formation during bone remodeling. In early OA, the osteoclasts are more active, resulting in increased bone resorption and loss of bone mass. However, in late OA, the osteoblasts are more active and show bone hyperplasia and hardening. Having been shown to be a potent chemokine of osteoblasts and osteoclasts, VEGF regulates osteoblast and osteoclast activity, thus affecting bone resorption and bone formation [33]. Invasion of osteoclasts by VEGF-mediated osteoclasts could result in absorption of the calcified matrix, osteoblast deposition in the degraded cartilage matrix, and endochondral ossification. Derived from osteoblasts, the VEGF paracrine acts on osteoclasts, which could cause the osteoclasts to undergo chemotaxis and increase the number of local osteoclasts, thereby promoting local bone resorption [34]. In animal experiments, synovial hyperplasia, articular cartilage calcification, and subchondral sclerosis could be observed in experimental groups through intra-articular injections of VEGF into healthy mice. However, these chang-

es did not occur in control groups. Results of the study indicate that VEGF played a catalytic role in subchondral bone sclerosis [35]. Hayami et al. [36] experimentally studied rat OA models and found that changes in early bone resorption were enhanced. Bone trabecula became smaller and the number of osteoclasts increased. Expression of VEGF increased when osteoclasts were in hypoxia or hypoxia stimulated the formation of osteoclasts. In addition, VEGF expression was upregulated during osteoclast differentiation by induction of hypoxia-inducible factor-1α by NF-kappaB [37]. VEGF could also regulate osteoclasts by autocrine effects. It has been found that RANKL stimulated osteoclasts to release VEGF, which could act on osteoclasts and increase their activity [38]. VEGF signaling was also crucial for skeletal remodeling. VEGF was also expressed during osteoblast differentiation and its expression level was increased by IGF, a stimulatory agent for osteoblast differentiation [39]. The role of VEGF on mature osteoclasts mainly involves the receptor VEGFR2 (Flk1, KDR) and receptor signaling using the PI3-kinase→Akt and MEK→ERK pathways [40-42]. Kim et al. [43] indicated that Src, PKC, and p38MAPK were reduced by inhibiting receptors of VEGFR1 and VEGFR2. These, in turn, reduced monocyte-induced differentiation into osteoclasts, thereby slowing bone remodeling of subchondral bone by osteoclasts.

The role of VEGF in synovitis

Synovitis is a pathological feature of OA. Angiogenesis and synovial inflammation are two processes tightly combined in OA, affecting disease progression. Macrophages are often found in most places where abnormal blood vessels are formed, such as in synovitis and tumors. To explore the relationship between synovial tissue inflammation and angiogenesis, Haywood et al. [44] found that VEGF immunoreactivity was localized in macrophages in the synovial membrane and VEGF expression was linearly related to the severity of synovial inflammation. VEGF, produced by the synovial cells and macrophages of the inflamed synovial tissue, induced angiogenesis. The resulting blood vessels provided the synovial tissue with the desired substances, further exacerbating synovitis and development of OA. Macrophages could also secrete inflammatory cytokines to stimulate other cells, such as endothelial cells and fibroblasts, and stimulated cells also expressed VEGF [45]. Inflammatory cells, such as macrophages and neutrophils, and proinflammatory cytokines, such as TNF-α and IL-β, could promote the production of VEGF. VEGF further stimulated angiogenesis, which led to the accumulation of more inflammatory factors, thereby further aggravating the development of inflammation and forming a vicious circle. Neural innervation could also be accompanied by vascularization of articular cartilage. Pressure and hypoxia could stimulate these new nerves and cause pain even after inflammation subsides [46]. In late OA, invasion of blood vessels from the subchondral growth plate, synovitis accompanied with angiogenesis, and formation of new bone callus could be observed. In the synovial cells, high expression of VEGF could be detected [13]. Angiogenesis and inflammation are important processes in the pathophysiology of OA. Inflammation and angiogenesis can cause pain. This is because the process of angiogenesis is accompanied by the entry of sensory nerves into the cartilage and inflammation can make the nerves present in the joints sensitive. Angiogenesis, inflammation, and innervation are highly interrelated and each of these factors can increase other factors. Inhibiting inflammation and angiogenesis could improve pain symptoms and delay joint damage, providing an effective method for treatment of OA [46]. Kasama et al. [47] studied the effects of synovial fluid neutrophils on elevated VEGF levels in arthritis, finding that synovial neutrophils contained significantly larger amounts of both VEGF protein and its mRNA than peripheral blood neutrophils from either RA patients or healthy controls. Therefore, synovial neutrophil-associated VEGF may be an indicator of both local and systemic inflammation of synovitis, contributing to neovascularization during synovitis.

The role of VEGF in the formation of osteophytes

Osteophytes, also called bone hyperplasia, are caused by a series of mechanical and biochemical factors that cause cartilage destruction, chondrocyte hyperplasia, and cartilage ossification. OA is characterized by cartilage damage, synovial fibrosis, and formation of osteophytes [48]. Hashimoto et al. [49] experimentally

studied a rabbit OA model. They found that hypertrophic chondrocytes in osteophytes expressed VEGF and that this could promote vascular invasion of cartilage. Yamairi et al. [50] studied the expression of VEGF in different OA models, finding that VEGF was not detected in normal articular cartilage and OA model articular cartilage at 1 week post-surgery. At 2 weeks post-surgery, VEGF expression was found in perichondrium and regenerated and hypertrophic chondrocytes. At 5 weeks post-surgery, expression of VEGF was enhanced in local chondrocytes where osteophytes formed. These results suggest that VEGF was related to osteophytes. Ray et al. [51] found that the inflammatory response transcription factor (SAF-1) could upregulate VEGF synthesis to stimulate angiogenesis, resulting in new bone formation in osteophytes. Results suggest that VEGF plays a catalytic role in the development of osteophytes in OA progression. Jeon et al. [52] found that TGF-β1 could activate mouse macrophages to overexpress VEGF and be overexpressed by Smad3/4 and hypoxia-inducible factor-1α/β. High expression of VEGF could lead to the formation of osteophytes. During osteophyte formation, hypertrophic chondrocytes produce VEGF, which induces angiogenesis into the avascular cartilaginous matrix. VEGF then recruits osteoblasts into the cartilage to release bone matrix. Bevacizumab, an anti-vascular endothelial growth factor antibody and an angiogenesis inhibitor, was found to have no negative effect on normal joints in rabbit OA model studies. Bevacizumab increased expression of type II collagen in articular cartilage and reduced the formation of osteophyte, synovitis, and cartilage degeneration. Experimental results showed that bevacizumab, which inhibited VEGF, had a certain effect on the treatment of OA and reduced osteophyte formation [53].

Conclusion

Highly expressed in synovial fluid and blood in OA, VEGF is involved in the pathological processes of OA, such as cartilage degeneration, synovitis, subchondral bone sclerosis and osteophyte formation. It plays an important role in the development and evolution of OA. Therefore, targeting VEGF may be a breakthrough in the study of OA. However, the specific mechanisms of VEGF in the pathogenesis of OA are intricate and complicated. Therefore, further experimental studies are needed.

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Disclosure of conflict of interest

None.

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