

Angiogenesis in Osteoarthritis

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Abstract: Articular cartilage is essentially avascular and in recent years the role of blood vessel formation in osteoarthritis has been increasingly recognized. Therefore, healthy cartilage most likely actively prevents vessel in growth although the underlying mechanisms have not been uncovered to date. Further, the role of inflammation in the degradative processes in osteoarthritis is increasingly recognized. An inflammation dependent angiogenesis is clearly involved in the pathophysiology of osteoarthritis. Vascular endothelial growth factor (VEGF) has evolved as a dominant mediator of angiogenesis. In addition an angiopoietin (Ang)-dependent signalling system as well as processes like hypoxia contribute to a complex signalling network that stimulates ingrowth of blood vessels and degradative processes in the cartilage tissue itself. It can be expected that additional players related to angiogenesis in osteoarthritis and/or antiangiogenesis in healthy cartilage will emerge in the future such as the CCN-family proteins. The cysteine rich protein 61 (CYR61/CCN1) represents an angiogenic inducer whereas the WNT1 inducible signalling pathway protein 3 (WISP3/CCN6) appears to be an antiangiogenic factor. The inhibition of inflammation dependent angiogenesis or solely angiogenesis appears to be a promising strategy in osteoarthritis. However, studies targeting angiogenesis (e.g. VEGF) are missing to date.

Keywords: Angiogenesis, osteoarthritis, VEGF, hypoxia.

INTRODUCTION

In this mini-review evidence for the causal role of angiogenesis in osteoarthritis is briefly summarized. Osteoarthritis is a group of joint diseases that represent a major burden for patients as well as societies as a whole. The progressive degenerative damage of articular cartilage observed in osteoarthritis is based on a complex etiology that is still insufficiently clear. Biochemical alterations, genetic and environmental factors converge to the manifestation of osteoarthritis [1, 2].

Accumulating evidence indicates that beyond cartilage surrounding tissues such as the subchondral bone and the synovium are involved in the initiation and progression of osteoarthritis [2-4]. In the recent years it has become increasingly clear that mechanisms of inflammation and angiogenesis contribute to osteoarthritis and might play an important causal role in the disease progression [5-7].

ANGIOGENESIS IN OSTEOARTHRITIS - MACROSCOPIC ASPECTS

Articular cartilage is essentially avascular and, therefore, requires adjacent highly vascularized structures for metabolic support which is mediated by the subchondral bone and the synovium. This is important for maintaining structural integrity of the tissue and to permit high mechanical loading [5]. Interestingly, articular cartilage explants are resistant to vascular invasion *in vitro* [8, 9]. Thus, healthy cartilage exhibits the ability to prevent blood vessel ingrowth. Although the underlying mechanisms are not well known the expression of antiangiogenic mediators likely contributes to this phenomenon.

The concept of involvement of angiogenesis in osteoarthritis has been developed several years ago [10-12], but did not receive much attention, while the role of angiogenesis in tumor growth became widely accepted. However, in recent years the importance of angiogenesis in osteoarthritis has been clearly recognized [5-7, 13-15].

In osteoarthritis the invasion of blood vessels from the subchondral bone is apparent even in early stages of the disease and subsequently leads to the loss of tidemark integrity [5, 16]. This is accompanied by new bone formation at the osteochondral junction [17], a process that is believed to originate as a tissue response due to the altered biomechanical environment in the diseased joint. In addition, sensory nerve formation is observed in osteoarthritis explaining the development of pain, based on the fact that angiogenesis and innervation are regulated by similar mechanisms [18-20]. Due to the lack of blood vessels, articular cartilage is characterized by a low oxygen tension. Since hypoxia is an important positive regulator of angiogenesis, healthy cartilage is considered to maintain a mechanism of hypoxia resistance although the underlying molecular events are unclear at present. Nevertheless, in osteoarthritis oxygen tension appears to be even lower compared to healthy cartilage [15, 21] which likely contributes to the angiogenic processes.

Recently, the role of inflammation in the degradative processes in osteoarthritis is increasingly recognized [6]. Histologically, chronic synovitis is observed in patients undergoing joint replacements. Macrophage infiltration, endothelial proliferation and increased angiogenesis in the synovium apparently contribute to the disease progression [6, 22, 23]. Inflammation and angiogenesis are closely linked in a synergistic manner and, at least in part, are based on similar mechanisms [6, 24, 25]. In osteoarthritis, inflammation induced angiogenesis has recently evolved as an important contributor to disease progression in a more exacerbated manner than previously thought.

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ANGIOGENESIS IN OSTEOARTHRITIS - MOLECULAR ASPECTS

VEGF

Angiogenesis - the generation of new blood vessels by sprouting from pre-existing blood vessels - depends on a complex network of activators and inhibitors that are regulated in a timely and sequentially order to mediate blood vessel formation [6, 26]. Principally, this leads to nutrient delivery, maintenance of oxygen homeostasis, supports the removal of waste products, allows for tissue regeneration and provides biological mediators. In the healthy adult angiogenic processes mainly represent a repair system (e.g. fracture healing), thus the ingrowth of blood vessels into articular cartilage can be viewed as a failed repair process.

Vascular endothelial growth factor (VEGF) has evolved as a dominant mediator of angiogenesis [27, 28]. At least seven members comprise this family of proteins with VEGF-A as the prominent member that often (and also in this review) is referred to as VEGF. Several isoforms based on alternative splicing vary in binding capabilities to the extracellular matrix and VEGF receptors. VEGF binds to the tyrosin kinase receptors VEGF receptor 1 (VEGFR-1) and 2 (VEGFR-2) [29, 30]. These receptors are characterized by seven extracellular immunoglobulin-like domains including a ligand-binding region, a membrane spanning domain and an intracellular tyrosine kinase domain. Activation of the kinase due to ligand binding stimulates angiogenesis, mitogenesis and cell survival [6, 26, 31].

VEGF-dependent signalling in embryonic development is important for the regulation of growth plate morphogenesis and the coupling between cartilage and bone formation. In line with this mice lacking VEGF in chondrocytes develop impaired embryonic bone development, angiogenesis and impaired removal of hypertrophic chondrocytes [32].

In healthy adult cartilage, however, VEGF is not expressed and the tissue is essentially avascular. The VEGF signalling mechanism in the adult is important for tissue repair processes such as fracture healing, however, in articular cartilage the expression of VEGF and subsequent VEGF dependent signalling in osteoarthritis can be viewed as a mis-attempt of the body in repair processes within the joint. Several reports revealed the expression of VEGF and the corresponding receptors in osteoarthritis [33-35]. Pufe *et al.* reported the expression of VEGF and receptors in tissue derived from osteoarthritis patients to be dependent on mechanical loading [36]. This indicates that the ingrowths of blood vessels correlates to an altered mechanical response of cartilage in osteoarthritis compared to healthy cartilage that permits a high degree of mechanical loading.

Besides VEGF, an angiopoietin (Ang)-dependent signalling system is important for angiogenesis [37]. This signalling system is connected to VEGF-signalling *via* the receptor Tie2 since Findley *et al.* [38] demonstrated that VEGF induced the proteolytic cleavage and shedding of Tie2 *via* a novel PI3K/Akt-dependent pathway.

VEGF expression has been associated with the induction of catabolic mediators that are involved in matrix degradation. The upregulation of matrix metalloproteinase 1 and 3 in particular was observed in chondrocytes derived from os-

teoarthritis patients [35] and in immortalized chondrocytes treated with VEGF [39]. However, these findings were not observed by others [15] indicating that the role of VEGF on MMPs and corresponding inhibitors is not sufficiently clear at present.

Apoptosis of chondrocytes has been described to contribute to matrix degradation in osteoarthritis [40]. In line with this the expression of the apoptosis inhibitor Bcl-2 was reported to be higher in normal cartilage compared to osteoarthritic cartilage [41]. Based on gene expression profiling studies further processes including cellular senescence have been proposed to contribute to the loss of matrix in osteoarthritis [42].

Hypoxia

The pronounced hypoxic situation in articular cartilage in osteoarthritis results in enhanced expression of hypoxia-induced factor 1 α (HIF-1 α), a positive stimulator of VEGF and Angiopoietins [15, 43, 44]. HIF-1 α appears to be a master regulator of hypoxia-regulated genes due to the presence of hypoxia response elements in the promoter of HIF-1 α dependent genes [45, 46].

Inflammation

Pro-inflammatory cytokines and chemokines such as interleukin family members, tumor necrosis factor α (TNF- α) and interferon are produced at inflamed sites in osteoarthritis and partly act *via* nuclear factor kappa-B (NF- κ B) to regulate a network of factors including matrix metalloproteinases resulting in catabolic processes. Infiltrating cells produce angiogenic factors such as VEGF, basic fibroblast growth factor (bFGF), platelet derived growth factor (PDGF) and others [6]. Inflammation and angiogenesis appear to rely on cross talk between NF- κ B and Angiopoietin signalling [6] (and references therein). Thus abundant inflammatory cells in the osteoarthritic synovium release factors that directly and indirectly stimulate VEGF expression. A complex network of VEGF-dependent and independent factors stimulates the ingrowth of blood vessels and degradative processes in the cartilage tissue itself.

Antiangiogenesis

Molecular pathways of antiangiogenesis, so far, mainly received attention in relation to cancer [47]. The antiangiogenic mechanisms responsible to maintain avascularity of healthy articular cartilage are not understood. It has been shown that explants of healthy human articular cartilage in the chorioallantoic membrane assay (CAM-assay) behave in an antiangiogenic manner compared to explants from osteoarthritic cartilage [9]. Conversely to the stimulation of angiogenesis in osteoarthritis, the loss of avascularity of healthy cartilage could be initiated by the loss of expression of antiangiogenic factors. It has been suggested that the expression of antiangiogenic factors such as troponin 1 mediates the resistance of cartilage to blood vessel ingrowth [48]. In a similar manner chondromodulin 1 appears to be an antiangiogenic player expressed in cartilage [49]. Thrombospondin 1 (TSP-1) could be of interest since it has been shown that TSP-1 inhibits VEGF-induced migration in human microvascular cells [50]. Antiangiogenic signalling mechanisms associated with factors such as endostatin, tumstatin, angiostatin and others have been elucidated [47] and

appear to be important in processes such as migration, proliferation/cell cycle arrest and survival/apoptosis. Future research is needed to elucidate related mechanisms of action of antiangiogenic factors in healthy cartilage and associated alterations in antiangiogenesis-dependent signalling in articular cartilage in osteoarthritis.

NOVEL CANDIDATE FACTORS IN ANGIOGENIC/ANTIANGIOGENIC PROCESSES IN OSTEOARTHRITIS

Despite the gain of knowledge on the role of angiogenesis in osteoarthritis and the increased molecular understanding of angiogenic signalling (e.g. VEGF mechanisms of action) in general, the elucidation of angiogenesis-related molecular events in the initiation and progression of osteoarthritis still is insufficiently clear. It can be expected that additional players related to angiogenesis in osteoarthritis and/or antiangiogenesis in healthy cartilage will emerge in the future. For example CCN-family proteins might evolve as important regulators in osteoarthritic cartilage. These matricellular proteins act in cell and tissue specific processes including angiogenesis in a non-redundant manner [51-53]. The Cysteine-rich protein 61 (CYR61/CCN1) is well known as an angiogenic inducer [50-53]. The wnt induced signalling protein 3 (WISP3/CCN6) appears to be able to stimulate chondrogenic markers (collagen type II and aggrecan) *via* the SOX9 pathway indicating a potential role of WISP3 for cartilage differentiation [54] and might play a cartilage protective role based on the observation of the development of pseudorheumatoid dysplasia in patients with WISP3 loss of function mutations [55]. The overexpression in WISP3 negative cells displayed a marked reduction in angiogenic and invasion characteristics in an *in vitro* matrigel assay [56], suggesting that WISP3 could play an antiangiogenic role.

SUMMARY

In osteoarthritis, angiogenesis and inflammation-induced angiogenesis play a causal role based on inflammatory signals, hypoxia as well as mechanical stress due to increased VEGF expression and catabolic mediators. The functional loss of disease-protective resistance mechanisms of healthy cartilage, which are incompletely understood to date, contributes to the subsequent degenerative loss of articular cartilage with the final necessity of joint replacement procedures.

THERAPEUTICAL CONSIDERATIONS

Treatment strategies aim to delay the need for joint replacement. Current developments, however, may be of limited success since these strategies addressing the tissue cartilage may aim at the wrong target. The inhibition of inflammation dependent angiogenesis or solely angiogenesis appears to be a promising strategy based on accumulating findings that angiogenesis plays a causal role in the progression of osteoarthritis. However, studies targeting angiogenesis (e.g. VEGF) in osteoarthritis are missing to date. Furthermore, research is needed to gain a better understanding of the "disease protective" mechanisms inherent to cartilage such as the hypoxia resistance and the pathways responsible for maintaining the avascular condition. A challenge for the future is to develop novel therapeutical procedures based on these considerations.

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