Osteoarthritis and Cartilage (2008) **16,** 279–286

doi:10.1016/j.joca.2007.09.003

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

The potential role of vascular endothelial growth factor (VEGF) in cartilage How the angiogenic factor could be involved in the pathogenesis of osteoarthritis?

M. Murata M.D., Ph.D., K. Yudoh M.D., Ph.D. and K. Masuko M.D., Ph.D.*

Department of Bioregulation and Proteomics, Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki, Japan

Summary

Although adult human cartilage is physiologically avascular tissue, angiogenesis can be observed during the process of endochondral bone development. Inflammation in articular joints can also lead to neovascularization in cartilage. In such conditions, the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), has been shown to play a key role, controlling not only angiogenesis but also chondrocyte metabolism. Here we review recent research findings concerning the potential role of VEGF in cartilage, focusing in particular on its possible involvement in the pathogenesis of osteoarthritis.

Key words: Vascular endothelial growth factor, Chondrocyte, Cartilage, Hypoxia, Osteoarthritis.

Introduction

Being composed of a network of extracellular matrices and scattered chondrocytes, mature articular chondrocytes in physiological condition are essentially devoid of vascular structure. Specifically, the proliferative and hypertrophic zones in the growth plate are not penetrated by vascular supply (Fig. 1)[1], leaving chondrocytes in these zones in an avascular milieu. The mechanisms by which mature articular cartilage might be maintained as avascular have not been fully clarified. However, as cartilage is suggested to be an enriched source of endogenous inhibitors of angiogenesis[2], an array of anti-angiogenic factors has been identified. For example, Moses *et al.* identified Troponin I (TnI) in cartilage, which is known as a contractile protein expressed in muscle[2]. In this study, TnI was shown to inhibit proliferation of capillary endothelial cells and embryonic angiogenesis *in vivo*, thus indicating the anti-angiogenic potential of TnI. In addition, a 25-kD glycoprotein, chondromodulin-I (ChM-I) was reported to play an important role in the resistance of cartilage to angiogenesis (reviewed in Ref. 3). As for ChM-I, Shukunami *et al.* demonstrated that the level of ChM transcript was maintained at a high level in the avascular zones of bone rudiments, including the resting, proliferating and early hypertrophic zones. In contrast, the ChM expression was abolished in the lower hypertrophic and calcified zones of cartilage, allowing vascular invasion to result in the initiation of endochondral bone formation[3].

*Address correspondence and reprint requests to: Dr Kayo Masuko, M.D., Ph.D., Department of Biochemistry, St. Marianna University School of Medicine, Kawasaki 216-8511, Japan. Tel: 81-44-977-8111; Fax: 81-44-976-7553; E-mail: kmarianna@mac.com

Received 13 April 2007; revision accepted 1 September 2007.

Despite this anti-angiogenic machinery occurring in cartilage, recent studies have revealed the important contribution made by a variety of pro-angiogenic factors in the cartilage metabolism, including vascular endothelial growth factor (VEGF). Here we review some recent reports on the representative angiogenesis-regulating factor (VEGF), focusing on its possible implication in the pathogenesis of osteoarthritis (OA).

VEGF

VEGF is known to be an important mediator of angiogenesis. The VEGF family comprises at least seven members: VEGF/VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PlGF)[4–6]. VEGF-A is often referred to as simply VEGF[7], is encoded by the *Vegf/Vegf-A* gene, which generates at least five isoforms of VEGF through alternative splicing. $VEGF_{189}$ and $VEGF_{206}$, which differ in their binding to the extracellular matrix and to the receptors[4,8]. In addition, recent studies have suggested that the selection of an alternate splice site may result in the generation of a sister family of isoforms, i.e., anti-angiogenic VEGF-A isoforms ($VEGF_{***}b$; e.g., $VEGF_{165}b$), in addition to pro-angiogenic isoforms ($VEGF_{***}$)[7].

Within the VEGF family, VEGF-A is the founding member and is thought to be of singular importance[4]. VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and PlGF share amino acid sequence homology with VEGF/VEGF-A and may have been classified as "VEGF-related proteins"[9]. Since most previous studies on VEGF in regard to its expression in chondrocytes have focused on VEGF/VEGF-A, and little is known about the involvement of other VEGF families, we hereafter focus on VEGF-A as the class of VEGF referred to in this review article.

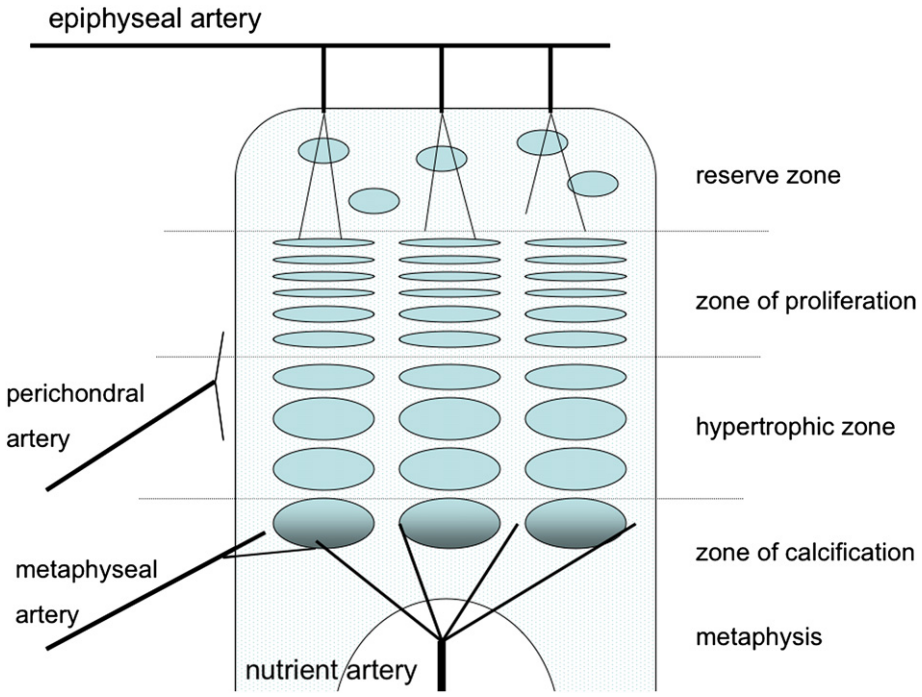

Fig. 1. Vascular supply in the growth plate (based on Ref. 1).

VEGF binds to surface tyrosine kinase receptors VEGFR-1 (or fms-like tyrosine kinase-1 (Flt-1) from mouse) and VEGFR-2 [or kinase domain region (KDR)/fetal liver kinase-1 (Flk-1)] which are strongly expressed by endothelial cells[10]. VEGFR-1 and VEGFR-2 are composed of seven extracellular immunoglobulin (Ig)-like domains containing the ligand-binding region, a short membrane-spanning sequence, and a conserved intracellular tyrosine kinase domain interrupted by a kinase insert sequence[5,9,10]. VEGFR-1 has the highest affinity for VEGF-A, and also binds PIGF and VEGF-B, whereas VEGFR-2 would seem to bind VEGF-C, VEGF-D, VEGF-E and VEGF-F, as well as VEGF-A[5,9]. VEGFR-1 and VEGFR-2 are structurally similar and share 43.2% sequence homology; more specifically, the extracellular domain displays 33.3% homology and the cytoplasmic region shares 54.6% between the two receptors[10,11]. Neuropilin-1, a receptor for semaphorins found in the nerve system, has also been found to bind VEGF[4,5,12]. VEGF-C and VEGF-D are known to bind to VEGFR-3, but binding of VEGF/VEGF-A to VEGFR-3 has not been found[5,9].

Evidence has revealed that the binding of VEGF to VEGFR leads to VEGF-induced angiogenesis, mitogenesis and cell survival (reviewed in Refs. 8,10). Under physiological conditions, a healthy adult organism does not exhibit any active angiogenic process, with the exception of a few cases, including that of the female reproductive system, in which hormonal regulation would appear to play a role also in the expression of VEGF and VEGFR[13,14]. VEGF expression is also detectable in certain adult organs and cell types, such as lung, kidney, liver and brain[10].

Expression of VEGF and subsequent angiogenesis is, on the other hand, an important process during tissue repair such as that of a fracture. For example, Street *et al.*[15] reported that the inhibition of VEGF using a soluble, neutralizing VEGF receptor impaired fracture repair in mice, while treatment with exogeneous VEGF promoted fracture repair as well as angiogenesis. The authors suggested that osteoblast played a role in the VEGF-mediated bone repairing mechanism[15].

Endochondral bone development and VEGF

Endochondral bone development is a complex process in which ordered phases of proliferation, maturation and apoptosis of chondrocytes occur in growth plate chondrocytes (reviewed in Ref. 16). Specifically, in immature skeleton, the growth plate is composed of three histologically distinct zones: reserve, proliferative, and hypertrophic (Fig. 1). Chondrocytes in the proliferative zone synthesize type II collagen; and after termination of proliferation, cells differentiate into hypertrophic chondrocytes that express type X collagen and produce alkaline phosphatase. The hypertrophic chondrocytes then mineralize the matrix, replacing the cartilaginous matrix with trabecular bone.

During this process, the growth plate allows distinct vascular supplies. More specifically, the epiphyseal artery enters the secondary center of ossification and its terminal branches terminate at the uppermost part of the proliferative zone. These vessels do not penetrate into the proliferative or hypertrophic zone. On the other hand, the metaphyseal artery enters at the mid-diaphysis and sends branches to each metaphysis without penetrating into the hypertrophic zone of the growth plate.

In regard to cartilage vascularization, Gerber *et al.*[17] clarified the essential role of VEGF in endochondral bone development by inactivating VEGF in systemic administration of a soluble receptor chimeric protein to mice. The results of the inhibition of VEGF activity showed impaired bone formation and the expansion of hypertrophic chondrocytes, along with an almost complete suppression of blood vessel invasion. The authors concluded that VEGF-mediated

vascularization is an essential signal to regulate growth plate morphogenesis through the induction of coupling resorption of cartilage with bone formation.

The important role of VEGF in cartilage vascularization and in bone development has been supported by other papers[18–21]. For example, Zelzer *et al.* generated mice lacking the VEGF gene (*Vegf-A*) in chondrocytes using a chondrocyte-specific promoter Col2a1, along with the Cre-loxP system[20]. The *Vegf-A* knockout mice showed impaired embryonic bone development, reduced angiogenesis, and reduced removal of terminally differentiated hypertrophic chondrocytes. There were also areas of massive cell death in the center of the bones of the *Vegf-A* knockout mice, suggesting that VEGF plays an essential role in chondrocyte survival during endochondral bone formation. Evidence of VEGF expression has also been found in human neonatal cartilage[22,23], indicating its potential implication in bone development in humans as well (Fig. 2).

Factors that induce VEGF expression

HYPOXIA AND HYPOXIA-INDUCIBLE FACTOR (HIF)-1

In the growth plate, oxygen tension in the reserve, proliferative and hypertrophic zones has been reported to be 20.5 ± 2.1, 57 ± 5.8 and 29.3 ± 2.4 mmHg, respectively[1]. Low oxygen in the reserve and hypertrophic zones is attributable to the lack of supply through vascularity.

It has been established that the hypoxia is a prime stimulus for angiogenesis[24]. More specifically, reduced oxygen tension induces expression of VEGF through induction of a transcription factor, HIF-1α. Specifically, HIF-1α leads to transcriptional activation of the VEGF gene in hypoxic cells by binding to a hypoxia response element (HRE) located 1 kb 5′ to the transcription initiation site[25,26]. A strong induction of VEGF by HIF-1α has been revealed in a wide range of tissues (Fig. 3)[5].

In this regard, accumulated data have revealed that HIF-1α is a master regulator of the expression of various hypoxia-induced genes. The target genes of HIF-1 are related not only to angiogenesis, but also to cell proliferation/survival, and glucose/iron metabolism[27]. Moreover, activation of HIF-1α has been closely associated with a variety of different tumors and oncogenic pathways[27].

With regard to the implication of hypoxia in arthritic conditions, hypoxic stress is thought to be one of the inflammation-modulating factors in articular joints. Specifically, a major survey in 1970 by Lund-Olesen reported that the mean oxygen tension (PO_2) in synovial fibroblasts (SF) of rheumatoid arthritis (RA) patients (27 mmHg) was significantly lower than in cases of acute traumatic effusion (63 mmHg). Knee SF from OA patients was also more hypoxic (mean 43 mmHg)[28]. These findings suggested that articular chondrocytes in inflammatory joints were supplied with lower levels of oxygen than under healthy conditions.

Consistent with the hypoxic condition of joints, it has been reported that expression of HIF-1α is upregulated in synovial cells of RA and in OA (Refs. 29,30, reviewed in Refs. 31,32). Giatromanolaki *et al.* showed that the high HIF-1α expression in OA synovial cells was associated with increased expression of ‘‘activated microvessel density (MVD)’’ assessed with positive staining of antibody 11B5, which stained VEGF bound to its receptor KDR[30]. They suggested that within the degenerative context of OA,

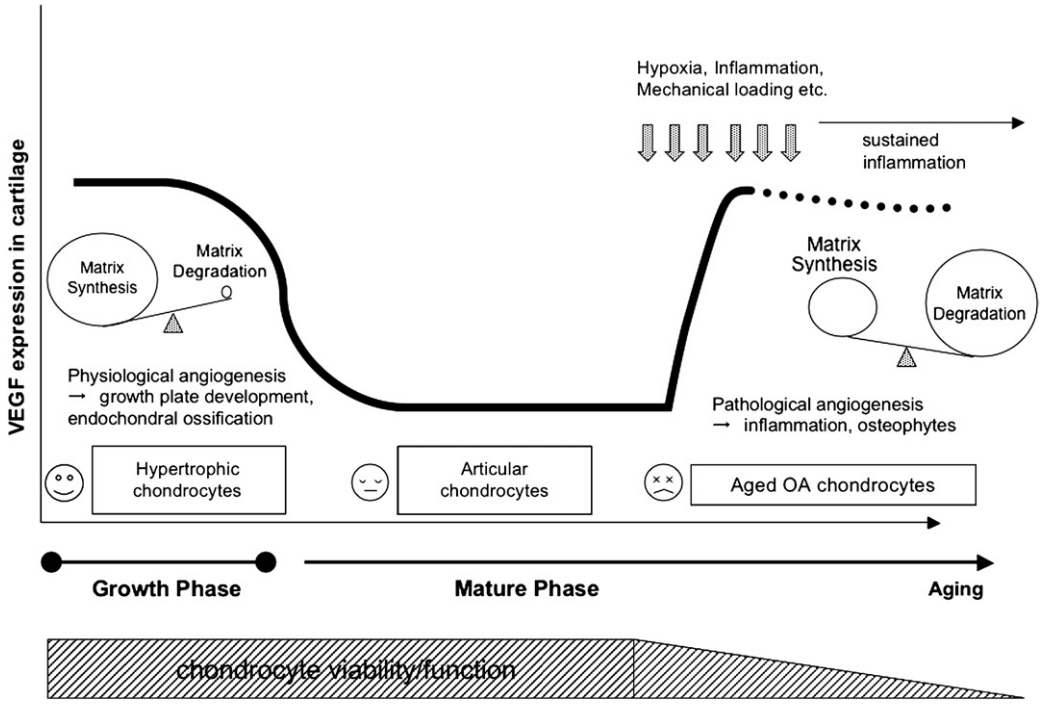

Fig. 2. Hypothetical kinetics of VEGF expression in human articular chondrocytes. During growth plate development (‘‘the growth phase’’), VEGF is expressed in chondrocytes playing a pivotal role in cartilage growth and endochondral ossification. In adult cartilage (‘‘the mature phase’’), expression of angiogenic factors is suppressed and blood vessel invasion is also almost completely suppressed, leaving mature cartilage avascular. Upon exposure to pathological stimulation such as inflammation or accumulating mechanical stress, VEGF would be re-upregulated in hypertrophic chondrocytes. Although the fate of VEGF expression in stressed chondrocytes has always been unclear, higher levels of VEGF expression in OA than in normal chondrocytes have been reported. Arbitrary levels of VEGF expression in chondrocytes are shown in the drawing.

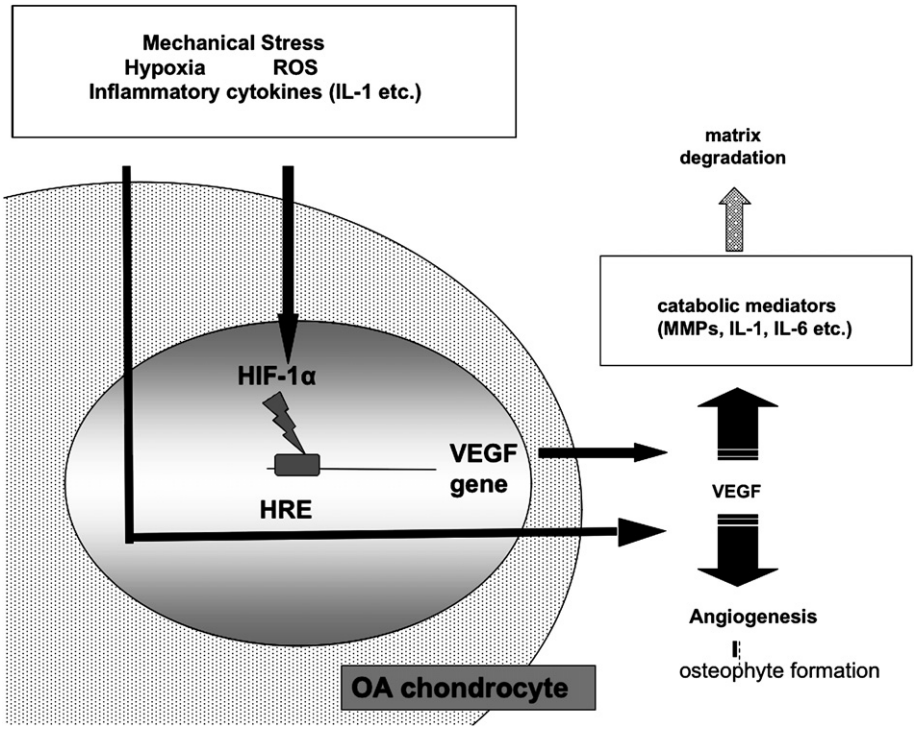

Fig. 3. The potential role of VEGF in OA (hypothesis). VEGF could be induced by an array of catabolic triggers in chondrocytes, and the expressed VEGF might affect these chondrocytes, e.g., *via* angiogenesis leading to osteophyte formation, *via* induction of matrix proteases, or *via* regulation of cellular viability through Bcl-2 activation.

impaired vascular homeostasis may result in focal, expanding hypoxic regions in the synovium, and that upregulation of HIF-1α together with resultant VEGF overexpression would lead to the genesis of a defective vascular network with poor survival ability, which would be consistent with the degenerative nature of OA. On the other hand, the same study demonstrated that in RA VEGF/KDR activation, MVD and expression of platelet-derived endothelial cell growth factor (PD-ECGF) did not depend on HIF-1α expression. The authors hypothesized that angiogenesis may not depend on the HIF reactivity and that hypoxia may not be the only up-regulating factor of the HIF/VEGF pathway in RA. Rather, inflammatory factors, such as interleukin-1 (IL-1) of tumor necrosis factor α (TNFα), may directly stimulate the VEGF or PD-ECGF overexpression in RA. It is therefore suggested that angiogenesis follows different pathogenic pathways in OA and RA[30].

As for the role of HIF-1α in cartilage, Schipani *et al.*[33] reported in 2001 that HIF-1α is essential for chondrocyte growth survival. In that paper, the authors developed mice lacking HIF-1α in growth plate chondrocytes, and showed that the lack of HIF-1α did not severely alter the differentiation process of chondrocytes *per se*. There was, however, a massive cell death in mutant growth plate chondrocytes indicating a requisite of HIF-1α for the survival of hypoxic chondrocytes[33,34]. Additional reports, including our own[35] and those of others[36], demonstrated the role of HIF-1α in cartilage matrix production. Specifically, we observed that HIF-1α-deficient chondrocytes did not maintain energy generation or cartilage matrix production under either normoxic or hypoxic conditions[35]. Furthermore, HIF-1α-deficient chondrocytes showed an acceleration of catabolic stress-induced apoptosis *in vitro*[35]. Taken together, low oxygen tension, or hypoxia, in inflamed synovial joints appears to evoke activation of HIF-1α, followed by expression of a plethora of downstream molecules including VEGF, that collectively alters the chondrocyte metabolism, possibly toward a catabolic response, hence modulating the pathophysiology of arthropathies.

MECHANICAL STRESS

It has been recognized that excessive mechanical stress, such as compression or shear, would cause deterioration of the cartilage metabolism through induction of catabolic factors, including matrix metalloproteinases (MMPs)[37–41]. In this regard, involvement of VEGF in the mechanical stress-induced cartilage degradation has been the subject of investigation. Specifically, Pufe *et al.* reported the effect of mechanical overload on VEGF expression in bovine cartilage disks[42]. The team demonstrated VEGF expression in overloaded disks but not in control disks. The overload-induced VEGF expression was accompanied by HIF-1α expression and also by co-expression of MMPs, suggesting that VEGF played a role in the destruction of stressed cartilage. In fact, the authors also reported expression of VEGF in the superficial zone of the tibial plateau in OA patients with degenerative changes, but not in healthy cartilage[43]. Although it is possible that VEGF exists in the deeper cartilage zones at below detectable levels, the predominance of VEGF in the superficial zone would appear to support enhanced expression after mechanical loading.

Of note, ''mechanical stress'' is usually composed of a complex mixture of tension, shear, compression and strain; and since each component might differentially regulate gene expression, the response *in vivo* would not be as simple as was observed in the *in vitro* experiments. Wong *et al.*[44], employing chondrocytes cultured in alginate beads,

assessed VEGF expression and found that the principal effect of hydrostatic pressure (HP) was to downregulate MMP-13 and type I collagen, while up-regulating tissue inhibitor of metalloproteinases (TIMP)-1. In their paper, the authors showed that VEGF was significantly upregulated by both cyclic tension and HP[44]. In any case, as Loeser suggested, abnormal mechanical stress might "awaken" the adult chondrocytes from low metabolic activity and stimulate each cell to produce inflammatory mediators, including VEGF, which increases the catabolic activity of chondrocytes[41].

MISCELLANEOUS

There are variety of other factors that are potent enough to induce VEGF (reviewed in Ref. 10); for example, IL-1, IL-17, TNFα, nitric oxide (NO) and reactive oxygen species (ROS) have all been reported to induce VEGF expression in cultured chondrocytes[8,45–47]. For example, in a study by Honorati *et al.* it was suggested that IL-1β was more potent in inducing VEGF in cultured OA chondrocytes than IL-17 or TNFα[46]. These mediators may utilize different signaling pathways to elicit VEGF expression, since we found that distinct signaling pathways are involved in hypoxia- and IL-1-induced VEGF expression in articular chondrocytes[47]. Kanata *et al.* recently demonstrated that a binding of oxidized low-density lipoprotein (ox-LDL) to lectin-like ox-LDL receptor-1 (LOX-1) upregulates VEGF expression in chondrocytes, suggesting that peroxisome proliferators-activated receptor (PPAR)-γ activation plays a role in this process[48].

It has been reported that ROS enhanced VEGF expression, including that in chondrocytes[49]. In this regard, a paper by Tomiyama *et al.* demonstrated that mechanical compression of articular cartilage induced ROS synthesis, whereas it inhibited proteoglycan synthesis[50]. Furthermore, Green *et al.* observed that impact injury led to chondrocyte death, even at a distant site from the injury, through the generation of NO[51]. Considering these findings together, it might be that mechanical stress induces ROS as a first response, and that factors such as VEGF are mobilized as a next step, altogether affecting chondrocyte viability and the cartilage metabolism[41].

Besides this, in experimental systems, sodium arsenite is also used to induce VEGF expression[52,53]. The arsenite-induced VEGF expression was reported to be mediated not by HIF-1, but rather by p38 Mitogen activated protein kinase (MAPK)[52].

Outcome of VEGF expression in cartilage—implications in OA?

Although the VEGF/VEGFR expression is critical in neonatal endochondral bone development, it is generally not found in adult human articular cartilage under physiological conditions[18,54]. Nevertheless, recent investigations have revealed expression of VEGF and its receptors in diseased cartilage, such as that of RA and OA (Fig. 2). For example, chondrocytes and cartilage tissue obtained from OA patients were reported to express VEGF and VEGF receptors to a higher degree than that found in healthy individuals[55–58]. Expression of VEGF was also found in OA synovium accompanied by angiogenesis and inflammation[59]. Nevertheless, the precise mechanism by which VEGF might be involved in the pathogenesis of OA is not clearly understood.

OSTEOPHYTES AND VEGF

Osteophytes are bony and cartilaginous structures in OA, which arise from the bony margins of the osseous components of the joint[60]. The formation of osteophytes has been interpreted as an adaptation of the joint to the altered biomechanics of OA joints[61]. In this connection, Hashimoto *et al.* reported that hypertrophic chondrocytes in osteophytes expressed VEGF after immunohistochemistry was used on experimental OA animals[61]. They suggested that VEGF plays a role in angiogenesis during osteophyte development; however, the precise mechanism by which VEGF might regulate osteophytes during OA development is still unclear.

INDUCTION OF MATRIX-DEGRADING CATABOLIC FACTORS BY VEGF

Several reports have hypothesized that VEGF might induce expression and production of proteinases that degrade extracellular matrix *in vitro* (Table I and reviewed in Ref. 62). For example, in a study by Enomoto *et al.* the authors incubated OA or normal chondrocytes with recombinant $VEGF_{165}$ protein (at 0, 10, or 50 ng/ml), and measured the levels of MMPs and TIMPs in the culture supernatants. The results showed that the recombinant $VEGF_{165}$ increased the production levels of MMP-1 and MMP-3 in OA chondrocytes, but not in normal chondrocytes. On the other hand, Pufe *et al.*[63] cultured immortalized human chondrocytic C28/I2 cells with recombinant $VEGF_{165}$ (10 ng/ml), and also showed a slight but significant increase in MMP levels. However, the increases in MMP-1 and MMP-3 shown in these experiments by VEGF were approx. 120–140% compared to samples without VEGF treatment (as 100%) even after 3 days' culture[57], and our experiments using OA, RA and normal samples did not show any significant increase in these MMPs, suggesting varied responsiveness to VEGF among patients and cells (Ref. 47, data not shown).

On the other hand, VEGF may have a synergistic role in regulating gene expression of catabolic mediators. Komiya *et al.*[64] using RA SF showed that VEGFR-2 expressing RA SF expressed higher levels of metalloproteinase ADAM (a disintegrin and metalloproteinase)-15 when treated sequentially with TNFα and $VEGF_{165}$, but not when stimulated by TNFα or $VEGF_{165}$ alone.

REGULATION OF CELL GROWTH AND APOPTOSIS BY VEGF

The important role played by VEGF in chondrocyte development and survival during bone development is borne out

Table I
Summary of the potential bioactivities of VEGF on cartilage. ↑: upregulate and ↓: downregulate

	MMP-1	MMP-3	TMP-1/-2	Others	Source of chondrocytes
Enomoto *et al.*[57]	↑	↑	→	MMP-2,-9,-13: →	OA chondrocytes
Pufe *et al.*[42]	↑	↑	↓	IL-1, Il-6: ↑; MMP-13, TNFα, NO_2: ↑	Human chondrocyte cell line C28/12
Pufe *et al.*[63]	↑	↑	↓	MMP-13: ↑	Bovine cartilage disks

by accumulating evidence[20,21]. It has also been suggested that apoptotic chondrocyte death plays a role in matrix breakdown in arthritis, including RA and OA, although the debate about the "apoptosis" of chondrocytes in OA has caused considerable controversy[65]. In this regard, Aigner *et al.*[66] have suggested in a recent comprehensive review that in the majority of cases OA chondrocytes might not undergo classical (apoptotic) cell death and remain viable, but instead degenerate as dysfunctional remnants. Furthermore, the precise mechanisms by which HIF-1α/VEGF-mediated signaling might be involved in apoptotic cell death in human OA have not been fully clarified.

It has been reported that VEGF promotes cell survival by inducing the expression of anti-apoptotic molecules such as Bcl-2[5,67,68]. In this context, some reports have suggested that Bcl-2 plays a role in regulation of chondrocyte apoptosis in OA[69–72], and further in regulation of matrix gene expression through Sox9[73,74]. For example, Kim *et al.* reported that Bcl-2 expression in normal cartilage was significantly higher than in OA cartilage, suggesting the involvement of Bcl-2 in OA pathogenesis[69]. Furthermore, a recent study by Surendran *et al.*[72] showed that *Bcl-2*-transfected chondrocytes were protected from NO-induced impairment of proteoglycan synthesis, implying the possible value of *Bcl-2* gene therapy in arthritis[72]. However, there has been no direct data to show the cell proliferating effect, or the upregulation of Bcl-2 expression by VEGF in human OA chondrocytes, and this issue awaits further investigation.

VEGF IN OA: A POTENTIAL THERAPEUTIC TARGET?

As described, there is evidence of higher levels of VEGF expression in OA chondrocytes than in nonarthritic chondrocytes. Thus VEGF might, at least in part, be participating in the pathophysiology of cartilage degeneration in OA. Targeting VEGF in OA could be beneficial for the suppression of osteophyte formation and/or downregulation of MMP production. The observations that VEGF might upregulate the expression of anti-apoptotic Bcl-2, and that apoptotic cell death has been suggested in OA (causing some controversy), seem to be inconsistent. However, as recent studies have suggested the occurrence of "senescence" or premature chondrocytes during aging as an important trigger of OA pathogenesis[66,75], cell death or degeneration of chondrocytes might already be in process when VEGF is re-upregulated by the stress trigger (Fig. 2). In these circumstances, suppression of VEGF could be a target of OA therapy, not as an anti-apoptotic but rather an "anti-inflammatory" strategy, since it has been suggested that OA is an inflammatory disease[76,77]. The potential effect (either direct or HIF-1-mediated) of VEGF on the metabolism of extracellular matrix components, such as collagens and proteoglycan, or in the cellular senescence of mature articular chondrocytes, should be further investigated.

Concluding remarks: not only angiogenesis, but more

Accumulating data are unveiling the importance of VEGF in physiological angiogenesis, as well as in growth plate development and endochondral ossification. However, it may still not be fully understood when and how the angiogenic factor is activated or functions in articular cartilage during aging, or in pathological situations such as arthritides. Targeting angiogenic factors, including VEGF, might open up a novel avenue toward establishing therapeutic strategies against cartilage degradation in OA.

References

1. Iannotti J, Goldstein S, Kuhn J, Lipiello L, Kaplan F, Zaleske D. The formation and growth of skeletal tissues. In: Orthopedic Basic Science. American Academy of Orthopaedic Surgeons 2000:77–109.
2. Moses MA, Wiederschain D, Wu I, Fernandez CA, Ghazizadeh V, Lane WS, *et al.* Troponin I is present in human cartilage and inhibits angiogenesis. Proc Natl Acad Sci USA 1999;96(6):2645–50.
3. Shukunami C, Oshima Y, Hiraki Y. Chondromodulin-I and tenomodulin: a new class of tissue-specific angiogenesis inhibitors found in hypovascular connective tissues. Biochem Biophys Res Commun 2005; 333(2):299–307.
4. Dvorak HF. Angiogenesis: update 2005. J Thromb Haemost 2005;3(8): 1835–42.
5. Roy H, Bhardwaj S, Yla-Herttuala S. Biology of vascular endothelial growth factors. FEBS Lett 2006;580(12):2879–87.
6. Bluteau G, Julien M, Magne D, Mallein-Gerin F, Weiss P, Daculsi G, *et al.* VEGF and VEGF receptors are differentially expressed in chondrocytes. Bone 2007;40(3):568–76.
7. Ladomery MR, Harper SJ, Bates DO. Alternative splicing in angiogenesis: the vascular endothelial growth factor paradigm. Cancer Lett 2007;249(2):133–42.
8. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. Faseb J 1999;13(1):9–22.
9. Robinson CJ, Stringer SE. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. J Cell Sci 2001;114(Pt 5): 853–65.
10. Thomas KA. Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem 1996;271(2):603–6.
11. Rahimi N. VEGFR-1 and VEGFR-2: two non-identical twins with a unique physiognomy. Front Biosci 2006;11818–29.
12. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. Cell 1998;92(6):735–45.
13. Shweiki D, Itin A, Neufeld G, Gitay-Goren H, Keshet E. Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. J Clin Invest 1993;91(5):2235–43.
14. Germeyer A, Hamilton AE, Laughlin LS, Lasley BL, Brenner RM, Giudice LC, *et al.* Cellular expression and hormonal regulation of neuropilin-1 and -2 messenger ribonucleic acid in the human and rhesus macaque endometrium. J Clin Endocrinol Metab 2005;90(3): 1783–90.
15. Street J, Bao M, deGuzman L, Bunting S, Peale FV Jr, Ferrara N, *et al.* Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci USA 2002; 99(15):9656–61.
16. Provot S, Schipani E. Molecular mechanisms of endochondral bone development. Biochem Biophys Res Commun 2005;328658–65.
17. Gerber H, Vu T, Ryan A, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. Nat Med 1999;5(6):623–8.
18. Carlevaro M, Cermelli S, Cancedda R, Cancedda F. Vascular endothelial growth factor (VEGF) in cartilage neovascularization and chondrocyte differentiation: auto-paracrine role during endochondral bone formation. J Cell Sci 2000;11359–60.
19. Maes C, Carmeliet P, Moermans K, Stockmans I, Smets N, Collen D, *et al.* Impaired angiogenesis and endochondral bone formation in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. Mech Dev 2002;111(1–2):61–73.
20. Zelzer E, Mamluk R, Ferrara N, Johnson RS, Schipani E, Olsen BR. VEGFA is necessary for chondrocyte survival during bone development. Development 2004;131(9):2161–71.
21. Maes C, Stockmans I, Moermans K, Van Looveren R, Smets N, Carmeliet P, *et al.* Soluble VEGF isoforms are essential for establishing epiphyseal vascularization and regulating chondrocyte development and survival. J Clin Invest 2004;113(2):188–99.
22. Horner A, Bishop N, Bord S, Beeton C, Kalsall A, Coleman N, *et al.* Immunolocalization of vascular endothelial growth factor (VEGF) in human neonatal growth plate cartilage. J Anat 1999;194(Pt 4): 519–24.
23. Kusafuka K, Hiraki Y, Shukunami C, Kayano T, Takemura T. Cartilage-specific matrix protein, chondromodulin-I (ChM-I), is a strong angioinhibitor in endochondral ossification of human neonatal vertebral tissues *in vivo*: relationship with angiogenic factors in the cartilage. Acta Histochem 2002;104(2):167–75.
24. Lin C, McGough R, Aswad B, Block JA, Terek R. Hypoxia induces HIF-1alpha and VEGF expression in chondrosarcoma cells and chondrocytes. J Orthop Res 2004;22(6):1175–81.

25. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, *et al*. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16(9):4604–13.
26. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, *et al*. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev 1998;12(2):149–62.
27. Lee JW, Bae SH, Jeong JW, Kim SH, Kim KW. Hypoxia-inducible factor (HIF-1) alpha: its protein stability and biological functions. Exp Mol Med 2004;36(1):1–12.
28. Lund-Olesen K. Oxygen tension in synovial fluids. Arthritis Rheum 1970; 13(6):769–76.
29. Hollander AP, Corke KP, Freemont AJ, Lewis CE. Expression of hypoxia-inducible factor 1alpha by macrophages in the rheumatoid synovium: implications for targeting of therapeutic genes to the inflamed joint. Arthritis Rheum 2001;44(7):1540–4.
30. Giatromanolaki A, Sivridis E, Maltezos E, Athanassou N, Papazoglou D, Gatter KC, *et al*. Upregulated hypoxia inducible factor-1alpha and -2alpha pathway in rheumatoid arthritis and osteoarthritis. Arthritis Res Ther 2003;5(4):R193–201.
31. Gaber T, Dziurla R, Tripmacher R, Burmester GR, Buttgereit F. Hypoxia inducible factor (HIF) in rheumatology: low O_2! See what HIF can do!. Ann Rheum Dis 2005;64(7):971–80.
32. Distler JH, Wenger RH, Gassmann M, Kurowska M, Hirth A, Gay S, *et al*. Physiologic responses to hypoxia and implications for hypoxia-inducible factors in the pathogenesis of rheumatoid arthritis. Arthritis Rheum 2004;50(1):10–23.
33. Schipani E, Ryan HE, Didrickson S, Kobayashi T, Knight M, Johnson RS. Hypoxia in cartilage: HIF-1alpha is essential for chondrocyte growth arrest and survival. Genes Dev 2001;15(21):2865–76.
34. Schipani E. Hypoxia and HIF-1 alpha in chondrogenesis. Semin Cell Dev Biol 2005;16(4–5):539–46.
35. Yudoh K, Nakamura H, Masuko-Hongo K, Kato T, Nishioka K. Catabolic stress induces expression of hypoxia-inducible factor (HIF)-1 alpha in articular chondrocytes: involvement of HIF-1 alpha in the pathogenesis of osteoarthritis. Arthritis Res Ther 2005;7(4):R904–14.
36. Pfander D, Cramer T, Schipani E, Johnson RS. HIF-1alpha controls extracellular matrix synthesis by epiphyseal chondrocytes. J Cell Sci 2003;116(Pt 9):1819–26.
37. Tanaka S, Hamanishi C, Kikuchi H, Fukuda K. Factors related to degradation of articular cartilage in osteoarthritis: a review. Semin Arthritis Rheum 1998;27(6):392–9.
38. Torzilli PA, Grigiene R. Continuous cyclic load reduces proteoglycan release from articular cartilage. Osteoarthritis Cartilage 1998;6(4): 260–8.
39. Fujisawa T, Hattori T, Takahashi K, Kuboki T, Yamashita A, Takigawa M. Cyclic mechanical stress induces extracellular matrix degradation in cultured chondrocytes *via* gene expression of matrix metalloproteinases and interleukin-1. J Biochem (Tokyo) 1999;125(5):966–75.
40. Akagi M, Nishimura S, Yoshida K, Kakinuma T, Sawamura T, Munakata H, *et al*. Cyclic tensile stretch load and oxidized low density lipoprotein synergistically induce lectin-like oxidized ldl receptor-1 in cultured bovine chondrocytes, resulting in decreased cell viability and proteoglycan synthesis. J Orthop Res 2006;24(8):1782–90.
41. Loeser RF. Molecular mechanisms of cartilage destruction: mechanics, inflammatory mediators, and aging collide. Arthritis Rheum 2006; 54(5):1357–60.
42. Pufe T, Lemke A, Kurz B, Petersen W, Tillmann B, Grodzinsky AJ, *et al*. Mechanical overload induces VEGF in cartilage discs *via* hypoxia-inducible factor. Am J Pathol 2004;164(1):185–92.
43. Pufe T, Kurz B, Petersen W, Varoga D, Mentlein R, Kulow S, *et al*. The influence of biomechanical parameters on the expression of VEGF and endostatin in the bone and joint system. Ann Anat 2005; 187(5–6):461–72.
44. Wong M, Siegrist M, Goodwin K. Cyclic tensile strain and cyclic hydrostatic pressure differentially regulate expression of hypertrophic markers in primary chondrocytes. Bone 2003;33(4):685–93.
45. Turpaev K, Litvinov D, Dubovaya V, Panasyuk A, Ivanov D, Prassolov V. Induction of vascular endothelial growth factor by nitric oxide in cultured human articular chondrocytes. Biochimie 2001; 83(6):515–22.
46. Honorati MC, Cattini L, Facchini A. IL-17, IL-1beta and TNF-alpha stimulate VEGF production by dedifferentiated chondrocytes. Osteoarthritis Cartilage 2004;12(9):683–91.
47. Murata M, Yudoh K, Nakamura H, Kato T, Inoue K, Chiba J, *et al*. Distinct signaling pathways are involved in hypoxia- and IL-1-induced VEGF expression in human articular chondrocytes. J Orthop Res 2006;24(7):1544–54.
48. Kanata S, Akagi M, Nishimura S, Hayakawa S, Yoshida K, Sawamura T, *et al*. Oxidized LDL binding to LOX-1 upregulates VEGF expression in cultured bovine chondrocytes through activation of PPAR-gamma. Biochem Biophys Res Commun 2006;348(3):1003–10.
49. Fay J, Varoga D, Wruck CJ, Kurz B, Goldring MB, Pufe T. Reactive oxygen species induce expression of vascular endothelial growth factor in chondrocytes and human articular cartilage explants. Arthritis Res Ther 2006;8(6):R189.
50. Tomiyama T, Fukuda K, Yamazaki K, Hashimoto K, Ueda H, Mori S, *et al*. Cyclic compression loaded on cartilage explants enhances the production of reactive oxygen species. J Rheumatol 2007;34(3): 556–62.
51. Green DM, Noble PC, Ahuero JS, Birdsall HH. Cellular events leading to chondrocyte death after cartilage impact injury. Arthritis Rheum 2006; 54(5):1509–17.
52. Duyndam MC, Hulscher ST, van der Wall E, Pinedo HM, Boven E. Evidence for a role of p38 kinase in hypoxia-inducible factor 1-independent induction of vascular endothelial growth factor expression by sodium arsenite. J Biol Chem 2003;278(9):6885–95.
53. Kao YH, Yu CL, Chang LW, Yu HS. Low concentrations of arsenic induce vascular endothelial growth factor and nitric oxide release and stimulate angiogenesis *in vitro*. Chem Res Toxicol 2003;16(4):460–8.
54. Sandal L, Heinegard D, Hering T. Cell biology, biochemistry, and molecular biology of articular cartilage in osteoarthritis. In: Osteoarthritis: Diagnosis and Medical/Surgical Management. Lippincott Williams & Wilkins 2007:73–106.
55. Pufe T, Petersen W, Tillmann B, Mentlein R. The splice variants VEGF121 and VEGF189 of the angiogenic peptide vascular endothelial growth factor are expressed in osteoarthritic cartilage. Arthritis Rheum 2001;44(5):1082–8.
56. Pfander D, Kortje D, Zimmermann R, Weseloh G, Kirsch T, Gesslein M, *et al*. Vascular endothelial growth factor in articular cartilage of healthy and osteoarthritic human knee joints. Ann Rheum Dis 2001;60(11): 1070–3.
57. Enomoto H, Inoki I, Komiya K, Shiomi T, Ikeda E, Obata K, *et al*. Vascular endothelial growth factor isoforms and their receptors are expressed in human osteoarthritic cartilage. Am J Pathol 2003;162(1): 171–81.
58. Shakibaei M, Schulze-Tanzil G, Mobasheri A, Beichler T, Dressler J, Schwab W. Expression of the VEGF receptor-3 in osteoarthritic chondrocytes: stimulation by interleukin-1 beta and association with beta 1-integrins. Histochem Cell Biol 2003;120(3):235–41.
59. Haywood L, McWilliams DF, Pearson CI, Gill SE, Ganesan A, Wilson D, *et al*. Inflammation and angiogenesis in osteoarthritis. Arthritis Rheum 2003;48(8):2173–7.
60. Mankin H, Mow V, Buckwalter J. Articular cartilage repair and osteoarthritis. In: Orthopaedic Basic Science. American Academy of Orthopaedic Surgeons 2000:471–88.
61. Hashimoto S, Creighton-Achermann L, Takahashi K, Amiel D, Coutts RD, Lotz M. Development and regulation of osteophyte formation during experimental osteoarthritis. Osteoarthritis Cartilage 2002; 10(3):180–7.
62. Mentlein R, Pufe T. New functions of angiogenic peptides in osteoarthritic cartilage. Curr Rheumatol Rev 2005;137–43.
63. Pufe T, Harde V, Petersen W, Goldring MB, Tillmann B, Mentlein R. Vascular endothelial growth factor (VEGF) induces matrix metalloproteinase expression in immortalized chondrocytes. J Pathol 2004; 202(3):367–74.
64. Komiya K, Enomoto H, Inoki I, Okazaki S, Fujita Y, Ikeda E, *et al*. Expression of ADAM15 in rheumatoid synovium: upregulation by vascular endothelial growth factor and possible implications for angiogenesis. Arthritis Res Ther 2005;7(6):R1158–73.
65. Sharif M, Whitehouse A, Sharman P, Perry M, Adams M. Increased apoptosis in human osteoarthritic cartilage corresponds to reduced cell density and expression of caspase-3. Arthritis Rheum 2004;50(2): 507–15.
66. Aigner T, Haag J, Martin J, Buckwalter J. Osteoarthritis: aging of matrix and cells—going for a remedy. Curr Drug Targets 2007;8(2):325–31.
67. Liu W, Ahmad SA, Reinmuth N, Shaheen RM, Jung YD, Fan F, *et al*. Endothelial cell survival and apoptosis in the tumor vasculature. Apoptosis 2000;5(4):323–8.
68. Kim WU, Kang SS, Yoo SA, Hong KH, Bae DG, Lee MS, *et al*. Interaction of vascular endothelial growth factor 165 with neuropilin-1 protects rheumatoid synoviocytes from apoptotic death by regulating Bcl-2 expression and Bax translocation. J Immunol 2006;177(8): 5727–35.
69. Kim HA, Lee YJ, Seong SC, Choe KW, Song YW. Apoptotic chondrocyte death in human osteoarthritis. J Rheumatol 2000;27(2):455–62.
70. Lee MS, Trindade MC, Ikenoue T, Goodman SB, Schurman DJ, Smith RL. Regulation of nitric oxide and bcl-2 expression by shear stress in human osteoarthritic chondrocytes *in vitro*. J Cell Biochem 2003;90(1):80–6.
71. Iannone F, De Bari C, Scioscia C, Patella V, Lapadula G. Increased Bcl-2/p53 ratio in human osteoarthritic cartilage: a possible role in regulation of chondrocyte metabolism. Ann Rheum Dis 2005;64(2):217–21.
72. Surendran S, Kim SH, Jee BK, Ahn SH, Gopinathan P, Han CW. Anti-apoptotic Bcl-2 gene transfection of human articular chondrocytes protects against nitric oxide-induced apoptosis. J Bone Joint Surg Br 2006;88(12):1660–5.

73. Yagi R, McBurney D, Laverty D, Weiner S, Horton WE Jr. Intrajoint comparisons of gene expression patterns in human osteoarthritis suggest a change in chondrocyte phenotype. J Orthop Res 2005;23(5):1128–38.
74. Yagi R, McBurney D, Horton WE Jr. Bcl-2 positively regulates Sox9-dependent chondrocyte gene expression by suppressing the MEK-ERK1/2 signaling pathway. J Biol Chem 2005;280(34):30517–25.
75. Dai SM, Shan ZZ, Nakamura H, Masuko-Hongo K, Kato T, Nishioka K, *et al*. Catabolic stress induces features of chondrocyte senescence through overexpression of caveolin 1: possible involvement of caveolin 1-induced downregulation of articular chondrocytes in the pathogenesis of osteoarthritis. Arthritis Rheum 2006;54(3):818–31.
76. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001;44(6):1237–47.
77. Masuko-Hongo K, Yudoh K. The role of inflammatory mediators in cartilage. Curr Rheumatol Rev 2005;1(2):119–24.