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Short Communication

Expression of Vascular Endothelial Growth Factor (VEGF) Associated with Histopathological Changes in Rodent Models of Osteoarthritis

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Abstract: Vascular endothelial growth factor (VEGF) and its receptors have recently reported to be expressed in human osteoarthritis (OA), suggesting that VEGF could be implicated in the pathogenesis of this disease. In the present study, expression of VEGF in the articular cartilage was determined in three different OA models: medial meniscectomy and monoiodoacetate (MIA) injection in rats and age-associated spontaneous joint cartilage destruction in guinea pigs. VEGF was detected by immunohistochemical analysis in the regenerative and hypertrophic chondrocytes, perichondrium and osteophyte areas and chondrocyte clones. Stain intensity of VEGF immunoreactivity increased simultaneously with the degree of cartilage destruction and reparation. These results suggest that VEGF is a key factor in the articular cartilage in human OA and animal OA models. (DOI: 10.1293/tox.24.137; J Toxicol Pathol 2011; **24**: 137-142)

Key words: Osteoarthritis, Vascular endothelial growth factor (VEGF), Immunohistochemistry, Medial meniscectomy, Monoiodoacetate (MIA)

Osteoarthritis (OA) is a degenerative disease of joint cartilage that occurs in a large proportion of elderly people. In OA, cartilage matrix is lost gradually, which eventually devastates functional joints. Joint pain and movement limitation are the primary symptoms associated with this disease. Contributing to OA pathogenesis, cartilage damage induces disease-related factors, including proteolytic enzymes of the matrix metalloproteinase (MMPs) and aggrecanase families, cytokines, chemokines and growth factors[1–7]. However, causal genes, the molecular biological background and the signal pathways of this disease are largely unknown. There are a number of OA models, such as anterior surgical ligament transection (ACLT), medial meniscectomy[1,7–9], collagenase injection[10], extracellular matrix loss[11,12], impact-induced trauma[13], monoiodoacetate (MIA) injection[14–16], the age-associated spontaneous OA-like model[17,18] and STR/OrtCrlj mice[19,20]. It is essential that these OA models are investigated in creating new drugs for OA disease and for each different stage in drug development, such as the screening and preclinical stages. However, each of these animal models likely reflects only a subset of cases due to the heterogeneity of human OA. Moreover, though human OA and these models actually have some common pathological appearances, analyses of histopathological similarities between human OA and these animal models have just started. Recently, it was reported that VEGF and its receptors are expressed in human OA accompanying the progression of this disease, and this suggested the possibility that a mechanism via VEGF is implicated for destruction of OA articular cartilage[21].

In the present study, we examined the expression of VEGF in the articular cartilage in OA-like models; rat medial meniscectomy, rat MIA injection and guinea pig age-associated spontaneous joint cartilage destruction. We then found that the immunoreactivity of VEGF in the cartilage commonly enhances with the degree of cartilage destruction and reparation in these models of rodents.

We used two OA-like models of the rat: medial meniscectomy and MIA injection. Moreover, an aged guinea pig model showing spontaneous joint cartilage destruction was also investigated. This is abbreviated here as the "SPOA model." In the medial meniscectomy model, twelve male Fischer rats (F344/DuCrlCrlj, 12 weeks old) were used, and the surgery was carried out at the facilities of Charles River Laboratories Japan Inc. (Yokohama, Japan). At 1, 2 and 5 weeks after medial meniscectomy and sham operation (n = 3 per group) as a control, rats were killed under anesthesia by isoflurane inhalation, and the right knee joints were removed for pathological evaluation. In the MIA model, four male Lewis rats (8 weeks old) were used and treated with a

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single intra-articular injection of 0.3 mg MIA (Wako Pure Chemical Industries, Ltd., Osaka, Japan). At 1 week after MIA or saline injection (n = 2 per group) as a control, rats were killed under anesthesia, and the right knee joints were removed for pathological evaluation. In the SPOA model, eight Hartley guinea pigs purchased from Charles River Laboratories Japan Inc. were used at 16 months of age and 10 weeks age as control (n = 4 per group), and their joints were examined. All animal experiments were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved in advance by the Committee of Animal Experiments in Research Laboratories of Mitsubishi Tanabe Pharma Corporation.

Knee joints in the three models were fixed in 10% neutralized buffered formalin, decalcified with buffered EDTA (10% ethylenediaminetetraacetic acid, pH 7.4), transected in the frontal or sagittal plane and embedded in paraffin. The sections included the tibial plateau and femoral condyle and were stained with safranin O or toluidine blue and evaluated for cartilage damage and osteophyte formation. Immunohistochemistry was employed to assess expression and localization of VEGF according to the histopathological progress of damage/repair of the articular cartilage. Tissue sections were incubated overnight at 4 °C with anti-VEGF (A-20) rabbit polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) followed by horseradish peroxidase-labeled goat antibody against rabbit IgG (Nichirei, Tokyo, Japan) and visualized with 3.3'-diaminobenzidine.

Initially, histopathological changes and VEGF immunohistochemistry were examined in the articular cartilage of the tibial medial plateau in the rat medial meniscectomy model. A knee joint from a sham-operated animal used as a control is depicted in Fig. 1, panels A and B. One week after medial meniscectomy, the articular cartilage showed fibrillation, loss and degeneration of chondrocytes (Fig. 1C). Two weeks after the surgery, a migrational response of the perichondrium and initial osteophyte formation were seen (Fig. 1E). Moreover, osteophyte development and reorganization of the regenerative articular cartilage were seen at 5 weeks after surgery (Fig. 1G). Expression of VEGF in the articular cartilage was not detected in normal rats and the animals studied one week after surgery (Fig. 1B, D). Two weeks after the surgery, VEGF was observed in the perichondrium and regenerative or hypertrophic chondrocytes (Fig. 1F). The stain intensity of VEGF became obvious as the chondrocytes in the osteophyte area increased at 5 weeks after surgery (Fig. 1H).

Histopathological changes and VEGF immunohistochemistry were then examined in the articular cartilage in rats of the MIA injection model. A knee joint from a saline-injected animal as a control is depicted in Fig. 2, panels A and B. At 1 week after MIA injection, loss and degeneration of chondrocytes and a reduction of safranin O-positive proteoglycan staining were observed in the articular cartilage without osteophyte formation (Fig. 2C, 2E). Although expression of VEGF was not detected in the articular cartilage of normal rats (Fig. 2B), some regenerative or hypertrophic chondrocytes showed positive immunoreaction 1 week after MIA injection (Fig. 2D, 2F).

Finally, histopathological changes and VEGF immunohistochemistry were examined in the articular cartilage in the guinea pig SPOA model. Examples of young guinea pigs (10 weeks old) are shown in Fig. 3, panels A and B. In aged guinea pigs (16 months old), the articular cartilage became thin and included advanced tidemark, chondrocytes were degenerated and proteoglycan staining was reduced (Fig. 3C). Fibrillation and osteophyte formation were seen in the tibial medial plateau in the frontal plane (data not shown). Moreover, appearance of chondrocyte clones were seen in the destructive cartilage matrix (Fig. 3E). Although expression of VEGF was not detected in the articular cartilage of young guinea pigs (Fig. 3B), some regenerative or hypertrophic chondrocytes showed positive immunoreaction against VEGF in the articular cartilage of aged guinea pigs (Fig. 3D). Moreover, the chondrocyte clones showed a strongly positive reaction against VEGF in the superficial and middle zone (Fig. 3F).

OA is pathologically characterized by fibrillation and erosion in cartilage, by chondrocyte proliferation and osteophyte formation at the joint margins and by sclerosis of subchondral bone. Destruction of articular cartilage (chondrocytes) involving catabolism of matrix proteins of chondrocytes, such as type 2 collagen and aggrecan, is caused by proteolytic enzymes of the matrix metalloproteinase (MMPs) and aggrecanase families. It is also reputed that MMPs or MMP13 inhibitors protect cartilage degeneration[2–4] and that mice deficient in a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS5) and MMP9 are protected from cartilage damage in some models[5,6]. Furthermore, other factors such as transforming growth factor-beta (TGF-β), cytokines, chemokines and cathepsin have been advocated for involvement in OA pathogenesis, but the relationship between these factors and articular cartilage destruction is still unknown.

In pathological conditions, such as rheumatoid arthritis and OA, damaged articular cartilage is frequently covered with and invaded by granulation tissue with high vascularity, the so- called pannus tissue. These findings under pathophysiological conditions suggest the involvement of angiogenic factors in the process. Among these, VEGF, which is produced by hypertrophic chondrocytes, is considered to be a coordinator of extracellular matrix (ECM) remodeling, angiogenesis and bone formation in the growth plate[22]. In fact, expression of VEGF was reported in human OA patients[21] and in the rabbit ACLT model[23].

In the present study, we found that some regenerative chondrocytes, perichondria and clones showed immunoreaction against VEGF in the MIA model, the medial meniscectomy model and the SPOA model. We demonstrated that expression of VEGF in cartilage was common to three different OA models established by different stress mechanisms. Indeed, stain intensity of VEGF was gradually enhanced simultaneously with histopathological severity, which was evaluated based on such things as regeneration and hypertrophy of the chondrocytes and clones, migrational response of perichondrium, as well as osteophyte forma-

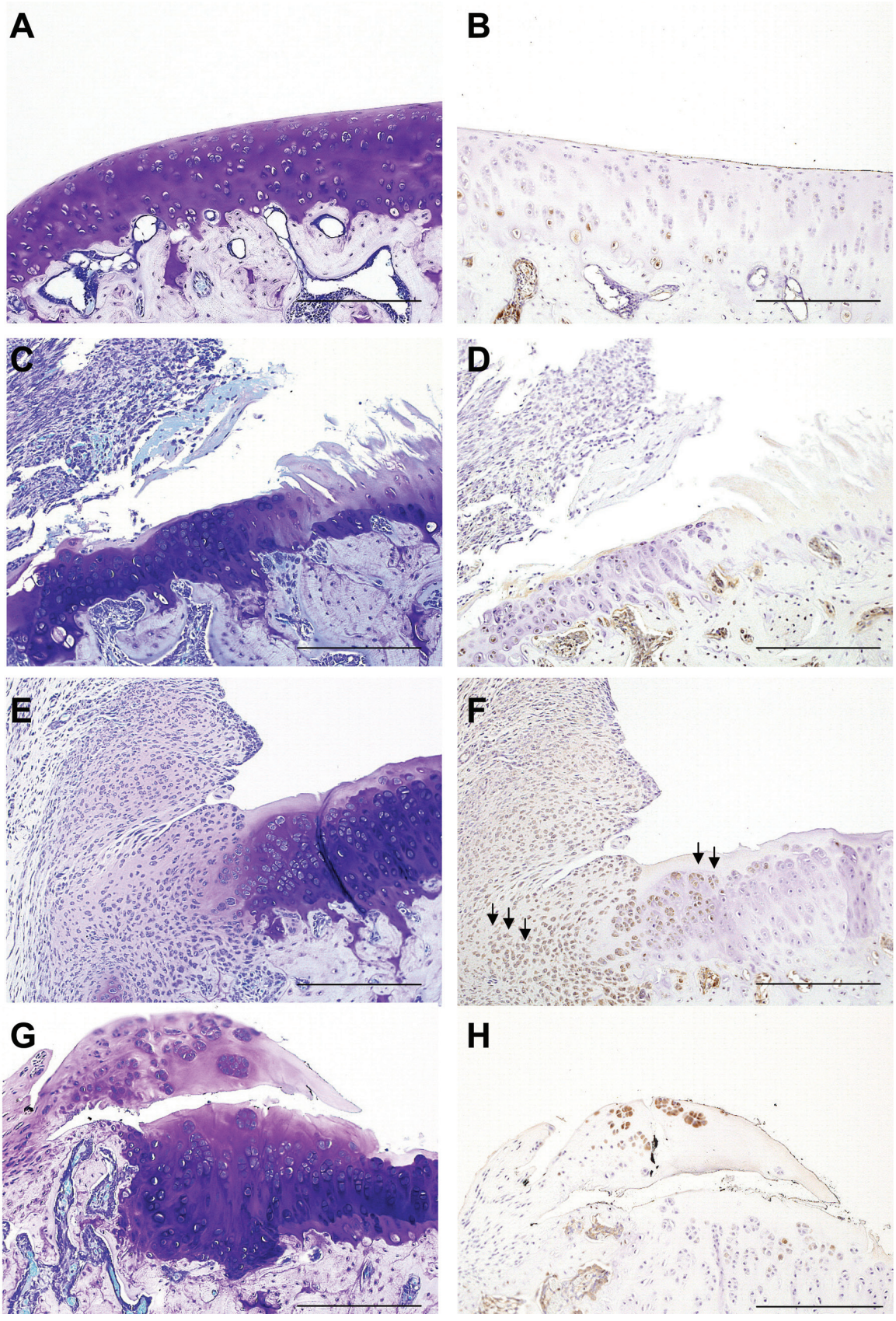

Fig. 1. Histopathological changes of the articular cartilage of the tibial medial plateau in the frontal plane. The bars indicate 0.5 mm. A, C, E, G: Toluidine blue staining. B, D, F, H: Immunohistochemical staining of VEGF. A, B: Sham operation (Control). C, D: One week after the medial meniscectomy. E, F: Two weeks after the medial meniscectomy. G, H: Five weeks after the medial meniscectomy. The arrows point to VEGF immunoactivity.

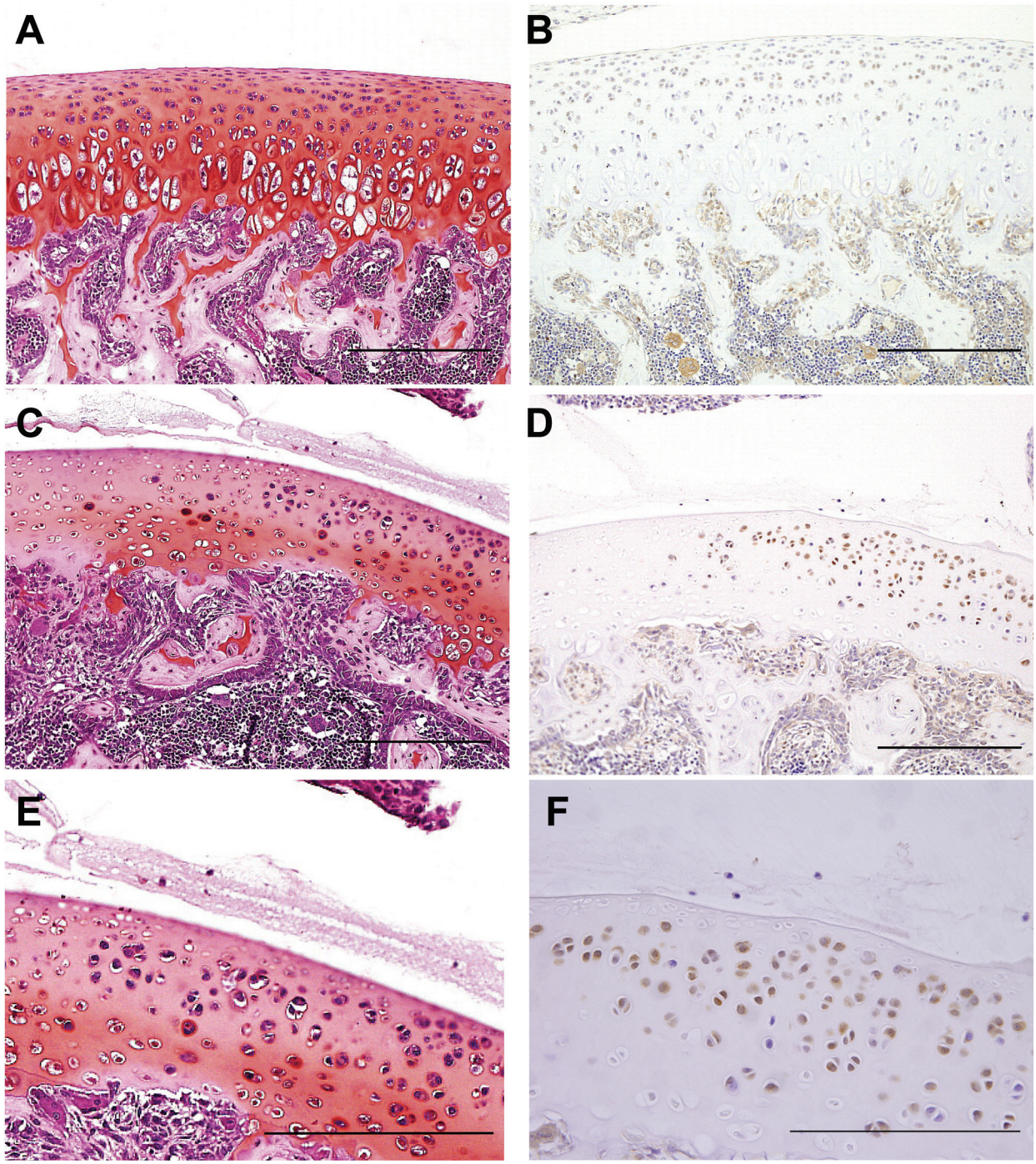

Fig. 2. Histopathological changes of the articular cartilage of the femoral condyle after monoiodoacetate (MIA) injection in the frontal plane. The bars indicate 200 µm. A, C: Safranin O staining. B, D: Immunohistochemical staining of VEGF. A, B: Saline injection (Normal). C, D: One week after monoiodoacetate (MIA).

tion and development after articular cartilage destruction. Some authors have found that regenerative chondrocytes or clones increase with the severity of OA[24]. The present data suggest a possible action of VEGF on the articular chondrocytes in OA-like models of rodents.

The primary function of VEGF was supposed to be that of angiogenesis. However, recent studies have demonstrated that the biological function of VEGF is dictated mainly by the expression of its receptors on the cells in various tissues besides blood vessels. Reportedly, at the cellular level, VEGF did not stimulate the proliferation of human OA chondrocytes[21]. However, VEGF stimulates OA chondrocytes to produce increased amounts of MMP-1 and MMP-3 without changing the production levels of TIMPs[21].

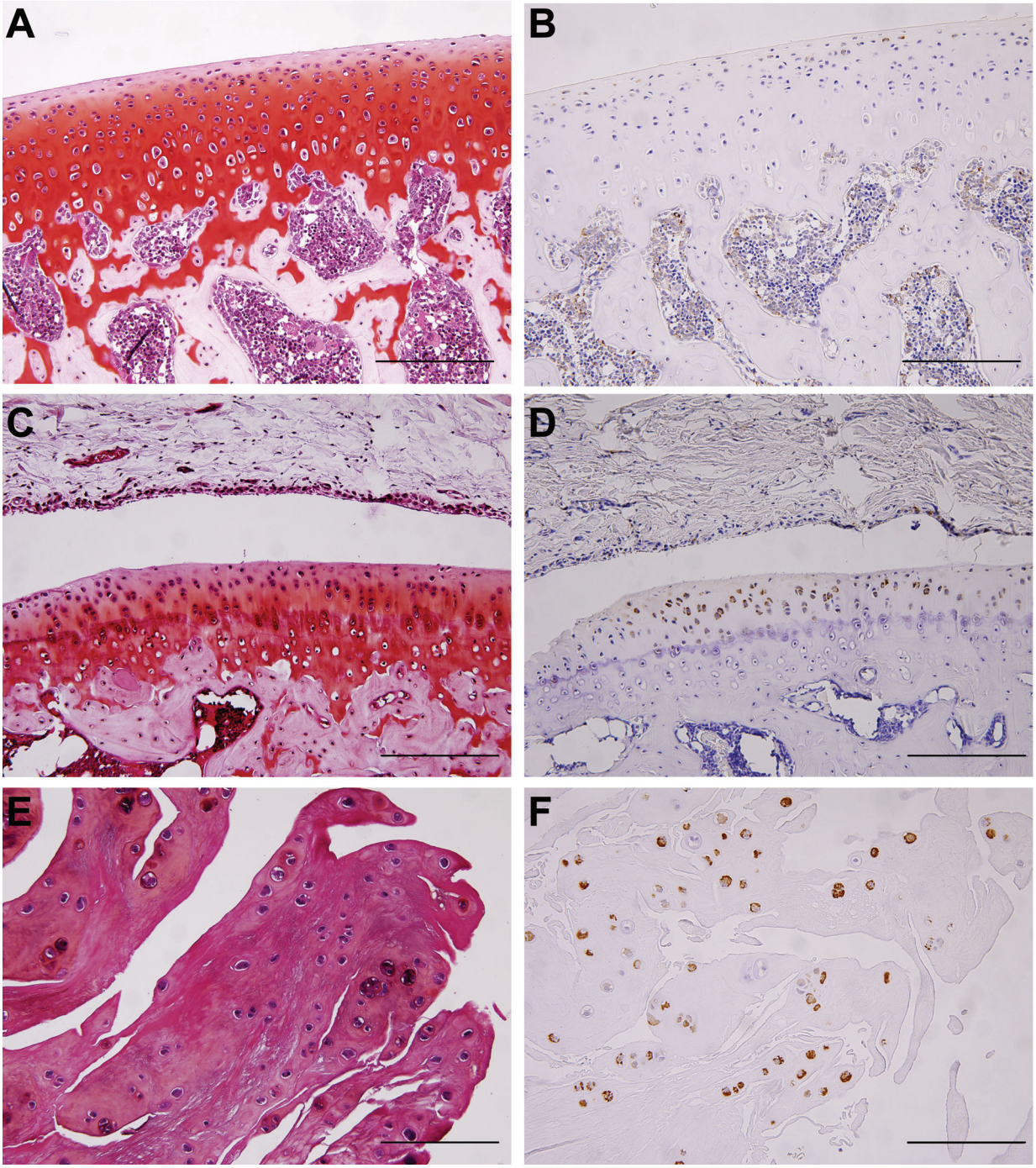

Fig .3. Histopathological changes of the articular cartilage on age-associated spontaneous OA in guinea pigs. The bars indicate 200 µm. A, C, E: Safranin O staining. B, D, F: Immunohistochemical staining of VEGF. A, B: 10 weeks old. C, D, E, F: 16 months old. A-D: Femoral condyle in the sagittal plane. E, F: Appearance of chondrocyte clones in the cartilage matrix of the tibial medial plateau in the sagittal plane.

In animals models, *in vivo*, regenerative chondrocytes or clones subjected to different forms of stress produce VEGF, and VEGF may then stimulate OA chondrocytes to produce increased MMPs and other proteolytic enzymes for destruction of articular cartilage.

In conclusion, we demonstrated that VEGF is expressed in the articular cartilage of OA-like models of rodents just as previously reported in human OA patients. Our data also suggest that VEGF is likely to be causally involved in the destruction of articular cartilage and in its repair. However, the intrinsic functions of VEGF in the pathogenesis of OA remain to be elucidated; in particular the localization and speci-

fication of its subtypes and receptors need to be clarified.

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