J. Oral Biosci. **47**(2)：105-114, 2005

REVIEW（JAOB/Lion Dental Research Award）

Osteoclast-forming Activity of Vascular Endothelial Growth Factor

Shumpei Niida §

Department of Bone and Joint Disease, Research Institute, National Center for Geriatrics and Gerontology（NCGG）
36-3 Gengo, Morioka, Obu 474-8522, Japan

Key words：osteoclast／VEGF／VEGF receptor／Flt-1／Flk-1

Abstract：Colony-stimulating factor-1（CSF-1）is an essential regulator of the differentiation, proliferation and survival of macrophage lineage cells including bone-resorbing osteoclasts. We have demonstrated that vascular endothelial growth factor（VEGF）, a known angiogenic factor, can act as a substitute for CSF-1 function in osteoclastogenesis through the VEGF receptor-1. Osteopetrotic *Csf1*^{op}/*Csf1*^{op}（*op*/*op*）mice exhibit severe osteoclast deficiency owing to the lack of CSF-1 function. However, the deficiency is gradually reversed with aging, suggesting the existence of an alternative factor supporting osteoclastogenesis. We have found that the administration of VEGF to *op*/*op* mice induces a sufficient number of osteoclasts to ameliorate the osteopetrosis. Estrogen deficiency induces the acceleration of osteoclastic bone resorption mediated by the upregulation of bone-resorbing factors including CSF-1. Ovariectomized *op*/*op* mice exhibited upregulation of VEGF expression and an increase in number of osteoclasts. VEGF antagonists inhibited both spontaneous osteoclast recruitment in the aging *op*/*op* mice and estrogen deficiency-dependent increases in osteoclasts in OVX-*op*/*op* mice. These results clearly demonstrate an ability of osteoclastogenic activity of VEGF.

Introduction

Osteoclast biology has been extensively studied over the last decade. It has been established that hematopoietic growth factor colony-stimulating factor-1（CSF-1, also known as M-CSF）is essential for the proliferation, differentiation, and survival of osteoclasts derived from monocyte-macrophage lineage cells[1,2]. The biological effects of CSF-1 are mediated through a cell-surface tyrosine kinase receptor c-Fms, which is one of the eight members of the platelet-derived growth factor receptor（PDGFR）family[3]. The critical role of CSF-1 in osteoclastogenesis has been proven in studies using osteopetrotic *Csf1*^{op}/*Csf1*^{op}（*op*/*op*）mice. Mice homozygous for a recessive *op* mutation on chromosome 3 exhibit a severe deficiency of osteoclasts, monocytes and tissue macrophages owing to a lack of functional CSF-1[4—6]. Yoshida, *et al*.[7] revealed that the loss of CSF-1 function in *op*/*op* mice is caused by a point mutation within the cording region of the *Csf1* gene. The administration of the recombinant human CSF-1（rhCSF-1）reversed the defects in *op*/*op* mice[8—10]. The expression of c-Fms in osteoclasts demonstrated the direct action of CSF-1 on osteoclast lineage cells[11,12]. However, severe osteopetrosis in *op*/*op* mice is evident only in juvenile mice. With aging, cells stained by tartrate-resistant acid phosphatase（TRAP）, an osteoclast marker, appear spontaneously in *op*/*op* mice bones and correct the osteopetrosis. In addition, only a single dose of rhCSF-1（$>5\,\mu$g/body）is sufficient to induce not only osteoclastogenesis but also continued

§ Corresponding author
E-mail：niida @ nils.go.jp

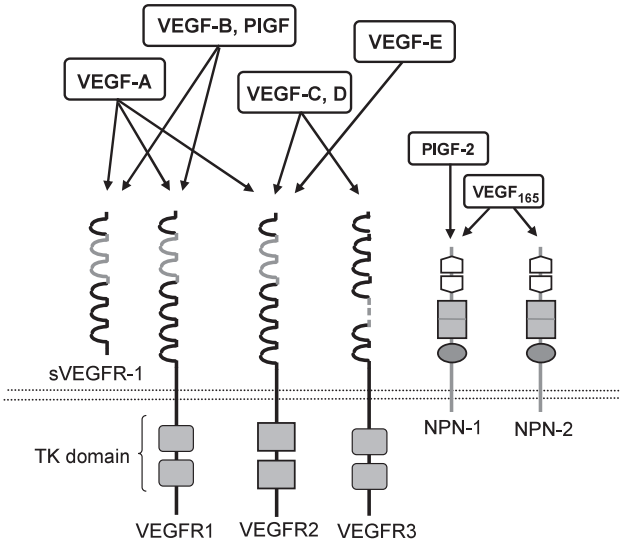

Fig. 1 VEGF families and their interactions with VEGF receptors. Osteoclasts and preosteoclasts express both VEGFR1 and VEGFR2.

active bone resorption in *op/op* mice[13,14]. These results suggest the existence of an alternative factor supporting osteoclastogenesis and survival in *op/op* mice.

Although evidence from *op/op* mice reveals an essential role for CSF-1 in osteoclast biology, it simultaneously raises the question：what induces osteoclastogenesis in *op/op* mice?. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has a function similar that of CSF-1 in the development of macrophage linage cells. However, GM-CSF is not responsible for the correction of osteoclast deficiency in the *op/op* mice[15,16]. We have previously demonstrated that congenital osteoclast deficiency in *op/op* mice can also be ameliorated by administration of a recombinant human vascular endothelial growth factor (rhVEGF)[17,18].

VEGF and Its Receptors

VEGF is a key regulator of the growth and differentiation of vascular and lymphatic endothelial cells[19], and is also known as vascular permeability factor (VPF)[20]. VEGF belongs to the PDGF supergene family and includes several members including VEGF-A, placenta growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. In addition, Orf-virus-derived VEGF-like polypeptide, VEGF-E, has been identified[21]. Human VEGF-A has multiple spliced isoforms including $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$[22—24]. In mice and rats, VEGF-A isoforms are shorter by one amino acid[19,25]. $VEGF_{121}$ fails to bind to heparin, while $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$ are heparin-binding proteins[26]. Recently, two other splice variants, $VEGF_{145}$ and $VEGF_{183}$, were identified in humans[27,28].

VEGF receptor 1 (VEGFR1/Flt-1) and VEGF receptor 2 (VEGFR2/Flk-1/KDR) are high-affinity receptos for VEGF-A and function as key mediators for angiogenesis (Fig. 1). These receptors have seven immunoglobulin (Ig)-like domains in their extracellular regions and an ~70 amino-acid-long tyrosine kinase (TK) domain in the cytoplasmic regions[29—32]. The fundamental structure of VEGFRs is very similar to that of PDGFR family members such as PDGFR/c-Fms/*c-kit*/Flt-3, although the PDGFRs have five instead seven Ig-like domains in their extracellular domains. VEGFR1 has a high affinity for $rhVEGF_{165}$[33] and as a decoy receptor for VEGF negatively regulates, at least in some circumstances, angiogenesis[34]. In addition, the *VEGFR1* gene encodes an alternatively spliced soluble form of VEGFR1 (sVEGFR1) lacking the seventh Ig-like domain and the cytoplasmic TK domain[29,31,35]. sVEGFR1 also has a high affin-