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Original article

Vascular endothelial growth factor receptor-1 (VEGFR-1) signaling enhances angiogenesis in a surgical sponge model.

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ABSTRACT

Background: Vascular endothelial growth factor (VEGF)-A binds to both VEGF receptor (VEGFR)-1 and VEGFR-2, thereby promoting angiogenesis. It is widely accepted that VEGF-A, especially VEGFR-2, is a central player in angiogenesis, however the role of VEGFR-1 in angiogenesis remains unclear. The present study was conducted to examine the role of VEGFR-1 signaling in angiogenesis, using a quantitative in vivo angiogenesis model.

Methods: Polyurethane sponge disks were implanted into dorsal subcutaneous tissue of mice. Angiogenesis was estimated by determining the number of $CD31^+$ vessels by immunohistochemical analysis. The expression of pro-angiogenic factors was quantified by reverse transcription quantitative polymerase chain reaction.

Results: Compared to control IgG-treated mice, the number of $CD31^+$ vessels in the sponge implant was significantly suppressed in anti-VEGF-A neutralizing antibody-treated mice. $CD31^+$ vessel counts were suppressed in VEGFR-1 tyrosine kinase knockout (TKKO) mice, at the same level as in VEGFR-2 tyrosine kinase inhibitor (ZD6474)-treated mice compared to wild-type (WT) mice. The accumulation of VEGFR-1^+ cells in granulation tissue was significantly suppressed in VEGFR-1 TKKO mice compared to WT mice. In addition, expression of the pro-angiogenic growth factors, VEGF-A, matrix metalloproteinase-2, interleukin-6, and basic fibroblast growth factor in granulation tissue was suppressed in VEGFR-1 TKKO mice. A bone marrow (BM) transplantation experiment showed that the number of VEGFR-1^+ BM-derived cells and angiogenesis were significantly suppressed in VEGFR-1 TKKO mice transplanted with green fluorescent protein $(GFP)^+$ VEGFR-1 TKKO BM compared to WT mice transplanted with GFP^+ WT BM.

Conclusions: These results suggest that the VEGFR-1 tyrosine kinase signaling has an effect on angiogenesis. A selective VEGFR-1 agonist/antagonist could be a candidate therapeutic agent to control angiogenesis with recruitment of BM cells.

1. Introduction

Angiogenesis, the growth of newly born capillary blood vessels, is an important process involved in many physiological and pathological conditions, such as embryogenesis, tumor growth, retinopathy, and chronic inflammation [1–3]. Angiogenesis plays a key role in the wound healing process and is controlled by a wide variety of pro- and anti-angiogenic chemical signals.

Vascular endothelial growth factor (VEGF)-A is a homodimeric glycoprotein that stimulates angiogenesis. VEGF-A binds to two receptor tyrosine kinases (TKs), VEGF receptor (VEGFR)-1 and VEGFR-2 [4]. VEGFR-2 is expressed mainly in endothelial cells. VEGFR-1 is expressed not only in endothelial cells, but also in hematopoietic stem cells and inflammatory cells [5–8].

VEGF-A induces angiogenesis, mainly via VEGFR-2 [9]. VEGFR-1 binds to VEGF-A with an affinity approximately 10-fold higher than that of VEGFR-2 [4]; however, the precise biological

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mechanism of VEGFR-1 signaling is not fully understood. VEGFR-2-null mice fail to develop blood vessels and die in utero, indicating that VEGFR-2 signaling is essential for development of the vascular system [10]. By contrast, VEGFR-1-null mice exhibit overgrowth and disorganization of blood vessels, which suggests that VEGFR-1 is a negative regulator of angiogenesis during embryonic development [4]. However, transgenic mice expressing a variant of VEGFR-1 that lacks the TK domain appear healthy with normal blood vessel formation [11]. The expression of VEGF-A and VEGFR-1 is elevated during the healing of blood flow recovery [12]. However, the precise mechanism of VEGFR-1 signaling in wound healing, especially angiogenesis, is not well understood.

The process of angiogenesis is regulated by various types of infiltrating cells. Currently, focus is directed at bone marrow (BM)-derived cells recruited to sites of ongoing angiogenesis. We previously reported that VEGFR-1 signaling is involved in homing of BM-derived cells to ischemic hind limbs and gastric ulcers [12,13]. The present study focuses on the angiogenic roles of these cells.

We employed a sponge implantation model that we developed previously [14–16]. This model displays granulation tissue formation and angiogenesis in the sponge, mimicking the host inflammatory responses driven primarily at the wound site. We used this model to determine whether VEGFR-1 signaling is involved in angiogenesis in skin.

We hypothesized that angiogenesis may be altered via modulation of VEGFR-1 signaling. Herein, we investigated the role of VEGFR-1 signaling in angiogenesis during the process of granulation tissue formation by using a domain-specific knockout mouse lacking the VEGFR-1 intracellular TK domain (VEGFR-1 TKKO). Angiogenesis was significantly suppressed in VEGFR-1 TKKO mice compared to WT mice, similar to the effect of VEGF-A/VEGFR-2 inhibition. We also confirmed that VEGFR-1 signaling in BM is crucial for the homing of VEGFR-1 expressing cells and subsequently for angiogenesis. These results indicate that VEGFR-1 is a key regulator of angiogenesis in skin.

2. Materials and methods

2.1. Animals and drugs

Male C57BL/6 mice (6–8 weeks old) were obtained from Oriental Yeast, Tokyo, Japan. VEGFR-1 TKKO mice with a C57BL/6 hybrid background were developed in our laboratory [11]. For the BM transplantation experiments, transgenic mice expressing green fluorescent protein ($GFP^{+/+}$) in a C57BL/6 background were designated as wild-type (WT) mice to confirm BM chimerism. VEGFR-1 TKKO and $GFP^{+/+}$ mice were crossed and the resultant heterozygous mice ($GFP^{+/-}$ TKKO) were interbred to obtain homozygous $GFP^{+/+}$ TKKO mice. Mice were maintained at constant humidity ($60 \pm 5\%$) and temperature ($20 \pm 1\,^{\circ}C$), and were continuously kept on a 12 h light/dark cycle. All animals were provided with food and water ad libitum. All experiments were performed in accordance with the guidelines for animal experiments of Kitasato University School of Medicine. A neutralizing antibody against VEGF-A (R&D Systems, Inc., Minneapolis, MN, USA) (10 μg/site) or their vehicle solution (physiological saline and isotype control IgG) was topically injected daily. ZD6474, a low molecular weight inhibitor of VEGFR-2 TK (AdooQ BioScience, Irvine, CA, USA), or vehicle solution (5% gum arabic prepared in distilled water) was orally administered (100 mg/kg, once daily).

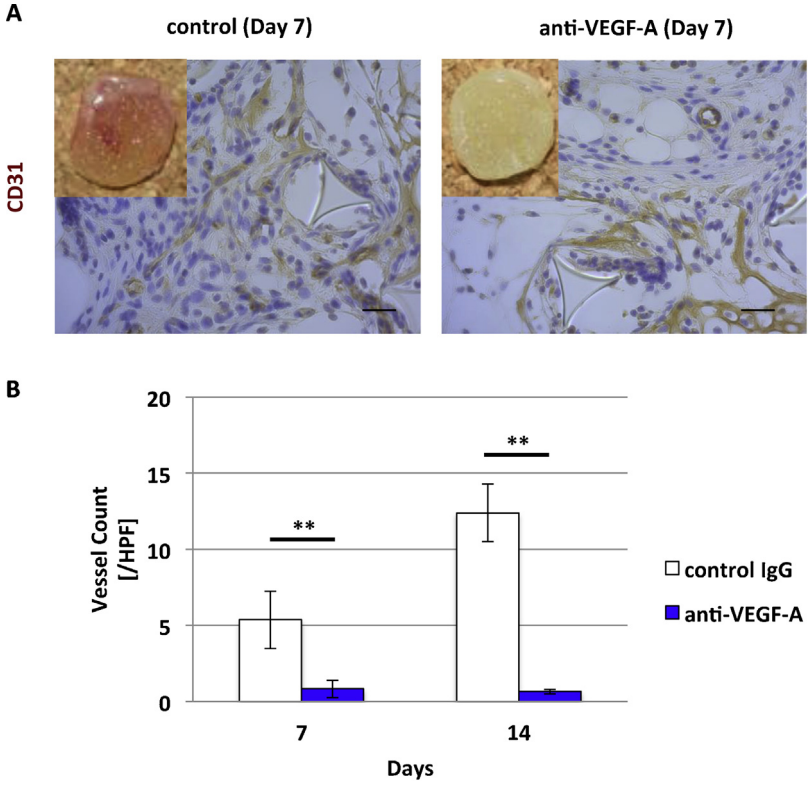

Fig. 1. Reduced angiogenesis in sponge granulation tissue in WT mice treated with an anti-VEGF-A antibody. (A) Typical appearance of the sponge implant and immunostaining of CD31 in sponge granulation tissue at 7 days after implantation. Microvessels are stained brown. Bars indicate 20 μm. (B) Blood vessel density in sponge granulation tissue. Density was determined by CD31 immunostaining. Treatment with an anti-VEGF-A antibody significantly decreased the blood vessel density in WT mice. Only very few vessels proliferated in WT mice treated with the antibody over the time course. Values are mean ± SEM ($n = 3$–4). $^{**}P < 0.01$ (Student's t test). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)