C-Terminal Heparin-Binding Peptide of Snake Venom VEGF Specifically Blocks VEGF-Stimulated Endothelial Cell Proliferation

Yasuo Yamazaki  Yuko Tokunaga  Koji Takani  Takashi Morita

Department of Biochemistry, Meiji Pharmaceutical University, Tokyo, Japan

Key Words
Vascular endothelial growth factor · Heparin · Heparin-binding peptide · Snake venom · KDR-binding protein · Lys49PLA₂

Abstract
Vascular endothelial growth factor-A165 (VEGF-A165) exhibits diverse biological effects through binding to its receptor KDR (VEGFR-2). Heparin-like molecules are known to modulate their interaction. There have been reports that VEGF-A lacking the C-terminal heparin binding region significantly reduced mitogenic activity. Recently, we found novel heparin-binding VEGFs from snake venoms, designated VEGF-Fs, which specifically recognize kinase domain containing receptor (KDR). The C-terminal heparin-binding region is almost completely absent in VEGF-Fs when compared with other heparin-binding VEGFs, despite their heparin-binding potential. In this congress, we report that the C-terminal heparin-binding region of VEGF-F specifically/preferentially interacts with the VEGF-bondable heparin/heparan sulfate, but not with those associated with bFGF or TFPI. We also present the identification of a VEGF receptor-binding protein from the venom of eastern cottonmouth (Agkistrodon piscivorus piscivorus). Sequence analysis revealed the isolated KDR-binding protein (designated KDR-bp) is identical to Lys⁴⁹PLA₂, an inactive PLA₂ homologue with strong myoxicity. KDR-bp binds to the extracellular domain of KDR with subnanomolar affinity. The interaction between KDR-bp and KDR was blocked by VEGF-A165, and KDR-bp specifically inhibited VEGF-A165-stimulated endothelial cell proliferation, indicating KDR-bp is an antagonistic ligand for KDR. This is the first observation demonstrating that an exogenous factor antagonizes the VEGF receptor, furthermore, it is the first identification of the target molecule of the myotoxic PLA₂ from viper venom.

VEGF-Blocking Effect of C-Terminal Heparin-Binding Peptide of Snake Venom-Derived VEGF

Vascular endothelial growth factor (VEGF) plays pivotal roles in physiologic and pathologic endothelial proliferative processes [1]. To date, several molecules have been identified as VEGF family proteins: VEGF-A to D, and PlGF from mammals, and viral VEGF (also denoted as VEGF-E) from Parapoxviruses. All of these subtypes exhibit distinct receptor selectivity and express diverse biological effects (fig. 1). Recently, we have identified two novel VEGFs in viper venoms and shown them to be specific ligands for KDR (fig. 1) [2]. Because of their structure [3] and strict receptor selectivity [2], we proposed a novel subtype name ‘VEGF-F’ for these snake venom-derived VEGF (fig. 1).
Vascular endothelial growth factor (VEGF-A165) is a heparin-binding protein that plays a pivotal role in physiologic and pathologic angiogenesis. VEGF-A165 is a ~45-kDa homodimeric glycoprotein composed of two domains: an N-terminal receptor-binding domain and a C-terminal heparin-binding domain. VEGF-A165 binds to its receptor KDR (kinase domain-containing receptor, VEGF receptor 2) and induces a range of biological effects, such as endothelial growth, vascular permeability and hypotension. It is reported that heparin greatly modulates the interaction of VEGF-A165 and its receptor KDR [4]. A C-terminal-deficient form of VEGF-A (110 residues) generated by plasmin-digestion is not retained on a heparin affinity column and displays markedly reduced potency of growth factor activity [5]. In contrast to VEGF-A165, snake venom-derived VEGF (VEGF-F) has a markedly short C-terminal region, despite its heparin-binding potential [2]. This region of VEGF-F is composed of 16–17 amino acid residues and is rich in basic residues. Recently, we have revealed that the heparin-binding region of VEGF-F is located in this region using a synthetic peptide (designated as peptide 1), and furthermore, that this peptide blocks VEGF-A165-stimulated endothelial cell proliferation [6]. In this study, we tested the specificity of peptide 1 for its VEGF-blocking effect.

We, first, examined whether the heparin-binding potential is involved in the biological activity of VEGF-F. In the presence of high concentrations of unfractionated heparin, both vammin (a VEGF-F from Vipera ammodytes ammodytes venom) and VEGF-A165-stimulated endothelial cell proliferation was completely abolished. These results indicate growth factor activity of VEGF-F is also mediated by its heparin-binding function. In order to investigate the specificity of peptide 1, its inhibitory effects against proliferation induced by bFGF, a well-characterized heparin-dependent growth factor [7], was assessed. Peptide 1 exhibited effects on VEGF-A165-induced proliferation, but not on bFGF-induced proliferation. Furthermore, the inhibitory effect of a synthetic peptide whose design was based on the heparin-binding sequence of tissue factor pathway inhibitor (TFPI) [8] was tested. Although the TFPI peptide displayed high affinity for heparin, it only weakly blocked VEGF-A165-induced endothelial cell growth when compared to peptide 1. These data indicate that peptide 1 specifically/preferentially interacts with the VEGF-bondable heparin/heparan sulfate structure [6].

**Identification of a VEGF Receptor-Binding Protein from the Venom of Eastern Cottonmouth**

Several snake venoms contain activator and inhibitor toxins that both target the same molecule: some vipers possess blood coagulation factor X activators, such as RVV-X, while others include factor X-binding protein (X-bp). X-bp binds the Gla domain of factor X and prevents blood coagulation. These activator and inhibitor combinations are also seen in the toxins that target platelet...
Specific Blockage of VEGF by C-Terminal Peptide of Snake Venom VEGF

References