T005

Everything You Always Wanted to Know About the Proteolytic Processing of VEGF-C

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Background

Vascular Endothelial Growth Factor-C (VEGF-C) is the central molecule that directs proliferation and migration of lymphatic endothelial cells (LECs) and thus VEGF-C is the primary growth factor for the lymphatic system. Many of these fundamental aspects of the biology of VEGF-C have only recently been uncovered, especially its activation by multiple different enzymes. The lymphatic system is integral to many disease processes. Understanding the biology of VEGF-C is therefore mandatory in the development of targeted therapeutic interventions.

Materials & Methods

We analyse molecular interactions using purified recombinant proteins, cell culture systems and in vivo models. Techniques include gene editing, cell-based protein interaction assays, protein chromatography and antibody-based methods (immunohistochemistry, Western blotting).

Take-home messages

1. ADAMTS3 is the major VEGF-C activating protease during development. Alternative proteases can activate VEGF-C (plasmin, KLK3/PSA) in the adult organism for specific purposes.

2. Both domains of CCBE1 play mutually independent roles (localization and acceleration) in the activation of VEGF-C.

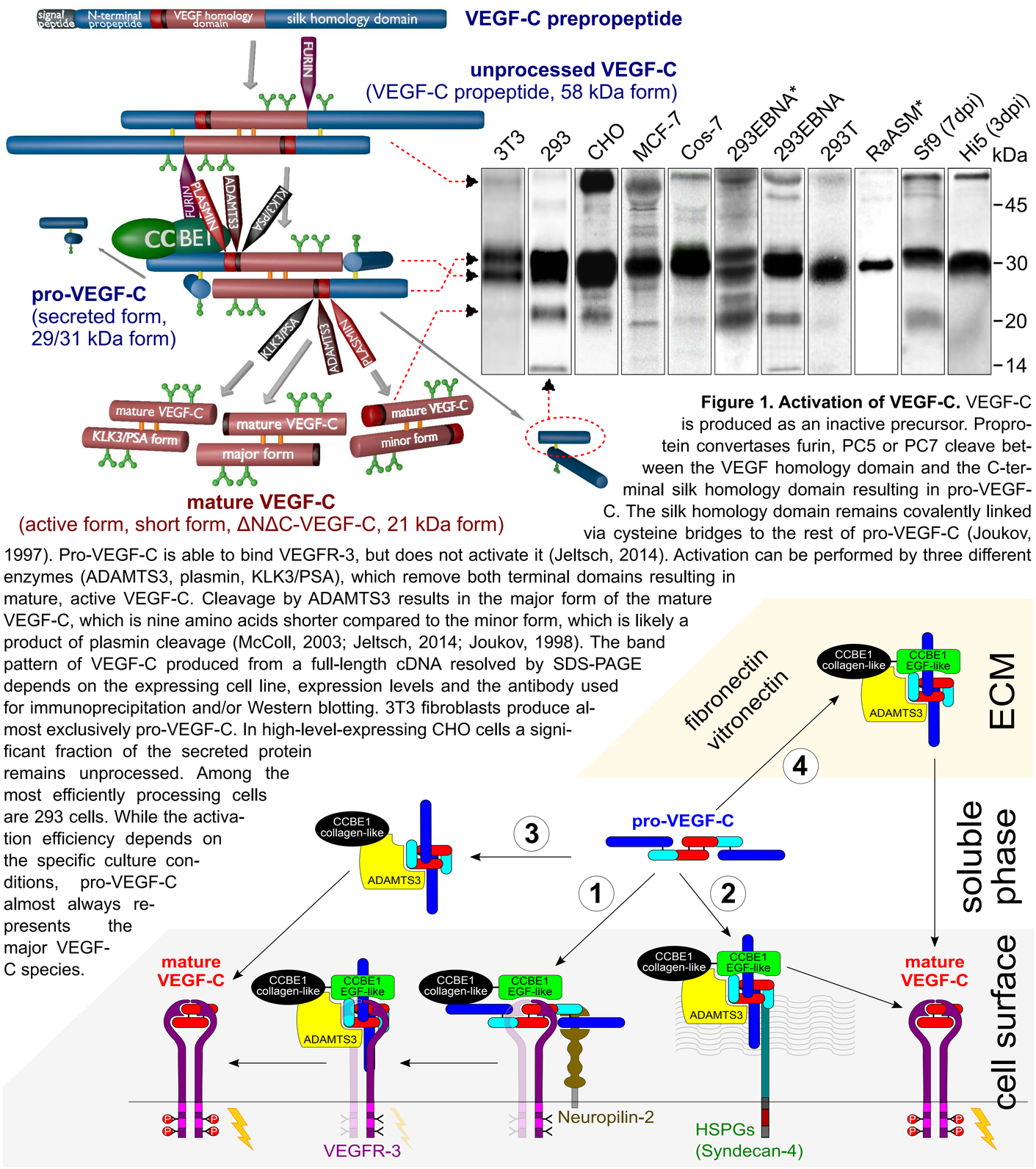

Figure 1. Activation of VEGF-C. VEGF-C is produced as an inactive precursor. Proprotein convertases furin, PC5 or PC7 cleave between the VEGF homology domain and the C-terminal silk homology domain resulting in pro-VEGF-C. The silk homology domain remains covalently linked via cysteine bridges to the rest of pro-VEGF-C (Joukov, 1997). Pro-VEGF-C is able to bind VEGFR-3, but does not activate it (Jeltsch, 2014). Activation can be performed by three different enzymes (ADAMTS3, plasmin, KLK3/PSA), which remove both terminal domains resulting in mature, active VEGF-C. Cleavage by ADAMTS3 results in the major form of the mature VEGF-C, which is nine amino acids shorter compared to the minor form, which is likely a product of plasmin cleavage (McColl, 2003; Jeltsch, 2014; Joukov, 1998). The band pattern of VEGF-C produced from a full-length cDNA resolved by SDS-PAGE depends on the expressing cell line, expression levels and the antibody used for immunoprecipitation and/or Western blotting. 3T3 fibroblasts produce almost exclusively pro-VEGF-C. In high-level-expressing CHO cells a significant fraction of the secreted protein remains unprocessed. Among the most efficiently processing cells are 293 cells. While the activation efficiency depends on the specific culture conditions, pro-VEGF-C almost always represents the major VEGF-C species.

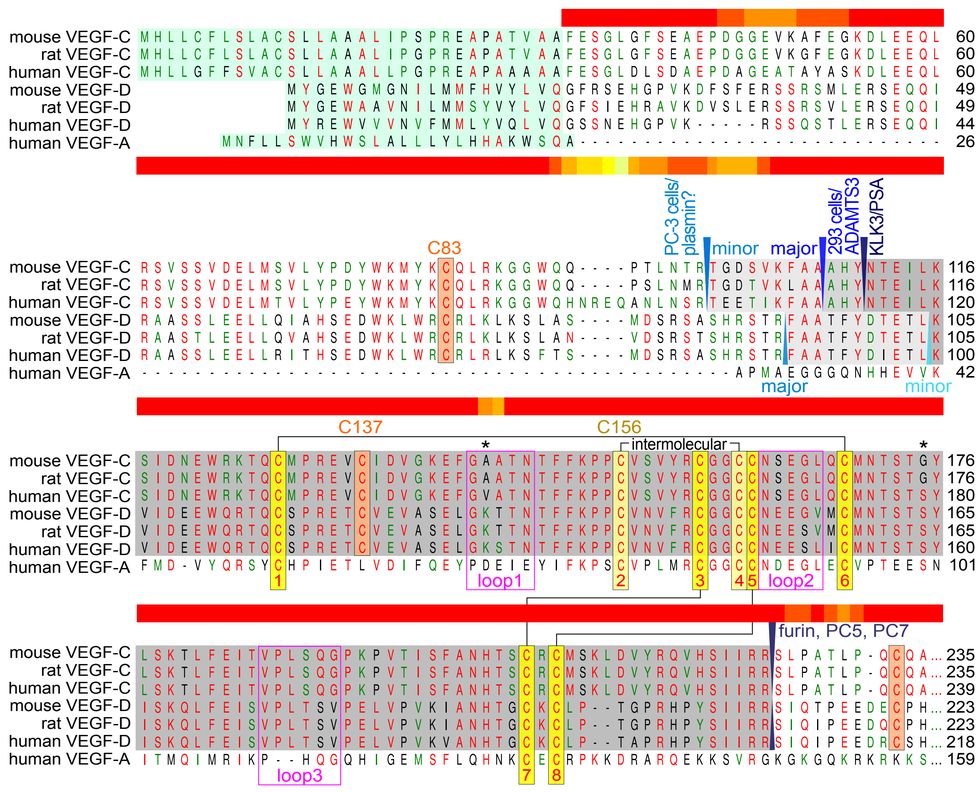

Figure 2. Alignment of VEGF-C/D with VEGF-A. The sequences of the active, mature VEGF-C/D are boxed grey. Proteolytic cleavage sites and enzymes are indicated in blue. The signal peptide is boxed green. The 8 conserved cysteines of the PDGF/VEGF signature (Muller, 1997) are boxed yellow and black connecting lines indicate intra- and intermolecular disulfide bridges. VEGF-C/D-specific conserved cysteine residues are boxed in orange. The asterisks denote the only two amino acid residues, that differ between mature mouse and human VEGF-C. Cys156, which is mutated to Ser in the VEGFR-3-monospecific variant VEGF-C_{C156S}, participates in the intermolecular cystine bridge (Joukov, 1998). When mature VEGF-C is produced from a truncated cDNA, Cys137 remains unpaired and decreases protein stability (Anisimov, 2009; Leppänen, 2010) or pairs with Cys156 and interfers with intermolecular disulfide bond formation and protein folding. This explains the relative abundance of single-linked dimers, non-covalent VEGF-C dimers and VEGF-C monomers (Chiu, 2014; Jeltsch, 2006; Joukov, 1996). The heat map above the alignment shows the areas of highest divergence.

Figure 3. Schematic view of CCBE1 action. CCBE1 acts on VEGF-C via two different mechanisms. It a) accelerates the activation of pro-VEGF-C by proteases and b) localizes the trimeric activation complex. Acceleration is mediated by the C-terminal domain of CCBE1, while localization by its N-terminal domain. Pro-VEGF-C is sequestered by the extracellular matrix (ECM), cell surface heparan sulfate proteoglycans (HSPGs), and inactive VEGFR-3. It can be mobilized by CCBE1-assisted proteolytic cleavage, which simultaneously activates VEGF-C. CCBE1 also promotes the translocation of pro-VEGF-C from the soluble phase to the cell surface. After proteolytic activation, VEGFR-3-bound VEGF-C can immediately start signaling, while HSPG-bound VEGF-C (Johns, 2016) first needs to translocate to VEGFR-3. Activation of VEGF-C does happen in solution (Bui, 2016), but the localization of pro-VEGF-C, CCBE1 and ADAMTS3 indicate that a significant fraction of the VEGF-C activation is associated with cell surfaces and the ECM.

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