Structure and function of VEGF-D by modeling and site directed mutagenesis

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Vascular development is largely mediate by of the angiogenic factors of the VEGF family. This is composed of several structurally and functionally related growth factors involved that includes the vascular endothelial growth factor (VEGF), the placental growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D and VEGF-E. All members of this family are angiogenic in vivo and able to stimulate proliferation of endothelial cells in vitro. Each member of the family recognizes and activates specific receptors on endothelial cells. In particolar, VEGF recognizes VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) while VEGF-D recognizes VEGFR-2 and VEGFR-3 (Flt-4). This latter is almost exclusively expressed in lymphatic vessels, suggesting that this factor, beside playing a role in angiogenesis is also involved in the formation of lymphatic vessels.
The extracellular portion of VEGF receptors is comprised of 7 immunoglobulin-like domains. From deletion experiments on VEGFR-1 is known that the minimal VEGF-binding fragment corresponds to residues 132-229 (D2 domain), while the receptor binding domain of human VEGF resides within the 110 N-terminal residues (VHD domain) (Keyt et al. JBC 1996).
Since the crystal structure of the complex between the VHD domain of VEGF (40-134) and the D2 domain of VEGF-R1 is available (Weismann et al. Cell 1997), we have generated a three dimensional model of the VHD domain of VEGF-D in complex with the D2 domain of both VEGFR-2 and VEGF-R3 by homology modelling, in to better identify the contact interaction between VEGF-D and its receptors.
The binding region of VEGF-Rs with VEGF-D is formed by two variable loops and an hydrophobic C-terminal region. On the other hand, VEGF-D interacts with its cognate receptors through three loops which show aminoacid variability among the members of the family and most likely account for the interaction with the receptors and VEGFs specificity. Additional contacts can been found at the C-terminal region of VDH domain. The first loop (residues 131-134), highly conserved between the members of the family, is faced to the C-terminal portion of the D2 receptor domain. The second one (residues 147-150), characterised by some sequence variability, contacts as expected its receptor in correspondence of one of the variable loops. The third loop, spanning from residues 169 to 177, has been also considered as candidate for binding. It results in fact highly exposed on the surface of the protein and, for this reason, could be potentially involved in the interaction with the adjacent immunoglobulin-like domain of the receptors.
Finally, the C-terminal region containing the K190 amino acid might play an important role for the receptor recognition, contacting a variable loop on the receptor surfaces.
From these indications, we have generated a series of VEGF-D mutants. Several of these, lying within the putative interaction surfaces, did show a reduced affinity with both receptors. In addition we also identified VEGF-D receptor specific mutants. Thus, we have identified VEGF-D mutants that bind specifically to R2 but not to R3 or to R3 but do not bind R2. Analysis of binding as well as functional data of the mutant VEGF-d will be discussed.