Structure/function relationships within the VEGF/VEGF receptor families

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Abstract

Members of the VEGF family of growth factors are central regulators of angiogenesis and lymphangiogenesis. There are many different VEGFs, but all bind to one or more VEGF receptors on the surface of mainly endothelial, but also a few other cell types. Every VEGF has a specific receptor binding pattern (Figure 1). Binding is mediated by the central VEGF homology domain (VHD), while additional auxiliary domains can contribute to specificity and further differentiate their function (Figure 7).

While the first member of the VEGF family has been crystallized 15 years ago, the follow-up with structures from related growth factors was relatively slow. In addition, only one VEGF structure complexed with its receptor had been solved at the time. However, in the recent few years most of the VEGF family members have been crystallized, a few together with a corresponding VEGF receptor.

VEGF-C and VEGF-D form a subfamily within the VEGF family based on their long N- and C-terminal auxiliary domains, their high homology in the VHD domain (Figure 5) and their similar biosynthesis (Figure 2). While the role of VEGF-C in developmental and adult lymphangiogenesis has been firmly established, the function of VEGF-D is still enigmatic, not least because the gene was knocked out in mice without significant effects.

Also computational analysis supports the notion that VEGF-D might be superfluous in mice (Figure 6). We have crystallized the VEGF-C/VEGFR-2(D2-3) complex (Leppänen & Prota et al. 2010) and VEGF-D (Figure 4, Leppänen & Jeltsch et al. 2011). We also analyzed receptor binding properties of several forms/mutants of the receptor and growth factors (Figure 8).

Our data provides deeper insight into the structural features that determine affinity and specificity within the VEGF/ VEGFR system. While our understanding of the affinity- and specificity-determining elements has increased, we are still lacking some crucial parts be able to draw a complete picture, notably the structure of VEGFR-3.

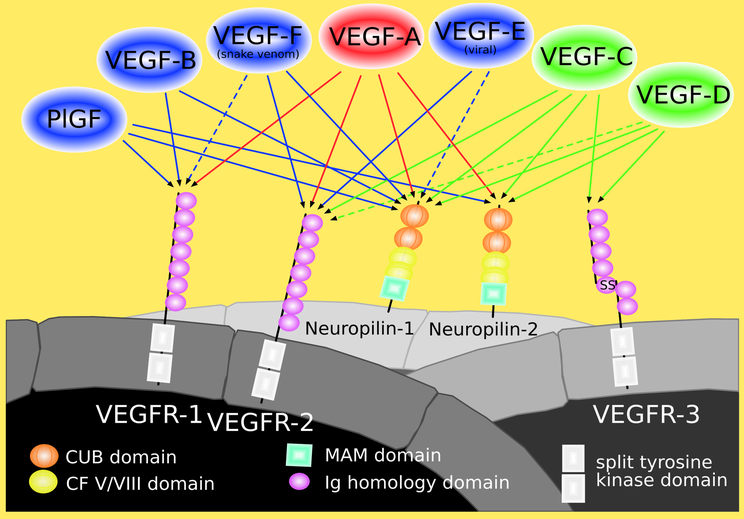

Fig 1. VEGFs and their receptors

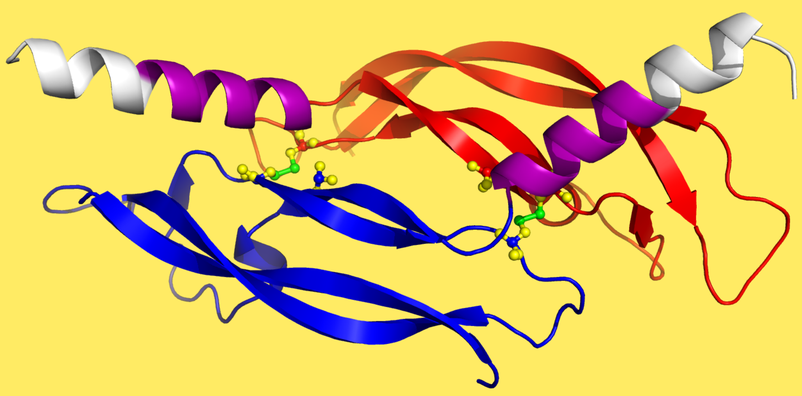

Fig 4. Cartoon representation of the VEGF-D(C117A) structure

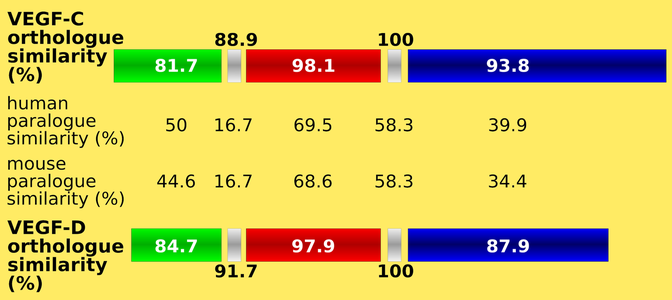

Fig 5. Homologies between VEGF-C and VEGF-D

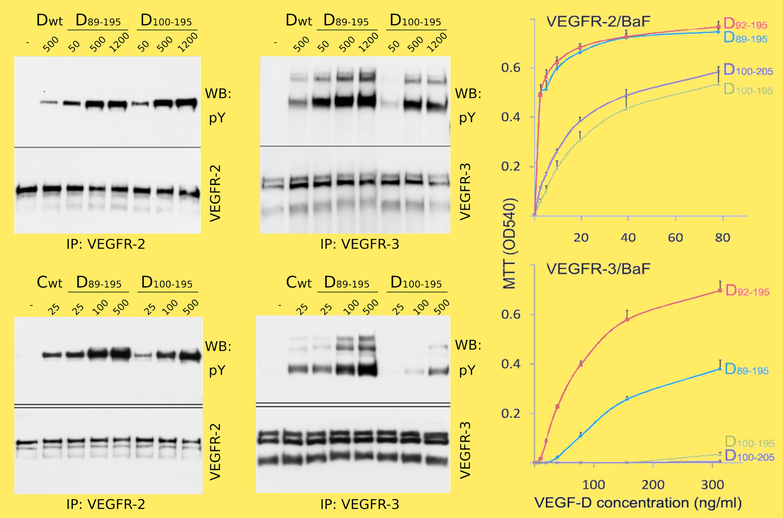

Fig 8. The mature minor form of VEGF-D doesn't bind VEGFR-2

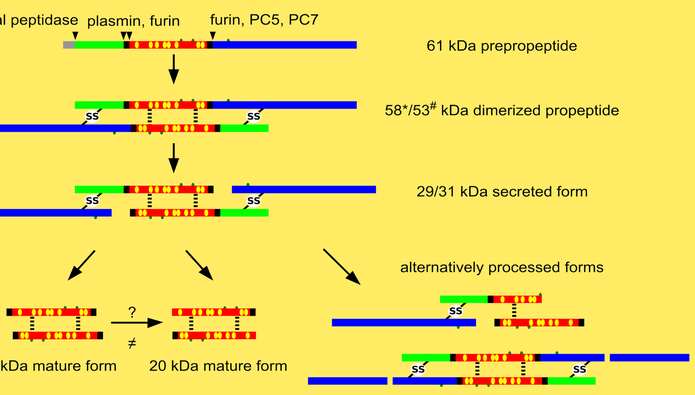

Fig 2. Proteolytic processing of VEGF-C and -D *VEGF-C, #VEGF-D

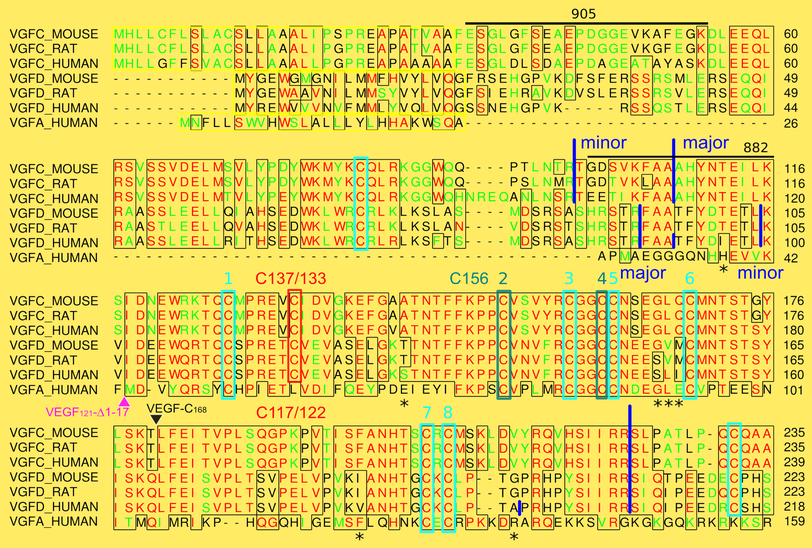

Fig 3. Alignment of the N-terminal and central regions of VEGF-C/VEGF-D

Fig 7. PDGFs and VEGFs are modular proteins

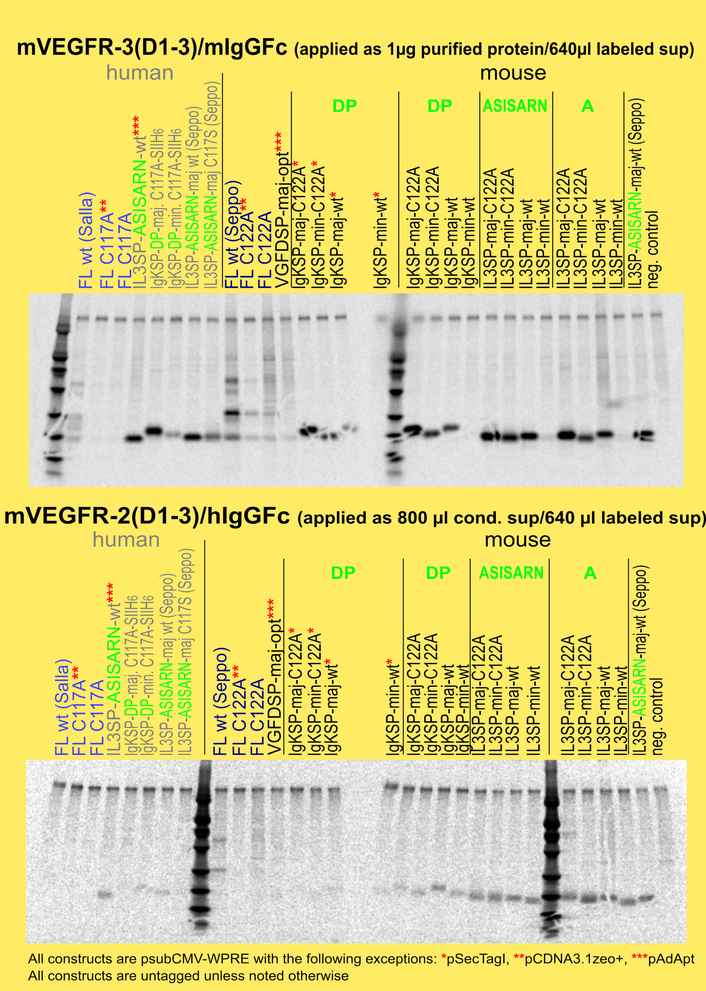

Fig 9. Differential interaction of mouse VEGF-D with mouse VEGFR-2

Results

Expectedly, the VEGF-D structure showed conserved overall features (including VEGFR-2 interaction residues) compared to VEGF-C (Figure 4).

However, both receptor binding and functional assays of N-terminally truncated VEGF-D polypeptides indicated that the residues between the proteolytic cleavage sites of the minor and the major form of VEGF-D (Figure 3) are important for binding and activation of VEGFR-3, but not of VEGFR-2 (Figure 8).

Despite the similarities in proteolytic cleavage of VEGF-C and VEGF-D, the N-terminal processing sites are not homologous.

Upon processing into the shorter (minor) mature form, VEGF-D looses a significant part of its N-terminal helix. This part seems to be essential for VEGFR-3 interaction, perhaps because it reaches towards the domain 1 of VEGFR-3.

The inability of mouse VEGF-D to bind mouse VEGFR-2 (Baldwin et al. 2001) can now be rationalized: Mainly the three amino acid differences between human and mouse VEGF-D (SLI vs. GVM, see Figure 3) seem to be responsible. However, the inability of mouse VEGF-D to interact with mouse VEGF-D is not complete and a dependence on the N-terminal residues preceding the N-terminal helix could be observed (Figure 9). Whether the observed interaction is significant in vivo, remains to be shown.

Bibliography

Baldwin et al. 2001, JBC 276, 19166
Leppänen & Prota et al. 2010, PNAS 107, 2425
Leppänen & Jeltsch et al. 2011, Blood 117, 1507

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