Dissecting Lymphangiogenesis and Angiogenesis

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Abstract

The ambivalent effects of VEGF-C have puzzled investigators since it was cloned: In some settings it behaves angiogenic, in other settings exclusively lymphangiogenic despite its ability to activate VEGF receptor-2. In the chorioallantoic membrane (CAM) both angiogenesis and lymphangiogenesis can be assayed simultaneously. Two strategies were used to obtain molecules suitable to dissect angiogenic and lymphangiogenic properties of VEGF-C:

1. Recombinant production and isolation of different VEGF-C forms.
2. Creation of VEGFs with novel receptor binding profiles by "non-random" DNA family shuffling.

Discussion

Proteolytic processing of VEGF-C

Proteolytic processing gives rise to different forms of VEGF-C. However, most analysed cell lines exhibited only limited capability to proteolytically process VEGF-C, the 29/31 kDa form being the dominant product. 293T cells processed VEGF-C more efficiently than all other tested cell lines.

Receptor binding profiles

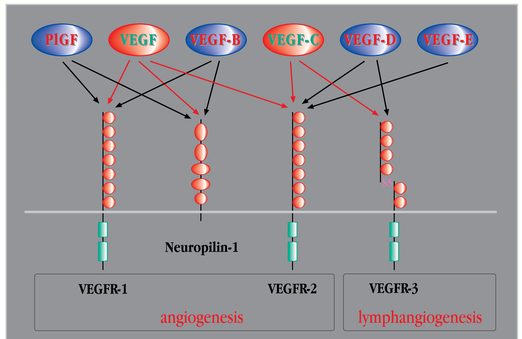
All forms of VEGF-C were capable of binding to VEGFR-2 and VEGFR-3, albeit with apparently different affinities. Exact receptor affinities of the different forms of VEGF-C are difficult to determine since despite extensive modification not all proteolytic processing can be inhibited. Interestingly proteolytic processing was increased when cell death was prominent (e.g. viral lysis, extended periods of labelling).

Super-VEGF

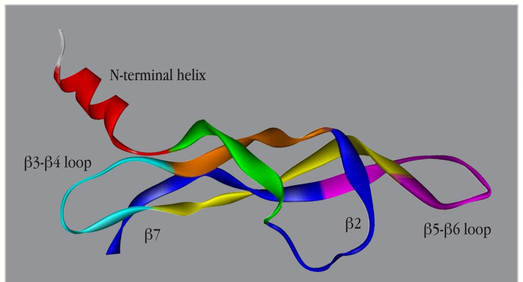
By exhaustive screening of a VEGF-VEGF-C mosaic library molecules with interesting receptor binding profiles were identified, including molecules that bind to and stimulate all three VEGF receptors ("super-VEGFs").

CAM assay

Selected mosaic VEGFs were expressed using the baculovirus system, purified and tested for their effects on endothelial cells in vivo using the chick (lymph)angiogenesis assay (Oh et al, 1997). Profound differences were observed between the in-vivo and in-vitro potencies of some mosaic molecules. Compared to VEGF and VEGF-C the biological effect of the tested mosaic VEGFs was weaker. Even the most potent mosaic VEGFs were applied in 5-10 times higher amounts to produce effects comparable to VEGF or VEGF-C.



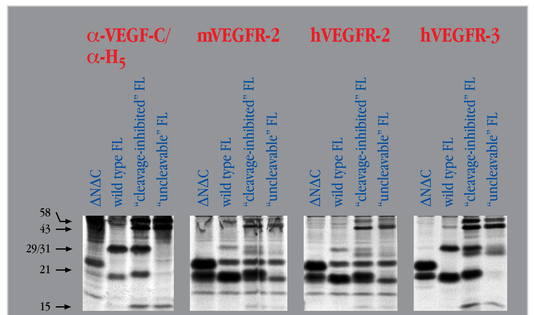
Receptor binding profiles of naturally occurring VEGFs.



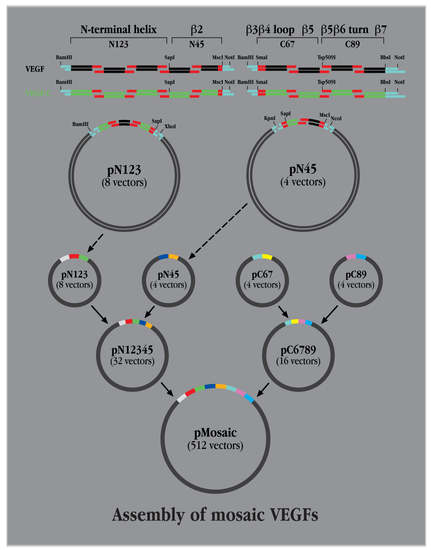
Fragmentation for "non-random" DNA family shuffling

possible mosaic molecules	$2^9 = 512$
secreted	400
binding profiles	
VEGFR-1 and -2 (VEGF analogues)	25
VEGFR-2 and -3 (VEGF-C analogues)	20
VEGFR-1 only	23
VEGFR-2 only	21
VEGFR-3 only	15
VEGFR-1 and -3 (but not VEGR-2)	6
all three VEGF receptors ("super VEGFs")	4

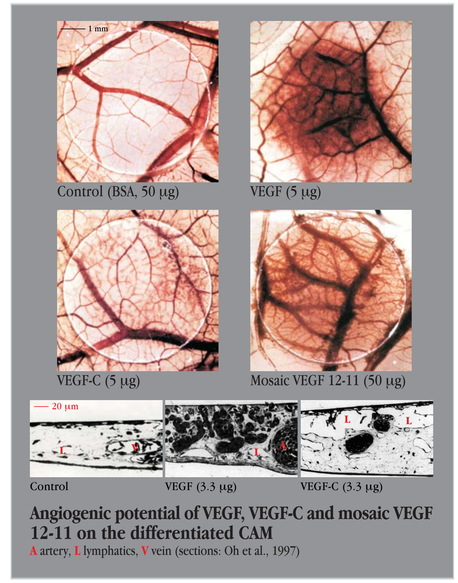
Summary of screening the hybrid library



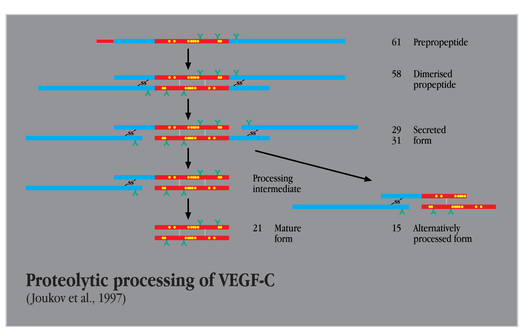
Binding profiles of different VEGF-C forms to receptor $IgGF_c$ bodies
(ΔNΔC: VEGF-C cDNA coding for the mature form, FL: full length cDNA)



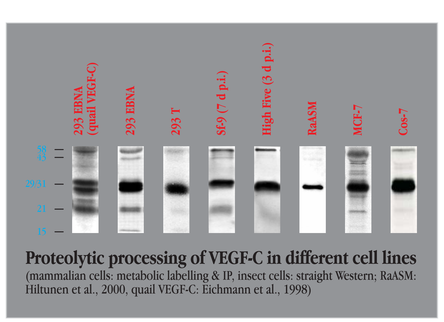
Assembly of mosaic VEGFs



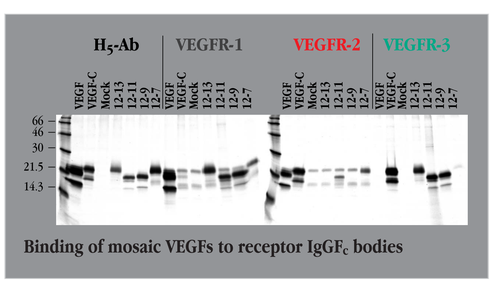
Angiogenic potential of VEGF, VEGF-C and mosaic VEGF 12-11 on the differentiated CAM
A artery, L lymphatics, V vein (sections: Oh et al., 1997)



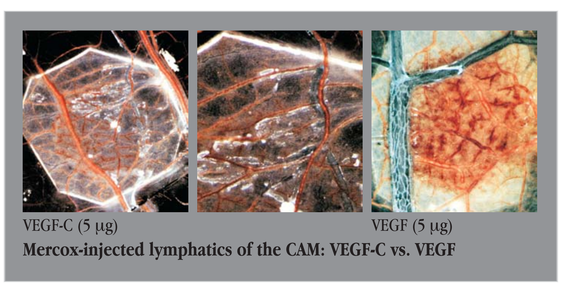
Proteolytic processing of VEGF-C
(Joukov et al., 1997)



Proteolytic processing of VEGF-C in different cell lines
(mammalian cells: metabolic labelling & IP, insect cells: straight Western; RaASM: Hiltunen et al., 2000, quail VEGF-C: Eichmann et al., 1998)



Binding of mosaic VEGFs to receptor $IgGF_c$ bodies



Mercox-injected lymphatics of the CAM: VEGF-C vs. VEGF