

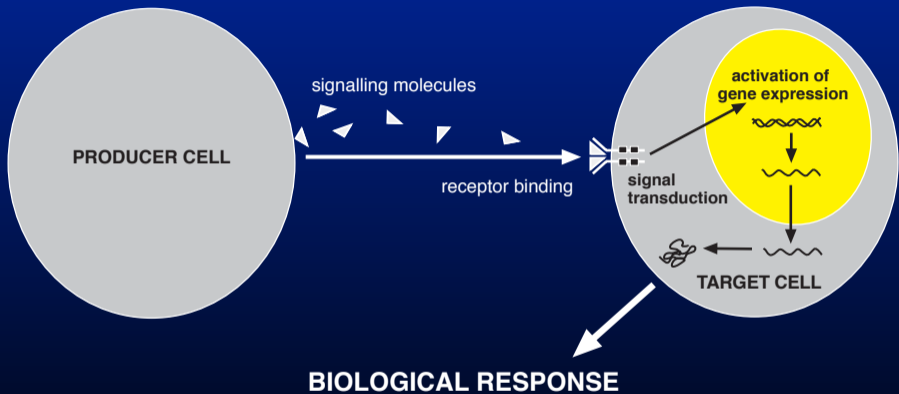
Functional Analysis of VEGF-B and VEGF-C

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Pro Gradu

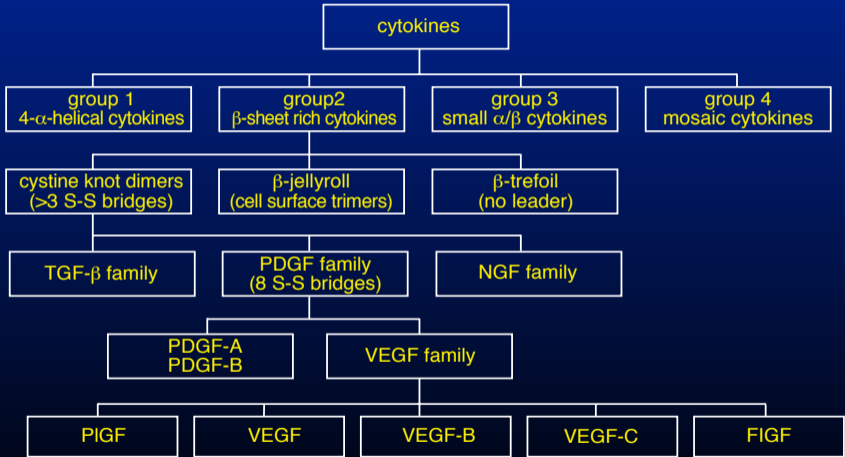
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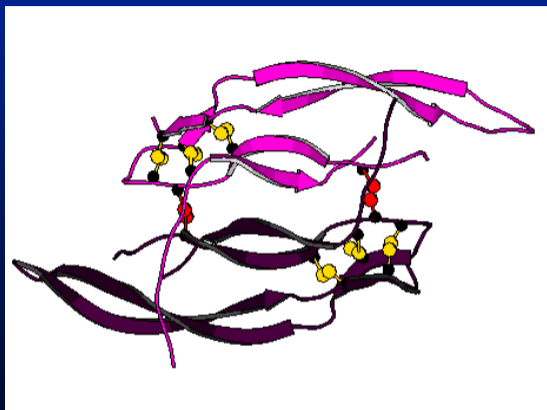
Faculty of Sciences
Division of Biochemistry

January 1997



THE CLASSIFICATION OF CYTOKINES





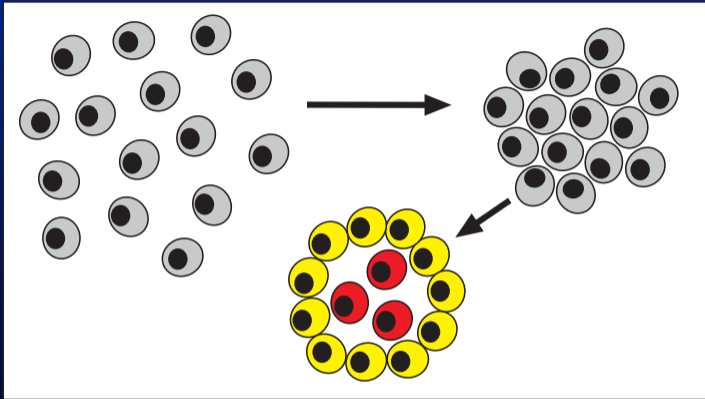
CRYSTAL STRUCTURE OF PDGF-BB

AMINO ACID SEQUENCE ALIGNMENT OF PDGF FAMILY MEMBERS

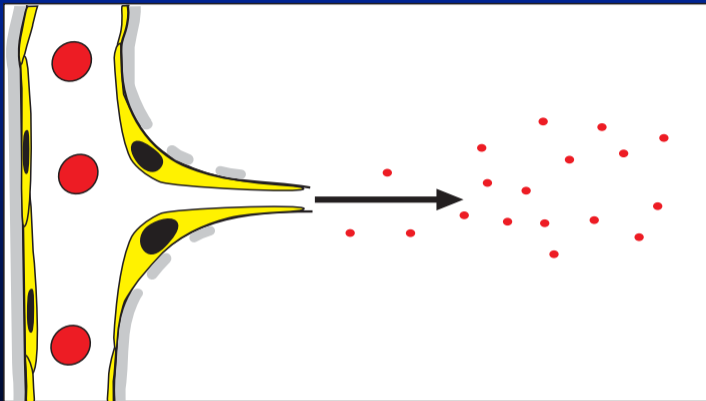
PDGF-AMRTLACL¹LLLLGCGYL²AHVLA³EEAEI⁴PREVIER⁵LARSQ⁶IHS⁷IRDL⁸Q⁹RLL¹⁰EID¹¹SVG¹²SEDSL¹³D¹⁴.TSL¹⁵.
 PDGF-BM¹NRCWA².L³FLSL⁴CCYL⁵RLVSA⁶EGDPI⁷PEELY⁸EMLSD⁹H¹⁰SIR¹¹SFDD¹²L¹³Q¹⁴RLL¹⁵HGDP¹⁶.GEEDGA¹⁷E¹⁸LDL¹⁹..
 PlGF-1MPVMRL¹FP²CC³.FL⁴Q⁵LL⁶AGL⁷LAL⁸...P
 VEGF165MN¹FLLS²..VW³HWSL⁴LALL⁵LYL⁶HH
 VEGF-B167MS¹PLLR²..RL³LLAAL⁴LQ⁵LAPA⁶Q
 VEGF-C MHL¹LG²FP³SVAC⁴SLLAA⁵ALL⁶PG⁷PR⁸.EAP⁹AAAA¹⁰AF¹¹ESGL¹²DL¹³SDA¹⁴EPD¹⁵AGEAT¹⁶AYASK¹⁷DLE¹⁸EQ¹⁹LR²⁰SV²¹SV²²DEL²³MT²⁴VLY²⁵PEY²⁶WK²⁷MY²⁸K²⁹Q³⁰CL³¹RK³²GG³³W³⁴Q
 FIGF MY¹REW²VVV³V⁴VM⁵ML⁶LY⁷Q⁸LV⁹Q¹⁰GSS¹¹NE¹²HG¹³PV¹⁴K¹⁵RS¹⁶SQ¹⁷ST¹⁸LE¹⁹RS.....EQ²⁰Q²¹IRA²²ASS²³LE²⁴EL²⁵LR²⁶ITH²⁷SE²⁸DW²⁹KL³⁰WR³¹CR³²LR³³LK³⁴SF³⁵.

PDGF-A ..AHG¹VHAT²K³HV⁴PE⁵K⁶RPL⁷..PI⁸RR⁹K¹⁰RSI¹¹.....EE¹²AV¹³PAV¹⁴CK¹⁵TR¹⁶TV¹⁷IYE¹⁸I¹⁹PRS²⁰Q²¹VD²²PTS²³AN²⁴FLI²⁵W²⁶PP²⁷CV²⁸EV²⁹K³⁰RC³¹TG³²CC³³NT³⁴SS³⁵VK³⁶C³⁷Q³⁸PS³⁹R⁴⁰VH
 PDGF-B ..M¹TRSH²SG³GE⁴LES.....L⁵ARG⁶RR⁷SL⁸G⁹SL¹⁰IA¹¹E¹²PAM¹³IA¹⁴E¹⁵CK¹⁶TR¹⁷TE¹⁸V¹⁹FEI²⁰SR²¹RL²²ID²³RT²⁴NAN²⁵FL²⁶V²⁷W²⁸PP²⁹CV³⁰EV³¹Q³²RC³³SG³⁴CC³⁵NN³⁶RV³⁷Q³⁸CR³⁹PT⁴⁰Q⁴¹V⁴²Q
 PlGF-1 AV¹PP²Q³QW.....AL⁴SAG⁵NS⁶SE⁷VE⁸V⁹PF¹⁰QE¹¹.VW¹²GR¹³SY¹⁴CR¹⁵ALER¹⁶L¹⁷VD¹⁸V¹⁹VE²⁰Y²¹PS²²..E²³VE²⁴H²⁵MF²⁶PS²⁷CV²⁸S²⁹LL³⁰RC³¹TG³²CC³³G³⁴DN³⁵L³⁶H³⁷CV³⁸P³⁹V⁴⁰ETA
 VEGF165 AK¹WS²QA.....PM³ABEG⁴GG⁵Q⁶NH⁷HE⁸V⁹V¹⁰K¹¹F¹²MD¹³.VY¹⁴QR¹⁵SY¹⁶CH¹⁷PI¹⁸ET¹⁹L²⁰VD²¹I²²F²³Q²⁴E²⁵Y²⁶PD²⁷..E²⁸I²⁹E³⁰Y³¹I³²F³³K³⁴PS³⁵CV³⁶PL³⁷MR³⁸CG³⁹CC⁴⁰N⁴¹DE⁴²GL⁴³EC⁴⁴VP⁴⁵TE⁴⁶ES
 VEGF-B167 AP¹VS²QP.....DAP³GH⁴QR⁵K⁶V⁷V⁸SW⁹ID¹⁰.VY¹¹TR¹²AT¹³CP¹⁴PRE¹⁵V¹⁶V¹⁷PL¹⁸T¹⁹VEL²⁰MG²¹..TV²²AK²³Q²⁴L²⁵V²⁶PS²⁷CV²⁸V²⁹Q³⁰RC³¹CG³²CC³³PD³⁴D³⁵GL³⁶EC³⁷VP³⁸T³⁹G⁴⁰H
 VEGF-C HN¹RE²QAN³LN⁴SR⁵TEE..TI⁶K⁷FAA⁸AH⁹Y¹⁰NT¹¹E¹²IL¹³KS¹⁴ID¹⁵NE¹⁶WR¹⁷K¹⁸T¹⁹QC²⁰MP²¹RE²²VC²³ID²⁴V²⁵G²⁶KE²⁷FGV²⁸..AT²⁹NT³⁰FF³¹K³²PP³³CV³⁴SV³⁵Y³⁶RC³⁷CG³⁸CC³⁹N⁴⁰SE⁴¹GL⁴²QC⁴³MT⁴⁴ST⁴⁵S
 FIGFT¹S²M³D⁴RS⁵S⁶ASH⁷R⁸STR⁹FA¹⁰AT¹¹F¹²Y¹³DI¹⁴ET¹⁵L¹⁶K¹⁷V¹⁸ID¹⁹E²⁰W²¹Q²²RT²³QC²⁴SP²⁵RET²⁶CE²⁷V²⁸EA²⁹SEL³⁰GK³¹..ST³²NT³³FF³⁴K³⁵PP³⁶CV³⁷N³⁸V³⁹FR⁴⁰CG⁴¹CC⁴²NE⁴³ES⁴⁴LI⁴⁵C⁴⁶MT⁴⁷ST⁴⁸S

PDGF-A HRS¹VK²VAK³VE⁴Y⁵VR⁶KK⁷PK⁸L⁹KE¹⁰V¹¹Q¹²VR¹³LEE¹⁴H¹⁵LE¹⁶CA¹⁷CA¹⁸T¹⁹TS²⁰LN²¹PD²²Y²³RE²⁴ED²⁵TD²⁶VR
 PDGF-B LRP¹VQ²VR³K⁴IE⁵IV⁶R⁷KK⁸P⁹IF¹⁰FK¹¹KAT¹²VT¹³LED¹⁴H¹⁵L¹⁶ACK¹⁷CE¹⁸T¹⁹VAA²⁰ARP²¹V²²TR²³SP²⁴GG²⁵S²⁶QE²⁷Q²⁸RA²⁹KT³⁰P³¹Q³²TR³³VT³⁴IR³⁵TV³⁶RV³⁷RR³⁸PP³⁹KG⁴⁰K⁴¹HR⁴²FK⁴³HT⁴⁴HD⁴⁵K⁴⁶TAL⁴⁷KE...
 PlGF-1 NVT¹M²QL³L⁴KIR⁵SG..DR⁶.SY⁷VEL⁸T⁹PS¹⁰QH¹¹VR¹²CE¹³CR¹⁴PL¹⁵RE¹⁶K¹⁷MK¹⁸PER¹⁹CG²⁰D²¹V²²AP²³RR
 VEGF165 NIT¹M²QIM³RI⁴K⁵PH⁶..QG⁷.HIG⁸EM⁹S¹⁰FL¹¹QH¹²NK¹³CE¹⁴CR¹⁵PK¹⁶K¹⁷DR.....AR¹⁸QEN¹⁹PC²⁰GP²¹CS²²ERR²³..HL²⁴F²⁵V²⁶Q²⁷D²⁸P²⁹T³⁰CK³¹CS³²CK³³NT
DS³⁴R³⁵CK³⁶AR³⁷Q³⁸L³⁹..EL⁴⁰..NERT⁴¹CR⁴²CK⁴³PR⁴⁴R
 VEGF-B167 QVR¹M²QIL³MIR⁴YP⁵SS⁶Q...LG⁷EM⁸S⁹LEE¹⁰H¹¹S¹²Q¹³CE¹⁴CR¹⁵PK¹⁶KK¹⁷D.....SA¹⁸V¹⁹K²⁰P²¹DS²²PR²³PL²⁴C²⁵PR²⁶CT²⁷QH²⁸H²⁹Q...RP³⁰..DP³¹RT³²CR³³CR³⁴RRR
S³⁵FL³⁶RC³⁷Q³⁸GR³⁹GL⁴⁰..EL⁴¹..NP⁴²DT⁴³CR⁴⁴CR⁴⁵KL⁴⁶RR
 VEGF-C YLS¹KT²L³FEI⁴TV⁵PL⁶S⁷Q⁸GP⁹.KP¹⁰VT¹¹IS¹²PAN¹³H¹⁴TS¹⁵CR¹⁶MS¹⁷K¹⁸LD¹⁹V²⁰.YR²¹Q²²V²³HS²⁴I²⁵IR²⁶RS²⁷.LP²⁸AT²⁹LP³⁰QC³¹QA³²ANK³³T³⁴CP³⁵T³⁶NY³⁷M³⁸NN³⁹HI⁴⁰CR⁴¹CLA⁴²QE
LA⁴³Q⁴⁴ED⁴⁵FM⁴⁶F⁴⁷SS⁴⁸D⁴⁹AG⁵⁰DD⁵¹ST⁵²D⁵³GF⁵⁴H⁵⁵DI⁵⁶CG⁵⁷.PNK⁵⁸..EL⁵⁹..DE⁶⁰ET⁶¹C⁶²Q⁶³CV⁶⁴CRAG
LR⁶⁵PAS⁶⁶CG⁶⁷.PHK⁶⁸..EL⁶⁹..DR⁷⁰NS⁷¹C⁷²Q⁷³CV⁷⁴CK⁷⁵KNK
LF⁷⁶PS⁷⁷Q⁷⁸CG⁷⁹.ANR⁸⁰..EF⁸¹..DENT⁸²C⁸³Q⁸⁴CV⁸⁵CK⁸⁶RT
CP⁸⁷.RNQ⁸⁸..PL⁸⁹..NPG⁹⁰K⁹¹CA⁹²CE⁹³CTES
P⁹⁴Q⁹⁵K⁹⁶CL⁹⁷L⁹⁸K⁹⁹GK¹⁰⁰..KF¹⁰¹..HH¹⁰²Q¹⁰³T¹⁰⁴CS¹⁰⁵Y¹⁰⁶RR
P¹⁰⁷CT¹⁰⁸NR¹⁰⁹Q¹¹⁰KA¹¹¹CE¹¹²PG¹¹³F¹¹⁴SY¹¹⁵SEE¹¹⁶V¹¹⁷CR¹¹⁸CV¹¹⁹PS¹²⁰Y¹²¹WK¹²²RP¹²³Q¹²⁴MS
 FIGF YIS¹K²Q³LF⁴FEI⁵S⁶V⁷PL⁸TS⁹V¹⁰P¹¹.EL¹²V¹³P¹⁴V¹⁵K¹⁶VAN¹⁷H¹⁸T¹⁹G²⁰CK²¹CL²²PT²³AP²⁴R²⁵HP²⁶Y...SI²⁷IR²⁸RS²⁹Q³⁰I³¹PE³²ED³³RC³⁴SH³⁵SK³⁶KL³⁷CP³⁸ID³⁹ML⁴⁰WD⁴¹SN⁴²CK⁴³CV
L⁴⁴.QE⁴⁵EN⁴⁶PLAG⁴⁷TE⁴⁸.D⁴⁹H⁵⁰SH⁵¹L⁵²Q⁵³EPAL⁵⁴CG⁵⁵.PHM⁵⁶..MF⁵⁷..DE⁵⁸DR⁵⁹CE⁶⁰CV⁶¹CK
TP⁶²CP⁶³.KDL⁶⁴..IQ⁶⁵..HP⁶⁶KN⁶⁷CS⁶⁸CF⁶⁹E⁷⁰CK
ES⁷¹LET⁷²CC⁷³Q⁷⁴.KHK⁷⁵..LF⁷⁶..HP⁷⁷DT⁷⁸CS⁷⁹CE⁸⁰ED⁸¹RC⁸²P
FH⁸³TR⁸⁴P⁸⁵CAS⁸⁶GKT⁸⁷.....ACA⁸⁸K⁸⁹H⁹⁰CR⁹¹FP⁹²KE⁹³KR...



VASCULOGENESIS



ANGIOGENESIS

Angiogenesis in the adult

Female reproductive cycle and pregnancy

Exercise-induced angiogenesis

Wound healing

Skin diseases

Rheumatoid arthritis

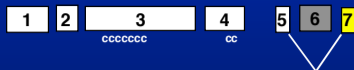
Ocular neovascularization

Ischemic heart and limbs

Tumor angiogenesis

Vascular endothelial growth factor (VEGF)	(Connolly, et al., JBC, 264: 20017-24, Leung, et al., Science, 246: 1306-9, Plouët, et al., EMBO J, 8: 3801-6)	Identity to VEGF in the VEGF homology domain
Placental growth factor (PlGF)	(Maglione, et al., Proc Natl Acad Sci USA, 88: 9267-71)	~53%
VEGF-B/VEGF-related factor (VRF)	(Grimmond, et al., Genome Res, 6: 124-131, Olofsson, et al., Proc Natl Acad Sci USA, 93: 2576-81, Olofsson, et al., JBC, 271: 19310-19317, Townson, et al., Biochem Biophys Res Com, 220: 922-8)	~43%
VEGF-C/VEGF-related protein (VRP)	(Joukov, et al., EMBO J, 15: 290-98, Lee, et al., Proc Natl Acad Sci USA, 93: 1988-1992)	~30%
C-fos-induced growth factor (FIGF)	(Orlandini, et al., Proc Natl Acad Sci USA, 93: 11675-80)	~27%
Flt-1 (VEGFR-1)	(Shibuya, et al., Oncogene, 5: 519-524)	Identity to Flt-1 in the extracellular domain
Flk-1/KDR (VEGFR-2)	(Matthews, et al., Proc Natl Acad Sci USA, 88: 9026-30, Terman, et al., Oncogene, 6: 1677-83)	~36%
Flt-4 (VEGFR-3)	(Galland, et al., Genomics, 13: 475-8, Pajusola, et al., Cancer Res, 52: 5738-43)	~33%

PIGF



VEGF



VEGF-B



VEGF-C



ccccccc

cc

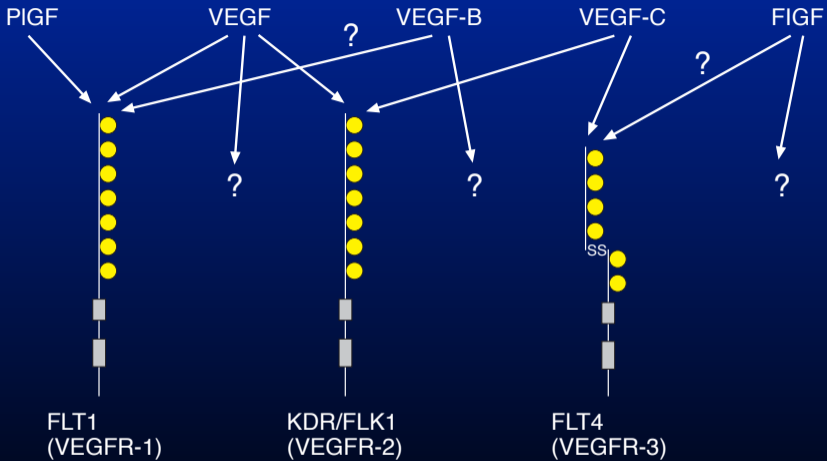
8 conserved cystein residues of the cystine knot consensus sequence (PDGF family subtype) C(24-26X)C(5X)CXXCC(6X)C32-36)CXC

cccc

4 conserved cysteins of the BR3P repeat C(10-14X)CXCXC

ccc

3 conserved cysteins of an incomplete BR3P repeat C(10-14X)CXC



Aim

Comparison of the biological effects of VEGF, VEGF-B and VEGF-C in vivo

Hypothesis

VEGF-C is a growth factor for lymphatic endothelial cells

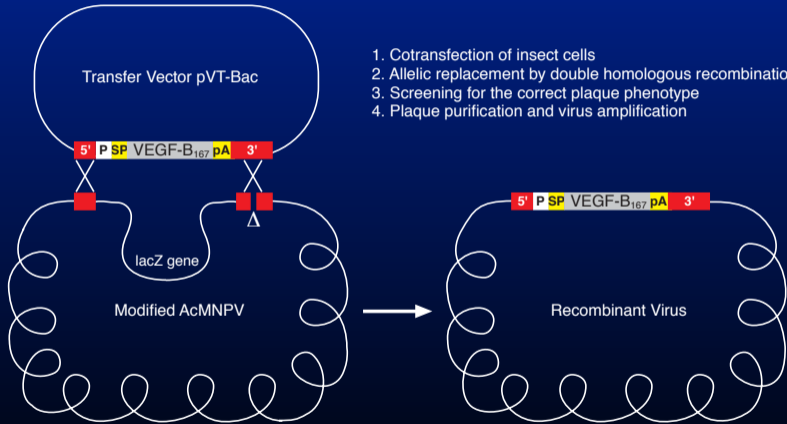
Approaches

Transgenic mice overexpressing VEGF-B and VEGF-C

Direct application of recombinant VEGF-B and VEGF-C

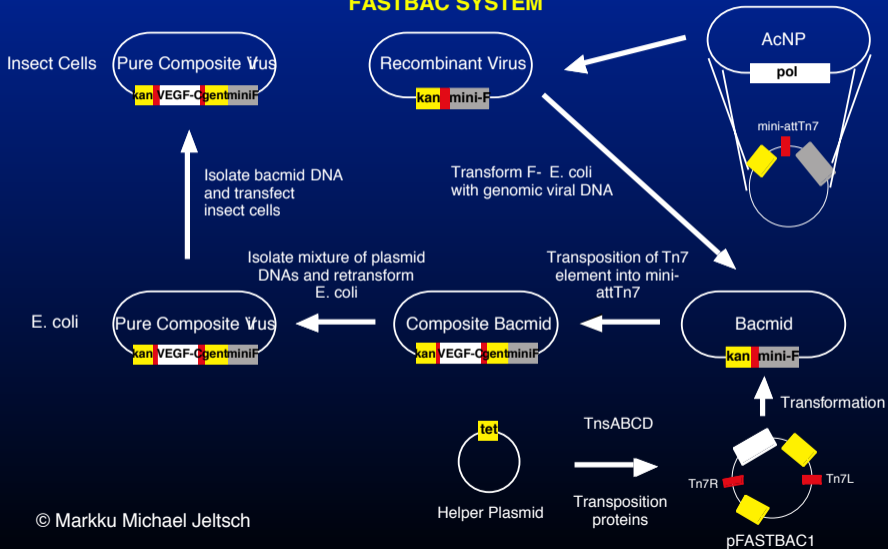
THE BACULOVIRUS PROTEIN EXPRESSION SYSTEM

ALLELIC REPLACEMENT IN INSECT CELLS



THE BACULOVIRUS PROTEIN EXPRESSION SYSTEM

FASTBAC SYSTEM



Infection of HF cells with recombinant virus



Harvest of cell supernatant



Concentration of cell supernatant by ultrafiltration



His₆-tag affinity chromatography (Ni²⁺ NTA)



Size exclusion chromatography (Sephadex G-25)



Quantitative, qualitative and functional assays

Coomassie Brilliant Blue staining

Western blotting & detection with VEGF-C specific antibody

Stimulation of receptor tyrosine phosphorylation in transfected NIH-3T3 cells

Applications

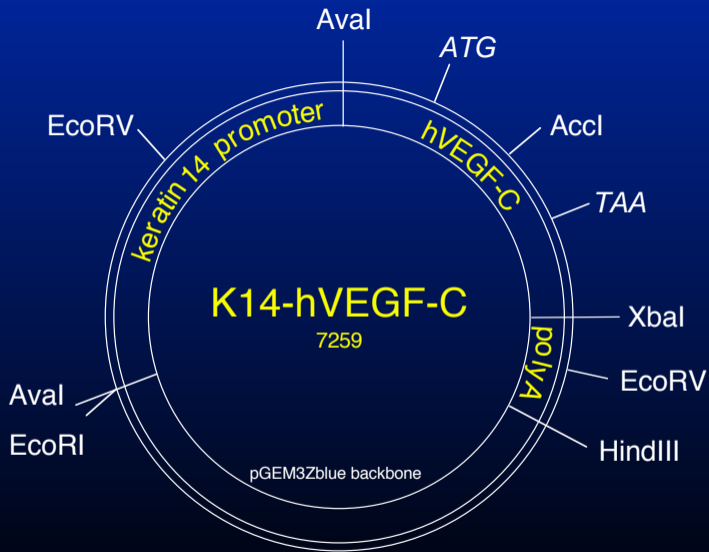
Chorioallantoic membrane assay

Rabbit cornea assay

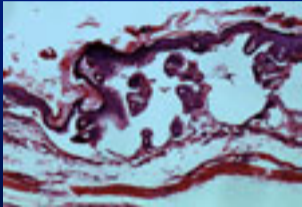
Injection into quail embryos

Effects on non-endothelial cells

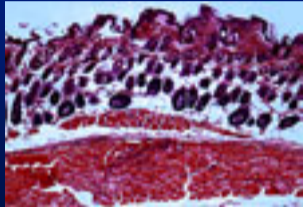
Antibody production



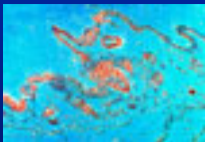




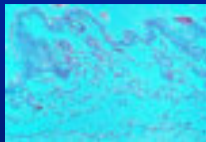
dorsal skin



control mouse



VEGF-C



VEGF-C, sense control



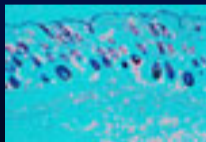
Flt-4



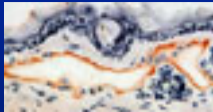
Flt-4, control mouse



FLK-2



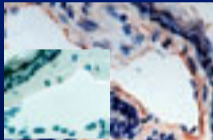
FLK-2, control mouse



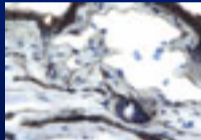
PECAM



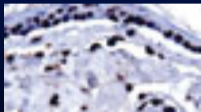
Desmoplakin I & II



Flt-4



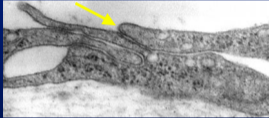
Collagen XVIII



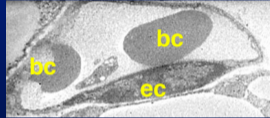
BrdU



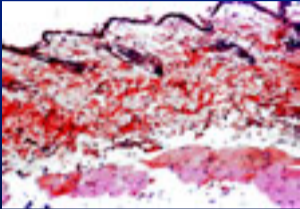
BrdU, control



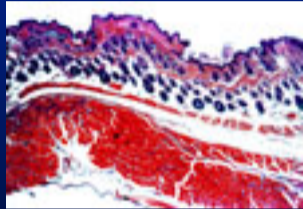
abnormal vessel



blood vessel



dorsal skin (VEGF-B₁₆₇
transgenic mouse)



control mouse

Conclusions

VEGF-C is a growth factor specific for lymphatic endothelial cells and causes hyperplasia of the lymphatic vasculature in vivo

VEGF-C acts in a paracrine fashion

The biological role of VEGF-B remains unknown and mysterious

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My collaborators from outside the Haartman Institute

Sirpa Kontusaari (University of Oulu): α -MHC mice

Stefano Mandriotta (University of Geneva): RIP mice

Xiao-Juan Meng, Merja Lakso and Heikki Rauvala (University of Helsinki, Viikki Biocentre): K14 mice

Ilkka Julkunen (National Public Health Institute, Helsinki): Expertise and practical help in the baculoviral work