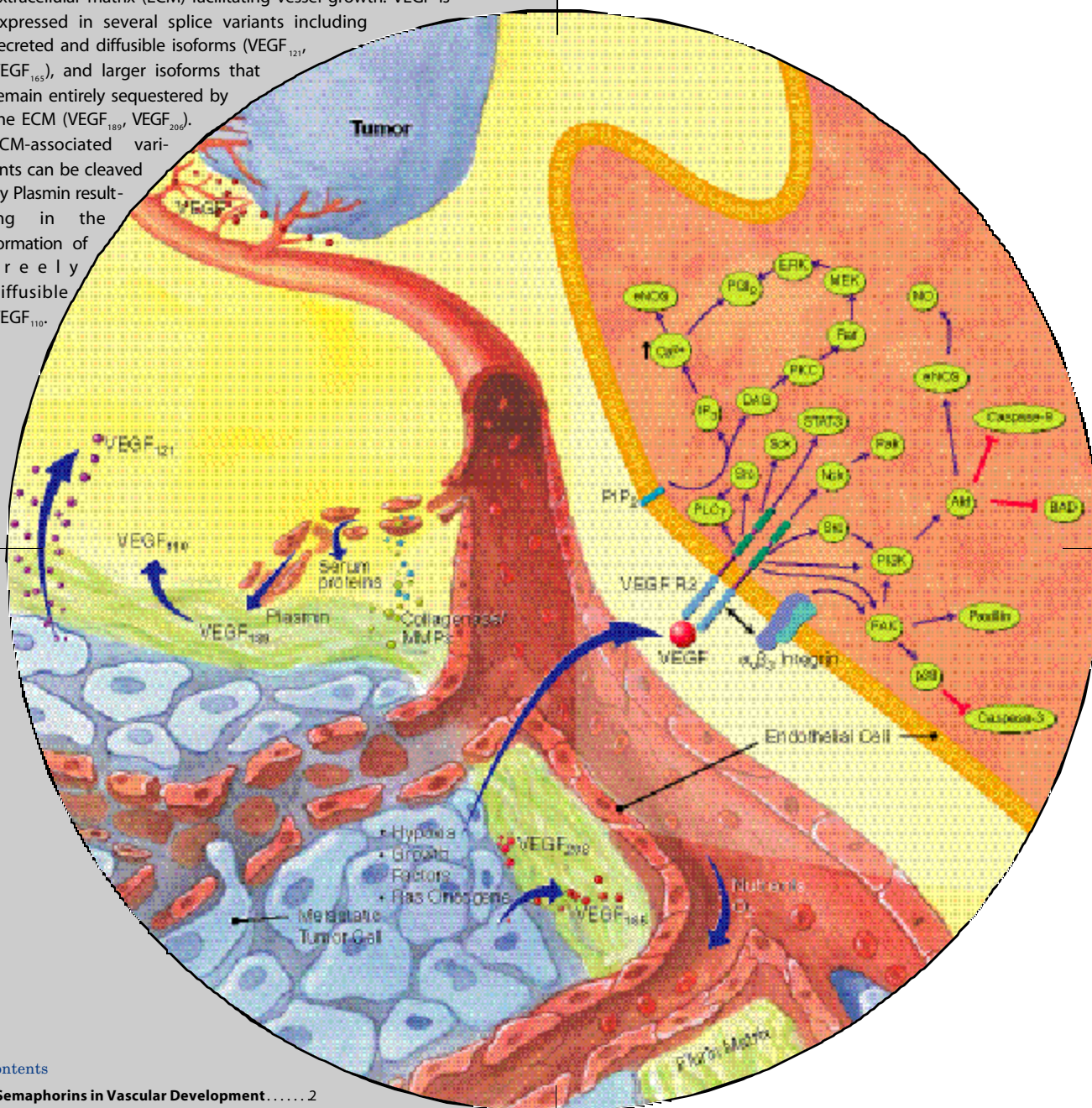


VEGF released from tumor cells stimulates angiogenesis from surrounding vessels. New vessels are leaky, resulting in the extravasation of serum proteins. This leads to the formation of a fibrin matrix that supports endothelial cell growth and migration. Also released are proteases that remodel the extracellular matrix (ECM) facilitating vessel growth. VEGF is expressed in several splice variants including secreted and diffusible isoforms (VEGF₁₂₁, VEGF₁₆₅), and larger isoforms that remain entirely sequestered by the ECM (VEGF₁₈₉, VEGF₂₀₆). ECM-associated variants can be cleaved by Plasmin resulting in the formation of freely diffusible VEGF₁₁₀.



Contents

Semaphorins in Vascular Development	2
Hemangioblast Identification	3
R&D Systems' Angiogenesis-related Products	4-5
VEGF-C and VEGF-D	6
IL-24 in Cancer Growth Suppression, Apoptosis, and Angiogenesis	7
Receptor Tyrosine Kinase DuoSet® IC ELISA Development Kits	8

Vascular Development Research Tools

Current research in vascular development focuses on the molecules involved in the shared patterning mechanisms between the nervous and vascular systems. R&D Systems offers the following antibodies applicable for studying three groups of molecules presently implicated in neurovascular congruency: Neuropilins, Semaphorins, and VEGF.

Antibodies and Applications Available

ANALYTE	Bioassay*	Western blot	ELISA	IHC	Flow Cytometry
Neuropilin-1	R	R	R		
Neuropilin-2		R	R		
Semaphorin 3A		H	H		
VEGF	H M R	H M R	H M R	H R	H
VEGF _{120/164}		M	M		
VEGF ₁₆₄		M	M		
VEGF-B		H	H		
VEGF-B _{167/186}		H M	H M		
VEGF-B ₁₈₆	H M	H M	H M	M	
VEGF-C		H	H	H	
VEGF-D	H	H M	H M	H	
VEGF R1 (Flt-1)	H M	H M	H M	H M	H
VEGF R2 (KDR)	H M	H M	H M	H M	H
VEGF R3 (Flt-4)		H M	H M	H	H M

*Neutralization and/or Adhesion Blockade

Legend: H human M mouse R rat

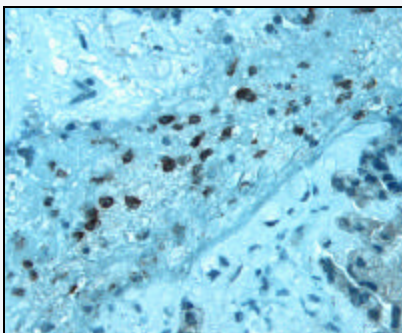


Figure 1. Detection of VEGF R1 (Flt-1) in paraffin-embedded human ovarian cancer tissue sections using R&D Systems' mouse anti-human VEGF R1 monoclonal antibody (Catalog # MAB321). Tissues were stained using R&D Systems' Mouse HRP-DAB Cell and Tissue Staining Kit (Catalog # CTS002; brown) and counter-stained with hematoxylin (blue). Antigen retrieval was performed using R&D Systems' Basic pH Retrieval Reagent (Catalog # CTS013).

Semaphorins in Vascular Development

The nervous and vascular systems exhibit striking anatomical, developmental, and molecular parallels. Both nerves and vessels form complex branching networks throughout the body, and cells from both systems form and retract filopodia to precisely guide their paths through areas of attractive and repulsive forces.¹⁻⁴ Nerves and vessels often follow similar routes during embryogenesis.^{1,2} One of the most prominent examples occurs in the vertebrate limb, where major nerves of the peripheral nervous system are co-localized with major arteries. These neurovascular bundles supply muscles and bones in a phenomenon termed neurovascular congruence.⁵ A hypothesis to explain neurovascular congruency suggests that the nervous and vascular systems use shared patterning mechanisms involving such signaling molecules as Semaphorins, Vascular Endothelial Growth Factor (VEGF), and their shared receptors, Neuropilins (Figure 1).⁵

Neuropilins are transmembrane proteins that function as receptors for the Class-3 Semaphorin subfamily as well as for certain splice forms of VEGF. Hence, Neuropilins have been shown to modulate both axon guidance and angiogenesis.^{6,7} VEGF₁₆₅, a well-known angiogenic factor, induces blood vessel growth and positively influences cell motility and the survival of endothelial cells.^{2,7} It also plays important roles in the nervous system such as encouraging neurogenesis and promoting events involved in nerve regeneration.^{8,9} What is not as well known, and garnering some recent research interest, is the role of Semaphorins in vascular development.

Semaphorins are key mediators of axon guidance: acting as inhibitors of axonal motility, repulsing neurite outgrowth, and collapsing growth cones.¹⁰ Semaphorins also impact cardiovascular development. Ectopic expression of Semaphorin 3A (Sema 3A) in developing chick forelimbs causes altered pathways of peripheral nerves and hypovascularization, and suggests that the role of Sema 3A is to refine neurovascular patterning.⁵ *In vitro* experiments demonstrate that Sema 3A impacts the development of the vasculature by inhibiting endothelial cell motility, preventing microvessel outgrowth and sprouting, and by disrupting lamellipodia formation and cytoskeletal organization.¹¹ These effects are dependent on Sema 3A binding Neuropilin-1 expressed on the surface of endothelial cells.¹¹ One way that Sema 3A may control endothelial cell migration is by competitive inhibition with VEGF₁₆₅. VEGF₁₆₅ and Sema 3A compete for Neuropilin-1 binding sites in both endothelial and dorsal root ganglion cells.¹¹ Another way that Sema 3A may inhibit endothelial cell motility is by suppressing integrin function, allowing for de-adhesion between endothelial cells and the extracellular matrix.¹² Even earlier in vascular development, Sema 3A regulates the pathway of migrating endothelial cell precursors (angioblasts) through the dorsal regions of the somites and is necessary (along with VEGF) for dorsal aorta formation in zebrafish.¹³ Neuropilin signaling via both VEGF and Class-3 Semaphorins in endothelial cells also helps coordinate various aspects of heart development. Septation of the outflow tract, topographic origin of the coronary arteries, and proper development of the atria are all dependent on Neuropilin signaling.¹⁴ Thus, a balance of Neuropilin signaling via Semaphorins and VEGF₁₆₅ serves to modulate endothelial cell migration, and ultimately contributes to the process of angiogenic remodeling and cardiovascular development.

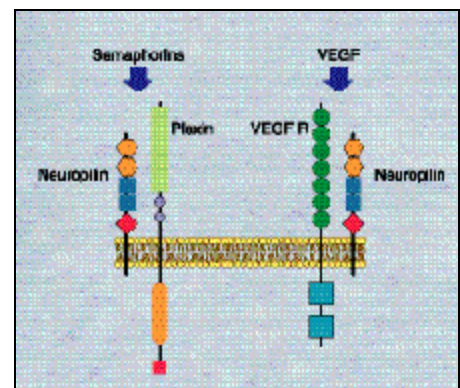


Figure 1. Semaphorins and VEGF signal through common co-receptors, Neuropilins, in vascular and nervous system development.

References

- Shima, D.T. & C. Mailhos (2000) *Curr. Opin. Genet. Dev.* **10**:536.
- Carmeliet, P. (2003) *Nat. Rev. Genet.* **4**:710.
- Dickson, B.J. (2002) *Science* **298**:1959.
- Gerhardt, H. *et al.* (2003) *J. Cell Biol.* **161**:1163.
- Bates, D. *et al.* (2003) *Dev. Biol.* **255**:77.
- Neufeld, G. *et al.* (2002) *Trends Cardiovasc. Med.* **12**:13.
- Klagsbrun, M. *et al.* (2002) *Adv. Exp. Med. Biol.* **515**:33.
- Sondell, M. *et al.* (2000) *Eur. J. Neurosci.* **12**:4243.
- Zhu, Y. *et al.* (2003) *FASEB J.* **17**:186.
- He, Z. *et al.* (2002) *Sci. STKE* **119**:RE1.
- Miao, H.-Q. *et al.* (1999) *J. Cell Biol.* **146**:233.
- Serini, G. *et al.* (2003) *Nature* **424**:391.
- Shoji, W. *et al.* (2003) *Development* **130**:3227.
- Gu, C. *et al.* (2003) *Dev. Cell* **5**:45.

Hemangioblast Identification

The hemangioblast is defined as a precursor cell with the potential to differentiate into both hematopoietic and endothelial cell lineages. The concept of the hemangioblast was first introduced more than 80 years ago based on histological analysis of the developing blood islands in the yolk sac.¹ It has since gained support from studies showing that the hematopoietic and endothelial lineages express a number of genes in common, such as CD34, VEGF R2 (also known as Flk-1 and KDR), SCL, Runx1 and GATA-1.²⁻⁶ The most direct evidence for the existence of the embryonic hemangioblast was provided by studies examining the *in vitro* differentiation of embryonic stem (ES) cells.^{7,8}

As hemangioblasts were more closely examined, additional specific markers were described (Table 1). VEGF R2 has been shown to play essential roles in vascular and hematopoietic development⁹ and its expression is used to define early embryonic hematopoietic precursors as well as vascular progenitors.^{5,10} Podocalyxin (also known as PODXL1), a sialoglycoprotein that is structurally related to CD34, has been used as a cell surface marker for identification of hemangioblasts from the aorta-gonad-mesonephros (AGM) region.¹¹ Recently, it has been shown that hemangioblast, blood cell, and vascular differentiation were impaired in ES cells deficient in the ephrin receptor, EphB4,¹² which suggests that EphB4 may be used as a functional marker to identify embryonic hemangioblasts. Adult hemangioblasts have also recently been discovered. Self-renewing adult hematopoietic stem cells with functional hemangioblast activity were characterized by their ability to clonally differentiate into all hematopoietic cell lineages as well as endothelial cells that revascularize adult retina.¹³ These bone marrow-derived and circulating postnatal progenitors appear to share similar antigenic determinants with embryonic hemangioblasts, such as CD34, VEGF R2, and CD133. Additionally, it has been shown that CD133-positive cells from granulocyte colony-stimulating factor-mobilized peripheral blood not only have hematopoietic potential, but also have the capacity to differentiate into endothelial cells.¹⁴

Hemangioblasts are frequently characterized by their ability to differentiate into hematopoietic and endothelial lineages as indicated by functionally related gene expression patterns. SCL, also known as TAL1, is selectively expressed in cells of hematopoietic, vascular and nervous system origin^{3,15} and is thought to be required for hemangioblast development and subsequent maturation to the hematopoietic program.^{6,16,17} Runx1, also known as CBFA2 and AML-1, belongs to a family of proteins known as core binding factors and is suggested to play functional roles at specific stages of hemangioblast development and commitment to the primitive and definitive hematopoietic lineages.^{18,19} The transcription factor GATA-1 is required to initiate blood formation in the embryo and appears to simultaneously promote differentiation and inhibit proliferation.^{20,21} CD31, also known as PECAM-1, is a member of the immunoglobulin superfamily and its expression is limited to endothelial cells, platelets, leukocytes, and their precursors. During angiogenesis, PECAM-1 is thought to be involved in the motility of endothelial cells and subsequent organization into vascular tubes.^{22,23}

MARKER	POLYCLONAL	MONOCLONAL	LABELLED
VE-Cadherin	H M	H	
CD31 (PECAM-1)	H	H	
CD133 (AC133)		H M	
EphB4	M	M	
GATA-1		H	
Podocalyxin (PODXL1)	H	H M	
Tie-2	H M Z	H Z	H
VEGF R2 (Flk-1/KDR)	H M	H M	H

Legend
 H Human
 M Mouse
 Z Zebrafish

Table 1. R&D Systems' antibodies to hemangioblast markers.

References

- Sabin, F.R. (1920) *Contrib. Embryol.* **9**:213.
- Millauer, B. *et al.* (1993) *Cell* **72**:835.
- Kallianpur, A.R. *et al.* (1994) *Blood* **83**:1200.
- Young, P.E. *et al.* (1995) *Blood* **85**:96.
- Kabrun, N. *et al.* (1997) *Development* **124**:2039.
- Robertson, S.M. *et al.* (2000) *Development* **127**:2447.
- Kennedy, M. *et al.* (1997) *Nature* **386**:488.
- Choi, K. *et al.* (1998) *Development* **125**:725.
- Shalaby, F. *et al.* (1995) *Nature* **376**:62.
- Yamashita, J. *et al.* (2000) *Nature* **408**:92.
- Hara, T. *et al.* (1999) *Immunity* **11**:567.
- Wang, Z. *et al.* (2004) *Hematopoiesis* **103**:10.
- Grant, M.B. *et al.* (2002) *Nat. Med.* **8**:607.
- Gehling, U.M. *et al.* (2000) *Blood* **95**:3106.
- Green, Q. *et al.* (1992) *Oncogene* **7**:653.
- Gering, M. *et al.* (1998) *EMBO J.* **17**:4029.
- Visvader, J.E. *et al.* (1998) *Genes Dev.* **12**:473.
- Wang, Q. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:3444.
- Lacaud, G. *et al.* (2004) *Blood* **103**:886.
- Briegleb, K. *et al.* (1996) *Development* **122**:3839.
- Fujiwara, Y. *et al.* (2004) *Blood* **103**:583.
- DeLisser, H.M. *et al.* (1997) *Am. J. Pathol.* **151**:671.
- Cao, G. *et al.* (2002) *Am. J. Physiol. Cell Physiol.* **282**:C1181.

MagCollect Products

R&D Systems' new MagCollect* technology is based on the use of Ferrofluids or magnetic nanoparticles that have no magnetic memory (superparamagnetic). Both complete kits and stand-alone MagCollect Ferrofluids are available. MagCollect cell isolation kits and reagents allow fast and easy separation of highly pure cell populations without the need for expensive instrumentation.

Kit Contents

- MagCollect Antibody Cocktail
- MagCollect Ferrofluid
- MagCollect 10X Buffer

Enrich for

- B Cells
- T Cells
- T Cell Subsets

Species

- Human • Mouse • Rat

MagCollect Magnet

designed to effectively work with all MagCollect Kits and Reagents and can accommodate six 12 x 75 mm (5 mL) or two 17 x 100 mm (15 mL) round bottom tubes.

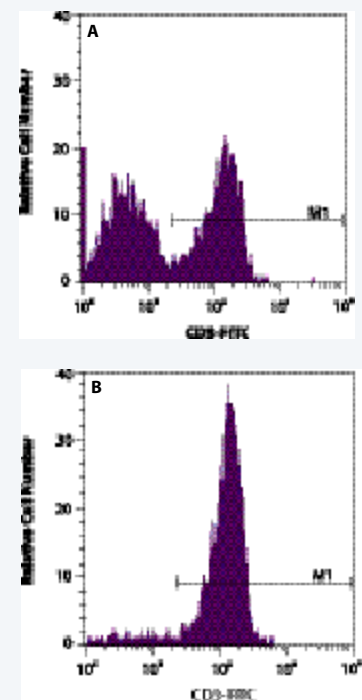


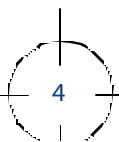
Figure 1. Mouse splenocytes before A) and after B) separation of CD3⁺ T cells using R&D Systems' MagCollect CD3⁺ T Cell Isolation Kit (Catalog # MAGM201). Histograms reflect viable cells stained with CD3-FITC. Purity of all isolated cells for this experiment was 98%.

*MagCollect products utilize and/or contain technology licensed from Immunicon Corporation, Huntingdon Valley, Pennsylvania 19006 USA, which is covered by one or more claims of United States and International patents and/or pending patent applications.

ANGIOGENESIS RESEARCH REAGENTS

from
R&D SYSTEMS

	Protein	Antibody	ELISA/ Assay	Primer Pair		Protein	Antibody	ELISA/ Assay	Primer Pair
ACE	H M	H	H		Ephrin-A1	M	M		
ADAMTS4				H	Ephrin-A2	M	M		
ADAMTS5				H	Ephrin-A3	H	H		
ALS		M			Ephrin-A4	H M	H M		
ANG	H	H	H		Ephrin-A5	H	H		
Ang-1	H	H		H M	Ephrin-B1	M	M		
Ang-2	H	H	H	H M	Ephrin-B2	M Z	M Z		
Ang-3	M	M			Ephrin-B3	H	H		
Ang-4	H	H			Epo	H M R	H M	H M	
Ang-like 3		M			Epo R	H M	H M		
Ang-like factor		H			ErbB2	H	H		
Angiostatin		H			ErbB3	H	H	H	
Artemin	M	M			ErbB4	H	H		
Cathepsin B	H M	H M	H		ERK1/MAPK3		H M R	Mu	
Cathepsin D	H M	H M	H		ERK1/ERK2		H M R Mu	Mu	
Cathepsin L	H M	H M			FGF acidic	H B	H B	H	
Cathepsin S	H	H			FGF basic	H B	H B	H	H
CCR1		H		H M R	FGF-BP	R	H R		
CCR3		H M		H M R	FGF R1	H	H		
CCR5		H		H M R	FGF R2	H M	H M		
CD31/PECAM-1	H	H			FGF R3	H M	H M	H	
CD133		H M			FGF R4	H	H M		
Collagen I	R B				Fibronectin	B			
Collagen IV	M				GATA-1		H		
Common γ Chain	H M	H M			gp130	H M	H M	H	
COX-1			H	H M R	HAI-1	H M	H M		
COX-2			H	H M R	HAI-2		H M		
CTGF		H			HGF	H	H	H	H
CXCR1/IL-8 RA		H		H M R	HGFA	H M	H M		
CXCR2/IL-8 RB		H		H M R	HGF R/c-Met	H M	H M		
CXCR4		H	H	H M	ICAM-1/CD54	H M R	H M R	H M R	H M R
DLL4	H M	M			ICAM-2/CD102	H M	H M		
E-Selectin	H M R	H M R	H M	H M R	IGF-I	H M	H M	H	
EGF	H	H	H		IGF-I R	H	H	H	
EGF R	H	H M	H Mu		IGFBP-1	H M	H M	H	
ENA-78/CXCL5	H	H	H		IGFBP-2	H M	H M	H M	
Endoglin/CD105	H M	H M	H		IGFBP-3	H M	H M	H M	
Endostatin		H M	H		IGFBP-4	H	H	H	
Endothelin-1			H		IGFBP-5	H M	H M	M	
Eotaxin/CCL11	H M	H M	H M		IGFBP-6	H M	H M	H M	
EphA1	H	H			IGFBP-rp1/IGFBP-7	H	H		
EphA2	M				IL-1 α /IL-1F1	H M R P CR	H M R P CR	H M R	H M R
EphA3	M	M			IL-1 β /IL-1F2	H M R P CR RM	H M R P CR	H M R P	H M R
EphA4	M	M			IL-1ra/IL-1F3	H M R P	H M P	H M	
EphA5	R	R			IL-1 RI/IL-1 R1	H M	H M	H	H
EphA6	M	M			IL-1 RII/IL-1 R2	H M	H M	H	H
EphA7	M	M			IL-2 R β	H	H M		
EphA8	M	M			IL-6	H M R P Ca CR	H M R P CR	H M R P	H M R
EphB1	R	R			IL-6 R	H	H	H	
EphB2	M	M			IL-8/CXCL8	H P Ca	H P	H P	H
EphB3	M	M			IL-10	H M R P Ca C R E F V	H M R P Ca C R F V	H M R P Ca F	H M R
EphB4	M	M							
EphB6	M	M							



Key

H Human
M Mouse

R Rat
P Porcine

B Bovine
Ca Canine

CR Cotton Rat
E Equine

F Feline
Mu Multi-species

RM Rhesus Macaque
V Viral

Z Zebrafish

	Protein	Antibody	ELISA/ Assay	Primer Pair
IL-10 R α	H M	H M		
IL-10 R β	H	H		
IL-12	H M P	H M P	H M	M R
IL-12 R β 1	H	H		
IL-15	H M	H M	H	H M R
IL-15 R α	H M	H M	M	
IL-17	H M	H M	H M	H M
IL-17 R	H	H M		
IL-18/IL-1F4	H M R P	H M R P	H M	
IL-18 R α /IL-1 R5	H	H M		
IL-18 R β /IL-1 R7	H	H M		H
Integrin α 4/CD49d		H		
Integrin α V/CD51		H		
Jagged 1	R	H R		
Kininogen	H			
Kininostatin	H	H		
Laminin I	M			
Leptin	H M R	H M	H M R	
Leptin R	H M	H M	M	
MCP-1/CCL2	H Ca	H	H	H
Midkine	H	H		
MIF	H	H	H	
MIP-1 α /CCL3	H M CR	H M CR	H M	
MMP-1	H	H	H	H
MMP-2	H M	H M	H M	H M
MMP-3	H M	H M	H M	H
MMP-7	H	H	H	H
MMP-9	H M	H M	H M	H
MMP-11				H
MMP-12	H	H		
MMP-13	H	H	H	
MMP-14	H	H		
MMP-24/MT5-MMP		H M		
Neuropilin-1	R	R		
Neuropilin-2	R	R		H M R
Nitrite/Nitrate			Mu	
Nitric Oxide (NO)			Mu	
eNOS		H	H	H M
iNOS		H	H M	H M
nNOS				H M
Notch-1	R			
Notch-2	R	R		
Notch-3	H M	H M		
NRG1- β 1/HRG- β 1	H	H	H	
Osteopontin	H M B	H M		
p38 α	H	H M R	Mu	
PD-ECGF	H	H		
PDGF	H P	H Mu		
PDGF-AA	H R	H R	H	
PDGF-AB	H R		H	
PDGF-BB	H R	H	H	
PDGF-CC	H			

	Protein	Antibody	ELISA/ Assay	Primer Pair
PDGF R α	H M	H M		H
PDGF R β	H M	H M	H	H M R
PEDF		H M		
PGE ₂			Mu	
Plasminogen Kringle 5		M		
PIGF	H	H	H	H M
PIGF-2	M	M	M	
PODXL-1		H M		
PTN	H	H		
RECK		H		H M
RSK1		H M R	Mu	
RSK2		H M R		
RSK3		Mu		
SDF-1/CXCL12	H M	H M	H M	
Semaphorin 3A	H	H		
Serpin C1	H M	M		
Sonic Hedgehog	H M	H M	M	
SPARC		H M		
STAT1		H M	H M	
STAT3		H M		
STAT4		H M	H	
TGF- α	H	H	H	
TGF- β 1	H P	Mu	H M R P	H M
TGF- β 1 LAP	H	H		
TGF- β 1 Latent	H		H	
TGF- β 1.2	H			
TGF- β 2	H P	Mu	H	H
TGF- β 3	H	Mu	H	
TGF- β RI/ALK-5	M	M		H M R
TGF- β RII	H M	H M		
TGF- β RIII	H	H		
Thrombin	H	H		
Tie-1	H	H		H M
Tie-2	H M Z	H M Z	H	H M
TIMP-1	H M R	H M R	H M R	H M R
TIMP-2	H	H	H	H M R
TIMP-3	H	H		H M R
TIMP-4	H	H		H M
TNF- α /TNFSF1A	H M R P Ca CR RM	H M R P CR RM	H M R P	H M
TNF RI/TNFRSF1A	H M	H M	H M	H M
TNF RII/TNFRSF1B	H M	H M	H M	H M
uPA	H	H		
uPAR	H M	H M	H	
VCAM-1/CD106	H M	H M	H M	H M
VEGF	H M R Ca Z	H M R	H M R	H M
VEGF-B	H	H M		H M
VEGF-C	H	H		H M
VEGF-D	H M	H M	H M	H M
VEGF R1/Flt-1	H M	H M	H M	H M
VEGF R2/KDR	H M	H M	H M Mu	H M
VEGF R3/Flt-4	H M	H M	H M	H M

Please visit www.RnDSystems.com/Angiogenesis for an up-to-date Angiogenesis product listing.

Key

H Human
M Mouse

R Rat
P Porcine

B Bovine
Ca Canine

CR Cotton Rat
E Equine

F Feline
Mu Multi-species

RM Rhesus Macaque
V Viral

Z Zebrafish

Human Endoglin Quantikine® ELISA

Endoglin (CD105) is a 180 kDa homo-dimeric co-receptor for members of the TGF- β superfamily that appears to play a key role in angiogenesis. Proliferating endothelial cells over-express Endoglin both *in vitro* and *in vivo*, and anti-Endoglin antibodies significantly reduce proliferation.^{1,2} Endoglin-deficient mice die of defective vascular development by 11.5 days gestation.³ In addition, Endoglin is a suitable target for the imaging of solid tumors and has been used to quantify intratumoral microvessel density, a prognostic factor in many cancers.^{1,4} Elevated levels of circulating soluble Endoglin have been observed in patients with both solid and hematological cancers, thus suggesting it may be an effective surrogate marker for angiogenic activity and/or a potential target for anti-angiogenic drugs.⁴⁻⁷

R&D Systems' Human Endoglin Quantikine ELISA has been validated for a variety of sample types including cell culture supernates, serum, and plasma. It has a sensitivity of 0.007 ng/mL and a linear range of 0.156 - 10 ng/mL (Figure 1).

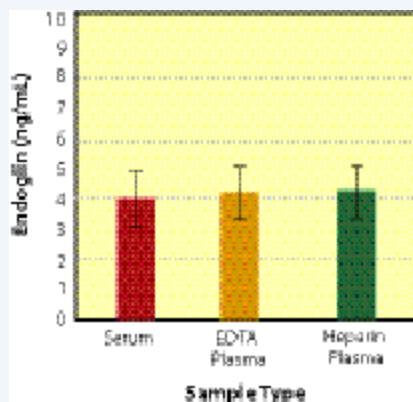


Figure 1. Endoglin detected in human serum and plasma using R&D Systems' Human Endoglin Quantikine ELISA kit (Catalog # DNDG00). Data are represented as average values of greater than 30 samples \pm standard deviation.

References

- Fonsatti, E. (2000) Clin. Cancer Res. **6**:2037.
- Maier, J.A. *et al.* (1997) Anticancer Drugs **8**:238.
- Li, D.Y. *et al.* (1999) Science **284**:1534.
- Fonsatti, E. *et al.* (2003) Oncogene **22**:6557.
- Li, C. *et al.* (2000) FASEB J. **14**:55.
- Takahashi, N. *et al.* (2001) Clin. Cancer Res. **7**:524.
- Calabro, L. *et al.* (2003) J. Cell. Physiol. **194**:171.

VEGF-C and VEGF-D

Full length versions of VEGF-C and -D contain a VEGF homology domain (VHD), flanking N- and C-terminal pro regions, and an N-terminal signal sequence (Figure 1).^{1,2} The C-terminal pro region is cysteine-rich, with two copies of a C₆C₁₀CRC motif and five nearly complete copies of a C₁₀CXCXC motif.¹ Three intrachain disulfide bonds create a cystine knot motif, which is a hallmark of all VEGF family proteins.² The VHD from VEGF-C and -D share approximately 60% identity with each other and 30% identity with VHD from other members of the family.

Processing of VEGF-C and -D preproteins begins intracellularly and continues after secretion (Figure 1). The proteins associate into antiparallel disulfide-linked homodimers that are then proteolytically cleaved. The C-terminal propeptides are removed late in the exocytic pathway or shortly after secretion, and the N-terminal propeptides are cleaved after secretion.³ There are multiple cleavage and association intermediates in the processing of both VEGF-C and -D. The proteases Furin, PC5, and PC7 have been shown to remove the propeptides of VEGF-C.⁴ Plasmin

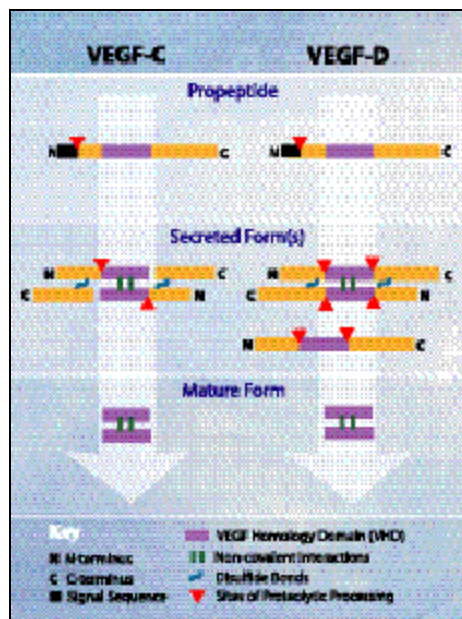


Figure 1. Schematic depiction of the proteolytic processing of VEGF-C and -D. [Note: figure adapted from Joukov, V. *et al.* (1998) EMBO J. **16**:3898 and Stacker, S.A. *et al.* (1999) J. Biol. Chem. **274**:32127.]

can remove the propeptides of both VEGF-C and -D.⁵ It is the proteolytic separation of the VHD that distinguishes VEGF-C and -D from other members of the VEGF family. VHD dimers derived from VEGF-C and -D are high affinity ligands for VEGF R2 (KDR) and VEGF R3 (Flt-4).² VHD dimers from other VEGF proteins can induce signaling through VEGF R2, but not through VEGF R3. As the proteolytic maturation of VEGF-C and -D progresses, their ability to bind and activate VEGF R3 is increased. The full-length dimeric factors can also activate VEGF R3, but with many fold lower potency. In contrast, VEGF-C and -D are not able to activate VEGF R2 until their maturation is further advanced.^{3,6} This difference leaves open the possibility that changes in the processing of VEGF-C and -D could differentially alter their ability to signal through VEGF R2 compared to VEGF R3.⁶ VEGF R2 is primarily expressed on endothelial cells lining blood and lymphatic vessels. VEGF R3 expression is found mainly on lymphatic vessel endothelium. VEGF R3 is expressed on embryonic venous as well as lymphatic endothelium, but becomes restricted to lymphatic and high endothelial venules in the adult.^{7,8} The ability of VEGF-C and -D to activate two receptors with different distribution patterns generates a complex picture of their effects. *In vivo*, VEGF-C overexpression in the skin induces lymphatic, but not vascular, endothelial cell proliferation and enlargement.⁹ Local delivery of VEGF-C induces sprouting of new blood vessels in a corneal implant model.¹⁰ Witzensbichler *et al.* reported that VEGF-C is mitogenic and chemotactic for microvascular endothelial cells and also promotes neovascularization in an ischemia model.¹¹ Mature VEGF-D apparently acts in a tissue specific manner, as it induces angiogenesis and lymphangiogenesis in a skin model, but only angiogenesis in a muscle model.¹² The angiogenic activity of VEGF-C and -D is presumed to be mediated by VEGF R2, and the lymphangiogenic activity by VEGF R3. The expression of VEGF-C and -D and their receptors often correlates with the metastatic capacity of various malignancies, in particular with their ability to spread via increased lymphatic drainage.¹³

References

- Chilov, D. *et al.* (1997) J. Biol. Chem. **272**:25176.
- Achen, M.G. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:548.
- Joukov, V. *et al.* (1998) EMBO J. **16**:3898.
- Siegfried, G. *et al.* (2003) J. Clin. Invest. **111**:1723.
- McColl, B.K. *et al.* (2003) J. Exp. Med. **198**:863.
- Stacker, S.A. *et al.* (1999) J. Biol. Chem. **274**:32127.
- Lymboussaki, A. *et al.* (1998) Am. J. Pathol. **153**:395.
- Kaipainen, A. *et al.* (1995) Proc. Natl. Acad. Sci. USA **92**:3566.
- Jeltsch, M. *et al.* (1997) Science **276**:1423.
- Cao, Y. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:14389.
- Witzensbichler, B. *et al.* (1998) Am. J. Pathol. **153**:381.
- Byzova, T. *et al.* (2002) Blood **99**:4434.
- Stacker, S.A. *et al.* (2002) Nat. Rev. Canc. **2**:573.

IL-24 in Cancer Growth Suppression, Apoptosis, and Angiogenesis

Melanoma differentiation-associated gene-7 (MDA-7) was originally identified as a molecule with elevated expression in terminally differentiated melanoma cells.¹ Based on structural and sequence similarity, it is now recognized as an IL-10 family member and has been renamed IL-24.^{2,3} This protein is known to suppress tumor growth,^{2,4,5} induce apoptosis,⁴ and inhibit angiogenesis,^{4,6} and it is currently being investigated as a possible therapeutic target.

IL-24 is a secreted glycoprotein belonging to the four helix bundle family of cytokine molecules, and is most closely related to the IL-10 subfamily.² Expression in normal tissues is restricted to melanocytes and cells of the immune system including monocytes, CD3-activated T cells, and subsets of B and NK cells.² IL-10 family cytokines signal via heterodimeric receptor complexes consisting of one alpha and one beta subunit. Within this family only three alpha subunits (IL-10 R α , IL-20 R α , and IL-22 R α) and two beta subunits (IL-20 R β and IL-22 R β) have been identified.⁷ Studies on cells transfected with different combinations of these receptor components demonstrate that IL-24 can signal through either IL-20 R α /IL-20 R β or IL-22 R α /IL-20 R β .⁷⁻⁹ Two other IL-10 family members, IL-19 and IL-20, also utilize the IL-20 R α /IL-20 R β complex, whereas only IL-20 and IL-24 signal via the IL-22 R α /IL-20 R β complex.⁷⁻⁹ Signaling through both complexes results in nuclear translocation of STAT3.^{8,9} Translocation of STAT1 was also observed at 100-fold higher doses of IL-24.⁹

Cultured melanoma cells undergo terminal differentiation and lose their proliferative capacity when treated with IFN- β and the protein kinase C activator, mezerein. IL-24 was initially identified because it was upregulated following this treatment.¹ Subsequent studies reveal that IL-24 is expressed in normal melanocytes, and expression levels fall progressively upon melanoma transformation and ultimate progression to metastatic disease.¹⁰ Ectopic expression of IL-24 via an adenovirus expression system (Ad~IL-24) in a variety of cultured tumor cell lines resulted in growth suppression and apoptosis.^{2,4,5} More than 50 different human cancer cell lines were tested and a majority was susceptible to IL-24 as opposed to normal control cells.^{2,4} In a nude mouse xenograft model system, subcutaneous human carcinomas were injected with Ad~IL-24 resulting in increased expression of TRAIL and elevated levels of tumor cell apoptosis.⁴ Further, a phase I clinical trial found that intratumoral injection of Ad~IL-24 induced apoptosis in a large percentage of tumor volume.¹¹

IL-24 has also been reported to inhibit angiogenesis. HUVECs (human umbilical vein endothelial cells), when cultured on appropriate substrates, will undergo differentiation resulting in the formation of capillary tube-like structures. Treatment of the HUVECs with soluble IL-24⁶ or with Ad~IL-24⁴ completely inhibited tube formation. An IL-22 R α neutralizing antibody restored tube formation indicating that this receptor mediates the inhibitory effect of IL-24 in this system.^{4,6} Similarly, injection of Ad~IL-24 into subcutaneous tumors in nude mice resulted in significantly fewer tumor cells that express CD31,⁴ a marker for neoangiogenesis. VEGF-dependent endothelial cell migration as measured in a transwell assay was also inhibited by soluble IL-24 in a dose dependent manner.⁶

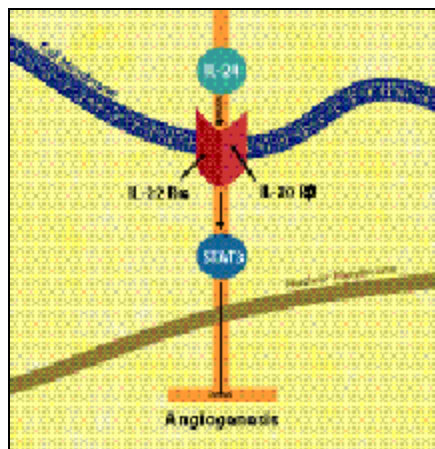


Figure 1. The IL-10 family member, IL-24, also known as melanoma differentiation-associated gene-7 (MDA-7), binds the IL-22 R α /IL-20 R β heterodimeric receptor complex to inhibit angiogenesis, possibly through a STAT3-dependent mechanism.

References

- Jiang, H. *et al.* (1995) *Oncogene* **11**:2477.
- Sauane, M. *et al.* (2003) *Cytokine Growth Factor Rev.* **14**:35.
- Fickenscher, H. *et al.* (2002) *Trends Immunol.* **23**:89.
- Saeki, T. *et al.* (2002) *Oncogene* **21**:4558.
- Jiang, H. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**:9160.
- Ramesh, R. *et al.* (2003) *Cancer Res.* **63**:5105.
- Wang, M. *et al.* (2002) *J. Biol. Chem.* **277**:7341.
- Dumoutier, L. *et al.* (2001) *J. Immunol.* **167**:3545.
- Parrish-Novak, J. *et al.* (2002) *J. Biol. Chem.* **277**:47517.
- Ekmekcioglu, S. *et al.* (2001) *Int. J. Cancer* **94**:54.
- Chanda, S. *et al.* (2001) *Cancer Gene Ther.* **8**:53.

Cell Culture Reagents

R&D Systems offers an array of cell culture reagents to support Angiogenesis research.

Growth factors

- Cytokines used in Cell Expansion
- Cytokines used in Cell Differentiation/Manipulation

Media Supplements

- N-2 Plus Media Supplement

Methylcellulose-based Reagents for Colony Forming Assays

- Methylcellulose Stock Solution
- Human Methylcellulose Base Media
- Human Methylcellulose Complete Media
- Human Methylcellulose Complete Media without Epo

Basement Membrane Extracts & Proteins

- Bovine Fibronectin
- Bovine Collagen I
- Rat Collagen I
- Mouse Collagen IV
- Mouse Laminin I
- Cultrex® Cell Staining Kit
- Cultrex® Basement Membrane Extracts

For a complete product listing, please visit www.RnDSystems.com/CellCulture

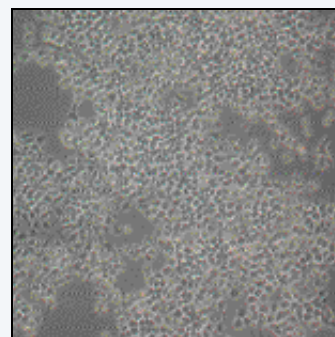


Figure 1. Rat cortical stem cells were cultured in a bovine fibronectin-precoated plate with N-2 Plus Media (Catalog # AR003) containing 20 ng/mL FGF-basic (Catalog # 233-FB-025).

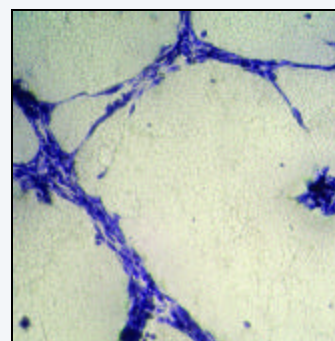


Figure 2. Capillary Formation. SEC4-10 cells incubated for 24 hours at 37 °C with 5% FBS and Cultrex® Basement Membrane Extracts.

Cultrex is a registered trademark of Trevigen, Inc.

Receptor Tyrosine Kinase

DuoSet® IC ELISA Development Kits

The platelet-derived growth factor (PDGF) family members contribute to cancer development and progression by both autocrine and paracrine signaling mechanisms. Four PDGF isoforms (designated A, B, C and D) exist as AA, AB, BB, CC, or DD disulfide-linked dimers. These species differentially bind two structurally related receptor tyrosine kinases (RTKs), PDGF R α and PDGF R β . PDGF R β binds PDGF-BB and -DD with high affinity, PDGF-AB with moderate affinity, and has no reported binding to PDGF-AA and -CC. Activation of the intrinsic tyrosine kinase activity of PDGF R involves ligand binding followed by receptor dimerization and tyrosine autophosphorylation. [Note: for a review, please see Li, X. & U. Eriksson (2003) Cytokine Growth Factor Rev. **14**:91.]

R&D Systems' DuoSet IC (Intracellular) ELISA Development Systems provide a rapid, economical, quantitative, and sensitive (Figure 1) alternative to Western blots that is specific (Figure 2 and 3) and is amenable to high-throughput analysis. The human phospho-PDGF R β DuoSet IC ELISA Kit (Catalog # DYC1767) measures the relative level of PDGF R β auto-phosphorylation.

Kit Contents*

- RTK-specific capture antibody
- pan anti-phospho-tyrosine HRP-conjugated detection antibody
- recombinant phospho-RTK control

*sufficient reagents for two or five 96-well microplates

Greater Sensitivity

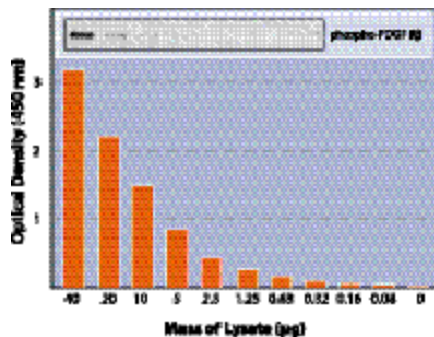


Figure 1. Immortalized human fibroblasts were treated with 100 ng/mL recombinant PDGF-BB (Catalog # 220-BB) for 5 minutes to induce tyrosine phosphorylation of PDGF R β . Serial dilutions of lysates were analyzed by R&D Systems' Phospho-PDGF R β DuoSet IC ELISA (Catalog # DYC1767) and by immunoprecipitation/Western blot (inset).

Phospho-specificity

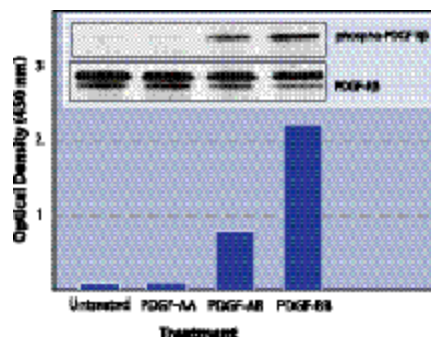


Figure 2. Immortalized human fibroblasts were untreated or treated with 100 ng/mL of recombinant PDGF-AA (Catalog # 221-AA), PDGF-AB (Catalog # 222-AB) and PDGF-BB (Catalog # 220-BB) for 5 minutes. Lysates were analyzed by R&D Systems' Phospho-PDGF R β DuoSet IC ELISA (Catalog # DYC1767) and by immunoprecipitation/Western blot (inset). This kit has not yet been tested with either PDGF-CC or -DD.

Receptor-specificity

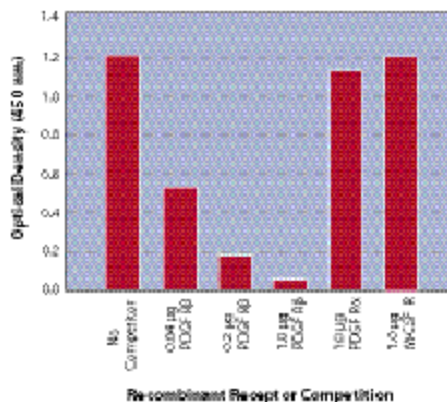


Figure 3. Lysates prepared from immortalized human fibroblasts were treated with 100 ng/mL of PDGF-BB for 5 minutes. The indicated amounts of PDGF R β (Catalog # 385-PR), PDGF R α (Catalog # 322-PR) or M-CSF R (Catalog # 329-MR) recombinant extracellular domains were added to the lysate and analyzed by R&D Systems' Phospho-PDGF R β DuoSet IC ELISA (Catalog # DYC1767).

For further information on R&D Systems' Receptor Tyrosine Kinase DuoSet IC ELISA Development Systems, including a current listing of available products, please see www.RnDSystems.com/RTKkits.



R&D Systems

614 McKinley Place N.E.
Minneapolis, MN 55413
Tel: (612) 379-2956
1-800-343-7475
Fax: (612) 656-4400
www.RnDSystems.com



Printed on recycled paper 10% post consumer waste.

PRSR STD
U.S. POSTAGE
PAID
R&D SYSTEMS

Change Service Requested