

VEGF receptors – key mediators of vascular formation and lymphangiogenesis

Vasculogenesis, the *de novo* formation of blood vessel from stem cells is an important process during development. Angiogenesis and lymphangiogenesis, the formation of blood vessel and lymphatics from preexisting vessel, is another vital step for further development of the embryo and a number of physiological and pathological conditions. All members of the Vascular Endothelial Growth Factor family (VEGF-A, VEGF-B, PlGF, VEGF-C, VEGF-D and viral VEGF-E) are potent mitogens and differentiation factors for vascular and lymphatic endothelium. They can induce angiogenesis and lymphangiogenesis *in vitro* and *in vivo* and some of them are potent vascular permeability inducers. These growth factors mediate their signals through three members of the receptor-type tyrosine kinase family called VEGFR-1 (flt-1), VEGFR-2 (KDR) and VEGFR-3 (flt-4). These trans-membrane receptors have the general structure of being a glycoprotein receptor with an extracellular ligand binding domain containing seven immunoglobulin-like domains and a split tyrosine kinase domain within their cytoplasmic region. An additional family of receptors, the neuropilins has also been identified and appears to function as co-receptors to modulate binding to other receptors without being active in signalling.

VEGFR-1 is the dominant VEGF receptor on monocytes and responsible for chemotaxis and tissue factor activation. It also plays an important role for signaling and recruitment of stem cells and endothelial precursor cells either alone or in combination with other VEGF receptors. The soluble splice product of this receptor is involved in the fine tuning of VEGF activity during the angiogenic cascade and a prognostic marker for tumour progression in breast cancer. Inactivation of the VEGFR-1 by homologous recombination in mouse has shown that VEGFR-1 is essential for vessel differentiation and maturation, as embryos die around E10 and lack functional blood vessel. Ligands for VEGFR-1 include several isoforms of PlGF, VEGF-A and VEGF-B.

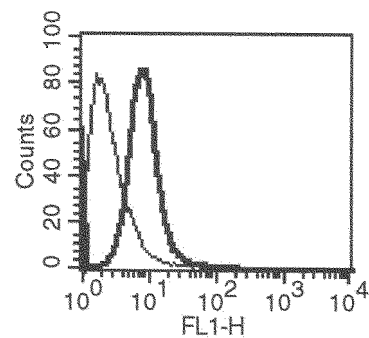
VEGFR-2 is the major receptor in terms of mitogenic signaling for vascular endothelial cells, and there is evidence to suggest that it also

plays a role in mediating the signals for vascular permeability. Inactivation of the VEGFR-2 gene in mouse embryonic stem cells has shown that VEGFR-2 signaling is absolutely required for vasculogenesis, as embryos lack both hematopoietic and endothelial precursor cells and die around E9. VEGFR-2 activation is also the key signaling pathway in a number of pathological conditions including cancer, where this receptor is frequently up-regulated on growing vessels. Numerous studies using specific inhibitors have shown that the VEGF/VEGFR-2 signaling pathway is critical for tumour angiogenesis and therefore solid tumor growth. Expression of VEGFR-2 in various cell types results in the ability to respond to ligands by the transduction of a mitogenic signal. Activation of VEGFR-2 results in the phosphorylation of numerous tyrosine residues within the cytoplasmic domain. This results in the association of a variety of signaling molecules including Shc, Grb2 and MAP kinase. Ligands for VEGFR-2, which include VEGF-A, viral VEGF-E and, at a significant lower affinity, VEGF-C and VEGF-D.

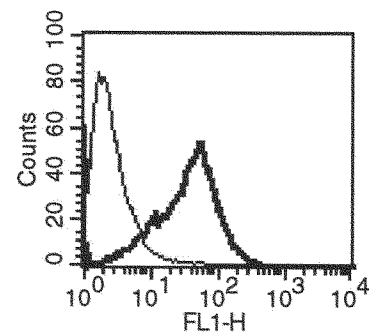
VEGFR-3 is the major receptor for lymphatic endothelial cells in terms of mitogenic signaling and differentiation of the lymph vessels. Mutations in the VEGFR-3 lead to severe pathological implications concerning the function of the lymphatics for fluid accumulation and plasma drainage. In early blood vessel formation VEGFR-3 is expressed together with VEGFR-1 and VEGFR-2 but later during development its expression is restricted to mature lymphendothelial cells. Therefore VEGFR-3 is a specific marker for lymphatics and can be used to isolate lymph endothelial from a mixture from different endothelial progenitor cells. So far ligands for VEGFR-3 include VEGF-C and VEGF-D, which are processed from precursor molecules. The further analysis of VEGFR-1 to VEGFR-3 and their functional relationship to other receptors, accessory proteins and co-receptors in development and diseases will be areas of much interest in vascular biology and lymphangiogenesis.

Our mouse monoclonal antibodies of human VEGFR-1 and VEGFR-2 should prove to be a valuable tool for investigating the receptors role in early blood and lymph vessel development. The antibodies were

raised against the extracellular domain of human VEGFR-1+ -2 and has been used successfully for flow cytometry, immunohistochemistry, Western blotting, ELISA and immunoprecipitations.



Detection of VEGFR-1 extracellular domain of human umbilical vein endothelial cells (HUVE) using monoclonal antibody VEGFR-1 (bold) versus control conditions (order no. 101-M26)



Detection of VEGFR-2 extracellular domain of human umbilical vein endothelial cells (HUVE) using monoclonal antibody VEGFR-2 (bold) versus control conditions (order no. 101-M20)

<u>Description</u>	<u>Applications</u>
anti-VEGFR-1 # EIC:	EL, IHC, FC
anti-VEGFR-1 # EWC:	EL, IB,
anti-VEGFR-2 # EIC:	EL, IHC, FC
anti-VEGFR-2 # EWC:	EL, IB, FC

IB= Immuno Blotting, Western
IHC= Immunohistochemistry
FC= FACS Analysis
EL=ELISA