

Werner Risau-Preis für Taija Mäkinen

Scientific career:

1994-1998	Biology studies at University of Helsinki, Finland
1997-1998	M.Sc. studies with Prof. Kari Alitalo, Molecular/Cancer Biology Laboratory, Haartman Institute and Biomedicum Helsinki, University of Helsinki, Finland
1998-2002	Ph.D. studies with Prof. Kari Alitalo, Molecular/Cancer Biology Laboratory, Haartman Institute and Biomedicum Helsinki, University of Helsinki, Finland
2003-	Post-doctoral studies with Dr. Rüdiger Klein, Molecular Neurobiology Laboratory, Max-Planck-Institute of Neurobiology, Martinsried, Germany



Grants and prizes:

1998	M.Sc. thesis prize from The Faculty of Science, University of Helsinki
2003	Ph.D. thesis prize from The Finnish Academy of Science and Letters
2003-2004	EMBO long-term fellowship
2004-	Human Frontier Science Program long-term fellowship

EphrinB2 is required for the development of lymphatic vasculature

Lymphatic vessels are essential for the maintenance of normal tissue fluid balance and immune surveillance. The unidirectional lymph flow recovers lymph from the periphery by blind-ended lymphatic capillaries, which drain into larger collecting lymphatic vessels and return the lymph to the cardiovascular system via the thoracic duct. Reflecting their functional specializations different types of lymphatic vessels can be distinguished morphologically; lymphatic capillaries have discontinuous basement membrane and loose intercellular junctions, making them highly permeable to large macromolecules. In contrast, collecting lymphatic vessels have a smooth muscle cell layer which helps to pump lymph forward, while the luminal valves prevent the backflow. During embryogenesis the development of lymphatic vessels starts after the establishment of blood vasculature. A subset of venous endothelial cells commits to the lymphatic endothelial cell lineage and sprouts from the major veins to form sac-like structures from which the vessels grow further by centrifugal sprouting. The homeodomain transcription factor Prox1 is required for the initial specification, while activation of VEGFR-3 by VEGF-C is essential for the migration

and proliferation of lymphatic endothelial cells. Recent studies also demonstrate that VEGFR-3 and its ligands are involved in the development of lymphedema, a disease characterized by hypoplasia or functional failure of the lymphatic vessels, and in lymphatic metastasis.

Neural guidance molecules of the ephrin-Eph ligand-receptor family are required for the establishment of arterial-venous identities and for the remodeling of the primitive capillary plexus into a hierarchical vessel network during the development of the blood vasculature, but their roles in lymphatic development have not been previously studied. Ephrins are transmembrane ligands for Eph receptor tyrosine kinases. This signaling functions bidirectionally; in addition to the activation of Eph tyrosine kinase activity ephrins have cytoplasmic domains with intrinsic signaling capacity and upon cell-cell contact mediated activation they are phosphorylated in their tyrosine residues, which then provide docking sites for intracellular signaling molecules. In addition, ephrins have a carboxyterminal motif for the binding of PDZ-domain containing proteins.

In order to get further insights into the mechanisms of ephrinB transduced signaling, we have

generated mutant mice expressing ephrinB2 lacking the carboxyterminal PDZ interaction site or ephrinB2 in which the five conserved tyrosine residues were replaced by phenylalanine to disrupt phosphotyrosine-dependent signaling events. Our analysis revealed that the homozygous mutant mice survived the requirement of ephrinB2 for embryonic angiogenesis. However, mice expressing ephrinB2 with a deficient PDZ target site developed chylothorax and exhibited major lymphatic defects, including a failure to remodel their primary lymphatic capillary plexus into a hierarchical vessel network consisting of lymphatic capillaries and collecting vessels, hyperplasia and lack of luminal valve formation. These abnormalities were largely rescued in mice expressing phosphotyrosine deficient ephrinB2. Our studies therefore define ephrinB2 as an essential component of postnatal lymphatic development, and indicate that interactions with PDZ domain effectors are required to mediate its functions. Furthermore, our results show that the previously uncharacterized lymphatic remodeling process is a critical step in the establishment of functional lymphatic vascular system

Selected publications:

- Dixelius, J., Mäkinen, T., Wirzenius M., Karkkainen, M.J., Wernstedt, C., Alitalo, K. and Claesson-Welsh, L. (2003) Ligand-induced vascular endothelial growth factor receptor-3 (VEGFR-3) heterodimerization with VEGFR-2 in primary lymphatic endothelial cells regulates tyrosine phosphorylation sites. *J. Biol. Chem.* 278, 40973-40979.
- Karkkainen, M.J., Mäkinen T. and Alitalo, K. (2002) Lymphatic endothelium: a new frontier of metastasis research? *Nat. Cell Biol.* 4, E2-E5. Review article.
- Mäkinen, T., Jussila, L., Veikkola, T., Karpanen, T., Kettunen, M.I., Pulkkanen, K.J., Kauppinen, R., Jackson, D.G., Kubo, H., Nishikawa, S.-I., Ylä-Herttuala, S. and Alitalo, K. (2001a) Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. *Nat. Med.* 2, 199-205.
- Mäkinen, T., Veikkola, T., Mustjoki, S., Karpanen, T., Catimel, B., Nice, E.C., Wise, L., Mercer, A., Kowalski, H., Kerjaschki, D., Stacker, S.A., Achen, M.G. and Alitalo, K. (2001b) Isolated lymphatic endothelial cells transduce growth, survival and migration signals via the VEGF-C/D receptor VEGFR-3. *EMBO J.* 20, 4762-4773.
- Mäkinen, T., Adams, R.A., Bailey, J., Lu, Q., Ziemiecki, A., Alitalo, K., Klein, R. and Wilkinson, G.A. (2005) PDZ interaction site in ephrinB2 is required for the remodeling of lymphatic vasculature. *Genes Dev.* 19, 397-410.
- Petrova, T.V., Mäkinen, T., Mäkelä, T.P., Saarela, J., Virtanen, I., Ferrell, R.E., Finegold, D.G., Kerjaschki, D., Ylä-Herttuala, S. and Alitalo, K. (2002) Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J.* 21, 4593-4599.
- Veikkola, T., Jussila, L., Mäkinen, T., Karpanen, T., Jeltsch, M., Petrova, T.V., Kubo, H., Thurston, G., McDonald, D.M., Jackson, D.G., Achen, M.G., Stacker, S.A. and Alitalo, K. (2001) Signaling via vascular endothelial growth factor receptor-3 is sufficient for lymphangiogenesis in transgenic mice. *EMBO J.* 20, 1223-1231.