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Fifteen years of molecular lymphangiogenesis - an interview with Kari Alitalo

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ABSTRACT Kari Alitalo is one of the leaders in the field of lymphangiogenesis. Research from his laboratory has contributed to the transformation of a largely descriptive field into a dynamic discipline, which now holds promise for the treatment of cancer, inflammation and vascular dysfunction. The interview intends to provide historical insights into these changes and hopefully inspiration to the young generation of vascular biologists.

KEY WORDS: *angiogenesis, lymphangiogenesis, tumor metastasis, lymphedema*

Dr. Kari Alitalo is a tenured Research Professor of the Finnish Academy of Sciences and Scientific Director of the Molecular/Cancer Biology Program and Centre of Excellence in the Biomedicum Helsinki research institute of the University of Helsinki. He obtained his M.D. and Ph.D. from the University of Helsinki in Finland. During his postdoctoral period in 1982-1983, Dr. Alitalo worked with Drs. J. Michael Bishop and Harold E. Varmus in San Francisco. Dr. Alitalo has discovered several novel receptor tyrosine kinases, important in particular in endothelial cells. He has shown that some of these receptors and their ligands play important roles in tumor angiogenesis. Among the original findings are the cloning and characterisation of a fibroblast growth factor receptor 4, the Bmx and Csk tyrosine kinases and the first endothelial specific receptor tyrosine kinase, Tie1. A significant achievement by Dr. Alitalo was the isolation and characterization of the first lymphangiogenic growth factor VEGF-C, its receptor VEGFR-3, and the isolation of lymphatic endothelial cells, opening up the lymphatic vascular system to molecular analysis after over a hundred years of descriptive pathology. The work from his laboratory was central in the characterization of VEGF-B, VEGF-C and VEGF-D and their receptors and signal transduction pathways and the function of VEGFR-3, showing that this receptor is required for angiogenesis and lymphangiogenesis. He has devised molecular therapies for lymphedema that are now entering clinical trials. These studies led to the demonstration of VEGF-C induced tumor angiogenesis and lymphangiogenesis, intralymphatic tumor growth, and VEGF-C association with tumor metastasis and its inhibition by blocking the VEGFR-3 signal transduction pathway. The inhibitors of these pathways have now entered phase I clinical trials.

Kari, could you tell us about your background and how you came into science?

At school I was interested in exact sciences, especially mathematics, and also biology. When studying at the Summer University of Helsinki during the summer breaks of my secondary school, I got my "Alitalo special" questions in the mathematics exams and after scoring a shared first in the national competition of mathematics, the expectations of my teachers were high that I would go and study mathematics at the university. However, my father was pragmatic enough to tell me that I cannot support a family as a mathematician. But what finally changed my plans was the reading of the novel "First Circle" by Alexander Solzhenitsyn. In this book, Nerzin, a mathematician prisoner trained in topology, "the stratosphere of the human mind", was forced to decode speech of telephone conversations for person identification. He regretted that he instead could not help mankind. He quotes Mephistopheles from Faust in the book:

"You get no word of suns and worlds from me.
How men torment themselves is all I see"

Being young with my aspirations, I was perhaps sensitive to this kind of reasoning, as I had suffered from quite severe asthma during my school years, which often forced me to quit sports and miss a lot of school, save for the exams of course. Thus I made a sudden last-minute decision to go to medical school. This surprised many, because a theoretical philosopher at the University of Helsinki had already recruited me as an assistant teacher of mathematical logic at the Summer University of Helsinki during my last years in secondary school.

Abbreviations used in this paper: VEGF, vascular endothelial growth factor.

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What was the subject of your PhD work and how did you choose what to do afterwards? What were the outstanding biology questions at that time?

I became interested in malignant transformation of cultured cells by tumor viruses. This was a hot topic at the time and I admired many of the pioneer scientists working on the topic. My mentor Dr. Antti Vaheri together with Dr. Erkki Ruoslahti had discovered fibronectin as an adhesion protein that was missing from transformed and tumor cells. I continued along these lines by identifying several novel types of procollagens from cultured tumor cells; the matrix was in general lost upon malignant transformation, although it was not known how this happens and how relevant this would be for human cancer or its eventual treatment possibilities (Alitalo, 1980; Alitalo *et al*., 1980a; Alitalo *et al*., 1982a; Alitalo *et al*., 1982b; Alitalo *et al*., 1980b; Alitalo *et al*., 1980c; Alitalo *et al*., 1980d).

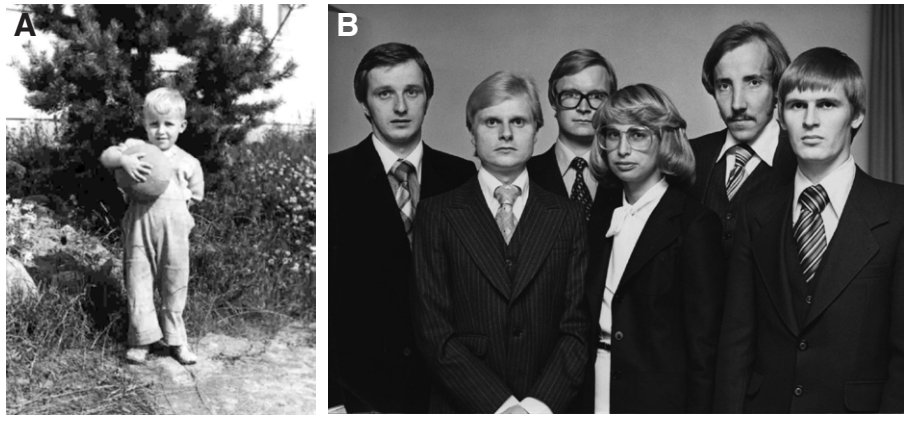


Fig. 1. Images of the growing Kari Alitalo. (A) *Early years.* **(B)** *Graduation from medical school (1977), Kari Alitalo is second from the right.*

After my dissertation, before getting the *Primus Doctorum* degree I had to answer in the tenth doctoral promotion of the faculty the question of how viruses have helped to understand the mechanisms behind human cancer. Luckily, the first publications showing insertional mutagenesis of a human proto-oncogene caused by avian leukemia viruses in chickens had just come out so that I could answer this question. My supervisor Antti Vaheri knew that I was highly interested in pursuing studies along these lines, and he kindly contacted Dr. Michael Bishop in San Francisco to inquire about the possibility of a postdoctoral training position for me. Unfortunately, there was a waiting list for the Bishop-Varmus lab, but I decided to work in the laboratory of Dr. Paul Bornstein at the University of Washington in Seattle during this waiting time. After just about nine months in Seattle I got a phone call from the Bishop-Varmus laboratory. Unexpectedly, they had an opening, and none of the other people on the list before me were available to fill that opening. I was ready to go, and talked with my supervisor who knew my plans and was very understanding and supportive. In the early morning hours of the following day, I was driving and already close to San Francisco, but so tired that the gas had run out in my car, and I had to walk to a nearby gas station to refill the tank! For this reason I was late for my first morning meeting with Michael Bishop, and I was afraid he would change his mind about my recruitment.

At that time, most scientists in the Bishop laboratory were working with chicken retroviruses and viral oncogenes, and they had produced thrilling data. I had a medical background and I had meanwhile read the book by George Stark about gene amplification, where he describes double minute chromosomes and homogeneously staining cromosomal regions as hallmarks of gene amplification to meet the need for increased gene expression. I proposed to Michael Bishop to buy all available tumor cell lines from the ATCC collection that showed signs of these chromosomal aberrations and screen them for oncogene amplification. Together with Manfred Schwab, a German postdoctoral fellow, we devised the screening strategy, a kind of rudimentary microarray using the available viral oncogene inserts on filter paper, hybridized with radioactive cDNA prepared from various tumour cell lines by reverse transcription. In the subsequent first Southern blot I had ever done, I found that the MYC-oncogene was amplified in a colon carcinoma cell line. It was obvious, that MYC had been amplified in the HSRs of this cell line (Alitalo *et al*., 1983a; Alitalo *et al*., 1983b; Alitalo *et al*., 1984; Schwab *et al*., 1983a; Schwab *et al*., 1983b). Some of the cross-reactive signals in our filters then led to the cloning of the NMYC gene by Manfred, and when the word got out, the discovery of other amplified oncogenes, among them MDM and LMYC. For several years thereafter NMYC amplification provided the best marker for poor prognosis of neuroblastoma in children.

At what point did you became interested in angiogenesis?

After returning to Finland, it was difficult for me to work in the gene amplification area, because at first I did not even have a bacterial shaker available, which is why the cloning work was severely delayed after arrival. However, in the Bishop lab I had learned a lot about signal transduction by tyrosine kinases, which led to another interesting project. The practical problem I addressed together with my wife, a hematologist, was related to bleeding problems in bone marrow stem cell transplanted hematological patients. When my wife was on call in the Fred Hutchinson hospital

Fig. 2. Not a fishing expedition! *David Baltimore in charge of the navigation! From left to right: Kari Alitalo and his heroes David Baltimore and Michael Bishop during a sailing trip organized by Dr. Eero Saksela in the Gulf on Finland.*

in Seattle, we often had to wake up because of problems with low plateled counts. When back in Finland, she studied megakaryoblastic differentiation of leukemia cells in culture, and using that model we decided to try and clone a tyrosine kinase receptor for a possible growth factor that could stimulate megakaryoblastic proliferation. This was done by RT-PCR using degenerate primers and to our surprise altogether 10 novel genes were obtained by a young graduate student Juha Partanen using this method. *In situ* hybridization for one of them, that we had named TIE1, showed expression exclusively in the vascular endothelium (Korhonen *et al.*, 1992a; Korhonen *et al.*, 1992b; Partanen *et al.*, 1992; Partanen *et al.*, 1990; Partanen *et al.*, 1991).

This was soon followed by the cloning of TIE2 by several groups and later by the isolation of their ligands, the angiopoietins. Despite intensive efforts, a ligand for TIE1 was not found, but we showed later that activated Tie receptors form *in trans* complexes at endothelial cell-cell junctions, a novel and exciting mechanism of receptor tyrosine kinase activation (Saharinen et al., 2008). Angiopoietin-2, which was a weaker activator of Tie phosphorylation than angiopoietin-1 in the endothelial cell-cell junctions has recently emerged as a promising target for anti-angiogenic tumor therapy (see Saharinen et al., 2011).

Fig. 4. Science is a lot of fun (sometimes). *With members of the laboratory in Helsinki, 1999. Photo by Henrik Duncker.*

Could you tell us more about the history of discovery of lymphangiogenic growth factors?

The crucial experiment which led to lymphangiogenesis, was carried out in 1994 by Arja Kaipainen, an M.D. working on her PhD thesis in my lab after we cloned our second endothelial-specific tyrosine kinase receptor, now known as vascular endothélial growth factor-3 (VEGFR-3), from megakaryoblastic cells (Aprelikova *et al.*, 1992; Armstrong *et al.*, 1993; Pajusola *et al.*, 1993; Pajusola *et al.*, 1992). Her *in situ* hybridization of 14.5 day mouse embryos indicated, that VEGFR-3 became restricted to the lymphatic vessels during development (Kaipainen *et al.*, 1995). When I saw it I realized that this would be a fairly specific signal transduction pathway for lymphangiogenesis, which was probably separate from angiogenesis.

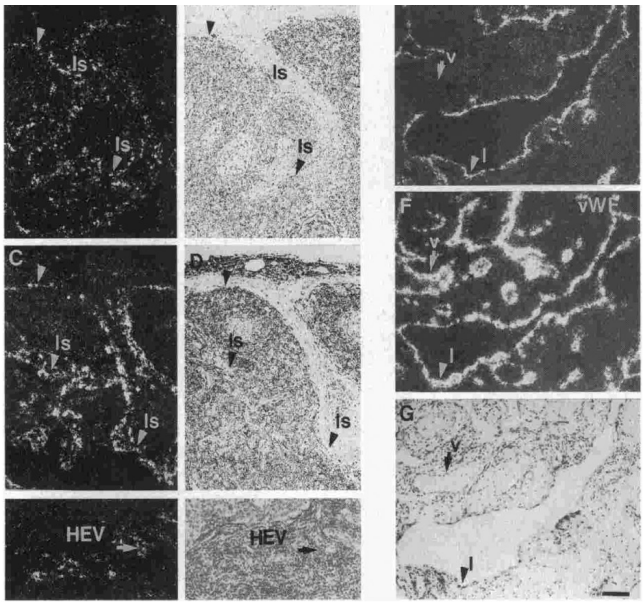
We quickly validated that VEGFR-3 does not bind vascular endothelial growth factor (VEGF-A), placenta growth factor (PLGF) or VEGF-B that was first detected as a "junk" clone in an unrelated yeast two-hybrid screen by Dr. Ulf Eriksson in Stockholm and then cloned and characterized in collaboration with him (Olofsson *et al.*, 1996). The first lymphangiogenic growth factor VEGF-C was isolated by my postdoctoral fellow Vladimir Joukov, an extremely disciplined researcher from The Kirov Military Medical Academy in Russia. He grew over a thousand plates of prostate carcinoma cells to obtain sufficient quantities of conditioned medium for the purification of the ligand activity using the extracellular domain of VEGFR-3 binding as a tool. We obtained and sequenced the corresponding cDNA clone with a failing sequencer during the long Finnish summer vacations, when all technicians were absent (Joukov *et al.*, 1996). Michael Jeltsch, then a graduate student in my lab, produced transgenic mice, where he overexpressed the newly cloned factor in the epidermis of the skin. These showed a tremendous growth of the lymphatic vessels in the skin, but only little effect on the blood vessels. That convinced everybody of the lymphangiogenic activity activity of VEGF-C (Jeltsch *et al.*, 1997).

Fig. 3. From the archive. (A) *On the importance of choosing the right direction. Time off during a Keystone meeting in 1995.* **(B)** *Arja Kaipainen and Vladimir Joukov, lead authors of articles reporting the lymphatic expression of VEGFR-3 (Kaipainen* et al., *1995) and cloning of the VEGFR-3 ligand VEGF-C (Joukov* et al., *1996)*

When was the concept of interfering with tumor metastasis by blocking tumor lymphangiogenesis formulated?

In 1995, I wrote letters to Dr. Douglas Hanahan and then to Dr. Gerhard Christofori, and requested the plasmid and transgenic mice where the SV40T-antigen oncogene is driven by the rat insulin promoter to produce islet sell carcinomas of the pancreas. I wanted to create mice that overexpress VEGF-C in the islets, and then cross these mice with the tumor mice, because I believed that an excess VEGF-C could drive lymphatic metastasis of cancers. We also started testing VEGF-C

A



B

C

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In conclusion, our present results show that the uniform venous and capillary expression of FLT4 becomes restricted to lymphatic vessels during mouse development, and in human adult tissues FLT4 is specifically expressed by lymphatic vessels and some HEVs. These results support the theories of the venous origin of lymphatic vessels. The major function of the lymphatic system is to provide fluid return from tissues and to transport many extravascular substances back to the blood (38). In addition, during the process of maturation, lymphocytes leave the blood, migrate through lymphoid organs and other tissues, and enter the lymphatic vessels, from which they return to the blood through the thoracic duct. Specialized venules, HEVs, bind lymphocytes again and cause their extravasation into tissues. Besides providing a marker for lymphatic vessels and some HEVs in human adult tissues, FLT4 may be actively involved in the genesis and maintenance of the lymphatic vessels.

Fig. 5. How it all started. (A) In situ *hybridization analysis in normal and inflamed lymph node demonstrates expression of Flt4 (VEGFR-3) in lymphatic vessels. From (Kaipainen et al., 1995).* **(B)** *Lymphatic vessels visualized by ink injection of a pig emrybro (Florence Sabin, 1904) or by X-gal staining of Vegfr3$^{lacz/+}$ mouse embryo (Dumont et al., 1998; Karkkainen et al., 2001).* **(C)** *Citation from Kaipainen et al., 1995, expressing the support for F. Sabin's hypothesis for venous origin of lymphatic vessels and potential role of Flt4. Copyright (1995) National Academyof Sciences, U.S.A.*

transfected tumor cells for increased metastasis. However, when we had cloned VEGF-C into the Rip-Tag plasmid, our animal facility developed an infection problem, and we could not proceed with the production of transgenic mice. At this time, I happened to mention the problem to Dr. Michael Pepper from University of Geneva, who offered to produce transgenic mice for us in a Swiss facility. Thus I sent the construct to him. When the transgenic mice were obtained, they became very interested in the mice and we finally worked with Michael and Gerhard trying to dissect what happens when VEGF-C is overexpressed in tumours. This was very slow, also because Dr. Pepper had to teach during the university semesters. Finally in 2000, we had solid evidence that VEGF-C greatly stimulates lymphatic metastasis in the transgenic mouse model. I gave the first talk on the findings in the NIH "Conquering Lymphatic Disease" Think Tank Conference held in May 11-13, 2000 in NIH Bethesda. A transcript of my talk was published (Editor Marlys Witte, The University of Arizona 2001). This promoted a lot of interest in the role of lymphangiogenesis in tumor metastasis. When we finally published our results in 2001, several other groups had already related data from tumor xenograft models (Mandriota et al., 2001). At that time I also published some studies I had been doing meanwhile as a proof of principle of lymphangiogenesis and metastasis inhibition using the extracellular soluble form of VEGFR-3 in a tumor xenograft model (Karpanen *et al.*, 2001). The ability to block lymphatic metastasis with VEGFR-3 inhibitors was not enough to get funding for clinical trials. But when we showed that blocking VEGFR-3 inhibits angiogenic vessel sprouts in a number of tumors, the pharmaceutical industry became interested (Tammela et al., 2008).

What, in your opinion, are the promises and challenges of anti-lymphangiogenic and pro-lymphangiogenic therapies?

We are moving to the clinical trials now with lymphatic vessel regeneration. In Finland a few patients have already been injected with adenoviral vector encoding VEGF-D by my collaborator Seppo Ylä-Herttuala. The obligatory toxicology tests on VEGF-C are ongoing in pigs and a clinical trial in non-cancer related lymphedema is going to be started next year. This has been an important goal for us, although when the developments move out of academia, it will be more difficult for us to follow and control all the steps. I am glad that our university strongly encouraged us by starting a company (that was later acquired elsewhere), which has made all this possible.

The other side, inhibition of lymphangiogenesis and lymphatic metastasis in cancer patients is based on the soluble VEGFR-3 extracellular domain and antibodies that block it, or that block its ligand VEGF-C or VEGF-D. VEGFR-3 blocking antibodies have now entered clinical trials. We envisage that a combination of antibodies blocking VEGFR-2 and VEGFR-3 will be a more effective inhibitor of tumor angiogenesis than current anti-VEGF or anti-VEGFR-2 alone. In our preclinical models all these blockers have been free of side-effects, probably because they are specific to their targets and active angiogenesis, whereas mature steady-state vessels are not affected.

The most time-consuming challenges in clinical translation of the discoveries ultimately seems to be related to funding and convoluted business processes that suddenly divert attention from the focus of bringing therapy to the clinics as fast as possible. Some of these delays, such as funding problems during economic recessions, company mergers or acquisitions, etc., are beyond the scope of most scientists.

What scientific controversies have you witnessed during the development of the lymphangiogenesis field?

One puzzling wave of papers concerned endothelial progenitor cell incorporation to angiogenic vessels. Although there still does not seem to be hard evidence for substantial numbers of such cells in the non-pregnant adult body, they live on in various journals, most often ill-defined just by flow cytometry.

Another interesting polemic concerned the question of whether functional intratumoral lymphatic vessels occur in various cancers. Again, I am not sure that both sides of the scientific exchange have used the same terms and definitions of the issues, and indeed the debate seems to be dying off.

Thirdly, for some time, the definition of a lymphangiogenic factor seemed to drift from a primary and quite specific regulator such as VEGF-C down the impact factor ladder to any factor that in darkfield light microscopy increased the number or area of entities staining for the lymphangiogenic marker Lyve-1. In many such publications, inflammation or overall regeneration in the tissue was of no concern and not analysed. In the hands of uncritical consumers, tumor cells also started to light up with certain commercial antibodies against VEGFR-3, suggesting to some that this receptor is a direct target on tumor cells that drives tumor progression.

Among some 400 papers you have published so far, could you name your three favorites?

This is difficult to answer. But maybe one trio to mention is a cluster of papers published almost simultaneously in 2001. They really set the clinical scenario that I envisioned in 1995, when seing the VEGFR-3 *in situ* results for the first time. These papers are:

- KARPANEN T. *et al.*, (2001). Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. *Cancer Res.* 61: 1786-1790.
- MANDRIOTA S.J. *et al.*, (2001). Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J.* 20: 672-682.
- MAKINEN T. *et al.*, (2001). Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. *Nature Med.* 7: 199-205.

Because of the title of the Cancer Res paper, few people noticed that it actually for the first time employs a systemic inhibitior of tumor spread into the lymphatics (Karpanen *et al.*, 2001). Also, as the the Acknowledgements of the EMBO J. paper indicate, the work on the genetic VEGF-C overexpressing tumor model was started several years earlier in my laboratory, and it took a long time to get the evidence from a genetic model as explained above, but that model gave more compelling results than the quickly growing tumor xenografts (Mandriota *et al.*, 2001). Finally, the Nature Medicine paper shows that it is only the growing lymphatic vessels that are affected, but not mature adult vessels, indicating thet there is a therapeutic safety window for use in humans (Mäkinen *et al.*, 2001).

Your home country Finland, joined the European Union in 1995, and we are all very much aware of the increasing role of the EU in shaping the future of science. What, in your opinion, are the main challenges that European scientists will face in the coming years?

My feeling is that in the EU-style top down management, we are still in a learning phase of scientific evaluations, funding decisions and success monitoring. It seems that in general the accountability for the delivery of results has infiltrated from business to academia in a way that is not perhaps in our best interest when thinking about the promotion of innovations. When we applied to the EU for six-year funding for our Lymphangiogenomics network, we were accountable for 118 deliverables and 61 milestones, and had to predict that some of these would be obtained during the sixth year of funding. In reality, technologies can change overnight, which is why we have to completely review and remodel plans ever so often. In the EU system, these events are called deviations, and it takes months to hear from Brussels if they have been approved. In creative and innovative science, this is not what you should do. Instead, one should expect the unexpected, and allow freedom to explore the unexplored, and the unknown.

Science to me is like an expedition into the jungle to find a treasure without a map. One has to first develop a vision, a big picture of where you are aiming, but then make constant decisions in uncertainty about how to go on; it is like climbing a tree: … does this branch bear my weight, does it bring me higher towards the top…, maybe that is why I would like to have more bottom-up initiatives also in science management. Too strict accountability for too many milestones and deliverables is like asking an artist to define in advance how many paintings of what colour, size etc. he/she is going to produce each year for the next six years. This is very damaging for the creative/innovative process; it is as if we should constantly prove our innocence to someone, who controls us with a short leash.

What do you think can be done to overcome these problems?

In addition to what I have just mentioned, we also have to get rid of the outsourcing of the overemphasized management of the scientists themselves! These days, scientists are expected to set up and recycle heavy management structures involving budgets in the eight figure range. The investigators are not always fully supported by their own institutions to do this, and this takes a lot of time out from research and discovery. These trends also seem to foster an entire fleet of companies that are in possession, not of the substance, but the required jargon to succeed with the applications. Recently, the calls for funding have also become directed to more special themes, perhaps as a result of lobbying by those who have the time for this kind of activity, and I don't think they are the top scientists. I am afraid that a trend for narrow and practical goals further restricts innovations.

In my own area, the science administration could perhaps interact with and learn from the National Institutes of Health of the US, where the more established grant culture is mature and well suited for biomedical scientists. But at the same time, it is encouraging that the relatively recently introduced ERC grants are directed to single labs, and that they allow proposals that are more bottom up than what we have been used to. I am optimistic that the learning curve for carrying out scientific discovery in Europe will be steep and that together, we will soon build better systems for the benefit of science, the EU, global health and especially the patients!

References

ALITALO, K. (1980). Production of both interstitial and basement membrane procollagens by fibroblastic WI-38 cells from human embryonic lung. *Biochem Biophys Res Commun* 93: 873-880.

ALITALO, K., BISHOP, J.M., SMITH, D.H., CHEN, E.Y., COLBY, W.W., and LEVINSON, A.D. (1983a). Nucleotide sequence to the v-myc oncogene of avian retrovirus MC29. *Proc Natl Acad Sci USA* 80: 100-104.

ALITALO, K., HOVI, T., and VAHERI, A. (1980a). Fibronectin is produced by human macrophages. *J Exp Med* 151: 602-613.

ALITALO, K., KESKI-OJA, J., HEDMAN, K., and VAHERI, A. (1982a). Loss of different pericellular matrix components of rat cells transformed with a T-class ts mutant of Rous sarcoma virus. *Virology* 119: 347-357.

ALITALO, K., KUISMANEN, E., MYLLYLA, R., KIISTALA, U., ASKO-SELJAVAARA, S., and VAHERI, A. (1982b). Extracellular matrix proteins of human epidermal keratinocytes and feeder 3T3 cells. *J Cell Biol* 94: 497-505.

ALITALO, K., KURKINEN, M., VAHERI, A., KRIEG, T., and TIMPL, R. (1980b). Extracellular matrix components synthesized by human amniotic epithelial cells in culture. *Cell* 19: 1053-1062.

ALITALO, K., KURKINEN, M., VAHERI, A., VIRTANEN, I., ROHDE, H., and TIMPL, R. (1980c). Basal lamina glycoproteins are produced by neuroblastoma cells. *Nature* 287: 465-466.

ALITALO, K., VAHERI, A., KRIEG, T., and TIMPL, R. (1980d). Biosynthesis of two subunits of type IV procollagen and of other basement membrane proteins by a human tumor cell line. *Eur J Biochem* 109: 247-255.

ALITALO, K., RAMSAY, G., BISHOP, J.M., PFEIFER, S.O., COLBY, W.W., and LEVINSON, A.D. (1983b). Identification of nuclear proteins encoded by viral and cellular myc oncogenes. *Nature* 306: 274-277.

ALITALO, K., SCHWAB, M., LIN, C.C., VARMUS, H. and BISHOP, J.M. (1983c). Homogeneously staining chromosomal regions contain amplified copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. *Proc Natl Acad Sci USA* 80: 1707-1711.

ALITALO, K., WINQVIST, R., LIN, C.C., DE LA CHAPELLE, A., SCHWAB, M., and BISHOP, J.M. (1984). Aberrant expression of an amplified c-myb oncogene in two cell lines from a colon carcinoma. *Proc Natl Acad Sci USA* 81: 4534-4538.

APRELIKOVA, O., PAJUSOLA, K., PARTANEN, J., ARMSTRONG, E., ALITALO, R., BAILEY, S.K., MCMAHON, J., WASMUTH, J., HUEBNER, K., and ALITALO, K. (1992). FLT4, a novel class III receptor tyrosine kinase in chromosome 5q33-qter. *Cancer Res* 52: 746-748.

ARMSTRONG, E., KASTURY, K., APRELIKOVA, O., BULLRICH, F., NEZELOF, C., GOGUSEV, J., WASMUTH, J.J., ALITALO, K., MORRIS, S., and HUEBNER, K. (1993). FLT4 receptor tyrosine kinase gene mapping to chromosome band 5q35 in relation to the t(2;5), t(5;6), and t(3;5) translocations. *Genes Chromosomes Cancer* 7: 144-151.

DUMONT, D.J., JUSSILA, L., TAIPALE, J., LYMBOUSSAKI, A., MUSTONEN, T., PAJUSOLA, K., BREITMAN, M., and ALITALO, K. (1998). Cardiovascular failure in mouse embryos deficient in VEGF receptor-3. *Science* 282: 946-949.

JELTSCH, M., KAIPAINEN, A., JOUKOV, V., MENG, X., LAKSO, M., RAUVALA, H., SWARTZ, M., FUKUMURA, D., JAIN, R.K., and ALITALO, K. (1997). Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. *Science* 276: 1423-1425.

JOUKOV, V., PAJUSOLA, K., KAIPAINEN, A., CHILOV, D., LAHTINEN, I., KUKK, E., SAKSELA, O., KALKKINEN, N., and ALITALO, K. (1996). A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J* 15: 1751.

KAIPAINEN, A., KORHONEN, J., MUSTONEN, T., VAN HINSBERGH, V.W., FANG, G.H., DUMONT, D., BREITMAN, M., and ALITALO, K. (1995). Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci USA* 92: 3566-3570.

KÄRKKAINEN, M.J., JUSSILA, L., FERRELL, R.E., FINEGOLD, D.N. and ALITALO, K. (2001). Molecular regulation of lymphangiogenesis and targets for tissue oedema. *Trends in Mol Med 7:* 18-22.

KÄRPANEN, T., EGEBLAD, M., KARKKAINEN, M.J., KUBO, H., YLÄ-HERTTUALA, S., JÄÄTTELÄ, M., and ALITALO, K. (2001). Vacular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. *Cancer Res* 61: 1786-1790.

MÄKINEN, T., JUSSILA, L., VEIKKOLA, T., KARPANEN, T., KETTUNEN, M.I., PULKKANEN, K.J., KAUPPINEN, R., JACKSON, D.G., KUBO, H., NISHIKAWA, S.-I*., et al.* (2001). Inhibition of lymphangiogenesis with resulting lymphedema in transgenic mice expressing soluble VEGF receptor-3. *Nat Med* 7: 199-205.

MANDRIOTA, S.J., JUSSILA, L., JELTSCH, M., COMPAGNI, A., BAETENS, D., PREVO, R., BANERJI, S., HUARTE, J., MONTESANO, R., JACKSON, D.G*., et al.* (2001). Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. *EMBO J* 20: 672-682.

OLOFSSON, B., PAJUSOLA, K., KAIPAINEN, A., VON EULER, G., JOUKOV, V., SAKSELA, O., ORPANA, A., PETTERSSON, R.F., ALITALO, K., and ERIKSSON, U. (1996). Vascular endothelial growth factor B, a novel growth factor for endothelial cells. *Proc Natl Acad Sci USA* 93: 2576-2581.

PAJUSOLA, K., APRELIKOVA, O., ARMSTRONG, E., MORRIS, S., and ALITALO, K. (1993). Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts. *Oncogene* 8: 2931-2937.

PAJUSOLA, K., APRELIKOVA, O., KORHONEN, J., KAIPAINEN, A., PERTOVAARA, L., ALITALO, R., and ALITALO, K. (1992). FLT4 receptor tyrosine kinase contains seven immunoglobulin-like loops and is expressed in multiple human tissues and cell lines. *Cancer Res* 52: 5738-5743.

PARTANEN, J., ARMSTRONG, E., MAKELA, T.P., KORHONEN, J., SANDBERG, M., RENKONEN, R., KNUUTILA, S., HUEBNER, K., and ALITALO, K. (1992). A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. *Mol Cell Biol* 12: 1698-1707.

PARTANEN, J., MAKELA, T.P., ALITALO, R., LEHVASLAIHO, H., and ALITALO, K. (1990). Putative tyrosine kinases expressed in K-562 human leukemia cells. *Proc Natl Acad Sci USA* 87: 8913-8917.

PARTANEN, J., MAKELA, T.P., EEROLA, E., KORHONEN, J., HIRVONEN, H., CLAESSON-WELSH, L., and ALITALO, K. (1991). FGFR-4, a novel acidic fibroblast growth factor receptor with a distinct expression pattern. *EMBO J* 10: 1347-1354.

SAHARINEN P, EKLUND L, MIETTINEN J, WIRKKALA R, ANISIMOV A, WINDERLICH M, NOTTEBAUM A, VESTWEBER D, DEUTSCH U, KOH GY *et al.* (2008) Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. *Nat Cell Biol.* 10: 527-537.

SAHARINEN P, EKLUND L, PULKKI K, BONO P and ALITALO K. (in press). VEGF and angiopoietin signaling in tumor angiogenesis and metastasis. *Trends Mol Med* DOI: 10.1016/j.molmed.2011.01.015

SCHWAB, M., ALITALO, K., KLEMPNAUER, K.H., VARMUS, H.E., BISHOP, J.M., GILBERT, F., BRODEUR, G., GOLDSTEIN, M., and TRENT, J. (1983a). Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature* 305: 245-248.

SCHWAB, M., ALITALO, K., VARMUS, H.E., BISHOP, J.M., and GEORGE, D. (1983b). A cellular oncogene (c-Ki-ras) is amplified, overexpressed, and located within karyotypic abnormalities in mouse adrenocortical tumour cells. *Nature* 303: 497-501.

TAMMELA T, ZARKADA G, WALLGARD E, MURTOMÄKI A, SUCHTING S, WIRZENIUS M, WALTARI M, HELLSTRÖM M, SCHOMBER T, PELTONEN R *et al.* (2008). Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. *Nature* 454: 656-660.

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