Pioneer in Vascular Endothelial Growth Factor Research: Kari Alitalo, MD, PhD

Characterising Vascular Endothelial Growth Factors and Investigating Their Role and Therapeutic Potential in Cardiovascular Disease and Cancer

On May 2, 2013, Kari Alitalo, MD, PhD, director, Wihuri Research Institute, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland, and academy professor of the Finnish Academy, was appointed a member of the National Academy of Sciences of the United States. Academy members include ≈500 Nobel laureates, are chosen for highly significant scientific merit and successful research, and represent the finest researchers in the world of science, 2179 from the United States and 437 from elsewhere.

This prestigious appointment recognises the immense contribution that Professor Alitalo has made to the understanding of vascular endothelial growth factors (VEGFs) and their role in cardiovascular disease and cancer. From 1996 to 2007, Professor Alitalo was Europe’s second-most cited author in cell biology. He says, “Progress in biomedical science has been humbling. I hope some of my work has lasting value, ultimately for patients.” His most enjoyable work is “the ongoing work and the next one after that!”

The work by Professor Alitalo and his group in the characterisation of VEGF-B as a coronary growth factor, VEGF-C and VEGF-D and their receptors and signal transduction pathways, and the function of VEGF receptor (VEGFR)-3, showing that these ligands and their receptor are required for angiogenesis and lymphangiogenesis, has been significant. They discovered mechanisms of lymphoedema and devised molecular therapies for its treatment, validated them in large animals in collaboration with Seppo Ylä-Herttuala, MD, PhD (see http://circ.ahajournals.org/content/126/24/f139), of the University of Eastern Finland, Kuopio, Finland, and transferred the tools to a company that has been funded for phase I clinical trials. The inhibitors of angiogenesis and lymphangiogenesis that emerged from the work have already entered phase I clinical trials in the United States and Australia.

“I Am Certain That We Can Engineer Growth Factors That Have the Desired Properties of Regenerating Vessels for Cardiac or Leg Ischaemia”

Professor Alitalo is last author of a recent article in Circulation titled, “Vascular Endothelial Growth Factor–Angiopoietin Chimera with Improved Properties for Therapeutic Angiogenesis.”

As a child, Professor Alitalo suffered severe asthma, which, he believes, shaped his career and fuelled his appetite for knowledge. The doctors recommended the family move to the coast to see whether the seaside climate would help his condition. “It didn’t help at all,” he says, “but I went to 8 different schools in various part of Finland before I settled in Helsinki and started my studies. The asthma was so severe that sometimes I was exempted from attending school classes and ended up reading a lot on my own. I learned to study on my own, starting with English-speaking books at a very early stage, and when I was in the last years of school, I was teaching mathematical logic in the summer at the University of Helsinki. I guess I compensated by favouring exact sciences, mathematics, and so on, where I performed well. I shared the first prize in the school for the countrywide mathematics competition.” Photo courtesy of Hyvinkää Sanomat newspaper archive (1971).
Based on an unmet need for proangiogenic therapeutic molecules for the treatment of tissue ischaemia in cardiovascular diseases, the study designed and tested the angiogenic properties of chimeric molecules consisting of receptor-binding parts of VEGF and angiopoietin-1. The research group’s work was aimed at combining the activities of both factors into a molecule for easy delivery and expression in target tissues. They found that the VEGF-angiopoietin-1 chimera (VA1) is a potent angiogenic factor that triggers a novel mode of VEGFR-2 activation, promoting less vessel leakiness, less tissue inflammation, and better perfusion in ischaemic muscle than VEGF, and that these properties of the VA1 make it an attractive therapeutic tool.

Professor Alitalo says, “I am certain that we can engineer growth factors that have the desired properties of regenerating vessels of the type needed in, for example, cardiac or leg ischaemia or lymphoedema. VA1 is an example of modular design that may improve therapeutic utility, and some of our present efforts are in this direction.” He believes that the ongoing work at the Wihuri Research Institute, a nonprofit biomedical research institute focused on the vascular system in various diseases, should expedite progress in this area of research.

Professor Alitalo was the first to identify, by molecular cloning, several of the receptors and growth factors that govern the development and maintenance of blood vessels and lymphatic vessels. Significant among his work is the cloning, isolation, and characterisation of the first lymphangiogenic growth factor, VEGF-C, and isolation of lymphatic endothelial cells, opening up the vascular system to molecular analysis, as well as, together with Professor Ulf Eriksson, PhD, the cloning of VEGF-B, which has been shown to be a highly cardiac-specific angiogenic factor.

“I Published the Myc Oncogene Sequence, Described Its Protein Product, and Found That This Gene Was Amplified in Homogeneously Staining Chromosomal Regions of Tumour Cells”

Professor Alitalo attended the University of Helsinki, initially with the intention of studying mathematics and theoretical philosophy. However, he was urged by his father to pursue a more traditional profession so he could “feed his family,” so he duly applied to the medical school. Research fired his imagination from an early stage, and he was particularly interested in the malignant transformation of cells into cancer cells.

Professor Alitalo graduated from the Faculty of Medicine in 1977 as an MD and completed his PhD thesis in 1980 at the University of Helsinki in the research group of Antti Vaheri, MD, PhD, when Professor Vaheri and his collaborator, Erkki Ruoslahti, MD, PhD, had just discovered the matrix protein that they named fibronectin. Professor Alitalo recalls, “They used tumour viruses to convert normal cells to cancerous cells and showed that fibronectin is lost from transformed cells.” I discovered that tumour cells secrete a variety of previously unknown matrix proteins.” One of Professor Alitalo’s most important friends and colleagues at this time was Ralf Pettersson, MD, PhD, from the Ludwig Institute in Stockholm, Sweden, “who sadly died of cancer in 2011.”

On completion of his thesis, the faculty appointed Professor Alitalo Primus Doctorum for the upcoming promotion of the faculty. This meant answering a key question before a large distinguished audience about the mechanisms that lead to malignant transformation. The experience inspired Professor Alitalo to pursue research in this area.

Following a period in the lab of Paul Bornstein, MD, Department of Biochemistry, University of Washington, Seattle, WA, Professor Alitalo did postdoctoral work at the University of California in San Francisco, San Francisco, CA, with Michael J. Bishop, MD, and Harold E. Varmus, MD, who were jointly awarded the Nobel Prize in Physiology or Medicine in 1989 “for their discovery of the cellular origin of retroviral oncogenes.” Working with Professors Bishop and Varmus influenced, impressed, and inspired Professor Alitalo. He says, “I learned their attitude of pursuing the most important questions and never giving up on the real scientific values. In Finland at the time when I published my thesis, researchers were evaluated much more based on the number of their publications, which was not really the way science should be evaluated. When I moved to the United States, I learned to emphasise the pursuit of important and high standard science and interactions between scientists across disciplines. I tried to accommodate this culture when I returned, and now we share this culture and have the same values.”

In San Francisco, Professor Alitalo became involved in the work on oncogenes and says, “I published the Myc oncogene sequence, described its protein product, and found that this gene was amplified in homogeneously staining chromosomal regions of tumour cells. Together with my collaborator, Manfred Schwab, PhD, we discovered N-myc as an amplified oncogene in neuroblastoma.”3–6
“We Isolated and Characterised the First Lymphangiogenic Growth Factor, VEGF-C, and Its Receptor, VEGFR-3, Which Allowed Us to Grow Lymphatic Endothelial Cells and Vessels, Opening Up the Lymphatic Vascular System for Detailed Molecular Analysis”

When he returned to Finland in 1983, Professor Alitalo was able to build on the advances in cloning and genetic analysis research he had made while working with Professors Bishop and Varmus. He established an independent lab and vascular research programme with his own research group, initially around the signal transduction research that he had learned during his postdoctoral period. For this, he teamed up with his wife, Riitta, who was working on megakaryoblastic differentiation of leukaemia cells in culture. Together, using that model, they tried to clone a tyrosine kinase receptor that would stimulate megakaryoblastic proliferation.

The team amplified 10 novel tyrosine kinase complementary DNAs and started looking at their sites of expression in mice. Eight were isolated by reverse transcriptase polymerase chain reaction using degenerate primers by a young graduate student, now Professor Juha Partanen, PhD. In situ hybridisation for one of these genes, which was named tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains 1 (TIE1), indicated that it was specific for the vascular endothelium.7

“This groundbreaking discovery was what I needed to continue investigating. I realised that these types of receptor, tyrosine kinases, could have a major impact in tumour angiogenesis,” says Professor Alitalo.

“At the same time, a completely different research group, and later, George Yancopoulos, MD, PhD, and colleagues isolated its ligands, the angiopoietins. As none of the angiopoietins bound TIE1, we studied its activation mechanism further and found later that TIE1 and TIE2 formed in transcomplexes at endothelial cell–cell junctions, which was an unexpected novel mode of activation for receptor tyrosine kinases.8 Antagonists of angiopoietin-2, which behaves as a weak activator or antagonist of the TIE receptors, are currently in phase III clinical trials in cancer patients.9

Professor Alitalo’s projects in this area have developed rapidly, and the results have included the cloning and characterisation of fibroblast growth factor receptor-4,10 the vascular endothelial growth factor receptor-1,11 and VEGF-C and VEGFR-3.12,13 This was followed, in collaboration with Ulf Eriksson, PhD, by the cloning and characterisation and determination of VEGF-1 and neuropilin-1 as VEGF-B receptors.14,15

Professor Alitalo recalls that the key experiment that led to the identification of lymphangiogenesis was carried out in 1994 by Arja Kaipainen, MD, PhD, who was working on her PhD thesis in his lab after they cloned VEGFR-3.16 The result showed that VEGFR-3 became confined to the lymphatic vessels during embryonic development.17

“The first glance of her in situ hybridisation image told me that there had to be a specific growth factor pathway for lymphangiogenesis, which is distinct from angiogenesis,” Professor Alitalo says. “Indeed, VEGFR-3 did not bind vascular endothelial growth factor-A, placenta growth factor, or the newly cloned VEGF-B.”

Professor Alitalo’s highly disciplined postdoctoral fellow, Vladimir Joukov, MD, PhD, from the Kirov Military Medical Academy, Saint Petersburg, Russia, grew >1000 plates of prostate carcinoma cells to purify enough of the first VEGF-3 ligand, VEGF-C, for sequencing and cloning.18 Professor Alitalo continues, “Transgenic mice that expressed the VEGF-C in the skin were made by Michael Jeltsch, PhD, then a graduate student in my lab. He showed that the mice have hyperplastic lymphatic vessels in the skin, but essentially no changes in the blood vessels, which confirmed the lymphangiogenic activity of VEGF-C.19,20 Thus, ≈360 years after the first description of the lymphatic vessels and after 100 years of descriptive histology and pathology of the lymphatic system, we had isolated and characterised the first lymphangiogenic growth factor VEGF-C and its receptor VEGFR-3, which allowed us to grow lymphatic endothelial cells and vessels, opening up the lymphatic vascular system for detailed molecular analysis.”

VEGF-D followed. It was characterised in collaboration with Associate Professors Marc Achen, PhD, and Steven Stacker, PhD, from Melbourne, Australia. All this opened up an entire field of lymphatic vascular biology research for molecular regulatory analysis and therapeutics development.21,22

Along with his central role in characterising VEGF-B, VEGF-C, and VEGF-D receptors and signal transduction pathways, Professor Alitalo discovered the function of VEGF-3, showing that this receptor is required for angiogenesis and later in lymphangiogenesis in embryos.23-29 He also developed a method for isolating human primary lymphatic and blood microvascular endothelial cells, and showed that these cells have interesting differences in gene
expression relevant for their distinct functions in vivo.\textsuperscript{36} His data provided important insights into the phenotypic diversity of endothelial cells and the possibility of transcriptional reprogramming of differentiated endothelial cells.\textsuperscript{31} “We developed a gene therapy model for human lymphoedema in mice,” he adds.\textsuperscript{32,33} His studies have led to the demonstration of VEGF-C-associated tumour lymphangiogenesis, intra-lymphatic tumour growth, and VEGF-C association with tumour metastasis and its inhibition by blocking the VEGFR-3 signalling transduction pathway.\textsuperscript{34–36}

Professor Alitalo says that this work with VEGF-C shows clinical translational potential, with interest from industry in a research area that has seen his group describe the molecular pathogenesis of the most common forms of human hereditary lymphoedemas.\textsuperscript{37,38} In collaboration with Professor Eriksson in Stockholm, Professor Alitalo and his research group cloned and characterised VEGF-B with the hope that it would be a candidate ligand for VEGFR-3, but they found that VEGF-B had its own distinct function. The ongoing work conducted on VEGF-B as a coronary vascular growth factor is among his most interesting projects. “It is our next translational target, which is also involved with cellular metabolism,” he says. “We are building various types of vessels in different tissues, and VEGF-B has an impressive effect on coronary vasculature. We are still trying to understand how it works, the reason for its specificity, and whether one can develop therapeutic tools based on this factor and the emerging knowledge.”

**“Be Willing to Question Previous Concepts and Always Be Critical About Medicine That Is Taught in Textbooks Because It Is Rapidly Changing”**

During the early stages of his career, Professor Alitalo combined weekend and night shifts in the clinics with his research work, but when he returned from the United States his research interests developed and took on a new momentum when he was offered a research fellow position with the Finnish Academy. He was also professor in medical biochemistry at the University of Turku, Turku, Finland, and professor of cancer biology at the University of Helsinki before being offered one of the few positions as a permanent research professor of the Finnish Academy at the University of Helsinki. “I was lucky to get this position,” he reflects, “and I have been very satisfied with it because it allows me a lot of freedom.”

From the start of 2013, Professor Alitalo took on responsibility as the director of the Wihuri Research Institute, which has been in existence for ≈70 years but has now moved to his own institute, Biomedicum Helsinki. He also does postgraduate teaching.

Professor Alitalo’s labs in the Biomedicum Helsinki Research Institute of the University of Helsinki focus on two areas: cardiovascular research and investigating the possibility of inhibiting cancer spread using blocking antibodies or soluble receptors for the vascular growth factor receptors. Professor Alitalo comments that if the group could inhibit blood or lymphatic vessel growth, they could also stimulate the growth and arterialisation of blood and lymphatic vessels.

He explains, “In ischaemic heart or in the leg, this could improve oxygenation by delivering more vessels to carry oxygen and nutrition, and more lymphatic vessels should decrease tissue oedema. This is what really led me to the field of cardiovascular biology. Gradually, we received more funding as our work became better known, and we had established more connections to other investigators in the vascular biology and cardiovascular fields internationally.”

“I am exceptionally happy every day when I can really concentrate on research and avoid too much administration and successfully combine my family life with the demanding lifestyle of a scientist,” adds Professor Alitalo. He says the roles he enjoys most are those as an independent research scientist, plus his “second role” as a grandfather.

One area of concern for Professor Alitalo, from the perspective of a scientist working in Finland, is how globalisation will influence science. He says, “I have encountered the misunderstanding in some scientific bodies that the character of a universal global ‘director’ is suitable for leadership of science in a company style. The global information highways are starting to exceed anyone’s capacity to keep abreast of the developments, and the danger is that we can no longer exercise good criticism of all the recent developments and instead start to operate on a more superficial level.”

Professor Alitalo is thankful for his numerous collaborators and colleagues, including those who have provided excellent infrastructure and valuable materials for his studies. His work currently receives funding from the Academy of Finland, Jenny and Antti Wihuri Foundation, European Research Council, Foundation Leducq, Seventh Framework Programme of the European Union, Marie Curie’s Initial Training Networks, the Juselius Foundation, Biocentrum Helsinki, Biocenter Finland, Helsinki University Central
therapeutics, and personalised medicine coming from the genome project and the subsequent functional genomics era in which we are now living. It is extremely important that people understand the basics of the new, more biological drugs."

Professor Alitalo also emphasises that good levels of communication should be maintained between those working in basic science and the clinics so the two do not "drift apart."

Professor Alitalo believes that future developments in his field will be in stem cells, reprogramming, biologicals, and gene therapy. He would like to develop further research connections across the world, ideally through an adjunct professorship with another university, and help future scientists, researchers, and clinicians in Finland continue to compete and collaborate with contemporaries in the world’s leading institutions. "I want to help the next generation here in Finland by staying connected, and I think the best I can do in my career is to help select and train a brilliant next generation in my home institute and help to further sciences in all contexts where I am involved," he says.

"It is not publications that count, but the people. Training somebody and making an investment in talented people is going to give much more than any of your single publications, no matter where they are published or quoted."

Over the years, Professor Alitalo has received many prizes, including the Louis-Jeantet Prize for Medicine in 2006 (see http://circ.ahajournals.org/content/124/8/f43), the InBev-Baillet Latour Health Prize (see http://circ.ahajournals.org/content/124/13/f73), the Jahre Prize in 2010, and the A. I. Virtanen Prize in 2013. He says, "The best grant so far was from the Louis-Jeantet Foundation, provided as a scientific prize for our research. The foundation essentially trusted my own judgement on the course of the research path to use the grant and this was fruitful. In other applications, we may have to commit ourselves by saying in advance exactly what we are going to do during the sixth year of funding, which isn’t always possible."

Professor Alitalo advises people wanting to follow a career in medicine/cardiology to be interactive and recognise how fast research is progressing. "Be willing to question previous concepts and always be critical about medicine that is taught in textbooks because it is rapidly changing," he says. "For innovative ideas, have an open mind and communicate with experts of a variety of disciplines. It would also be good for people to prepare for the future by having a solid basic sciences background, with more and more diagnostics, therapeutics, and personalised medicine coming from the genome project and the subsequent functional genomics era in which we are now living. It is extremely important that people understand the basics of the new, more biological drugs."

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