Looking For Biomarkers That May Predict Individualised Cardiovascular Risk

Karsten Sydow, MD, senior physician, Department of General and Interventional Cardiology, University Heart Center, Hamburg, Germany, talks to Mark Nicholls.

For more than a decade, Karsten Sydow, MD, senior physician at the Department of General and Interventional Cardiology, University Heart Center, Hamburg, Germany, has investigated endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine (ADMA) as a biomarker for cardiovascular risk. This research culminated in a 2012 article in *Circulation*, in which he and his colleagues showed that ADMA profoundly impairs nitric oxide synthesis of polymorphonuclear neutrophils resulting in increased polymorphonuclear neutrophil adhesion to endothelial cells, superoxide generation, release of leukocyte-derived haemoprotein myeloperoxidase (MPO), and subsequent impairment of dimethylarginine dimethylaminohydrolase activity. These data highlighted the previously unrecognised cytokine-like properties of ADMA and identified MPO as a regulatory switch for ADMA bioavailability under inflammatory conditions.

Dr Sydow’s interest in ADMA began in Hannover in 1996 and 1997 with his doctoral thesis, titled “Interactions Between the L-Arginine-NO and Homocysteine Pathway: Examinations in Cultured Human Endothelial Cells and Patients With Peripheral Arterial Disease.” That interest continued in his early days in Hamburg and was sustained when he spent time at Stanford University, Stanford, CA, using transgenic mice models where he collaborated with Masashi Tanaka, MD, of the Department of Cardiothoracic Surgery during a postdoctoral fellowship. “With the mice models we were able to determine some underlying mechanisms by which ADMA was acting on the vasculature and even inflammatory processes,” he says. “It was amazing to see Masashi Tanaka performing the heterotopic heart transplantations in these tiny animals and how we were able to transfer our hypotheses into an in vivo model.”

Since 2009, Dr Sydow has been a senior physician in the Department of General and Interventional Cardiology, where his clinical work takes ≈80% of his time and includes interventional cardiology, coronary angiography, right heart catheterisation, echocardiography, rounds, and consultations. He is involved in the Department’s move towards mitral valve replacement therapy later this year. “It will be interesting to see how the interventional mitral valve will perform,” he says. “This may have the potential to change or even revolutionise the cardiovascular field.” Photograph courtesy of Dr Sydow.

On other pages...

**Funding:** British Heart Foundation Intermediate Basic Science Research Fellowships

Three recipients of British Heart Foundation Intermediate Basic Science Research Fellowships describe their research and fellowship, which provides 4 to 5 years of funding to postdoctoral cardiovascular scientists who aspire to become research leaders in the United Kingdom.
In my opinion, ADMA plays an important role in modulating and may be even initiating cardiovascular diseases, but I am uncertain whether ADMA will make the step to become a clinically relevant biomarker that predicts individualized cardiovascular risk.” This recognition that ADMA may not discriminate patients with high risk and low risk because the cutoff between the pathologic and physiologic is too narrow has prompted a change in Dr Sydow’s research direction. Though still working with biomarkers that may identify patients at individual risk of coronary artery disease, hypertension, and heart failure, Dr Sydow will be looking for those that may predict individualized cardiovascular risk using magnetic resonance and computed tomography imaging and genomics.

Dr Sydow’s most important research so far has been an investigation into how low-density lipoprotein cholesterol upregulates ADMA synthesis in human endothelial cells. He explains, “We were able to show a pathway by which ADMA was synthesised. At the time, ADMA had been an emerging risk factor shown to be associated with cardiovascular risk factors and diseases. However, the understanding about its origin, synthesis, and degradation was only marginal.” While working on this project, Dr Sydow was inspired by a 1992 article by Patrick Vallance, MD, FRCP, FMedSci, who was the first to show that ADMA is increased in patients with renal insufficiency and that exogenous administration of ADMA impaired vasodilation and increased blood pressure, and developed his interest in basic research and academic medicine.

Dr Sydow’s research work is funded by the Deutsche Forschungsgemeinschaft and the Department of General and Interventional Cardiology. He has won a number of awards, including the Doctoral Thesis Awards of the German Society of Angiology in 2001, the Young Investigator Awards Competition at the 15th Annual Meeting of the Society of Vascular Medicine and Biology, Anaheim, CA, in 2004, and a Young Investigator Award for an outstanding presentation at the 4th International Conference on ADMA, Bregenz, Austria, in 2008.

“The Huge Variety of Interventions Was the Most Exciting Thing for Me”

Dr Sydow was born in Hildesheim, Germany, and studied at Hannover Medical School in Hannover, Germany, from 1993 to 2000. He had an early fascination for the electrocardiogram. “That was what opened my eyes to cardiology. I was fascinated how, through the electrocardiogram, you can see the function of the heart on written paper and extrapolate from that what may be wrong with the organ,” he says. “As I gained more experience at medical school, I became fascinated by the variety of interventional approaches you can choose in cardiology: from percutaneous coronary intervention, pacemaker and defibrillator implantation, electrophysiology, to renal denervation therapy, and now interventional valve treatment. The huge variety of interventions was the most exciting thing for me. What is really fascinating is that you see patients with an acute myocardial infarction and you are able to help them immediately.”

On leaving medical school, Dr Sydow became a medical resident at Hamburg University Heart Center in the Department of General and Interventional Cardiology headed by Professor Thomas Meinertz, MD, FESC, FACC (see http://circ.ahajournals.org/content/123/12/I67), who was a major influence with his “tremendous clinical expertise, remarkable soft skills, and always placing the patient...
and his particular needs at the centre of things.” Professor Rainer Böger, MD, head of the Institute of Clinical Pharmacology and Toxicology at Hamburg University Medical School was Dr Sydow’s doctoral adviser in Hannover and supported his interest in basic research and an academic cardiovascular career. He helped Dr Sydow obtain a position as an internal medicine fellow at Hamburg University Heart Center in 2000.

From 2003 to 2004, Dr Sydow worked as a postdoctoral cardiovascular medicine fellow at Stanford University Medical School with Professor John P. Cooke, MD, PhD. “He taught me to focus on the most relevant findings and projects and also that sometimes it is hard to cut some projects in which you are really interested and in which you have put so much effort and time, but whose hypotheses do not seem to work,” says Dr Sydow. “However, to contribute significantly, you have to cut these bridges sometimes. He was always looking for the next but one step.”

Dr Sydow also has responsibilities in teaching medical and pharmacology students, and he continues his basic and clinical research. At the university hospital, he is coordinator for the Internal Medicine/Pharmacology/Pathology module, coordinator for the Cardiology module, and responsible for the Cardiovascular/Pulmonary/Emergency Medicine module of the reform curriculum at Hamburg University Medical School, which starts in October 2012. He says, “I enjoy cardiology lessons, bedside teaching, and teaching in the cath lab. Teaching is important in our department, and there is a lot of enthusiasm for it. In addition, the students often ask questions we need to find the answers to, so there is a big benefit to us.

“The Hamburg University Heart Center is a great place to work. I work in a great team, it is a great place for innovative cardiology, there is a great collaborative atmosphere with the Department of Electrophysiology and Department of Heart Surgery, and I can mix clinical work, science, and teaching.”

In Hamburg, Professor Stephan Baldus, MD, has supported Dr Sydow with intellectual discussions and clinical expertise, and more recently Professor Stefan Blankenberg, MD, head of the Department of General and Interventional Cardiology, has proved inspirational. “He has taught me to think ‘big’ with a large number of patient cohorts and again to focus on the most relevant issues,” says Dr Sydow.

References

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Intermediate Basic Science Research Fellowships

Providing 4 to 5 Years of Funding to Postdoctoral Cardiovascular Scientists Who Aspire to Become Research Leaders in the United Kingdom

Three recipients of British Heart Foundation Intermediate Basic Science Research Fellowships describe their fellowship and research to Jennifer Taylor, BSc, MSc, MPhil.

The British Heart Foundation (BHF) awards Intermediate Basic Science Research Fellowships to scientists who aspire to become research leaders in the United Kingdom. Grants are for 4 years, with a possible 1-year extension, and may include up to 1 year abroad. Applicants who should normally be a national of the European Economic Area should have completed 3 to 6 years of postdoctoral work and published a number of high-impact articles. Awards may include the fellow’s salary, the salary of a technician or research assistant, research consumables, and research equipment.

Investigating the Role of Hira During Neural Crest Cell Differentiation at the Epigenetic Level

Ariane Chapgier, PhD, BHF Intermediate Basic Science Research Fellow at the Institute of Child Health, University College London, London, England, received the fellowship in 2010. Her project is to investigate the role of Hira in the epigenetic events involved during cardiac neural crest cell differentiation in vitro and during cardiac development. She is being supervised by Professor Peter Scambler, MD.

Cardiac neural crest cells develop into melanocytes, neurons, cartilage, and connective tissue (of the third, fourth, and sixth pharyngeal arches), and they produce the entire musculoskeletal tissue wall of the large arteries as they arise from the heart and contribute to the septum that separates the pulmonary circulation from the aorta. Defects in neural crest cell development contribute to, among other congenital diseases, DiGeorge syndrome, which is being studied in Professor Scambler’s lab.

Hira deposits the histone variant H3.3 in a replication-independent manner on the gene body of transcriptionally active and repressed genes. Chromatin remodelling in the cardiac neural crest cells is vital for cardiovascular morphogenesis, and studies in chickens suggest that in mammals, Hira might be required in neural crest cell differentiation.

Dr Chapgier is investigating the role of Hira during neural crest cell differentiation at the epigenetic level. More broadly, she plans to describe the epigenetic mechanisms involved in cardiac neural crest cell differentiation. “To this aim, I am developing an in vitro culture model of neural crest cells deficient for Hira. In addition, I am analysing mouse conditional mutants deficient for Hira in the neural crest cell lineage,” she says. “Furthermore, cardiac neural crest cells are present as dormant multipotent cells in the adult heart and are capable of differentiating into cardiomyocytes, so my long-term work would potentially help future stem cell therapy for heart disease.”

Before being awarded this fellowship, Dr Chapgier worked as a postdoctoral research fellow using a Biotechnology and Biological Sciences Research Council fellowship in Professor Scambler’s lab. She studied the role of Hira during gastrulation in Hira-null embryos, and embryonic stem cell differentiation. Embryonic stem cells are taken into the blastocyst from the inner cell mass before gastrulation starts, and their differentiation towards a mesodermal lineage in vitro mimics the first stages of gastrulation.

Dr Chapgier has developed a collaboration with the lab of Professor C. David Allis, PhD, at the Rockefeller University in New York, NY, and used a European Molecular Biology Organization short-term fellowship to learn about epigenetics in his lab for 4 months. She looked at H3.3 deposition at the genome-wide level and compared this deposition in wild type versus Hira null cells in undifferentiated and mesodermally differentiated embryonic stem cells. She then correlated the differences in H3.3 deposition with the gene expression changes observed between Hira null and wild type embryonic stem cells.

Similar to embryonic stem cells, neural crest cells are pluripotent cells that can give rise to different tissues, so this previous experience of working on epigenetic changes during differentiation events is helpful in her current project. Before the fellowship, Dr Chapgier also gained experience in developing conditional mice that allow the knockdown of Hira expression in neural crest cells specifically.

“I think I was successful in my fellowship application because I had previously developed all the tools to answer my
project in the right environment, and my articles show a proven track record,” says Dr Chagpier. “Neural crest cells are present as potential stem cells in the heart, so my investigation of the epigenetic mechanisms of their differentiation may contribute to clinical therapies for heart diseases.”

She adds, “This fellowship gives me the nice opportunity of developing my own team and area of research on the mechanisms of stem cell differentiation for the use of stem cell therapy to treat heart diseases.”

**Reference**


**Investigating Whether Vascular Endothelial Growth Factor (VEGF)-C Can Regulate VEGF-A-Induced Changes in Glomerular Endothelial Cell Barrier Properties in Health and Disease**

Rebecca R. Foster, PhD, BHF Intermediate Basic Science Research Fellow, School of Clinical Sciences, University of Bristol, Bristol, England, was awarded a BHF fellowship in 2010. Her 4-year project asks, “Can VEGF-C regulate VEGF-A-induced changes in glomerular endothelial cell barrier properties in health and disease?”

Early predictors of heart failure improve mortality rates and reduce healthcare costs. Systemic endothelial dysfunction is 1 such early predictor. It is associated with urinary microalbumin secretion. Microalbuminuria of 30 to 300 mg/day indicates a disruption in the renal glomerular filtration barrier, occurs in various renal pathologies, and precedes overt diabetic nephropathy.

Vascular endothelial growth factor (VEGF-A) is an angiogenic protein that is highly expressed by epithelial cells including specialised epithelial cells (podocytes) within the renal microvasculature system (glomerulus). Changes in the levels of its expression are associated with systemic and glomerular disease (eg, hyperfiltration in diabetic nephropathy), but anti-VEGF therapies are renotoxic. Dr Foster has shown that VEGF-C, a lymphangiogenic protein also expressed by podocytes, can block VEGF-A-induced increases in glomerular endothelial permeability in a human immortalised cell line.

Dr Foster is investigating the VEGF receptor (VEGFR) activation profiles and changes in glomerular endothelial cell barrier properties in response to VEGF-C and VEGF-A using in vitro systems and then explored further in podocyte-specific diabetic and nondiabetic VEGF-C-inducible mice. The work involves a previously developed approach of manipulating glomerular VEGF-A to treat diabetic nephropathy. However, manipulation of VEGF-A using VEGF-C, a member of the same family of proteins and one that appears to compensate for VEGF-A physiologically, has not yet been explored.

“I aim to dissect the effects of VEGF-C on glomerular endothelial cell barrier properties and decipher whether VEGF-C can ameliorate the effects of VEGF-A on increased glomerular albumin permeability in diabetic and nondiabetic conditions, both in vitro, using human cell lines, and in vivo, using transgenic mouse models,” says Dr Foster. “This project will establish the effects of VEGF-C on glomerular endothelial cell barrier properties and assess its clinical potential in diabetic nephropathy and other systemic diabetic pathologies, as well as its potential for reduced renotoxic effects.”

The aims of the project are to examine how VEGF-C activation of VEGFRs differs to, and modulates, VEGF-A; to identify which aspect of the glomerular endothelial cell barrier that VEGF-C acts on to block the effects of VEGF-A; and to determine whether VEGF-C can block the adverse effects of increased macromolecular passage due to VEGF-A in a diabetic mouse model.

VEGF-A is highly expressed in the podocytes of mature glomeruli despite a distinct lack of angiogenesis. Much of Dr Foster’s early research has been devoted to explaining this paradox. In 2003, she demonstrated that VEGF-A could promote survival in human podocytes in an autocrine manner,1 and she later showed that this was dependent on a podocyte-specific protein (nephrin).2 She also demonstrated that VEGF-C is expressed by podocytes and that it too could promote survival in podocytes in an autocrine manner.3

Other groups have focused on the effects of transgenic manipulation of podocyte VEGF-A, and it is now widely accepted that VEGF-A is critical in glomerular endothelial cell maintenance in the mature glomerulus. Taking this a step further, Dr Foster investigated how VEGF-C may manipulate glomerular endothelial cell behaviour.4 She and her colleagues demonstrated that VEGF-C could phosphorylate the main signalling receptor for VEGF-A, VEGFR2, in glomerular endothelial cells but not VEGFR3, which is the typical signalling receptor for VEGF-C. The phosphorylation of VEGFR2 by VEGF-C was over a later time course than VEGF-A and VEGF-C and resulted in a comparatively much reduced increase in intracellular calcium. “Importantly we went on to show that VEGF-C dose-dependently increased monolayer integrity and decreased protein permeability in contrast to VEGF-A,” says Dr Foster.

Together, these articles gave rise to the hypothesis of Dr Foster’s fellowship project—that increased VEGF-C will counteract the barrier effects of increased VEGF-A exposure on glomerular endothelial cells. Dr Foster says, “This fellowship has given me the fantastic opportunity to direct my own project and develop my own research team and has been an invaluable boost to my career.”
Dr Chung completed her PhD on the physics of complex magnetic systems at the University of Warwick, West Midlands, England. Afterwards, she was keen to apply her physics research background to problems in the biological sciences. She joined the National Health Service in 2004 as a medical physicist based at Leicester Royal Infirmary, and she quickly became involved with Doppler embolus detection research led by Professor David Evans, PhD, and published a series of articles on the detection of emboli by human observers. “As part of my clinical duties, I performed routine Doppler ultrasound measurements during carotid endarterectomy, which was crucial in helping me understand the problems and limitations of the transcranial Doppler technique,” she says.

In 2007, Dr Chung proposed the first computational model of embolic stroke,1,2 which later formed the basis of her fellowship proposal. She also performed lab experiments to investigate binding of ultrasound contrast agents targeted to thrombus emboli,3 explain anomalous Doppler signals associated with vascular occlusion,4 and map the trajectories of solid emboli on encountering the circle of Willis.5 In 2007, she received a EUROSON Young Investigator prize, and she was a short-listed nominee for a UK L’Oréal-Unesco Woman in Science award. Recent highlights include giving a talk for World Stroke Day 2011 and having an image from her research5 featured on the April 2010 cover of Stroke.

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