

3rd International Symposium

NEW FRONTIERS in CARDIOVASCULAR RESEARCH

8-9 March 2016

8:00 AM to 7:00 PM

Lecture Theatre, Level 7

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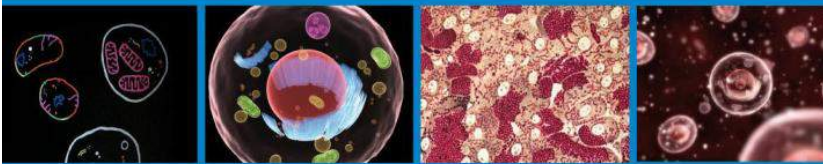


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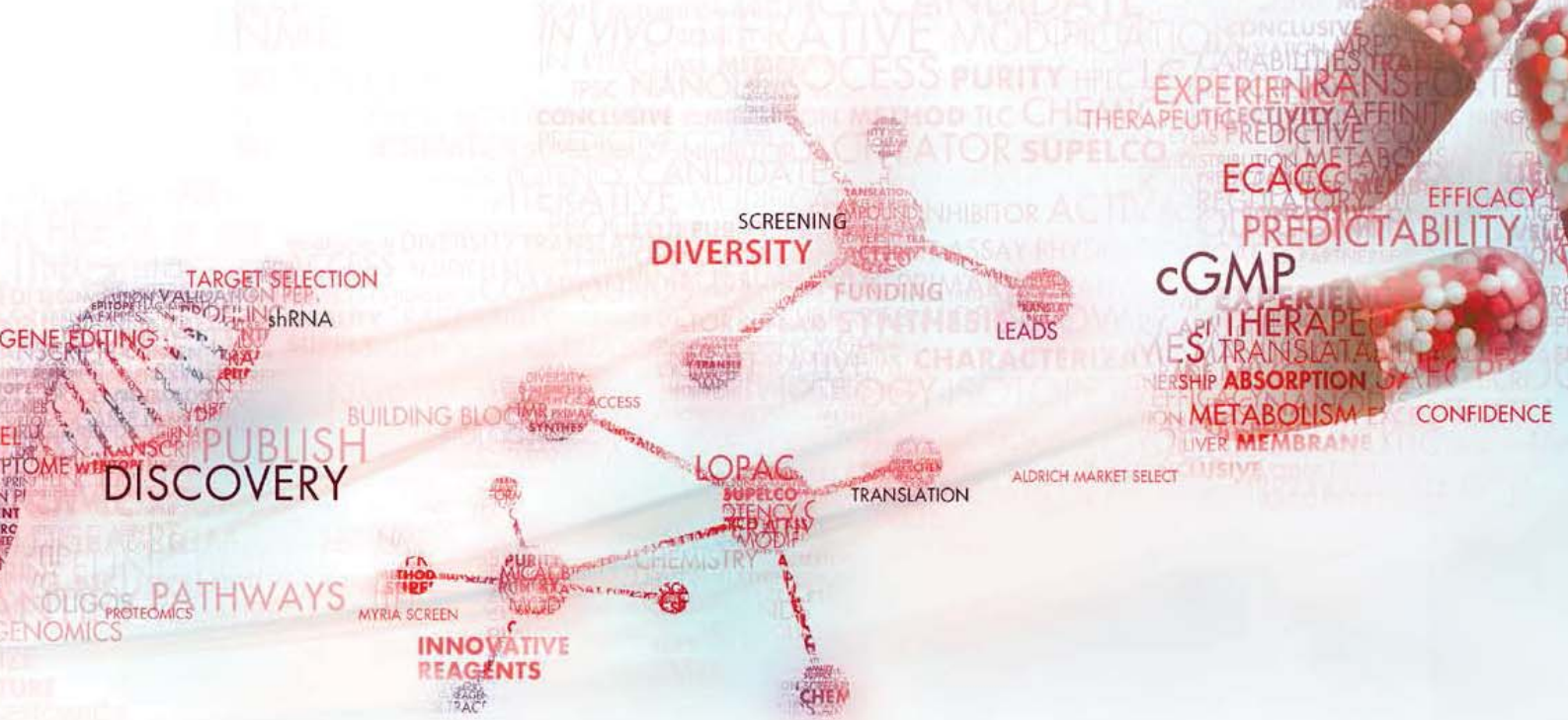
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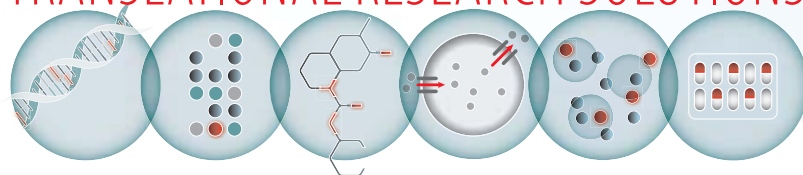


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SCIENTIFIC PROGRAM

Tuesday, March 8, 2016

08:00 *Registration at NHCS Atrium Level 7, Lecture Theatre*

08:30 *Welcome Address*

Terrance Chua - Medical Director of National Heart Centre Singapore (NHCS)

Stuart Cook - Director of National Heart Centre Institute of Singapore

Director of the Program in Cardiovascular & Metabolic Disorders, Duke-NUS, Singapore

Welcome and Introduction

Derek J. Hausenloy (Singapore-UK) and **Klaus T. Preissner (Giessen, Germany)**

Session 1

Protecting Your Heart – Basic

08:45 – 10:45

Chair: **Derek Yellon (London, UK), Stuart Cook (Duke-NUS, Singapore)**

Gerd Heusch (Essen, Germany)

Remote ischemic preconditioning and its signal transduction

Peter Ferdinandy (Budapest, Hungary)

Why do we still not have cardio-protective drugs? Need for unbiased “omics” approach and co-morbidity models to find valid targets

Sandrine Lecour (Capetown, South Africa)

Novel therapies to activate the SAFE path for cardio-protection

David Garcia-Dorado (Barcelona, Spain)

Combined therapies against myocardial reperfusion injury in STEMI

Hans J Schnittler (Munster, Germany)

The two faces of Junction Associate Intermittent Lamellipodia (JAIL) in sealing and remodeling of cell junctions

10:45 – 11:30

Breakfast at NHCS Atrium Level 7, Lecture Theatre

Session 2

Protecting Your Heart – Clinical

11:30 – 13:30

Chair: **Klaus T. Preissner (Giessen, Germany), Carl-Wilhelm Vogel (Hawaii, USA)**

Hans Bøtker (Aarhus, Denmark)

Remote ischemic conditioning: From laboratory bench to hospital bedside

Andreas Boening (Giessen, Germany)

Remote ischemic preconditioning: Magic trick or helpful tool?

Saadah Suleiman (Bristol, UK)

The use of Propofol cardioplegia in cardiac surgery: Experimental & clinical evidence

Michael Marber (London, UK)

Measurement of Cardiac Myosin Binding Protein C in the systemic circulation

Alan Fong (Sarawak, Malaysia)

ACS in South East Asia: Insights into a multi-ethnic population

13:30 – 14:30

Lunch at NHCS Atrium Level 7, Lecture Theatre

Session 3

Cardiovascular Scheherazade

14:30 – 16:30

Chair: Wei He Ming (NHCS, Singapore), Jean-Paul Kovalik (Duke-NUS, Singapore)

Winston Shim (NHCS, Singapore)

Induced pluripotent stem cells in cardiac arrhythmias

Karl Tryggvason (Duke-NUS, Singapore)

Xeno-free laminin-based protocol for generating clinical quality cardio-myocytes from hES cells

Manvendra Singh (Duke-NUS, Singapore)

Hippo signaling mediators Yap and Taz are required in the epicardium for coronary vasculature development

Erich Gnaiger (Innsbruck, Austria)

To the heart of the Amazon: Comparative cardiac mitochondrial physiology

Malcolm Walker (London, UK)

Cardio-oncology: A new opportunity for translation; a new target for cardio-protection

16:30 – 17:00

Coffee break at NHCS Atrium Level 7, Lecture Theatre

Session 4

Inside – Outside Vessels

17:00 – 19:00

Chair: Ye Lei (NHCS, Singapore), Mark Chan (NUS, Singapore)

Jürgen Bernhagen (Munich, Germany)

MIF family cytokines in cardiovascular disease: Update and new members

Bernd Engelmann (Munich, Germany)

Microvascular thrombosis: pathological relevance in vascular disease

Daniel Sedding (Hannover, Germany)

Inhibiting miR-146a augments angiogenesis and myocardial regeneration

Felix B. Engel (Erlangen, Germany)

Challenges in cardiac regeneration based on cardio-myocyte proliferation

Carolyn Lam (Duke-NUS, Singapore)

Understanding Heart Failure With Preserved Ejection Fraction

Concluding remarks (day 1)

19:00

Wine and cheese reception at NHCS Atrium Level 8, Lecture Theatre

Tetsuji Miura (Sapporo, Japan)

Enlargement of myocardial infarct size by chronic kidney disease:
A novel mechanism of inhibiting protective signaling to mitochondria

Paul Yen (Duke-NUS, Singapore)

Hormonal control of lipophagy and mitophagy

Rainer Schulz (Giessen, Germany)

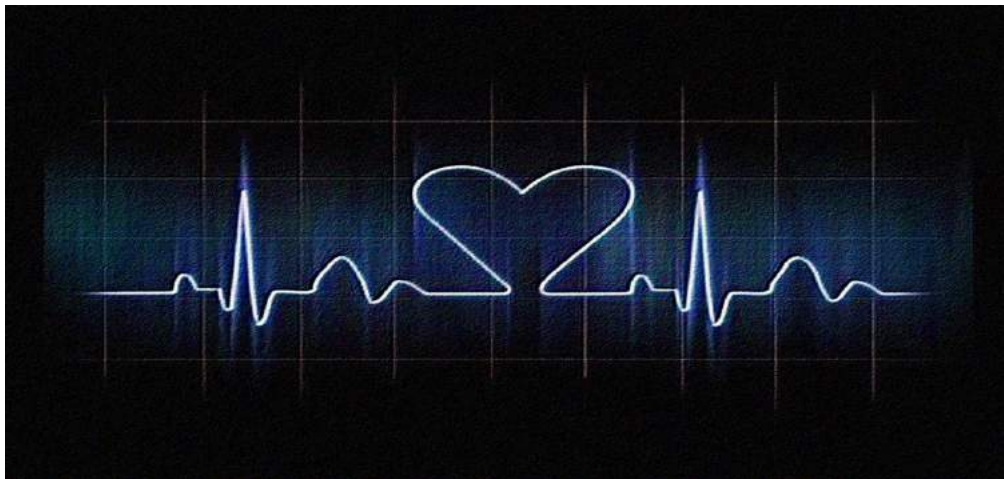
Importance of mitochondrial proteins for myocardial ischemia/reperfusion
injury and protection from it

16:15 Concluding remarks

Thomas Coffman - Dean, Duke-NUS Graduate Medical School, Singapore

Patrick Casey - Senior Vice Dean Research, Duke-NUS Graduate Medical School, Singapore

16:30 End of Scientific Meeting



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A B S T R A C T S

(in scientific program order)

Remote ischemic conditioning and its signal transduction

Gerd Heusch, Petra Kleinbongard

Institute for Pathophysiology, West German Heart and Vascular Center,
University of Essen Medical School, Germany

In remote ischemic conditioning (RIC), brief and fully reversible episodes of ischemia/reperfusion in one vascular bed, tissue or organ confer a global protective phenotype and render remote tissues and organs resistant to ischemia/reperfusion injury. This phenomenon has been confirmed in different experimental models and species, including humans. Cardioprotection by RIC has been documented as reduced infarct size in experimental animals. Patients undergoing elective or primary percutaneous coronary interventions or coronary artery bypass grafting had reduced biomarker (CK-MB, troponin) release or reduced infarct size on imaging when undergoing RIC before the intervention. The signal transduction of RIC is not yet clear in detail. The peripheral stimulus can be chemical, mechanical or electrical in nature and appears to involve the activation of peripheral sensory nerves. The transfer of the protective signal from the periphery to the heart or other organs is through neuronal and humoral communications. Protection can be transferred, even across species, with plasma or a plasma-derived dialysate and involves nitric oxide, stromal-derived factor-1 α , microRNA-144, but also other, not yet identified factors. The signal transduction within the myocardium involves adenosine, bradykinin, cytokines and chemokines and the activation of their specific receptors. The RISK- and SAFE-kinase programs are activated by RIC. In human left ventricles with prior RIC, STAT 5 phosphorylation is increased, and mitochondrial function is improved. Further and more comprehensive understanding of the molecular mechanisms underlying RIC will help overcome confounders and facilitate translation to patient care.

For review see: JACC 65, 2015, 177-95 and Circ Res 116, 2015, 674-99.

Supported by the German Research Foundation (He 1320/18-3; SFB 1116 B8), the Dr. Heinz-Horst Deichmann Foundation, the Hans and Gertie Fischer Foundation and the German Heart Foundation.

*Why do we still not have cardioprotective drugs?
Need for unbiased “omics” approach and co-morbidity models to find valid targets*

Péter Ferdinandy

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; Pharmahungary Group, Szeged, Hungary

Ischemic heart disease is the leading cause of mortality worldwide, therefore, identification of valid drug targets for cardioprotection is of great importance. However, we still do not have cardioprotective drugs on the market. The discovery of ischemic preconditioning (3 decades ago), postconditioning, and remote conditioning triggering endogenous cardioprotective mechanisms that render the heart more resistant to lethal ischemic-reperfusion injury gave much hope to identify cardioprotective drug targets. However, it seems that major cardiovascular co-morbidities such as hyperlipidemia, diabetes, and their co-medications interfere with most of the known cardioprotective mechanisms. Ischemia reperfusion injury and cardioprotection by conditioning have been shown to affect global myocardial gene expression profile at the transcript level. Moreover, fine tuning regulators of mRNA expression, miRNAs also contribute to cardioprotective gene expression response of the heart. Cardiovascular co-morbidities have been also shown to affect global cardiac gene expression profile. Further understanding and the comprehensive analysis of the cardioprotective gene expression fingerprint at the transcript and protein level in normal, protected, and in comorbid conditions may lead to identification of novel molecular targets for cardioprotection.

Novel therapies to activate the SAFE path for cardioprotection

Sandrine Lecour

Cardioprotection Group, Hatter Institute for Cardiology Research in Africa,
University of Cape Town, South Africa

An important emerging concept is that the heart has an innate immune-related protective mechanism. In the context of ischemic heart disease, our latest research showed that Tumor Necrosis Factor alpha (TNF-alpha), a major player of the immune system, initiates the activation of a cardioprotective signalling pathway that involves the activation of the signal transducer and activator of transcription 3 (STAT-3). We have named this path the SAFE (Survivor Activating Factor Enhancement) pathway (Lecour, J Mol Cell Cardiol, 2009). Our current research aims to better characterize this novel path which represents a novel opportunity in the development of new drug therapies for ischemic heart disease. Recent discoveries within the group suggest that toll-like receptor 4, sphingosine-1 phosphate and activation of specific microRNAs form part of this pathway (Nduhirabandi et al, J Pin Res, 2015).

The activation of the SAFE pathway is made possible with dietary melatonin. Indeed, we have recently demonstrated that melatonin, given at a concentration found in red wine, can protect against ischemic heart disease and most importantly, confers cardioprotection against pulmonary hypertension, a disease with currently inexistent efficient therapy (Lamont et al, J Pin Res, 2010; Lamont et al, BBRC, 2015; Maarman, J Pin Res, 2015).

High density lipoproteins can also activate the SAFE pathway to protect against ischemia-reperfusion injury. In 2002, we were first to report that sphingosine-1 phosphate can protect against reperfusion injury (Lecour et al, J Mol Cell Cardiol, 2002). Sphingosine-1 phosphate is a major component of high density lipoproteins, often referred to as the “good” cholesterol. Using various reconstituted HDL (synthesized by our research collaborators in Switzerland), we have demonstrated that sphingosine-1 phosphate contributes to the cardioprotective effect of HDL against reperfusion injuries by activating the SAFE pathway and ultimately regulating the mitochondrial function (Brulhart-Meynet MC et al, Plos One, 2015). With the recent acquisition of the lipoprint system, we are now able to explore the subfractions, composition and functionality of lipoproteins in different pathophysiological conditions such as obesity, hypertension and heart failure.

Combined therapies against myocardial reperfusion injury in STEMI

David Garcia-Dorado

Head, Department of Cardiology, Vall d'Hebron University Hospital & Research Institute,
Universitat Autònoma de Barcelona, Spain

Patients with STEMI continue to have a significant mortality and a high rate of development of heart failure despite receiving reperfusion therapy as early as possible. Preclinical studies and some proof of concept clinical trials indicate that attenuation of reperfusion injury by different interventions applied immediately before or during pPCI may limit infarct size and could improve prognosis in STEMI patients. However, the beneficial effects of these interventions are often small and in some cases inconsistent in different trials. It has been proposed that longer ischemic intervals, comorbidities, and co-medications may reduce the efficacy of interventions. presence studies. A potential approach to increase the efficacy of cardioprotective interventions is the combined use of two of them. Pre-clinical studies have shown that some of the interventions producing most promising results in clinical trials exert their protective actions through different pathways. Moreover, the susceptibility to the different comorbidities and the interaction with co-treatments appears to vary between cardioprotective interventions. Combination therapies may benefit thus from additive effects as well as from a reduced risk of reduced efficacy due to comorbidities. We will discuss the rationale and potential usefulness of different combination therapies including ischemic conditioning interventions and cardioprotective drugs in patients with STEMI receiving reperfusion therapy.

The two faces of Junction Associate Intermittent Lamellipodia (JAIL) in sealing and remodeling of cell junctions

Hans J Schnittler

Institute of Anatomy and Vascular Biology, WWU-Münster, Münster, Germany

The entire functionality of the vascular endothelium largely depends on cell layer integrity implemented by distinct protein complexes characteristic for adherens, tight and gap junctions. By 3D structured illumination microscopy and direct stochastic optical reconstruction microscopy we demonstrate the supra-molecular organization of endothelial cell adherens junctions including the junction associated intermittent lamellipodia (JAIL). These highly dynamic structures maintain both integrity and allow dynamic remodeling of mature junction and allow cell movement within sheet forming cell layers at the same time without alteration of the cell layer integrity. JAIL are WAVE-WASP/ARP2/3 complex controlled and actin driven small plasma membrane protrusions that preferentially appear locally at junction sites with less local VE-cadherin concentrations. We show the impact of JAIL in Notch/VEGF-receptor mediated angiogenesis as well as in fluid shear stress induced change in endothelial barrier function in vitro.

Remote ischaemic conditioning: from laboratory bench to hospital bedside

Hans Erik Bøtker

Professor of Cardiovascular Medicine and Interventional Cardiology
Department of Cardiology, Aarhus University Hospital Skejby,
DK-8200 Aarhus N, Denmark

Conditioning the heart to resist ischemia-reperfusion injury has become a core focus in cardiovascular research. In remote ischemic conditioning (RIC), brief, reversible episodes of ischemia with reperfusion in one vascular bed, tissue, or organ confer a global protective phenotype and render remote tissues and organs resistant to ischemia/reperfusion injury. RIC is obtained by intermittent inflation of a blood pressure cuff to 200 mm Hg for 5 minutes 4 times alternating with 5 minutes of reperfusion. RIC has been utilized in a number of clinical settings and with most promising results in patients with acute ST-elevation myocardial infarction. The intervention can be initiated in the ambulance during transportation to primary percutaneous intervention (PCI) and increases myocardial salvage leading to improved left ventricular function and improved outcome when used as an adjunct to primary PCI. Studies in subgroups of patients have demonstrated that the cardioprotective effect of RIC is most pronounced in patients with prolonged health-care system delay and in patients with collateral blood flow. RIC may improve outcome in patients with stroke.

The signal transfer to the heart or other organs is through neuronal and humoral communications. Protection can be transferred, even across species, with plasma-derived dialysate and involves nitric oxide, stromal derived factor-1 α , microribonucleic acid-144, but also other, not yet identified factors that may attenuate the inflammatory response and endothelial function seem to be involved. Intracardiac signal transduction involves: adenosine, bradykinin, cytokines, and chemokines, which activate specific receptors; intracellular kinases; and mitochondrial function. Also, the cardioprotective effect of ischaemic conditioning is preserved during mild hypothermia suggesting that a variety of cardioprotective modalities may be combined and protect against reperfusion injury. Even though experimental studies at the bench may provide mechanistic insight, there are challenges in the translation from experimental studies when we move from standardized infarct protocols in healthy experimental animals to patients with risk factors and comorbidity because there are many confounders and pitfalls that may affect the results. Real patients do not have completely normal coronary arteries but may have residual stenosis that provide more gentle reperfusion, which may by itself attenuate reperfusion injury and hence the possibility to demonstrate a beneficial effect of any cardioprotective intervention. Microembolization must be appropriately addressed as partial reperfusion may attenuate the cardioprotective intervention. Finally, concomitant medical therapy may influence the potential for cardioprotection in clinical practice and comorbidity such as diabetes may alter the response to remote ischaemic conditioning. In the clinical setting we have found RIC effective in patients with diabetes mellitus.

No significant adverse effects have been reported until now. However, properly sized randomized clinical trials are essential to clarify whether RIC affords clinically relevant prognostic benefits to the patients and whether RIC may have further perspectives by an extended application for four weeks in the post infarction period to prevent cardiac remodeling and a potential for achievement of global organ protection in cardiac arrest patients.

Remote ischemic preconditioning: magic trick or helpful tool?

Boening A¹, Niemann B¹, Grieshaber P¹, Simsekyilmaz S²,
Preissner KT², Cabrera-Fuentes HA^{3,4}

¹Department of Cardiovascular Surgery, ²Institute of Biochemistry, Medical School, Justus-Liebig-University, Giessen, Germany; ³Cardiovascular and Metabolic Disorders Program, Duke-National University of Singapore, Singapore; ⁴National Heart Research Institute Singapore, National Heart Centre Singapore, Singapore;

Objective: Remote ischemic preconditioning (RIPC) is a non-invasive and virtually cost-free strategy for protecting the heart against acute ischemia-reperfusion injury (IRI). Although RIPC worked well experimentally, the results of two big randomized studies in patients were disappointing. In previous studies, we have shown a possible functional pathway for RIPC.

Material and methods: Experimentally, we have identified an eRNA-RNase pathway as possible mediator for RIPC. Clinically, 14 patients underwent cardiac surgery with previous RIPC (4 x 5 min limb ischemia) or a sham procedure. Circulating eRNA and RNase were quantified in plasma from arterial and coronary sinus blood obtained from patients undergoing cardiac surgery. The potential effect of RNase alone was investigated in Langendorff hearts using an eRNA-free model. To test the influence of medical ischemic preconditioning, the influence of Buprenorphine, isoflurane, and etomidate on the eRNA/RNase pathway was investigated.

Results: Before surgery, eRNA levels were similar in both groups (14.12 ± 6 ng/ml). In patients without RIPC, arterial eRNA levels rose during (87 ± 12 ng/ml) and peaked after (127 ± 11 ng/ml) surgery, while eRNA levels in coronary sinus blood were significantly higher (206 ± 32 ng/ml). Interestingly, applying a RIPC protocol significantly increased levels of plasma endogenous vascular RNase1 (>7-fold) with the results that the arterial (22 ± 6 ng/ml) and coronary sinus (27 ng/ml) circulating eRNA became substantially hydrolyzed. In an eRNA-free Langendorff model, we could not show any positive RNase effects. In animals receiving buprenorphine or isoflurane, RNase 1 rose significantly when compared to RIPC without these drugs. Etomidate did not affect RNase levels.

Conclusion: Our findings imply a significant contribution of the RIPC-dependent endothelial RNase1 release for improving the outcome of cardiac ischemia, while the exact mechanism of RNase1-induced cardioprotection still remains to be uncovered. It seems that medical preconditioning exhausts the preconditioning capacity of mammals, so that RIPC can not promote additional preconditioning as such.

Saadeh Suleiman

School of Clinical Sciences, Faculty of Medicine & Dentistry, University of Bristol, UK.

Propofol is a general anaesthetic widely used for the induction and maintenance of anaesthesia during cardiac surgery and in postoperative sedation. It is used as emulsion for injection using an Intralipid vehicle containing: soybean, glycerol & egg lecithin. The anaesthetic effect is due to potentiating the inhibitory effects of the neurotransmitter GABA and possibly due to inhibiting the sodium channels. By 1995 there was a strong experimental evidence to support a role for the drug in cardioprotection in a variety of experimental models. The proposed underlying mechanisms for this effect included the ability of propofol to inhibit the sarcolemmal L-type calcium channels and to scavenge free radicals. However, these reports were controversial as propofol was used in high doses and therefore would have an additional systemic effect.

This presentation will briefly report work that started in 1995 and aimed at formulating and investigating the cardioprotective efficacy of propofol cardioplegia. In particular data will be presented showing that propofol is cardioprotective in normal and in diseased heart when used in cardioplegia in a variety of *in vitro* experimental models and using clinically relevant concentration. Furthermore, this drug was found to protect the heart by inhibiting the mitochondrial permeability transition pore.

The cardioprotective efficacy was also demonstrated in a pig model of normothermic blood cardioplegic arrest and cardiopulmonary bypass. Subsequently a single-centre randomized controlled trial was designed to investigate the effects of propofol cardioplegia on blood and myocardial biomarkers of stress and injury in patients with isolated coronary artery bypass grafting or aortic valve replacement using cardiopulmonary bypass. Key background information on the process of translating a drug for a new medicinal purpose and the regulatory and ethical issues will be presented. This trial has recently been completed and some of the data (published and unpublished) will be discussed.

Measurement of Cardiac Myosin Binding Protein C in the systemic circulation

Marjot J, Anand A, Shah A, Kaier T, Weber E, Mills N, **Marber M.**

Department of Cardiology, The Rayne Institute, St Thomas' Campus, King's College London, London, UK.

Cardiac myosin binding protein C (cMyC) is an abundant sarcomeric protein with a unique cardiac isoform. We created a panel of mouse monoclonal antibodies to the unique N-terminal C0-C1 domains of the protein and triaged those with the highest binding affinity based on surface plasmon resonance on immobilised cMyC. From these monoclonal antibodies two high affinity binders were chosen based on close proximity of their epitopes based peptide mapping and lack of steric interaction on sequential exposure to immobilised cMyC. These antibodies were tested as a capture-detection pair and an immunoassay created and optimised on the Singulex Errena detection platform. The assay for cMyC had a lower limit of detection of 0.4ng/L, a lower limit of quantification of 1.2ng/L (LLoQ at 20% CV) and reasonable recovery (107.1+/-3.7%; mean+/-SD), dilutional linearity (101.0+/-7.7%) and intra- (CV 11+/-3%) and inter- (CV 13+/-3%) series precision. In 360 stable patients, cMyC was quantifiable in 359 and compared to cardiac Troponin T (cTnT) and cTnI measured using contemporary high sensitivity assays. cMyC concentration (median 12.23ng/L, IQR 7.87-21.15ng/L) was linearly correlated with those for cTnT (median <3.0ng/L, IQR <3.0-4.88ng/L; R=0.56, p<0.01) and cTnI (median 2.10ng/L, IQR 1.30-4.20ng/L; R=0.77, p<0.01) and showed similar dependencies on age and renal and left ventricular function. In a separate population of patients with aortic stenosis cMyC was related to fibrosis on cardiac magnetic resonance imaging and clinical outcome. In conclusion, we have developed a high sensitivity assay for cMyC and circulating concentrations correlate with underlying pathology in ambulatory patients.

Acute Coronary Syndrome: A Malaysian perspective

Alan Fong

Department of Cardiology, Sarawak Heart Centre, Malaysia

Acute coronary syndrome (ACS) is the leading cause of mortality in Malaysia. In 2006, the National Cardiovascular Disease (NCVD) Registry was launched, with the primary data capture structures being for Acute Coronary Syndrome (NCVD-ACS) and Percutaneous Coronary Intervention (NCVD-PCI). Despite being a voluntary data capture system, substantial amount of data was accumulated, leading to publication of three reports of the NCVD-ACS Registry, the most recent being in 2015. In a multi-ethnic population, reflecting the major ethnic groups of Asia, and drawn from a patient pool that are from both urban and rural areas, we have developed a greater insight into ACS as a disease and its impact on the local population. In a developing country, where advanced tertiary centres of cardiology care are established only in the major urban centres, strategies to improve mortality and morbidity in the suburban and rural areas must be developed in parallel. Various aspects of ACS management drawn from our NCVD experience will be discussed, with the aim of generating important and viable research directions.

Induced Pluripotent Stem Cell in Cardiac Arrhythmia

Winston Shim

Principal Scientist, National Heart Centre Singapore

The advent of human induced pluripotent stem cell-derived cardiomyocytes presents a tremendous opportunity for the study of cardiac arrhythmias in vitro. Our group together with colleagues at the NHCS have characterized cellular models for major subtypes of inherited channelopathy caused by dysfunctional potassium and sodium channels that contribute to Long QT Syndrome (LQTS) 1, LQTS2 and LQTS3. In particular, we showed that corrective trafficking of KCNH2 (LQTS2) potassium channel through intra-cellular mechanisms restored hERG currents and reduced arrhythmia in LQTS2 patient-derived cardiomyocytes. This novel approach diverges from the challenging drug discovery strategy for channelopathy where biophysical interference of the plasmalemmal/sarcolemmal ion channels is traditionally employed. In addition, we share our data in using human stem cell-derived cardiomyocytes in studying drug-induced LQTS, cardiotoxicity and rhythm disturbance. We highlight the implications of human stem cell-derived cardiomyocytes as a tool in meeting the new initiatives of the pharmaceutical industry in early prediction of cardiac arrhythmias in drug development program.

*Xeno-free laminin-based protocol for generating clinical quality
cardiomyocytes from hES cells*

Lynn Yap & **Karl Tryggvason**

Cardiovascular and Metabolic Disorders Program, Duke-NUS, Singapore

Basement membrane (BM) laminins (LN) are highly cell type specific proteins important for adhesion, differentiation and phenotype stability. We have produced most of the 16 known laminins as recombinant proteins and revealed several important properties and applications. Using LN-511 and LN-521, we have clonally derived new hES cell lines in defined and xeno-free conditions, without a need to destroy the embryo cell source. Importantly, laminins are useful as cell culture substrata when generating chemically defined and xeno-free differentiation protocols, such as for making cardiomyocytes and endothelial cells (EC). The heart ventricle contains LN-221 and LN-521 as major laminin isoforms, in addition to lesser amount of LN-211, as well as vascular BM laminins LN-411 and LN-421. Here, we show that recombinant human LN-221 and LN-521 can mimic the *in vivo* cardiomyocyte matrix to allow robust differentiation of hES cells to cardiomyocytes. We have characterized the cells at different differentiation stages using RNAseq, immunostaining, electrophysiology and cytotoxicity. The progenitors continue to mature for up to 120 days into spontaneously beating cardiomyocytes expressing >80 % *TNNT2* and expression signature of highly mature cardiomyocytes. We are in the process of testing different progenitors for myocardial repair in SCID mice.

Supported by the National Medical Research Council of Singapore.

Hippo signaling mediators Yap and Taz are required in the epicardium for coronary vasculature development

Manvendra K. Singh

Cardiovascular and Metabolic Disorders Program, Duke-NUS, Singapore

Formation of the coronary vasculature is a complex and precisely coordinated morphogenetic process that begins with the formation of epicardium. The epicardium gives rise to many components of the coronary vasculature, including fibroblasts, smooth muscle cells and endothelium. Hippo signaling components have been implicated in cardiac development and regeneration. However a role of Hippo signaling in the epicardium has not been explored. Employing a combination of genetic and pharmacological approaches, we demonstrate that inhibition of Hippo signaling mediators Yap and Taz leads to impaired epicardial epithelial-to-mesenchymal transition (EMT) and a reduction in epicardial cell proliferation and differentiation into coronary endothelial cells. We provide evidence that Yap and Taz control epicardial cell behavior, in part by regulating Tbx18 and Wt1 expression. Our findings show a previously unidentified role for Hippo signaling in epicardial cell proliferation, EMT and cell fate specification during cardiac organogenesis.

To the heart of the Amazon: comparative cardiac mitochondrial physiology

Erich Gnaiger^{1,2}, Ramon B Baptista³, Adalberto L Val³, Vera M Fonseca de Almeida e Val³

¹D. Swarovski Research Lab, Dept Visceral, Transplant Thoracic Surgery, Medical Univ Innsbruck; ²OROBOROS INSTRUMENTS, Innsbruck, Austria; ³Instituto Nacional de Pesquisas de Amazonia, Laboratório de Ecofisiologia e Evolução Molecular, Manaus, Brazil

Advances in the diagnosis of mitochondrial (mt) function made it possible to perform OXPHOS analyses for mt-phenotyping on small samples of skeletal or cardiac muscle biopsies [1,2]. Comparison of mt-respiratory control in human and rodent tissues reveals a surprising diversity of diagnostic OXPHOS patterns between tissues and species. With respect to substrate control, comprehensive information has become available on OXPHOS capacity with NADH-linked or N-type substrates (pyruvate, glutamate, malate) versus succinate supported respiration (S-type; succinate and rotenone). N&S exert an additive effect on respiratory flux when supplied in combination to reconstitute TCA cycle function in mt-preparations. NS-additivity varies greatly and may be linked to the tightness of metabolic channelling through supercomplexes of the electron transfer system (ETS) [3]. Although marked differences in N- versus S-linked OXPHOS capacity between mammalian heart and liver mitochondria have been described for decades, no conceptual framework or hypothesis emerged on the adaptive significance of these different NS-substrate control patterns.

The topic of coupling has long been restricted to discussions of the proton leak across the inner mt-membrane. Only after introducing complex substrate-uncoupler-inhibitor titration (SUIT) protocols, studies of mt-respiratory control revealed entirely contrasting coupling control with respect to protonophore-stimulated, noncoupled ETS capacity, E , which is in varying excess over maximally ADP-stimulated OXPHOS capacity, P [3]. In mouse heart, OXPHOS capacity (coupled) and ETS capacity (noncoupled) are nearly identical, indicating a matching of the capacity of the phosphorylation and electron transfer systems, in contrast to the excess ETS capacity in the human heart [1]. Similarly, mouse skeletal muscle and human skeletal muscle differ with respect to the control exerted by the phosphorylation system [2]. The functional implications of these differences on coupling control patterns are not known, yet have to be considered in applications of animal models.

Comparative physiology may provide a key towards delineating mitochondrial respiratory control patterns in different species and tissues, as a basis to classify mitochondrial risk factors as causes or consequences of disease. In the present study we explored large-scale evolutionary variations of cardiac mt-respiratory control patterns, to evaluate mitochondrial fitness in terms of adaptations to different environments and life styles. Heart mitochondria were studied from Amazon fish species which are highly adapted to a fluctuating hypoxic environment, contrasting with the hypoxia-intolerant mammalian and trout hearts. Surprisingly, mt-respiratory control in the heart appeared to be evolutionarily conserved among the fish species studied, irrespective of diverse environmental challenges. A conceptual understanding of the mouse-human divergence remains elusive, but alterations in heart failure [1] do not support a neutral model of evolution.

Large-scale screening of mitochondrial fitness in comparative physiology and clinical diagnostics requires the cooperation of multiple centers. Quantitative comparability of results can only be based on standardization of tissue preparation, incubation conditions, SUIT protocols and application of general criteria for instrumental quality control. Our studies are designed as an attempt towards establishing a basic protocol for OXPHOS control analysis, to provide a diagnostic tool for evaluation of the role of mitochondria in health and disease in the heart and other tissues, in humans and animal models.

Contribution to the K-Regio project MitoFit and COST Action MITOEAGLE.

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Cardio-oncology: a new opportunity for translation: a new target for cardioprotection

Malcolm Walker

Consultant Cardiologist, University College Hospital London, Clinical Director Hatter
Cardiovascular Institute

In the UK there are about 340,000 new cases of cancer per year; with recent advances in therapy 50% of these patients will expect to survive for 10 years or more. It has been known for some years that some cancer treatments are associated with cardiovascular complications. These may be acute, inducing cardiac dysfunction, heart failure and arrhythmia, threatening survival at worst and, in less severe cases, affecting the capacity to deal effectively with the cancer. Other manifestations of cardiovascular involvement are more indolent, with heart failure developing months to years after the cancer therapy has ended and sometimes even after the patient has been deemed "cured". Of the 620,000 heart failure cases in the UK it is estimated that between 30,000 to 60,000 may have their origin in previous cancer therapy.

The anthracyclines are amongst the most effective and commonly used drugs in oncology and have been the drugs most commonly associated with cardiotoxicity, which often limits their optimal usage. Many of the conditions where anthracyclines are especially effective, affect children and young adults, who appear particularly sensitive to cardiovascular toxicity. The cardiotoxicity induced by anthracycline therapy has been the subject of investigation for many years and many of the mechanisms involved have been described; some aspects of cardio-toxicity have analogies to injury induced by ischaemia reperfusion.

Newer, agents, angiogenesis inhibitors and tyrosine kinase inhibitors have transformed the outlook for many cancers and they are often used in combinations. There is therefore an ever evolving field of potential cardio toxic targets, which is adding a new dimension of complexity to clinical management. This has led to the concept of a new area of clinical and scientific investigation, the field of cardio-oncology .

We present here a newly initiated trial designed to investigate the potential for remote ischaemic pre-conditioning to protect the heart in the context of oncology patients undergoing treatment which includes anthracyclines, the ERIC-ONC trial.

Jürgen Bernhagen

Department of Vascular Biology, Institute for Stroke and Dementia Research, Klinikum der Universität München Ludwig-Maximilians-Universität (LMU) München

MIF is an inflammatory cytokine with chemokine-like functions that interacts with the type II receptor CD74 and the chemokine receptors CXCR2 and CXCR4. Recent data also shows that MIF interacts with the chemokine scavenger receptor CXCR7. The MIF-CXCR2 and –CXCR4 axes potently promote atherogenic monocyte and T cell recruitment, respectively, while the role of MIF/CXCR7 and the MIF homolog MIF-2/DDT has remained unknown. Collectively, MIF pathways drive atheroprotection and plaque instability and dual blockade of CXCR2 and CXCR4 by anti-MIF mAbs even confers some degree of plaque regression. Furthermore, MIF controls the maturation and migration of B cells, an adaptive immune cell type more recently implicated in atherosclerosis. Counter-intuitively, MIF has cardioprotective effects in myocardial ischemia/reperfusion injury via the CD74/AMPK pathway, anti-oxidative effects, but also cardiomyocyte-expressed CD74/CXCR2 complexes. One mechanistic explanation for this complex scenario has been the occurrence of phase-dependent effects in resident myocardial versus recruited inflammatory cells, but we also hypothesize that the context-dependent generation of specific post-translational variants of MIF as well as the production of the MIF homolog MIF-2/DDT or the shedding of CD74 may underlie the complex contribution of MIF proteins in cardiovascular disease. I will present the available evidence and discuss these possibilities and outline potential therapeutic options to target MIF proteins and/or diagnostic options for stratification.

Microvascular thrombosis: pathological relevance in vascular disease

Bernd Engelmann^{a)}, Susanne Pfeiler^{a)}, Konstantin Stark^{b)}, Steffen Massberg^{b)}

^{a)} Institut für Laboratoriumsmedizin, ^{b)} Medizinische Klinik und Poliklinik I, Ludwig-Maximilians-Universität, Munich, Germany

Microvascular thrombosis has been found to be associated with many vascular diseases, including stroke, myocardial infarction, thrombotic microangiopathies, infections and cancer. For several of these disease groups, the pathogenetic role of microvascular thrombosis has remained enigmatic. Exceptions are microangiopathies such as haemolytic-uremic syndrome and disseminated intravascular coagulation during sepsis for both of which the pathogenetic relevance has been clearly demonstrated. It has been assumed that the failure to assign a clear pathogenetic role to microvascular thrombosis in many diseases is due to difficulties in their detection and in the inability to assess the efficacy of antithrombotic treatments in the clinical situation. We have recently shown that during systemic bacterial infections microvascular thrombosis under certain conditions acts as an instrument of intravascular immunity. In organs such as the liver and spleen, fibrin-rich microthrombi support the containment and elimination of *E. Coli* inside blood vessels which prevents the tissue invasion and dissemination of the pathogens. This mechanism has been termed immunothrombosis. Immunothrombosis is suggested to form a major biological basis of pathological microvascular and macrovascular thrombosis (especially deep vein thrombosis), together with the physiological mechanism arresting bleeding (hemostasis). Immunothrombosis is a transient process as it appears to be normally resolved within two days. Pathological forms of microvascular thrombosis during infections such as disseminated intravascular coagulation are likely caused by an excessive activation of immunothrombosis and/or by its impaired resolution. Most probably, the formation of microvascular thrombi under non-infectious conditions might equally be able to protect the intravascular compartment from damage such as caused by immune complexes, circulating cell fragments or endothelial damage. This could suggest that the beneficial nature of microvascular thrombosis may also apply to non-infectious conditions. Hence the failure to document a pathological role for microvascular thrombosis under several pathological conditions may be due to the fact that it represents a rather general host-protective mechanism.

Inhibiting miR-146a Augments Angiogenesis and Myocardial Regeneration

Daniel Sedding

Department of Cardiology and Angiology, Hannover Medical School, Hannover, Germany

Background: Myocardial infarction leads to cardiac remodeling and the development of heart failure. Myocardial remodeling and regeneration rely on myocardial capillary density and thus on effective neovascularization after myocardial infarction. However, the mechanisms underlying myocardial angiogenesis and specifically the regulation of neovascularization by microRNAs are not well understood.

Methods and Results: Here, we showed that the small noncoding RNA microRNA-146a (miR-146a) was upregulated in the ischemic myocardium in C57BL/6 mice following ligation of the left anterior descending (LAD) artery, as well as in the ischemic hind limb following ligation of the femoral artery in a time-dependent manner. *In vitro*, the overexpression of miR-146a significantly attenuated endothelial cell proliferation and migration, abolished endothelial capillary network formation, and inhibited cell sprouting from endothelial spheroids. In contrast, knocking down miR-146a augmented endothelial cell proliferation, migration, network formation, and sprouting significantly. Mechanistically, NOX4, NOTCH1, and nRAS were identified and validated as direct targets of miR-146a in endothelial cells according to mRNA and protein expression profiles, as well as luciferase gene reporter assays. *In vivo*, blocking the upregulation of endothelial miR-146a using specific inhibitors (antagomirs) significantly enhanced angiogenesis and re-vascularization in the infarcted myocardium and ischemic hind limb. This was accompanied by a significantly reduced myocardial infarct size and preserved cardiac function.

Conclusions: Our findings identify miR-146a as a critical regulator of angiogenesis during myocardial regeneration. Moreover, miR-146a may represent an attractive target for future therapeutic interventions for the treatment of ischemic heart disease.

Challenges in cardiac regeneration based on cardiomyocyte proliferation

Felix B. Engel

Experimental Renal and Cardiovascular Research, Department of Nephropathology, Institute of Pathology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

The adult zebrafish and the newt regenerate their heart by cardiomyocyte proliferation. Recently, it has been reported that also newborn mice can regenerate their heart after injury. Moreover, several independent laboratories have reported that they can induce adult mouse or rat heart regeneration based on cardiomyocyte proliferation. In addition, it has been suggested by pathologists since over a century that also adult human cardiomyocytes can proliferate contributing to homeostasis. Yet, it remains unclear whether cardiomyocyte proliferation is a real option for human cardiac regeneration. First, we have found that mammalian cardiomyocytes, in contrast to zebrafish and newt cardiomyocytes, utilize centrosome disassembly to achieve their post-mitotic state. Second, the currently available data on newborn as well as adult mammalian regeneration is controversial and a critical analysis of the literature demonstrates that the efficiency in inducing cardiomyocyte proliferation decreases proportionally to the age of the cardiomyocytes. Third, in the majority of cases the utilized methods cannot demonstrate that a certain therapy indeed resulted in cardiomyocyte division instead of binucleation or polyploidization. Finally, no study has proven that the observed improvement after a therapy is indeed due to an increase in cardiomyocyte number. Taken together, our own data on centrosome disassembly and neonatal heart regeneration and the analysis of the literature suggest that only a subpopulation of cardiomyocytes maintains the ability to proliferate.

Understanding Heart Failure With Preserved Ejection Fraction

Carolyn Lam

Cardiovascular & Metabolic Disorders Program, Duke-NUS Graduate Medical School Singapore

The term “diastolic HF” was first coined to reflect the leading pathophysiologic factor believed to cause the syndrome – left ventricular diastolic dysfunction. In the landmark study by Zile et al, abnormalities in left ventricular relaxation and compliance were uniformly demonstrated in 47 cases of HF despite a normal ejection fraction. However, population-based studies also showed that left ventricular diastolic dysfunction was present in a large proportion of community-based adults without HF, and that patients with “systolic HF” were even more likely to have moderate/severe diastolic dysfunction compared to patients with so-called “diastolic HF”. Nonetheless, progression of left ventricular diastolic dysfunction was found to be a major mechanism distinguishing HFpEF from age-, sex- and body size-matched healthy controls and hypertensive individuals without HF in the general community. Other mechanistic studies challenged the concept that HFpEF was a uniform syndrome of “diastolic HF”. These studies described various abnormalities beyond diastolic dysfunction, including abnormal ventricular-arterial coupling with exercise, impaired systemic vasodilator reserve, chronotropic incompetence, myocardial contractile dysfunction despite a normal ejection fraction, left atrial dysfunction, pulmonary hypertension with intrinsic pulmonary vascular disease, endothelial dysfunction, and volume overload (related to extra-cardiac causes such as obesity, chronic kidney disease, or anemia). It is possible that each of these mechanistic studies selected a specific subset of patients with HFpEF; indeed evidence is mounting that HFpEF is not a single homogeneous syndrome, but is rather a heterogeneous condition consisting of several pathophysiological sub-types.

It has in particular been proposed that three subtypes of HFpEF patients exist: those with exercise induced diastolic dysfunction, those with chronic volume overload and those with associated right HF and/or pulmonary hypertension. The importance of recognizing the heterogeneity of the pathophysiology in HFpEF is highlighted by the fact that a “one size fits all” approach for clinical trials in HFpEF has been disappointing and that treatments directed at HFpEF as a large undifferentiated group have failed to improve outcomes. Understanding the heterogeneity of HFpEF and improved phenotypic characterization of mechanistic sub-types might therefore allow the design of more targeted HFpEF clinical trials. Most recently, a new paradigm has been put forward based on observation of specific myocardial structural and functional changes observed in HFpEF. This paradigm emphasizes the role of a pro-inflammatory state with widespread endothelial dysfunction, leading to reduced nitric oxide (NO) bioavailability in cardiomyocytes, reduced myocardial cyclic guanosine 3', 5'-monophosphate (cGMP) content and low protein kinase-G activity (PKG). The central role of the NO-cGMP-PKG pathway is described in this paradigm: Endothelial dysfunction has been shown to be highly prevalent and independently predictive of survival in HFpEF, suggesting that it plays a major role in the pathophysiology of HFpEF.

Endothelial dysfunction occurs in diabetes and hypertension, both important risk factors for HFpEF, and causes oxidative stress with high levels of reactive oxygen species which interfere with NO production in endothelial cells. This leads to reduced NO bioavailability to adjacent cells such as cardiomyocytes. cGMP is the second messenger that plays a role in various key physiologic pathways, including cardiovascular homeostasis, cellular growth and contractility, and inflammation. Guanylate cyclases are enzymes that catalyze the conversion of guanosine-5'-triphosphate to cGMP.

Membrane-bound particulate guanylate cyclase (pGC) serves as a receptor for natriuretic peptides, whereas soluble guanylate cyclase (sGC) acts as a receptor for NO. Subsequently, cGMP effectors include cGMP-dependent protein kinases, such as PKG. The disruption of the NO-cGMP-PKG signalling pathway can therefore explain the development of concentric LV remodelling, increased stiffness of the cardiomyocyte through hypo-phosphorylation of titin, and increased collagen deposition in HFpEF. Progress has been made in the understanding of the pathophysiology of this condition, and there is increasing emphasis on therapeutic strategies aimed at altering specific signalling pathways. It is critical for future clinical trials to ensure a proper characterization of the phenotype of patients to be tested. Several novel approaches appear promising in pre-clinical or early clinical studies, but need to be tested in properly designed clinical trials.

Karlheinz Peter

Baker IDI Heart & Diabetes Institute, Melbourne, Australia

The detection and therapy of thrombi/emboli and inflammation is a major challenge in clinical practice. Site-directed therapy and molecular imaging targeting platelet-specific antigens is a highly promising approach. One of the most abundant epitopes on platelets is the surface receptor GPIIb/IIIa (alphaIIb/beta3, CD41/CD61). In addition upon activation of platelets, as it is the case in thrombosis and inflammatory reactions, the GPIIb/IIIa receptor undergoes a conformational change exposing receptor epitopes that then can be used as clot-specific targets. We used phage-display of PCR-cloned human single-chain antibodies (scFv) and generated several clones that are highly specific for activated GPIIb/IIIa and thus for activated platelets. We then developed various genetic, chemical and biological coupling strategies to fuse either therapeutic drugs, contrast particles, or the combination of both to these clot-specific scFvs. Targeting various anticoagulants, anti-platelet drugs and fibrinolytics to the clot, we can achieve effective thrombolysis and prevention of thrombi/emboli without prolongation of bleeding times in mice. Using newly designed/generated, targeted ultrasound microbubbles, magnetic resonance nano/microparticles, and positron emission tomography (PET) tracers we can detect thrombi/emboli/inflammation with high sensitivity/specificity in the respective imaging modalities. In a unique theranostic approach we can combine detection/imaging of thrombi/inflammation, immediate effective/side-effect poor treatment, and monitoring of success/failure of therapy.

HIF oxygen-sensing metabolic pathways in heart biology

Daniel Tello¹ and **Julián Aragonés**¹

Research Unit, Santa Cristina Hospital, Research Institute Princesa (IP), Autonomous University of Madrid, Madrid 28009, Spain.

O₂ supply to cardiomyocytes is compromised not only in ischemic diseases but also during embryonic development *in utero*. Cells respond to oxygen fluctuation by activation of hypoxia-inducible factors (HIF1 and HIF2), which rewire metabolism to adequate cellular functions to oxygen supply. Indeed, we recently identified critical factors controlling amino acid metabolism through the amino acid carrier SLC7A5 and mTORC1 pathway as well as cardiac glucose metabolism. We will discuss the role of these metabolic pathways in heart biology during development as well as in adulthood.

*Toll-like receptors in myocardial inflammation during
reperfusion injury and adverse remodeling*

Dominique de Kleijn

Dept of Surgery NUS, Singapore. Dept of Cardiology UMC Utrecht, the Netherlands

There is increasing evidence that engagement of molecules released after injury modulate inflammation. It is postulated that these molecules serve as 'danger signals' or endogenous ligands for leukocytes. Until recently, it remained unclear by which mechanisms cells could recognize endogenous ligands. The identification of Toll-like receptors (TLR) shed new light in the activating properties of endogenous ligands in innate immune responses after cardiac ischemia. Originally described as receptors that serve as a first-line defense against bacteria, virus etc., several TLRs have been shown to play a pivotal role in non-infectious pathological cardiovascular conditions (e.g. atherosclerosis, ischemia/reperfusion injury, heart failure). In this presentation, we show the involvement of several TLRs in ischemia/reperfusion injury and adverse remodeling after myocardial infarction as well as after pressure overload and discuss possible interventions for therapy.

Pharmacological heart preconditioning in vivo with minimal side-effects

Adelina Curaj¹, Sakine Simsekyilmaz², Mareike Staudt¹, Zhuojun Wu¹, Xiaofeng Li¹,
Andreas Götzenich³, Andreea Urs⁴, Mircea Leabu⁴, Elisa A. Liehn¹

¹Institute for Molecular Cardiovascular Research, RWTH University Hospital Aachen, Germany

²Institute for Biochemistry, Medical Faculty, Justus-Liebig-University Gießen, Germany

³Institute of Biochemistry and Molecular Cell Biology, RWTH University Hospital Aachen, Germany

⁴Department of Morphological Sciences, "Victor Babes" National Institute of Pathology, Bukarest, Romania

Despite considerable progress over the last period, acute myocardial infarction continues to remain the major cause of morbidity and mortality worldwide. Phosphatidylserine (**PS**) is a phospholipid component of the inner leaflet of cell membranes and seems to be a messenger for the cellular death and an important destruction signal for the macrophages. On the other hand, **PS** has demonstrated some usefulness in treating cognitive impairment but also speeded up recovery, prevented muscle soreness, improved well-being, and might possess ergogenic properties.

The effect of **PS** was tested *in vitro* on isolated cardiomyocytes undergoing hypoxia. After 3 hours of preincubation with, the cardiomyocytes increased significantly their protection, indicating no stress signals as analysed by Alamar blue staining after 5 hours of ischemia.

Using a mouse model of myocardial infarction, we tested the effect of oral administration of **PS** on myocardial function and biology. The pre-treated with **PS** one week before myocardial infarction induction showed a significantly preservation of the heart function and reduced infarction size. No differences in apoptosis were detected, as measured by TUNEL staining in infarcted myocardium. Inflammation was also reduced, do probably reduced tissue damages after **PS** administration. mRNA extraction from isolated cardiomyocytes after hypoxia and myocardium after myocardial infarction showed a significant up-regulation of main player of preconditioning program, as protein kinase C type β , cyclooxygenase-2, aldose reductase, Mn superoxide dismutase.

As already known, **PS** side effects are rare and include only mild gastrointestinal discomfort. When taken together with other blood thinners drugs (such as warfarin, aspirin, pentoxifylline, clopidogrel, ticlopidine, garlic and vitamin E), thinning the blood was also reported. However, to exclude any major changes in lipid composition, lipid were extracted from heart, kidney, liver, lung after **PS** administration and fatty acids were analysed. We did not detect any significant changes in fatty acids or lipid composition after one week **PS** administration in any analysed organs. Moreover, the blood analysis showed normal and unchanged parameters after in all groups.

We have demonstrated that **PS** can be used to activate preconditioning program of the heart, to assure a significant protection from ischemia *in vitro* and *in vivo*. We strongly believe that **PS** can be used in the treatment of heart failure to protect the cardiomyocytes and to adapt them to chronic hypoxic conditions, preserving their function. Comparing with other methods used in present, as remote ischemia or opioide administration, **PS** administration seems to be easy to performe with minimal side effects, no professional supervision beeing necessary. Since **PS** are already in clinical use for some cognitive diseases, we believe that our findings would be for a high relevance for the cardiovascular community, with immediately clinical implication.

Circulating MicroRNAs: Evolving Players in the Field of Medicine

Manuel Mayr

King's British Heart Foundation Centre, King's College London, London, United Kingdom

Plasma microRNAs are highly correlated. Platelets contain and release microRNAs and are a major source of abundant microRNAs in plasma. Besides microRNAs, a strong platelet dependency was observed for YRNA fragments. There was a striking correlation of microRNAs and YRNA fragments with platelet activation markers in the general population. Platelet microRNAs and YRNA fragments in plasma correlate with indices of platelet function in patients on dual anti-platelet therapy. MicroRNA-126, previously considered to be endothelial specific, is present in platelets and in a human megakaryoblastic cell line. A single nucleotide polymorphism that facilitates processing of microRNA-126 increases plasma levels of platelet activation markers. Treatment with antagomiRs to microRNA-126 reduces platelet activation in mice. MicroRNAs may not just be markers of platelet activity but also alter their function, most probably by influencing gene expression in megakaryocytes.

Systems biology approaches to identify novel regulators of heart development

Fulvia Ferrazzi¹, Felix B. Engel²

¹Institute of Human Genetics, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

²Experimental Renal and Cardiovascular Research, Department of Nephropathology,
Institute of Pathology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

Understanding heart development will help to identify the underlying causes of congenital heart disease and improve diagnostics. In addition, several important biological processes occur during heart development that, if reactivated postnatally, have a great potential to improve heart function in heart disease, such as after myocardial infarction. For example, it is known that the mammalian heart loses its ability to regenerate, largely due to the fact that after birth cardiomyocytes fail to undergo cytokinesis. Instead, they exit the cell cycle after karyokinesis resulting in bi-nucleated cells. Thus, understanding how cardiomyocyte cytokinesis is regulated during development might provide new approaches towards cardiac regeneration and also to repress cancer growth. Here, we will discuss how systems biology approaches can identify novel regulators of cardiac development and suggest regulatory networks. In particular, computational analyses integrating large scale expression datasets addressing the same question from different angles together with prior knowledge improve our ability to dissect regulatory mechanisms. As an example the joint analysis of a high-resolution temporal expression dataset describing heart development and another transcriptomic dataset describing FGF1/p38i-induced cardiomyocyte proliferation will be presented. This analysis resulted in the identification of novel candidate cytokines genes that are currently being experimentally validated. Taken together, our systems biology approach is able to identify novel key players in development with therapeutic potential.

Fighting Fat with Fat: Fatty Acid-Derived Mediators in the Regulation of Metabolism and Vascular Homeostasis

Timo Frömel, Nicole Mangels, Jiong Hu, **Ingrid Fleming**

Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe-University, Frankfurt am Main, Germany.

Over the last 20 years it has become clear that cytochrome P-450 (CYP) enzymes generate a spectrum of bioactive lipid mediators from endogenous substrates. However, studies focused on the determining biological activity of the CYP system have focused largely on the metabolites generated by one substrate i.e. arachidonic acid. However, epoxides and diols derived from other endogenous substrates such as linoleic acid, eicosapentaenoic acid and docosahexenoic acid may be generated in higher concentrations and potentially be a more physiological relevance. Recent studies that used a combination of phenotyping and lipid array analyses revealed that rather than being inactive and product's fatty acid diols play important roles in a number of biological processes including inflammation, angiogenesis and metabolic regulation.

In the retina the deletion of the soluble epoxide hydrolase (sEH; the enzyme that metabolises fatty acid epoxides to diols) significantly delayed angiogenesis, tip cell and filopodia formation, a phenomenon associated with activation of the Notch signalling pathway. Lipid profiling revealed that sEH deletion decreased retinal and Müller cell levels of 19,20-dihydroxydocosapentaenoic acid (DHDP), a diol of docosahexenoic acid (DHA). 19,20-DHDP suppressed endothelial Notch signalling *in vitro* by via inhibition of the γ -secretase and the redistribution of presenilin 1 from lipid rafts. In the diabetic retina the situation is reversed and sEH expression is markedly elevated and 19,20-DHDP levels increased. Via a similar mechanism the diol affects the presence of VE-cadherin and N-cadherin in lipid rafts thus affecting pericyte coverage and endothelial permeability. Treating mice with a sEH inhibitor effectively reduced diol production and prevented the diabetes induced retinopathy.

Diabetes is also associated with an increase in sEH expression in other tissues including the liver. Interestingly sEH^{-/-} mice are largely protected against the development of type 2 diabetes and the associated hypertension when fed a high fat diet. This occurs at the expense of the liver as the sEH controls the expression of key enzymes involved in lipid metabolism e.g CLOCK, MTP, ApoE and ApoB thus markedly affecting liver lipid transport.

Thus, inhibitors of the soluble epoxide hydrolase that increase epoxide but decrease diol levels have potential for the treatment of the metabolic syndrome/type 2 diabetes and its complications.

A landscape of circular RNA expression in the human heart

Roger Foo

Genome Institute of Singapore

Circular RNA (circRNA) is a newly validated class of single-stranded RNA ubiquitously expressed in mammalian tissues and possessing key functions including acting as microRNA sponges and as transcriptional regulators by binding to RNA-binding proteins. While independent studies confirm the expression of circRNA in various tissue types, circRNA expression in the heart has yet to be reported.

We performed deep RNA-sequencing on ribosomal-depleted RNA isolated from 14 human hearts, 10 mouse hearts and across a 12-week differentiation time-course of human embryonic stem cell-derived cardiomyocytes (hESC-CM). Using purpose-designed bioinformatics tools, we uncovered a total of 16,416 and 2,300 cardiac circRNA with in human and mouse, respectively. Top highly expressed circRNA corresponded to key cardiac genes including *TTN*, *RYR2* and *DMD*. The most abundant cardiac-expressed circRNA is a cytoplasmic localized single-exon *circSLC8A1*. The longest human gene Titin (*TTN*) alone generates up to 253 different circRNA isoforms, the majority (77%) of which originates from the I-band domain. Finally, we confirmed the expression of circRNA by RT-PCR, Sanger sequencing and RNA-fluorescence in-situ hybridization.

Our data provides the first circRNA expression landscape in hearts. There is a high abundance of specific cardiac-expressed circRNA. These findings open up a new avenue for future investigation into this emerging class of RNA.

Role of the mitochondrial calcium uniporter in myocardial ischemia/reperfusion injury

Marika Campesan, Tania Zaglia, Diego De Stefani, Rosario Rizzuto, Marco Mongillo,
Fabio Di Lisa

Department of Biomedical Sciences, University of Padova, Italy

Background: The role of mitochondrial calcium uniporter (MCU) has been evaluated mostly using a pharmacological approach suggesting that MCU inhibition elicits protection against cardiac injury induced by ischemia and reperfusion (I/R). However, MCU deletion has been shown not to affect the cardiac susceptibility to I/R. Here, we aimed at investigating whether changes in MCU expression affect I/R injury and formation of reactive oxygen species (ROS).

Methods and results: Neonatal rat ventricular myocytes (NRVMs) overexpressing MCU by adenovirus infection showed a reduction in I/R-induced cell death as compared to wild type (wt) cells ($41.82\% \pm 8.37$ vs $60.44\% \pm 11.68$, $p < 0.05$). The in vitro evidence of cardioprotection was confirmed also ex vivo in perfused hearts overexpressing MCU ($65.3\% \pm 3.35$ vs basal) by means of adenoassociated virus infection. Indeed, reperfusion after 40 min of global ischemia resulted in a significant decrease of lactate dehydrogenase release as compared to wt hearts (16.14 ± 11.69 vs 67.01 ± 0.07). This increased tolerance to I/R injury was associated with a large decrease in levels of reactive oxygen species (ROS) upon reperfusion. However, starting at 12 h after infection NRVMs displayed a slight increase in ROS levels associated with an increase (2.8 ± 0.26 fold) in phosphorylation of protein phosphatase 2A (PP2A) that is known to decrease its activity.

Conclusion: MCU overexpression reduced I/R-induced cell death both in NRVMs and intact hearts. The decreased susceptibility to reperfusion injury appears to represent a preconditioning-like effect might resulting from a slight increase in mitochondrial ROS levels in response to the augmented Ca^{2+} uptake induced by MCU overexpression. In particular, the ROS-induced decrease in PP2A activity is likely to enhance the activity of survival kinases.

Mitochondrial (dys)function in the aged and failing myocardium: some common features

Marisol Ruiz-Meana

Vall d'Hebron Institut de Recerca, University Hospital Vall d'Hebron, Barcelona

Experimental data indicate that mitochondria play a causative role in the functional deterioration of senescent myocardium, increasing its vulnerability to ischemic damage. The detrimental role of mitochondria in the functional decline of aging cardiomyocytes is due, in part, to the development of a disrupted communication with sarcoplasmic reticulum that leads to defective calcium exchange and a concomitant mismatch between energy demand and energy supply. Interestingly, the development of a bioenergetic mismatch and altered calcium handling has also been described in failing cardiomyocytes. In both aged and failing hearts the presence of an increased subcellular prooxidative environment appears to further aggravate alterations in mitochondrial respiration and integrity. However, the mechanism by which increased ROS production participates in the pathophysiology of heart failure and aging is not clearly established, and other factors, like an increased dicarbonyl stress and glycation may be involved.

Enlargement of myocardial infarct size by chronic kidney disease: a novel mechanism of inhibiting protective signaling to mitochondria

Tetsuji Miura, Toshiyuki Tobisawa, Toshiyuki Yano, Masaya Tanno

Department of Cardiovascular, Renal and Metabolic Medicine, Sapporo Medical University
School of Medicine

Chronic kidney disease (CKD, chronic reduction of glomerular filtration rate and/or proteinuria) is a major risk factor of cardiovascular events and mortality after myocardial infarction. CKD increases myocardial infarct size after ischemia/reperfusion, but its mechanism remains unclear. Our previous studies showed that cytoprotective signaling leading to inhibition of mitochondrial permeability transition is disrupted at distinct steps by different co-morbidities, including hypertension and diabetes (Miki et al. *Circulation* 2000, Miki et al. *Diabetes* 2009, Hotta et al. *Circ Res* 2010, Yano et al. *Hypertension* 2011). Here we systematically analyzed the effect of CKD on cytoprotective signaling by use of a rat model of CKD, two-stage 5/6 nephrectomy. Infarct size after 20-min ischemia/2-h reperfusion in vivo was larger by 30% in CKD than in the control. CKD increased the level of Thr308 phosphorylation in Akt at baseline by 375%, though its levels upon reperfusion were similar in CKD and the control. In contrast, phosphorylation of Akt at Ser473 and also phosphorylation of p70S6K and GSK3-beta upon reperfusion were significantly suppressed by CKD, though their baseline phosphorylation levels were unaffected. Inhibition of Akt-Ser473 phosphorylation upon reperfusion by Ku-0063794, an mTOR inhibitor, significantly enlarged infarct size in control rats. Protein levels of PDK1 and mTORC2, which phosphorylate Thr308 and Ser473 in Akt, respectively, were not changed by CKD. However, of PP2A regulatory subunits, B55alpha, a subunit targeting Thr308 in Akt, was selectively reduced by 24% in CKD. By overexpressing HA-tagged wild-type Akt and phospho-Thr308-mimetic mutant Akt (T308D) in HEK293 cells, we found that constitutive phosphorylation of Akt-Thr308 negatively regulates the response of Akt-Ser473 phosphorylation to its upstream signaling. These results indicate that CKD suppresses Akt activation upon reperfusion by intramolecular inhibition of Ser473 phosphorylation, in which reduction in B55alpha-mediated Thr308 dephosphorylation in Akt is involved, and that insufficient activation of Akt-GSK3beta signaling to mitochondria upon reperfusion contributes to infarct size enlargement by CKD.

Thyroid hormone control of lipophagy and mitophagy

Paul Yen

Cardiovascular & Metabolic Disorders Program, Duke-NUS Graduate Medical School
Singapore

We previously showed that thyroid hormone (TH) is a potent stimulator of hepatic lipophagy and beta oxidation of fatty acids. As such, TH plays important roles in lipid metabolism and its decrease activity in the liver may contribute to NAFLD. TH also has long been known to stimulate mitochondrial function, but its role in mitochondrial turnover remains unclear. Mitochondria play an essential role in oxidative phosphorylation and fatty acid oxidation. However, prolonged mitochondrial activity produces reactive oxygen species (ROS) that inflict oxidative damage leading to mitochondrial dysfunction that can be associated with metabolic disorders. Here, we show that TH increases “mitophagy” in hepatic cells in association with increased oxidative phosphorylation. This was evident from co-localization of autophagy/autolysosomal markers and mitochondria using confocal and electron microscopy. Furthermore, we found that T₃ induced a concomitant increase in ROS that was crucial for TH-induced mitophagy through the AMPK/ULK1 pathway. Similar results were seen in primary hepatocytes and *in vivo*. Our results describe a novel mechanism of TH-induced mitochondrial turnover through mitophagy and mitochondrial biogenesis. This coordinated turnover, which is dependent upon ROS generation, enables TH to maintain oxidative phosphorylation and beta oxidation in the face of high flux of fatty acids induced by TH. Our preliminary evidence suggests that TH also can induce autophagy in a number of tissues including, skeletal muscle, brown adipose tissue, and heart. We currently are characterizing autophagy in these tissues as well as its functional and metabolic consequences.

Importance of mitochondrial proteins for myocardial ischemia/reperfusion injury and protection from it

Rainer Schulz, MD

Institute of Physiology, Justus-Liebig University Giessen, Giessen, Germany

Acute myocardial infarction with subsequent left ventricular dysfunction and heart failure continues to be a major cause of morbidity and mortality in the Western world. Rapid advances in the treatment of acute myocardial infarction, mainly through timely reperfusion, have substantially improved outcomes in patients presenting with acute coronary syndrome, although reperfusion itself contributes to ultimate infarct size. Brief episodes of nonlethal ischemia/reperfusion before or during sustained ischemia or at the onset of reperfusion can reduce ischemia/reperfusion injury. This so called "ischemic conditioning" also reduces infarct size when the brief episodes of ischemia/reperfusion are applied to organs or tissue distal to the heart. The conditioning protocols recruit complex signal cascades of activation of sarcolemmal receptors and intracellular enzymes, reactive oxygen/nitrosative species, mitochondrial stabilisation and finally inhibition of death signalling.

Firstly, the importance of the gap junction protein connexin 43 in the context of ischemia/reperfusion injury and protection from it will be discussed. Apart from being present at gap junctions connexin 43 is also located at mitochondria and is involved in mitochondrial respiration, ATP generation and mitochondrial potassium influx. Blockade of connexin 43-formed channels reduce ischemia/reperfusion injury but at the same time abolishes cardioprotection by ischemic preconditioning. Secondly, the importance of p66shc for ischemia/reperfusion injury and protection from it will be addressed. Thus, more research is needed to understand intracellular connexin 43 and p66shc turnover to potentially use it as a therapeutic target.