„Cardiac Regeneration and Vascular Biology“ Conference 2015
Convention Centre San Servolo, Venice, Italy
June 17th – 19th 2015
Conference Program

Wednesday June 17th 2015

12:00 Welcome address
Christian Weber, Christian Kupatt

Session I: Novel vascular functions
Chairs: Florian Limbourg (Hannover)
       Michael Potente (Bad Nauheim)

12:00 – 12:45 Reinhardt Fässler (Munich)
          Mechano-sensation and Mechano-transduction

12:50 - 13:10 Michael Potente (Bad Nauheim)
          The link between angiogenesis and endothelial metabolism

13:15 – 13:35 Qi Chen (Münster)
          Origin of cardiac pericytes

Session II: Molecular Therapy of Cardiomyopathy
Chairs: Bernd Fleischmann (Bonn)
        Oliver Söhnlein (Munich)

13:40 – 14:00 Arne Hansen (Hamburg)
          Human engineered heart tissue - a versatile in vitro model

14:05 – 14:25 Johannes Backs (Heidelberg),
          Novel twists of HDAC4

14:30 – 14:45 Rabea Hinkel (Munich)
          Vascular basis of diabetic cardiomyopathy
14:50 – 15:15  Coffee Break

Session III:  Inflammatory vascular remodeling
Chairs: Sabine Steffens (Munich)
        Daniel Sedding (Hannover)

15:15 – 15:30  Oliver Söhnlein (Munich),
Mechanisms of neutrophil instructed monocyte recruitment

15:35 – 15:50  Sabine Steffens (Munich)
Novel aspects of sterile postischemic inflammation

15:55 – 16:10  Esther Lutgens (Amsterdam)
Co-stimulation atherosclerosis: novel players and insights

16:15 – 16:30  Andrés Hidalgo (Matrid)
Neutrophil-platelet crosstalk in vascular inflammation

16:35 – 16:50  Maliheh Nazari-Jahantigh (Munich)
Role of microRNAs in macrophages during atherosclerosis

16:50 – 17:05  Coffee Break

17:10 – 18:10  Keynote Lecture
Chair: Christian Weber (Munich)
Sek Kathiresan (Boston)
Developing medicines that mimic the natural successes of the human genome

19:30  Dinner at Westin Europe and Regina, Venice
Thursday June 18th 2015

Session IV: Leducq Network CVGeneF(x)
Chairs: Christian Weber (Munich)
       Dan Rader (Philadelphia)

9:00 – 9:20 Dan Rader (Philadelphia)
Functional genomics of new pathways regulating lipid metabolism revealed through human genetics

9:25 – 9:45 Thomas Quetermous (Stanford)
Vascular wall disease pathways from coronary disease GWAS loci

9:50 - 10:10 Endre Kiss-Toth (Sheffield)
Tribbles at the Crossroads... Modulation of immunity and lipid homeostasis via Tribbles-1 dependent mechanisms

10:15 - 10:35 Christian Weber (Munich)
Role of the CXCR4/CXCL12 axis in atherosclerosis

10:40 - 11:00 Coffee Break

11:00 - 11:20 Heribert Schunkert (Munich)
Linking coronary risk loci with underlying mechanisms

Session V: miRNAs and heart disease
Chairs: Thomas Thum (Hannover)
        Gianluigi Condorelli (Milan)

11:35 – 11:55 Thomas Thum (Hannover)
Long noncoding RNA hits in the heart

12:00 – 12:20 Manuel Mayr (London)
From MicroRNA Biomarkers to Function

12:25 - 12:45 Stefan Engelhardt (Munich)
Role of microRNAs in myocardial remodeling

13:00 – 15:00 Lunch Break and Poster walk
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Friday June 19th 2015

Session VII: Structural Heart Disease
Chairs: Christoph Schmitz (Berlin)
       Christian Kupatt (Munich)

9:00 – 9:25 Derk Frank (Kiel)
         TAVI – Sapien 3 real world

9:30 – 09:55 Sabine Bleiziffer (Munich)
         Is transapical TAVI really so bad?

10:00 – 10:25 Christoph Schmitz (Berlin)
            Growing valves

10:30 – 10:45 Coffee Break

Session VIII: Translational session
Chairs: Sabine Bleiziffer (Munich)
       Michael Näbauer (Munich)

10:45 – 11:10 Georg Lutter:
            Development of transcatheter mitral valve implants

11:15 -- 11:40 Achim Büttner (Bad Krozingen)
            State of the art: Chronic total occlusions (CTOs)

12:15 Closing remarks
        Christian Kupatt, Christian Weber

Departure
**Poster Index**

**Session I: Angiogenesis, Arteriogenesis, Vessel Function**  
*Chairs: Bernd Fleischmann*

**Poster 1**  
Yu Ting  
Role of Taz in vessel shape and size

**Poster 2**  
Markus Kraus  
rAAV.MRTF-A induced therapeutic neovascularization: role of pericyte recruitment

**Poster 3**  
Jorge Andrade  
Unraveling the role of Foxo1 in Zebrafish vascular development

**Poster 4**  
Veronika Kaczmarek  
Cardioprotective potential of Thymosin β4 in a model of ischemic cardiomyopathy with additional risk factors

**Poster 5**  
Tamar Kapanadze  
Regulation of ischemic kidney inflammation by Notch-signaling

**Poster 6**  
Dario Bongiovanni  
Thymosin-β4 and MRTF-A pathway attenuates microcirculatory and hemodynamic destabilization in sepsis

**Poster 7**  
Donato Santovito  
Role of apoptosis and autophagy in differential of miR-126-3p and miR-126-5p in endothelial cells

**Poster 8**  
Sascha dAlmeida  
Sphingosine-1-Phosphat as a potential therapeutic target in LPS-induced sepsis
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Lack of Dicer expression in smooth muscle cells promotes neointima formation

Poster 10 Richard Michael Blay
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Poster 11 Joana Viola
Pushing towards resolution: lipid mediator intervention in atherosclerosis

Poster 12 Almudena Ortega-Gomez
Neutrophil Serine Proteases in early atherosclerosis

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Plasma MicroRNA levels are associated with platelet function: effects of medication and genetic variation

Session III: Stem cells and iPS technology
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Poster 15 Anika Eike Benzin
Disease modelling of phospholamban mutations in human iPSC-derived cardiomyocytes

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Human induced pluripotent stem cells for tissue-engineered cardiac repair

Poster 17 Britta Husse
Characterization of Ca2+-handling in Tbx3-programmed ESC-derived pacemaker-like cells

Poster 18 Markus Krane
Postnatal induction of embryonic Nkx2.5 enhancer positive cardiac cells after myocardial infarction

Poster 19 Christian Rimmbach
Generation of highly pure physiologically and pharmacologically functional Sinus-Nodal-Bodies from Pluripotent Stem Cells
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Chairs: Norbert Frey

Poster 20  Maria-Teresa Piccoli
Identification of functional long noncoding RNAs in cardiac fibrosis

Poster 21  Franziska Schöttmer
Paracrine transfer of regulatory non-coding RNAs via extracellular vesicles in the cardiovascular system

Poster 22  Giuseppina Mastrototaro
Ablation of paladin in adult cardiac muscle causes dilated cardiomyopathy

Poster 23  Michael Horckmans
Role of cannabinoid receptor 2 on innate response activator B cells in myocardial infarction healing response

Poster 24  Ng Judy King Man
Generation of a conditional Tb4-overexpressing mouse model to study its cell-specific beneficial effects

Poster 25  Deepak Ramanujam
Inhibition of miR-21 prevents myocardial remodelling in a swine model of Ischaemia/reperfusion injury
Abstracts

Disease modeling of phospholamban mutations in human iPSC-derived cardiomyocytes

Anika Benzin1, Ingra Vollert1, Birgit Klampe1, David Letuffe-Brenière1, Sandra Laufer2, Aya Shibamiya2, Ingrid van Rijsingen3, Yigal Pinto3, Sian Harding4, Thomas Eschenhagen1, Arne Hansen1
1 Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Centre, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
2Hamburg Centre for Experimental Therapy Research (HEXT), Stem Cell Core Unit, Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Centre, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; 3Department of Cardiology, Heart Failure Research Centre, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands; 4Faculty of Medicine, National Heart & Lung Institute, Imperial College, London, Great Britain

Introduction: Phospholamban (PLN) is regulating calcium re-uptake into the cardiomyocyte’s sarcoplasmatic reticulum. PLN mutations are associated with dilated cardiomyopathy (DCM). In this study we want to establish an in vitro model for the PLN-R14Del mutation with disease-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) in three-dimensional, force-generating engineered heart tissues (EHT) and define disease-specific contractility pattern.

Methods: PLN-R14Del carrying hiPSCs were reprogrammed from skin fibroblasts by sendai virus-mediated reprogramming. Differentiation into cardiomyocytes was performed with an embryoid body, growth factor-based protocol. Dissociated cardiomyocytes were used to generate strip-format, fibrin EHTs. Expression of calcium handling proteins (PLN, Serca2, L-type calcium channel, calsequestrin, Na+/Ca2+-Exchanger1, Na+/K+-ATPase, Na+/H+-Exchanger1) was verified by western blot. Characterization of contractile force was performed by automated video-optical recording and analysis.

Results: Baseline characterization of electrically paced (1.5-2.0 Hz) PLN-R14Del-EHTs showed significantly lower force (0.09 vs. 0.21 mN), longer contraction - (0.128 vs. 0.115 s) and relaxation time (0.179 vs. 0.139 s) compared to unrelated control EHTs. PLN-R14Del-EHTs beat spontaneously at significantly lower frequency (26 vs. 58 bpm) and showed irregular beating pattern. Long-term incubation (9 hours) revealed a concentration-and time-dependent increase in irregular beating pattern (IBP) with increasing extracellular
calcium concentration (1.0, 1.8, 3.0 mM). Similar observations were made in the presence of L-type calcium channel agonist BayK-8644 (100 nM). Verapamil (1000 nM) transiently suppressed IBP under 3.0 mM extracellular calcium. **Conclusion:** This preliminary data set suggests that a disease-specific contractility pattern can be demonstrated for PLN-R14Del in hiPSC-derived EHTs. Further studies will focus on establishing isogenic controls.
Deletion of miR-21 in hematopoietic cells reduces atherosclerotic lesion formation in Apoe<sup>−/−</sup> mice by increasing apoptosis of lesional macrophages

Richard M. Blay<sup>1</sup>, Maliheh Nazari-Jahantigh<sup>1,2</sup>, Lucia Natarelli<sup>1</sup>, Kathrin Heyll<sup>1</sup>, Claudia Geißler<sup>1</sup>, Eric N. Olson<sup>3</sup>, Christian Weber<sup>1,2</sup>, Andreas Schober<sup>1,2</sup>

<sup>1</sup>Experimental Vascular Medicine (EVM), Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University, Munich, Germany; <sup>2</sup>DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany; <sup>3</sup>Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Background: We have previously shown that miR-155 in macrophages promotes atherosclerosis by increasing inflammatory activation and inhibiting efferocytosis. In addition to miR-155, both miR-21 strands, miR-21 and miR-21*, belong to a small set of miRNAs up-regulated in atherosclerotic plaques. MiR-21 protects from apoptosis and reduces inflammation by targeting PDCD4. We hypothesized that expression of the miR-21 gene in macrophages may play a role in atherogenesis.

Methods and Results: To identify the localization of miR-21 and 21* in atherosclerotic lesions, in situ PCR and Mac2 immunostaining was performed on aortic root sections from miR-21<sup>+/+</sup>Apoe<sup>−/−</sup> and miR-21<sup>−/−</sup>Apoe<sup>−/−</sup> mice fed a high cholesterol diet (HCD) for 12 weeks. We found that miR-21 and 21* are expressed in lesional macrophages from miR-21<sup>+/+</sup>Apoe<sup>−/−</sup>, whereas negligible background staining was observed in miR-21<sup>−/−</sup>Apoe<sup>−/−</sup> mice. To study the role of miR-21 in macrophages during atherogenesis, miR-21<sup>+/+</sup>Apoe<sup>−/−</sup> mice were subjected to X-ray radiation and the bone marrow (BM) was reconstituted with either miR-21<sup>+/+</sup>Apoe<sup>−/−</sup> (miR-21<sup>+/+</sup> BM) or miR-21<sup>−/−</sup>Apoe<sup>−/−</sup> (miR-21<sup>−/−</sup> BM) bone marrow cells. After feeding the mice a HCD for 12 weeks, atherosclerotic lesions were significantly reduced in miR-21<sup>−/−</sup> BM mice compared to miR-21<sup>+/+</sup> BM mice as determined in en face-prepared aorta by oil red-o staining and staining of aortic root sections with Elastic van Gieson stain.

Moreover, the necrotic core area in the aortic root was significantly reduced in miR-21<sup>−/−</sup> BM mice compared to miR-21<sup>+/+</sup> BM mice. The lesional macrophage content and apoptosis was assessed by Mac2 immunostaining in combination with staining for activated Caspase-3 or TUNEL staining of aortic root sections. Our results show that the macrophage cell number is reduced and the number of apoptotic macrophages is increased in the lesions from mice transplanted with miR-21<sup>−/−</sup> BM. Moreover, we found by immunostaining and qRT-PCR of laser microdissected lesional macrophages that the pro-apoptotic gene X chromosome-linked inhibitor of apoptosis protein (XIAP)-associated factor 1 (Xaf1) is highly upregulated in atherosclerotic lesions of miR-21<sup>−/−</sup> BM mice compared to miR-21<sup>+/+</sup> BM mice.

Conclusion: Our results demonstrate that miR-21 deletion in hematopoietic cells reduces atherosclerosis presumably through the upregulation of Xaf1, which induces macrophage apoptosis leading to decreased macrophage accumulation and necrotic core formation.
Thymosin β4 attenuates microcirculatory and hemodynamic destabilization in sepsis

D. Bongiovanni¹,², T. Ziegler¹, S. D’Almeida¹, Zhang T¹, R. Hinkel¹,², C. Kupatt¹,²
¹Medizinische Klinik und Poliklinik I, Klinikum der Universität München
²Medizinische Klinik I, Klinikum Rechts der Isar der TU München

Introduction: Thymosin β4 (Tβ4) was first identified as a regulator in actin polymerization. In recent years, Tβ4 has been shown to be involved in angiogenesis, wound healing, cell survival and anti-inflammatory responses. We have previously shown, that Tβ4 is capable of recruiting pericytes, supporting cells in the microvasculature stabilizing endothelial barrier function (Hinkel et al., Nat Comm. 2014). Here we analyzed, whether treatment with Tβ4 is able to reduce the pericytes loss in LPS-induced sepsis sepsis and to improve the hemodynamic function and survival in C57BL/6 mice.

Methods: LPS (20mg/kg) was injected intraperitoneally into C75BL/6 mice. 14 days before sepsis induction, groups were transfected with an adenoassociated virus carrying the Tβ4 (rAAV.Tβ4) or LacZ gene (rAAV.LacZ). A sepsis-severity-score was assessed after 12, 24, 36, 48, 72 and 96 hours. At the timepoints 12 and 24 hours non-invasive hemodynamic measurements were performed. After completion of the experiment, heart and muscle samples were analyzed for PECAM-1⁺ capillaries and NG2⁺ pericytes.

Results and Conclusion: At 36 hours there was a decrease of sepsis severity in rAAV.Tβ4-treated animals as compared to control (Tβ4:18.7±1.2 vs. LacZ:22.7±0.8 points, p=0.01). Furthermore, rAAV. Tβ4- treated animals displayed a higher blood pressure compared to rAAV.LacZ treated animals as early as 12 hours after sepsis induction (Tβ4:63±6 vs. LacZ:44±33mmHg, p=0.01). Of note, the rAAV.Tβ4 group showed higher pericytes counts in both, the heart and peripheral muscle samples (heart: 2315±205 cells/mm² for rAAV.Tβ4 vs. 1659±96 cells/mm² for rAAV.LacZ, p=0.02; peripheral muscle: 664±44 cells/mm² for rAAV.Tβ4 vs. 400±41 for rAAV.LacZ, p=0.001). Finally Tβ4 treatment resulted in a dramatic reduction of mortality compared to control animals (rAAV.Tβ4: 33% survival vs. rAAV.LacZ: 0% survival, p=0.005). These early data indicate a potential role of Thymosin β4 in preventing hemodynamic changes in septic hypercirculation and highlight Tβ4 as a potential therapeutic target in severe sepsis.
Human induced pluripotent stem cells for tissue-engineered cardiac repair

Kaja Breckwoldt¹, Florian Weinberger¹, Simon Pecha², Allen Kelly³,⁴, Birgit Geertz¹, Jutta Starbatty¹, Godfrey Smith³,⁵, Arne Hansen¹, Thomas Eschenhagen¹

¹ Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck; ² Department of Cardiovascular Surgery, Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; DZHK (German Center for Cardiovascular Research), partner site Hamburg/Kiel/ Lübeck
³ K.G. Jebsen Center of Exercise in Medicine, Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway; ⁴ Norwegian Council on Cardiovascular Disease, Norway; ⁵ Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, Scotland

Myocardial infarction causes an unrecoverable loss of cardiomyocytes. Engineered heart tissue (EHT) is an in vitro model of three-dimensional, force generating cardiomyocyte network with morphological and functional similarity to native heart tissue. In this study, we transplanted EHTs from human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes on guinea-pig hearts after cryoinjury-mediated myocardial infarction and investigated if hEHTs support left ventricular function.

Differentiation of hiPSCs to cardiomyocytes and endothelial cells was performed by an embryoid body-based three-stage differentiation protocol. EHTs were created from 5x10⁶ hiPS-CM and 2x10⁶ hiPS-EC and cultivated for 3 weeks under auxotonic stretch between flexible silicone posts. Development of contractile force was monitored prior to transplantation. Left ventricular myocardial cryoinjury was induced in adult guinea pigs (n=26). 7 days after injury, EHTs (18x2x2 mm, 2 per animal, n=14) or cell-free fibrin constructs (n=12) were implanted. Animals received ciclosporin and methylprednisolon for immunosuppression. Functional parameters were examined by echocardiography at baseline, before and 28 days after transplantation. Electrophysiological coupling of the implanted hEHTs to the host myocardium was assessed by 2P microscopy of di-4-ANEPPS-stained Langendorff-perfused hearts. Hearts were explanted 28 days after transplantation and analyzed histologically.

Cryo-injury resulted in large transmural scars (~25% of left ventricular wall), which were verified histologically. Immunohistochemical staining for dystrophin and MLC2v showed the formation of large islets of cross-striated muscle tissue in the scar. The human origin was demonstrated by fluorescent-in-situ-hybridization. The new myocardium was vascularized with endothelium partly being of human origin. Echocardiographic evaluation 7 days after injury showed a decline in FAS (24±7% vs. 43±8% at baseline) without dilation (7.9±0.9 mm vs. 7.9±1.0 mm at baseline) of the left ventricle. 28 days after transplantation, left ventricular function remained essentially unchanged in animals that had received cell-free constructs and left ventricular dimensions increased. hEHT transplantation attenuated left ventricular dilatation (8.6±1.3 mm vs. 9.4±2.0 mm cell-free) and improved FAS (39±12% vs. 27±2% cell-free). Evidence of electrical coupling was successfully demonstrated in 1 of 4 hEHT hearts. Within the remote myocardium, hEHT-transplanted hearts demonstrated a significantly improved electrophysiological phenotype (4 of 4 hEHT hearts), with shorter AP duration and faster transmural conduction velocity in hEHT-transplanted compared to control hearts, compatible with a reversal of the heart failure phenotype in the infarcted hearts.

This study demonstrated for the first time that hEHTs are able to electrically couple to non-rat rodent host myocardium and improve left-ventricular function after transplantation.
Origin of perivascular cells in the heart

Qi Chen, Yang Liu, Monica Corada, Elisabetta Dejana, Ralf H. Adams

1 Max Planck Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, and University of Muenster, Faculty of Medicine, Muenster, Germany
2 Max Planck Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, 48149 Muenster, Germany
3 IFOM Fondazione, FIRC Institute of Molecular Oncology, 20139 Milan, Italy
4 IFOM Fondazione, FIRC Institute of Molecular Oncology, 20139 Milan, Italy and Department of Biosciences, University of Milan, 20133 Milan, Italy

Perivascular cells are essential supporting cells in the cardiovascular system and help to maintain the integrity and function of blood vessels. In the heart, coronary perivascular cells are required for heart regeneration and were recently reported to function as progenitors of myofibroblasts in a model of heart disease. While previous work has focused on epicardium-derived progenitors as a source of coronary perivascular cell, there was also data indicating that other sources might exist. Here, we find that coronary perivascular cells originate from mesenchymal progenitors in the embryonic atrioventricular canal and outflow tract. By using reporter mice and genetic clonal analysis, we uncovered the formation of PDGFRβ+ NG2+ PDGFRα- perivascular cells in the heart from primitive PDGFRβ+ NG2- PDGFRα+ mesenchymal cells present in early embryonic stages. Tissue-specific and inducible gene knockout experiments also revealed that canonical Wnt signaling in PDGFRβ cells was necessary for coronary perivascular cell development. Absence of Frizzled4 and β-catenin in PDGFRβ cells impaired their ability to form perivascular cells in the compact myocardium. Blockade of Wnt secretion from endothelial cells also inhibited coronary perivascular cell development indicating the necessity of endothelial-derived Wnt signaling in this developmental process. Our work identifies a novel origin of perivascular cells in the heart and elucidates a new molecular mechanism for the development of the coronary vasculature. This knowledge improves our understanding of perivascular cell development and may be relevant for cardiovascular disease and regeneration.
Thymosin ß4 improves chronic myocardial ischemia in diabetic pigs

V. Kaczmarek², A. Howe¹, R. Hinkel¹,²,³, S. Straub¹, W. Husada¹, S. Lee¹, S. Renner⁴, E. Wolf⁴, C. Kupatt¹,²

¹ Medizinische Klinik und Poliklinik I, Klinikum der LMU München
² 1. Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar der TU München
³ IPEK, Klinikum der LMU München
⁴ Lehrstuhl für Molekulare Tierzucht und Biotechnologie, Genzentrum der LMU München

Diabetes mellitus is one of the major risk factors for developing cardiovascular disease. Diabetic microcirculatory rarefaction potentially impairs responsiveness to coronary revascularization. Novel pro-angiogenic factors like Thymosin ß4, a small 4.9 kDa peptide, have yet to be assessed in preclinical models of diabetic cardiomyopathy.

Methods: In wildtyp and transgenic pigs displaying diabetes mellitus typ I (due to a C94Y mutation in the porcine insulin gene), vascularization and myocardial function were analyzed at basal conditions. In a second set of experiments, chronic myocardial ischemia was induced in wildtyp and transgenic pigs via reduction stent graft in the circumflex artery, gradually inducing a total occlusion on day 28 (d28). Retroinfusino of rAAV Tß4 (5x10E12 viral particles) was performed at day 28. Global myocardial function (EF, LVEDP) was obtained at day 28 and 56. In addition subendocardial segment shortening (SES) in the ischemic region and post mortem angiography (collateral growth) were examined on day 56. Histological analysis of PECAM-1 positive cells (capillaries / high power field (c/hpf),) and vessel maturation (pericyte coverage, NG-2 positive cells) was performed in the ischemic tissue.

Results: Analysis of non-ischemic wt and diabetic animals revealed capillary rarefaction in the myocardium (234±8 in wt vs. 163±14 c/hpf in diabetic hearts). Analysis of myocardial function in non-ischemic hearts detected a trend towards loss of ejection fraction in diabetic pigs. In chronic myocardial ischemia, rAAV.Tß4 overexpression in wt animals significantly enhanced capillary density (278±6 vs. 148±6 c/hpf) and collaterals (9±1 vs. 3±1) in the ischemic area compared to control wt animals. Furthermore, rAAV.Tß4 improved ejection fraction (47±4% vs. 29±3 % in controls) as well as SES (at 150 beats/min: 73±5 vs. 10±6 % of non-ischemic area). In diabetic pigs, blood glucose levels were elevated (305±12 mg/dL at day 28 and 353±10 mg/dL at day 56). Here, rAAV.Tß4 still induced angiogenesis (190±4 c/hpf (Tß4) vs. 120±5 c/hpf in wildtype hearts) as well as collateral growth (4±1 (Tß4) vs. 2±1 in control hearts). Moreover, EF increased in rAAV.Tß4 diabetic hearts (32±2 % vs. 27±1 % in controls)

Conclusion: Thymosin ß4 transduction induces therapeutic neovascularization and thereby improves the myocardial function in the presence of type I diabetes mellitus. Therefore, rAAV.Tß4 appears suitable for treatment of ischemic cardiomyopathy associated with this cardiovascular risk factor.
rAAV.MRTF-A induced therapeutic neovascularization: Role of pericyte recruitment

M. Kraus¹, R. Hinkel¹, ³, A. Howe¹, T. Trenkwalder¹,², F. Gesenhues¹, S. Lee³, C. Kupatt¹, ³

¹Internal Medicine I, Klinikum Grosshadern, Munich, Germany; ²Walter-Brendel-Centre for
experimental Medicine, Munich, Germany; ³DZHK (German Center for Cardiovascular research),
partner site Munich Heart Alliance, Munich, Germany

Introduction: Thymosin beta 4 (Tb4), a small G-actin sequestering protein is known to
induce angiogenesis and acts as a cardioprotective agent limiting myocardial infarction
and ischemia reperfusion injury. Besides or in addition to a AKT mediated signaling of
Tb4, it is capable of induce transcription activation via MRTF/SRF pathway. The family
of MRTFs (myocardin-related transcription factors) transmit changes of G-actin
concentration into transcriptional activity. Therefore, we investigated the potential of
MRTF-A transduction in a rabbit hindlimb ischemia model and the role of pericyte
recruitment.

Methods: Female rabbits (New Zealand White, 2-2,5 kg body weight; n=5) underwent
a complete excision of the right femoral artery on day 0 as well as rAAV application (5
x 10¹² virus particles) via i.m. injection into the right hindlimb. Viruses encoded for LacZ,
MRTF-A or Angiopoietin 2 (Ang2). To further elucidate the timeframe for pericyte
recruitment a tet-off.Ang2 rAAV was used to control expression in vivo. Angiography
and fluorescent microspheres application was performed on day 7 and 35 to quantify
collateral growth (% of day 7) and regional blood flow (% of day 7). 5 weeks after
excision tissue was harvested for immunostaining (PECAM-1 for capillaries, NG2 for
pericytes) to quantify capillaries (c = capillaries) and pericytes (p/c = pericytes/capillaries).

Results: Compared to rAAV.LacZ control animals rAAV.MRTF-A application
significantly increased capillarization (39 ± 2 c) compared to control animals (26 ± 2 c)
as well as pericyte recruitment (0,56 ± 0,03 p/c vs. 0,34 ± 0,03 p/c in controls). This
increased angiogenesis and vessel maturation translated in an enhanced collateral
formation (153 ± 5 % vs. 104 ± 6 % in controls) and perfusion (202 ± 23 % vs. 108 ±
12 % in controls) in ischemic limbs. Co-transduction of constitutive active Ang2
significantly reduced the pericyte recruitment but had no influence on capillary density.
This reduction in vessel maturation abrogated the MRTF-A effect on collateral
formation (110 ± 5 %) and perfusion (119 ± 4 %). The MRTF-A induced pro-
angiogenetic effect could be enhanced by early vessel destabilization (52 ± 4 c) via
rAAV.tet-off.Ang2 (overexpression till day 14). The impact on the pericyte coverage
was enhanced slightly by this therapy (0,65 ± 0,04 p/c) but did not translate in an
enhanced collateral formation (138 ± 14 %) and perfusion in ischemic limbs.
Heme oxygenase 1 provides cardioprotection after ischemia/reperfusion injury via inhibition of postischemic inflammation

J. Ng12&, R. Hinkel123&, E. Gottlieb2, B. Petersen4, Q. Di2, P. Lange2, S. Lee2, P. Wenzel5, H. Niemann4, C. Kupatt1&
1 Medizinische Klinik und Poliklinik, Klinikum rechts der Isar, TU Munich, Germany; 2 Medizinische Klinik und Poliklinik I, Klinikum der Universität München, Campus Großhadern, München, Germany; 3 Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany; 4 Department of Biotechnology, Friedrich-Loeffler-Institute, Institute of Farm Animal Genetics, Neustadt a.Rbge., Germany; 5 2.Medizinische Klinik und Poliklinik, Universitätsklinikum and Zentrum für Thrombose und Hämostase, Mainz, Germany; & DZHK (German Center for Cardiovascular research), partner site Munich Heart Alliance, Munich, Germany

Heme oxygenase 1 (HO-1) is stress-induced and degrades heme into carbon monoxide (CO), free iron (Fe2+) and biliverdin, which is then converted into bilirubin. These reaction products attenuate oxidative stress, inflammation and apoptosis, all of which are involved in ischemia/reperfusion injury. We investigate the cardioprotective potential of recombinant adeno-associated virus (rAAV) – encoding HO-1 in mice and pigs after acute myocardial ischemia and reperfusion.

Methods: human HO-1 overexpressing transgenic pigs (TG) were produced by somatic cell nuclear transfer. Pigs (wt ± rAAV-hHO-1, hHO-1-TG ± inhibitor ZnPP) underwent LAD occlusion (I, 60 min) and reperfusion (R, 24 h), and apoptosis, leukocyte influx, capillary density, infarct size, and myocardial function were assessed. Mice (HO-1 +/-, +/-, -/-, rAAV-injected) underwent 45 min I/ 24 h R, and global myocardial function (via Millar pressure tip catheter), infarct size (via SPECT and TTC staining) and inflammation were analyzed.

Results: In porcine hearts, hHO-1 overexpression significantly reduced inflammation in the ischemic area (TG 874±66, rAAV 888±72 vs. wt 1611±180 leukocytes/mg tissue) and reduced infarct size (TG 36%±2, rAAV 43%±3 vs. wt 58%±4). Left ventricles of hHO-1 overexpressing pigs displayed a smaller loss of function (TG 17%, rAAV 16% vs. wt 25%, TG+ZnPP 28%) and did not show increasing end diastolic pressure as in control hearts (Δp 0.5mmHg in hHO-1Tg and 1.5mmHg in rAAV.hHO-1 vs. 3.5mmHg in controls and 4.0mmHg in hHO-1TG+ZnPP). Moreover, apoptosis rate was reduced and capillary density was enhanced in the ischemic tissue upon hHO-1 overexpression. These effects were sensitive to the specific HO-1 inhibitor ZnPP. In murine ischemia and reperfusion (I/R), although infarct size and ejection fraction at rest were indistinguishable between HO-1-/- and HO-1+/- mice, functional reserve was found to be lower in HO-1-/- mice under norepinephrine stimulation. Furthermore, the postischemic influx of CD45+ leukocytes, Ly-6Chigh monocytes and Ly-6G+ neutrophils observed in HO-1+/- mice was further exacerbated in HO-1-/- hearts. In cell culture, overexpression of hHO-1 in cardiomyocytes enhanced their survival and also reduced endothelial cell apoptosis.

Conclusion: We have demonstrated that heme oxygenase 1 overexpression curtails pro-inflammatory activation and infarct expansion, and thereby is cardioprotective after acute myocardial infarction. Enhancing HO-1 activity in early reperfusion is a potential approach to treat post-ischemic/reperfusion injury.
Plasma MicroRNA Levels Are Associated with Platelet Function: Effects of Medication and Genetic Variation

Philipp Skroblin¹, Dorothee Kaudewitz¹, Peter Willeit²,³, Allison C. Morton⁴, Lukas Bender¹, Nicholas Sunderland¹, Karin Willeit¹,³, Raimund Pechlaner¹,³, Anna Zampetaki¹, Robert F. Storey⁵, Stefan Kiechl³, Manuel Mayr¹

¹ King’s British Heart Foundation Centre, King’s College London, UK
² Department of Public Health and Primary Care, University of Cambridge, UK
³ Department of Neurology, Medical University Innsbruck, Austria
⁴ Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK
⁵ Department of Cardiovascular Science, University of Sheffield, UK

Background. Platelets shed microRNAs (miRNAs). Plasma miRNAs change upon platelet inhibition. It is currently unclear if plasma miRNA levels correlate with function.

Methods and Results. Plasma miRNAs were measured in 155 patients with a history of ACS (STEMI, NSTEMI or unstable angina) who had undergone detailed assessment of platelet function 30 days after the acute event. Using custom-made quantitative real-time polymerase chain reaction plates, 92 miRNAs were assessed in ACS patients on four different anti-platelet therapies (aspirin, clopidogrel+aspirin, prasugrel+aspirin, ticagrelor+aspirin). MiR-126 and miR-223 plus other key platelet-related plasma miRNAs were correlated with two platelet function tests (the VerifyNow P2Y12 assay and vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay). Significant positive associations were obtained for miR-126 with the VerifyNow (rp=0.347, n=39, P=0.033) and VASP assay (rp=0.224, n=123, P=0.013). MiR-223 (rp=0.231, P=0.003) and other abundant platelet miRNAs also showed significant positive correlations with the VASP assay. To further proof their platelet origin, platelet poor plasma was spiked with platelets. MiR-126 was among the miRNAs, which showed the greatest dependency on platelets and its plasma levels strongly correlated to soluble P-selectin, platelet factor 4 and platelet basic protein in the population-based Bruneck study (year 2000 follow-up, n=685). The primary sequence of human miR-126 contains a single nucleotide polymorphism (dbSNP: rs4636297) downstream of the pre-miR sequence: pri-miR-126 encoded by the major “G” allele is processed to a lesser extent than pri-miR-126 encoded by the minor “A” allele. Using a recessive model, the “AA” genotype accounted for a significant rise of circulating miR-126 levels and a corresponding increase in P-selectin, platelet factor 4 and platelet basic protein in the Bruneck cohort.

Conclusions. Levels of platelet-related plasma miRNAs correlate with platelet function tests in ACS patients and platelet activation markers in the general population. This study provides proof-of-principle that circulating miRNA levels are, at least in part, genetically determined and that SNPs in miRNAs can affect platelet reactivity.
Lack of Dicer expression in smooth muscle cells promotes neointima formation

Farima Zahedi1, Maliheh Nazari-Jahantigh1,4, Zhe Zhou2, Stefan Offermanns3, Christian Weber1,4, Andreas Schober1,4
1Experimental Vascular Medicine (EVM), Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University, Munich, Germany
2Institute for Molecular Cardiovascular Research, RWTH Aachen University, Aachen, Germany
3Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
4DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany.

The main cause for restenosis following coronary intervention is neointimal hyperplasia due to accumulation of smooth muscle cells (SMCs) with an immature and synthetic phenotype. Small, non-coding microRNAs (miRNAs) generated by the RNase Dicer play an important role in SMCs during development and SMC differentiation. Following vascular injury, several upregulated miRNAs, such as miR-21 and miR-221, promote neointima formation by increasing SMC proliferation. However, the role of Dicer in SMCs in neointima formation is unclear.

To study the effect of Dicer in SMCs on neointima formation, smooth muscle myosin heavy chain (SMMHC)-Cre/Dicer+/-apolipoprotein E (Apoe)-/- (smooth muscle [SM]-DicerWT) and SMMHC-Cre/Dicerflx/flxApoe-/- (SM-DicerKO) mice were fed a high fat diet, treated with tamoxifen to induce the deletion of Dicer in SMCs, and subjected to wire injury of the left carotid artery. Neointima formation, the neointimal SMC content, and the SMC proliferation was increased in SM-DicerKO mice after 14d and 28d compared to SM-DicerWT mice as quantified by Elastic van Gieson stain and Ki67/smooth muscle actin (SMA) immunostaining.

To identify miRNA-mRNA interactions in SMCs that regulate neointima formation, the miRNA expression and the genome-wide gene expression profiles in carotid arteries were compared between SM-DicerKO mice and SM-DicerWT mice at 14d after vascular injury. Integrative target prediction analysis of the 84 miRNAs and 460 mRNAs that were down-regulated and up-regulated in SM-DicerKO mice, respectively, predicted binding sites for miR-27a in ten genes, including the conserved binding sites in the Rho guanine nucleotide exchange factor (GEF) 26 (ARHGEF26), carbohydrate sulfotransferase1 (CHST1), and SH3 domain binding glutamate-rich protein like 2 (SH3BGRL2) mRNAs. Moreover, miR-27a suppressed ARHGEF26 but not CHST1 or SH3BGRL2 mRNA expression in human SMCs by targeting the predicted binding site in the ARHGEF26 3'UTR as demonstrated by GW182 immunoprecipitation (MirTrap) and luciferase 3'UTR reporter assays. In contrast to SM-DicerKO mice, combined in situ PCR and SMA immunostaining revealed that miR-27a is expressed in medial and neointimal SMCs in SM-DicerWT mice. The number of neointimal SMCs expressing ARHGEF26 protein was significantly increased in SM-DicerKO mice as detected by ARHGEF26/SMA immunostaining. Inhibition of miR-27a or the miR-27a binding site in the ARHGEF26 3'UTR using LNA-inhibitors increased the proliferation of human SMCs as determined by Ki67 immunostaining.
In conclusion, miRNA biogenesis by Dicer in SMCs reduced neointima formation by suppressing SMC proliferation. This effect of Dicer is partly due to the expression of miR-27a, which inhibits SMC proliferation by targeting ARHGEF26, a guanine exchange factor that promotes growth factor signaling. Hence, local treatment with miR-27a is a promising therapeutic strategy for restenosis.