Late-Breaking
Basic Science
Abstracts

from the
American Heart Association
Scientific Sessions 2005

Dallas, Texas
November 13 – 16, 2005

Abstract Topics Include:

- Stem Cell and Cardiac Cell Replacement
- Arrhythmia Mechanisms
- Cellular Signaling
Abstracts

Late-Breaking Basic Science Abstracts From the American Heart Association Scientific Sessions 2005.

November 13–16, 2005
Dallas Convention Center Dallas, TX

Late-Breaking Basic Science: Stem Cells and Cardiac Cell Replacement

Adult Mouse Spermatogonial Stem Cells Differentiate into Cardiovascular Lineages and Generate Functional Cardiomyocytes

Kazumi Guan, Department of Cardiology and Pneumology, Heart Center, Georg-August University of Goettingen, Goettingen, Germany; Karim Nayemina, Institute of Human Genetics, Georg-August University of Goettingen, Goettingen, Germany; Lars S Maier, Stefan Wagner, Frieder Wolf, Department of Cardiology and Pneumology, Heart Center, Georg-August University of Goettingen, Goettingen, Germany; Marpuy Li, Wolfgang Engel, Institute of Human Genetics, Georg-August University of Goettingen, Goettingen, Germany; Gerd P Hasenfuss, Department of Cardiology and Pneumology, Heart Center, Georg-August University of Goettingen, Goettingen, Germany

Adult stem cells with the pluripotency of embryonic stem cells (ESCs) would be ideal for organ regeneration strategies. Previous studies in neonatal mice suggest that the germline lineages may retain the ability to generate pluripotent cells. We aimed to evaluate the pluripotency and plasticity of adult spermatogonial stem cells (SSCs), which are responsible for maintaining spermatogenesis throughout life in the male. Using a genetic approach we established the culture condition for isolation of SSCs from adult mouse testis (success rate ~ 27% of mice). These isolated SSCs respond to the culture conditions and acquire ESC properties. They are able to spontaneously (without co-culture) differentiate into functional cardiomyocytes expressing cardiac-specific genes (α-sat, GATA4, Nkx2.5, MEF2C). α-MHC, MLC2v, ANF, SERCA2a and NCX) and proteins (sarcromeric HMC, cardiac troponin T and connexin 43). Four major types of action potentials (APs) characteristic for specialized cells of the ventricle, sinus node, Purkinje fibers and atrium are found in these cardiomyocytes. Functional maturity of ventricle-like cells (n = 11 from 3 independent experiments) is shown by AP characteristics: upstroke velocity (dV/dt) being 55.8 ± 4.8 V/s, amplitude (APA) being 115.8 ± 3.3 mV, duration at 90% of repolarisation (APD90) being 288.9 ± 30.6 ms and maximum diastolic potential (MDP) being -8.3 ± 5.0 V. The amplitudes of calcium transients (fluo4) measured in SSC-derived ventricle-like cardiomyocytes (n = 19) are similar to those measured in ESC-derived cardiomyocytes (n = 6; 464 ± 77 nm vs. 287 ± 69 nm, respectively). This is also comparable to adult cardiac myocytes. Besides cardiomyocytes, SSCs give rise to vascular smooth muscle (expressing smooth muscle α-actin, vascular smooth muscle MHC) and endothelial cells (expressing E-PECAM-1, VEGFR2, and VWF). SSCs also differentiate into other somatic cell types in vitro. Furthermore, we show that SSCs are able to differentiate into cardiac cells and most other somatic cells in vivo after blastocyst microinjection. Thus, adult spermatogonial stem cells exhibit pluripotency like embryonic stem cells and may offer new possibilities in cell based cardiac regeneration strategies.
Late-Breaking Abstracts

5004 Transplanted Human Cord Blood Derived Unrestricted Somatic Stem Cells (ussc) Improve Left-ventricular Function And Prevent Left-ventricular Dilatation And Scar Formation After Acute Myocardial Infarction

Arjan Ruipanwar, Hannover Medical School, Hannover, Germany; Alireza Ghodsiadad, Heinrich-Heine-Universität, Duesseldorf, Germany; Michael Niehause, Hannover Medical School, Hannover, Germany; Gesine Koegler, Peter Wernet, Heinrich-Heine-University, Duesseldorf, Germany; Christoph Bara, Matthias Karck, Theo Koldis, Mike Makou, Ulrich Martin, Nawid Khaladi, Michael Mengel, Hannover Medical School, Hannover, Germany; Emmeran Garms, Hans Michael Klein, Heinrich-Heine-University, Duesseldorf, Germany; Axel Haverich, Hannover Medical School, Hannover, Germany

Objectives: Intra-myocardial transplantation of adult stem cells as a therapy of heart failure is increasingly being discussed and performed in animal and human studies. Human unrestricted somatic stem cells (USSC) are newly discovered cord blood-derived cells with pluripotent differentiation potential. We aimed to assess whether intra-myocardial transfer of USSC would improve global left-ventricular function in a porcine model of myocardial infarction (MI). Methods: USSC were isolated from cord blood (CB) of the umbilical cord vein. 13 to 10 6 cells were transplanted into the acutely ischaemic lateral wall of the left ventricle (n = 5). Control animals (n = 5) received medium injection. LV function and dimension were assessed by transthoracic echocardiography (TEE) immediately before MI, after MI followed by cell transplantation and after 6 weeks. The hearts were examined for cell survival, cardiac differentiation and scar formation. Results: Global LVEF after MI was 32 ± 6 % in controls and 36 ± 9 % in the cell treated group. After 2 months, mean global LVEF had decreased to 27 ± 5 % in the control group and increased to 52 ± 2 % in the treated group (p < 0.05). Left-ventricular end-diastolic diameter (LVEDD) after two months was 77 ± 4 ml in the control group compared to 26 ± 2 ml in the cord blood group (p < 0.01). Myocardial scar was present only in the control group. Conclusion: Transplantation of USSC after myocardial infarction significantly improves LV function, reduces infarct size and prevents LV dilatation.

5005 Reduction Of Fibulin-4 Expression Affects Elastogenesis And Results In Aortic Aneurysm And Dissection

Katsuhito Hanada, Marcel Vermeij, George A Garinis, Monique C de Waard, Maurice G Kunze, Dirk J Dunker, Carel Meijers, Roland Kanaar, Jeroen Essers, Erasmus MC, Rotterdam, The Netherlands

The Fibulins are a six-member protein family, prominently expressed in blood vessels and hypothesized to function as intermolecular bridges that stabilize the organization of extracellular matrix structures such as elastic fibers and basement membranes. To examine a potential role of Fibulin-4/EFEMP2 in elastic fiber assembly and cardiovascular disease we generated loss-of-function mice. Four-week old Fibulin-4-/- mice have thickened aortic valvular leaflets that are associated with an increased systolic pressure-gradient between left ventricular pressure (154 ± 4 mmHg) and aortic pressure (117 ± 7 mmHg) and transvalvular blood flow velocity suggestive for the presence of a mild aortic valve stenosis. Altogether Fibulin-4 heterozygous and homozygous mice provide a unique model to follow the pathogenetic sequence for aneurysm. In addition, we implemented a functional genomics approach and determined the aorta transcriptome of wild type, Fibulin-4-/- and Fibulin-4-/- mice using full mouse genome Affymetrix arrays. This approach enables us to identify those biological processes that were significantly over represented including apoptosis, blood pressure and coagulation and extracellular matrix organization as well as to identify several gene targets implicated in the response to aortic failure.

5006 Elevated Plasma Transforming Growth Factor-Beta 1 (TGF-β1) Reduces Aortic Atherosclerosis, Aortic Root Dilation and Pseudoxenuremy in Formation in Apolipoprotein E-null (ApoE−/) Mice

Andrew D Fridkin, Goro Otsuka, Ming Xiao, University of Washington, Seattle, WA; Sunyoung Lee, UCLA, Los Angeles, CA; Helen L Dichek, David A Dichek, University of Washington, Seattle, WA

INTRODUCTION The “protective cytokine” hypothesis predicts that elevated plasma TGF-β1 would limit atherogenesis. But, in humans high plasma TGF-β1 has been associated with both protection against and predisposition to severe atherosclerosis. An animal model of regulable TGF-β1 expression would permit a direct test of the hypothesis. METHODS We generated ApoE−/− mice that express active TGF-β1 in a “tet-off” system (Mx CRE-TRA TGF-β1-KO). Dietary doxycycline (dox) suppresses tranngene expression. Removal of dox from the diet at 6 weeks of age activates cardiac-specific expression of the TGF-β1 transgene and yields elevated plasma TGF-β1 within two weeks. We compared atherosclerosis in doubly transgenic mice off dox (DT-Off) to doubly transgenic mice on dox (DT-On) and singly transgenic mice off dox (ST-Off). All mice were littermates, ApoE−/−, and fed-fat from age 6 - 18 weeks. TGF-β1 transgene expression was evaluated by Northern analysis and by ELISA of plasma (n = 7–14). Plasma size and histology were analyzed in aortic root sections (n = 14–17). Plasma lipid measurements and FPLC were performed (n = 8–13). RESULTS Only the hearts of DT-Off mice expressed TGF-β1 transgene mRNA. Hearts of DT-Off mice secreted more total TGF-β1 to yield a 10–fold elevation of total plasma TGF-β1. DT-Off mice had significant reductions in aortic root plasma (area (~20%) and lipid content (~15%), aortic root circumference (~10%), and fewer pseudoxenuresmy (~80%). CONCLUSIONS Elevated plasma TGF-β1 retards atherogenesis, limits aortic root dilation, and inhibits pseudoxenuremy formation. Eludication of the mecha- nisms of these effects may suggest novel therapies for atherosclerosis and aneurysms.

Late-Breaking Basic Science Poster Abstracts

5005 Reduction Of Fibulin-4 Expression Affects Elastogenesis And Results In Aortic Aneurysm And Dissection

Katsuhito Hanada, Marcel Vermeij, George A Garinis, Monique C de Waard, Maurice G Kunze, Dirk J Dunker, Carel Meijers, Roland Kanaar, Jeroen Essers, Erasmus MC, Rotterdam, The Netherlands

The Fibulins are a six-member protein family, prominently expressed in blood vessels and hypothesized to function as intermolecular bridges that stabilize the organization of extracellular matrix structures such as elastic fibers and basement membranes. To examine a potential role of Fibulin-4/EFEMP2 in elastic fiber assembly and cardiovascular disease we generated loss-of-function mice. Four-week old Fibulin-4-/- mice have thickened aortic valvular leaflets that are associated with an increased systolic pressure-gradient between left ventricular pressure (154 ± 4 mmHg) and aortic pressure (117 ± 7 mmHg) and transvalvular blood flow velocity suggestive for the presence of a mild aortic valve stenosis. Altogether Fibulin-4 heterozygous and homozygous mice provide a unique model to follow the pathogenetic sequence for aneurysm. In addition, we implemented a functional genomics approach and determined the aorta transcriptome of wild type, Fibulin-4-/- and Fibulin-4-/- mice using full mouse genome Affymetrix arrays. This approach enables us to identify those biological processes that were significantly over represented including apoptosis, blood pressure and coagulation and extracellular matrix organization as well as to identify several gene targets implicated in the response to aortic failure.

5007 Scavenger Receptor Bi Prevents Nitric Oxide-induced Cytotoxicity And Endotoxin-induced Animal Death

Xiang-An Li, Ling Guo, University of Kentucky, Lexington, KY; Reto Asmus, University of Texas Health Science Center, San Antonio, TX; Eric J Smart, University of Kentucky, Lexington, KY

Nitric oxidative stress contributes to a variety of diseases, such as atherosclerosis, Alzheimer’s disease and diabetes. Although numerous mechanisms have been described controlling the production of nitric oxide (NO); for example, endothelial nitric oxide synthase (eNOS) activity is regulated by membrane-targeted nitric oxide synthase (eNOS) activity, which allows for NO to be released in the NO enzyme-catalyzed reaction. NO is important because NO can induce cell death at high level. However, in the context of age-related cardiovascular disease, NO is required for cell survival. In this study we examined the role of the NR-BI in preventing NO-induced cytotoxicity in vitro. We demonstrate that NO inhibits NO synthase activity, and inhibits pro-inflammatory cytokine release, and that the active site responsible for NR-BI's anti-NO cytotoxicity is a conserved CXXS redox motif. In conclusion, we demonstrate a novel regulatory mechanism in aorta to prevent NO-induced oxidative stress, which must likely will affect human cardiovascular diseases.

5008 ApoE-Null Mice Lacking Thrombospondin-1 or Its Receptor CD47 Are Protected Aga Inst Neointimal Thickening of Injured Arteries But Not Against Diet-induced Atherosclerosis

Loretta K. Pappan, Ming-Ping Wu, Dan Ye, Anna Oldenborg, Clay F. Semenkovich, Washington University, Saint Louis, MO; Jack Lawler, Kathryn J. Moore, Loretta K. Pappan, Ming-Ping Wu, Dan Ye, Anna Oldenborg, Clay F. Semenkovich, Lexington, KY

ApoE-Null mice lacking Thrombospondin-1 or its receptor CD47 are protected against intimal thickening of injured arteries but not against diet-induced atherosclerosis.
Engraffment, Migration And Functional Improvement In Ischemic Mouse Hearts Injected With Human Cardioblast-derived Stem Cells

Lucio Barle, Jhu, Baltimore, MD; Elisa Mesenita, University of Rome “La Sapienza”, Rome, Italy; Raul Marzo, H. de Leppo, H. de Leppo, H. de Leppo, H. de Leppo; Mariella Abraham, Mark Pittenger, Jhu, Baltimore, MD; Alessandro Giacomelli, University of Rome “La Sapienza”, Rome, Italy; Eduardo Marban, Jhu, Baltimore, MD

Introduction: Cardiac stem cells self-assembly into cardiospheres can which expand in monolayers to yield cardioblast-derived cells (CDCs). CDCs express high levels of progenitor cell markers such as c-kit, MDRT1 and CD105, but their potential for cardiomyoplasty is unclear.

Aims: We quantified the cardiac regenerative capacity and functional consequences of human cardiospheres injected in vivo (hereafter called cardiosphere injection). Method: Emergency endomyocardial biopsy specimens in primary culture yielded cardiac stem cells which were further passaged to produce CDCs. Mice were produced in Scid mice by ligation of the mid-LAD and 10 CDCs (transduced with adenovirus or lentivirus expressing β-galactosidase) were injected into the border zones to assess engraftment, migration, cardiac function and fibrosis. CDCs were injected and hearts harvested at 8, 15 and 20 days (n=4-5 in each group). To quantify functional effects and regeneration, 10 CDCs (n=8) or control human dermal fibroblasts (n=4) were injected with echos and histology at 20 days. We demonstrated engraftment in CDC-injected mice, but no staining was evident in the PBS-injected group. At day 0, cells were located at injection sites in the border zone, but at day 8 and day 20 transduced cells were mainly distributed within the MI area, forming islands or continuous bands of β-Gal positive tissue. At 20 days, the CDC-treated group exhibited a significantly higher left ventricular ejection fraction (mean 38.8±4.7%) as compared to either the fibrblast-treated group (24.3%, p<0.05) or the PBS-treated group (27.4%, p<0.05), but the two control groups were not significantly different. Underlying the observed functional improvement, Masson trichrome staining at 20 days revealed a higher fraction of viable fuchsian-positive tissue within the MI zone in CDC-injected (20.4±5.5%) than in fibrblast-injected hearts (14.7±1.5%, p<0.008). Conclusion: Human CDCs injected into the border zone of mouse MI's engraft, migrate into the MI zone, partially replace the scar and improve left ventricular function. These data show that engineering effects of human CDCs in mice motivate longer-term large-animal studies of CDCs, as a next step to therapeutic applications in patients.

5002 Association Between Mobilization of CXCR4+/CD34+ Stem Cells Early in Acute Myocardial Infarction and Left Ventricular Ejection Fraction and NT-proBNP Levels After 1 Year of Follow-Up

Wojciech Wojakowski, Rafal Wyderka, Silesian Medical Academy, Katowice, Poland; Anna Zielbka, Polish-American Children's Hospital Jagiellonian University, Krakow, Poland; Joanna Czermak, Polish-American Medical Centre, Jagiellonian University, Krakow, Poland; Krzysztof Sapienza', Rome, Italy; Eduardo Marban, Jhu, Baltimore, MD

Background: The mobilization of circulating stem cell populations has been associated with a variety of beneficial outcomes in patients with acute myocardial infarction (AMI). Unfortunately, there is no data regarding the long-term follow-up.

Aim: The study was to correlate the early mobilization of CD34+/CD45−/CD133−/CD34+ stem cells early in STEMI with the peak number of CD34+/CD133−/CD34+ cells as well as higher levels of NT-proBNP.

Methods and Results: 40 patients were enrolled. Stem cells numbers and concentrations of NT-proBNP, SDF-1, G-CSF, VEGF, IL-6 and IL-8 were measured on admission and after 1 year and 2-year follow-up. Echocardiography was carried out on admission and after 1 year and 2-year follow-up. Echocardiography was performed on admission and after 1 year and 2-year follow-up.

Conclusion: The peak number of mobilized CD34+/CD45−/CD133−/CD34+ was positively correlated with the peak number of CD34+/CD133−/CD34+ cells as well as higher levels of NT-proBNP. The peak number of mobilized CD34+/CD45−/CD133−/CD34+ was positively correlated with the peak number of CD34+/CD133−/CD34+ cells as well as higher levels of NT-proBNP. The peak number of mobilized CD34+/CD45−/CD133−/CD34+ was positively correlated with the peak number of CD34+/CD133−/CD34+ cells as well as higher levels of NT-proBNP.

5003 Novel Injectable Alginate Biomaterial Attenuates Progressive Infract Expansion and Preserves Left Ventricular Systolic and Diastolic Function Late After Myocardial Infarction

Natalie Landa, Michala S Feinberg, Radka Holbova, Liron Miller, Tel Aviv University; Tel-Hasmer, Israel; Smadar Cohen, Ben Gurion university, Beer-Sheva, Israel; Jonathan Leor, Tel Aviv University, Tel-Hasmer, Israel

Background: Cessation or reversal of progressive left ventricular (LV) dysfunction is a major aim of heart failure therapy. We developed a cross-linked concentrated alginate solution, which...
upon solidification in tissue becomes a hydrogel. The aim of the present study was to test the hypothesis that an injection of this novel alginate solution mimicking the extracellular matrix (ECM) into an old scar tissue, late after myocardial infarction (MI), will provide physical and biological scaffolding, promote tissue rejuvenation and will prevent progressive LV dysfunction.

**Methods and results:** Rats (n=40) were subjected to extensive anterior myocardial infarction (MI). Two months later, the survivors were randomized to injection of cross-linked alginate solution (n=15) or PBS (control, n=12) into myocardial scar. Four months after MI and two months after injection, postmortem morphometric analysis and histological examination of the hearts revealed that alginate solution injection promoted increased neovascularization and increased vessel density (106±2 vs 43±9 mm2; p<0.0001), increased scar thickness (0.73±0.27 cm vs 1.63±0.31 cm; p=0.01) and significantly reduced expansion index (0.58±0.08 vs. 1.05±0.26; p=0.0001), as compared with controls. Serial echocardiography studies showed that alginate solution injection attenuated the progressive deterioration in LV fractional shortening, as compared with control (12±6% vs. 37±9%; p<0.05). Furthermore, while control animals developed restrictive LV filling pattern, as assessed by Doppler echocardiogram, diastolic function improved in alginate-treated hearts (E/A wave ratio of 1.73±0.09 for control vs. 3.34±0.5 for control; p<0.0001). Conclusions: Our work shows, for the first time, that injection of an in situ gelification of cross-linked alginate into an old scar tissue provides physical and biological scaffolding, and preserves LV systolic and diastolic function. Our work enables a minimally invasive, catheter-based, acellular option to repair old scar tissue and to prevent heart failure.
fluorescence and confocal microscopy to examine the T-tubular localization of DAP components in the myocardium of F1B (control) and T02 hamsters at 8–15 wks of age. These components included: β3-dystroglycan (β3DG), dystrophin, δ- or αSG and caveolin-3. Sarcollemal β3DG was observed at high levels in F1B and T02 groups. An increase in T-tubular hypertyrophy and β3DG T-tubular localization was observed in the myocardium of 12–15 wk hamsters. This translocation of β3DG co-localized with the T-tubular markers, DHR 1-9 and the mediator of T-tubulogenesis, Bin1. The T-tubular localization of these markers was not altered in the F1B or T02 groups. These events corresponded to the development of chamber dilatation, wall thinning, calcifications and fibro-necroses in the 12–15 wk T02 groups. T-tubular hypertyrophy and increased phosphorylation was observed in the 8–10 wk T02 or 8–15 wk F1B groups. The observed loss of β3DG was accompanied by a loss of αSG and dystrophin from myocardial T-tubules in the T02 groups. Myocardial T-tubular localization of caveolin-3 was not altered in the F1B or T02 groups. The interaction of β3DG and Bin1 in the 15 wk T-tubular group was very similar to that observed in the F1B group and this suggests an association of Bin1 with β3DG, presumably via the SH3 domain of Bin1 and the proline rich, C-terminal region of β3DG.

Discussion: This study suggests that the T-tubular interaction of β3DG with Bin1 in the 102 group initiates T-tubular hypertyrophy, potentially by reversing an attenuation of T-tubular growth in mature adult hearts. T-tubular hypertyrophy might contribute to increased diffusion of ion fluxes, disrupting physiologic calcium handling and initiating the development of dilated cardiomyopathy.
activity of the NHE-1, a causal link between the decrease in the NHE-1 expression and the protective effect of PDE5A inhibition may be suggested. 

**TABLE. LVDD and LVSd: LEFT VENTRICULAR DIASTOLIC AND SYSTOLIC DIAMETER RESPECTIVELY.**

<table>
<thead>
<tr>
<th></th>
<th>LVM (mg)</th>
<th>CSA (μm²)</th>
<th>FS (%)</th>
<th>dP/dtL/mmHg/s</th>
<th>dP/dtP/mmHg/s</th>
<th>LVdD (mmHg)</th>
<th>LVsd (mmHg)</th>
<th>BNP (pg/mL)</th>
<th>NHE-1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>655±25</td>
<td>181.8±0.8</td>
<td>45±3.2</td>
<td>675±987</td>
<td>89±6±7</td>
<td>6.0±3.3</td>
<td>3.4±0.2</td>
<td>166±32</td>
<td>267±11</td>
</tr>
<tr>
<td>n=5</td>
<td>n=2</td>
<td>n=7</td>
<td></td>
<td>n=6</td>
<td>n=6</td>
<td>n=6</td>
<td>n=7</td>
<td>n=6</td>
<td>n=5</td>
</tr>
<tr>
<td>MI+5</td>
<td>618±40</td>
<td>162.0±3.4</td>
<td>46.4±4</td>
<td>5501±800</td>
<td>112.1±2</td>
<td>6.2±0.2</td>
<td>2.2±0.2</td>
<td>75.1±14</td>
<td>179.19</td>
</tr>
<tr>
<td>n=4</td>
<td>n=6</td>
<td>n=5</td>
<td></td>
<td>n=4</td>
<td>n=4</td>
<td>n=4</td>
<td>n=4</td>
<td>n=4</td>
<td>n=2</td>
</tr>
</tbody>
</table>

*Indicates P<0.05 vs MI.

**Regulation Of Mitochondrial Biosynthesis Is By The Serine Threonine Kinase, AKT3 In Primary Endothelial Cells Gary L. Wright, Juanita Eldridge, Kelley M. Arggraves, Lina M. Obeid, Robin C. Muise-Helmericks, Medical University of South Carolina, Charleston, SC**

Precise mechanisms regulating angiogenesis remain to be delineated, it is generally accepted that VEGF is the main inducer of angiogenesis, promoting endothelial cell (EC) proliferation, migration and tube formation. AKT3 is a member of the Akt family of serine threonine kinases of which there are three, Akt1, Akt2 and Akt3. The delineation of the distinct functions of these three kinases is only beginning to be determined and it has been widely assumed that these kinases are functionally redundant. We have uncovered a novel VEGF signaling pathway that leads to the regulation of AKT3 through the sphingosine-1-phosphate (S1P) receptor, Edg3, a G-protein coupled transmembrane receptor involved in the regulation of angiogenesis. This pathway is independent of other Akt family members. Gene blocking has defined a discrete role for AKT3 downstream of VEGF signaling, namely, the control of mitochondrial biogenesis. RNAi inhibition of AKT3 expression, but not Akt1 inhibition, results in a decrease in mitochondrial gene expression, decreased mitochondrial respiration, and a perinuclear mitochondrial distribution. Stimulation of EC by VEGF results in an increase in total mitochondrial mass per cell, indicating a growth factor induced increase in mitochondrial biogenesis. This increase is blocked by RNAi directed against Akt3. This signaling pathway may then be important in coordinating mitochondrial biogenesis with cellular energy demands as they relate to processes such as angiogenesis. Potential downstream targets of the Akt3 kinase will be discussed.

**Late-Breaking Basic Science: Arrhythmia Mechanisms and Cellular Signaling**

**Mutant Caveolin-3 Alters Cardiac Sodium Channel Function and is Associated with Congenital Long QT Syndrome**

Matteo Vatta, Baylor College of Medicine, Houston, TX; Michael J Ackerman, Mayo Clinic College of Medicine, Rochester, MN; Jonathan C Makiecki, University of Wisconsin, Madison, WI; David J Tester, Mayo Clinic College of Medicine, Rochester, MN; Bin Yu, Ravi C Baijajlaggi, Jason D Foell, Timothy J Kemp, University of Wisconsin, Madison, WI; Jeffrey A Towbin, Baylor College of Medicine, Houston, TX

**Background:** Mutations in the alpha subunits of potassium channels and sodium channels account for approximately 70% of congenital long QT syndrome (LQTS, LQT1–3) while only 1% of LQTS are due to mutations involving kainonyquin or potassium channel beta subunits (LQT4–6). Recent evidence shows that SCN5A colocalizes with caveolae and that caveolae acts beta adrenergic transduction. Caveolae are specific omega-shaped microdomains of the sarcolemma that are organized by the structural protein caveolin, and are critically involved in cell signaling. We hypothesize that caveolin-3 (CAV3), the major component of caveolae in striated muscle, may be implicated in modulating the cardiac SCN5A-encoded sodium channel activity. We therefore speculated that CAV3 may be a candidate gene for LQTS. **Methods:** Using denaturing high performance liquid chromatography and direct DNA sequencing, open reading frame/splice site mutational analysis was performed on CAV3 on 84 unrelated patients referred for LQTS genetic testing. Caveolin-3 mutations were engineered by site-directed mutagenesis and functionally phenotyped by transient heterologous expression into stable expressing SCN5A cell lines. **Results:** Overall, 6 non-synonymous single nucleotide polymorphisms were identified in 17 patients (2%), including the common black-specific polymorphisms, G565S and C722W previously linked to limb-girdle muscular dystrophy (LGMD), having heterozygote frequencies of 25% and 2% respectively in this study. The other 4 variants were absent in more than 200 controls, were conserved across species, and were present in 5 subjects (0.6%) lacking mutations in all known LQTS-associated genes. Co-expression of caveolin-3 mutations with SCN5A resulted in a 4-6 fold increase in late sodium current compared to expression of wild-type caveolin-3 in HEK293 cells. This is consistent with sustained sodium current observed in prolonged QT interval in patients with SCN5A alterations. **Conclusions:** We provide the first molecular and functional evidence implicating caveolin-3 in the pathogenesis of LQTS. LQTS-caused caveolin-3 mutations perturb sodium channel function and elicit an LQTS phenocopy by producing a gain-of-function increase in late sodium current.

**Cardiac-Specific Loss of N-cadherin Leads to Alteration in Connexins with Conduction Slowing and Arrhythmogenesis**

Jilen Li, Igor Kostetskii, Center for Research on Reproduction and Women’s Health, University Pennsylvania School of Medicine, Philadelphia, PA; Vickas V Patel, Department of Medicine, University Pennsylvania School of Medicine, Philadelphia, PA; Yanning Xiong, Center for Research on Reproduction and Women’s Health, University Pennsylvania School of Medicine, Philadelphia, PA; Cindy Yu, Gregory E Morley, The Leon H. Charney Division of Cardiology, New York University School of Medicine, New York, NY; Jeffrey D. McKerrnent, Division of Molecular Cardiovascular Biology, Children’s Hospital Medical Center, Cincinnati, OH; Ganu L. Radice, Center for Research on Reproduction and Women’s Health, University Pennsylvania School of Medicine, Philadelphia, PA

The remodeling of ventricular gap junctions, as defined by changes in size, distribution, or function, is a prominent feature of diseased myocardium. However, the regulation of assembly and maintenance of gap junctions remains poorly understood. The cell adhesion molecule, N-cadherin, mediates strong cell-cell adhesion via linkage to the actin cytoskeleton. N-cadherin has been implicated in the assembly of gap junctions in cultured cardiomyocytes. To determine whether N-cadherin function is required for maintaining gap junctions in the working myocardium, we utilized a floxed N-cadherin gene in conjunction with a cardiac-specific tamoxifen-inducible Cre transgene. The mutant animals appeared healthy and active until their sudden death about two months after deleting N-cadherin from the heart. Ambulatory ECG monitoring captured the abrupt onset of spontaneous ventricular tachycardia confirming that the deaths were arrhythmic in nature. Electrophysiologic analysis revealed abnormal conduction in the ventricles of mutant animals including diminished QRS complex amplitude consistent with loss of electrical coupling in the myocardium. Hypophosphorylation of CaV3 preceded a significant reduction in gap junction proteins, Cx43 and Cx40, in N-cadherin depleted cardiomyocytes. Real-time PCR demonstrated an increase in Cx43 mRNA levels indicating that N-cadherin regulates Cx43 expression posttranscriptionally. Altered connexin function resulted in decreased ventricular conduction velocity as determined by optical mapping. Our data suggest that perturbation of the N-cadherin/catenin complex in heart disease may be an underlying cause leading to establishment of the arrhythmogenic substrate by destabilizing gap junctions at the cell surface.

**Atrial NAD(P)H Oxidase Activity Predicts The Development Of Atrial Fibrillation After On-pump Coronary Artery Bypass Graft Surgery**

Young M Kim, University Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford, Oxford, United Kingdom; Hassan Katib, Chand N Rathurangani, Ravi Pillai, Department of Cardiotrohca, John Radcliffe Hospital, Oxford, United Kingdom; Keith Channon, Barbara Casadei, University Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford, United Kingdom

**Background:** Atrial fibrillation (AF) is a frequent complication following coronary artery bypass graft (CABG) surgery and recent evidence associates enhanced human atrial NAD(P)H oxidase...
activity with chronic AF. To determine whether enhanced intrinsic atrial oxidative stress was also involved in the pathophysiology of post-operative AF, we investigated whether atrial NAD(P)H oxidase activity could predict the occurrence of AF following CABG surgery. Methods and Results: We prospectively measured atrial NAD(P)H oxidase activity in the right atrial appendage (RAA) obtained before cardiopulmonary bypass (CPB) of 172 patients undergoing first time on-pump CABG surgery using SuM lucigenin-enhanced chemiluminescence. In a sub-group of patients we compared both basal and NADPH-stimulated superoxide (O$_2^-$) release in samples of RAA obtained before CPB and 1–3 minutes after reperfusion which revealed no differences, gained data from 23 patients in RLU/sec/mg protein: pre- v. post-CPB basal 97 ± 13 v. 105 ± 22, P = 0.7 and pre- v. post-CPB NADPH stimulated 7224 ± 690 v. 7693 ± 684; P = 0.6). Thiobarbituric acid reactive substances and carbonyls were measured in pre and post-operative blood samples to assess both lipid and protein oxidation in the plasma. Patients were genotyped for the C242T polymorphism of the p22phox subunit of NAD(P)H oxidase using standard RFLP techniques. 74 patients (43%) developed AF post-operatively and NADPH-dependent atrial O$_2^-$ release was significantly higher in this group compared with patients remaining in sinus rhythm (SR), (in RLU/sec/mg protein: 4374 ± 162 v. 3809 ± 144 (n=98 in SR), P = 0.03). Multivariate logistic regression indicated that NADPH-dependent atrial O$_2^-$ generation was the only significant predictor of post-operative AF (OR, 2.64; 95% CI, 1.02–7.58; P = 0.05) independent of age, β-blocker use and other risk factors. Plasma markers of oxidative stress and the C242T polymorphism of p22phox had no bearing on the occurrence of post-operative AF. Conclusions: These findings suggest that atrial NAD(P)H oxidase activity, but not plasma markers of oxidative stress, is a main independent predictor of post-operative AF in patients undergoing CABG surgery.

**5028**

**Impaired Angiogenesis In β2 Adrenergic Receptor (ar) Knock Out Mice**

Michele Ciccarelli, Federico II University Naples, Napoli, Italy; Rui-Hai Zhou, Walter J Koch, Thomas Jefferson University, Philadelphia, PA; Bruno Trimmer, Federico II University, Naples, Napoli, Italy; Andrea Eckhart, Thomas Jefferson University, Philadelphia, PA; Guido Iaccarino, Federico II University, Naples, Napoli, Italy

We have recently shown in rats that beta 2 adrenergic receptor gene delivery by adenovirus (ADβ2AR) to the endothelium ameliorates the angiogenic response after hindlimb (HL) ischemia. To point out the role of endothelial β2AR in neangiogenesis, we removed the right common femoral artery in 6 twelve-week old b2AR knockout (b2KO) mice and 6 wildtype controls (WT) and evaluated for 15 days blood flow (BF) through both ischemic and non ischemic tibial posterior artery by means of echodoppler probe (100 Mhz, Vevo 770, GE). After 15 days, BF was also assessed by injecting 108 beads into the abdominal aorta and measuring the release of dye in digested muscles of the ischemic and non ischemic HLs. We also counted the capillary (~10 micron diameter) per muscle fiber density on tibial muscle sections stained with CD31 antibody. β2KO and WT presented similar doppler profiles in the non ischemic HL (max, mm/sec/b2KO: 64 ± 5; WT: 83 ± 5, n.s.) BF was not assessable in the ischemic HL one day after surgery in both strains. On day 15, WT showed partial restoration of doppler Vmax which was 75 ± 15% of the contralateral non ischemic HL. This was significantly less in b2KO (5 ± 5%, p < 0.02 vs WT). The impaired angiogenic response of b2KO vs WT was also illustrated by a greater occurrence of blistersing of the ischemic HL (83% vs 17%, p < 0.05, chi squared), reduced dyed microsphere dilution (24 ± 15% vs 59 ± 15%, p < 0.05), and capillary density. In another group of 3 b2KO, at the time of the femoral artery resection we performed intra-arterial ADβ2AR delivery (10^6 PFU) in the ischemic HL, increasing 45% βAR density as compared to b2KO mice (fnl/mg; b2KO: 31 ± 6; b2KO + ADβ2AR: 133 ± 41, p < 0.05). By day 15, BF (720 ± 20%, ns vs WT) and capillary density were increased while blistering was reduced (0%, ns vs WT). Our results suggest that vascular β2ARs play a major role in the regulation of the angiogenic response to ischemia.

**5029**

First Molecular Evidence that Inositol Trisphosphate Signaling Contributes to Infarct Size Reduction with Ischemic Preconditioning

Karin Przyklenk, Michelle Maynard, Peter Whittaker, UMMS Medical School, Worcester, MA

Stimulation of G-protein-coupled receptors, followed by production of diacylglycerol (DAG) and activation of protein kinase C, have been identified as key mechanisms contributing to infarct size reduction with preconditioning (PC). Production of DAG is, however, accompanied by the 'in parallel' generation of the second messenger inositol 1,4,5-trisphosphate (IP3). While IP3 signaling (generation of IP3, followed by binding to IP3 receptors) is well-recognized to participate in the regulation of calcium homeostasis, the role of IP3 signaling in PC-induced cardioprotection is, at present, unknown. Methods: To investigate this issue, hearts were harvested from: (1) adult Itpr-1/- mice displaying spontaneous mutation of the IP3 receptor gene and, thus, reduced expression of IP3 receptor mRNA and protein (heterozygotes were used, as homozygotes die soon after birth); and (2) age-matched adult C57BL/6J mice (parent strain). All hearts were buffer-perfused in Langendorff mode, and randomized to receive: 2 5-min episodes of PC ischemia; pre-treatment with D-myo-IP3 (sodium salt of native IP3: 6 μM); or no intervention (controls). After the treatment phase, all hearts underwent 30 min global ischemia + 2 h reperfusion, and infarct size was delineated by tetrazolium staining. Results: Significant cardioprotection was seen with both PC and exogenous D-myo-IP3 in the C57 parent strain (p < .01 vs C57-Controls). There were no differences in baseline hemodynamics or heart weight/body weight ratios in Itpr-1/- vs C57BL/6J mice (p-values > .15). However, both PC and D-myo-IP3 failed to limit infarct size in IP3 receptor null mice. Post-hoc experiments further revealed that, even when the PC stimulus was amplified to 4 5-min cycles of ischemia, Itpr-1-/- mice remained refractory to PC-induced protection (infarct size: 63%). Conclusion: These data provide novel molecular evidence that IP3 signaling contributes to infarct size reduction with preconditioning.
Late-Breaking Basic Science Abstracts From the American Heart Association's Scientific Sessions 2005, Dallas, Texas, November 13-16

doi: 10.1161/01.RES.0000196463.09130.2b

\textit{Circulation Research} is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/97/11/1199.citation

\textbf{Permissions:} Requests for permissions to reproduce figures, tables, or portions of articles originally published in \textit{Circulation Research} can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

\textbf{Reprints:} Information about reprints can be found online at:
http://www.lww.com/reprints

\textbf{Subscriptions:} Information about subscribing to \textit{Circulation Research} is online at:
http://circres.ahajournals.org/subscriptions/