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ERK1/2 Regulate the Balance between Eccentric and Concentric Growth of the Heart

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Introduction: The myocardium undergoes cellular and chamber remodeling as a means of maintaining cardiac output in response to increased workload. An increase in cardiac afterload typically produces concentric hypertrophy characterized by an increase in cardiomyocyte width, while volume overload results in eccentric growth, characterized by cellular elongation and addition of sarcomeres in series. Concentric and eccentric growth likely result from orchestrated activation of specific intracellular signaling pathways.
Material and Methods: To determine the role of extracellular signal-regulated kinases 1/2 (ERK1/2) in regulating the cardiac hypertrophic response we used mice lacking all ERK1/2 protein in the heart by crossing Erk1-/- mice with Erk2fl/fl targeted mice and a cardiac Cre-recombinase expressing line. We also studied mice expressing activated MEK1 in the heart to induce ERK1/2 signaling and used mechanistic experiments in cultured myocytes to assess cellular growth characteristics associated with this signaling pathway.
Results: While loss of all ERK1/2 from the heart did not block the cardiac hypertrophic response per se, it did dramatically alter how the heart grew. For example, adult myocytes from hearts of Erk1-/-;Erk2fl/fl-Cre mice showed preferential eccentric growth (lengthening) while myocytes from MEK1 transgenic hearts showed concentric growth (width increase). Isolated adult myocytes acutely inhibited for ERK1/2 signaling by adenoviral gene transfer showed spontaneous lengthening while infection with an activated MEK1 adenovirus promoted constitutive ERK1/2 signaling and increased myocyte thickness.
Conclusions: Taken together these data demonstrate that the ERK1/2 signaling pathway uniquely regulates the balance between eccentric and concentric growth of the heart. Thus, the MEK1-ERK1/2 pathway may be the first identified signaling pathway capable of specifically directing the mode of cardiomyocyte hypertrophy.

Establishing a System for Viral Delivered Gene Therapy as a Treatment for CPVT

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Catecholaminergic polymorphic ventricular tachycardia (CPVT), a lethal human arrhythmia provoked by exercise or emotional stress. Beta-adrenergic blockers are the therapy of choice for human CPVT but they only achieve complete arrhythmia control in less than 50% of cases. Therefore alternative therapy is obligated. Gene therapy has become atractive treatement for genetic disorders.

We established a new delivery system in CASQ2 knock-out mice. AAV2 recombinant plasmids were generated by cloning the gene of interest into pAAV-IRES-hrGFP vector. The expression plasmid was co-transfected into the AAV-293 cells with pHelper (carrying adenovirus-derived genes) and pAAV-RC (carrying AAV-2 replication and capsid genes), which together supplied all the transacting factors required for AAV replication and packaging in the AAV-293 cells. Viral particles were purified from crude cell lysates and first examined for infectivity and transgene expression in neonatal rat cardiomyocytes by GFP fluorescence. Based on the success of the viral infection technique, we succeeded to inject the vector to a murine model of recessively-inherited CPVT. Viral particles were concentrated and injected into the left ventricle of 16-week-old mice. GFP expression in various tissues was observed using cryo sections. Cardiac muscle and lung tissues were infected with AAV and GFP and were seen in the sections 4 weeks after injection ($p=0.05$). Other tissues that were examined for infection did not show any GFP expression (liver and spleen). Control mice had no GFP expression in any of the tissues ($n=4$). This method has proven as a good delivery method for viral particles to the heart muscle and is being used now for different vectors that can attenuate the sevirity of the arrhythmia.

PPARA and NRF2 Polymorphisms are Associated with Exercise Capacity in Trained Heart Failure Patients

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Background: Peroxisome proliferator-activated receptors (PPARs) and nuclear respiratory factors (NRFs) are associated with abnormal substrate metabolism and oxidative phosphorylation in heart failure.

Aims: the aim of the present study was to investigate whether polymorphisms in the PPARA (rs1800206, rs4253778), PPARD (rs2016520) and NRF2 (rs12594956) genes have an impact on exercise tolerance at baseline and in response to exercise training in HF patients.

Methods: A total of 61 patients with HF completed a 6-month exercise-training programme. Exercise tolerance (METs) was assessed before and after exercise training. Polymorphisms were detected with restriction fragment length polymorphism analysis.

Results: At baseline, there were no differences between genotypes in exercise tolerance. In contrast, training-induced increase in exercise tolerance was more pronounced in PPARA 162Val carriers than in 162Leu homozygotes (0.88 ± 0.81 METs vs. 0.41 ± 0.51 METs, P = 0.032). Moreover, exercise training increased exercise tolerance in NRF2 AA (from 3.9 ± 1.3 to 4.5 ± 1.5 METs, P<0.05) and AC genotype carriers (from 4.0 ± 1.0 to 4.6 ± 1.3 METs, P<0.05), but not in G allele homozygotes (from 4.5 ± 0.7 to 4.7 ± 0.8 METs, n.s). No differences were found for the other polymorphisms.

Conclusions: This study suggests that PPARA Leu162Val and NRF2 A/C SNPs are associated with the training response in heart failure patients.

Vascular Inflammation and Endothelial Dysfunction in Fracture Healing

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Several in vitro studies showed that angiogenesis is involved in the first stages of bone healing but it has not been demonstrated in vivo.
Methods: 20 young healthy patients that were admitted with traumatic long bone fractures were studied for vascular responsiveness and markers of inflammation (osteopntin, E-selectin and vascular endothelial growth factor) in the first week of the study and after 1 month.
Results: Patients (13 men, 7 women, mean age of 40.63±11.13 years old) had severe endothelial dysfunction [FMD% of -2.5%±7.5%]. Neither of them was smoking, had diabetes mellitus, hypertension, or hypercholesterolemia, and their fractures were caused by a traumatic event. Healthy volunteers (10 men and 10 women, mean age of 41.86±11.34 years old) that had no risk factors for cardiovascular disease serevd as the control group. Their endothelial function was normal [FMD% of +15.0±6.5%] with a significant difference (p=0.0001) compared with the patients' FMD%. High osteopontin levels were found in the first week, but after 1 month levels decreased towards normal [from 77.19±52.10 ng/ml to 30.73±17.49 ng/ml; p=0.001]. E-selectin levels were high in the first week, then decreased [from 28.15±14.88 ng/ml to 21.50±10.75 ng/ml; p=0.01]. VEGF levels were high in the first week of the fracture and stayed high without change during the first month [from 449.81±191.38 pg/ml to 400.81±323.19 pg/ml; p=0.5].
Conclusions: Long bones' fractures caused intense vascular and inflammatory responses represented by high levels of osteopontin, E-selectin, and VEGF. In vivo measurements have demonstrated severe endothelial dysfunction that could support the idea of recruitment of the vascular system to build new blood vessels that will support bone regeneration.

Late Apoptosis after Radiofrequency Ablation in the Heart Muscle

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Background: Radiofrequency (RF) catheter ablation has become routine treatment of most cardiac arrhythmias. During the last decade complex ablation involves massive ablation mass and previously negligible effect has become significant. Apoptosis contributes to wanted effect in oncologic radiofrequency ablation in different tissues. The purpose of this study was to evaluate the timing and the extent of apoptosis after catheter ablation in a rat-model. Methods: Epicardial RF ablation was performed in 35 rats, 3 moths old, 250-325 gram weight. The RF energy was delivered for 20 seconds on the epicardial surface of the left ventricle at 50-55 C° and 50 Watts power. The rats were scarified after 0, 0.5, 3, 12 and 24 hours and 7 and 14 days (5 rats in each group). Total caspase and activated caspase was determined with Western Blot and apoptosis was quantified with TUNEL staining.
Results: There was a significant increase in the 17k activated caspase level and the apoptotic bodies count increased gradually from 0.7±0.9 at 0 time to 47.6±42.6 after 14 days. The 17k caspase/actin increased from 0.09 at 0 time to 0.47 after 14 days. The figure shows TUNEL staining after 14 days.
Conclusions: Apoptosis develops gradually with maximal presentation after 14 days. This phenomenon may explain the late complications after extensive and complex RF ablation (atrial fibrillation and ventricular tachycardia ablation).

Nitric Oxide is Involved in Cardiac Resistance to Ischemic Injury in Calorically Restricted Mice

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Caloric restriction (CR) exerts a variety of health benefits in mammals including protection against ischemic damage in the heart. Adiponectin, an adipokine that increases under starvation and senses the cellular energy status, has been implicated in cardioprotection induced by CR. Factors such as pAMPK, eNOS (endothelial Nitric Oxide Synthase) and STAT3 have been previously linked to adiponectin-induced cardioprotection. CR-induced cardiprotection has been studied so far in ex vivo and in vitro models for cardiac ischemic stress, but rarely in in vivo models. Here we studied this issue after ligation in vivo of left descending (LAD) coronary artery. We compared C57Bl mice fed 65% of their normal food intake for 2 weeks to control mice fed ad libitum. Cardiac functions were tested and blood and tissue samples were taken before and 24 hours after ligation.

The results demonstrated that CR significantly improved left ventricular functions. CR also reduced infarct size, inflammatory cytokines, the number of neutrophils and apoptotic cells and levels of caspase 3 and pJun, suggesting the involvement of both inflammation and apoptosis in the damage to the cardiac tissue. CR also significantly increased (45%) the levels of adiponectin in the serum of non-treated CR mice. In addition, phosphorylation of AMPK, eNOS, STAT3, and AKT as well as the levels of VEGF were higher in the ischemic heart of CR mice. The difference in cardiac functions between LAD-treated CR and control mice was abrogated after pretreatment with L-NAME, an inhibitor of NO formation by NOS.

These results show that short-term CR can confer resistance against ischemic damage in the heart using, for the first time, an in vivo model for myocardial infarction. Adiponectin and several signaling factors correlated with this resistance. The finding that L-NAME could abrogate the improvement in ventricular functions indicates that NO is involved in CR-induced cardioprotection.

Upregulation of Isl1, A Transcription Factor Mastering Embryonic Cardiogenesis, in AMI Patients

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Background: The transcription factor Isl1 plays a crucial role in the embryonic development of the myocardium and its vasculature. While a population of cardiac Isl1+ adult stem cells was found , their role in ischemic heart disease is yet unknown.

Objective: The aim of the current study was to examine whether human hematopoietic stem cells express Isl1, and whether Isl1 expression is up regulated in patients suffering from acute myocardial infarction, or from chronic atherosclerotic disease.

Methods: Patients diagnosed with acute myocardial infarction, three vessel atherosclerotic disease and their referent population, were matched for gender, age and atherosclerosis risk factors. Mononuclear cells were extracted from whole blood samples taken from all patients. Numbers of hematopoietic stem cells expressing Isl1 were studied by FACS analysis and by real time PCR.

Results: We show for the first time an expression of Isl1 within a distinct population of human circulatory hematopoietic stem cells. Moreover, an abrupt increase in Isl1 expression was demonstrated in patients experiencing acute myocardial infarction compared to patients with normal coronaries.

Conclusions: These data imply to up regulation and mobilization of Isl1+ stem cells under the setting of acute myocardial infarction.

Endothelial Dysfunction is Reversible in Helicobacter Pylori Positive Subjects

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Background: Epidemiological studies have shown an association between Helicobacter Pylori (HP) infection and atherosclerosis. Although epidemiological studies have suggested a relationship between HP infection and atherosclerosis it is not clear whether HP eradication will improve vascular inflammation, reverse endothelial damage and prevent future cardiovascular events.

Methods: 31 subjects (16 males, 15 females; 50.8±16.7 y) with dyspepsia were diagnosed as HP positive using antral histopathologic evalutaion. 11 subjects with dyspepsia (5 males, 6 females; 55.4±9.3 y) that were negative to HP served as controls.

Vascular measurements (ABI and endothelial function [FMD%]) were done on entry to the study and 3 months afterwards. HP+ subjects were treated with the triple therapy.

Results: HP+ subjects had severe endothelial dysfunction (FMD% of -1.26±8.4%) that improved significantly after treatment (8.4±9.0%) (p=0.001). HP- subjects had endothelial dysfunction (FMD% of 1.9±9.7%) that was not improved (5.6±8.3%) (p=0.41). Neither HP+ nor HP- subjects changed their ABI (p=0.46) and (p=0.51).

Conclusions: HP eradication can improve endothelial dysfunction significantly and may prevent atherosclerosis and future cardiovascular events.

	HP+	HP -
FMD % P-value	Before Rx 3 m after -1.26±8.4 8.4±9.0 0.001	Before Rx 3 m after 1.9±9.7 5.6±8.3 0.41
ABI P-value	1.2±0.2 1.2±0.2 0.46	1.2±0.2 1.3±0.2 0.51

< HP+ HP - Before Rx 3 m after Before Rx 3 m after FMD % -1.26±8.4 8.4±9.0 1.9±9.7 5.6±8.3 P-value 0.001 0.41 ABI 1.2±0.2 1.2±0.2 1.2±0.2 1.3±0.2 P-value 0.46 0.51