Late-Breaking Basic Science I

Validation Of Circulating MicroRnas As Biomarkers In Heart Failure In Two Large Independent Cohorts
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Background: Small studies suggested circulating microRNAs (circmiRs) as biomarkers for Heart Failure (HF). However, standardized approaches and quality assessment are not established, and results have been inconsistent, with little replication between studies. We aimed to implement quality standards to enable comparison between cohorts and assess which circmiRs may add prognostic information in HF.

Methods: We measured 15 circmiRs in two independent cohorts totaling >2000 subjects. Cohort I (Barcelona) comprised of n=843 chronic HFpEF patients. Cohort II from Detroit comprised of n= 1384 chronic HF patients (892 HFrEF, 492 HFP EF). Each sample was measured in duplicate, and normalized to an abundant and stable circmiR (mir-486-5p). Algorithms were installed to define each circmiR measurement as "valid", "unmeasurable" or "invalid". This allowed inclusion of valid low-level circmiR measurements while reducing noise from false amplification signals. Results: In general, between 20–40% of measurements were "invalid", while mir-499b_5p and -208a were "unmeasurable" in the majority of patients in both cohorts. Higher levels of circmiRs-133b, -1254, -622, -208a and -499a_5p were significantly associated with risk of death in both cohorts, with hazard ratios ranging from 1.103 to 1.365 per log fold (p-values 0.001 to 0.05). However, adding these circmiRs to established predictors (age, renal function and NT-proBNP) did not further augment the c-stat beyond 0.71 (cohort I) or 0.78 (cohort II). Conclusion: We developed a stringent quality assessment for circmiR testing, and for the first time robustly validate the association of circmiRs 208a, -499a_5p, -133b, -1254 and -622 with risk of death in HF patients. However, circmiR levels failed to incrementally improve prognostication offered by current biomarkers, possibly due to the relative high number of invalid measurements. This highlights the shortcomings of current PCR-based technology. Novel technologies under study that improve signal-noise ratios may enhance the prognostic performance of circmiRs.

Key Words: MicroRNA; Heart Failure; Biomarkers

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Key Words: MicroRNA; Heart Failure; Biomarkers

Validation Of Circulating MicroRnas As Biomarkers In Heart Failure In Two Large Independent Cohorts

Downregulation Of TNF-alpha by In Vivo CRISPR-Cas9 Genome Editing to Treat Cardiac Fibrosis in Diabetic Mice
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Introduction: Cardiac fibrosis is the leading cause of cardiac dysfunction in patients with diabetes, renal currently, there is no effective treatment to prevent the onset of this condition. Inflammation is a hallmark of diabetes associated with heart diseases and TNF-alpha is a key factor involved in inflammation. We tested whether downregulation of TNF-alpha could prevent cardiac fibrosis in diabetic mice. Methods and Results: Diabetes was induced in C57BL/6 mice by injecting streptozotocin (STZ, 55 mg/kg/d) for 5 days. Citrate buffer was injected as control. One week after STZ injection, blood glucose levels significantly increased in diabetic C57BL/6 mice (336 ± 26 mg/dl) compared to control (108 ±21 mg/dl). The increased blood glucose was accompanied by increased cardiac fibrosis (MassonTricrime), and increased TNF-alpha mRNA and protein expression in heart tissue. To assess whether genome editing using a clustered regularly interspersed short palindromic repeats (CRISPR/Cas9) system can efficiently introduce loss-of-function mutation into the endogenous TNF-alpha gene in vivo, we constructed lentivirus expressing CRISPR-Cas9 and a CRISPR guide RNA targeting TNF-alpha. The lentivirus particles were transfected into the mouse myocardium by direct intramyocardial injection at the time of initial induction of diabetes. Within 5 days of administration of the lentivirus, the mutagenesis rate of TNF-alpha in the heart was as high as >50% by surveyor assay. No off-target mutagenesis was detected in other tissues such as lung and liver. The CRISPR/Cas9 based knockdown approach resulted in decreased cardiac fibrosis (MassonTricrime), accompanied by reduced expression of TNF-alpha in heart assessed by PCR, Western blot and immunostain- ing. No significant change of the inflammatory cytokines including IL-6 was observed in the hearts. Furthermore, the CRISPR/Cas9 approach did not alter cell proliferation (BrdU staining) and cell survival (TUNEL assay) in heart. Conclusion: This study demonstrates that inflammation contributes to cardiac fibrosis via TNF-alpha signaling pathway, and suggests that diabetes related cardiac fibrosis may be prevented by downregulation of TNF-alpha via novel CRISPR-Cas9 approach.

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Key Words: CRISPR/Cas9; Inflammation; cardiac fibrosis; TNF-alpha

Cardiac-Specific Knock-out of the Histone Methyltransferase Smyd1 Leads to Coordinated Downregulation of Energy Metabolism
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Background: Global regulatory mechanisms controlling the complex metabolic remodeling which occurs during the onset of heart failure are still poorly understood. However, recent advances have suggested a synergistic link between epigenetic regulation and cellular metabolism, although how this interplay is carried out in the heart is largely unknown. Our recent analyses of chromatin binding proteins differentially regulated during cardiac hypertrophy and failure identified the histone methyltransferase Smyd1. To determine the role of Smyd1 in the adult myocardium we generated inducible, cardiac-specific Smyd1 knockout (Smyd1-KO) mice, which exhibit cellular hypertrophy, chamber remodeling and cardiac dysfunction. In addition, bioinformatics analysis of transcripts differentially expressed in Smyd1-KO heart tissue, before heart function declined, showed that cellular metabolism was the most perturbed biological process in these animals and suggests that Smyd1 may be a key regulator of energy metabolism.

Methods and Results: To investigate this hypothesis, we carried out metabolite and gene expression analysis of Smyd1-KO heart tissue to comprehensively characterize the abundance of energetics-related transcripts and metabolites in these animals. Interestingly, our results revealed systemic dysfunction in energy substrate metabolism characterized by downregulation of fatty acid ß-oxidation (observed as a decrease in PPAR-a, carnitine-palmitoyltransferase I, carnitine transporter OCTN2 and myocardial carnitine content [43% reduction]) and branched-chain amino acid (BCAA) oxidation (observed as a 2-fold accumulation of all BCAAs and the decreased expression of PP2Cm, an key activator of BCAA catabolism). In addition, our results identified a dramatic increase in myocardial lactate and alanine (340% and 170% respectively), concomitant with decreased expression of pyruvate dehydrogenase E1 ß, indicative of glycolytic impairment. Conclusion: Overall, this study identifies a novel role for Smyd1 in regulating energy metabolism in the heart and provides key insights into the epigenetic mechanisms modulating metabolic disorders such as heart failure.


Key Words: Cardiac hypertrophy/failure; Smyd1 (histone methyltransferase); Epigenetics; Cardiac energetics; Metabolomics/Transcriptomics

Circling miR-1 And miR-133b Correlate With Subclinical Myocardial Injury In Breast Cancer Patients Under Doxorubicin Treatment
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Introduction: Myocardial injury is one of the major concerns when doxorubicin (DOX) is chronically administered for several weeks and may lead to cardiomyopathy and heart failure. Recently, circulating microRNAs (c-miRNAs) have been suggested as potential biomarkers of myocardial injury and drug-induced cardiotoxicity. However, the potential c-miRNAs as biomarkers of DOX-induced cardiotoxicity in a clinical setting was not assessed. Therefore, the aim of this study is to evaluate the cardiotoxic effects of DOX on the circulating levels of miR-1, miR-133b, miR-146a, miR-208a, miR-208b and miR-423-5p in breast cancer patients. Methods: In brief, 59 female patients (50.02±8.64 age) received 4 cycles of chemotherapy with cumulative doses of 60 mg/m2 DOX during 12 weeks. Cardiac troponin I (cTnI), LVEF and c-miRNAs were measured before the treat- ment and every 3 weeks after DOX administration. Results: miR-208a and miR-208b
Late-Breaking Basic Science II

A Novel TRPV4-dependent Mechanotranscription Pathway Regulates Cardiac Fibrosis Following Pressure-Overload

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Cardiac fibroblast (CF) differentiation into highly contractile and hypersecretory myofibroblasts (mFibs) is critical for reparative fibrosis following myocardial infarct. However, excessive and remote area fibrosis by mFibs can lead to cardiac dysfunction and eventual heart failure. Recently, we have shown that a mechanosensitive ion channel TRPV4 mediates CF differentiation into mFibs in vitro. In the present study, we investigated the underlying molecular mechanism and the physiological role of TRPV4 during cardiac remodeling following pressure overload (transverse aortic constriction, TAC), in wild type (WT) and TRPV4 knockout (TRPV4KO) mice. We found that TRPV4KO mice exhibited not only improved survival rates compared to WT, but cardiac function analysis showed preserved ejection fraction and fractional shortening in TRPV4 null mice, post-TAC surgeries. Importantly, we found that TAC induced both interstitial and peripheral fibrosis in WT hearts, which was significantly reduced in TRPV4KO hearts. To understand the molecular mechanisms, we isolated CFs from WT and TRPV4KO mouse hearts (mFibs). In vitro, we found that TGF-β1 induced differentiation was completely attenuated in TRPV4KO mFibs compared to WT. Further, TGF-β1 treatment induced activation of Rho in WT mCFs, which was inhibited by pre-treatment with TRPV4 antagonist, AB1519908, suggesting that Rho is downstream of TRPV4 in CF differentiation to mFibs. Further, our recent results found that both TGF-β1 and pharmacological TRPV4 activator GSK1016790A induced the activation of cardiomyocyte-related transcription factor-A (MRTF-A) (nuclear translocation), that may lead to the development of biomarkers to monitor DOX-induced subclinical myocardial injury and heart failure.

Cardiac Fibrosis Following Pressure-Overload


Key Words: microRNA; Cardiotoxicity; Doxorubicin; Myocardial Injury; Breast Cancer

DNA-repair In Cardiomyocytes Is Critical For Maintaining Cardiac Function

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Aims: DNA in every cell is continuously damaged and DNA repair systems are essential for protection from DNA damage induced cancer and aging- related diseases. Here we studied the role of DNA repair in cardiomyocytes in maintaining normal cardiac function. DNA repair-deficient full body Xpg- and cardiomyocyte-specific DNA repair-deficient full body Xpg- and cardiomyocyte-specific DNA repair-deficient full body Xpg- and cardiomyocyte-specific DNA repair-deficient full body Xpg- and cardiomyocyte-specific

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DNA-repair In Cardiomyocytes Is Critical For Maintaining Cardiac Function


Key Words: Mitochondria; Adenergetic; Physical activity and exercise; Mitochondrial energetics; Muscle, cardiac

Physiological Mitochondrial Fragmentation is a Cardiac Adaptation to Increased Energy Demand

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Current paradigms hold that, in the heart, mitochondrial fission and fragmentation are the result of pathologic stresses such as ischemia, are an indicator of poor mitochondrial health, and lead to mitophagy and cell death. However, recent studies demonstrate that

Physiological Mitochondrial Fragmentation is a Cardiac Adaptation to Increased Energy Demand


Key Words: TRPV4; cardiac fibroblast; myofibroblast; mechanical signaling; fibrosis

inhibiting fission also results in cardiac impairment, suggesting that fission is important for maintaining normal mitochondrial homeostasis. As these studies have relied on genetic models of dynamic regulation, the role of fission in normal cardiac physiology is still unclear. In this study, we identify a novel role for mitochondrial fragmentation, as a normal physiologic adaptation to meet the energetic demands of exercise. During acute exercise in mice, there is significant "physiologic" mitochondrial fragmentation in the heart, demonstrated by a 30% increase in mitochondrial number and a 20% decrease in area (electron microscopy). Whereas pathologic fragmentation leads to impaired respiration, physiologic fragmentation increases both basal and maximal respiration by 20% (seahorse oxymetry). Similar to pathologic fragmentation, physiologic fragmentation is induced by translocation of Drp1 to the mitochondria; however, unlike pathologic fragmentation, mitochondrial membrane potential and reactive oxygen species are maintained and regulators of mitophagy (PKNI1, Parkin, LC3) are downregulated. Inhibition of Drp1 during exercise (using either the Drp1-Fis1 inhibitor P110 or the Drp1 GSTase inhibitor mi6iv) prevents mitochondrial fragmentation in the heart, increases glycolytic flux and results in a 40% decrease in exercise capacity, demonstrating the requirement for physiologic mitochondrial fragmentation to meet the energetic demands of exercise. In summary, we have shown that cardiac mitochondrial fragmentation is not limited to pathologic states and can be a component of normal physiologic regulation of mitochondrial function during stresses such as exercise. Whether pathologic mitochondrial fragmentation can be harnessed as a treatment of cardiovascular disease remains to be determined, although some of the cardiovascular benefit of exercise could be mediated by such a mechanism.

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Author Disclosures: Michael Coronado, Giovanni Fajardo, Kim Nguyen, Mingming Zhao, Kristina Bezold Kookier, Gwanyung Jung, Dong-Qing Hu, Suraima Reddy, Erik Sandalov, Aleksandr Stotland, Roberta Gottlieb, Daniel Bernstein; 1Stanford Univ, Stanford, CA; 2Cedars-Sinai Med Ctr, Los Angeles, CA.

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Xpg−/− mice were used to study left ventricular (LV) geometry and function. Methods: Xpg−/− (n=14) and control wildtype (wt, n=14) mice were sacrificed at the age of 16 wks. LV geometry and function were measured and hypertrophy marker genes were determined by qPCR. Superoxide (O2−) production and NOX activity were studied using lucigenin-enhanced chemiluminescence. NO synthesis (NOS) and NADPH Oxidase (NOX) - dependent O2− production were assessed with L-NAME and VAS2870. LV Xpg−/− and wt mice (both n=4), molecular imaging was performed to determine apoptosis in the in vivo heart using near infrared fluorescent Annexin V probe. Results: Xpg−/− mice showed reduced growth, followed by body weight loss and shortened lifespan (16 wks). LV-Xpg−/− mice exhibited normal growth and body weight gain, but also reduced lifespan (28 wks). At 16 wks, LV function had deteriorated in both Xpg−/− and Xpg−/− mice compared to wt (Table 1). Total and NOS-dependent O2− production was only increased in Xpg−/−. In the presence of NADPH, NOX activity was elevated in both groups, but NOX-dependent O2− generation was higher only in Xpg−/−. The relative RNA expression level of atrial natriuretic peptide was increased in both groups, but particularity in Xpg−/−. Moreover, Xpg−/− showed a marked increase in LV end-diastolic lumen diameter and displayed a marked increase in in vivo cardiac apoptosis (27±2% vs. 6±1% in wt; p<0.05). Conclusion: Mice with cardiomyocyte-restricted loss of DNA-repair protein XPG display a heart failure phenotype, demonstrating that intact repair in cardiomyocytes is critical for maintaining normal cardiac function.


Key Words: cardiac function; DNA repair; XPG; oxidative stress; mice

Dysregulation of the Nonmyocyte Microenvironment Promotes Rapid Load-Induced Heart Failure via Activation of the ERK Signaling Cascade

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Background: Little is known about how perturbations in the ECM microenvironment regulate nonmyocyte function. This study was designed to assess whether deficiency of fibrillin-1, a ubiquitous ECM molecule that is expressed primarily in fibroblast-like cells, renders the heart more vulnerable to mechanical stress. Methods and Results: Baseline cardiac structure and function were indistinguishable between WT and fibrillin-1 deficient (Fbn1C1039G+/−) mice. However, after only 1 week of pressure overload induced by transverse aortic constriction (TAC), Fbn1C1039G+ hearts showed dramatic dilation and dysfunction which progressed in the ensuing 3 weeks. Fbn1C1039G+/−TAC heart mass was profoundly increased compared with WT:TAC (14.1±1.8 vs. 6.2±0.1 mg/g; p<0.05) as was myocyte area (224±3 vs 206±4 μm2; p<0.05) and fibrosis (fold change to SHAM, 3.2±0.2 vs 1.3±0.3; p<0.05). Though Fbn1 expression increased in Fbn1C1039G+/−TAC vs. WT:TAC hearts, fibrillin-1 deposition in the ECM microenvironment increased only in WT:TAC which notably had enhanced function at study end. Intriguingly, Fbn1F1C1039G+/− hearts, ePKCα/2 was enhanced selectively in vimentin-positive nonmyocytes, whereas enhanced pSmad2 was found in both nonmyocyte and myocyte cell compartments. Remarkably, treatment pre- and post-TAC with a selective MEK inhibitor of ERK1/2 phosphorylation reversed dysfunction despite chronic load. Furthermore, not only was ERK1/2 normalized but also pSmad2 was normalized in both the nonmyocyte and myocyte cell compartments, fibrosis was reduced, and myocyte area returned to normal, implicating Erk-mediated TGFβ-dependent autocrine/paracrine pathways at play. Lastly, in the WT heart, while Fbn1 expression and deposition were increased during compensated hypertrophy, Fbn1 was significantly decreased in the transition to decompensated function which correlated with increased TGFβ signaling and expression of TGFβ targets. Conclusion: Fibrillin-1 deposition in the nonmyocyte microenvironment is an important cardioprotective adaptation that opposes decompensated heart failure. These findings suggest that manipulation of cell-specific microenvironments may represent a therapeutic target for ameliorating heart failure.


Key Words: fibrillin-1; microenvironment; nonmyocyte; MAPK; transforming growth factor beta

Late-Breaking Basic Science Posters

Novel Role Of MicroRNA-532 In Vascular Function

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Recent evidence indicates the importance of microRNAs in the control of vascular homeostasis. However, little is known about the expression of microRNAs in human peri-vascular cells, namely microvascular and adventitial pericytes. The latter are attracting much attention owing to their potential pro-healing activity, which reportedly involves miR-132. Here, we focus on microRNA-532 as a novel regulator of pericyte function. Adventitial pericytes were isolated from vein leftovers of coronary artery bypass graft surgery (n=7 patients). Using a miRCURY Universal RT miRNA PCR Human array, we found 175 microRNAs expressed in all samples, with 19 of them being differentially regulated by hypoxia. By qPCR, we confirmed that microRNA-532 is downregulated by hypoxia (P<0.0001). We next used a Taqman anti-miR inhibitor to reduce microRNA-532 expression to levels similar to those seen under hypoxia. Inhibition of microRNA-532 resulted in enhanced migratory activity as assessed by scratch assays (1.5-fold, P<0.05), without affecting pericyte proliferation or apoptosis. In vitro Matrigel assay showed microRNA-532-inhibited pericytes have reduced capacity to promote network formation by HUVECs, which was associated with prevalent localization of Dll-labelled pericytes around network branches rather than notches. Furthermore, conditioned media from microRNA-532-inhibited pericytes increase the permeability of HUVEC monolayers. Among microRNA-532 candidate targets, we found leptin to be upregulated following pericyte exposure to hypoxia or microRNA-532 silencing. Studies using leptin-silencing confirmed contribution of this hormone in induction of vascular permeability by hypoxic pericytes. On the other hand, angiopoietin-1, a potent vascular stabilizer produced by pericytes, was remarkably downregulated following microRNA-532 silencing. This effect is indirect as angiopoietin-1 is not a direct target microRNA-532. In summary, microRNA-532 is modulated by hypoxia and involved in the control of pericyte motility, angiogenic activity and modulation of vascular permeability. These data reveal a previously unreported role of microRNA-532 in vascular biology.


Key Words: microRNA; Vascular; Hypoxia

Functionality Consequences of a Rare TBX5 Variant In Familial Brugada Syndrome

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Introduction: The Brugada syndrome (BrS) is a channelopathy with a distinctive ECG pattern reflecting decreased sodium current and increased risk of sudden death. Loss of function variants in the cardiac sodium channel gene, SCN5A, account for ~20% of cases. Reduced channel expression has been proposed as a mechanism in other cases of BrS, but functional experiments that directly link regulatory variants to altered ionic current in BrS or other channelopathies have not been reported. Methods and Results: We identified a family with multiple individuals displaying the BrS ECG, but no rare coding region variants in SCN5A. However, Sanger sequencing of other candidate genes identified a previously unreported non-synonymous variant of unknown significance (G145R) in TBX5; TBX5 haploinsufficiency in mice produces multiple phenotypes, including reduced Scn5a expression in the BrS myocytes compared to multiple control lines. Peak sodium current at -30 mV (from a holding potential of -100 mV) was -97.1±36.5 pA/pF (n=8) in the BrS myocytes compared to -211±36.0 pA/pF (n=13 from two unique donors; p<0.05) in controls (Figure). Compared to wild-type protein. To further establish the functional consequences of TBX5-G145R, we studied cardiomyocytes differentiated from induced pluripotent stem cells (iPSC-CMs) derived from mutation carriers in the family. BrS iPSC-CMs studied 35 days after cardiomyocyte induction, SCN5A transcript abundance was decreased compared to multiple control lines. Peak sodium current at -30 mV (from a holding potential of -100 mV) was -87.1±36.5 pA/pF (n=8) in the BrS myocytes compared to -211±36.0 pA/pF (n=13 from two unique donors; p<0.05) in controls (Figure). Conclusion: These data provide strong evidence that TBX5-G145R causes the Brugada syndrome phenotype in this family by reducing cardiac sodium channel expression. The development of iPSC-CMs has allowed us for the first time to directly link altered ion channel gene expression to a channelopathy, and this approach will therefore enable definition of the role of regulatory elements as causes and modulators of cardiac arrhythmia susceptibility.

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Introduction to the session: Dysregulation of the nonmyocyte microenvironment is a hallmark of cardiac failure and a potential therapeutic target. Studies from our lab and others (e.g., 1, 2, 3) have allowed us for the first time to directly link altered ion channel gene expression to a channelopathy, and this approach will therefore enable definition of the role of regulatory elements as causes and modulators of cardiac arrhythmia susceptibility.

Author Disclosures: None.

Key Words: fibrillin-1, microenvironment, nonmyocyte, MAPK, transforming growth factor beta

Control (n=3) BrS (n=8)

Control

Current density (pA/pF)

1,000 pA

0

p<0.05

10 msec
Insufficient Mitophagy Is Associated With Exaggerated Inflammamae Activation And Adverse Post-infarct Ventricular Remodeling In Type 2 Diabetic Mice

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Background: Diabetes is a major risk factor for cardiovascular diseases. Type 2 diabetic patients have a significantly increased mortality after myocardial infarction compared to non-diabetics, though the underlying mechanisms remain poorly understood. Methods and Results: We investigated the cellular and molecular mechanism of post-infarct left-ventricle remodeling by lightig left-anterior descending artery (LAD) in 12–14 month old T2DM and control mice. Cardiac MRI imaging was performed at baseline, day 7 and day 14 post-LAD i-gation. Global left ventricular ejection fraction was significantly decreased (21.30 ± 4.8% (n=8) vs 35.17 ± 8.25% (n=15) for T2DM and controls, P<0.001) in parallel with increased mortality in T2DM mice compared to controls. Genome-wide mRNA-sequencing analysis (Fold change >1.5 and a P-value of <0.005) identified 1609 genes to be differentially expressed (568 upregulated and 1041 downregulated) in the infarcted myocardium of T2DM mice compared to controls. Pathway analysis of differentially expressed genes were enriched for mitochondrial dysfunction, TCA cycle and fatty acid oxidation. Transcription factor analysis showed inhibition of PGC-1α, PGC-1β, ERRα, ERRβ and TFAM in infarcted mycardium of T2DM mice. Transmission electron microscopy (TEM) analysis showed an altered mitochondrial dynamics (fission and fusion), impaired mitophagy and altered cardiomyocyte death modality in infarcted myocardium of T2DM mice compared to controls. Furthermore, Aim 2 and NLR4 inflammasome were highly activated in infarcted myocardium of T2DM mice compared to controls. Cardiac leukocytes exhibited an altered phenotype as assessed by flow cytometry, associated with impaired neovascularization and increased collagen deposition as assessed by fourier-transform infra-red spectroscopy in infarcted myocardium of T2DM mice. Conclusion: We conclude that an altered mitochondrial dynamics, exaggerated inflammasome activation together with impaired neovascularization responses may contribute to an increased mortality after myocardial infarction in T2DM. Modulation of mitochondrial dynamics together with inhibition of inflammasome activation may offer a novel therapeutic target.


Mechanosensitive Ion Channel TRPV4 Negatively Regulates Angiogenesis Via Modulation Of Rho/Rho Kinase Pathway

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Angiogenesis, the growth of new capillaries from pre-existing ones, is regulated by a balance of both mechanical and soluble factors. Important for natural processes such as wound healing and reproduction, insufficient or excessive angiogenesis causes a variety of pathologi-problems that affects more than one billion people worldwide. While the mechanism by which angiogenesis occurs is well established, how this process is regulated, more specifically, negatively regulated, is not well studied. Here, we show mechanosensitive ion channel TRPV4 negatively regulates angiogenesis. First, we cultured aortic ring explants from wild-type (WT) and TRPV4 KO mice, finding a significant increase in the sprouting from TRPV4KO aortic rings. Next, we found that endothelial cells (EC) isolated from TRPV4KO mice (TRPV4KO EC) exhibited increased proliferation, migration, as well as abnormal angiogenesis in vitro compared to their WT counterparts. Further, in vivo Matrigel plug assays revealed an increase in vascular growth in TRPV4KO mice. To determine the molecular mechanism by which TRPV4 regulates angiogenesis, we next focused on Rho/Rho kinase pathway. Intriguingly, we found that TRPV4KO EC showed significantly increased basal Rho activity compared to WT EC. Therefore, we pharmacologically inhibited the Rho pathway with Rho kinase (ROK) inhibitor, Y-27632, which normalized the abnormal angiogenesis exhibited by TRPV4KO EC in vitro. Finally, Y-27632 treatment, in conjunction with Cisplatin, reduced abnormal tumor angiogenesis and growth exhibited by TRPV4KO mice injected with Lewis Lung Carcinoma cells, suggesting that ROX inhibition may normalize the tumor vasculature and improved the delivery of Cisplatin. Taken together, these data suggest that TRPV4 regulates angiogenesis via modulation of Rho/Rho kinase pathway and the loss of TRPV4 negatively contributes to both physiological and pathological angiogenesis. This research has received full or partial funding support from the American Heart Association, Yes.


Key Words: TRPV4; angiogenesis; endothelial; Rho; mechanosensitive

Uncoupling Protein 2 Inhibits Vascular Smooth Muscle Cell Proliferation and Neointimal Formation

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Objective: Mitochondrial membrane potential and oxidative stress play important roles in vascular smooth muscle cell (SMC) proliferation. We sequenced and investigated potent mitochondrial transporters in SMC proliferation and neointimal formation. Methods And Results: The wire-injured carotid arteries and uninjured arteries of C57BL6 mice were collected at 5 days post-injury to perform the transcriptome array. We found that uncoupling protein 2 (UCP2) was significantly upregulated in wire-injured arteries. The gPCR results
showed that the wire-injury caused an initial increase of UCP2 from day 1 to day 7 post-injury and followed by a decrease below basal level at 14, 21 and 28 days. Treatment of cultured aortic SMCs with PDGF caused gradual downregulation of UCP2. The PDGF-induced proliferation and migration of SMCs were augmented in UCP2−/− cells, while attenuated by adenosine (Ad)-mediated overexpression of UCP2. Similarly, PDGF-stimulated mitochondrial hyperpolarization and superoxide production were also enhanced in UCP2−/− SMCs but blunted by UCP2 overexpression. TEMPOL abolished UCP2 ablation-elicted increases in SMC proliferation and migration. By screening the PDGF pathway, we found the phosphorylation of IκB was decreased in Ad-UCP2-transfected SMCs. Western blot results showed that UCP2 overexpression inhibited the NF-κB nuclear translocation. Neointima/media ratios at 14, 21 and 28 days post-injury were higher in UCP2−/− mice than wild-type mice, whereas the intimal hyperplasia was similar at 7 and 14 days comparable between the two groups. Oral administration of TEMPOL blocked UCP2 ablation-induced increases in neointimal hyperplasia. Local delivery of Ad-UCP2 inhibited the neointimal formation in wire-injured carotid arteries of C57BL/6 mice and also in balloon-injured carotid arteries of Sprague Dawley rats and New Zealand white rabbits. Furthermore, delivery of Ad-UCP2 to stented arterial wall by using a local drug infusion balloon catheter inhibited in-stent restenosis of swine coronary arteries. Finally, we found that overexpression of UCP2 inhibited neointimal hyperplasia in an organ culture model of human saphenous veins. Conclusions: UCP2 inhibits SMC proliferation, neointimal hyperplasia and restenosis.

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Key Words: neointimal hyperplasia; restenosis; uncoupling protein 2; vascular smooth muscle cell; mitochondria

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The Serpin Domain of Angiotensinogen Has Novel Biological Functions

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We found that angiotensinogen (AGT) led to body weight gain and liver steatosis independent of angiotensin (AngII). To identify regions potentially critical for the AngII-independent functions of AGT, sequences of AGT were aligned from human, rat, mouse, and zebrafish, and a bioinformatic analysis was performed by mapping conserved sequences onto the structure of AGT. As shown in the image, in addition to the well conserved N-terminal AngI encoding region (Green) and core hydrophobic residues (Purple) necessary for stabilizing the protein fold, three highly conserved surface regions were identified in the serpin domain (i.e. des(AngI)AGT). One conserved region directly contacts renin (Blue) and therefore may contribute to AGT and renin interaction. The face distal to the renin binding surface also has selected regions of high conservation. Three conserved residues (Red) on the central beta-sheet face, K253, H274, and E422, are remote in sequence, but spatially co-located. Furthermore, the loop formed by residues 291–301 (Gold) contains strictly conserved solvent accessible hydrophobic residues W292 and V299 along with strictly conserved S298. These conserved regions bear the hallmarks of functional protein interaction. To directly determine effects of the core serpin domain of AGT, we infected hepatocyte-specific AGT−/− mice, in an LDL receptor−/− background, with an adeno-associated viral vector expressing mouse des(AngII)AGT. These mice were fed a Western diet for 12 weeks, starting 2 weeks after AAV injections. des(AngII)AGT expression had no effect on plasma renin concentrations or atherosclerosis, indicating lack of effects on AngII-dependent functions. In contrast, des(AngII)AGT expression increased body weight gain, liver weight and liver triglyceride content. These data demonstrate that the core serpin domain of AGT mediates body weight gain and liver steatosis, newly identified AngII-independent functions of AGT.


Key Words: angiotensinogen; atherosclerosis; obesity; liver steatosis; angiotensin

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Lysosphatidic Acid Signaling Is Required For The Normal Proliferation Of Postnatal But Not Embryonic Cardiomyocytes

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Introduction: Increasing evidence demonstrate that cardiomyocytes still has the potential of proliferation after birth. However, it remains unknown whether regulatory mechanisms of proliferation after birth is the same with that of embryonic period. Lysosphatidic acid (LPA) is a small glycerophospholipid with diverse function including promoting cell proliferation by binding to its receptors: LPA1-LPA6. Our previous study found that expression of LPA1 and LPA3 significantly peaked during the early postnatal period, and decreased rapidly thereafter compared to other receptors. In this study, we investigated the role of LPA signaling in cardiomyocyte proliferation before- and after-birth. Methods and Results: By using immunofluorescence and high-content analysis, we determined the number of H3P and K67 positive cardiomyocytes in the hearts of Lpar1−/− and Lpar3 knockout mice on E14.5, postnatal day 1(p1), p4, p7, p14 and p21 respectively. The results showed that proliferation of cardiomyocytes decreased significantly in the Lpar3 knockout mice compared to wild-type at the day during first week (70% on p4) but not on E14.5, p14 and p21. Moreover, cardiac function evaluated by echocardiography showed that Lpar3−/− mice were significantly larger than wild type mice. These results demonstrated that LPA3 is required for normal cardiomyocyte proliferation during the first week after birth but not before period, which may important for the fully-functional heart in adult. Similarly, H3P, K67 and BrdU positive cardiomyocytes in neonatal SD rats showed a 70% reduction by intraperitoneal injection of K186425 (LPA1/LPA3 inhibitor). Meanwhile, we found LPA promoted proliferation of cultured rat immature cardiomyocytes (postnatal day 1 and 4) in vitro. By using specific siRNA for LPA3, we obtained additional support showing that LPA promotes cardiomyocyte proliferation through LPA3. Furthermore, we identified LPA activated YAP, a newly defined transcriptional coactivator playing essential roles in cardiac development, to induce cardiomyocytes proliferation. Conclusions: LPA signaling is important in promoting normal cardiomyocytes proliferation after birth and suggests its specific effect on postnatal but not embryonic heart.

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Key Words: cardiomyocytes; proliferation; lysosphatidic acid; LPA receptor; YAP

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Trophoblast Oncocotefal Glycoprotein Antigen is required for human pericyte migration and pro-angiogenic effects

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Background: Pericytes from microvasculature and adventitia of large vessels are thought to provide a new source of regenerative stem cells. Mechanisms underpinning pericyte migration and pro-angiogenic activities are poorly understood. Here, we investigate the role of Trophoblast Oncocotefal Glycoprotein Antigen (TPBG), which induces a migratory phenotype in human trophoblast and is linked to a poor prognostic marker of embryonic stem cell differentiation. However, the distribution and impact of TPBG within the vascular system to date has not been addressed.

Methods and Results: Immunofluorescence staining of human saphenous veins (SV) identified the co-expression of TPBG with the pericyte markers CD31 and C034 in cells surrounding the adventitial vasa vasorum. Flow cytometry confirmed SV-derived adventitial pericytes (SV-APCs) universally express intracellular TPBG (n=11 biological replicates). Exposure of SV-APCs to hypoxia increased transcriptional expression of TPBG compared to normoxia-matched controls by 4.7 times (p<0.001 n=4). This was associated with induction of TPBG transactivator. Additionally, under hypoxia or low density seeding, TPBG was translocated to the plasma membrane and associated with...
filopodia. Tri-lineage differentiation of SV-APCs coincided with the up-regulation of TPBG transcripts at day 3 post-induction (p<0.05 n=3). SiRNA silencing of TPBG in adventitial, cardiac and skeletal muscle pericytes reduced wound closure in a scratch assay by 47% (p<0.0001 n=5), 38% (p<0.01 n=3), 38% (p<0.05 n=3) respectively compared to controls. Additionally, SiRNA silencing of TPBG in SV-APCs reduced their ability to support network formation by human umbilical vein endothelial cells in a Matrigel assay (40% reduction p<0.01 n=4). Crucial pro-angiogenic molecules MMP2 and MMP9 transcripts were also reduced in the TPBG siRNA-silenced SV-APCs compared to control cells (80% reduction p<0.01 n=4).

**Conclusion:** In this study we have shown for the first time the presence of TPBG within the cardiovascular system and TPBG importance in pericyte migration and promotion of endothelial network formation. TPBG may participate in biological functions of human pericytes instrumental to reparative processes.

**Author Disclosures:**


Key Words: Stem cell biology; Cell signaling; Angiogenesis; Cardiovascular