Animal Models of Human Disease

The Editors

The following articles are being highlighted as part of Circulation: Cardiovascular Genetics’ Topic Review series. This series will summarize the most important manuscripts, as selected by the editors, published in the Circulation portfolio and Circulation: Cardiovascular Genetics, in particular. The studies included in this article represent the most read manuscripts published on the topic of animal models of human disease in 2010 and 2011. (Circ Cardiovasc Genet. 2012; 5:e28-e36.)

Reciprocal Transcriptional Regulation of Metabolic and Signaling Pathways Correlates With Disease Severity in Heart Failure

Summary: Congestive heart failure (HF) is a leading cause of morbidity and mortality worldwide. Despite phenotypic similarities characterized by ventricular dilatation and reduced contractility, the extent of common and divergent gene expression between different forms of HF remains a matter of intense debate. Focusing on molecular pathways, we demonstrate that myocardial gene expression in 28 experimental models of nonischemic (pressure overload, tachypacing, chronic isoproterenol infusion, Chagas disease, and transgenic HF models) and ischemic HF is consistently characterized by downregulation of major metabolic pathways and concomitant upregulation of cell signaling pathways, thus recapitulating a fetal gene expression program. In contrast to this uniform transcriptional response observed in animal models, human HF microarray studies displayed a greater variability, with some studies even showing a reversed pattern. The reasons for this divergence are almost certainly multifactorial and include the lack of true nonfailing human control tissue (significant cardiac disease in nonfailing samples and differences in tissue preservation techniques, leading to a fetal gene expression pattern) and the effect of medication, namely angiotensin-converting enzyme (ACE) inhibitors and β-blockers, partially reversing the fetal gene expression pattern in failing samples. These results highlight the difficulties of interpreting results from real world human clinical samples and stress the importance of well-controlled animal models to elucidate disease mechanisms. We conclude that, irrespective of the etiology of HF, gene expression in failing myocardium is characterized by downregulation of metabolic transcripts and concomitant upregulation of cell signaling pathways, thus providing a unifying concept for the heterogeneous transcriptional response observed in phenotypically similar models of HF.

Conclusions: Irrespective of the etiology, gene expression in failing myocardium is characterized by downregulation of metabolic transcripts and concomitant upregulation of cell signaling pathways. Gene expression changes along this metabolic-signaling axis in mammalian myocardium are a consistent feature in the heterogeneous transcriptional response observed in phenotypically similar models of HF.

Downregulation of Kv7.4 Channel Activity in Primary and Secondary Hypertension

Summary: Hypertension is a major risk factor for a number of cardiovascular diseases and is the leading cause of mortality worldwide. Hypertension is characterized by an increase in peripheral resistance and is associated with remodeling of the blood vessel architecture, which contributes to the maintenance of elevated blood pressure in the longer term. Recently, voltage-dependent potassium channels encoded by the KCNQ gene family (Kv7.1 through Kv7.5) have been identified in rodent and human vascular smooth muscle, in which they are important regulators of the membrane potential and hence vascular contractility. The present study shows that, in normotensive rats and mice, structurally different Kv7 activators relaxed mesenteric resistance vessels and thoracic aorta and improved coronary perfusion considerably. Strikingly, the vasorelaxant effects of these agents were markedly attenuated in tissues from spontaneously hypertensive rats and angiotensin II-infused hypertensive mice, and the effect on coronary perfusion was negligible. These impaired functional responses were associated with a downregulation of KCNQ4 gene expression and reduced production of Kv7.4 protein. Downregulation of KCNQ4 and the loss of this antispasmodic mechanism appear to be a common feature of hypertensive blood vessels, which provides considerable new insight into the pathogenesis of hypertension. Strategies for restoring KCNQ4 could be therapeutically beneficial.

Conclusions: In 2 different rat and mouse models of hypertension, the functional impact of Kv7 channels was dramatically downregulated.

High-Resolution Identity by Descent Mapping Uncover the Genetic Basis for Blood Pressure Differences Between Spontaneously Hypertensive Rat Lines

Summary: Blood pressure and essential hypertension are influenced by genetic factors. Identification of the genetic factors influencing blood pressure and hypertension in human populations has had only limited success. This undoubtedly arises from genetic complexity such as interaction between genetic variants to cause changes in blood pressure, the relatively small effects of each genetic factor alone, the possibly large number of genetic factors dispersed in the population, and the effect of environmental variation to obscure underlying genetic effects. For this reason, animal models of hypertension may be useful because environmental variables can be controlled, genetic complexity can be reduced (using inbred strains), and selective breeding can be performed to map genes. In the present study, we examined 2 closely related spontaneously hypertensive rat lines to determine whether the genetic basis for their hypertension was identical. We concluded that it was not. Next, we used single nucleotide polymorphism (SNP) markers to ask how related are these lines. The 2 lines had inherited ~87% of their genome from the same ancestors. Genetic difference in blood pressure must be limited to the 13% of the genomes that came from different ancestors, further reducing genetic complexity. Our mapping experiments found a single locus containing genetic variation affecting blood pressure.

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We used gene expression data to identify <10 genes in this region that were expressed at different levels in the kidneys of the 2 spontaneously hypertensive rat lines. Among these genes may be those that are able to influence blood pressure. Sequence analysis of these genes has begun to identify possible genetic variation that may contribute to elevated blood pressure.

Conclusions: Thus hypertension in SHR-A3 and –B2 appears to arise from an overlapping set of susceptibility alleles, with SHR-A3 possessing an additional hypertensive locus that contributes to further increase blood pressure.1

Connexin 37 Limits Thrombus Propensity by Downregulating Platelet Reactivity

Summary: Platelets play a key role in the pathogenesis and the acute complications of atherosclerosis such as myocardial infarction and stroke. Current antiplatelet drugs are the cornerstone of the treatment of this widespread disease, but their clinical benefit is relatively limited. Innovative research toward new targets in platelets for drug development is therefore mandatory. The present study reports that platelets and megakaryocytes express connexin 37, which belongs to the family of gap junction proteins. Gap junctions provide a pathway for direct communication between neighboring cells and thereby enable intercellular coordination of tissue activity. Deletion of the connexin 37 gene in mice shortens bleeding time and increases platelet aggregation and thrombus propensity. Aggregation of human platelets is also increased when treated with gap junction blockers, which effectively dampen the communication between platelets during the formation of an aggregate. The importance of functional gap junction channels between platelets is further supported by the association between a known polymorphism in the human connexin 37 gene (GJA4) and platelet aggregation responses. Connexin 37-built gap junctions between platelets therefore provide a mechanism to limit thrombus propensity by downregulating platelet aggregation and aggregation. We propose that transfer between platelets of an anti-aggregating factor occurs after the initiation of thrombus formation following platelet activation. Interestingly, the GJA4 polymorphism has been associated with atherosclerosis and myocardial infarction in previous studies. Our observations should open new avenues for the development of antiplatelet drugs that target thrombus propensity.

Conclusions: We propose that the establishment of gap junctional communication between Cx37-expressing platelets provides a mechanism to limit thrombus propensity. To our knowledge, these data provide the first evidence incriminating gap junctions in the pathogenesis of thrombosis.4

Increased MicroRNA-1 and MicroRNA-133a Levels in Serum of Patients With Cardiovascular Disease Indicate Myocardial Damage

Summary: Recently, it was reported that levels of muscle-specific microRNA (miRNA or miR) increased in the plasma or serum of patients with acute myocardial infarction (MI); however, it is still poorly understood from where or under what conditions miRNAs are released into the bloodstream. We first show that muscle-specific miR-1 and miR-133a increased in the serum of patients with acute coronary syndrome, and these microRNA levels were elevated in the early phase after the onset of acute MI, when there was no increase of serum cardiac Troponin T. The expression levels of these miRNAs were correlated with the serum cardiac Troponin T levels. We also indicated that the miR-133a levels increased in the serum of patients with not only acute MI but also unstable angina pectoris and Takotsubo cardiomyopathy. Next, we attempted to determine the tissue distribution of miR-133a in a mouse model of MI and revealed that not only the infarcted region but also the border zone is the source of circulating miR-133a. Furthermore, in vitro experiments indicated that stimulation of calcium ionophore increased miR-133a release from cardiac myoblasts only at concentrations in which cell death was observed and the released miRNA was functional. Taken together, our data suggest that circulating miR-133a, which is derived from injured myocardium, can be used as a sensitive, early diagnostic biomarker for myocardial damage. Additionally, because released microRNAs can regulate gene expression in other cells, the present study may provide a new insight into the function of miRNA in the pathophysiology of MI.

Conclusions: These results suggest that elevated levels of circulating miR-133a in patients with cardiovascular diseases originate mainly from the injured myocardium. Circulating miR-133a can be used as a marker for cardiomyocyte death, and it may have functions in cardiovascular diseases.5

Inhibition of Small-Conductance Ca²⁺-Activated K⁺ Channels Terminates and Protects Against Atrial Fibrillation

Summary: Among cardiac arrhythmias, atrial fibrillation (AF) is the most common and is an established risk factor for stroke and premature death. The lifetime risk for development of AF is approximately 25% in the general population. It has been estimated that 2.3 million people in the United States and 6 million in the European Union have paroxysmal or persistent AF. Current options for antiarrhythmic therapy of AF are limited by marginal efficacy and toxicity, including proarrhythmia, making new drug development crucial. New investigational agents that target atrial-specific ion channels offer promising new treatment approaches that may have improved risk-benefit profiles. We have provided evidence that pharmacological inhibition of small-conductance Ca²⁺-activated K⁺ (SK) channels show antiarrhythmic potential and does not result in undesirable proarrhythmic side effects on the ventricle. Provided that its efficiency can be demonstrated in humans, small-conductance Ca²⁺-activated K⁺ channel inhibition might be a realistic therapeutic approach to treat atrial fibrillation pharmacologically.

Conclusions: Inhibition of SK channels prolongs atrial effective refractory period without affecting QT interval and prevents and terminates AF ex vivo and in vivo, thus offering a promising new therapeutic opportunity in the treatment of AF.6

Marked Variability in Susceptibility to Ventricular Fibrillation in an Experimental Commotio Cordis Model

Summary: The data reported in this article demonstrate for the first time that an individual susceptibility to commotio cordis exists. Whether individuals are more or less susceptible to chest wall blow-induced ventricular fibrillation has been a clinical question for quite some time, and individual susceptibility may partially explain the rarity of the condition. Yet, despite its rarity, commotio cordis is the second leading cause of sudden cardiac death in the playing field for young athletes. It also is of major concern to parents, coaches, and organizations such as US Lacrosse and Little League Baseball. These data also have clinical implications for the decision of whether return to sports for individuals who have survived a commotio cordis event is wise.

Conclusions: Swines display a wide range of individual vulnerability to VF triggered by chest wall impact, with a distinct minority being uniquely susceptible. Mild abnormalities in cardiac depolarization and repolarization might underlie this susceptibility. Such individual susceptibility may also be present in humans and contribute to the rarity of commotio cordis.7

A ZASP Missense Mutation, S196L, Leads to Cytoskeletal and Electric Abnormalities in a Mouse Model of Cardiomyopathy

Summary: Dilated cardiomyopathies are often genetic and associated with arrhythmias and sudden cardiac death. The links between
Role of Reactive Oxygen Species in Hyperadrenergic Hypertension: Biochemical, Physiological, and Pharmacological Evidence From Targeted Ablation of the Chromogranin A (Chga) Gene

Summary: Oxidative stress, in which reactive oxygen species (ROS) outstrip antioxidant defenses, contributes to cardiovascular disease. In the present investigation, we studied derangements of ROS in the development of a hyperadrenergic model of hereditary hypertension: targeted ablation (knockout [KO]) of chromogranin A (Chga) in the mouse. In the KO mouse, blood pressure (BP) elevation was accompanied by not only catecholamine excess but also by increased ROS (H₂O₂) and isoprostane levels (index of lipid peroxidation). Renal transcript analyses implicated changes in several redox enzymes. KO alterations in BP as well as biochemical traits could be abrogated by inhibition of either sympathetic outflow or of NADPH oxidase. In cultured renal podocytes, H₂O₂ production was augmented by epinephrine (probably through β1 receptors). Thus, ROS may play an important role in the development of hyperadrenergic hypertension in this experimental model, in a process mechanistically linking elevated BP with catecholamine excess, renal transcriptional responses, ROS elevation, lipid peroxidation, and nitric oxide depletion. Overall, our results demonstrate the existence of novel pathophysiological links between the adrenergic system and oxidative stress and suggest new strategies to probe the role and actions of ROS in this setting.

Conclusions: ROS appear to play a necessary role in the development of hyperadrenergic hypertension in this model, in a process mechanistically linking elevated BP with catecholamine excess, renal transcriptional responses, ROS elevation, lipid peroxidation, and NO depletion. Some of the changes appear to be dependent on transcription, whereas others are immediate. The cycle could be disrupted by inhibition of either sympathetic outflow or NADPH oxidase. Because common genetic variation at the human CHGA locus alters BP, the results have implications for antihypertensive treatment as well as prevention of target-organ consequences of the disease. The results document novel pathophysiological links between the adrenergic system and oxidative stress and suggest new strategies to probe the role and actions of ROS within this setting.

Selective Molecular Potassium Channel Blockade Prevents Atrial Fibrillation

Summary: Atrial fibrillation (AF) is the most common arrhythmia found in clinical practice, affecting 2 to 5 million people in the United States and several million more worldwide. The presence of AF substantially increases individual risk of stroke, heart failure, and death. A principal limitation to clinical practice is the lack of safe, effective therapies for this pervasive arrhythmia. We previously reported a gene-painting method capable of 100% transmural gene transfer to all parts of the atria accessible from an open-chest pericardium approach. In the present report, we used this method to transduce the atria with KCNH2-G628S, a mutation that blocks the rapid component of the delayed rectifier potassium current. This current is also blocked by class III antiarrhythmic drugs, but those drugs affect atrial and ventricular myocytes alike. The painting method is specific to atrial myocytes. We found that KCNH2-G628S gene transfer prolonged atrial action potential and prevented AF. This effect correlated with the time course of transgene expression. The method should be directly applicable to the problem of postoperative AF. With modifications to increase duration of gene expression and to reduce the invasive nature of delivery, the method should also be applicable to general AF. Formal preclinical testing is required before clinical investigation.

Conclusions: Gene therapy with KCNH2-G628S eliminated AF by prolonging atrial action potential duration. The effect duration correlated with transgene expression.

Protein Kinase D2 Controls Cardiac Valve Formation in Zebrafish by Regulating Histone Deacetylase 5 Activity

Summary: Defective development of the heart valves occurs in 20% to 30% of congenital malformations; however, in most cases, the underlying causes have not been identified. There is increasing evidence that the regulatory mechanisms governing normal valve development also contribute to human valve pathology. In searching for novel molecular signaling pathways that orchestrate vertebrate heart valve development, we isolated the molecular cause of the ethynitrosourea (ENU)-induced recessive embryonic-lethal zebrafish mutant bungee (bung), which shows defective endocardial cushion development and subsequently impaired heart valve formation. We found that the bung mutation selectively impairs Protein kinase D2 kinase activity, which leads to reduced Histone deacetylase 5 phosphorylation, nuclear export, and inactivation. As a result of enhanced Histone deacetylase 5 repressor activity, Notch signaling is severely impaired in bungee-mutant embryos. Accordingly, expression of the well-known Notch target genes Hey1, Hey2, and HeyL is decreased in bung-mutant embryos. Hence, it will be interesting to evaluate in future studies whether mutations in components of this novel signaling pathway such as Protein kinase D2, Histone deacetylase 5, Krüppel-like factor, Notch1, and members of the Hey family are also involved in human congenital heart disease, especially those that arise from defective endocardial cushion development, such as septal and valvular defects. Furthermore, our findings might affect future strategies aiming to engineer human heart valve tissue for improved therapeutics or replacement strategies.

Conclusions: We demonstrate for the first time that proper heart valve formation critically depends on Protein kinase D2-Histone deacetylase 5-Krüppel-like factor signaling.

Noninvasive Assessment of Murine Pulmonary Arterial Pressure: Validation and Application to Models of Pulmonary Hypertension

Summary: Despite the development of new therapeutic strategies such as endothelin-receptor antagonists, phosphodiesterase type-5 inhibitors, and prostanoid, the prognosis of patients with pulmonary arterial hypertension (PAH) remains poor. Advances in understanding the pathophysiological mechanisms that contribute to PAH are critical to the discovery of new therapeutic targets. In this setting, small rodent models (in particular, genetically modified mice) offer the unique opportunity to study the signaling pathways involved in PAH and to evaluate the effectiveness of therapeutic interventions.
Indeed, the ability to study pulmonary artery pressure or right ventricular systolic pressure (RVSP) in mice underexpressing or overexpressing a gene will help to elucidate the functional role of this particular gene in the regulation of pulmonary pressure. In mice, right heart catheterization is the only available method to measure RVSP and is a terminal procedure. The absence of a noninvasive technique that would allow serial assessment of RVSP in mice has significantly undermined progress in the field of PAH, preventing the rapid evaluation and development of mouse PAH models. In the present study, pulmonary artery flow measurements obtained using transthoracic echocardiography detect acute and chronic increases in RVSP with high sensitivity and specificity and identify the effect of treatment on RVSP. Transthoracic echocardiography may allow the characterization of the evolution of PAH and the evaluation of therapeutic interventions noninvasively in mice.

Conclusions: Right ventricular systolic pressure can be estimated noninvasively in mice. Echocardiography is able to detect acute and chronic increases in RVSP with high sensitivity and specificity, as well as to assess the effects of treatment on RVSP. This noninvasive technique may permit the characterization of the evolution of pulmonary arterial hypertension in genetically modified mice.

**Int6/eIF3e Silencing Promotes Functional Blood Vessel Outgrowth and Enhances Wound Healing by Upregulating Hypoxia-Induced Factor 2α Expression**

**Summary:** During the past decades, several studies have tried to trigger revascularization by exogenously applying angiogenic factors into the tissues of interest. A common obstacle was that the application of the specific angiogenic factor alone or an unbalanced combination with others led to the development of unphysiological and incomplete leaky blood vessels. In our research, we first found that hypoxia-inducible factor 2α is under subtype-specific and negative regulation of INT6, which directly binds to hypoxia-inducible factor 2α and triggers its degradation through the proteasome pathway. Further in vivo experiments revealed that Int6 silencing led to enhancement of hypoxia-inducible factor 2α activity even under normoxic condition, temporarily inducing a physiological and potent neovascularization. In mouse skin, we determined subcutaneous fibroblasts as the major source of angiogenic factors. The fibroblasts treated ex vivo with siRNA-Int6 demonstrated that transplantation into the skin also led to the same strong induction of physiological neovascularization. Further application of siRNA-Int6 in a wound healing model (normal and db/db mice) showed a significantly enhanced wound repair, with concomitant formation of new vessels. These results prefigure an encouraging therapeutic value of siRNA-Int6 for treating delayed wound healing, especially in patients with diabetes with impaired microcirculation. Therefore, siRNA-Int6–transfected fibroblast cell treatment or direct application of siRNA-Int6 might be of clinical value in treating ischemic diseases such as heart and brain ischemia, skin injury, and diseases involving obstructed vessels.

**Conclusions:** We suggest that the pathway involving INT6/HIF2α acts as a hypoxia-independent master switch of functional angiogenesis; therefore, siRNA-Int6 application might be of clinical value in treating ischemic diseases such as heart and brain ischemia, skin injury, and diseases involving obstructed vessels.

**Reversal of Hyperlipidemia With a Genetic Switch Favorably Affects the Content and Inflammatory State of Macrophages in Atherosclerotic Plaques**

**Summary:** The ultimate cure for atherosclerosis would be the regression of arterial plaques. Discovery research toward this goal has been hampered by limited and sometimes cumbersome animal models. The Reversa mouse combines a standard model of human atherosclerosis, the hyperlipidemic low-density lipoprotein receptor-deficient mouse, with a genetic switch that electively shuts off low-density lipoprotein production. In the present study, arterial plaques were allowed to develop in Reversa mice to a stage mimicking advanced human coronary artery disease, and then the elevated low-density lipoprotein level was severely reduced, thereby simulating aggressive lipid management. The major findings after such lipid reduction were decreases in the content and inflammatory state of the central cell of plaques, macrophages, with the change in total plaque size more modest because of compensatory increases in collagen content. The improvement in macrophage inflammatory status was augmented by treatment with pioglitazone, consistent with the effects of peroxisome proliferator-activated receptor-γ agonists on macrophages in vitro. The results may explain why plaque-volume decreases have been modest in recent statin trials despite significant reduction in events and may provide one basis for the cardioprotective effects of pioglitazone in clinical studies. Continued study of this convenient model should lead to an improved understanding of plaque regression at the molecular level.

**Conclusions:** The Reversa mouse is a new model of atherosclerosis regression. After lipid lowering, favorable changes in plaque composition were independent of changes in size. In addition, plaque CD68+ cells became less inflammatory, an effect enhanced by treatment with pioglitazone.

**Novel Nonmajor Histocompatibility Complex-Linked Loci From Mouse Chromosome 17 Confer Susceptibility to Viral-Mediated Chronic Autoimmune Myocarditis**

**Summary:** The identification of susceptibility genes for virus-induced chronic myocarditis and dilated cardiomyopathy is an evolving field. Chromosome substitution strain mice are a unique and powerful resource that allows the investigator to quickly identify genetic determinants associated with select disease. Chromosome substitution strain mice contain 1 chromosome from the disease-susceptible A/J strain on an otherwise resistant C57BL/6 background. Using these mice and mice congenic for smaller segments of chromosome 17, we identified 4 susceptibility loci on chromosome 17 for virus-induced autoimmune myocarditis. Two of these loci are novel with regard to the disease association and are located in the proximal portion of chromosome 17. These 2 loci encompass 35 and 69 possible susceptibility genes, respectively. A third locus encodes a number of genes associated with disease susceptibility, including the major histocompatibility complex locus. Characterization of the expression and function of these identified candidate genes within the disease-associated loci will allow us to determine the true susceptibility gene for virus-induced myocarditis and will facilitate the study of how disease risk is conferred. Identification of virus-induced myocarditis susceptibility genes will allow patients to be identified as susceptible to heart disease and to be more efficiently monitored for disease progression. With identification of patients with early-onset disease, treatment could potentially be started at the initiation of disease and thereby limit the damage to the myocardium and arrest or slow disease progression to dilated cardiomyopathy. Further, better identification of patients with increased susceptibility to viral myocarditis could lead to the practical development and use of a vaccine to reduce the development of myocarditis later in life.

**Conclusions:** We have identified 4 loci that confer susceptibility of viral-induced chronic myocarditis. Of these loci, 3 were distinct from the major histocompatibility complex locus and thus represent novel susceptibility loci. The close proximally of the 2 novel loci with susceptibility loci for other autoimmune diseases such as type 1 diabetes and chronic experimental autoimmune thyroiditis suggests the presence of global autoimmune susceptibility genes.
Changes in Ion Channel Gene Expression Underlying Heart Failure-Induced Sinoatrial Node Dysfunction

Summary: It is estimated that >20 million people have heart failure (HF) worldwide, and bradyarrhythmias account for about half of the sudden deaths of patients with HF. Consistent with the high incidence of bradyarrhythmic deaths, HF is known to cause dysfunction of the cardiac conduction system, including the sinoatrial node (SAN). The dysfunction of the SAN is likely to be the result of a remodeling of the ion channels and related proteins responsible for the pacemaker activity of the SAN, and the aim of the study was to investigate this. HF was induced in rats by the ligation of the proximal left coronary artery. In the HF animals, there was an increase in the left ventricular diastolic pressure and a decrease in the left ventricular systolic pressure and SAN dysfunction, the intrinsic heart rate was reduced, and the corrected SAN recovery time was increased. Quantitative polymerase chain reaction was used to measure gene expression in the SAN and surrounding atrial muscle. There was a widespread remodeling of ion channels, gap junction channels, calcium-, sodium-, and proton-handling proteins, and receptors in the SAN. The decrease of the intrinsic heart rate can be explained by an upregulation of various potassium channels. Curiously, the atrial muscle was much less sensitive to HF. Of the 91 genes studied, 41% changed in the SAN, but only 7% changed in the atrial muscle. The elucidation of the mechanisms responsible for SAN dysfunction in HF opens the way to the development of new treatments.

Conclusions: SAN dysfunction is associated with HF and is the result of an extensive remodeling of ion channels; gap junction channels; Ca2+, Na+, and H+–handling proteins; and receptors in the SAN. This will provide a biological rationale for the association between heart failure and atrial fibrillation. The present study showed that reduced levels of the cardiac isoform of PITX2, pitx2c, are sufficient to provoke AF in adult mice. Furthermore, our findings demonstrate that reducing pitx2c levels shortens the left atrial action potential and causes dysregulation of genes involved in calcium handling, cell-cell contacts, and melanogenesis. Shortening of the atrial action potential, secondary to electric remodeling or conferred by genetic variants in ion channel genes, is one of the main causes of AF. These observations provide a biological rationale for the association between PITX2 and AF and suggest that pitx2c has a function in the adult left atrium.

Conclusions: These findings demonstrate a physiological role for PITX2 in the adult heart and support the hypothesis that dysregulation of PITX2 expression can be responsible for susceptibility to AF.

Striking in Vivo Phenotype of a Disease-Associated Human SCN5A Mutation Producing Minimal Changes in Vitro

Summary: A conventional approach to characterize the function of ion channel mutations is to compare wild-type and variant channel function by heterologous expression in mammalian, noncardiac cells such as Chinese hamster ovary or human embryonic kidney cells. The cardiac sodium channel mutation D1275N has been reported in multiple individuals and families with a range of phenotypes, including arrhythmias and dilated cardiomyopathy; however, conventional heterologous expression studies have not identified major differences between wild-type and D1275N function. Thus, it has even been uncertain whether this mutation causes the clinical phenotypes with which it has been associated. In this study, we addressed this issue by studying mice in which the cardiac sodium channel locus had been disrupted and replaced with full-length human wild-type or D1275N-mutant sodium channels. We observed slowed and disordered cardiac conduction and decreased contractile function in mice bearing the mutation; mice with 2 D1275N alleles displayed worse phenotypes than those with 1 variant allele. In vitro electrophysiological studies identified reduced peak cardiac sodium current as a key defect, and this is consistent with the observed reduced conduction velocity. The major clinical implication of these findings is that heterologous expression may be insufficient to assess mutant channel function. In addition, the data lend support to the concept that sodium channel mutations are associated not only with arrhythmias but also with dilated cardiomyopathy phenotypes. The mutant mice will be of invaluable tool to dissect mechanisms underlying these findings.

Conclusions: Although D1275N produces near-normal currents in multiple heterologous expression experiments, our data establish this variant as a pathological mutation that generates conduction slowing, arrhythmias, and a dilated cardiomyopathy phenotype by reducing cardiac sodium current.

Heterogeneity of Genetic Modifiers Ensures Normal Cardiac Development

Summary: Individuals who share the same underlying basis for congenital heart disease can have presentations ranging from normal to life-threatening. Understanding the basis of such wide variability
could suggest prognostic and therapeutic strategies focused not on the causes but on the modifiers of disease. We thus characterized the effect of genetic modifiers on the incidence of heart defects associated with mutation of the cardiac transcription factor Nkx2-5. Quantitative analyses of multiple inbred mouse strain crosses reveal the profound effect of polymorphic genetic modifiers. Protective and susceptibility alleles of modifier loci directly manifest the occurrence of 1 or more types of heart defects, suggesting that they affect the sensitivity of specific cardiac developmental pathways to a perturbation. The modifiers alter the risk of a particular phenotype either independently or via genetic interactions with other loci. The results intertwine the genetic basis of health and congenital heart disease, providing a conceptual framework to understand common clinical observations related to incomplete penetrance and pleiotropy. We propose that stabilizing selection generated a diverse set of polymorphisms so that, in a genetically heterogeneous population, the predominant effect of modifier genes is to ensure the robustness of cardiac development.

Conclusions: Alleles of modifier genes can either buffer perturbations on cardiac development or directly manifest a defect. In a genetically heterogeneous population, the predominant effect of modifier genes is health.

Genes Within the MHC Region Have a Dramatic Influence on Radiation-Enhanced Atherosclerosis in Mice

Summary: In this investigation, we report an unexpected finding that genes within the major histocompatibility complex (MHC) have a dramatic influence on radiation-enhanced atherosclerosis in mice. The mouse strain C3H/HeJ (C3H) is extremely resistant to atherosclerosis, with development of much smaller lesions than the strain C57BL/6 (B6) when the mouse is deficient in apolipoprotein E (apoE−/−) or fed an atherogenic diet. The 2 inbred strains differ in the MHC haplotype, with B6 having H2b and C3H having H2k. C3.SW is a congenic strain of C3H/HeJ in which the H2k haplotype is replaced with the H2b haplotype. C3.SW mice that underwent bone marrow transplantation after lethal irradiation exhibited a 21-fold increase in atherosclerotic lesion size as compared with C3H apoE−/− mice receiving the same treatment, demonstrating the huge influence of H2 haplotypes on radiation-enhanced atherosclerosis. Radiation-induced tissue damage depends on radiation dose, tissue volume treated, and an unknown genetic predisposition. The ability to identify which patients are at risk for radiation-induced complications could facilitate the development of patient-specific treatment regimens toward maximizing therapeutic efficacy while minimizing the incidence of side effects.

Conclusions: These results indicate that gene(s) within the H2 region have a dramatic impact on radiation-enhanced atherosclerosis, and their effect is conveyed partially through bone marrow-derived cells.

Altered Hepatic Gene Expression Profiles Associated With Myocardial Ischemia

Summary: Acute coronary syndrome (ACS) is accompanied by systemic changes in inflammation, coagulation, and metabolism, which may affect the outcome and prognosis of ACS. These systemic reactions are not explained by cardiac events alone. Several lines of evidence suggest that patients with fatty liver disease have a high risk of developing cardiovascular diseases, and it is possible to speculate that the liver is involved in a systemic reaction that modifies the pathogenesis of ACS; however, the relation between liver and myocardial ischemia in the acute ischemic phase has not been elucidated so far. In this investigation, we simultaneously analyzed the gene expression profiles of the liver and heart during acute myocardial ischemia in mice and observed the presence of humoral factors that intervened between the heart and liver. These humoral factors were released from the heart and influenced the liver to secrete important tissue remodeling factors. One of these humoral factors, osteopontin, a widely expressed glycoprotein, was increased in the ischemic heart and altered the gene expression of hepatocytes to produce important tissue-remodeling factors (such as vascular endothelial growth factor-A). Our observations suggest that hepatic gene expression is potentially regulated by humoral factors of cardiac origin provoked by myocardial ischemia, and we provide direct evidence that the liver is involved in a systemic reaction that accompanies ACS. Our findings provide potential new insights into the pathophysiology of ACS.

Conclusions: Hepatic gene expression is potentially regulated by cardiac humoral factors under myocardial ischemia. These results provide new insights into the pathophysiology of acute coronary syndrome.

Protein Aggregates and Novel Presenilin Gene Variants in Idiopathic Dilated Cardiomyopathy

Summary: Heart failure is a progressive and, ultimately, fatal disease, which represents a leading public health problem worldwide. Despite substantial advances in the clinical management of heart failure, the only current permanent therapeutic option is heart transplantation; however, the limited supply of functional organs for transplantation and the age threshold for surgical intervention constrain the scope of this treatment modality. The majority of cases of heart failure are ischemic in origin. The second most frequent origin of nonischemic heart failure is idiopathic dilated cardiomyopathy, in which no other causative events can be recognized. Recent studies highlight the recognition of the public health importance of a large number of diseases associated with defects in the ability of proteins to fold, leading to the accumulation of cytotoxic protein deposits. This group of diseases includes amyloidosis and various neurodegenerative disorders such as Alzheimer disease. Preliminary evidence suggests that idiopathic dilated cardiomyopathy may be included among these misfolding diseases. In addition, mutations in the same genes causing Alzheimer disease in a significant percentage of cases can also be at the origin of idiopathic dilated cardiomyopathy. Interestingly, a dual mechanism seems to mediate the effect of these genetic variations: (1) changes in the control of Ca2+-handling proteins causing defect in the contractile function; and (2) cell damages associated with the protein aggregation process. The incidence, prognosis, and therapeutic options for idiopathic dilated cardiomyopathy may therefore be greatly advanced by establishing a fundamental understanding of key factors leading to the disease.

Conclusions: On the basis of these findings, we propose that 2 mechanisms may link protein aggregation and cardiac function: oligomer-induced changes on Ca2+ handling and a direct effect of PSEN1 sequence variants on excitation-contraction coupling protein function.

Genetic and Pharmacological Hydrogen Sulfide Therapy Attenuates Ischemia-Induced Heart Failure in Mice

Summary: Heart failure continues to be a major health problem as evidenced by a rise in the number of hospitalizations for heart failure, the number of deaths attributed to heart failure, and the ever-increasing costs associated with care. Therapeutic strategies designed to coincide with the standard means of care are, therefore, needed to combat the development and progression of heart failure. Hydrogen sulfide (H2S) is an endogenous gaseous signaling molecule with a diverse physiological profile that has recently been shown to be cardioprotective in various models of cardiac injury. In the present study, we found that either the modulation of endogenous H2S production or direct pharmacological H2S administration significantly reduced mortality and attenuated the severity of ischemia-induced heart failure in mice. Importantly, the present study demonstrates that although a single administration of H2S at the time of reperfusion is beneficial in attenuating infarct size, this alone is not sufficient to improve cardiac function significantly. On the other hand, daily H2S therapy for the first 7 days of reperfusion or increased endogenous H2S production provided significant improvements.
in cardiac function, suggesting that multiple therapeutic interventions are paramount for improvements in outcome. Together, these findings further support the emerging concept that H$_2$S therapy may be of clinical importance in the treatment of cardiovascular disease and may have a practical clinical use after myocardial infarction to reduce the morbidity and mortality associated with ischemia-induced heart failure.

Conclusions: The results of the present study suggest that either the administration of exogenous H$_2$S or the modulation of endogenous H$_2$S production may be of therapeutic benefit in the treatment of ischemia-induced heart failure.24

Nox Activator 1: A Potential Target for Modulation of Vascular Reactive Oxygen Species in Atherosclerotic Arteries

Summary: After decades of investigation, there remains a need for preventive strategies that can reduce atherosclerosis and atherothrombosis, which are the most common causes of death and disability in the United States. Despite many important advances in the treatment of cardiovascular diseases, elucidation of specific therapies that target vascular wall cells has been elusive. An important limitation of targeting intracellular signaling pathways in dysfunctional vascular cells is that most of them are present ubiquitously and are necessary for normal cellular function. Many investigators have studied the hypothesis that regulated production of reactive oxygen species and oxidative stress in vascular cells are important in atherogenesis and that it may be possible to specifically inhibit upregulation of reactive oxygen species production in vascular cells and, in doing so, limit atherogenesis and atherothrombosis. The pathways that produce reactive oxygen species (NADPH oxidase, xanthine oxidase, cyclooxygenase, lipoprotein, and others) are necessary, however, for normal cellular function. The experiments described here were designed to test the strategy of targeting a specific component of NADPH oxidase, arguably the most important regulated system for reactive oxygen species production in vascular cells. To do so, we examined modulation of NoxA1, a NADPH oxidase component necessary for upregulation of superoxide production in vascular smooth muscle cells. Our studies indicate that NoxA1 is critically important in the NADPH oxidase-mediated overexpression of reactive oxygen species characteristic of vascular diseases. Although specific inhibitors of NoxA1 are not known, this work suggests that the strategy of cell-specific modulation of NADPH oxidase function is a therapeutic approach worthy of further investigation.

Conclusions: NoxA1 is the functional homolog of p67phox in VSMCs that regulates redox signaling and VSMC phenotype. These findings support the potential for modulation of NoxA1 expression as a viable approach for the treatment of vascular diseases.25

Inhibition of Hyaluronan Synthesis Accelerates Murine Atherosclerosis: Novel Insights Into the Role of Hyaluronan Synthesis

Summary: Hyaluronan is an integral extracellular matrix component that plays crucial roles in, for example, development and homeostasis of cartilage and skin; however, increased hyaluronan production is associated with tumor progression and vascular disease. Hyaluronan accumulates during neointimal thickening in atherosclerotic plaques and restenotic lesions. In the neointima, it contributes to volume expansion and supports the proliferative and secretory phenotype of vascular smooth muscle cells. Therefore, inhibition of hyaluronan synthesis has been considered as a strategy to limit neointimal thickening and atheroprosoposis. On the other hand, recent research has established hyaluronan on the luminal surface of vascular endothelial cells to be a critical constituent of the endothelial glyocalyx, which has strong vasoprotective functions. In the present study, it is shown in a murine model of atherosclerosis that inhibition of hyaluronan synthesis by an oral hyaluronan synthesis inhibitor surprisingly enhances inflammatory and thrombotic responses and, in the long term, increases atherosclerosis. This adverse effect was attributed to a partial loss of the endothelial glyocalyx. Of note, hyaluronan synthesis inhibitors are effective in inhibiting tumor progression in mouse models and may be tested clinically to enhance the response to antitumor strategies. In light of the present results, it may be crucial to avoid adverse effects on the endothelial glyocalyx because damage of the glyocalyx may lead to increased atherothrombotic risk and enhance inflammatory cell recruitment.

Conclusions: The data suggest that systemic inhibition of hyaluronan synthesis by 4-MU interferes with the protective function of the endothelial glyocalyx, thereby facilitating leukocyte adhesion, subsequent inflammation, and progression of atherosclerosis.26

Unique Properties of the ATP-Sensitive K⁺ Channel in the Mouse Ventricular Cardiac Conduction System

Summary: Cardiovascular ATP-sensitive K⁺ channels have both protective and deleterious roles in the heart. A clear beneficial role for these channels in cardiac pathophysiological states has long been identified; opening of K_{ATP} channels protects against ischemic events by reducing the infarct size and participates as one of the triggers for ischemic preconditioning; however, K_{ATP} channel opening may also be detrimental. For example, their opening during myocardial ischemia may promote K⁺ efflux and reduce the action potential duration. The ensuing electric heterogeneity creates a substrate for reentrant arrhythmias. Our present study characterizes the K_{ATP} channel in the specialized conduction system and found them to have biophysical and pharmacological properties that differ from those of the ventricular K_{ATP} channel. Their molecular composition is also different, and they express subunits normally abundant in noncardiac cells. Numeric simulation studies predict these channels to be more sensitive to metabolic stress than their ventricular counterparts, suggesting that they may preferentially open during the early phases of cardiac ischemia. We demonstrate that K_{ATP} channel blockade mitigates electric conduction slowing during ischemia, suggesting a contribution of the cardiac conduction system K_{ATP} channels to ischemia-induced conduction disturbances and arrhythmias.

Conclusions: These data imply a differential electrophysiological response (and possible contribution to arrhythmias) of the ventricular cardiac conduction system to K_{ATP} channel opening during periods of ischemia.27

Conditional Transgenic Expression of Fibroblast Growth Factor 9 in the Adult Mouse Heart Reduces Heart Failure Mortality After Myocardial Infarction

Summary: Paracrine mechanisms are thought to contribute to the beneficial effects of bone marrow cells on perfusion and function of the infarcted heart that have been demonstrated in experimental studies and some clinical trials. We have previously identified fibroblast growth factor 9 (FGF9) as 1 of >100 paracrine factors that are secreted from bone marrow cells after myocardial infarction (MI). In the embryonic heart, FGF9 is expressed by the epicardium and endocardium and provides an epithelial-to-mesenchymal signal to stimulate coronary artery formation and cardiomyoblast expansion in the developing myocardium. FGF9, however, is expressed only at very low levels in the adult heart. Using a tetracycline-responsive binary transgene system based on the ε-myosin heavy chain promoter, we show here that conditional expression of FGF9 in the adult mouse myocardium supports functional adaptation and survival after MI. Transgenic FGF9-stimulated left ventricular hypertrophy with microvessel expansion, reduced interstitial fibrosis, attenuated fetal gene expression, preserved diastolic but improved systolic function, and markedly reduced heart failure mortality. A single injection of an adenoviral vector encoding FGF9 promoted similar improvements in
left ventricular systolic function and survival after MI. Mechanistically, FGF9 acted primarily on endothelial cells to promote angiogenesis and paracrine release of prohypertrophic factors, including bone morphogenetic protein 6. These observations support the idea that secretome analyses in bone marrow cells can lead to the identification of secreted factors with therapeutic activity after MI. Specifically, the data suggest a previously unrecognized therapeutic potential for FGF9 after MI.

Conclusions: Conditional expression of FGF9 promotes myocardial vascularization and hypertrophy with enhanced systolic function and reduced heart failure mortality after MI. These observations suggest a previously unrecognized therapeutic potential for FGF9 after MI.28

Genetic Deficiency of Plasminogen Activator Inhibitor-1 Promotes Cardiac Fibrosis in Aged Mice: Involvement of Constitutive Transforming Growth Factor-β Signaling and Endothelial-to–Mesenchymal Transition

Summary: Cardiac fibrosis, defined as the proliferation of interstitial fibroblasts and accumulation of extracellular matrix components in the heart, is a common consequence of cardiovascular disease, including acute myocardial infarction and hypertension. Cardiac fibrosis contributes to the development of ventricular dysfunction (systolic and diastolic), heart failure, and arrhythmias. Although fibrosis is predictably identified in end-stage heart disease, the origin of fibroblasts contributing to the excessive synthesis of collagen in the fibrotic heart is controversial. At present, there is no effective treatment to prevent or to reverse cardiac fibrosis. The present study elucidates the molecular basis of cardiac fibrosis using a murine model of age-dependent spontaneous cardiac fibrosis that develops in the absence of the plasminogen activator inhibitor-1 gene. The present study suggests that the cardiac fibrosis in plasminogen activator inhibitor-1–deficient mice is due to increased inflammation, elevated levels of transforming growth factor-β, and induction of transforming growth factor-β–induced profibrotic responses. Plasminogen activator inhibitor-1–deficient endothelial cells appear to be more susceptible to the phenomenon called endothelial-mesenchymal transition in response to transforming growth factor-β via induction of both Smad and ERK1/2 MAPK pathways. These findings provide new insights into molecular mechanisms of cardiac fibrosis and suggest that physiological plasminogen activator inhibitor-1 levels help to protect the heart from age-dependent fibrogenesis. Thus, specific disruption of activated Smad and ERK1/2 MAPK signaling pathways with small-molecule inhibitor(s) may be useful in limiting endothelial-mesenchymal transition and may inform a novel therapeutic approach to prevent and treat cardiac fibrosis in humans.

Conclusions: These results indicate that spontaneous activation of both Smad and non-Smad transforming growth factor-β signaling may contribute to profibrotic responses in aged PAI-1–deficient mice hearts and establish a possible link between endothelial-to–mesenchymal transition and cardiac fibrosis in PAI-1–deficient mice.29

PITX2 Insufficiency Leads to Atrial Electric and Structural Remodeling Linked to Arrhythmogenesis

Summary: Atrial fibrillation (AF) is the most frequent cardiac arrhythmia, leading to a high risk of mortality and morbidity. Though its prevalence is high, genetics of AF has remained rather elusive, with sporadic reports on point mutations in a wide variety of ion channel-encoding genes. Recently, genome-wide association studies have unraveled genetic variants (associated with AF risk) that are located close to the homeobox transcription factor PITX2 in a large proportion of patients with AF. In the present investigation, we corroborated these findings in a small cohort of patients with AF. We also provided evidence that PITX2 is downregulated in patients with AF and experimentally demonstrated that PITX2 insufficiency results in cellular and molecular changes leading to atrial electric and cellular remodeling linked to atrial arrhythmogenesis. Thus, these findings provide insights into signaling pathways that are implicated in the pathogenesis of AF.

Conclusions: This study thus identifies PITX2 as an upstream transcriptional regulator of atrial electric function, the insufficiency of which results in cellular and molecular changes leading to atrial electric and structural remodeling linked to arrhythmogenesis.30

Nonmuscle Myosin Light-Chain Kinase Deficiency Attenuates Atherosclerosis in Apolipoprotein E-Deficient Mice via Reduced Endothelial Barrier Dysfunction and Monocyte Migration

Summary: Endothelial dysfunction and monocyte migration have been implicated in the pathogenesis of atherosclerosis. Nonmuscle myosin light chain kinase (nmMLCK) is known to contribute to inflammation-associated endothelial barrier dysfunction by activating the cytoskeletal contractile response via its kinase activity on myosin light chain phosphorylation. The specific contribution of nmMLCK to atherosclerotic injury and its mechanism of action have not been evaluated. In this study, we tested the hypothesis that nmMLCK promoted atherosclerotic lesion development by altering endothelial barrier properties. In the aorta of apolipoprotein E-deficient mice fed an atherogenic diet, nmMLCK deficiency significantly reduced lesion size, intimal hyperplasia, and macrophage deposition in the vascular wall, indicating a pathogenic role of nmMLCK in atherosclerosis. Consistent with the in vivo observations, nmMLCK expression was detected in both aortic endothelial cells and peripheral monocytes, and nmMLCK deficiency attenuated endothelial hyperpermeability and monocyte transeendothelial migration caused by atherosclerosis-relevant inflammatory stimuli, including thrombin, oxidized low-density lipoprotein, tumor necrosis factor-α, and monocyte chemoattractant protein-1. Further mechanistic studies demonstrated that, in addition to myosin light chain phosphorylation, Src signaling contributed to nmMLCK-induced cellular responses. Pharmacological blockade or genetic manipulation of Src inhibited nmMLCK-mediated hyperpermeability and monocyte transmigration. Taken together, the data suggest a novel function of nmMLCK in atherosclerosis that involves a nonconventional signaling pathway independent of myosin light chain phosphorylation. Further characterization of specific cellular responses to isofrom-specific MLCK kinase activity and kinase-independent mechanisms would contribute to the development of new therapeutic targets for treating atherosclerosis.

Conclusions: Nonmuscle myosin light-chain kinase contributes to atherosclerosis by regulating endothelial barrier function and monocyte migration via mechanisms involving not only kinase-mediated MLC phosphorylation but also Src activation.31

Calcineurin Splicing Variant Calcineurin Aβ1 Improves Cardiac Function After Myocardial Infarction Without Inducing Hypertrophy

Summary: Because the adult mammalian heart has limited regenerative capacity, the considerable loss of cardiomyocytes after myocardial infarction (MI) leads to the formation of a permanent scar and the onset of numerous pathological events that culminate in heart failure. Modulating this response may lessen the consequences of MI and offers opportunity for therapeutic intervention. In this work, we explored the potential benefit of cardiac overexpression of calcineurin Aβ1 (CnAβ1), an alternative splicing isoform of the phosphatase calcineurin found in both progenitor cells and regenerative tissues. Although other calcineurin isoforms play a detrimental role in heart disease, a unique domain in the carboxy-terminus of the protein, confers CnAβ1 the ability to activate a protective pathway in the cardiomyocyte. Mice overexpressing CnAβ1 show significantly improved cardiac function and reduced scar formation after infarction. We identified the underlying molecular mechanism to be interaction between CnAβ1 and mTOR complex 2 and subsequent
activation of the Akt pathway and amino acid biosynthesis response. This work suggests that alternative splicing isoforms of the same protein can have opposite effects on heart pathophysiology and highlights manipulation of alternative splicing, in general, and induction of CnAβ1, in particular, as a future therapeutic approach.

**Conclusions:** Calcineurin Aβ1 shows a unique mode of action that improves cardiac function after MI, activating different cardioprotective pathways without inducing maladaptive hypertrophy. These features make CnAβ1 an attractive candidate for the development of future therapeutic approaches.

**References**


