MicroRNA-181b Inhibits Atherogenesis (p 32)

MicroRNA-181b inhibits vascular inflammation and atherosclerosis in mice, report Sun et al.

Atherosclerosis is a chronic inflammatory vascular disease that is in part driven by the activation of the transcription factor NF-κB. In vascular endothelial cells, oxidized low-density lipoproteins, angiotensin II and other pro-atherogenic factors activate NF-κB. These factors promote the transfer of NF-κB from the cytoplasm to the nucleus where it increases the transcription of pro-inflammatory genes. Recently, miR-181b was found to repress importin-α3, the protein responsible for transferring NF-κB to the nucleus. But whether this miR could actually suppress atherosclerosis was unknown. This prompted Sun and colleagues to inject atherosclerosis-prone mice with a version of miR-181b. They found that miR-181b reduced NF-κB activity as well as the expression of importin-α in the aortic arches of the mice. Importantly, atherosclerotic lesion formation was also reduced, and the lesions contained fewer macrophages and T cells. Interestingly, the team also found that patients with coronary artery disease had reduced levels of miR-181b in their blood. Taken together these results indicate that increasing miR-181b levels in patients could be a potential therapy for diminishing atherogenesis.

Lasting Effects of AAV1/SERCA2a in Heart Failure (p 101)

A gene therapy trial for heart failure patients shows promising long-term results, say Zsebo et al.

A non-fatal heart attack often leads to progressive tissue damage that results in heart failure. Indeed, despite recent advances,
heart failure remains a leading cause of death worldwide. An important factor that contributes to the condition is reduction in the calcium pumping activity of sarcoplasmic reticulum ATPase, or SERCA2a. Previous studies have shown that boosting SERCA2a activity improves cardiac function in animal models of heart failure. Based on these findings, a gene therapy trial was initiated in which the SERCA2a gene was delivered to the coronary arteries of 39 heart failure patients. Twelve months later, patients who received the gene had lower rates of cardiac events including worsening heart failure, myocardial infarction, heart transplant and death compared with those who received the placebo. Zsebo and colleagues now report that, after three years, these patients still showed low rates of cardiac events, and still expressed the transgene in their heart tissue. And because there were no safety concerns with the treatment, these findings suggest that a single dose of SERCA2a gene therapy could have positive long-lasting effects in heart failure patients. Researchers can therefore be somewhat optimistic about the large-scale international trial of the treatment that is currently underway.

Stress-Induced Microparticle Release (p 109)3

Levels of circulating microparticles rise in response to cardiac stress, report Augustine et al.

Microparticles are small—0.1 to 1µm diameter—vesicles released from the surfaces of cells, and are thought to participate in a large variety of physiological processes including cell-to-cell communication and waste disposal. Certain subtypes of these vesicles—identified by the expression of cell-of-origin surface markers—are known to be abundant in the blood of patients with cardiovascular disease. However, similar increases can also be observed in healthy people after strenuous exercise. Thus, Augustine and colleagues measured microparticle levels in the blood of patients following an exercise-free, drug-induced cardiac stress test called a dobustine stress echocardiogram (DSE). Of the 119 patients referred for DSE, 25 had a positive result indicating ischemic heart disease. The remaining 94 patients had negative DSE results. Interestingly, both negative and positive DSE patients had comparable baseline levels of microparticles prior to the stress test, but afterwards only those patients with a negative DSE result showed an increase in circulating microparticles, which returned to baseline approximately an hour later. The authors propose that this rise and fall in microparticles is a normal physiological response to stress and might even be protective against vascular disease. If so, procedures to boost and then clear microparticles may be useful disease prevention strategies.

Cardiosplenic Axis in Heart Failure (p 266)4

Ismahil et al report changes in inflammatory cells in mice with chronic heart failure.

Persistent inflammation is a characteristic feature of heart failure. Indeed in humans increased levels of pro-inflammatory cytokines correlate with severity of heart failure and, in animal models, inflammation has been shown to contribute to ventricular remodeling and contractile dysfunction. However, in clinical trials cytokine antagonism has failed to provide benefits to patients. Given that cytokines are produced by, and target, immune cells, studying the behavior of these cells during chronic heart failure might provide additional insights into the condition. Ismahil and colleagues found that pro-inflammatory macrophages as well as dendritic cells are increased in the hearts of mice with chronic heart failure, while pro-inflammatory monocytes were more abundant in the blood. In addition, they also found that the spleens of the mice displayed increased numbers of dendritic cells, cytotoxic T cells and helper T cells. Strikingly, removal of the spleen reversed the remodeling and inflammation observed in the failing mouse hearts. Furthermore, when spleen cells from mice with heart failure were transferred into healthy mice, the recipient’s hearts began to fail. These results suggest that targeting specific immune cell populations in the heart and or the spleen might engender more effective therapeutic outcomes in chronic heart failure than targeting cytokines alone.

Carotid Intima-Media Thickness in Children With FH (p 307)5

Children with familial hypercholesterolemia show evidence of artery thickening from a very young age, report Kusters et al.
Familial hypercholesterolemia (FH) is characterized by elevated LDL levels in the blood from birth onwards. The condition elevates the risk of atherosclerosis and cardiovascular disease later in life, but sub-clinical thickening of the carotid artery wall—indicative of atherosclerosis—has been observed in children with FH as young as 12. This early wall thickening is the basis of an ongoing safety and efficacy trial of the LDL-lowering drug rosuvastatin in children. Using ultrasound measurement of the carotid artery, Kusters and colleagues have now examined the wall, or intima-media, thickness in even younger children. They recruited 196 children with FH aged between six and 17 as well as 64 of their unaffected siblings. The ultrasounds revealed that, in general, children with FH had significantly thicker intima-media than their unaffected siblings. Furthermore, this significant difference was discernable even before the age of eight. Thus, the new report extends the findings of the previous study to younger children. It also highlights the importance of testing LDL-lowering therapies, like rovustatin, in children of a very young age—the aim being to stop atherogenesis before it starts.

Bone Marrow and Critical Limb Ischemia (p 311)6

Critical limb ischemia is associated with vascular and neuropathic changes in the bone marrow, say Teraa et al. Critical limb ischemia (CLI) is the most advanced stage of peripheral artery disease and is associated with a high risk of amputation, and even death. CLI risk is increased by diabetes and the condition is associated with impaired growth of new blood vessels in the lower limbs, which may be due in part to the reduction in circulating progenitor cells observed in these patients. This reduction in progenitor cell levels suggests that CLI patient bone marrow—the source of circulating progenitor cells—may be dysfunctional. To examine whether CLI damages the bone marrow and how this is affected by diabetes, Teraa and colleagues took bone marrow biopsies from 33 patients with CLI, 13 of whom had diabetes, and 12 of whom did not. Their histological analyses revealed that bone marrow from CLI patients had fewer microvessels—capillaries and sinusoids—and fewer nerve terminals than that from healthy controls. However, the team saw no differences in the microvessel density or the degree of innervation between diabetic and non-diabetic CLI patients. These findings demonstrate that independent of diabetes, CLI is associated with altered bone marrow histology, and suggest such alterations may be the root cause of the diminished circulating progenitor cell numbers.

Imaging Ca2+ Nanosparks in Heart (p 412)7

Shang et al develop a new calcium-sensitive probe to measure calcium nanosparks in dyads of the sarcoplasmic reticulum and the surface membrane.

During excitation-contraction (EC) coupling, the influx of calcium through the voltage-gated calcium channel triggers the release of calcium from the intracellular stores via the ryanodine receptor. This process of calcium-induced calcium release also controls calcium influx in the myocyte via retrograde coupling. Such transient changes in myocyte calcium levels have been extensively studied using calcium-sensitive bioluminescent probes and fluorescent indicators. While the use of these diffusible probes has provided detailed information about the nature of the calcium transient, they do not accurately reveal the behavior of the ryanodine receptor in the microdomain of dyads formed by the close apposition of the surface membrane and the sarcoplasmic reticulum (SR). Hence, to more accurately
measure calcium changes in these nanoscopic dyads, Cheng and coworkers developed a new calcium biosensor in which a calcium-sensitive protein (GCaMP6f) is fused to other proteins (triadin 1 or junction) that localize to the junctional SR. By genetically targeting the probe to dyadic clefts, the authors were able to obtain high resolution, ultra-sensitive images of junctional calcium transients, or “calcium nanosparks,” within a volume that was 50 times smaller than those accessible with conventional diffusible indicators. Because conditions such as heart failure and cardiac arrhythmias are often associated with dysynchronous calcium release, the use of these probes could provide more accurate measurements of disease-induced changes in local calcium release events.

The Hippo Pathway in Arrhythmogenic Cardiomyopathy (p 454)*

Adipogenic differentiation of cardiac myocytes during arrhythmogenic cardiomyopathy is linked to the activation of the Hippo pathway, say Chen et al.

Arrhythmogenic Cardiomyopathy (AC) is a hereditary disease in which cardiac myocytes, mainly those in the right ventricle, are replaced by fibro-adipocytes. The disease often leads to cardiac arrhythmias, heart failure and even sudden cardiac death. Mutations in genes that encode desmosome proteins have been identified as the underlying cause; however, the molecular mechanism by which these mutations lead to disease phenotype remains unknown. Marian and colleagues now report that the so-called Hippo pathway is activated in human hearts with AC, as well as in mouse models of the disease. They found extensive changes in the expression and localization of several proteins in the intercalated discs (IDs) in AC hearts. Not only the activation of the Hippo pathway linked, in part, to a reduced junctional localization of PKCalpha, but it was also associated with the suppression of the canonical Wnt signaling, which regulates cell fate and differentiation. The authors propose that these changes alter the myogenic program and increase the expression of adipocyte genes in cardiac myocytes. As a whole, these findings provide new insights into the role of ID proteins in regulating cell fate and differentiation, and how their molecular remodeling changes myocyte phenotype, as well as the cell-cell interactions that lead to the pathological manifestations of AC.

3-Hydroxykynurenine and Endothelial Dysfunction (p 480)*

Wang et al report that activation of tryptophan metabolism contributes to endothelial dysfunction by increase the generation of reactive oxygen species by NADPH oxidase.

Endothelial dysfunction is a key contributing factor in the development of cardiovascular disease. It is characterized by increased apoptosis of endothelial cells and decreased endothelium-dependent relaxation of blood vessels. While many factors have been shown to contribute to impaired endothelial function, several studies have linked an increase in the local generation of reactive oxygen species (ROS) to endothelial dysfunction. Now, Zou and colleagues report new evidence showing that endothelial dysfunction may actually be linked to the activation of the kynurenine (Kyn) pathway, the major route for tryptophan metabolism in mammals. They found that treatment with angiotensin II, a major inducer of endothelial dysfunction, increased Kyn production via an interferon-gamma-dependent mechanism. This increase in Kyn production was associated with increased activation of the NADPH oxidase, which induced endothelial cell apoptosis by generating high levels of superoxide. Because endothelial injury was diminished in mice lacking components of NADPH oxidase or the kynurenine pathway, the authors suggest that the activation of tryptophan metabolism leads to an up-regulation of NADPH oxidase, which in turn causes endothelial injury. Based on these findings they propose that inhibiting Kyn formation may be a potentially gainful strategy for preventing endothelial dysfunction in cardiovascular disease.

Genetics of Collateral Circulation and Stroke (p 660)*

Sealock et al identify a locus in the mouse genome influencing recovery from ischemic injury.
The severity of tissue damage that results from the occlusion of blood vessels is influenced by the number and size of collateral vessels in the tissue. Collateral vessels link arterioles to one another and hence can provide alternative routes for blood flow should one arteriole become blocked. Recently, a genetic locus that controls collateral size and abundance was discovered within a 27 megabase region on chromosome 7 in the mouse. Through genetic mapping techniques, Sealock and colleagues have now narrowed this region from 27 to 0.7 megabases containing just 28 protein-coding genes. Although the specific gene regulating collateral growth has not been identified, the team showed that a version of the locus associated with increased number and size of collaterals also confers improved recovery from ischemic injury in models of stroke and peripheral artery disease. Importantly, the chromosome 7 locus is partially syntenic with a region of chromosome 16 in man. If this chromosome 16 region also has effects on collaterals, and the gene responsible can be identified, it may provide useful information for assessing a person’s risk for ischemia and their ability to recover from it.

Levels of C reactive protein (CRP) in the blood have long been associated with cardiovascular disease risk. And many of the known factors that cause atherosclerosis also cause levels of CRP to rise. These findings have led to speculation that CRP itself might play a causative role in atherosclerosis. Indeed, studies examining the effect of CRP on human cells in culture, on animals and even on human volunteers have indicated that CRP has a proinflammatory effect. But, Lane and colleagues say that the CRP used in most earlier studies was, a recombinant protein prepared from bacterial cultures. Thus the proinflammatory effects of recombinant CRP may have been caused by bacterial impurities. To test this idea, the team isolated and purified CRP from human plasma and infused this clinical-grade preparation at varying doses into seven healthy adults. By measuring cytokine levels and counting the numbers of neutrophils and platelets in the volunteers after CRP infusion, it was clear that none of the recipients showed any evidence of inflammation. The authors conclude that CRP itself is not proinflammatory and therefore is unlikely to be a useful target for anti-atherosclerosis treatments.

**Interferon and Pulmonary Arterial Hypertension (p 677)**

Interferon prompts the development of pulmonary arterial hypertension in mice, report George et al.

Pulmonary arterial hypertension (PAH), characterized by increased resistance in the lungs’ vasculature, causes strain to the right side of the heart. Left untreated the condition can ultimately lead to heart failure and death. PAH has been linked to HIV, systemic sclerosis and other conditions in which blood levels of the cytokine interferon are elevated. Furthermore, PAH is also associated with therapeutic use of interferon. Despite the accumulating circumstantial evidence, however, no causative role for interferon in PAH has been established. George and colleagues have now found more evidence of a link between interferon and PAH, showing that systemic sclerosis patients with PAH have higher levels of interferon in the blood and increased expression of an interferon receptor in the lungs of PAH patients when compared with patients without PAH. More importantly,
they have also found evidence that interferon signaling is involved in the pathology of PAH. Their experiments showed that mice that lacked an interferon receptor were protected against experimentally induced PAH. These findings provide an explanation for why PAH can develop in patients receiving interferon therapy, and suggest that the interferon pathway may be a useful target for the development of new PAH treatments.

**Imaging Inflammation in Atherosclerotic Lesions (p 770)**

Chèvre et al capture high-resolution images of inflammatory cell dynamics in the mouse carotid artery.

Inflammation plays an important role in atherogenesis from the initiation of lesion formation to the development of unstable plaques. Thus, visualizing the dynamics of inflammatory cells at different stages of plaque growth would provide key mechanistic insights into the pathological process. But in two of the most critical blood vessels—the carotid artery and the aorta—such imaging is technically challenging because the vessels exhibit considerable pulsatile and respiratory movements. Chèvre and colleagues have now developed a method for stabilizing the carotid artery. In this method, they lay an anesthetized mouse on its back, surgically expose the carotid artery and then sandwich the vessel between a metal plate and a microscope coverslip. The mouse and its stabilized artery are then placed under a microscope for imaging. Using this method together with fluorescent labeling of infiltrating cells the team visualized real time dynamics of neutrophils, T cells and other inflammatory cells in the atherosclerotic lesions of mice. Among their findings, they observed that a high fat diet increased the numbers of rolling leukocytes—indicating attachment to the artery wall—and that the recruitment of platelets to the artery wall required interaction with already-recruited myeloid cells. The new system should prove useful for imaging not only atherosclerotic lesions, but a variety of other arterial pathologies, say the authors.

**β-arrestin1 and Processing of MicroRNAs (p 833)**

β-arrestins are regulatory proteins that desensitize G protein-coupled β-adrenergic receptors (βARs) by preventing G-protein mediated signaling. However, β-arrestins can also transduce βAR signals independently of G protein pathways—a recently-discovered function known as biased signaling. Via their G protein-mediated signals, βARs are known to activate a number of cardiac microRNAs (miRs)—small non-coding RNAs that regulate gene expression. Kim and colleagues therefore wondered if βARs could also activate miRs via β-arrestin-mediated biased signaling. They found that a biased signaling agonist—the β-blocker drug carvedilol—could indeed activate subsets of miRs in both human cells and mouse hearts. Rather than upregulating miR transcript expression directly, however, carvedilol upregulated the miR maturation process—by promoting the interaction of β-arrestin with the miR processor Drosha in the nucleus. Carvedilol, which is used to treat congestive heart failure, is a non-specific βAR blocker and a weak activator of the biased signaling pathway. The work by Kim and colleagues lays the foundation for the development of new and more potent drugs that target β-arrestin biased signaling or the downstream miRs for the treatment of cardiovascular disease.

**APOL1 Genetic Variants and CV Disease (p 845)**

Variants of APOL1 commonly carried by African Americans confer an increased risk for cardiovascular disease, say Ito et al.
APOL1 is a major component of the high-density lipoprotein cholesterol transporter in the blood, but can also act as a trypanolytic factor—part of the body’s innate response against trypanosome infections. For example, many African Americans carry versions of the APOL1 gene that protects them against infection with Trypanosoma brucei, an insect-borne trypanosome that causes sleeping sickness. But this benefit comes at a price. Carriers of the protective APOL1 forms are more likely to suffer from chronic kidney disease (CKD). And as Ito and colleagues now show, they are also more likely to suffer from cardiovascular disease. The team sequenced the APOL1 genes of nearly 2000 African Americans and found that individuals carrying two CKD-risk alleles had a two-fold greater risk of cardiovascular events such as myocardial infarction or stroke. By studying a cohort without CKD, the team also showed that the cardiovascular risk was independent of CKD. Somewhat surprisingly, carriers did not differ from other participants with regard to diabetes status, hypertension, left ventricular function or cholesterol levels. Thus, the pathological basis for the increased cardiovascular risk is as yet unknown. Nevertheless, screening African Americans for the risk alleles might help prevent cardiovascular disease, or guide its treatment.

PAD Inhibition and Atherosclerosis (p 947)

Preventing neutrophils from casting nets curbs atherosclerosis development, say Knight et al.

Like Spiderman spinning a web to catch a villain, neutrophils cast chromatin NETs (neutrophil extracellular traps) to capture pathogens. But while this innate immune tactic is useful for defeating foreign infiltrators, it can also damage blood vessels, both by stimulating inflammation and by promoting blood clots. Indeed, these NETs have been found to aggravate the development of atherosclerotic plaques. The formation of NETs by neutrophils is regulated by the enzyme peptidylarginine deiminase. Thus, to determine whether inhibition of this enzyme might attenuate atherosclerotic lesion formation, Knight and colleagues gave atherosclerosis-prone mice a high-fat diet and daily injections of the PAD inhibitor-CI-amidine. After 11 weeks, the CI-amidine treated mice produced fewer NETs and had significantly smaller atherosclerotic lesions. The team also confirmed that the effect of CI-amidine was neutrophil dependent. While neutrophil depletion alone decreased atherogenesis CI-amidine treatment offered no further protection suggesting the inhibitor treatment decreased lesion formation by inhibiting NET formation. The specificity of PAD inhibition—eliminating NETs, but not other immune functions—makes this enzyme an appealing target for possible atherosclerosis prevention.

YAP and Cardiovascular Development (p 957)

YAP is a critical regulator of cardiovascular development, report Wang et al.

YAP is a major effector protein of the Hippo pathway, which regulates organ growth and size in animals. Indeed over-expression of YAP in the embryonic mouse heart causes it to become enlarged, while inactivation of YAP leads to cardiac hypoplasia. In the adult mouse, YAP promotes proliferation of vascular smooth muscle cells (VSMCs) following blood vessel injury. Wang and colleagues therefore wondered whether YAP might also promote VSMC proliferation during vasculogenesis. To test this, they genetically engineered mouse embryos lacking YAP in both cardiomyocytes and VSMCs and observed thinning of the right and left ventricle walls, as well as thinning of the walls of the carotid and thoracic arteries, both of which were associated with reduced cell proliferation. They also found that VSMCs isolated from the engineered mice proliferated less in culture and had increased expression of cell cycle arrest genes. In one of those genes, Gpr132, the team found a binding site for TEAD—a protein that interacts with YAP to silence genes—and increased recruitment of histone deacetylase—a chromatin modifier involved in gene silencing—in the absence of YAP. Given YAP’s role in cardiovascular development, further studies on this protein could provide new understanding of mechanisms underlying congenital cardiovascular disorders.

St3Gal4-Deficiency and Atherosclerosis (p 976)

Döring et al discover that inhibiting sialyltransferase activity in myeloid cells reduces atherosclerosis.
The recruitment of white blood cells to the endothelium is a first step in atherosclerotic plaque development. Deciphering how leukocytes are recruited and how it could be stopped are therefore key goals of atherosclerosis research. It is known that chemokine receptors on the surface of the leukocytes interact with ligands on the vessel wall endothelium, causing the leukocytes to stick and roll along the wall, finally coming to a stop. It is also known that the addition of a sialic acid moiety—sialylation—to one particular leukocyte chemokine receptor is required for interaction with its endothelial ligands. However, whether sialylation is a requirement for other receptor-ligand interactions is not known. Döring and colleagues found that mouse myeloid cells that lacked the sialyltransferase enzyme St3Gal4 had impaired interactions between chemokine Ccl5 and its receptors, while interactions between Ccl2 and its receptor were unaffected. Importantly, the deficiency in Ccl5 interaction was enough to reduce atherosclerotic plaque development in atherosclerosis-prone mice. Blocking sialyltransferase activity in leukocytes may therefore be an effective therapeutic strategy for slowing or stopping atherosclerotic lesion progression, suggest the authors.

Activated Platelet Targeted Fibrinolysis (p 1083)¹⁹

Wang et al develop a low dose, low risk thrombolytic fusion protein for busting blood clots.

Thrombolytic drugs such as tissue plasminogen activator are used to breakdown blood clots in the event of a heart attack or stroke, but because these drugs act systemically, they carry a high risk of causing excessive bleeding. If such drugs could be targeted specifically to blood clots, it would both increase their potency and decrease their associated bleeding risk. Wang and colleagues therefore created a new fusion protein between a plasminogen activator and an antibody that specifically targets clot-initiating activated platelets. They found that the fusion protein inhibited platelet aggregation in culture, and maintained blood flow velocity more effectively than an equivalent dose of commercial untargeted plasminogen activator when given to mice that were induced to develop thrombi. A high dose of the commercial drug gave results similar to the fusion protein, but it dramatically increased bleeding times following tail transections. Based on the ability to the fusion protein to breakdown clots at a lower dose, without drastically increasing bleeding risk, Wang and colleagues suggest that such antibody targeted clot-busting strategies might be used not only as thrombolytic agents but also as a preventative treatment in patients at high risk of thrombosis.

O-GlcNAcylation and Vascular Calcification (p 1094)²⁰

Heath et al find a causative link between O-GlcNAcylation and vascular calcification in diabetes.

Vascular calcification is a common complication of diabetes and is associated with an increased risk of life-threatening cardiovascular events. Determining how calcification occurs and how to prevent it are therefore major goals of current diabetes research. Previous work has shown that proteins in the calcified blood vessels of patients with diabetes exhibit high levels of a post-translational modification called O-GlcNAcylation, in which N-acetylglucosamine moieties are linked to serine or threonine residues. But whether and how O-GlcNAcylation might cause calcification was unknown. Heath and colleagues, therefore, experimentally induced O-GlcNAcylation in mouse vascular smooth muscle cells (VSMCs) and in the blood vessels of diabetic mice and found that both were subject to increased calcification. Furthermore, they found that O-GlcNAcylation directly modified AKT, a protein kinase known to promote VSMC calcification. Mutational analysis revealed that the O-GlcNAcylation occurred on threonine 430 and 479 of AKT, which in turn promoted phosphorylation of serine 473; thereby enhancing AKT activity and activating the downstream calcification pathway. Blocking O-GlcNAcylation of AKT may therefore be a novel strategy for preventing vessel calcification in diabetes, say the authors.
Hwang et al. investigate how different calmodulin mutants affect ryanodine receptor 2 calcium channels.

Calmodulin (CaM) is a calcium-binding protein that regulates several cell functions, including calcium sensing and calcium release from intracellular stores. Mutations in the CaM gene have been linked to cardiac arrhythmia, although different mutations cause different types of irregularity. For example, two specific CaM mutants, associated with stress-induced polymorphic ventricular tachycardia, are referred to as CPVT-CaMs, while another three associated with long QT syndrome are referred to as LQTS-CaMs. In the heart, wild type CaM binds to and inhibits the sarcoplasmic reticulum calcium channel ryanodine receptor 2 (RyR2), reducing the frequency of calcium release. Hwang and colleagues have now discovered that CPVT-CaMs bind with higher efficiency to RyR2 and that they increase rather than reduce the frequency of calcium release. LQTS-CaMs on the other hand tend not to affect RyR2 function, suggesting an alternative mechanism of arrhythmogenesis. CPVT-CaMs dysregulated RyR2 even in the presence of an 8-fold excess of wild type protein. This potency of the mutant proteins explains their dominant phenotypic effects. Altogether the results explain the distinct types of arrhythmia caused by the different CaM mutations, which in turn may help in the development of appropriate treatment strategies for carriers of the mutations.

Götz et al. visualize cGMP dynamics in living cardiomyocytes.

In the heart, cyclic guanosine monophosphate (cGMP) regulates contractility as well as hypertrophy. Indeed, elevated cardiac levels of cGMP in mice have been shown to prevent hypertrophic remodeling and improve heart function. To learn more about this cardioprotective molecule, Götz and colleagues have developed a system that allows real-time visualization of cGMP in living cells. They have genetically engineered mice to express a recently developed, highly sensitive cGMP biosensor called cGES-DE5 specifically in the heart. This biosensor emits green fluorescence, but binding to cGMP alters its conformation and switches its fluorescence emission from green to red—a process known as Förster resonance energy transfer (FRET). Using cardiomyocytes isolated from the mice, the team showed that a vasodilator called C-type natriuretic peptide was a strong stimulator of cGMP synthesis while the enzyme phosphodiesterase 3 (PDE3) was a potent degrader of cGMP. In a mouse model of hypertrophy, another phosphodiesterase, PDE5, also contributed to cGMP degradation. Thus, by revealing the real time dynamics of cGMP this new transgenic system could allow for a more rapid evaluation of interventions and pharmacological agents that affect cGMP levels in the heart.

Yano et al. discover a cardioprotective positive feedback mechanism involving mTORC2 and ribosomal protein S6.

After a myocardial infarction, reperfusion therapy is essential for saving heart tissue and, as a result, the patient’s life. But often reperfusion alone is not enough, and additional cardioprotective treatment strategies are needed to minimize the loss of viable tissue. Researchers have therefore been looking at the protein kinase Akt, which is known to protect heart cells from death. Full activation of Akt requires phosphorylation by a protein called mTORC2, but details of how mTORC2 and Akt are regulated remain unclear. Yano and colleagues show that cardiac preconditioning—a process that increases
the resistance of cardiac tissue to ischemic injury—leads to mTORC2-induced phosphorylation of Akt. Furthermore, inhibiting mTORC2 abolished the cardioprotective effects of preconditioning. The team also discovered that the ribosomal protein Rps6 is a downstream target of Akt, and yet also activates mTORC2-induced Akt phosphorylation. Thus Rps6 both sustains and amplifies the cardioprotective signal. Targeting the mTORC2/Akt/Rps6 positive feedback loop may therefore be an effective complement to reperfusion therapy, suggest the authors.

**Analysis From the POSEIDON Trial (p 1292)**

Injection site matters when it comes to stem cell treatments for heart failure, say Sunicon et al.

Injection of mesenchymal stem cells (MSCs) into the heart can reduce scar size and improve heart function according to the results of a recent clinical trial, but how far from the injection site the beneficial effects spread is unclear. In the trial—called POSEIDON (Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis)—patients with myocardial infarction were injected with stem cells at 10 sites around the infarct border zone. Now, using a high tech imaging approach, Sunicon and colleagues have studied the patients’ hearts more closely and examined whether injected tissue responded differently from nearby non-injected tissue. They found that there was significant scar reduction at tissue segments that received injections. Non-injected sites also exhibited scar reduction, but to a far lesser degree. In terms of muscle function, however, while injected sites showed significant increases in contractility, non-injected sites displayed no improvements at all. Interestingly, the biggest improvements in contractility were seen at those injected tissue segments that were initially the most damaged by infarction. Based on these results, the authors suggest that tailoring injection strategies to an individual patient’s infarct may yield better clinical outcomes.

**CHRF Regulates Hypertrophy (p 1377)**

Wang et al identify two noncoding RNAs that regulate cardiac hypertrophy.

After a myocardial infarction, heart cells enlarge in order to compensate for damaged cells. But if this hypertrophic growth becomes permanent it can actually weaken the heart, leading to heart failure and eventually death. Therefore a better understanding of the factors that control cardiac hypertrophy is needed to develop more effective strategies for prevention and treatment. Noncoding RNAs are known to be important regulators of cellular processes, so Wang and colleagues decided to search for noncoding RNAs involved specifically in hypertrophy. They discovered a noncoding, 23-nucleotide microRNA (miR) called miR-489 that was downregulated in cardiomyocytes treated with Ang-II—a potent inducer of hypertrophy. The team went on to show that knocking down miR-489 in cardiomyocytes caused the cells to become larger, while overexpression of the miR prevented the cells from growing and expressing hypertrophy markers. Transgenic mice that overexpressed miR-489 also displayed a reduced response to hypertrophy induction. Upon further investigation, the team discovered that miR-489 was itself regulated by another noncoding RNA—a 1843-nucleotide RNA they call cardiac hypertrophy related factor (CHRF). Unlike miR-489, CHRF was upregulated during hypertrophy. CHRF, it turned out, directly bound and inhibited miR-489, thus promoting hypertrophy. The team suggests that both CHRF and miR-489 might be suitable targets for developing new therapies for heart failure.

**MALAT1 and Endothelial Cell Function (p 1389)**

A long noncoding RNA called MALAT1 promotes endothelial cell proliferation, report Michalik et al.
The growth of new blood vessels, or angiogenesis, is required for the regeneration of tissues damaged by ischemic injury. On the other hand, preventing angiogenesis can inhibit the growth of life-threatening tumors. There is thus a continuing quest to find factors that promote or reduce angiogenesis. In their own search for such factors, Michalik and colleagues decided to investigate regulatory noncoding RNAs. They found that MALAT1 (metastasis-associated lung adenocarcinoma transcript 1), a long noncoding RNA, was highly expressed in endothelial cells in culture and that its expression was profoundly increased by hypoxia. In vitro, the silencing of MALAT1 increased endothelial cell migration but impaired proliferation. Indeed, the team showed that MALAT1 downregulated the expression of several genes that control the cell cycle. They also found that mice lacking MALAT1 exhibit impaired blood vessel extension and reduced vessel density in their retinas. Taken together, these results suggest that increasing the expression of MALAT1 might be a potentially useful strategy for preventing tissue ischemic injury, while inhibiting MALAT1 might prevent tumor growth.

In heart cells, tight control of calcium release and re-uptake from the sarcoplasmic reticulum (SR) is critical for establishing sturdy excitation-contraction coupling. Indeed, beat-to-beat variations in SR calcium release can lead to beat-to-beat variations in heart cell repolarization—known as alternans. Studies of SR calcium release dynamics and alternans have largely relied on single cell measurements. Wang et al now report simultaneous measurements of intracellular calcium release and transmembrane potential in the intact heart. Using a fluorescent calcium sensor, together with a voltage-sensitive fluorescent dye, they showed that as arrhythmia was experimentally induced, SR calcium release alternans preceded action potential alternans. Furthermore, the onset of alternans varied across the heart. The team also showed that ryanodine receptors (RyR)—calcium release channels of the SR—were to blame for the alternans. RyRs naturally remain closed, or refractory, after calcium release, allowing the SR stores to replenish. But if this refractoriness was pharmacologically reduced—by caffeine treatment—the hearts became less susceptible to alternans. These findings suggest that targeting the refractoriness of RyR could be used for the treatment of some arrhythmias, and that whole heart analysis could be a useful tool for assessing those treatments.

Kumarswamy et al discover an RNA biomarker for heart failure.

Patients that survive a heart attack often develop cardiac remodeling and heart failure, but there are few indicators that help to determine which patients are most at risk. In cancer research, extracellular RNAs have been identified in blood plasma as
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potential biomarkers of disease. Whether heart failure patients might also have clinically informative plasma RNA profiles, however, has not been investigated. Thus, Kumarswamy and colleagues decided to analyze long non-coding RNAs in the plasma of patients who had suffered myocardial infarctions. Using ECG data, the team compared 15 patients who exhibited the most extreme heart remodeling with 15 who exhibited no remodeling at all. They found seven candidate RNAs that differed between these two extreme groups, but after examining the candidates in an additional 216 patients, only two consistently correlated with heart remodeling. They picked one, called LIPCAR (Long Intergenic non-coding RNA Predicting Cardiac Remodeling) and went on to examine its levels in patients with chronic heart failure. Not only was the level of LIPCAR even higher in these patients, but it was able to reliably predict those patients most at risk of suffering fatal cardiovascular events. Although it is not clear exactly why LIPCAR levels increase as heart failure worsens, the RNA still has the potential to be a valuable marker for both tracking and predicting disease progression.

Platelet TLR4 and Pulmonary Hypertension (p 1596)29

The receptor TLR4 on platelets drives the development of pulmonary hypertension, according to a report by Bauer et al.

Pulmonary hypertension—increased blood pressure in the lung vasculature—is a severe and often fatal disease. It was traditionally thought to be triggered by vasoconstriction; however, it is now known that other mechanisms, including thrombosis and inflammation, can also contribute to and even initiate the disease. Indeed, recent evidence suggests that the innate immune receptor TLR4 is involved in the pathology of pulmonary hypertension, although its exact role remains unclear. Bauer and colleagues have now shown that mice lacking the receptor were more resistant to experimentally induced pulmonary hypertension. They also discovered that specific deletion of the receptor from platelets conferred this resistance, but that its deletion from myeloid cells had no effect. Unlike their wild-type counterparts, TLR4-lacking platelets failed to activate during experimental pulmonary hypertension and, in turn, this prevented the characteristic vascular remodeling—such as vessel wall thickening and increased vessel muscularization—associated with the disease. Taken together, the results suggest that blocking interactions between TLR4 and its endogenous ligands could be an effective strategy for treating pulmonary hypertension.

Monocyte and Macrophage Responses in MI (p 1611)30

Hilgendorf et al investigate the macrophage and monocyte milieu after myocardial infarction.

After a heart attack, the removal of damaged tissues and debris is important, but so too is minimizing the scar tissue, which can diminish the functionality of the myocardium. Therefore, an initial inflammatory phase involving recruitment of monocytes expressing high levels of the surface glycoprotein Ly-6C gives way to a reparative phase directed by cells expressing low levels of Ly-6C. It is unclear whether this transition from high to low Ly-6C expression is due to the recruitment of new cells or to the differentiation of high Ly-6C expressing monocytes into low-expressing macrophages. Hilgendorf et al now report that the latter is, in fact, responsible. They found that mice lacking the transcription factor Nr4a1—which is essential for producing low Ly-6C-expressing monocytes, but not macrophages—could still accumulate low Ly-6C-expressing macrophages in the heart after infarction. Since these mice do not produce low-expressing monocytes at all, the low-expressing macrophages must have been derived from high-expressing monocytes. The team also showed that, coincident with transition to the repair phase, Nr4a1 levels increased in the heart, which reduced the recruitment of high Ly-6C-expressing inflammatory monocytes. Thus, because Nr4a1 controls the transition from inflammation to repair, the transcription factor could prove a valuable target of future treatment strategies aimed at minimizing the development of scar tissue in heart failure.

Atherosclerosis Induced by PCSK9 Gene Transfer (p 1684)31

Bjorklund et al describe a faster and easier method for inducing atherosclerosis in mice.
Genetically engineered mice lacking the Apoe or Ldlr gene are the go-to models for studying hypercholesterolemia and atherosclerosis. However, numerous rounds of breeding are required to study the effects of other genetic modifications in these strains. Such procedures are not only time consuming and costly, but they involve the use of a large number of animals. Now Bjørklund and colleagues have devised a new method to induce hypercholesterolemia and atherosclerosis in wild-type mice and hamsters by a single injection of a viral vector encoding proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein known to increase levels of LDL, or “bad” cholesterol in humans and mice. When placed on a high-fat diet the injected animals displayed persistent hypercholesterolemia and atherosclerosis. The team then used this technique to examine the effect of diabetes on atherosclerosis. They found that when injected with the PCSK9 vector, diabetic mice developed more severe hypercholesterolemia and atherosclerosis than non-diabetic mice. The speed and simplicity of this new approach should expedite atherosclerosis research not only in mice and hamsters, but other mammals as well.

Sorafenib Cardiotoxicity Increases Mortality (p 1700)32

Duran et al discover how the anticancer drug sorafenib causes cardiac injury, and how to protect against its cardiotoxic effects.

Sorafenib is a protein kinase inhibitor that has proven to be an effective treatment for solid tumors such as renal cell carcinoma. However, in clinical trials the use of sorafenib is associated with significant cardiotoxicity. Duran and colleagues have now confirmed the cardiotoxicity of this drug in animals; showing that mice given sorafenib are more likely to die after myocardial infarction. The team reports that treatment with sorafenib induces necrosis in myocytes in vitro and in vivo, while causing pathological hypertrophy in surviving myocytes. Moreover, in both in vitro and in vivo experiments, the drug inhibited stem cell proliferation, which the authors suggest could diminish the healing process and thereby exacerbate cardiac injury. Indeed, they found fewer proliferating cells in infarct border zones of sorafenib-treated mice versus untreated mice. Importantly, treatment with the beta-blocker, metoprolol reduced the cardiotoxic effects of sorafenib. On the basis of these observations the authors suggest that treating cancer patients on sorafenib with metoprolol might be a simple way to protect against heart damage.

Ferroxidase I Activity and Heart Failure (p 1723)33

Low ferroxidase activity is a predictor of mortality in heart failure patients, report Cabassi et al.

Heart failure is associated with aberrant production of damaging free radicals derived from both oxygen and nitric oxide. In the body, iron(II) catalyzes the production of these free radicals, while iron(III) is relatively less reactive and less toxic. The enzyme ferroxidase, which is part of the serum protein ceruloplasmin (Cp), converts iron(II) to its safer form iron(III) but, surprisingly, higher levels of Cp have been associated with heart failure, myocardial infarction and mortality. Cabassi and colleagues have now found that while Cp levels were indeed increased in a cohort of 96 elderly heart failure patients, ferroxidase activity was significantly decreased and was lowest in those with most severe symptoms. Ferroxidase was, in fact, a strong predictor of mortality and its activity was inversely related to the level of nitrotyrosine-bound Cp—a modification to the protein known to inhibit its ferroxidase activity. This modification, the team showed, was caused by peroxynitrite—a potent oxidizing and nitrating agent—which is known to be increased in heart failure patients. Altogether the results suggest that low ferroxidase and high nitrotyrosine-bound...
Cp levels may be good indicators of heart failure prognosis, and that increasing ferroxidase activity might be a worthy aim of future therapies.

*Circulation Research, vol 115, 2014*

**miR-33 and Hepatic Lipoprotein Secretion (p 10)**

*Allen et al* advise against targeting miR-33 for decreasing atherosclerotic lesions.

MicroRNAs (miRs) are small RNAs—approximately 22 nucleotides in length—that bind to and suppress the expression of target mRNAs. A number of these have been implicated in regulating several cardiovascular processes. miR-33, for example has been reported to be pro-atherogenic and it has been shown that short-term suppression of this miR in mice increases plasma levels of HDL—the “good” cholesterol. However, contradictory results for miR-33 have also been reported, and now findings by Allen and colleagues support this contradiction. They found that long-term suppression of miR-33 in mice promoted liver secretion of very low-density lipoproteins (VLDLs), or “bad” cholesterol, and that, overexpression of miR-33 in hepatocytes reduced LDL secretion. The team showed that the mRNA encoding NSF, a key lipoprotein vesicle trafficking factor, was directly targeted by miR-33 and that suppression of miR-33 increased NSF expression. The authors conclude that together miR-33 and NSF control the lipoprotein secretory pathway in hepatocytes, but it remains unclear why miR-33 suppression would in one context increase HDL secretion and, in another, VLDL secretion. Nonetheless, the results suggest that simply targeting miR-33 in anti-atherogenic therapies may not be enough to yield desirable outcomes.

**Inhibition of mNCE Prevents Heart Failure (p 44)**

Inhibiting a mitochondrial sodium-calcium exchanger prevents heart failure, arrhythmia and sudden death in guinea pigs, report *Liu et al*.

After a myocardial infarction, heart cells increase in size (hypertrophy) to compensate for the injured, dysfunctional tissue. But chronic hypertrophy can eventually lead to dilated myocardium, impaired contractility, arrhythmia and even sudden cardiac death. Mitochondrial dysfunction is considered to be responsible, at least in part, for the failing myocardium because oxidative stress and limited energy production—both regulated by mitochondria—are associated with the condition. It is thought that elevated levels of cytosolic sodium in failing heart cells diminish mitochondrial calcium via the mitochondrial sodium-calcium exchanger, mNCE. Importantly, inhibition of mNCE in cultured cardiomyocytes not only increases energy production but also decreases the levels of reactive oxygen species triggered by high intracellular sodium. Liu et al now show that inhibition of mNCE can also do this in heart cells from a guinea pig model of heart failure. Moreover, they found that the mNCE inhibitor CGP also attenuated cardiac remodeling and preserved heart function in these animals and protected them from arrhythmia and sudden death. 61 percent of the untreated guinea pigs with heart failure suffered sudden death, while only 14 percent of those receiving continuous mNCE inhibition died. Taken together, these results suggest that blocking mNCE activity could be a possible approach to the treatment of heart failure.

**T<sub>reg</sub> and Macrophage Differentiation Post-MI (p 55)**

Boosting T<sub>reg</sub> activity after myocardial infarction increases survival in mice, report *Weirather et al*.

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**In This Issue** Anthology e105
After a heart attack, immune cells rush to the injury site in an effort to remove dead and damaged myocardial tissue. If this inflammation is exaggerated, however, it can lead to poor healing and overt remodeling. Regulatory T cells or Tregs are immune-suppressors and thus contribute to the resolution of inflammation. Recent studies have reported that these cells in lymph nodes close to the heart are activated after myocardial infarction but Weirather and colleagues wanted to know whether Tregs are also recruited to the heart itself and whether they contribute to the healing process. They found that myocardial infarctions in mice did indeed result in Treg recruitment to the infarct zone and improved functional recovery. Genetic ablation of Tregs in mice increased infarct sizes and left ventricle dilation. Experimentally increasing Treg activity after infarction, on the other hand, improved scar formation and, importantly, increased survival as well—from 47 percent to 77 percent after eight weeks. Increasing Treg activity also led to the production of M2 macrophages in the heart, which are known to be anti-inflammatory and to promote wound healing. These findings suggest that Treg activation in patients with myocardial infarction could be a novel therapeutic strategy.

Benign HCM-Causing Mutation Can Become Malignant (p 227)

An apparently benign mutation in β-MHC turns deadly in the presence of another β-MHC mutation, Blankenburg et al report.

The Notch Pathway and Sox17 Expression (p 215)

Notch suppresses Sox17 expression to inhibit vascular branching, report Lee et al.
cardiomyopathy. Mice that were heterozygous for the R453C mutations, however, displayed progressive hypertrophy and fibrosis, but lived a normal lifespan. Despite lack of symptoms with V606M alone, when crossed with R453C animals, the heterozygous mice had massive fibrosis, rapidly progressing hypertrophy and premature death. Although it remains unclear how the V606M mutation exacerbates disease in the presence of the R453C mutation, these findings suggest that patients found to be carrying one β-MHC mutation should be checked for others.

**MiR-133 Targets β₁AR Pathway (p 273)**

MicroRNA-133 represses components of the β₁-adrenergic signaling pathway, report Castaldi et al.

Hyperactivity of β-adrenergic signaling in cardiomyocytes is associated with heart disease. Indeed, β-blockers are commonly prescribed to treat congestive heart failure. One of the damaging effects of overt β-adrenergic signaling is the promotion of cardiomyocyte apoptosis. A microRNA called miR-133, on the other hand, suppresses cardiomyocyte apoptosis and tends to be expressed at reduced levels in cardiac hypertrophy patients. Castaldi and colleagues now link these findings by showing that miR-133 directly targets and suppresses the expression of the β₁-adrenergic receptor (β₁AR). Bioinformatic searches for candidate miR-133 targets revealed β₁AR, adenylyl cyclase and other components of the signaling cascade, while in vitro experiments confirmed them as targets. In vivo experiments with transgenic mice that overexpressed miR-133 also confirmed the ability of miR-133 to suppress the expression of β₁AR. And, importantly, when cardiac hypertrophy and heart failure were experimentally induced in these mice, they fared much better than control mice—cardiac function was preserved, remodeling of the myocardium was attenuated, and fibrosis was reduced. Based on these findings, the authors suggest that boosting the level of miR-133 in hypertrophy patients where it is reduced could be a novel strategy for the treatment of heart failure.

**Therapeutic Window for Cardiac ROS Suppression (p 348)**

Antioxidant therapies may not always be beneficial, say Song et al.

Mitochondria, when damaged, produce reactive oxygen species (ROS) such as hydrogen peroxide, which at high concentrations could induce substantial cell injury. Therefore, damaged and dysfunction mitochondria are removed by a process called mitophagy. Mitophagy is mediated by a protein called mitofusin, and mice lacking mitofusin in their hearts exhibit reduced mitophagy and increased ROS production. These mice also develop cardiomyopathy, but whether the ROS themselves are responsible for this deterioration was unknown. To test the role of hydrogen peroxide, the team gave a low dose of catalase to the mitofusin-lacking mice. As expected, they found that cardiomyopathy was attenuated—there was evidence of reduced hypertrophy and improved left ventricle function. Mitochondrial dysfunction also decreased, suggesting that ROS cause additional damage to the mitochondria themselves. Unexpectedly however, a high dose of catalase worsened cardiomyopathy, exacerbating heart enlargement and accelerating functional decline. Importantly, the team observed that mitofusin-lacking cells utilized a secondary disposal pathway for eradicating damaged mitochondria. The authors suggest that ROS are the signals for both mitophagy and this secondary pathway and that overt suppression of ROS inhibits these signals, allowing damaged mitochondria to accumulate and interfere with efficient energy production. Caution should be exercised in the treatment of chronic diseases with antioxidants, the authors warn.
Increase in Cardiomyocyte Proliferation by YAP

Activating YAP improves heart function after a myocardial infarction, report Lin et al.

The transcriptional co-activator Yes-Associated Protein, or YAP, is essential for controlling cell proliferation and organ growth in the developing mammalian embryo. Indeed, YAP activation in mouse fetal cardiomyocytes drives proliferation of these cells, while heart-specific deletion of YAP causes cardiac hypoplasia. Lin and colleagues therefore wondered whether inducing YAP in adult heart might provide a means of generating new cardiomyocytes to replace those destroyed by ischemia. To test this, the team generated mice in which YAP expression could be induced specifically within the heart. First, they confirmed that YAP activation did indeed induce adult cardiomyocyte proliferation. Then they found that activating YAP one week after experimental myocardial infarctions led to both a reduction in infarct size and better preservation of cardiac function compared with control mice. Similar results were obtained when the team performed gene therapy with a viral vector containing YAP. Taken together these results suggest that boosting YAP activity could be a novel strategy for diminishing the impact of ischemic injury and other forms of myocardial insults.

Premature Cardiac Failure With Pim Deletion

Loss of Pim kinases promotes premature cardiac aging, report Din et al.

Aging is associated with gradual cardiac deterioration. In the aged hearts, fewer cardiomyocytes are generated, ventricular hypertrophy develops and metabolism switches from mainly fatty acid oxidation to mainly glucose utilization, as seen in heart failure. It was shown recently that overexpression of the protein kinase Pim-1 in aged human cardiac stem cells decreases the expression of senescence markers, and promotes proliferation and survival. Pim-1 is also known to promote mitochondrial integrity and thus protect cells against damaging ROS. Din et al have now examined the effects of Pim deletion on the heart. They generated transgenic mice lacking all three Pim kinases and showed that the mice exhibited premature cardiac hypertrophy—with increased fibrosis and left ventricle enlargement at just 6 months of age. Cardiomyocytes of the mice also showed evidence of senescence, with shorter telomeres, disrupted morphology, reduced ATP production, and a switch to glucose metabolism. Altogether the results corroborate the previous evidence that Pim-1 promotes heart cell rejuvenation and suggest that boosting this kinase might be a useful treatment for attenuating pathogenic changes associated with cardiac aging.

Locus Confined Control of Cardiac Tbx3 Expression

van Weerd et al examine the 3D chromatin architecture of the Tbx3/5 locus.

The abundance, location, and timing of the expression of transcription factors such as Tbx3 and Tbx5 are critical for the precise development of the cardiac conduction system. Recently, common variations in genomic sequence surrounding the Tbx3/5 locus have been linked to conduction system anomalies that pose a risk for heart failure and sudden death in humans. To investigate whether this region contains important Tbx3/5 regulatory elements, Weerd et al used a chromatin conformation capturing technique called 4C-seq, which identifies genomic regions that interact with one another in the 3D nuclear environment. Using this technique, they found that both the Tbx3 and Tbx5 promoters interact with DNA sequences upstream and downstream of the genes themselves. But crucially, there was almost no overlap between the interaction zones for the two genes. In addition to this strict division of interactions, the team found two enhancers upstream of Tbx3 that were essential and sufficient for correct Tbx3 expression...
patterns in the developing mouse heart. Surprisingly, however, these enhancers interacted with the Tbx3 promoter regardless of the cell type. These findings not only further our understanding of the chromatin architecture and transcriptional control of Tbx3 and Tbx5 but also provide insight into how the conduction system develops and how the nearby genetic variations can lead to conduction anomalies.

**Arginase 2 Translocation by OxLDL (p 450)**

Blocking arginase 2 activity reduces severity of atherosclerosis, report Pandey et al.

The amino acid L-arginine is a substrate of nitric oxide synthase (NOS) used in the production of the important vasodilator nitric oxide. But L-arginine is also a substrate for arginase, suggesting that arginase might regulate NOS activity by means of substrate competition. Oxidized low-density lipoprotein (OxLDL, or bad cholesterol) is known to increase arginase 2 activity in human vascular endothelial cells, which could therefore decrease NO production and lead to vascular injury and dysfunction. Nevertheless, how OxLDL increases arginase 2 is unclear. Pandey et al have now shown that OxLDL prompts arginase 2 to relocate from its depot in the mitochondria to the cytoplasm where activity of the enzyme is higher. They also showed that this translocation requires cleavage of arginase 2 by the mitochondrial processing peptide, MPP. The team confirmed that OxLDL led to upregulation of MPP and showed that in atherosclerosis-prone mice, deletion of the arginase 2 gene led to smaller plaques as well as increased NO production and decreased reactive oxygen species in the aortic intima. Collectively, these results suggest that inhibiting arginase 2, by the activation of MPP or other means, could be a novel strategy for anti-atherosclerosis therapy.

**SEMA3A in Brugada Syndrome (p 460)**

Neuronal factor semaphorin 3A plays a novel role in cardiac conduction, report Boczek et al.

Semaphorins are developmental factors that guide the growth and migration of neurons, including those that innervate the heart. But intriguingly, semaphorin 3A (SEMA3A) has a domain that is highly similar to hanatoxin—found in the venom of tarantulas. Hanatoxin is an inhibitor of voltage-gated potassium channels, leading Boczek and colleagues to wonder whether SEMA3A could do the same. In cell culture experiments, they showed that SEMA3A did indeed reduce the current produced by potassium channel K,4.3. And in human and mouse heart tissue preparations, they found that SEMA3A and K,4.3 interact with each other. They also found that of 198 patients with Brugada syndrome—characterized by abnormal electrical activity in the heart and increased risk of arrhythmia—10 had mutations in SEMA3A, and two of these mutations were absent from approximately 20,000 control individuals, suggesting these mutations might be pathological. Indeed, studies showed that cells containing these mutant versions of SEMA3A had higher K,4.3 activity compared with cells containing wild-type SEMA3A. Since an increase in K,4.3 activity has been described in some Brugada syndrome patients, Boczek and colleagues suggest targeting the SEMA3A/K,4.3 interaction as a novel therapeutic strategy.

**Genome Editing of PCSK9 In Vivo (p 488)**

With a single injection Ding et al reduce cholesterol levels in mice.

High levels of low-density lipoprotein (LDL) in the blood are one of the primary risk factors for coronary heart disease, and even though statins can decrease LDL levels, their use does not
Completely mitigate the risk of heart disease. Moreover, some patients are intolerant of statin therapy. Hence, new strategies for reducing LDL levels are highly desirable. In humans, LDL levels are regulated by the liver enzyme PCSK9, and individuals with loss-of-function mutation in the PCSK9 gene are healthy but have lower LDL levels and a lower risk of coronary heart disease. Ding and colleagues therefore wondered whether mutating the Pcsk9 gene artificially in mice would mimic the benefits seen in human PCSK9 mutants. To mutate Pcsk9, the team performed gene-editing—a process whereby specially designed nucleases are directed to cut a particular DNA sequence of interest. They packaged a Pcsk9–specific nuclease into a liver-homing viral vector, and then injected it into mice. Four days after injection, they found that 50 percent of the liver cells contained the mutated protein. Importantly, the treated mice had lower levels of plasma PCSK9 enzyme and lower cholesterol levels. This proof-of-principle experiment paves the way for developing clinical-grade gene-editing strategies for targeting PCSK9 in humans.

AMPK–ACC Signaling and Fatty Acid Oxidation (p 518)47

Theories of fatty acid metabolism in the heart require a rethink, say Zordoky et al.

The kinase AMPK phosphorylates a variety of intracellular targets including the enzyme acetyl-CoA carboxylase (ACC), which is an inhibitor of fatty acid oxidation. AMPK phosphorylation of ACC inhibits the activity of this enzyme and thus AMPK indirectly promotes fatty acid metabolism. In the liver, inactivation of ACC by AMPK is essential for fat metabolism, but Zordoky and colleagues have now discovered that this is not the case in the heart. The team examined fatty acid metabolism in the hearts of genetically engineered mice expressing a version of ACC that was incapable of being phosphorylated by AMPK. They discovered that fatty acid oxidation was unaffected at both low and high workloads and even under ischemic conditions—known to specifically raise AMPK activity. These results indicate that while AMPK may be capable of promoting fatty acid metabolism in the heart it is certainly not required, and that other pathways must be involved. Discovering those pathways may be particularly relevant to the field of ischemia research, because fatty acid metabolism is thought to be the primary energy source in the heart during reperfusion.

Ceramide Changes the Mediator of FID (p 525)48

Ceramide promotes hydrogen peroxide production in the vessels of patients with coronary artery disease, report Freed et al.

In response to shear stress, endothelial cells lining the blood vessels release factors that trigger vasodilation. In general, this flow-induced dilation (FID) is mediated by nitric oxide (NO), but in patients with coronary artery disease the endothelium switches from NO to hydrogen peroxide (H2O2) production. Both factors act as vasodilators, but while NO has anti-inflammatory, anti-thrombotic and anti-proliferative effects, H2O2 is pro-inflammatory, pro-thrombotic and pro-atherogenic. It is not clear why or how this switch occurs, but Freed and colleagues suspected that ceramide might be involved. Ceramide is not only a risk factor for atherosclerosis, but it also promotes mitochondrial production of reactive oxygen species, such as H2O2. Hence, they incubated healthy human arterioles with ceramide and showed that FID switched from being NO-dependent to being H2O2-dependent. They also showed that in arterioles from patients with coronary artery disease, inhibiting ceramide synthesis led to the replacement of H2O2 production by NO production. Together these results suggest that ceramide is a pivotal regulator of damaging H2O2 production and that targeting ceramide or its associated pathways may be a novel therapeutic strategy for the treatment of coronary artery disease.

Mutations in STAP1 Associate With ADH (p 552)49

Fouchier et al discover a new gene associated with autosomal dominant hypercholesterolemia.

In most afflicted individuals, autosomal dominant hypercholesterolemia (ADH) is caused by a mutation in one of three genes—LDLR, APOB or PCSK9, but in others the causative mutation is unknown. Fouchier and colleagues have now performed genetic linkage analysis and exome sequencing on members of a family who have ADH without mutations in LDLR, APOB or PCSK9. In such individuals, they identified a new mutation in the gene STAP1. They found that this mutation was absent from 400 healthy controls of similar ancestry as well as numerous other control exome and genome sequences—thus suggesting the mutation is not merely a harmless variation. Indeed, analysis of STAP1 coding sequences in a cohort of ADH patients revealed another individual with the same mutation and three individuals with alternative mutations in STAP1—one of which were
found in the 400 healthy controls. The team observed that carriers of the \textit{STAP1} mutations had higher levels of both total and LDL cholesterol compared with non-carriers, while levels of HDL were unaltered. Figuring out \textit{STAP1} function, which is currently unknown, should provide novel insight into the pathological pathways of hypercholesterolemia and might even suggest new treatment modalities.

**Human iPSC-CMs as a Model for Viral Myocarditis (p 556)**

Heart cells derived from human induced pluripotent stem cells can be used to study viral infection, report Sharma \textit{et al}.

Between 30 and 50 percent of cases of myocarditis—heart inflammation—are caused by Group B coxsackieviruses. Infections with the viruses can cause heart failure, arrhythmia, and even sudden death, however, at present clinical options for treating the disease are limited. Hence, more in-depth understanding of the underlying mechanism is needed to develop new therapies. But for this, a steady supply of human heart cells is needed. Unfortunately, obtaining heart cells from patients is invasive and expensive, so Sharma and colleagues set out to develop these cells from skin cells, which are relatively easier to get and maintain. Using cells from tiny scraps of skin from healthy people, they induced these cells to become pluripotent stem cells and were then directed them to differentiate into cardiomyocytes. The differentiated cells expressed cardiomyocyte markers including the receptor used by coxsackieviruses for gaining entry. They also exhibited spontaneous regular contractions. Upon infection with a specially constructed luminescent coxsackievirus, the cells began beating erratically and, after approximately 12 hours, they ceased beating altogether. The luminescent virus allowed the researchers to track infection, to gauge viral replication and also to observe the effects of antiviral agents. As such, these cells represent a powerful resource for studying coxsackievirus infection and for high-throughput testing of novel antiviral therapeutics, say the authors.

**Gain-of-Function LDLR for Treating FH (p 591)**

Somanathan \textit{et al} create degradation-resistant LDLR variants to improve the chances of successful gene therapy in familial hypercholesterolemia.

Loss-of-function mutations in the LDL receptor gene cause familial hypercholesterolemia. Indeed, patients who carry two copies of the mutant gene develop early-onset, life-threatening cardiovascular disease. It has been suggested that these patients may benefit from gene therapy using an LDLR-expressing viral vector, but researchers fear that the transfected receptor may be rapidly degraded by PCSK9 and IDOL, proteins that naturally degrade LDLR in the body, thereby minimizing any potential benefits of LDLR gene therapy. Hence to generate a more stable, and less degradable LDLR, Somanathan \textit{et al} created vectors expressing PCSK9- and IDOL-resistant variants of LDLR. They screened a panel of mutant LDLRs to find one that escaped PCSK9 degradation. They also mutated two amino acids in LDLR known to be required for IDOL-induced degradation. Then they incorporated all three mutations into one LDLR gene, cloned the gene into a viral vector, and administered the vector to mice that expressed both human PCSK9 and IDOL. Compared with wild-type LDLR, the mutated LDLR protein avoided both PCSK9 and IDOL degradation in the mice. Levels of intact LDLR were increased and, more importantly, levels of serum cholesterol were suppressed in transfected mice. Based on these data, the authors suggest that inclusion of these mutations in clinical-grade versions of LDLR-containing vectors might increase the efficacy of LDLR gene therapy for familial hypercholesterolemia.

**Fibroblast Behavior Upon Injury (p 625)**

Ali \textit{et al} report that birthplace does not influence future potential—at least for cardiac fibroblasts.
Myocardial infarction or pressure-overload leads to the accumulation of fibrotic scar tissue that hinders the function of the cardiac muscle. Hence, minimizing fibrosis could prevent negative remodeling of the heart and improve cardiac function after injury. However, in the heart, scar formation is driven by fibroblasts, which are a heterogeneous mixture of cells of different origins and it is unclear which of these contribute to injury-induced fibrosis. Therefore, Ali and colleagues performed lineage tracing experiments to track fibroblasts from different origins. They showed that the vast majority of fibroblasts in the adult mouse heart are derived from the epicardium, while a significant proportion comes from the endothelium. A small number of fibroblasts also arise from the neural crest. While these different origins influenced the ultimate location of the cells in the heart, there was no difference in their behavior. Regardless of origin, the cells proliferated at similar rates and exhibited similar gene expression patterns both in vitro and in the hearts of mice subjected to pressure-overload. Thus, therapies aimed at reducing fibrosis in the heart should target pathways common to all fibroblasts, say the authors.

VSMC Plasticity in Atherosclerosis (p 662)$^{53}$

In atherosclerotic plaques, most macrophages come from muscle, say Feil et al.

![Image](image_url)

Atherosclerosis is a chronic inflammatory condition in which the walls of the blood vessels develop plaques filled with fatty deposits and immune cells. Upon plaque rupture, blood flow is interrupted by an occlusive thrombus, which can result in a heart attack or stroke. Indeed, atherosclerosis is a leading cause of death in the developed world. Besides immune cells, smooth muscle cells (SMCs) in the blood vessel walls can also expand and contribute to plaque formation. Interestingly, recent research showed that some cells in plaques show characteristics of both SMCs and macrophages, leading Feil and colleagues to investigate how such cells arise. The team labeled adult differentiated SMCs in mice that are prone to developing atherosclerosis and then tracked the progeny of the labeled cells as lesions developed. They observed that by clonal expansion SMCs contribute to the formation of new plaques and start to express macrophage markers. They found that such SMC-derived macrophages account for the majority of macrophages in the lesions. Based on these findings, the authors say that the extent of SMC plasticity and their contribution to lesion formation may have been vastly underestimated, and that targeting transdifferentiation of SMCs to macrophages could be an important future strategy for slowing the progression of atherosclerotic lesions.

lncRNAs and Myocardial Infarction (p 668)$^{54}$

Vausort et al investigate blood levels of long non-coding RNAs after myocardial infarctions.

![Graph](graph_url)

Although 80 percent of the human genome is transcribed into RNA only two percent is translated into proteins. In scratching the surface of what the remaining 78 percent might do, scientists have discovered that microRNAs are important regulators of gene expression. They have also recently discovered that long non-coding RNAs (lncRNAs)—greater than 200 nucleotides—may play important regulatory roles. Recent studies have shown that lncRNA regulate a variety of physiological functions and they have also been implicated in cardiac disease processes, such as heart failure, hypertrophy and infarction. In addition, levels of lncRNA could also be used as biomarkers of disease risk, progression, or outcomes. Vausort and colleagues therefore analyzed levels of such RNAs in the blood of 414 myocardial infarction patients. Of the five RNAs they investigated—MALAT1, MIAT, aHIF, ANRIL and KCNQ1OT1—MIAT showed no discernable difference between patients and controls, while MALAT1, aHIF and KCNQ1OT1 were all higher in patients than controls and ANRIL was lower. Importantly, they found that two of the RNAs—KCNQ1OT1 and ANRIL—were predictors of left ventricular dysfunction at a four-month follow-up. These findings pave the way for larger-scale analyses both to confirm the usefulness of these lncRNAs as biomarkers and to discover new ones.

14q32 miRs in Neovascularization (p 696)$^{55}$

Welten et al suppress microRNAs to boost neovascularization after ischemia.
Neovascularization is a crucial process for restoring blood supply after injury. Hence, finding factors that promote neovascularization is an important goal in promoting tissue recovery after ischemic insults. Welten and colleagues analyzed the sequences of 127 neovascularization genes, searching for putative binding sites for microRNAs that regulate their mRNAs. They found a large number of possible sites, but they also found an unusually high proportion of sites that were predicted to bind miRs clustering at one particular chromosomal location: 14q32. Microarray analysis of ischemic tissue confirmed the upregulation of 14q32 miRs, and they chose four of these miRs for further study. Using specific gene silencing oligonucleotides, they suppressed each of the four miRs in mice and, then subjected the mice to ischemic injury. Suppression of the four miRs improved blood flow recovery to the affected tissue after the ischemia—both increasing the number of large collateral arteries and, in three of the four cases, increasing capillary density. While further analysis of other potential ischemia-related miRs in the 14q32 cluster is warranted, inhibiting the four miRs studied so far may be a useful approach for future neovascularization therapies.

**FOXF1 Stimulates Vascular Development (p 709)**

The transcription factor FOXF1 is a crucial regulator of vascular development, report Ren et al.

Heterozygous deletions and point mutations of the gene encoding transcription factor FOXF1 account for approximately 40 percent of cases of alveolar capillary dysplasia with misalignment of pulmonary veins (ACD/MPV), which is a fatal congenital disorder characterized by abnormalities in the pulmonary vasculature. Haploinsufficiency of FOXF1 in mice has been shown to cause ACD/MPV-like abnormalities, confirming the importance of this transcription factor in vascular development. To investigate the role of FOXF1 in vasculogenesis, Ren and colleagues created mice in which FOXF1 was specifically deleted in endothelial cells, where the transcription factor is normally expressed during embryogenesis. As a result, the endothelial cells exhibited decreased proliferation and increased apoptosis, while the mice themselves had severe cardiovascular defects, growth retardation, and died in utero.

The team discovered that FOXF1 regulated the transcription of Flk1 and Flt1—receptors for vascular endothelial growth factor (VEGF)—and thus, in the absence of functional FOXF1, endothelial cells were unresponsive to VEGF stimulation. The results indicate that FOXF1 or its downstream targets, such as Flk1 and Flt1, may be promising pharmaceutical targets to treat ACD/MPV patients.

**Basigin in Pulmonary Hypertension (p 738)**

Cyclophilin A and its receptor basigin promote pulmonary hypertension in mice, report Satoh et al.

Pulmonary hypertension (PAH) is a severe, often life-threatening, disease characterized by vasoconstriction, remodeling of the lung vasculature, vascular smooth muscle cell (VSMC) proliferation and perivascular inflammation. Hypoxia is a characteristic feature of PAH pathogenesis. It induces VSMCs to secrete cyclophilin A (CyPA), which in turn stimulates...
VSMC proliferation and attracts inflammatory cells. Satoh and colleagues discovered that both CyPA and its extracellular receptor basigin are strongly expressed in the remodeled pulmonary arteries of PAH patients. Furthermore, high levels of CyPA in patient plasma were associated with poor outcome, suggesting that CyPA could be used as a biomarker of disease progression. The team also found that deficiency of either CyPA or basigin in mice ameliorated the development of hypoxia-induced PAH. VSMCs from basigin-deficient mice proliferated less than those derived from wildtype mice, and produced lower levels of inflammatory cytokines, the team showed. Together the results suggest that inhibiting the activity of CyPA and/or basigin could be a therapeutic strategy for future PAH treatments.

Anti-miR33 Improves Plaque Regression in Diabetes (p 759)58

Distel et al suggest an additional therapy for treating atherosclerosis in people with diabetes.

The second heart field (SHF), discovered a little over a decade ago, is a cell population in the early embryo that contributes to the development of the heart. The transcription factor TBX1 regulates proliferation and differentiation of SHF cells and is also thought to drive migration of SHF cells to the arterial pole of the developing heart, including what will later become the outflow tract. Indeed, Rana and colleagues discovered that, unlike wild type mouse embryos, TBX1-/- embryos lacked labeled SHF-derived cells in their developing outflow tracts. However, at stages E14.5 to E18.5 the TBX1-/- embryos also exhibited atrioventricular septal defects, which are indicative of problems arising from the venous pole of the heart. Genetic tracing experiments revealed that unlike in wild-type embryos, in the TBX1-/- embryos the SHF cells failed to contribute to venous pole structures—suggesting that TBX1 regulates the migration of progenitor cells to this region. These results provide new information about the origin of heart cells as well as the underlying mechanisms that control their ultimate destinations, information that is important for understanding the etiology of congenital heart malformations and for developing potential preventive strategies.

Cell-based revascularization therapies may benefit from a boost in activin A, say Merfeld-Clauss et al.
Cell therapies that combine endothelial cells (ECs) and adipose stromal cells (ASCs) have been suggested as potential treatments to promote the growth of new blood vessels in ischemic tissue. The ability of these two cells to form vascular networks in vitro is associated with the induction of smooth muscle actin (αSMA)—a marker of vascular smooth muscle cells (VSMCs)—in ASCs following their contact with ECs. Merfeld-Clauss and colleagues have now shown that this EC-ASC contact is in fact essential for the differentiation of ASCs into VSMCs and for the development of vascular networks. Indeed, when separated from ECs, ASCs cultured in the same dish do not differentiate. Investigating the underlying mechanism, the team found that when ASCs come in contact with ECs, their activin A, which induces αSMA expression, is increased. These cells also secrete activin A, which enables nearby ASCs (not in contact with ECs) to differentiate. Given that activin A appears to be a master regulator of VSMC differentiation, the authors suggest that modulating activin A activity could enhance the outcomes of cell therapies comprising ECs and ASCs.

miR-195 and Aortic Aneurysms (p 857)61

MicroRNA-195 is a biomarker, but not a target, for aortic aneurysmal disease, report Zampetaki et al. Abdominal aortic aneurysm (AAA) is the ballooning of the aorta, creating a risk of life-threatening rupture. The condition is associated with a loss of elastin and increased turnover of collagen in the vessel wall. Previous work has shown that miR-29 inhibits the expression of both elastin and collagen and, that inhibition of miR-29 consistently reduces aortic aneurysm in mice. Zampetaki and colleagues now show that miR-195 also targets elastin. They found that overexpression of miR-29 or miR-195 caused a comparable decrease in elastin levels in vitro, while inhibiting miR-195 in mice prompted an increase in elastin expression. Unlike miR-29 inhibition, however, the inhibition of miR-195 did not protect mice from aortic aneurysm. This appears to be related to the upregulation of matrix metalloproteinase enzymes that degrade the collagen and elastin containing extracellular matrix, upon miR-195 inhibition. The team also found that miR-195 levels in human plasma were inversely correlated with aortic diameter and with the occurrence of AAA. Therefore, even though miR-195 may not be a target for inhibition in AAA therapies, it could serve as a useful biomarker of the condition, say the authors.

Hypoxia and IL-1β Production in Macrophages (p 875)62

Hypoxia boosts inflammation in atherosclerotic plaques, say Folco et al.

Atherosclerosis is primarily an inflammatory condition associated with the influx of a large number of macrophages and the production of inflammatory cytokines—such as IL-1β—within plaques. Now Folco and colleagues report that not only does inflammation drive atherosclerosis,
the conditions within the plaques make inflammation even worse, perpetuating the problem. Most cells within plaques experience a moderate to severe lack of oxygen, and the team found that this hypoxia drives IL-1β production. They exposed human macrophages to 2% oxygen for 24 hours and discovered that, when activated, the cells could produce and secrete approximately three times more IL-1β protein than macrophages exposed to normal oxygen levels. IL-1β mRNA levels in the cells remained similar indicating post-transcriptional control. Indeed, they found that hypoxia stabilized IL-1β by limiting degradation of the protein in autophagosomes—a waste disposal system within cells. The team went on to show that the most hypoxic regions of dissected human plaques accumulated IL-1β. The results indicate that neutralizing IL-1β, which is currently being tested in patients with coronary artery disease, could help reduce chronic inflammation in atherosclerosis.

De Novo CNVs in Congenital Heart Disease (p 884)63

Glessner and colleagues detect increased copy number variations and novel candidate loci involved in congenital heart disease.

Congenital heart disease (CHD) is one of the most common birth defects, but in a majority of cases the etiology remains unknown. Several genome-wide studies to find loci associated with CHD have been conducted; however, they generally employed low-resolution methods such as comparative genomic hybridization and low-density single nucleotide polymorphism (SNP) analysis. Such studies have identified broad regions of the genome that may be involved in the disorders, but not specific genes. Glessner and colleagues have therefore performed high-density SNP analysis and/or whole-exome sequencing on a total of 538 CHD trios (affected offspring and their parents). They confirmed previous findings that locus copy number variations (CNVs) tend to be increased in these individuals and identified a total of 65 novel CNV sites. Importantly the higher resolution approaches led to the identification of candidate genes associated with CHD. For example, the recurrent CNV at chromosome location 15q11.2 incorporates three genes: CYFIP1, NIPA1, and NIPA2. These and the other new loci might act both as new diagnostic markers of CHD as well as possible targets for therapy.

Nox1 Phosphorylation Mediates NoxA1 Association (p 911)64

Streeter et al suggest a way to inhibit the vascular disease-causing enzyme NADPH oxidase.

Nox1 is the catalytic subunit of the enzyme NADPH oxidase, which transfers electrons from NADPH to oxygen to create damaging reactive oxygen species (ROS). The enzyme is present in a number of cell types such as vascular smooth muscle cells (VSMCs) and an increase in its activity has been linked to the development of atherosclerosis and other vascular diseases. Streeter and colleagues now show that activation of Nox1, which requires interaction with subunit NoxA1, depends on Nox1 phosphorylation. The team discovered that in the arteries of animals with vascular disease or injury—monkeys fed an atherogenic diet and rodents that sustained vessel injuries—Nox1 phosphorylation was significantly increased. They localized this phosphorylation event to tyrosine residue 429 of the protein, and showed by mutational analyses that phosphorylation of this residue was required for both association with NoxA1 and for enzyme activity. Cells containing a non-phosphorylatable version of Nox1 produced fewer ROS than those containing the wild-type protein. Together the results indicate that preventing tyrosine 429 phosphorylation of Nox1 might be a novel mechanism for diminishing vascular oxidative stress.

Ankyrin-G Signaling Platform (p 929)65

Makara et al report the role of ankyrin-G in cardiomyocyte excitability.
The voltage gated sodium channel Nav1.5 is critical for excitation and conduction in cardiomyocytes. Indeed mutations to the gene encoding Nav1.5 (SCN5A) are associated with atrial fibrillation, ventricular arrhythmias and other conduction abnormalities. But surprisingly little is known about the regulation of this channel. Ankyrin proteins target ion channels to membranes in a variety of excitable cells and ankyrin-G has been shown to associate with Nav1.5 in cultured cells. To examine whether this association occurs in vivo and to assess its significance, Makara and colleagues engineered mice with a conditional heart-specific knock out of ankyrin-G. Heart cells from these mice showed a loss of Nav1.5 localization to intercalated disks—specialized junctions between heart cells where ankyrin-G and Nav1.5 both normally reside. The cells also exhibited abnormal Nav1.5-dependent sodium currents. The kinase CAMKIIδ, which phosphorylates Nav1.5, was also absent from intercalated discs and, as a result, Nav1.5 phosphorylation was reduced. The team also found that the engineered mice had cardiac conduction abnormalities and were more prone to induced arrhythmias than control mice. The study provides greater insight into the cell biology of Nav1.5, which in turn should help with the design of treatments for Nav1.5-associated arrhythmias.

CD133+ Progenitor Cells to Promote Angiogenesis
(p 950)

Jimenez-Quevedo et al present the final two-year follow-up results of the PROGENITOR trial for ischemic heart disease.

Amp II protein is required for correct formation of transverse tubules in the heart, say Caldwell et al.

Transverse (t) tubules are plasma membrane invaginations that stretch across cardiomyocytes and are closely associated with sarcoplasmic reticulum (SR). This t-tubule network is required for synchronizing the release of calcium from the SR during heart muscle contraction. Indeed, loss of t-tubules is associated with heart failure. Despite their essential function, little is known about how t-tubules are generated and the
mediators regulating their formation have not been identified. One candidate mediator is Amp II, which is thought to direct calcium channels to t-tubules and is decreased in heart failure, much like t-tubules themselves. Caldwell and colleagues now show that Amp II expression not only correlates with t-tubule density, but that it also participates in their formation. In large mammals, such as humans and sheep, t-tubules are less extensive in atria than ventricles, while in mice and rat atria t-tubules are almost entirely absent. Caldwell’s team showed, that Amp II expression is lower in atria than ventricles of both large and small mammals. They then showed that silencing Amp II expression in rat ventricular cells reduced t-tubule density. It also decreased the amplitude and synchronicity of calcium release in these cells. The results suggest that boosting Amp II expression might be a way to restore t-tubules and synchronize contractility in failing hearts.

Thymidine Phosphorylase and Thrombosis (p 997)68

Li et al uncover a novel target protein for anti-thrombotic therapies.

Cardiac progenitors differentiate with the help of Abi3bp protein, report Hodgkinson et al.

Cardiac progenitor cells ultimately differentiate into cardiac myocytes, smooth muscle cells and endothelium, but the factors and processes driving this differentiation have not been completely identified. The progenitors share many features with mesenchymal stem cells (MSCs), leading Hodgkinson and colleagues to wonder whether the protein Abi3bp, which prompts differentiation in MSCs, might play the same role in cardiac progenitors. They observed that expression of Abi3bp was 100-fold lower in isolated cardiac progenitors than in cardiac myocytes, but that its expression was increased upon differentiation. Moreover, cardiac progenitors from Abi3bp-lacking mice failed to differentiate correctly in culture, exhibiting aberrant expression levels of cardiac myocyte differentiation markers. This impaired differentiation also correlated with impaired recovery after myocardial infarction in the Abi3bp-lacking mice. The team also found that Abi3bp interacts with integrin-β, and that this partnership, together with the downstream phosphorylation of kinases PKC and Akt, is important for Abpi3bp-driven cardiac myocyte differentiation. The finding that Abi3bp promotes cardiac progenitor differentiation and improves heart tissue recovery after injury may be important for designing future progenitor-based therapies, say the authors.
References

1. Sun X, He S, Wara AKM, Icli B, Shvartz E, Tesmenitsky Y, Belkin N, Li D, Blackwell TS, Sukhova GK, Croce K, Feinberg MW. Systemic delivery of microRNA-181b inhibits nuclear factor-kB activation, vascular inflammation, and atherosclerosis in apolipoprotein E-deficient mice. Circ Res. 2014;114:32–40. doi: 10.1161/CIRCRESAHA.113.302089.

2. Zseo B, Yaroshinsky A, Rudy JJ, Wagner K, Greenberg B, Jessup M, Hajar RJ. Short-term effects of AAV1/SIRC4a2 gene transfer in patients with severe heart failure: analysis of recurrent cardiovascular events and mortality. Circ Res. 2014;114:101–108. doi: 10.1161/CIRCRESAHA.113.302421.

3. Augustin D, Ayers LV, Lima E, Newton L, Lewandowski AJ, Davis EF, Ruggiero A, Willerson JT, Marian AJ. The Hippo pathway is activated and is a causal mechanism for adipogenesis in murine models of atherosclerosis. Circ Res. 2014;114:947–956. doi: 10.1161/CIRCRESAHA.114.303265.

4. Ismahil MA, Hamid T, Bansal SS, Patel B, Kingery JR, Prabhu SD. The role of tumor necrosis factor alpha in the development of diabetic cardiomyopathy. Circ Res. 2014;114:307–310. doi: 10.1161/CIRCRESAHA.114.301430.

5. Vlcek R, Gonzalez-Granado JM, Megens RT, Sreeramkumar V, Choudhry K, Baliga JS, Balliss MB, Wang SQ, Cheng H. Imaging Ca2+ nanosparks in heart with a new targeted biosensor. Circ Res. 2014;114:423–432. doi: 10.1161/CIRCRESAHA.114.303265.

6. Chedeville R, Gervasi-Granado JM, Megen RT, Steenbakkers V, Ziemiecki D, Sandoval KM, Langenbahn DM, Sorensen K, Tesch P, Abraham TP, O'Rourke B. Inhibiting mitochondrial Na+/Ca2+ exchange abrogates short circuit-mediated myocardial calcium influx and prevents arrhythmia. Circ Res. 2014;114:357–366. doi: 10.1161/CIRCRESAHA.114.303265.

7. Schuster W, Spentzos D, Linder H, Gunther W, Reisner J, Pfeffer MA, Högstedt B, Kasper W, Pabst M. Reduced expression of microRNA-181b inhibits nuclear factor-kB activation and prevents atherosclerotic lesion development. Circ Res. 2014;114:1596–1600. doi: 10.1161/CIRCRESAHA.114.303312.

8. Duran JM, Makarewich CA, Trappensee D, et al. Sorafenib cardiototoxicity increases mortality after myocardial infarction. Circ Res. 2014;114:1700–1712. doi: 10.1161/CIRCRESAHA.114.303200.

9. Cabassi A, Bimbo SM, Tedeschi S, Ruzicka V, Dancelli S, Rocco R, Vicini V, Coghi P, Regolisti G, Montanari A, Fiaccadori E, Govoni P. PTC124 reduces cardiac injury in heart failure with preserved ejection fraction. Circ Res. 2014;114:1684–1689. doi: 10.1161/CIRCRESAHA.114.303937.

10. Allen RM, Marquart TJ, Jesse JJ, Ittzke D, Baldon JD, Cheng H. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res. 2014;114:1777–1786. doi: 10.1161/CIRCRESAHA.114.303662.

11. Wang X, Palusubramaniam J, Gkanatsas Y, Hohmann JD, Westein E, Kanojia R, Alt K, Huang D, Jia F, Ahrens I, Medcalfe RL, Peter K, Hambry-Gouzy CE. Towards effective and safe thrombolysis and thromboprolylaxis: preclinical testing of a novel antibody-targeted recombinate plasminogen activator directed against activated platelets. Circ Res. 2014;114:1083–1093. doi: 10.1161/CIRCRESAHA.114.303514.

12. Heath JM, Sun Y, Yuan K, Bradley W, Litovsky S, Della Italia LJ, Chatham JC, Wu H, Fré Tu, Yang Y, Wallewke K, Pereira L, Johnson CN, Faggioni M, Chazin WJ, Laver D, George AL Jr, Cornea RL, Berts DM, Knollmann BC. Divergent regulation of rydanoendorceptor 2 calcium release channels by arhythmogenic human calmodulin missense mutants. Circ Res. 2014;114:1114–1124. doi: 10.1161/CIRCRESAHA.114.303391.

13. Wang Q, Ding Y, Zhang W, Wang Q, Zhang W, Song P, Zou MH. The role of tumor necrosis factor alpha in the development of diabetic cardiomyopathy. Circ Res. 2014;114:1684–1689. doi: 10.1161/CIRCRESAHA.114.302937.

14. Wang Q, Zhang W, Ding Y, Wang Q, Zhang W, Song P, Zou MH. NF-kappaB activation, vascular inflammatory responses in carriers of APOL1 genetic variants. Circ Res. 2014;114:101–108. doi: 10.1161/CIRCRESAHA.114.303312.

15. Kamarswamy R, Bauters C, Volkman I, Maury F, Fetisch J, Holzmann T, Lemesle G, Grooste P, Fitting F, Tham T. Circulating long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res. 2014;114:1377–1388. doi: 10.1161/CIRCRESAHA.114.302476.

16. Kim HJ, Lee WJ, Lee WK, Shin YW, Park JH, et al. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489. Circ Res. 2014;114:1777–1786. doi: 10.1161/CIRCRESAHA.114.303204.
exchange prevents sudden death in a guinea pig model of heart failure. Circ Res. 2014;115:45–54. doi: 10.1161/CIRCRESAHA.115.303062.

36. Weirather J, Hofmann UD, Beyerdsdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerka T, Frantz S. Fogo3–CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. Circ Res. 2014;115:55–67. doi: 10.1161/CIRCRESAHA.115.303095.

37. Lee SH, Lee S, Yang H, Song S, Kim K, Saunders TL, Yoon JK, Koh GY, Kim I. Notch pathway targets proangiogenic regulator Sox17 to restrict angiogenesis. Circ Res. 2014;115:215–226. doi: 10.1161/CIRCRESAHA.115.303142.

38. Blankenburg R, Hackert K, Wurster S, Deenen R, Seidman JG, SeidmanSeidman CE, Lohse MJ, Schmitt JP. β-myosin heavy chain variant Val606Met causes very mild hypertrophic cardiomyopathy in mice, but exacerbates HCM phenotypes in mice carrying other HCM mutations. Circ Res. 2014;115:227–237. doi: 10.1161/CIRCRESAHA.115.303178.

39. Castaldi A, Zagli T, Di Mauro V, et al. MicroRNA-133 modulates the β1-adrenergic receptor transduction cascade. Circ Res. 2014;115:273–283. doi: 10.1161/CIRCRESAHA.115.303252.

40. Song M, Chen Y, Murphy E, Rabinovitch PS, Dorn GW II. Superoxide suppression of mitochondrial reactive oxygen species signaling impairs compensatory autophagy in primary mitochondrial cardiomyopathy. Circ Res. 2014;115:348–353. doi: 10.1161/CIRCRESAHA.115.303384.

41. Lin Z, von Gise A, Zhou P, Gu F, Ma Q, Jiang J, Yau AL, Buck JN. Ceramide changes the mediator of flow-induced vasodilation from nitric oxide to hydrogen peroxide in the human microcirculation. Circ Res. 2014;115:552–555. doi: 10.1161/CIRCRESAHA.115.303441.

42. Din S, Konstandin MH, Johnson B, Emathinger J, van de Werken CA, Rader DJ, Musunuru K. Permanent alteration of PCSK9 with in vivo CRISPR-Cas9 genome editing. Circ Res. 2014;115:556–566. doi: 10.1161/CIRCRESAHA.115.303836.

43. van Weerd JH, Badi I, van den Boogaard M, Stefanovic S, van de Werken CA, Barnett P, Christoffels VM. A large permissive regulatory domain consistent with premature aging results from deletion of Pim kinases. Circ Res. 2014;115:591–599. doi: 10.1161/CIRCRESAHA.115.303794.

44. Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff K, Schaller M, Feil K. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells contributing atherogenesis. Circ Res. 2014;115:662–667. doi: 10.1161/CIRCRESAHA.115.304634.

45. Vausort M, Wagner DR, Devaux Y. Long noncoding RNAs in patients with acute myocardial infarction. Circ Res. 2014;115:668–677. doi: 10.1161/CIRCRESAHA.115.303836.

46. Welten SM, Bastiaansen AJ, de Jong RC, de Vries MR, Peters EA, Boonstra MC, Sheikh SP, La Monica NL, Kandimalla ER, Quax, PH. Nossent AJ. Inhibition of 14G32 monoclonal antibody activity by mr329, mr487b, mr494, and mr495 increases neovascularization and blood flow recovery after ischemia. Circ Res. 2014;115:696–708. doi: 10.1161/CIRCRESAHA.114.304747.

47. Ren X, Ustyan V, Pradhan A, Cai Y, Havrilak JA, Bolte CS, Shannon JM, Kalin TV, Kalinichenko VV. FOXF1 transcription factor is required for formation of embryonic vasculature by regulating VEGF signaling in endothelial cells. Circ Res. 2014;115:709–720. doi: 10.1161/CIRCRESAHA.115.304382.

48. Satoh K, Satoh T, Kikuchi N, et al. Basigin mediates pulmonary hypertension by promoting inflammation and vascular smooth muscle cell proliferation. Circ Res. 2014;115:738–750. doi: 10.1161/CIRCRESAHA.115.304563.

49. Distel E, Barrett TJ, Chung K, Girgis NM, Parathath S, Essau CC, Murphy AJ, Moore KJ, Fisher EA. miR33 inhibition overcomes deleterious effects of diabetes mellitus on atherosclerosis plaque regression in mice. Circ Res. 2014;115:759–769. doi: 10.1161/CIRCRESAHA.115.304591.

50. Rana MS, Théveniau-Ruissy M, De Bono C, Mesbah K, Francou A, Rammah M, Domínguez JN, Roux M, Lalonge B, Anderson RH, Mohun T, Zaffran S, Christoffers VL, Kelly RG. Tbx1 coordinates adaption of posterior second heart field progenitor cells to the arterial and venous poles of the heart. Circ Res. 2014;115:790–799. doi: 10.1161/CIRCRESAHA.115.305020.

51. Manders-Claus S, Lupp J, Lu H, Feng D, Compton-Craig P, March KL, Traktuev DO. Adipose stromal cells differentiate along a smooth muscle lineage pathway upon endothelial cell contact via activation of activin A. Circ Res. 2014;115:800–809. doi: 10.1161/CIRCRESAHA.115.304026.

52. Zampetaki A, Attia R, Mayr U, Gomes RMS, et al. Role of miR-195 in aortic aneurysmal disease. Circ Res. 2014;115:857–866. doi: 10.1161/CIRCRESAHA.115.304361.

53. Fesole EJ, Sukhova GK, Quillard T, Libby P. Moderate hypoxia potentiates interleukin-β production in activated human macrophages. Circ Res. 2014;115:875–883. doi: 10.1161/CIRCRESAHA.115.304437.

54. Glessner JT, Bick AG, Ito K, et al. Increased frequency of de novo copy number variants in congenital heart disease by integrative analysis of single nucleotide polymorphism array and exome sequence data. Circ Res. 2014;115:884–896. doi: 10.1161/CIRCRESAHA.115.304588.

55. Steiner J, Schikling KM, Jiang S, Stanic B, Thiel WH, Gakkar L, Houten JC, Miller FJ Jr. Phosphorylation of Nox1 regulates association with NoxA1 activation domain. Circ Res. 2014;115:919–918. doi: 10.1161/CIRCRESAHA.115.304267.

56. Makara MA, Curran J, Little SC, Musa H, Polina I, Smith SA, Wright PJ, Unudurthi SD, Snyder J, Bennett V, Hund TJ, Mohler PJ. Ankyrin-G coordinates intercellular signaling to restrict angiogenesis. Circ Res. 2014;115:929–938. doi: 10.1161/CIRCRESAHA.115.305154.

57. Jimenez-Quevedo P, Gonzalez-Ferrer JJ, Sabate M, et al. Selected CD133+ progenitor cells to promote angiogenesis in patients with refractory angina: final results of the PROGENITOR randomized trial. Circ Res. 2014;115:950–960. doi: 10.1161/CIRCRESAHA.115.303463.

58. Caldwell JL, Smith CE, Taylor RF, Kinimoto A, Eisser DA, Dibb KM, Trafford AW. Dependence of cardiac transverse tubules on the BAR domain protein amphiphysin II (BIN-1). Circ Res. 2014;115:986–996. doi: 10.1161/CIRCRESAHA.115.304164.

59. Li W, Gigante A, Perez-Perez MJ, Yue H, Hirano M, McIntyre TM, Silverstein RL. Thymidine phosphorylase participates in platelet signaling and promotes thrombosis. Circ Res. 2014;115:997–1006. doi: 10.1161/CIRCRESAHA.115.304591.

60. Hodgkinson CP, Gomez JA, Payne AJ, Zhang L, Wang X, Dal-Pra S, Prasad R, Dzau VJ, Dzau VJ. SIRT6 regulates cardiac progenitor cell proliferation and differentiation. Circ Res. 2014;115:1007–1016. doi: 10.1161/CIRCRESAHA.115.304216.