Over the past several years, the development of new technologies and techniques has continuously accelerated the pace of discovery and translation in cardiovascular genetics research and the year 2014 turned out to be no exception. Many of the great scientific advances done in the year 2014 were characterized by being multidisciplinary collaborative efforts using an array of skills and knowledge aiming to increase our understanding of molecular, cellular, and genetic determinants of cardiovascular disease, drug responses, and patient outcomes.

The American Heart Association Functional Genomics and Translational Biology Council (www.my.americanheart.org/fgtbcouncil) provides an international and multidisciplinary platform for advancing discoveries from genetics, systems, and translational biology and facilitates their application in global cardiovascular health and disease to build healthier lives, free of cardiovascular diseases and stroke. With input from the Early Career Committee of the Council of Functional Genomics and Translational Biology, we considered many great advances published during 2014 and selected 10 outstanding ones. In this Special Report, we summarize these advances.

The scientific understanding of how cells respond to environmental changes and maintain a dynamic equilibrium took a large leap forward with the discovery of large intergenic noncoding RNAs (lincRNAs). These key regulators of cellular processes are defined as nonprotein coding transcripts longer than 200 nucleotides whose identification has been made possible by advances in RNA sequencing. Han et al. identified a family of such lincRNA transcripts in the Myh6/7 locus of normally functioning myocytes, which they named myosin heavy-chain–associated RNA transcripts (Mhrt). These lincRNAs were present in myocardium but not endocardium or epicardium by in situ RNA analysis, and were reduced in response to stress, such as transverse aortic constriction. The authors demonstrated a cardioprotective effect of this lincRNA by inducing expression of Mhrt779 (the most abundant of the family of lincRNAs identified at the locus) at the time of transverse aortic constriction stress, and impressively 2 weeks after initiation of stress. In both the cases, there was a significant reduction in hypertrophy and improved fractional shortening with Mhrt779 expression. The authors concluded by identifying a negative feedback loop between Mhrt and the Brg1-Hdac-Parp chromatin repressor complex, which explains not only the reduction in expression of Mhrt in settings of myocardial stress but also the role of Mhrt in sequestering Brg1 from its genomic DNA targets to prevent chromatin remodeling. Taken together, these data convincingly demonstrate the epigenetic importance of a novel lincRNA, and clarify the mechanism by which it could be used to prevent pathological myocardial hypertrophy.

Most genome-wide association studies are conducted in large homogenous populations. The value of using founder populations despite their smaller size is the increased ability to find deleterious mutations—the so called goldilocks’ alleles of large effect and frequency. Moltke et al. presented evidence that an early truncation mutation in TBC1D4 shows strong association with type 2 diabetes mellitus (D2M) and postprandial serum glucose levels in particular. In a study limited to individuals from Greenland, one nonsense single-nucleotide polymorphism on chromosome 13 (rs7330796) was identified for genome-wide association with serum glucose levels 2 hours after an oral glucose load. Exome sequencing of 9 trios identified 1 truncation mutation in high linkage disequilibrium with rs7330796. This truncation (p.Arg684Ter) showed robust association with glucose and insulin levels 2 hours after an oral glucose load and with risk for D2M; however, no association with hemoglobin A1C and only minimal association with fasting insulin and glucose levels were observed. A second peculiarity related to this specific truncation mutation is that it only affects the long isoform or TBC1 domain family member 4 (TBC1D4), and this study identified the skeletal muscle as the predominant cell type in which the long isoform is expressed. Other plausible tissues such as adipose, liver, and pancreatic islets showed minimal expression of the long isoform. In skeletal muscle biopsies, the authors showed a clear relationship between genotype and TBC1D4 transcript and protein level, with the carriers of the truncation mutation having lower gene expression. Although this gene was known to affect glucose homeostasis as a mediator of Akt-induced glucose uptake, the knockout mice have this phenotype in both the skeletal muscle and adipose. As a result, the gene has been associated with decreased basal plasma glucose levels. The identification of the p.Arg684Ter truncation mutation which leads to a skeletal muscle-specific reduction in TBC1D4 shows the importance...
of postprandial glucose in risk for D2M, despite no appreciable effect on other traditional risk factors, such as fasting glucose or HgbA1C. This genotype–phenotype relationship is unique to the Greenlandic population where the truncated allele is present in 17% of individuals, but is virtually absent in all the other cohorts. This study wonderfully demonstrates the promise of disease-associated variant identification in founder populations.

Obesity and elevated levels of circulating fatty acids are associated with increased insulin sensitivity and glucose intolerance and are features that define D2M. However, recent studies have found that certain types of adipose tissue (eg, functional human brown adipose tissue) can, in fact, also induce positive metabolic effects through increased energy expenditure and decreased risk of developing obesity and D2M. Using advanced quantitative mass spectrometry, Yore et al3 identified a new class of endogenous lipids which they refer to as fatty acid esters of hydroxyl fatty acids (FAHFAs) that was upregulated among genetically modified diabetes mellitus–resistant AG40X mice (AG40X mice have elevated circulating fatty acids and adiposity, yet have enhanced glucose tolerance) compared with normal wild-type mice. The most strongly upregulated FAHFAs were referred to as palmitic acid-hydroxy stearic acids (16- to 18-fold upregulation). Additional analysis showed that palmitic acid-hydroxy stearic acids correlated strongly with insulin sensitivity and were reduced in adipose tissue of serum and insulin-resistant humans. In mouse models, administration of FAHFAs resulted in more efficient clearance of glucose from the blood because of increased insulin secretion and sensitivity through activation of the GRP120 receptor and the glucagon-like peptide-1. Moreover, in vivo models showed that FAHFA activation of GPR120 also resulted in a reduced anti-inflammatory response. The importance of this study lies in the identification of FAHFAs (and palmitic acid-hydroxy stearic acids) as a new endogenous lipid class with both positive anti-inflammatory and metabolic effects and represents a possible novel pharmacological target to treat D2M.

Genome-wide association studies have reproducibly identified hundreds of associated variants with cardiovascular disease. One of the most robust associations is between a group of noncoding variants in 47-kb region of the fat mass and obesity-associated (FTO) gene. Each copy of the risk haplotype containing a bacterial artificial chromosome of the region, the associated variants and FTO gene expression or function until now. Taking advantage of recent breakthroughs in the importance of spatial organization in the human genome. Recent evidence suggests that miRNAs can be selectively released into the extracellular environment and taken up by neighboring or distant cells. To investigate this phenomenon in the heart, Bang et al1 performed a comprehensive series of cell culture experiments to test whether miRNAs derived from cardiac fibroblasts can be taken up by cardiomyocytes and induce cardiomyocyte hypertrophy. They demonstrated that rat cardiac fibroblasts secrete exosomes that are enriched in miRNAs and contain a surprisingly high abundance of miRNA passenger strands (also known as star miRNAs), which would normally be degraded in cells. Next, they showed that one of these passenger miRNAs, miR-21*, could be taken up by rat cardiomyocytes via a mechanism that was both actin- and temperature-dependent. Uptake of fibroblast-derived miR-21* by cardiomyocytes led to downregulation of multiple proteins, including 2 miR-21* targets, SORBS2 and PDLIM5, and induced cardiomyocyte hypertrophy. The authors then went on to show that miR-21* was present at higher levels in pericardial fluid from mice with cardiac hypertrophy induced by transverse aortic constriction compared with sham-operated control mice. Finally, in mice with angiotension II–induced cardiac hypertrophy, they demonstrated that pharmacological inhibition of miR-21* with a specific miR-21* antagonist attenuated both heart/body weight ratio and cardiomyocyte diameter, compared with mice treated with a scrambled antagonist. This study is noteworthy as it provides the first evidence of a miRNA/exosome-mediated mechanism for cross-talk between cardiac fibroblasts and cardiomyocytes and identifies miR-21* as a paracrine regulator and potential therapeutic target for cardiomyocyte hypertrophy.

Although complex diseases have received a lot of attention in recent years, studies of monogenic diseases have helped to shape the way we think of classic and modern genetics and contributed to our understanding of disease mechanisms, pathogenic mutations, and gene regulation. However, the rarity and severity of some monogenic diseases (eg, Barth syndrome, a rare X-linked disorder caused by mutations in the tafazzin gene [TAZ] characterized by dilated cardiomyopathy, neutropenia, skeletal myopathy, and early mortality) has made in vivo research practically impossible which means that much of the underlying disease pathology remains unknown, until now. Taking advantage of recent breakthroughs in the

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technology of reprogramming mature cells into induced pluripotent stem cells (iPSC), Wang et al. generated iPSC cardiomyocytes (iPSC-CM) cell lines from 2 Barth syndrome patients carrying TAZ mutations to uncover underlying disease mechanisms. First, they showed that the iPSC-CM cell lines displayed known characteristics of the Barth syndrome, including impaired cardiolipin acetylation (cardiolipin is an important component of the inner mitochondrial membrane with important electron transport complex stabilizing properties). Next, they tested the mitochondrial function in iPSC-CM compared with controls. Here, they showed that the Barth syndrome iPSC-CM had proton leak across the mitochondrial membrane which in turn lead to radical oxygen species, increased O2 consumption, and subsequent ATP depletion. Confirming that the TAZ mutations were indeed responsible for the Barth phenotype, Wang et al rescued the mitochondrial phenotype by introducing stable TAZ mRNA into the Barth iPSC-CM, but also induced the Barth mitochondrial phenotype in iPSC-CM controls by introducing the TAZ mutations through clustered regularly interspaced short palindromic repeats (CRISPR)-associated 9 genome editing. Contractile deficits associated with Barth syndrome were subsequently reproduced using the heart-on-chip technology after which the phenotype was rescued in a similar fashion as mentioned above. Interestingly, the authors also evaluated translational properties by testing small molecule interventions; the most favorable effect was seen with the cardiolipin precursor, linoleic acid. This study not only elegantly provides new insights into the mechanistic underpinnings of Barth syndrome by using iPSC-CM cell lines to link TAZ mutations with excess radical oxygen species, and contractile deficiency, but also suggests that in vitro iPSC modeling may provide key insights into new treatment strategies.

Cardiac cell therapy aims to replace damaged myocardial cells with de novo cardiomyocytes and has shown promise as a strategy for improving cardiac function in small animal models. To test whether cardiac cell therapy may be safe and feasible in humans, Chong et al. developed a method to generate viable human embryonic-stem-cell–derived cardiomyocytes (hESC-CMs) on a clinical scale and examined whether these cells could engraft and electrically couple with host myocardium in a nonhuman primate model of myocardial infarction. First, they generated batches of >1 billion hESC-CMs with good viability by using established cell cryopreservation techniques. Next, in 4 pigtail macaques who had undergone myocardial ischemia-reperfusion, they showed that injection of cryopreserved hESC-CMs into the infarct region and surrounding border zones led to extensive remuscularization of the infarcted area, with hESC-CM grafts making up 40% of the total infarct volume. The authors observed frequent connections between host and graft cells and demonstrated that the host vasculature had perfused the graft within 12 weeks of cell treatment. Most importantly, all cell-treated hearts showed regular cardiac transients that were perfectly synchronized with the host ECG, providing strong evidence for electromechanical coupling between engrafted hESC-CMs and the host myocardium. However, because of the small sample size, they were unable to demonstrate a significant improvement in left ventricular function. Furthermore, all cell-treated macaques experienced nonfatal arrhythmias after treatment, highlighting that additional challenges need to be overcome before this technology can be translated into the clinic. Nevertheless, by demonstrating that hESC-CM transplantation may be feasible on a large scale, this study represents a significant step toward clinical translation and paves the way for further studies using animal models with a similar heart size and heart rate to humans.

Taking an alternative approach to Chong et al. and Ong et al. sought to improve the efficacy of cardiac cell therapy although enhancing the survival of transplanted cardiac progenitor cells (CPCs) by modulating the host microenvironment. They hypothesized that co-delivery of CPCs with nonviral microparticles carrying hypoxia-inducible factor-1 (HIF1), an oxygen-sensitive transcription factor that promotes cell survival, would increase the potency of CPCs for cardiac repair in the infarcted heart. Using a mouse model of myocardial infarction, the authors demonstrated that survival of transplanted CPCs was significantly better when they were delivered with microparticles carrying HIF1 (MC-HIF1) than with microparticles carrying green fluorescent protein, during 6 weeks follow-up. Compared with mice treated with saline- or MC-green fluorescent protein, mice cotreated with CPCs and MC-HIF1 had reduced infarct sizes, improved cardiac function, enhanced vascularity, and increased expression of proangiogenic genes in peri-infarct areas, with mice treated with MC-HIF1 alone showing intermediate effects. The authors then undertook an extensive series of cell culture experiments to demonstrate that the prosurvival effects of MC-HIF1 are mediated, at least in part, through cross-talk with host cardiac endothelial cells via an miRNA/exosome mechanism. Specifically, they demonstrated that cardiac endothelial cells secrete miRNA-containing exosomes and that levels of several exosomal miRNAs, including miR-126 and miR-210, were increased in response to HIF1. Furthermore, they showed that these miRNAs can be taken up by CPCs, leading to altered expression of miR-126 and miR-210 target genes and an increased tolerance to ischemic stress. Finally, in mice, they confirmed that intravenous delivery of exosomes from endothelial cells overexpressing HIF1 directly enhanced CPC survival in vivo. The significance of this study lies in the potential to improve cardiac cell therapy through modulating the host microenvironment and enhancing beneficial cross-talk between host endothelial cells and transplanted CPCs, leading to increased cell survival.

One of the most inspiring examples of rapid bench-to-bedside translation has been the development of proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitory antibodies for the reduction of serum low-density lipoprotein and prevention of coronary artery disease. One logical expectation would be for a permanent disruption of the PCSK9 gene, rendering continued therapy unnecessary as the naturally occurring and protective loss-of-function mutation could be given as a 1-time treatment. Ding et al. presented evidence that the technology for this now exists with genome editing and the CRISPR/Cas9 genome editing. CRISPR/Cas9-associated 9 nucleases. CRISPR/Cas9-associated 9 is the latest nuclease with demonstrated ability to generate double-strand breaks in mammalian cells at a specified target site, and thereby create frame-shift mutations when error-prone repair processes insert or delete nucleotides.
at the break site. In this proof-of-concept article, the authors first designed efficient synthetic guide RNAs (gRNA) targeting exons 1 and 2 of the PCSK9 gene and introduced them through adenoviruses expressing CRISPR-associated 9, the CRISPR-associated nuclease derived from Streptococcus pyogenes, via tail vein injection in mice. A single injection resulted in a >5-fold reduction of plasma PCSK9 levels and a 35% to 40% reduction in total cholesterol. Many technical hurdles about efficient delivery, immunogenicity, and off-target effects of the nuclease must be addressed before this sort of gene-editing approach can be used in the clinical setting. Even so, the rapid rate of innovation in the field and the dramatic effect seen in this study make for an exciting future for the therapeutic potential of genetic editing.

Alternative splicing and processing greatly increases the biodiversity of proteins that can be encoded for by a single gene. However, alterations in splicing factors or their target can disrupt the final gene product and lead to various genetic diseases. Although genetic variation in RNA-binding motif 20 (RBM20) has previously been associated with altered RNA splicing and dilated cardiomyopathy, the mechanisms are unclear. Maat et al10 used high throughput RNA sequencing, immunoprecipitation techniques, and proteomics to delineate how RBM20 regulates alternative splicing in the heart of rat and humans. Using these techniques in concert, they identified the presence of a unique RNA-recognition element localized predominantly to intronic-binding sites which was also linked to exon splicing with RBM20 near both 3′ and 5′ splice sites. Proteomic analysis showed that RBM20 also interacted with small nuclear ribonucleic particles and that RBM20-dependent splicing repression was mediated by spliceosome stalling. Moreover, evidence of conservation across species for the RBM20-binding site and regulation was also identified and evidence of a negative selection was identified using various human populations from the 1000 genomes project. Additional analysis aimed to identify direct protein–protein interacting partners with RBM20 revealed several genes that have previously been implicated with dilated cardiomyopathy and also showed how reduced RBM20 expression affected splicing. Overall, this study wonderfully describes how RBM20 acts as a cellular molecular switch and how it is heavily implicated in coordinating splicing and isoform transitions for an array of genes with important cardiac function.

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References

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