The Ten Most Read Articles Published in Circulation Research in 2011

Roberto Bolli, for the Editors

The Editors are pleased to announce yet another new feature of the journal: a list of the 10 most read original articles published in Circulation Research in 2011. We intend to publish this list annually. We realize that the number of citations is the most conventional parameter used to gauge interest by the readership; however, providing this metric would require a few years, by which time the articles may have lost their novelty and appeal. Consequently, we have selected the articles on the basis of the number of Full Text/PDF downloads, which we hope will offer a reasonable estimate of the level of interest among our readers.

Our motivation in compiling this list is multifarious. By highlighting the most popular articles, we wish to direct the attention of our readers to new information that may be of particular interest to a large fraction of the community of cardiovascular scholars. In addition, a synopsis of the most popular articles can be a useful indicator of burgeoning areas of research that are likely to dominate the landscape for years to come. This “honor roll” is also meant to acknowledge the outstanding work of the authors and their efforts in advancing the frontiers of cardiovascular science. Furthermore, we believe that the articles highlighted below represent paradigms of scientific excellence, particularly with respect to the three criteria that we value most at Circulation Research: conceptual and/or mechanistic novelty, scientific impact, and methodological rigor. Finally, we hope that this list will provide tangible evidence of the high (and rising) level of scientific excellence of the work published in Circulation Research.

It should be noted that 4 of these 10 articles report human data (Williams et al, Chinetti-Gbaguidi et al, Losordo et al, and Boon et al). Publication of these studies is a further manifestation of our keen interest in promoting the dissemination of translational/clinical work. As discussed in a previous Editor’s Note,1 the Editors strongly encourage submission of studies that utilize human subjects or human tissues to illuminate the basis and mechanisms of cardiovascular disease.

The following represent a selection of the most read Circulation Research articles published between January 2011 and December 2011, presented in their order of publication. Articles were selected based on the number of Full Text/PDF downloads, adjusted to compensate for differences in the length of time articles have been available online.

From the February 18, 2011 issue:

Myocardial Injection With GSK-3β–Overexpressing Bone Marrow–Derived Mesenchymal Stem Cells Attenuates Cardiac Dysfunction After Myocardial Infarction

Jaeyeon Cho, Peiyong Zhai, Yasuhiro Maejima, Junichi Sadoshima

Abstract

Rationale: Glycogen synthase kinase (GSK)-3β upregulates cardiac genes in bone marrow–derived mesenchymal stem cells (MSCs) in vitro. Ex vivo modification of signaling mechanisms in MSCs may improve the efficiency of cardiac cell-based therapy (CBT).

Objective: To test the effect of GSK-3β on the efficiency of CBT with MSCs after myocardial infarction (MI).

Methods and Results: MSCs overexpressing either GSK-3β (GSK-3β–MSCs), LacZ (LacZ-MSCs), or saline was injected into the heart after coronary ligation. A significant improvement in the mortality and left ventricular (LV) function was observed at 12 weeks in GSK-3β–MSC–injected mice compared with in LacZ-MSC– or saline-injected mice. MI size and LV remodeling were reduced in GSK-3β–MSC–injected mice compared with in LacZ-MSC– or saline-injected ones. GSK-3β increased survival and increased cardiomyocyte differentiation of MSCs, as evidenced by activation of an Nkx2.5-LacZ reporter and upregulation of troponin T. Injection of GSK-3β–MSCs induced Ki67-positive myocytes and c-Kit–positive cells, suggesting that GSK-3β–MSCs upregulate cardiac progenitor cells. GSK-3β–MSCs also increased capillary density and upregulated paracrine factors, including vascular endothelial growth factor A (Vegfa). Injection of GSK-3β–MSCs in which Vegfa had been knocked down abolished the increase in survival and capillary density. However, the decrease in MI size and LV remodeling and the improvement of LV function were still observed in MI mice injected with GSK-3β–MSCs without Vegfa.

Conclusions: GSK-3β significantly improves the efficiency of CBT with MSCs in the post-MI heart. GSK-3β not only increases survival of MSCs but also induces cardiomyocyte differentiation and angiogenesis through Vegfa-dependent and -independent mechanisms.2

1 Correspondence to Roberto Bolli, Editor-in-Chief, Circulation Research. Email circulation.research@circresearch.com
2 (Circ Res. 2012;110:e24-e28.)
3 © 2012 American Heart Association, Inc.
4 Circulation Research is available at http://circres.ahajournals.org
5 DOI: 10.1161/RES.0b013e31824c38b7
From the April 1, 2011 issue:

Intramyocardial Stem Cell Injection in Patients With Ischemic Cardiomyopathy: Functional Recovery and Reverse Remodeling

Adam R. Williams, Barry Trachtenberg, Darcy L. Velazquez, Ian McNiece, Peter Altman, Didier Rouy, Adam M. Mendizabal, Pradip M. Pattany, Gustavo A. Lopera, Joel Fishman, Juan P. Zambrano, Alan W. Heldman, Joshua M. Hare

Abstract
Rationale: Transcatheter, intramyocardial injections of bone marrow-derived cell therapy produces reverse remodeling in large animal models of ischemic cardiomyopathy.

Objective: We used cardiac MRI (CMR) in patients with left ventricular (LV) dysfunction related to remote myocardial infarction (MI) to test the hypothesis that bone marrow progenitor cell injection causes functional recovery of scarred myocardium and reverse remodeling.

Methods and Results: Eight patients (aged 57.2 ± 13.3 years) received transendocardial, intramyocardial injection of autologous bone marrow progenitor cells (mononuclear or mesenchymal stem cells) in LV scar and border zone. All patients tolerated the procedure with no serious adverse events. CMR at 1 year demonstrated a decrease in end diastolic volume (208.7 ± 20.4 versus 167.4 ± 7.3 mL; \(P = 0.03\)), a trend toward decreased end systolic volume (142.4 ± 16.5 versus 107.6 ± 7.4 mL; \(P = 0.06\)), decreased infarct size (\(P < 0.05\)), and improved regional LV function by peak Eulerian circumferential strain in the treated infarct zone (−8.1 ± 1.0 versus −11.4 ± 1.3; \(P = 0.04\)). Improvements in regional function were evident at 3 months, whereas the changes in chamber dimensions were not significant until 6 months. Improved regional function in the infarct zone strongly correlated with reduction of end diastolic volume (\(r^2 = 0.69\), \(P = 0.04\)) and end systolic volume (\(r^2 = 0.83\), \(P = 0.01\)).

Conclusions: These data suggest that transcatheter, intramyocardial injections of autologous bone marrow progenitor cells improve regional contractility of a chronic myocardial scar, and these changes predict subsequent reverse remodeling. The findings support the potential clinical benefits of this new treatment strategy and ongoing randomized clinical trials.³

From the April 1, 2011 issue:

Mitochondrial Oxidative Stress Mediates Angiotensin II-Induced Cardiomyopathy: Functional Recovery and Reverse Remodeling

Objective: We evaluated the contribution of mitochondrial reactive oxygen species (ROS) to cardiac hypertrophy and failure by using genetic mouse models overexpressing catalase targeted to mitochondria and to peroxisomes.

Methods and Results: Angiotensin II increases mitochondrial ROS in cardiomyocytes, concomitant with increased mitochondrial protein carbonyls, mitochondrial DNA deletions, increased autophagy, and signaling for mitochondrial biogenesis in hearts of angiotensin II-treated mice. The causal role of mitochondrial ROS in angiotensin II-induced cardiomyopathy is shown by the observation that mice that overexpress catalase targeted to mitochondria, but not mice that overexpress wild-type peroxisomal catalase, are resistant to cardiac hypertrophy, fibrosis and mitochondrial damage induced by angiotensin II, as well as heart failure induced by overexpression of Gaq. Furthermore, primary damage to mitochondrial DNA, induced by zidovudine administration or homozygous mutation of mitochondrial polymerase γ, is also shown to contribute directly to the development of cardiac hypertrophy, fibrosis, and failure.

Conclusions: These data indicate the critical role of mitochondrial ROS in cardiac hypertrophy and failure and support the potential use of mitochondrial-targeted antioxidants for prevention and treatment of hypertensive cardiomyopathy.⁴

From the April 15, 2011 issue:

Human Atherosclerotic Plaque Alternative Macrophages Display Low Cholesterol Handling but High Phagocytosis Because of Distinct Activities of the PPARγ and LXRα Pathways

Giulia Chinetti-Gbaguidi, Morgane Baron, Mohamed Amine Bouhliel, Jonathan Vanhoutte, Corinne Copin, Yasmine Sebti, Bruno Derudas, Thérèse Mayi, Gaël Bories, Anne Tailleux, Stéphane Haulon, Christophe Zawadzki, Brigitte Jude, Bart Staels

Abstract
Rationale: A crucial step in atherogenesis is the infiltration of the subendothelial space of large arteries by monocytes where they differentiate into macrophages and transform into lipid-loaded foam cells. Macrophages are heterogeneous cells that adapt their response to environmental cytokines. Th1 cytokines promote monocyte differentiation into M1 macrophages, whereas Th2 cytokines trigger an "alternative" M2 phenotype.

Objective: We previously reported the presence of CD68⁺ mannose receptor (MR)⁺ M2 macrophages in human atherosclerotic plaques. However, the function of these plaque CD68⁺MR⁺ macrophages is still unknown.

Methods and Results: Histological analysis revealed that CD68⁺MR⁺ macrophages locate far from the lipid core of the plaque and contain smaller lipid droplets compared to CD68⁺MR⁻ macrophages. Interleukin (IL)-4-polarized CD68⁺MR⁺ macrophages display a reduced capacity to handle and efflux cellular cholesterol because of low expression levels of the nuclear receptor liver X receptor (LXRα) and its target genes, ABCA1 and apolipoprotein E, attributable to the high 15-lipoxygenase activity in CD68⁺MR⁺ macrophages.
By contrast, CD68^{+}MR^{+} macrophages highly express opsonins and receptors involved in phagocytosis, resulting in high phagocytic activity. In M2 macrophages, peroxisome proliferator-activated receptor (PPAR)γ activation enhances the phagocytic but not the cholesterol trafficking pathways.

**Conclusions:** These data identify a distinct macrophage subpopulation with a low susceptibility to become foam cells but high phagocytic activity resulting from different regulatory activities of the PPARγ-LXRα pathways.5

**From the June 10, 2011 issue:**

**Exercise Protects Against Myocardial Ischemia–Reperfusion Injury via Stimulation of β3-Adrenergic Receptors and Increased Nitric Oxide Signaling: Role of Nitrite and Nitrosothiols**

John W. Calvert, Marah E. Condit, Juan Pablo Aragón, Chad K. Nicholson, Bridgette F. Moody, Rebecca L. Hood, Amy L. Sandler, Susheel Gundewar, Douglas R. Seals, Lili A. Barouch, David J. Lefer

**Abstract**

**Rationale:** Exercise training confers sustainable protection against ischemia–reperfusion injury in animal models and has been associated with improved survival following a heart attack in humans. It is still unclear how exercise protects the heart, but it is apparent that endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) play a role.

**Objective:** To determine the role of β3-adrenergic receptors (β3-ARs), eNOS activation, and NO metabolites (nitrite and nitrosothiols) in the sustained cardioprotective effects of exercise.

**Methods and Results:** Here we show that voluntary exercise reduces myocardial injury in mice following a 4-week training period and that these protective effects can be sustained for at least 1 week following the cessation of the training. The sustained cardioprotective effects of exercise are mediated by alterations in the phosphorylation status of eNOS (increase in serine 1177 and decrease in threonine 495), leading to an increase in NO generation and storage of NO metabolites (nitrite and nitrosothiols) in the heart. Further evidence revealed that the alterations in the eNOS phosphorylation status and NO generation were mediated by β3-AR stimulation and that in response to exercise a deficiency of β3-ARs leads to an exacerbation of myocardial infarction following ischemia–reperfusion injury.

**Conclusions:** Our findings clearly demonstrate that exercise protects the heart against myocardial ischemia–reperfusion injury by stimulation of β3-ARs and increased cardiac storage of nitric oxide metabolites (ie, nitrite and nitrosothiols).6

**From the June 24, 2011 issue:**

**Growth of Engineered Human Myocardium With Mechanical Loading and Vascular Coculture**

Nathaniel L. Tulloch, Veronica Muskholi, Maria V. Razu- mova, F. Steven Korte, Michael Regnier, Kip D. Hauch, Lil Pabon, Hans Reinecke, Charles E. Murry

**Abstract**

**Rationale:** The developing heart requires both mechanical load and vascularization to reach its proper size, yet the regulation of human heart growth by these processes is poorly understood.

**Objective:** We seek to elucidate the responses of immature human myocardium to mechanical load and vascularization using tissue engineering approaches.

**Methods and Results:** Using human embryonic stem cell and human induced pluripotent stem cell-derived cardiomyocytes in a 3-dimensional collagen matrix, we show that uniaxial mechanical stress conditioning promotes 2-fold increases in cardiomyocyte and matrix fiber alignment and enhances myofibrillogenesis and sarcomeric banding. Furthermore, cyclic stress conditioning markedly increases cardiomyocyte hypertrophy (2.2-fold) and proliferation rates (21%) versus unconditioned constructs. Addition of endothelial cells enhances cardiomyocyte proliferation under all stress conditions (14% to 19%), and addition of stromal supporting cells enhances formation of vessel-like structures by 10-fold. Furthermore, these optimized human cardiac tissue constructs generate Starling curves, increasing their active force in response to increased resting length. When transplanted onto hearts of athymic rats, the human myocardium survives and forms grafts closely apposed to host myocardium. The grafts contain human microvessels that are perfused by the host coronary circulation.

**Conclusions:** Our results indicate that both mechanical load and vascular cell coculture control cardiomyocyte proliferation, and that mechanical load further controls the hypertrophy and architecture of engineered human myocardium. Such constructs may be useful for studying human cardiac development as well as for regenerative therapy.7

**From the August 5, 2011 issue:**

**Intramyocardial, Autologous CD34+ Cell Therapy for Refractory Angina**


**Abstract**

**Rationale:** A growing number of patients with coronary disease have refractory angina. Preclinical and early-phase clinical data suggest that intramyocardial injection of autologous CD34+ cells can improve myocardial perfusion and function.

**Objective:** Evaluate the safety and bioactivity of intramyocardial injections of autologous CD34+ cells in patients with refractory angina who have exhausted all other treatment options.

**Methods and Results:** In this prospective, double-blind, randomized, phase II study (ClinicalTrials.gov identifier: NCT00300053), 167 patients with refractory angina received 1 of 2 doses (1×10^5 or 5×10^5 cells/kg) of mobilized autologous CD34+ cells or an equal volume of diluent
(placebo). Treatment was distributed into 10 sites of ischemic, viable myocardium with a NOGA mapping injection catheter. The primary outcome measure was weekly angina frequency 6 months after treatment. Weekly angina frequency was significantly lower in the low-dose group than in placebo-treated patients at both 6 months (6.8±1.1 versus 10.9±1.2, P=0.020) and 12 months (6.3±1.2 versus 11.0±1.2, P=0.035); measurements in the high-dose group were also lower, but not significantly. Similarly, improvement in exercise tolerance was significantly greater in low-dose patients than in placebo-treated patients (6 months: 139±151 versus 69±122 seconds, P=0.014; 12 months: 140±171 versus 58±146 seconds, P=0.017) and greater, but not significantly, in the high-dose group. During cell mobilization and collection procedures were associated with cardiac enzyme elevations consistent with non-ST segment elevation myocardial infarction. Mortality at 12 months was 5.4% in the placebo-treatment group with no deaths among cell-treated patients.

**Conclusions:** Patients with refractory angina who received intramyocardial injections of autologous CD34+ cells (10^5 cells/kg) experienced significant improvements in angina frequency and exercise tolerance. The cell-mobilization and -collection procedures were associated with cardiac enzyme elevations, which will be addressed in future studies.

**From the August 5, 2011 issue:**

**Small-Molecule Inhibitors of the Wnt Pathway Potently Promote Cardiomyocytes From Human Embryonic Stem Cell–Derived Mesoderm**

Erik Willems, Sean Spiering, Herman Davidovics, Marion Lanier, Zebin Xia, Marcia Dawson, John Cashman, Mark Mercola

**Abstract**

**Rationale:** Human embryonic stem cells can form cardiomyocytes when cultured under differentiation conditions. Although the initiating step of mesoderm formation is well characterized, the subsequent steps that promote for cardiac lineages are poorly understood and limit the yield of cardiomyocytes.

**Objective:** Our aim was to develop a human embryonic stem cell-based high-content screening assay to discover small molecules that drive cardiogenic differentiation after mesoderm is established to improve our understanding of the biology involved. Screening of libraries of small-molecule pathway modulators was predicted to provide insight into the cellular proteins and signaling pathways that control stem cell cardiogenesis.

**Methods and Results:** Approximately 550 known pathway modulators were screened in a high-content screening assay, with hits being called out by the appearance of a red fluorescent protein driven by the promoter of the cardiac-specific MYH6 gene. One potent small molecule was identified that inhibits transduction of the canonical Wnt response within the cell, which demonstrated that Wnt inhibition alone was sufficient to generate cardiomyocytes from human embryonic stem cell-derived mesoderm cells. Transcriptional profiling of inhibitor-treated compared with vehicle-treated samples further indicated that inhibition of Wnt does not induce other mesoderm lineages. Notably, several other Wnt inhibitors were very efficient in inducing cardiogenesis, including a molecule that prevents Wnts from being secreted by the cell, which confirmed that Wnt inhibition was the relevant biological activity.

**Conclusions:** Pharmacological inhibition of Wnt signaling is sufficient to drive human mesoderm cells to form cardiomyocytes; this could yield novel tools for the benefit of pharmaceutical and clinical applications.

**From the September 2, 2011 issue:**

**miR-15 Family Regulates Postnatal Mitotic Arrest of Cardiomyocytes**

Enzo R. Porrello, Brett A. Johnson, Arin B. Aurora, Emma Simpson, Young-Jae Nam, Scot J. Matkovich, Gerald W. Dorn II, Eva van Rooij, Eric N. Olson

**Abstract**

**Rationale:** Mammalian cardiomyocytes withdraw from the cell cycle during early postnatal development, which significantly limits the capacity of the adult mammalian heart to regenerate after injury. The regulatory mechanisms that govern cardiomyocyte cell cycle withdrawal and binucleation are poorly understood.

**Objective:** Given the potential of microRNAs (miRNAs) to influence large gene networks and modify complex developmental and disease phenotypes, we searched for miRNAs that were regulated during the postnatal switch to terminal differentiation.

**Methods and Results:** Microarray analysis revealed subsets of miRNAs that were upregulated or downregulated in cardiac ventricles from mice at 1 and 10 days of age (P1 and P10). Interestingly, miR-195 (a member of the miR-15 family) was the most highly upregulated miRNA during this period, with expression levels almost 6-fold higher in P10 ventricles relative to P1. Precocious overexpression of miR-195 in the embryonic heart was associated with ventricular hypoplasia and ventricular septal defects in β-myosin heavy chain–miR-195 transgenic mice. Using global gene profiling and argonauta-2 immunoprecipitation approaches, we showed that miR-195 regulates the expression of a number of cell cycle genes, including checkpoint kinase 1 (Chek1), which we identified as a highly conserved direct target of miR-195. Finally, we demonstrated that knockdown of the miR-15 family in neonatal mice with locked nucleic acid-modified anti-miRNAs was associated with an increased number of mitotic cardiomyocytes and derepression of Chek1.

**Conclusions:** These findings suggest that upregulation of the miR-15 family during the neonatal period may be an important regulatory mechanism governing cardiomyocyte cell cycle withdrawal and binucleation.

**From the October 28, 2011 issue:**

**MicroRNA-29 in Aortic Dilation: Implications for Aneurysm Formation**

Reinier A. Boon, Timon Seeger, Susanne Heydt, Ariane Fischer, Eduard Hergenreider, Anton J.G. Horrevoets, Manlio Vinciguerra, Nadia Rosenthal, Sergio Sciaccia, Michele Pi-
Abstract

Rationale: Aging represents a major risk factor for coronary artery disease and aortic aneurysm formation. MicroRNAs (miRs) have emerged as key regulators of biological processes, but their role in age-associated vascular pathologies is unknown.

Objective: We aim to identify miRs in the vasculature that are regulated by age and play a role in age-induced vascular pathologies.

Methods and Results: Expression profiling of aortic tissue of young versus old mice identified several age-associated miRs. Among the significantly regulated miRs, the increased expression of miR-29 family members was associated with a profound downregulation of numerous extracellular matrix (ECM) components in aortas of aged mice, suggesting that this miR family contributes to ECM loss, thereby sensitizing the aorta for aneurysm formation. Indeed, miR-29 expression was significantly induced in 2 experimental models for aortic dilation: angiotensin II-treated aged mice and genetically induced aneurysms in Fibulin-4R/R mice.

Conclusion: In conclusion, miR-29-mediated downregulation of ECM proteins may sensitize the aorta to the formation of aneurysms in advanced age. Inhibition of miR-29 in vivo abrogates aortic dilation in mice, suggesting that miR-29 may represent a novel molecular target to augment matrix synthesis and maintain vascular wall structural integrity.

References


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