The 10 Most Read Articles Published in Circulation Research in 2012

Roberto Bolli, for the Editors

Continuing the initiative launched last year, we are pleased to provide the list of the 10 most read original articles published in Circulation Research in 2012. 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, as we did last year, 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 3 criteria that we value most at Circulation Research: conceptual 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 5 of these 10 articles report studies of stem cells/cardiac regeneration (Jayawardena et al, Kara et al, Ferreira-Martins et al, Inagawa et al, and Yaniz-Galende et al). The high number of downloads of these studies and their disproportionate representation in the top 10 papers is further evidence of the enormous level of interest that stem cell biology and regenerative medicine attract in the cardiovascular research community. As outlined in our editorial manifesto,1 rapid advances in these fields may transform cardiovascular medicine. We view them as an important focus of the journal, and we are proud of the fact that Circulation Research has been at the forefront of the explosive growth in stem cell research, both at the basic and at the translational levels.

—Roberto Bolli

The following represent a selection of the most read Circulation Research articles published between January 2012 and December 2012, 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 time since publication.

From the January 6, 2012 issue:

Fetal Cells Traffic to Injured Maternal Myocardium and Undergo Cardiac Differentiation

Abstract

Rationale: Fetal cells enter the maternal circulation during pregnancy and may persist in maternal tissue for decades as microchimeras.

Objective: Based on clinical observations of peripartum cardiomyopathy patients and the high rate of recovery they experience from heart failure, our objective was to determine whether fetal cells can migrate to the maternal heart and differentiate to cardiac cells.

Methods and Results: We report that fetal cells selectively home to injured maternal hearts and undergo differentiation into diverse cardiac lineages. Using enhanced green fluorescent protein (eGFP)-tagged fetuses, we demonstrate engraftment of multipotent fetal cells in injury zones of maternal hearts. In vivo, eGFP+ fetal cells form endothelial cells, smooth muscle cells, and cardiomyocytes. In vitro, fetal cells isolated from maternal hearts recapitulate these differentiation pathways, additionally forming vascular tubes and beating cardiomyocytes in a fusion-independent manner; ≈40% of fetal cells in the maternal heart express Caudal-related homeobox2 (Cdx2), previously associated with trophoblast stem cells, thought to solely form placenta.

Conclusions: Fetal maternal stem cell transfer appears to be a critical mechanism in the maternal response to cardiac injury. Furthermore, we have identified Cdx2 cells as a novel cell type for potential use in cardiovascular regenerative therapy.2

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News & Views

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**From the March 2, 2012 issue:**

**Cardiomyogenesis in the Developing Heart Is Regulated by C-Kit–Positive Cardiac Stem Cells**

João Ferreira-Martins, Barbara Ogórek, Donato Cappetta, Alex Matsuda, Sergio Signore, Domenico D’Amaro, James Kostyla, Elisabeth Steadman, Noriko Ide-Iwata, Fumihiro Sanada, Grazia Iaffaldano, Sergio Ottolenghi, Toru Hosoda, Amarosa Leri, Jan Kajstura, Piero Anversa, Marcello Rota

**Abstract**

**Rationale:** Embryonic and fetal myocardial growth is characterized by a dramatic increase in myocyte number, but whether the expansion of the myocyte compartment is dictated by activation and commitment of resident cardiac stem cells (CSCs), division of immature myocytes or both is currently unknown.

**Objective:** In this study, we tested whether prenatal cardiac development is controlled by activation and differentiation of CSCs and whether division of c-kit–positive CSCs in the mouse heart is triggered by spontaneous Ca\(^{2+}\) oscillations.

**Methods and Results:** We report that embryonic-fetal c-kit–positive CSCs are self-renewing, clonogenic, and multipotent in vitro and in vivo. The growth and commitment of c-kit–positive CSCs is responsible for the generation of the myocyte progeny of the developing heart. The close correspondence between values computed by mathematical modeling and direct measurements of myocyte number at E9, E14, E19, and 1 day after birth strongly suggests that the organogenesis of the embryonic heart is dependent on a hierarchical model of cell differentiation regulated by resident CSCs. The growth-promoting effects of c-kit–positive CSCs are triggered by spontaneous oscillations in intracellular Ca\(^{2+}\), mediated by IP3 receptor activation, which condition asymmetrical stem cell division and myocyte lineage specification.

**Conclusions:** Myocyte formation derived from CSC differentiation is the major determinant of cardiac growth during development. Division of c-kit–positive CSCs in the mouse is promoted by spontaneous Ca\(^{2+}\) spikes, which dictate the pattern of stem cell replication and the generation of a myocyte progeny at all phases of prenatal life and up to 1 day after birth.\(^3\)

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**From the March 30, 2012 issue:**

**Nanobodies Targeting Mouse/Human VCAM1 for the Nuclear Imaging of Atherosclerotic Lesions**

Alexis Broisat, Sophie Hernot, Jakub Toczek, Jens De Vos, Laurent M. Riou, Sandrine Martin, Mitra Ahmadi, Nicole Theilens, Ulrich Wernery, Vicky Caveliers, Serge Voos, Laurent M. Riou, Sandrine Martin, Mitra Ahmadi, Nicole Theilens, Ulrich Wernery, Vicky Caveliers, Serge Muyldermans, Tony Lahoute, Daniel Fagret, Catherine Ghezzi, Nick Devoogdt

**Abstract**

**Rationale:** A noninvasive tool allowing the detection of vulnerable atherosclerotic plaques is highly needed. By combining nanomolar affinities and fast blood clearance, nanobodies represent potential radiotracers for cardiovascular molecular imaging. Vascular cell adhesion molecule-1 (VCAM1) constitutes a relevant target for molecular imaging of atherosclerotic lesions.

**Objective:** We aimed to generate, radiolabel, and evaluate anti-VCAM1 nanobodies for noninvasive detection of atherosclerotic lesions.

**Methods and Results:** Ten anti-VCAM1 nanobodies were generated, radiolabeled with technetium-99 m, and screened in vitro on mouse and human recombiant VCAM1 proteins and endothelial cells and in vivo in apolipoprotein E-deficient (ApoE\(^{-/-}\)) mice. A nontargeting control nanobody was used in all experiments to demonstrate specificity. All nanobodies displayed nanomolar affinities for murine VCAM1. Flow cytometry analyses using human umbilical vein endothelial cells indicated murine and human VCAM1 cross-reactivity for 6 of 10 nanobodies. The lead compound cAbVCAM1-5 was cross-reactive for human VCAM1 and exhibited high lesion-to-control (4.95±0.85), lesion-to-heart (8.30±1.11), and lesion-to-blood ratios (4.32±0.48) (P<0.05 versus control C57Bl/6J mice). Aortic arch atherosclerotic lesions of ApoE\(^{-/-}\) mice were successfully identified by single-photon emission computed tomography imaging. 99mTc-cAbVCAM1-5 binding specificity was demonstrated by in vivo competition experiments. Autoradiography and immunohistochemistry further confirmed cAbVCAM1-5 uptake in VCAM1-positive lesions.

**Conclusions:** The 99mTc-labeled, anti-VCAM1 nanobody cAbVCAM1-5 allowed noninvasive detection of VCAM1 expression and displayed mouse and human cross-reactivity. Therefore, this study demonstrates the potential of nanobodies as a new class of radiotracers for cardiovascular applications. The nanobody technology might evolve into an important research tool for targeted imaging of atherosclerotic lesions and has the potential for fast clinical translation.\(^4\)

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**From the April 27, 2012 issue:**

**Nox4 Is a Protective Reactive Oxygen Species Generating Vascular NADPH Oxidase**

Katrin Schröder, Min Zhang, Sebastian Benkhoff, Anja Mieth, Rainer Pliquett, Judith Kosowski, Christoph Kruse, Peter Luedike, U. Ruth Michaelis, Norbert Weisssmann, Stefanie Dimmeler, Ajay M. Shah, Ralf P. Brandes

**Abstract**

**Rationale:** The function of Nox4, a source of vascular H2O2, is unknown. Other Nox proteins were identified as mediators of endothelial dysfunction.

**Objective:** We determined the function of Nox4 in situations of increased stress induced by ischemia or angiotensin II with global and tamoxifen-inducible Nox4\(^{-/-}\) mice.

**Methods and Results:** Nox4 was highly expressed in the endothelium and contributed to H2O2 formation. Nox4\(^{-/-}\) mice exhibited attenuated angiogenesis (femoral artery ligation), and PEG-catalase treatment in control mice had a similar effect. Tube formation in cultured Nox4\(^{-/-}\) lung endothelial cells (LECs) was attenuated and restored by low concentrations of H2O2, whereas PEG-catalase attenuated tube formation in control LECs. Angiotensin II infusion was used as a model of oxidative stress. Compared with wild-type, aortas from inducible Nox4-deficient animals had development of increased inflammation, media hypertrophy, and endothelial dysfunction. Mechanistically, loss of Nox4 resulted in reduction of endothelial nitric oxide synthase
expression, nitric oxide production, and heme oxygenase-1 (HO-1) expression, which was associated with apoptosis and inflammatory activation. HO-1 expression is controlled by Nrf-2. Accordingly, Nox4-deficient LECs exhibited reduced Nrf-2 protein level and deletion of Nox4 reduced Nrf-2 reporter gene activity. In vivo treatment with heme, an inducer of HO-1, blocked the vascular hypertrophy induced by Nox4 deletion in the angiotensin II infusion model, and carbon monoxide, the product of HO-1, blocked the Nox4-deletion–induced apoptosis in LECs.

**Conclusion:** Endogenous Nox4 protects the vasculature during ischemic or inflammatory stress. Different from Nox1 and Nox2, this particular NADPH oxidase may therefore have a protective vascular function.6

**From the May 25, 2012 issue:**

**MicroRNA-Mediated In Vitro and In Vivo Direct Reprogramming of Cardiac Fibroblasts to Cardiomyocytes**

Tilanthi M. Jayawardena, Bakhytbek Egennazarov, Elizabeth A. Finch, Lunan Zhang, J. Alan Payne, Kumar Pandya, Zhiping Zhang, Paul Rosenberg, Maria Mirottsou, Victor J. Dzau

**Abstract**

**Rationale:** Repopulation of the injured heart with new, functional cardiomyocytes remains a daunting challenge for cardiac regenerative medicine. An ideal therapeutic approach would involve an effective method at achieving direct conversion of injured areas to functional tissue in situ.

**Objective:** The aim of this study was to develop a strategy that identified and evaluated the potential of specific micro (mi)RNAs capable of inducing reprogramming of cardiac fibroblasts directly to cardiomyocytes in vitro and in vivo.

**Methods and Results:** Using a combinatorial strategy, we identified a combination of miRNAs 1, 133, 208, and 499 capable of inducing direct cellular reprogramming of fibroblasts to cardiomyocyte-like cells in vitro. Detailed studies of the reprogrammed cells demonstrated that a single transient transfection of the miRNAs can direct a switch in cell fate as documented by expression of mature cardiomyocyte markers, sarcomeric organization, and exhibition of spontaneous calcium flux characteristic of a cardiomyocyte-like phenotype. Interestingly, we also found that miRNA-mediated reprogramming was enhanced 10-fold on JAK inhibitor I treatment. Importantly, administration of miRNAs into ischemic mouse myocardium resulted in evidence of direct conversion of cardiac fibroblasts to cardiomyocytes in situ. Genetic tracing analysis using Fsp1Cre-traced fibroblasts from both cardiac and noncardiac cell sources strongly suggests that induced cells are most likely of fibroblastic origin.

**Conclusions:** The findings from this study provide proof-of-concept that miRNAs have the capability of directly converting fibroblasts to a cardiomyocyte-like phenotype in vitro. Also of significance is that this is the first report of direct cardiac reprogramming in vivo. Our approach may have broad and important implications for therapeutic tissue regeneration in general.6

**From the June 8, 2012 issue:**

**Nuclear miRNA Regulates the Mitochondrial Genome in the Heart**

Samarjit Das, Marcella Ferlito, Oliver A. Kent, Karen Fox-Talbot, Richard Wang, Delong Liu, Nalini Raghavachari, Yanqin Yang, Sarah J. Wheelan, Elizabeth Murphy, Charles Steenbergen

**Abstract**

**Rationale:** Mitochondria are semiautonomous cellular organelles with their own genome, which not only supply energy but also participate in cell death pathways. MicroRNAs (miRNAs) are usually 19 to 25 nt long, noncoding RNAs, involved in posttranscriptional gene regulation by binding to the 3′-untranslated regions of target mRNA, which impact on diverse cellular processes.

**Objective:** To determine whether nuclear miRNAs translocate into the mitochondria and regulate mitochondrial function with possible pathophysiological implications in cardiac myocytes.

**Methods and Results:** We find that miR-181c is encoded in the nucleus, assembled in the cytoplasm, and finally translocated into the mitochondria of cardiac myocytes. Immunoprecipitation of Argonaute 2 from the mitochondrial fraction indicates binding of cytochrome c oxidase subunit 1 (mt-COX1) mRNA from the mitochondrial genome with miR-181c. Also, a luciferase reporter construct shows that mi-181c binds to the 3′-untranslated regions of mt-COX1. To study whether miR-181c regulates mt-COX1, we overexpressed precursor miR-181c (or a scrambled sequence) in primary cultures of neonatal rat ventricular myocytes. Overexpression of miR-181c did not change mt-COX1 mRNA but significantly decreased mt-COX1 protein, suggesting that miR-181c is primarily a translational regulator of mt-COX1. In addition to altering mt-COX1, overexpression of miR-181c results in increased mt-COX2 mRNA and protein content, with an increase in both mitochondrial respiration and reactive oxygen species generation in neonatal rat ventricular myocytes. Thus, our data show for the first time that miR-181c can enter and target the mitochondrial genome, ultimately causing electron transport chain complex IV remodeling and mitochondrial dysfunction.

**Conclusions:** Nuclear miR-181c translocates into the mitochondria and regulates mitochondrial genome expression. This unique observation may open a new dimension to our understanding of mitochondrial dynamics and the role of miRNA in mitochondrial dysfunction.7

**From the September 28, 2012 issue:**

**Dealcoholized Red Wine Decreases Systolic and Diastolic Blood Pressure and Increases Plasma Nitric Oxide**

Gemma Chiva-Blanch, Mireia Urpi-Sarda, Emilio Ros, Sara Arranz, Palmira Valderas-Martínez, Rosa Casas, Emilio Sacanella, Rafael Llorach, Rosa M. Lamuela-Raventos, Cristina Andres-Lacueva, Ramon Estruch

**Abstract**

**Rationale:** Experimental studies have shown a potential blood pressure (BP) lowering effect of red wine polyphenols,
whereas the effects of ethanol and polyphenols on BP in humans are not yet clear.

**Objective:** The aim of the present work was to evaluate the effects of red wine fractions (alcoholic and nonalcoholic) on BP and plasma nitric oxide (NO) in subjects at high cardiovascular risk.

**Methods and Results:** Sixty-seven men at high cardiovascular risk were studied. After a 2-week run-in period, subjects were randomized into 3 treatment periods in a crossover clinical trial, with a common background diet plus red wine (30 g alcohol/d), the equivalent amount of dealcoholized red wine, or gin (30 g alcohol/d), lasting 4 weeks each intervention. At baseline and after each intervention, anthropometrical parameters, BP and plasma NO were measured. Systolic and diastolic BP decreased significantly after the dealcoholized red wine intervention and these changes correlated with increases in plasma NO.

**Conclusions:** Dealcoholized red wine decreases systolic and diastolic BP. Our results point out through an NO-mediated mechanism. The daily consumption of dealcoholized red wine could be useful for the prevention of low to moderate hypertension. Trial registered at controlled-trials.com: ISRCTN88720134.

**From the October 12, 2012 issue:**

**Induction of Cardiomyocyte-Like Cells in Infarct Hearts by Gene Transfer of Gata4, MeF2c, and Tbx5**

Kohei Inagawa, Kazutaka Miyamoto, Hiroyuki Yamakawa, Naoto Muraoka, Taketaro Sadahiro, Tomohiko Umei, Rie Wada, Yoshinori Katsumata, Ruri Kaneda, Koji Nakade, Chitose Kurihara, Yuichi Obata, Koichi Miyake, Keiichi Fukuda, Masaki Ieda

**Abstract**

**Rationale:** After myocardial infarction (MI), massive cell death in the myocardium initiates fibrosis and scar formation, leading to heart failure. We recently found that a combination of 3 cardiac transcription factors, Gata4, MeF2c, and Tbx5 (GMT), reprograms fibroblasts directly into functional cardiomyocytes in vitro.

**Objective:** To investigate whether viral gene transfer of GMT into infarcted hearts induces cardiomyocyte generation.

**Methods and Results:** Coronary artery ligation was used to generate MI in the mouse. In vitro transduction of GMT retrovirus converted cardiac fibroblasts from the infarct region into cardiomyocyte-like cells with cardiac-specific gene expression and sarcomeric structures. Injection of the green fluorescent protein (GFP) retrovirus into mouse hearts, immediately after MI, infected only proliferating noncardiomyocytes, mainly fibroblasts, in the infarct region. The GFP expression diminished after 2 weeks in immunocompetent mice but remained stable for 3 months in immunosuppressed mice, in which cardiac induction did not occur. In contrast, injection of GMT retrovirus into α-myosin heavy chain (αMHC)-GFP transgenic mouse hearts induced the expression of αMHC-GFP, a marker of cardiomyocytes, in 3% of virus-infected cells after 1 week. A pooled GMT injection into the immunosuppressed mouse hearts induced cardiac marker expression in retrovirus-infected cells within 2 weeks, although few cells showed striated muscle structures. To transduce GMT efficiently in vivo, we generated a polycistronic retrovirus expressing GMT separated by 2A self-cleaving peptides (3F2A). The 3F2A-induced cardiomyocyte-like cells in fibrotic tissue expressed sarcomeric α-actinin and cardiac troponin T and had clear cross striations. Quantitative RT-PCR also demonstrated that FACS-sorted 3F2A-transduced cells expressed cardiac-specific genes.

**Conclusions:** GMT gene transfer induced cardiomyocyte-like cells in infarcted hearts.

**From the October 12, 2012 issue:**

**Overexpression of Endothelial Nitric Oxide Synthase Prevents Diet-Induced Obesity and Regulates Adipocyte Phenotype**


**Abstract**

**Rationale:** Endothelial dysfunction is a characteristic feature of diabetes mellitus and obesity in animal models and humans. Deficits in nitric oxide production by endothelial nitric oxide synthase (eNOS) are associated with insulin resistance, which is exacerbated by high-fat diet. Nevertheless, the metabolic effects of increasing eNOS levels have not been studied.

**Objective:** The current study was designed to test whether overexpression of eNOS would prevent diet-induced obesity and insulin resistance.

**Methods and Results:** In db/db mice and in high-fat diet–fed wild-type C57BL/6J mice, the abundance of eNOS protein in adipose tissue was decreased without significant changes in eNOS levels in skeletal muscle or aorta. Mice overexpressing eNOS (eNOS transgenic mice) were resistant to diet-induced obesity and hyperinsulinemia, although systemic glucose intolerance remained largely unaffected. In comparison with wild-type mice, high-fat diet–fed eNOS transgenic mice displayed a higher metabolic rate and attenuated hypertrophy of white adipocytes. Overexpression of eNOS did not affect food consumption or diet-induced changes in plasma cholesterol or leptin levels, yet plasma triglycerides and fatty acids were decreased. Metabolomic analysis of adipose tissue indicated that eNOS overexpression primarily affected amino acid and lipid metabolism; subpathway analysis suggested changes in fatty acid oxidation. In agreement with these findings, adipose tissue from eNOS transgenic mice showed higher levels of PPAR-α and PPAR-γ gene expression, elevated abundance of mitochondrial proteins, and a higher rate of oxygen consumption.

**Conclusions:** These findings demonstrate that increased eNOS activity prevents the obesogenic effects of high-fat diet without affecting systemic insulin resistance, in part, by stimulating metabolic activity in adipose tissue.
Stem Cell Factor Gene Transfer Promotes Cardiac Repair After Myocardial Infarction via In Situ Recruitment and Expansion of c-kit+ Cells

Elisa Yaniz-Galende, Jiqiu Chen, Elie Chemaly, Lifan Liang, Jean-Sebastien Hulot, LaTronya McCollum, Teresa Arias, Valentin Fuster, Krisztina M. Zsebo, Roger J. Hajjar

Abstract

Rationale: There is growing evidence that the myocardium responds to injury by recruiting c-kit+ cardiac progenitor cells to the damage tissue. Even though the ability of exogenously introducing c-kit+ cells to injured myocardium has been established, the capability of recruiting these cells through modulation of local signaling pathways by gene transfer has not been tested.

Objective: To determine whether stem cell factor gene transfer mediates cardiac regeneration in a rat myocardial infarction model, through survival and recruitment of c-kit+ progenitors and cell-cycle activation in cardiomyocytes, and explore the mechanisms involved.

Methods and Results: Infarct size, cardiac function, cardiac progenitor cells recruitment, fibrosis, and cardiomyocyte cell-cycle activation were measured at different time points in controls (n=10) and on stem cell factor gene transfer (n=13) after myocardial infarction. We found a regenerative response as a result of stem cell factor overexpression characterized by an enhancement in cardiac hemodynamic function—an improvement in survival; a reduction in fibrosis, infarct size and apoptosis; an increase in cardiac c-kit+ progenitor cells recruitment to the injured area; an increase in cardiomyocyte cell-cycle activation; and Wnt/β-catenin pathway induction.

Conclusions: Stem cell factor gene transfer induces c-kit+ stem/progenitor cell expansion in situ and cardiomyocyte proliferation, which may represent a new therapeutic strategy to reverse adverse remodeling after myocardial infarction.11

References

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