Circulating MicroRNAs as Novel Biomarkers for Platelet Activation

Peter Willeit, Anna Zampetaki, Katarzyna Dudek, Dorothee Kaudewitz, Alice King, Nicholas S. Kirkby, Roxanne Crosby-Nwaobi, Marianna Prokopi, Ignat Drozdov, Sarah R. Langley, Sobha Sivaprasad, Hugh S. Markus, Jane A. Mitchell, Timothy D. Warner, Stefan Kiechl, Manuel Mayr

Abstract
Rationale—MicroRNA (miRNA) biomarkers are attracting considerable interest. Effects of medication, however, have not been investigated thus far.

Objective—to analyze changes in plasma miRNAs in response to antiplatelet therapy.

Methods and Results—Profiling for 377 miRNAs was performed in platelets, platelet microparticles, platelet-rich plasma, platelet-poor plasma, and serum. Platelet-rich plasma showed markedly higher levels of miRNAs than serum and platelet-poor plasma. Few abundant platelet miRNAs, such as miR-24, miR-197, miR-191, and miR-223, were also increased in serum compared with platelet-poor plasma. In contrast, antiplatelet therapy significantly reduced miRNA levels. Using custom-made quantitative real-time polymerase chain reaction plates, 92 miRNAs were assessed in a dose-escalation study in healthy volunteers at 4 different time points: at baseline without therapy, at 1 week with 10 mg prasugrel, at 2 weeks with 10 mg prasugrel plus 75 mg aspirin, and at 3 weeks with 10 mg prasugrel plus 300 mg aspirin. Findings in healthy volunteers were confirmed by individual TaqMan quantitative real-time polymerase chain reaction assays (n=9). Validation was performed in an independent cohort of patients with symptomatic atherosclerosis (n=33), who received low-dose aspirin at baseline. Plasma levels of platelet miRNAs, such as miR-223, miR-191, and others, that is, miR-126 and miR-150, decreased on further platelet inhibition.
Conclusions—Our study demonstrated a substantial platelet contribution to the circulating miRNA pool and identified miRNAs responsive to antiplatelet therapy. It also highlights that antiplatelet therapy and preparation of blood samples could be confounding factors in case-control studies relating plasma miRNAs to cardiovascular disease.3

From the April 12, 2013 issue:

Discovery and Characterization of Alamandine: A Novel Component of the Renin–Angiotensin System


Abstract

Rationale—The renin–angiotensin system (RAS) is a key regulator of the cardiovascular system, electrolyte, and water balance. Here, we report identification and characterization of alamandine, a new heptapeptide generated by catalytic action of angiotensin-converting enzyme-2, angiotensin A, or directly from angiotensin-(1–7).

Objective—To characterize a novel component of the RAS, alamandine.

Methods and Results—Using mass spectrometry, we observed that alamandine circulates in human blood and can be formed from angiotensin-(1–7) in the heart. Alamandine produces several physiological actions that resemble those produced by angiotensin-(1–7), including vasodilation, antifibrosis, antihypertensive, and central effects. Interestingly, our data reveal that its actions are independent of the known vasodilator receptors of the RAS, Mas, and angiotensin II type 2 receptor. Rather, we demonstrate that alamandine acts through the Mas-related G-protein–coupled receptor, member D. Binding of alamandine to Mas-related G-protein–coupled receptor, member D, is blocked by D-Pro2-angiotensin-(1–7), the Mas-related G-protein–coupled receptor, member D ligand β-alanine and PD123319, but not by the Mas antagonist A-779. In addition, oral administration of an inclusion compound of alamandine/β-hydroxypropyl cyclodextrin produced a long-term antihypertensive effect in spontaneously hypertensive rats and antifibrotic effects in isoproterenol-treated rats. Alamandine had no noticeable proliferative or antiproliferative effect in human tumoral cell lines.

Conclusions—The identification of these 2 novel components of the RAS, alamandine and its receptor, provides new insights for the understanding of the physiological and pathophysiological role of the RAS and may help to develop new therapeutic strategies for treating human cardiovascular diseases and other related disorders.3

From the June 7, 2013 issue:

Control of Cholesterol Metabolism and Plasma High-Density Lipoprotein Levels by microRNA-144

Cristina M. Ramírez, Noemi Rotllan, Alexander V. Vlassov, Alberto Dávalos, Mu Li, Leigh Goedeke, Juan F. Aranda, Daniel Cirera-Salinas, Elisa Araldi, Alessandro Salerno, Amalyis Wanschel, Jiri Zavadil, Antonio Castrillo, Jungsu Kim, Yajaira Suárez, Carlos Fernández-Hernando

Abstract

Rationale—Foam cell formation because of excessive accumulation of cholesterol by macrophages is a pathological hallmark of atherosclerosis, the major cause of morbidity and mortality in Western societies. Liver X nuclear receptors (LXRs) regulate the expression of the adenosine triphosphate–binding cassette (ABC) transporters, including adenosine triphosphate–binding cassette transporter A1 (ABCA1) and adenosine triphosphate–binding cassette transporter G1 (ABCG1). ABCA1 and ABCG1 facilitate the efflux of cholesterol from macrophages and regulate high-density lipoprotein (HDL) biogenesis. Increasing evidence supports the role of microRNA (miRNAs) in regulating cholesterol metabolism through ABC transporters.

Objective—We aimed to identify novel miRNAs that regulate cholesterol metabolism in macrophages stimulated with LXR agonists.

Methods and Results—To map the miRNA expression signature of macrophages stimulated with LXR agonists, we performed an miRNA profiling microarray analysis in primary mouse peritoneal macrophages stimulated with LXR ligands. We report that LXR ligands increase miR-144 expression in macrophages and mouse livers. Overexpression of miR-144 reduces ABCA1 expression and attenuates cholesterol efflux to apolipoproteinA1 in macrophages. Delivery of miR-144 oligonucleotides to mice attenuates ABCA1 expression in the liver, reducing HDL levels. Conversely, silencing of miR-144 in mice increases the expression of ABCA1 and plasma HDL levels. Thus, miR-144 seems to regulate both macrophage cholesterol efflux and HDL biogenesis in the liver.

Conclusions—miR-144 regulates cholesterol metabolism via suppressing ABCA1 expression, and modulation of miRNAs may represent a potential therapeutic intervention for treating dyslipidemia and atherosclerotic vascular disease.4

From the July 19, 2013 issue:

A Novel Mouse Model of Atherosclerotic Plaque Instability for Drug Testing and Mechanistic/Therapeutic Discoveries Using Gene and MicroRNA Expression Profiling

Yung-Chih Chen, Anh Viet Bui, Jeannine Diesch, Richard Manasseh, Christian Hausding, Jennifer Rivera, Izhak Haviv, Alex Agrotis, Nay Min Htun, Jeremy Jowett, Christoph Eugen Hagemeeyer, Ross D. Hannan, Alex Bobik, Karlheinz Peter

Abstract

Rationale—The high morbidity/mortality of atherosclerosis is typically precipitated by plaque rupture and consequent thrombosis. However, research on underlying mechanisms and therapeutic approaches is limited by the lack of animal models that reproduce plaque instability observed in humans.
Methods and Results—On the basis of flow measurements and computational fluid dynamics, we applied a tandem stenosis to the carotid artery of apolipoprotein E-deficient mice on high-fat diet. At 7 weeks postoperatively, we observed intraplaque hemorrhage in ≈50% of mice, as well as disruption of fibrous caps, intraluminal thrombosis, neovascularization, and further characteristics typically seen in human unstable plaques. Administration of atorvastatin was associated with plaque stabilization and downregulation of monocyte chemotactrant protein-1 and ubiquitin. Microarray profiling of mRNA and microRNA (miR) and, in particular, its combined analysis demonstrated major differences in the hierarchical clustering of genes and miRs among nonatherosclerotic arteries and stable and unstable plaques, and allows the identification of distinct genes/miRs, potentially representing novel therapeutic targets for plaque stabilization. The feasibility of the described animal model as a discovery tool was established in a pilot approach, identifying a disintegrin and metalloprotease with thrombospondin motifs 4 (ADAMTS4) and miR-322 as potential pathogenic factors of plaque instability in mice and validated in human plaques.

Conclusions—The newly described mouse model reflects human atherosclerotic plaque instability and represents a discovery tool toward the development and testing of therapeutic strategies aimed at preventing plaque rupture. Distinctly expressed genes and miRs can be linked to plaque instability.5

From the August 16, 2013 issue:

Bone-Derived Stem Cells Repair the Heart After Myocardial Infarction Through Transdifferentiation and Paracrine Signaling Mechanisms

Objective—Development and use of a mouse model of plaque rupture that reflects the end stage of human atherosclerosis.

Methods and Results—On the basis of flow measurements and computational fluid dynamics, we applied a tandem stenosis to the carotid artery of apolipoprotein E-deficient mice on high-fat diet. At 7 weeks postoperatively, we observed intraplaque hemorrhage in ≈50% of mice, as well as disruption of fibrous caps, intraluminal thrombosis, neovascularization, and further characteristics typically seen in human unstable plaques. Administration of atorvastatin was associated with plaque stabilization and downregulation of monocyte chemotactrant protein-1 and ubiquitin. Microarray profiling of mRNA and microRNA (miR) and, in particular, its combined analysis demonstrated major differences in the hierarchical clustering of genes and miRs among nonatherosclerotic arteries and stable and unstable plaques, and allows the identification of distinct genes/miRs, potentially representing novel therapeutic targets for plaque stabilization. The feasibility of the described animal model as a discovery tool was established in a pilot approach, identifying a disintegrin and metalloprotease with thrombospondin motifs 4 (ADAMTS4) and miR-322 as potential pathogenic factors of plaque instability in mice and validated in human plaques.

Conclusions—The newly described mouse model reflects human atherosclerotic plaque instability and represents a discovery tool toward the development and testing of therapeutic strategies aimed at preventing plaque rupture. Distinctly expressed genes and miRs can be linked to plaque instability.5

From the September 13, 2013 issue:

Jmjd3 Controls Mesodermal and Cardiovascular Differentiation of Embryonic Stem Cells

Kisho Ohtani, Cong Zhao, Gergana Dobreva, Yosif Manavskii, Britta Kluge, Thomas Braun, Michael A. Rieger, Andreas M. Zieher, Stefanie Dinnmeler

Abstract

Rationale—The developmental role of the H3K27 demethylase, Jmjd3, especially its epigenetic regulation at target genes in response to upstream developmental signaling, is unclear.

Objective—To determine the role of Jmjd3 during mesoderm and cardiovascular lineage commitment.

Methods and Results—Ablation of Jmjd3 in mouse embryonic stem cells does not affect the maintenance of pluripotency and self-renewal but compromised mesoderm and subsequent endothelial and cardiac differentiation. Jmjd3 reduces H3K27me3 marks at the Brachyury promoter and facilitates the recruitment of β-catenin, which is critical for Wnt signal–induced mesoderm differentiation.

Conclusions—These data demonstrate that Jmjd3 is required for mesoderm differentiation and cardiovascular lineage commitment.7

From the October 12, 2013 issue:

Endothelial Cell–Dependent Regulation of Arteriogenesis

Filipa Moraes, Julie Paye, Feilim Mac Gabhann, Zhen W. Zhuang, Jiasheng Zhang, Anthony A. Lanahan, Michael Simons

Abstract

Rationale—Arteriogenesis is the process of formation of arterial conduits. Its promotion is an attractive therapeutic strategy in occlusive atherosclerotic diseases. Despite the functional and clinical importance of arteriogenesis, the biology of the process is poorly understood. Synectin, a gene previously implicated in the regulation of vascular endothelial cell growth factor signaling, offers a unique opportunity to determine relative contributions of various cell types to arteriogenesis.
Objective.—We investigated the cell-autonomous effects of a synectin knockout in arterial morphogenesis.

Methods and Results.—A floxed synectin knockin mouse line was crossed with endothelial–specific (Tie2, Cdh5, Pdgfb) and smooth muscle myosin heavy chain–specific Cre driver mouse lines to produce cell type–specific deletions. Ablation of synectin expression in endothelial, but not smooth muscle, cells resulted in the presence of developmental arterial morphogenetic defects (smaller size of the arterial tree, reduced number of arterial branches, and collaterals) and impaired arteriogenesis in adult mice.

Conclusions.—Synectin modulates developmental and adult arteriogenesis in an endothelial cell–autonomous fashion. These findings show for the first time that endothelial cells are central to both developmental and adult arteriogenesis and provide a model for future studies of factors involved in this process.

From the October 25, 2013 issue:

MicroRNA-663 Regulates Human Vascular Smooth Muscle Cell Phenotypic Switch and Vascular Neointimal Formation
Pan Li, Ni Zhu, Bing Yi, Nadan Wang, Ming Chen, Xiaohua You, Xiaoxian Zhao, Charalambos C. Solomides, Yongwen Qin, Jianxin Sun

Abstract

Rationale.—Abnormal phenotypic switch of vascular smooth muscle cell (VSMC) is a hallmark of vascular disorders such as atherosclerosis and restenosis after angioplasty. MicroRNAs (miRNAs) have emerged as important regulators for VSMC function, and we recently identified miR-663 as critical for controlling human aortic smooth muscle cell proliferation.

Objective.—To investigate whether miR-663 plays a role in human VSMC phenotypic switch and the development of neointima formation.

Methods and Results.—By using quantitative reverse-transcription polymerase chain reaction, we found that miR-663 was significantly downregulated in human aortic VSMCs on platelet-derived growth factor treatment, whereas expression was markedly increased during VSMC differentiation. Furthermore, we demonstrated that overexpression of miR-663 increased expression of VSMC differentiation marker genes, such as smooth muscle 22α, smooth muscle α-actin, calponin, and smooth muscle myosin heavy chain, and potently inhibited platelet-derived growth factor–induced VSMC proliferation and migration. We identified the transcription factor, JunB, and myosin light chain 9 as downstream targets of miR-663 in human VSMCs because overexpression of miR-663 markedly inhibited expression of JunB and its downstream molecules, such as myosin light chain 9 and matrix metalloproteinase 9. Finally, we showed that adenoviral–miR-663 markedly suppressed the neointimal lesion formation by ≥50% in mice after vascular injury induced by carotid artery ligation, specifically via decreased JunB expression.

Conclusions.—These results indicate that miR-663 is a novel modulator of human VSMC phenotypic switch by targeting JunB/myosin light chain 9 expression. These findings suggest that targeting miR-663 or its specific downstream targets in human VSMCs may represent an attractive approach for the treatment of proliferative vascular diseases.

From the November 8, 2013 issue:

Synaptojanin-2–Binding Protein Stabilizes the Notch Ligands DLL1 and DLL4 and Inhibits Sprouting Angiogenesis
M. Gordian Adam, Caroline Berger, Anja Feldner, Wan-Jen Yang, Joycelyn Wüstehube-Lausch, Stefanie E. Herberich, Marcel Pinder, Sabine Gesierich, Hans-Peter Hammes, Hellmut G. Augustin, Andreas Fischer

Abstract

Rationale.—The formation of novel blood vessels is initiated by vascular endothelial growth factor. Subsequently, DLL4–Notch signaling controls the selection of tip cells, which guide new sprouts, and trailing stalk cells. Notch signaling in stalk cells is induced by DLL4 on the tip cells. Moreover, DLL4 and DLL1 are expressed in the stalk cell plexus to maintain Notch signaling. Notch loss-of-function causes formation of a hyperdense vascular network with disturbed blood flow.

Objective.—This study was aimed at identifying novel modifiers of Notch signaling that interact with the intracellular domains of DLL1 and DLL4.

Methods and Results.—Synaptojanin-2–binding protein (SYNJ2BP, also known as ARIP2) interacted with the PDZ-binding motif of DLL1 and DLL4, but not with the Notch ligand Jagged-1. SYNJ2BP was preferentially expressed in stalk cells, enhanced DLL1 and DLL4 protein stability, and promoted Notch signaling in endothelial cells. SYNJ2BP induced expression of the Notch target genes HEY1, lunatic fringe, and ephrin-B2, reduced phosphorylation of ERK1/2, and decreased expression of the angiogenic factor vascular endothelial growth factor. It inhibited the expression of genes enriched in tip cells, such as angiopoietin-2, ESM1, and Apelin, and impaired tip cell formation. SYNJ2BP inhibited endothelial cell migration, proliferation, and VEGF-induced angiogenesis. This could be rescued by blockade of Notch signaling or application of angiopoietin-2. SYNJ2BP-silenced human endothelial cells formed a functional vascular network in immunocompromised mice with significantly increased vascular density.

Conclusions.—These data identify SYNJ2BP as a novel inhibitor of tip cell formation, executing its functions predominately by promoting Delta–Notch signaling.

From the December 6, 2013 issue:

MicroRNA-223 Antagonizes Angiogenesis by Targeting β1 Integrin and Preventing Growth Factor Signaling in Endothelial Cells
Lei Shi, Beate Fisslthaler, Nina Zippel, Timo Frömel, Jiong Hu, Anaro Elghazawi, Heinrich Heide, Rüdiger Popp, Ingrid Fleming

Abstract

Rationale.—Endothelial cells in situ are largely quiescent, and their isolation and culture are associated with the switch to a proliferative phenotype.
**Objective**—To identify antiangiogenic microRNAs (miRNA) expressed by native endothelial cells that are altered after isolation and culture, as well as the protein targets that regulate responses to growth factors.

**Methods and Results**—Profiling studies revealed that miR-223 was highly expressed in freshly isolated human, murine, and porcine endothelial cells, but those levels decreased in culture. In primary cultures of endothelial cells, vascular endothelial cell growth factor and basic fibroblast growth factor further decreased miR-223 expression. The overexpression of precursor-miR-223 did not affect basal endothelial cell proliferation but abrogated vascular endothelial cell growth factor–induced and basic fibroblast growth factor–induced proliferation, as well as migration and sprouting. Inhibition of miR-223 in vivo using specific antagomirs potentiated postnatal retinal angiogenesis in wild-type mice, whereas recovery of perfusion after femoral artery ligation and endothelial sprouting from aortic rings from adult miR-223−/y animals were enhanced. MiR-223 overexpression had no effect on the growth factor–induced activation of ERK1/2 but inhibited the vascular endothelial cell growth factor–induced phosphorylation of their receptors and activation of Akt. β1 integrin was identified as a target of miR-223, and its downregulation reproduced the defects in growth factor receptor phosphorylation and Akt signaling seen after miR-223 overexpression. Reintroduction of β1 integrin into miR-223–overexpressing cells was sufficient to rescue growth factor signaling and angiogenesis.

**Conclusions**—These results indicate that miR-223 is an antiangiogenic microRNA that prevents endothelial cell proliferation at least partly by targeting β1 integrin.11

**References**

The 10 Most Read Articles Published in *Circulation Research* in 2013

Roberto Bolli
for the Editors

*Circ Res.* 2014;114:765-769
doi: 10.1161/CIRCRESAHA.114.303612

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/114/5/765

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/