



Published in final edited form as:

Circ Res. 2011 October 28; 109(10): . doi:10.1161/CIRCRESAHA.111.256388.

Modulating the MicroRNA Architecture of an Aging Aorta

Jason R. O'Rourke and Eric N. Olson

Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX

Keywords

Editorials; aging; aorta; miR-29; extracellular matrix; coronary aneurysm

The 17th century British physician Thomas Sydenham is credited with saying, "A man is as old as his arteries." Indeed, aging is associated with myriad structural and functional changes in the cardiovascular system.¹ In particular, thoracic aortic aneurysms are commonly found in people during the sixth or seventh decade of life and at a frequency of nearly 1 in 16 000 people per year.² An aneurysm is a balloon-like bulge that is characterized by thinning and weakness of the vascular wall. Enlarged aortic aneurysms are prone to rupture and often result in death.

Maintaining the structural integrity of the aorta is critical to its function and is dictated by the biomechanical properties of the 3 different wall layers: the adventitia, media, and intima. Smooth muscle and extracellular matrix within the media are the largest components of the aortic vascular wall. Smooth muscle cell death or dysfunction and perturbations in extracellular matrix deposition are often associated with aging and aneurysms.³ Advances in surgical procedures to treat aortic aneurysms increase life expectancy and reduce morbidity in the young population but are more risky in the elderly because of cardiovascular comorbidity and surgery-related lethality. Therefore, a comprehensive understanding of the changes in molecular signaling events during aortic aging is necessary to develop novel therapeutics for preventing and treating aneurysm formation.

Recent studies have pointed to microRNAs (miRNAs) as key regulators of a variety of cardiovascular disorders and biomarkers of disease progression.^{4,5} miRNAs negatively regulate target mRNAs through Watson-Crick base pairing with complementary sequences in 3' untranslated regions of mRNAs and often target collections of mRNAs that encode proteins with related functions.⁶ In this issue of *Circulation Research*, Stefanie Dimmeler and colleagues provide novel insights into miRNA-mediated pathogenic mechanisms that govern aorta aging.⁷ Boon et al⁷ profiled mRNAs and miRNAs in young (6 weeks old) and elderly (18 months old) mouse aortas. They then used 2 independent bioinformatics tools to identify miRNAs whose predicted targets are reciprocally regulated during aging. The only miRNA that met this criterion was miR-29. miR-29 is elevated in old aortas in which extracellular matrix proteins with predicted miR-29 binding sites are decreased. On the basis of these findings, Boon et al⁷ argued that miR-29 is the only miRNA that significantly

© 2011 American Heart Association, Inc.

Correspondence to Eric N. Olson, Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9148. eric.olson@utsouthwestern.edu.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

This manuscript was sent to Ali J. Marian, Consulting Editor, for review by expert referees, editorial decision, and final disposition.

Disclosures

Eric Olson is a cofounder of miRagen Therapeutics, which is developing microRNA-based drugs for cardiovascular disease.

affects mRNA expression during aorta aging; this assumes that the bioinformatics algorithms they used accurately predict all miRNA targets. Nonetheless, the authors chose to investigate how miR-29 functions during aortic aging and dilation.

The miR-29 family of miRNAs contains 3 members (29a, 29b, and 29c) that are encoded by 2 distinct loci that give rise to bicistronic precursor miRNAs (miR-29a/b1 and miR-29b2/c). The elevation of miR-29 expression in aged aortas is consistent with a recent report demonstrating that miR-29 is upregulated in multiple tissues during aging and in a progeria mouse model of premature aging.⁸ Strikingly, the authors observed that only the miR-29a/b1 precursor pri-miRNA was upregulated in aged aortas. This suggests that miR-29c, which is encoded by the miR-29b2/c cluster, must increase via a posttranscriptional mechanism during aging and warrants further investigation. Another unusual finding was that different miR-29 family members displayed distinct patterns of expression in multiple settings of aortic dilation. For example, miR-29a, miR-29b, and miR-29c were all upregulated in aneurysms of fibulin-4 knockout mice, but only miR-29b was elevated after angiotensin II infusion, which causes vascular distention. In addition, miR-29b was the only family member elevated in biopsy samples from patients who displayed thoracic aortic aneurysms. Therefore, it appears as if miR-29b is most sensitive to aortic perturbation. These data suggest that miR-29b is preferentially processed or stabilized during aortic dilation.

miR-29 has been shown to regulate extracellular matrix protein levels in multiple cellular contexts.^{9–12} Indeed, Boon and colleagues⁷ demonstrated that the mRNAs that encode collagens 1A1 and 3A1 and elastin are all decreased in old aortas. The critical experiment required to transform these phenomenological observations into functionality was performed by blocking miR-29 in angiotensin II-treated mice. Locked nucleic acid-modified 16mer oligonucleotides complementary to miR-29 (anti-miR-29) were used to inhibit miR-29 activity. Anti-miR locked nucleic acids are thought to function by sequestering miRNAs from their mRNA targets.¹³ The authors demonstrated that all miR-29 family members were reduced in anti-miR-29-treated mice. Importantly, a scrambled locked nucleic acid control, which is often lacking in such studies, was used to confirm specificity. Angiotensin II-treated mice that received anti-miR-29 displayed increases in extracellular matrix gene expression and extraordinary reduction in aorta dilation. It is noteworthy that there were no changes in fibrosis on histological examination.

There are several aspects of the study by Boon and colleagues⁷ that warrant future investigation. First, it is surprising that such a modest elevation in miR-29 (~1.5-fold) during aging is associated with a more pronounced reduction in extracellular matrix components (~3-fold). These findings suggest remarkably precise titration of mRNAs by miR-29 and imply that the ratio of miR-29 to its targets establishes a sharp threshold for regulation. An alternative explanation is that non-miR-29 regulatory mechanisms exist that control extracellular matrix deposition during aging and dilation. It is also striking that a small change in extracellular matrix components (~1.5–2-fold) that was observed in anti-miR-29-treated mice resulted in an almost complete reversal of the angiotensin II-mediated aortic dilation. Are the beneficial effects solely explained by a marginal increase in matrix proteins, or is it possible that miR-29 may have extracellular matrix-independent functions that reduce aorta dilation? It is also noteworthy that the authors chose to use old (18 months) mice for the angiotensin II infusion experiments, because this would be an aged environment in which extracellular matrix remodeling has already occurred. It would be interesting to know whether anti-miR-29-treated mice show aortic improvement in the absence of angiotensin II and during normal aging. Finally, Loeys-Dietz is a recently described aortic aneurysm syndrome that is caused by transforming growth factor-mutations.¹⁴ Transforming growth factor- signaling has been shown to regulate miR-29 and extracellular matrix proteins in multiple tissues and cell lines.^{9,10,12,15–17} It might be

worthwhile to investigate whether miR-29 dysfunction results in inherited disorders in which the aorta is primarily affected.

The extended longevity in modern societies has resulted in an increase in aging-associated disorders. Furthermore, the rising prevalence in aortic disease underscores the need for novel diagnostics and therapeutics. miR-29 may prove to be a biomarker for age-related disease, and blocking miR-29 has potential to treat age-induced aortic aneurysms. Modulation of miRNA activity has emerged as an auspicious therapeutic strategy. The ability of miRNAs to modulate complex biological processes by regulating collections of targets, as exemplified by the control of extracellular matrix production by miR-29, represents a new strategy for therapeutic intervention. The possibility of delivering anti-miRs or miRNA mimics directly to the vessel wall via coated stents offers possibilities for direct manipulation of vascular disease and avoids possible toxicities associated with systemic delivery. While there are clearly many challenges to the development of miRNA-based therapeutics, the central roles of miRNAs in cardiovascular disease, combined with recent advances in oligonucleotide chemistries with demonstrated therapeutic efficacy in primates and humans, raise many opportunities for future disease modification.

Acknowledgments

Sources of Funding

J.R.O. is supported by a National Research Service Award F32 postdoctoral fellowship from the National Institutes of Health (National Institute of Arthritis and Musculoskeletal and Skin Diseases). Work in the laboratory of E.N.O. is funded by grants from the National Institutes of Health, the Donald W. Reynolds Center for Clinical Cardiovascular Research, the American Heart Association, the Jon Holden DeHaan Foundation, the Leducc Foundation, and the Robert A. Welch Foundation.

References

- O'Rourke MF. Arterial aging: pathophysiological principles. *Vasc Med.* 2007; 12:329–341. [PubMed: 18048471]
- Isselbacher EM. Thoracic and abdominal aortic aneurysms. *Circulation.* 2005; 111:816–828. [PubMed: 15710776]
- Ince H, Nienaber CA. Etiology, pathogenesis and management of thoracic aortic aneurysm. *Nat Clin Pract Cardiovasc Med.* 2007; 4:418–427. [PubMed: 17653114]
- Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. *Nature.* 2011; 469:336–342. [PubMed: 21248840]
- Edwards JK, Pasqualini R, Arap W, Calin GA. MicroRNAs and ultra-conserved genes as diagnostic markers and therapeutic targets in cancer and cardiovascular diseases. *J Cardiovasc Transl Res.* 2010; 3:271–279. [PubMed: 20560048]
- Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009; 136:215–233. [PubMed: 19167326]
- Boon RA, Seeger T, Heydt S, Fischer A, Hergenreider E, Horrevoets AJ, Vinciguerra M, Rosenthal N, Sciacca S, Pilato M, van Heijningen P, Essers J, Brandes RP, Zeiher AM, Dimmeler S. MicroRNA-29 in aortic dilation: implications for aneurysm formation. *Circ Res.* 2011; 109:1115–1119. [PubMed: 21903938]
- Ugalde AP, Ramsay AJ, de la Rosa J, Varela I, Marino G, Cadinanos J, Lu J, Freije JM, Lopez-Otin C. Aging and chronic DNA damage response activate a regulatory pathway involving mir-29 and p53. *EMBO J.* 2011; 30:2219–2232. [PubMed: 21522133]
- Cushing L, Kuang PP, Qian J, Shao F, Wu J, Little F, Thannickal VJ, Cardoso WV, Lu J. Mir-29 is a major regulator of genes associated with pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2011; 45:287–294. [PubMed: 20971881]

10. Qin W, Chung AC, Huang XR, Meng XM, Hui DS, Yu CM, Sung JJ, Lan HY. TGF-beta/SMAD3 signaling promotes renal fibrosis by inhibiting miR-29. *J Am Soc Nephrol.* 2011; 22:1462–1474. [PubMed: 21784902]
11. Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, Janssen J, Koppe C, Knolle P, Castoldi M, Tacke F, Trautwein C, Luedde T. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. *Hepatology.* 2011; 53:209–218. [PubMed: 20890893]
12. van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, Hill JA, Olson EN. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A.* 2008; 105:13027–13032. [PubMed: 18723672]
13. Obad S, dos Santos CO, Petri A, Heidenblad M, Broom O, Ruse C, Fu C, Lindow M, Stenvang J, Straarup EM, Hansen HF, Koch T, Pappin D, Hannon GJ, Kauppinen S. Silencing of microRNA families by seed-targeting tiny LNAs. *Nat Genet.* 2011; 43:371–378. [PubMed: 21423181]
14. Van Hemelrijk C, Renard M, Loeys B. The Loeys-Dietz syndrome: an update for the clinician. *Curr Opin Cardiol.* 2010; 25:546–551. [PubMed: 20838339]
15. Kwiecinski M, Noetel A, Elfimova N, Trebicka J, Schievenbusch S, Strack I, Molnar L, von Brandenstein M, Tox U, Nischt R, Coutelle O, Dienes HP, Odenthal M. Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA-29 induction. *PLoS One.* 2011; 6:e24568. [PubMed: 21931759]
16. Villarreal G Jr, Oh DJ, Kang MH, Rhee DJ. Coordinated regulation of extracellular matrix synthesis by the microRNA-29 family in the trabecular meshwork. *Invest Ophthalmol Vis Sci.* 2011; 52:3391–3397. [PubMed: 21330653]
17. Winbanks CE, Wang B, Beyer C, Koh P, White L, Kantharidis P, Gregorevic P. TGF-beta regulates miR-206 and miR-29 to control myogenic differentiation through regulation of HDAC4. *J Biol Chem.* 2011; 286:13805–13814. [PubMed: 21324893]