ORIGINAL ARTICLE

Novel Association of miR-451 with the Incidence of TEVG Stenosis in a Murine Model

Narutoshi Hibino, MD, PhD,¹ Cameron A. Best, BS,² Alyson Engle, BS,³ Svetlana Ghimbovski, PhD,^{4,5} Susan Knoblach, PhD,^{4,5} Dilip S. Nath, MD,⁶ Nobuyuki Ishibashi, MD,⁶ and Richard A. Jonas, MD⁶

The development of a tissue-engineered vascular graft (TEVG) holds great promise for advancing the field of cardiac surgery. Despite the successful translation of this technology, previous reports identify the primary mode of graft failure as stenosis secondary to intimal hyperplasia. MicroRNAs (miRNAs) regulate gene expression by interfering with mRNA function and recent research has suggested miRNA as a potential therapeutic target. The role of miRNAs in TEVGs during neotissue formation is currently unknown. In this study, we investigated if miRNAs regulate the inhibition of graft stenosis. Biodegradable PGA-P(LA/CL) scaffolds were implanted as inferior vena cava interposition grafts in a murine model ($n=14$). Mice were sacrificed 14 days following implantation and TEVGs were harvested for histological analysis and miRNA profiling using Affymetrix miRNA arrays. Graft diameters were measured histologically, and the largest grafts (patent group) and smallest grafts (stenosed group) were profiled ($n=4$ for each group). Cell population in each graft was analyzed with immunohistochemistry using antismooth muscle actin (SMA) and antimacrophage (F4/80) antibodies. The graft diameter was significantly greater in the patent group (0.63 ± 0.06 mm) than in the stenosed group (0.17 ± 0.06 mm) ($p < 0.01$). Cell proliferation was significantly greater in the stenosed grafts than in patent grafts ($p < 0.01$: SMA [187 ± 11 vs. 77 ± 8 cells] vs. $p = 0.025$: F4/80 [245 ± 23 vs. 187 ± 11 cells]). MiRNA array of 1416 genes showed that in stenosed grafts, mir-451, mir-338, and mir-466 were downregulated and mir-154 was upregulated. Mir-451 exhibited the greatest difference in expression between stenosed and patent grafts by -3.1 -fold. Significant negative correlation was found between the expression of mir-451 and cell proliferation (SMA: $r = -0.86$, $p = 0.003$; F4/80: $r = -0.89$, $p = 0.001$). Our data, along with previous evidence that mir-451 regulates tumor suppressor genes, suggest that downregulation of mir-451 promotes acute proliferation of macrophages and smooth muscle cells, thereby inducing TEVG stenosis. Adequate expression of mir-451 may be critical for improving TEVG patency.

Introduction

CONGENITAL HEART DISEASE (CHD) affects nearly 1% of live births each year and is a leading cause of death in the newborn period.^{1,2} A quarter of infants born with CHD require invasive treatment within their first year of life, and complications arising from the use of synthetic graft materials such as GoreTex or Dacron in cardiovascular reconstruction, repair, or patching are a major source of morbidity and mortality.³ Similarly, in the absence of viable autologous tissue,

synthetic vascular conduits used in small-diameter (<6 mm) bypass procedures demonstrate poor patency and poor long-term durability and are prone to calcification, thrombus formation, and infection or host rejection.⁴

A promising alternative to traditional synthetic vascular conduits is a tissue-engineered vascular graft (TEVG) produced by seeding autologous stem cells onto a biodegradable scaffold. TEVGs are not limited in supply and offer immunologic compatibility, resistance to thrombosis, growth potential, and the capacity for remodeling and self-repair.

¹Department of Cardiac Surgery, Johns Hopkins Hospital, Baltimore, Maryland.

²Tissue Engineering Program and Surgical Research, Nationwide Children's Hospital, Columbus, Ohio.

³George Washington University School of Medicine and Health Sciences, Washington, District of Columbia.

⁴Department of Integrative Systems Biology, George Washington University School of Medicine and Health Sciences, Washington, District of Columbia.

⁵Research Center for Genetic Medicine, Children's National Medical Center, NW Washington, District of Columbia.

⁶Department of Cardiovascular Surgery, Children's National Medical Center, NW Washington, District of Columbia.

First-generation TEVGs seeded with autologous bone marrow mononuclear cells have been successfully translated to the pediatric population for the treatment of single ventricle physiology; however, stenosis due to chronic intimal hyperplasia was reported as the primary graft-related complication.⁵ Current research is aimed at identifying the underlying cellular and molecular mechanisms of vascular neotissue formation, TEVG remodeling upon scaffold degradation, and the development of TEVG stenosis to rationally design a second-generation TEVG with off-the-shelf availability for clinical translation.^{2,6,7}

The transition from biodegradable scaffold to vascular neotissue is an inflammation-mediated remodeling process dependent upon the activity of infiltrating host-derived monocytes and macrophages.^{8,9} Paracrine signaling from inflammatory cells within the scaffold in the form of growth factors, cytokines, and chemokines induces migration of neighboring native smooth muscle and endothelial cells, which take residence in the scaffold and proliferate to form the medial and intimal layers of the neovessel as scaffold materials degrade.^{8–10} Simultaneously engrafted macrophages and fibroblasts in the scaffold deposit extracellular matrix (ECM) precursors (MMP-2, MMP-9) that over time result in the formation of a robust and organized vascular ECM that determines the mechanical characteristics of the TEVG.^{11,12} The degree of macrophage infiltration has been shown to correlate with the incidence of stenosis, and one role of bone marrow mononuclear cell seeding is to mitigate the host's initial foreign body response to the scaffold, thereby preventing acute stenosis and promoting tissue regeneration.⁹ Interestingly, patent TEVGs in a C57BL/6 model have been reported without the use of cell seeding; however, the incidence of neointimal hyperplasia and stenosis in unseeded (cell-free) TEVGs after 2 weeks of implantation is high (64%).⁹ In the clinical setting, the use of cell-free TEVGs would obviate the need for bone marrow harvest and *in vitro* graft seeding and offers the potential of a truly off-the-shelf TEVG.² Current efforts are therefore aimed at elucidating the mechanisms by which the development of TEVG stenosis is regulated to progress toward the clinical translation of a second-generation cell-free TEVG.

The latest research is focusing on the regulatory role that miRNAs play in the development of disease and their potential as diagnostic tools and therapeutic targets.^{13–15} miRNAs are endogenous, tissue-specific, small (~22 nucleotide) noncoding RNAs that negatively regulate mRNA function by binding to the 3' untranslated region of target mRNA and either repress the target mRNA translation or cause its degradation.^{13,15–17} Two critical features of miRNA function are (1) tissue and cell specificity and (2) miRNAs can regulate multiple mRNA targets and the translation of one mRNA can be inhibited by multiple miRNAs.^{18,19} It has been demonstrated that aberrant miRNA expression is a signature of many human malignancies, and basic and clinical studies have identified specific miRNAs as oncogenes and tumor suppressors; furthermore, miRNAs are now understood as essential to cell processes such as proliferation, differentiation, migration, and apoptosis.^{13,14,19–23} Interestingly, miRNAs are also linked to regulation of vascular cell function and phenotype in response to injury, and specific miRNAs (i.e., miR-21, miR-121, miR-122, miR-143, and miR-145) are critical to the progression of proliferative vascular disease such as neointimal lesion formation and restenosis.^{15,16,22,24}

Since the formation of a patent neovessel from a TEVG scaffold is considered as an intricate balance between cellular migration, proliferation, differentiation, and apoptosis, miRNA regulation of these cellular events may be involved in either maintaining a patent TEVG or contributing to neointimal hyperplasia and graft stenosis/occlusion. In the present report, we hypothesized that specific miRNA may be responsible for the regulation of TEVG stenosis and investigated the relative expression of all currently known miRNAs between patent and stenosed cell-free TEVGs after 2 weeks of implantation in a C57BL/6 mouse model.

Materials and Methods

Graft, implantation, harvest

Biodegradable vascular scaffolds (inner diameter: 0.7 mm, wall thickness 0.3 mm, length: 3.0 mm) were fabricated from a nonwoven polyglycolic acid (PGA) felt sealed with a copolymer solution (1:1 weight:volume) of L-lactide and ε-caprolactone (P[LA/CL]) (Gunze Corporation, Tokyo, Japan). Vascular scaffolds were implanted as inferior vena cava (IVC) interposition grafts in 14 female C57 BL/6 wild-type mice, 8–12 weeks old, weighing 18–20 g (Jackson Laboratories, Bar Harbor, ME). Using the aseptic microsurgical technique, a midline incision was made and the IVC was exposed. A 2.0-mm section of the IVC between the left renal vein and the iliac bifurcation was clamped and excised. Vascular scaffolds were placed as interposition grafts (end-to-end anastomoses) using a running 10-0 nylon suture. Mice were sacrificed 14 days after implantation and the TEVGs were harvested for either histology and immunohistochemistry or miRNA assay. The Institutional Animal Care and Use Committee and the Institutional Review Board at Children's National Medical Center and Nationwide Children's Hospital approved all animal procedures and experimental protocols reported in this study.

Histology and immunohistochemistry

TEVG samples harvested for standard histology and immunohistochemical staining were perfusion fixed in formalin (1:10 dilution in phosphate-buffered saline [PBS]), stored overnight at 4°C, and embedded in optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Torrance, CA). To determine graft size and patency, sections were stained with hematoxylin and eosin (H&E) and visualized with light microscopy, then analyzed with ImageJ software (NIH, Bethesda, MD). Two sample groups were established from the H&E sections for further analysis: four patent grafts of the largest diameter composed the patent group and four stenotic grafts of the smallest diameter composed the stenosed group.

To determine cellular infiltration and proliferation within the biodegradable scaffold, immunohistochemical staining was performed. Macrophages were stained using an anti-F4/80 primary antibody (1:100 dilution in PBS; Abcam, Cambridge, UK), and smooth muscle cells were stained with an anti-α-smooth muscle actin (αSMA) primary antibody (1:500 dilution in PBS; Dako, Carpinteria, CA). Sections were incubated with primary antibodies at 4°C overnight. Sections were then incubated with a species-appropriate biotinylated secondary antibody (Dako), followed by binding of horseradish peroxidase

TABLE 1. TAQMAN MICRORNA-451 ASSAY AND SNO RNA202 SEQUENCING

Assay type	Assay name	miRNA sequence	miRBase accession number
muture miRNA	mmu-miR-451	CUUGGGAAUGGCGAGGAAACCGUUACCAUACUGAGU UUAGUAAUGGUAACGGUUCUUGCUGCUCCCA	MI0001730
muture miRNA	mmu-miR-154	GAAGAUAGGUUAUCCGUGUUGCCUUCGCUUUAUUCGUG ACGAAUCAUACACGGUUGACCUAUUUUU	MI000176
muture endocontrol	snoRNA202	GCTGTACTGACTTGATGAAAGTACTTTTGAACCCCTTTTCCA TCTGATG	AF357327

miRNA, microRNA.

streptavidin (Dako) and chromogenic detection with 3, 3'-diaminobenzidine (DAB, Vector Laboratories, Burlingame, CA). Finally, a hematoxylin counterstain (Gill's Formula, Vector) was used to identify nuclei. Cells positive for either α -SMA or F4/80 antibodies from grafts within the patent and stenosed groups were visualized with light microscopy and quantified with ImageJ software.

RNA extraction

Mouse IVC tissue samples were subjected to the total RNA isolation procedure using the mirVana™ miRNA Isolation Kit (Applied Biosystems/Ambion, Austin, TX). Concentration of each RNA sample was determined by NanoDrop® spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE). The quality of RNA samples was assessed with Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA).

Gene expression profiling

An aliquot of 200 ng of high-quality total RNA from each sample was used for miRNA expression profiling, using the

Affymetrix GeneChip® approach (Affymetrix, Santa Clara, CA). Total RNA samples, containing low-molecular-weight RNA, were biotin labeled with the FlashTag™ Biotin HSR RNA Labeling Kit (Genisphere LLC, Hatfield, PA). The quality of the biotin labeling process was confirmed by the enzyme-linked oligosorbent assay (ELOSA) protocol (Genisphere LLC); 21.5 μ L of each high-quality biotin-labeled miRNA sample was hybridized to Affymetrix GeneChip miRNA 2.0 array for 16 h according to the Affymetrix protocol. The arrays were washed and stained on the Affymetrix Fluidics station 400 and scanned with a Hewlett Packard G2500A gene Array Scanner. Affymetrix® miRNA QC Tool v 1.1.1.0 (Affymetrix) was used for data summarization and microarray quality control.

Expression profiling data analyses

To generate miRNA expression values, Affymetrix GeneChip-derived CEL intensity files were imported into the Partek Genomics Suite, version 6.5 (Partek, St. Louis, MO), to determine differently expressed genes, statistics, and data visualization analyses. During this transaction, a normalization

FIG. 1. Lumen diameter from hematoxylin and eosin (H&E) staining of patent and stenosed tissue-engineered vascular grafts (TEVGs). Diameter measurements obtained from H&E staining of TEVG cross sections revealed a significant difference in lumen diameter between patent (**a**: 0.63 $\pm$ 0.06 cm) and stenosed (**b**: 0.17 $\pm$ 0.06 cm) TEVGs ($p < 0.01$). Color images available online at www.liebertpub.com/tea

TABLE 2. miRNAs WITH THE GREATEST DIFFERENCE BETWEEN PATENT AND STENOSED GROUPS

<i>microRNA ID</i>	<i>p</i>	<i>Fold change in stenosis</i>
miR-154	0.0459	1.7
miR-1940	0.0376	1.3
miR-292-3p	0.0152	1.3
miR-330	0.0374	1.3
miR-465c-5p	0.0043	1.3
miR-717	0.0053	1.2
miR-200c-star	0.0274	1.2
miR-145	0.0235	1.2
miR-125b-star	0.0071	1.2
miR-325	0.0211	1.2
miR-761	0.0429	1.1
miR-677	0.0398	1.1
miR-181a-2-star	0.0047	1.1
let-7i-star	0.0016	-1.2
miR-450a-5p	0.0143	-1.2
miR-872	0.0153	-1.2
miR-124-star	0.0167	-1.2
miR-292-5p	0.0184	-1.2
miR-1948	0.0326	-1.3
miR-574-5p	0.0209	-1.3
miR-673-5p	0.0409	-1.4
miR-466e-3p	0.0225	-1.4
miR-483-star	0.0021	-1.4
miR-1966	0.0432	-1.4
miR-466i	0.0279	-1.5
miR-338-5p	0.0247	-1.6
miR-451	0.0361	-3.1

Comparison of miRNA expression between stenosed and patent TEVGs indicates that miR-451, miR-338, and miR-466 were the most downregulated and miR-154 was the most upregulated miRNA in the presence of stenosis. The greatest difference in miRNA expression was found in miR-451 by -3.12-fold.

TEVG, tissue-engineered vascular graft.

algorithm, Robust Multiarray Average (RMA), was applied to the data, which performed quantile normalization, background correction, and $\log_2$ transformation for the generated expression values. One-way ANOVA statistical test was applied to the profiles to verify significance of the comparative results, and only expression values with $p < 0.05$ were considered for further analyses. Unsupervised hierarchical clustering analysis for selected significantly changed miRNAs was performed in Partek Genomics Suite, version 6.5, using the average linkage algorithm and Euclidean distance metric (two-way clustering).

Gene expression data validation through quantitative PCR

MicroRNA expression validation was performed using the TaqMan microRNA assay approach (Life Technologies Co., Applied Biosystems, Carlsbad, CA), according to the manufacturer's protocols. TaqMan microRNA, as well as endogenous control assays, consists of an RNA-specific stem-looped RT primer and TaqMan assay (forward and reverse primers and FAMTM dye-labeled MGB probe). Reverse transcription (RT) was performed with the TaqMan microRNA reverse transcription kit (Life Technologies Co., Applied Biosystems) both for miRNA-451 and endogenous

control snoRNA-202. Following RT, each RT reaction product (1.5 μ L) was mixed with TaqMan Universal PCR, No AmpErase UNG, Master Mix, 2 $\times$ (10 μ L), and specific TaqMan miRNA assay—target miRNA or endogenous control, respectively, 20 $\times$ (1 μ L). Table 1 represents the sequence for both assays. Standard TaqMan thermocycling conditions were applied: 10 min at 95°C, 45 cycles of 20 s at 95°C, and 45 s at 60°C, with polymerase chain reaction (PCR) volume of 20 μ L. All samples were run in triplicate with both assays. Relative quantification of gene expression was determined using the comparative Ct method, as previously described.²⁵ Student's *t*-test was applied to determine statistical significance of the results.

Results

Diameter measurements obtained from H&E staining of TEVG cross sections revealed a significant difference in lumen diameter between patent (0.63 ± 0.06 cm) and stenosed (0.17 ± 0.06 cm) groups ($p < 0.01$) (Fig. 1).

Using microarray analysis of explanted TEVG tissue for all currently known miRNA (1416 genes), 27 mature miRNAs significantly changed between patent and stenosed TEVGs ($p < 0.05$) are presented in Table 2 and Figure 2. Comparison of miRNA expression between stenosed and patent TEVG groups indicated that miR-451, miR-338, and miR-466 were the most downregulated and miR-154 was the most upregulated miRNA in the presence of stenosis. The greatest difference in miRNA expression was found in miR-451 by -3.1-fold (Table 2). Quantitative immunohistochemistry for α -SMA and F4/80 confirmed that cellular proliferation in stenosed TEVG was significantly greater than in patent TEVG (α -SMA: 229 ± 21 vs. 77 ± 8 positive cells, $p < 0.01$; F4/80: 245 ± 23 vs. 187 ± 11 positive cells, $p < 0.067$) (Fig. 3). Finally, a significant negative correlation between miR-451 expression and cellular proliferation was identified. (α -SMA: $r^2 = -0.086$, F4/80: $r^2 = -0.089$; $p < 0.005$) (Fig. 4).

Discussion

The current study describes the novel association of miR-451 with the regulation of stenosis in small-diameter TEVGs. Cell-free, bioresorbable porous scaffolds fabricated from FDA-approved polymers were implanted as IVC interposition grafts in a well-established murine model for a duration of 2 weeks,^{9,10,12} and upon explant, H&E staining of frozen sections was used to quantify graft patency based on lumen diameter. Samples classified as patent or stenosed were then subject to miRNA microarray analysis of all currently known 1416 miRNAs. We report a 3.1-fold difference between the expression of miR-451 in patent and stenosed TEVGs, suggesting that this miRNA may be critical to maintaining graft patency and promoting vascular neotissue formation over the time course of scaffold degradation. We characterized the degree of cellular infiltration and proliferation in patent and stenosed TEVGs, demonstrating a significantly greater quantity of macrophages and smooth muscle cells in stenosed grafts than in patent grafts. These results confirmed previous reports.⁹ Furthermore, we observed a significant negative correlation between the degree of macrophage and smooth muscle cell engraftment

FIG. 2. microRNA (miRNA) microarray of TEVG tissue samples. Unsupervised hierarchical clustering for 27 mature miRNAs significantly changed between stenosis and patent grafts (one-way, ANOVA, $p < 0.05$). *Cluster 1*: miRNAs downregulated in patent and upregulated in stenosis samples. *Cluster 2*: miRNAs upregulated in patent and downregulated in stenosis samples. Color images available online at www.liebertpub.com/tea

and the expression of miR-451 in our TEVG scaffolds at 2 weeks, suggesting a key role of this specific miRNA in the regulation of cell proliferation and migration in TEVGs. Our data indicate that dysregulation of miR-451 is implicated in the formation of neointimal hyperplasia due to excessive macrophage infiltration and overproliferation of vascular smooth muscle cells, leading to acute TEVG stenosis. In our data, the stenosed TEVGs demonstrate upregulation of miR-154 and downregulation of miR-451, miR-338, and miR-466. miR-154 is a known tumor suppressor of colorectal and prostate cancers,^{26–30} but aberrant upregulation of miR-154 has only been associated with pulmonary fibrosis.³¹ In this context, it is understood to induce overproliferation of lung fibroblasts and its expression may be regulated by TGF- β 1,³¹ a molecule that has previously been implicated in the

development of TEVG stenosis.³² Downregulation of miR-338 has been associated with progression and severity of human gastric, colorectal, and hepatocellular carcinomas; however, the potential role of miR-338 in our model of TEVG stenosis is unclear.^{33–35} On the other hand, miR-466 may regulate components of the innate immune system (IL-10 and IFN- α),^{36,37} which we have demonstrated to be a critical determinant of TEVG patency,³⁸ and is therefore a potential target for further study.

While the aberrant expression of miR-154, -338, and -466 may provide insight on the complex miRNA network contributing to the development of a functional neovessel from a cell-free TEVG scaffold, miR-451 shows the largest relative change in magnitude of expression, suggesting that it may be a promising therapeutic target and warrants further

FIG. 3. Cellular proliferation in TEVG. Quantitative immunohistochemistry for α -smooth muscle actin (α -SMA) and F4/80 confirmed that cellular proliferation in stenosed TEVG was significantly greater than in patent TEVG (α -SMA: 229 ± 21 vs. 77 ± 8 positive cells, $p < 0.01$; F4/80: 245 ± 23 vs. 187 ± 11 positive cells, $p < 0.067$).

FIG. 4. miR-451 expression correlates with cellular proliferation and incidence of stenosis. A significant negative correlation between miR-451 expression and cellular proliferation was identified (α -SMA: $r^2 = -0.086$, $F4/80$: $r^2 = -0.089$; $p < 0.005$).

investigation. MiR-451 is perfectly conserved between mice and humans, and clinical investigations have demonstrated that miR-451 is underexpressed in many human tumor types.^{19,39–45}

Current research indicates that expression of miR-451 plays an important role in tumor suppression, and *in vitro* administration of miR-451 oligonucleotide mimics has been shown to reduce cellular proliferation, impair cell cycle progression, suppress cellular migration, and induce apoptosis in human glioblastoma, liposarcoma, colorectal carcinoma, osteosarcoma, and nasopharyngeal carcinoma cell lines.^{39,40,43,45,46}

Although the tumor-suppressive role of miR-451 is well defined, mechanistic studies regarding its specific mRNA targets are ongoing. Recently, macrophage migration inhibitory factor (MIF), a pleiotropic proinflammatory cytokine, has been identified as a direct target of miR-451.^{35,38} Specifically, miR-451 has been shown to suppress MIF mRNA and protein expression, and MIF mRNA overexpression has been correlated with abnormal cellular proliferation and migration.^{44,47} MIF is well characterized as a potent upstream regulator of the innate immune system and has been implicated in atherosclerosis, neointimal lesion formation, and restenosis.^{48,49} In the context of vascular wound healing, MIF is directly involved in monocyte recruitment, macrophage activation, and neointimal thickening at the site of vascular injury.^{50–52}

Using miR-451 to target MIF and thereby regulate the TEVG scaffold remodeling *in vivo* could be a promising approach to mitigate the host inflammatory response and create an ideal environment for neovessel formation. We have previously demonstrated that implanted TEVGs transform into functional neovessels by an inflammation-mediated process of growth and remodeling.⁸ The primary cause of TEVG failure is stenosis secondary to intimal hyperplasia, and the incidence of stenosis correlates with the degree of monocyte and macrophage infiltration in TEVG scaffolds.⁹ Proinflammatory signaling such as MIF from infiltrating monocytes and macrophages in cell-free TEVGs is thus a critical determinant of TEVG patency and neotissue formation.

In conclusion, we demonstrated for the first time a significant correlation between the expression of miR-451 and TEVG stenosis in a murine model. MicroRNA-451 is known to function as a tumor suppressor, and a direct regulatory link between miR-451 and MIF has been characterized. Based on these data and the research implicating MIF in the progression

of vascular disease, we suggest that the miR-451/MIF pathway may be both a novel tool for detection and a therapeutic target for treatment of intimal hyperplasia and stenosis in second-generation TEVGs.

Acknowledgments

This work was partially supported by the Baier-Hills Cardiac Surgery Fund at Children's National Medical Center, NIH NCMRR/NINDS 2R24HD050846-06 (NCMRR-DC Core Molecular and Functional Outcome Measures in Rehabilitation Medicine), NIH NCRR UL1RR031988 (GWU-CNMC CTSI), and NIH NINDS (Intellectual and Developmental Disabilities Research Center 1P30HD040677-06). Contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Disclosure Statement

No competing financial interests exist.

References

- Hoffman, J.I., and Kaplan, S. The incidence of congenital heart disease. *J Am Coll Cardiol* **39**, 1890, 2002.
- Patterson, J.T., *et al.* Tissue-engineered vascular grafts for use in the treatment of congenital heart disease: from the bench to the clinic and back again. *Regen Med* **7**, 409, 2012.
- Roger, V.L., *et al.* Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation* **125**, e2, 2012.
- Dohmen, P.M., *et al.* Six years of clinical follow-up with endothelial cell-seeded small-diameter vascular grafts during coronary bypass surgery. *J Tissue Eng* **4**, 2041731413504777, 2013.
- Hibino, N., *et al.* Late-term results of tissue-engineered vascular grafts in humans. *J Thorac Cardiovasc Surg* **139**, 431, 436.e1–2, 2010.
- Cleary, M.A., *et al.* Vascular tissue engineering: the next generation. *Trends Mol Med* **18**, 394, 2012.
- Naito, Y., *et al.* Vascular tissue engineering: towards the next generation vascular grafts. *Adv Drug Deliv Rev* **63**, 312, 2011.
- Roh, J.D., *et al.* Tissue-engineered vascular grafts transform into mature blood vessels via an inflammation-mediated process of vascular remodeling. *Proc Natl Acad Sci U S A* **107**, 4669, 2010.

9. Hibino, N., *et al.* A critical role for macrophages in neovessel formation and the development of stenosis in tissue-engineered vascular grafts. *FASEB J* **25**, 4253, 2011.
10. Hibino, N., *et al.* Tissue-engineered vascular grafts form neovessels that arise from regeneration of the adjacent blood vessel. *FASEB J* **25**, 2731, 2011.
11. Naito, Y., *et al.* Beyond burst pressure: initial evaluation of the natural history of the biaxial mechanical properties of tissue-engineered vascular grafts in the venous circulation using a murine model. *Tissue Eng Part A* **20**, 346, 2014.
12. Naito, Y., *et al.* Characterization of the natural history of extracellular matrix production in tissue-engineered vascular grafts during neovessel formation. *Cells Tissues Organs* **195**, 60, 2012.
13. Bushati, N., and Cohen, S.M. microRNA functions. *Annu Rev Cell Dev Biol* **23**, 175, 2007.
14. Iorio, M.V., and Croce, C.M. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. *EMBO Mol Med* **4**, 143, 2012.
15. Zampetaki, A., and Mayr, M. MicroRNAs in vascular and metabolic disease. *Circ Res* **110**, 508, 2012.
16. Ji, R., *et al.* MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ Res* **100**, 1579, 2007.
17. Bartel, D.P. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281, 2004.
18. Krek, A., *et al.* Combinatorial microRNA target predictions. *Nat Genet* **37**, 495, 2005.
19. Pan, X., Wang, R., and Wang, Z.X. The potential role of miR-451 in cancer diagnosis, prognosis, and therapy. *Mol Cancer Ther* **12**, 1153, 2013.
20. Hwang, H.W., and Mendell, J.T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* **94**, 776, 2006.
21. Zhang, B., *et al.* microRNAs as oncogenes and tumor suppressors. *Dev Biol* **302**, 1, 2007.
22. Song, Z., and Li, G. Role of specific microRNAs in regulation of vascular smooth muscle cell differentiation and the response to injury. *J Cardiovasc Transl Res* **3**, 246, 2010.
23. Lu, J., *et al.* MicroRNA expression profiles classify human cancers. *Nature* **435**, 834, 2005.
24. Xin, M., *et al.* MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev* **23**, 2166, 2009.
25. Livak, K.J., and Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402, 2001.
26. Xin, C., Zhang, H., and Liu, Z. miR-154 suppresses colorectal cancer cell growth and motility by targeting TLR2. *Mol Cell Biochem* **387**, 271, 2014.
27. Kai, Y., *et al.* Decreased miR-154 expression and its clinical significance in human colorectal cancer. *World J Surg Oncol* **13**, 195, 2015.
28. Zhu, C., *et al.* miR-154 inhibits prostate cancer cell proliferation by targeting CCND2. *Urol Oncol* **32**, 31.e9-16, 2014.
29. Formosa, A., *et al.* MicroRNAs, miR-154, miR-299-5p, miR-376a, miR-376c, miR-377, miR-381, miR-487b, miR-485-3p, miR-495 and miR-654-3p, mapped to the 14q32.31 locus, regulate proliferation, apoptosis, migration and invasion in metastatic prostate cancer cells. *Oncogene* **33**, 5173, 2014.
30. Zhu, C., *et al.* miR-154 inhibits EMT by targeting HMG2 in prostate cancer cells. *Mol Cell Biochem* **379**, 69, 2013.
31. Milosevic, J., *et al.* Profibrotic role of miR-154 in pulmonary fibrosis. *Am J Respir Cell Mol Biol* **47**, 879, 2012.
32. Duncan, D.R., *et al.* TGFbetaR1 inhibition blocks the formation of stenosis in tissue-engineered vascular grafts. *J Am Coll Cardiol* **65**, 512, 2015.
33. Huang, X.H., *et al.* Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated. *Hepatol Res* **39**, 786, 2009.
34. Sun, K., *et al.* miRNA-338-3p suppresses cell growth of human colorectal carcinoma by targeting smoothed. *World J Gastroenterol* **19**, 2197, 2013.
35. Peng, Y., *et al.* MicroRNA-338 inhibits growth, invasion and metastasis of gastric cancer by targeting NRP1 expression. *PLoS One* **9**, e94422, 2014.
36. Li, Y., *et al.* MicroRNA-4661 inhibits antiviral innate immune response by targeting interferon-alpha. *Cell Mol Immunol* **9**, 497, 2012.
37. Ma, F., *et al.* MicroRNA-4661 upregulates IL-10 expression in TLR-triggered macrophages by antagonizing RNA-binding protein tristetraprolin-mediated IL-10 mRNA degradation. *J Immunol* **184**, 6053, 2010.
38. Hibino, N., *et al.* The innate immune system contributes to tissue-engineered vascular graft performance. *FASEB J* **29**, 2431, 2015.
39. Gal, H., *et al.* MIR-451 and Imatinib mesylate inhibit tumor growth of Glioblastoma stem cells. *Biochem Biophys Res Commun* **376**, 86, 2008.
40. Nan, Y., *et al.* MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Res* **1359**, 14, 2010.
41. Godlewski, J., *et al.* MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell* **37**, 620, 2010.
42. Wang, X.C., *et al.* The expression and function of miRNA-451 in non-small cell lung cancer. *Cancer Lett* **311**, 203, 2011.
43. Xu, H., *et al.* Tumor-suppressing effects of miR451 in human osteosarcoma. *Cell Biochem Biophys* **69**, 163, 2014.
44. Bandres, E., *et al.* microRNA-451 regulates macrophage migration inhibitory factor production and proliferation of gastrointestinal cancer cells. *Clin Cancer Res* **15**, 2281, 2009.
45. Gits, C.M., *et al.* MicroRNA expression profiles distinguish liposarcoma subtypes and implicate miR-145 and miR-451 as tumor suppressors. *Int J Cancer* **135**, 348, 2014.
46. Li, H.Y., *et al.* MicroRNA-451 inhibits growth of human colorectal carcinoma cells via downregulation of Pi3k/Akt pathway. *Asian Pac J Cancer Prev* **14**, 3631, 2013.
47. Liu, N., *et al.* MiR-451 inhibits cell growth and invasion by targeting MIF and is associated with survival in nasopharyngeal carcinoma. *Mol Cancer* **12**, 123, 2013.
48. Calandra, T., and Roger, T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* **3**, 791, 2003.
49. Asare, Y., Schmitt, M., and Bernhagen, J. The vascular biology of macrophage migration inhibitory factor (MIF).

- Expression and effects in inflammation, atherogenesis and angiogenesis. *Thromb Haemost* **109**, 391, 2013.
50. Schober, A., and Weber, C. Mechanisms of monocyte recruitment in vascular repair after injury. *Antioxid Redox Signal* **7**, 1249, 2005.
51. Schober, A., *et al.* Stabilization of atherosclerotic plaques by blockade of macrophage migration inhibitory factor after vascular injury in apolipoprotein E-deficient mice. *Circulation* **109**, 380, 2004.
52. Chen, Z., *et al.* Evidence for a role of macrophage migration inhibitory factor in vascular disease. *Arterioscler Thromb Vasc Biol* **24**, 709, 2004.

Address correspondence to:
Narutoshi Hibino, MD, PhD
Department of Cardiac Surgery
Johns Hopkins Hospital
1800 Orleans Street
Baltimore, Maryland 21287
E-mail: nhibino1@jhmi.edu

Received: April 3, 2015
Accepted: September 17, 2015
Online Publication Date: December 15, 2015