MicroRNA-503 and the extended microRNA-16 family in angiogenesis

Citation for published version:
Caporali, A & Emanueli, C 2011, 'MicroRNA-503 and the extended microRNA-16 family in angiogenesis'
Trends in cardiovascular medicine, vol 21, no. 6, pp. 162-6. DOI: 10.1016/j.tcm.2012.05.003

Digital Object Identifier (DOI):
10.1016/j.tcm.2012.05.003

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Trends in cardiovascular medicine

Publisher Rights Statement:
Copyright © 2011 Elsevier Inc.
This document may be redistributed and reused, subject to certain conditions.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
MicroRNA-503 and the Extended MicroRNA-16 Family in Angiogenesis
Andrea Caporali and Costanza Emanueli*

MicroRNAs (miRs) are post-transcriptional inhibitory regulators of gene expression acting by direct binding to complementary messenger RNA (mRNA) transcripts. Recent studies have demonstrated that miRs are crucial determinants of endothelial cell behavior and angiogenesis. We have provided evidence of the prominent role of miR-503 in impairment of postischemic reparative angiogenesis in the setting of diabetes. Because miR-503 belongs to the miR-16 extended family of miRs, in this review, we describe the cardiovascular functions of miR-503 and other members of the miR-16 family and their impact on angiogenesis. (Trends Cardiovasc Med 2011;21:162-166) © 2011 Elsevier Inc. All rights reserved.

Introduction

MicroRNAs (miRs) are small, noncoding RNAs that regulate gene expression, mainly by binding 3’ untranslated regions (3’ UTRs) of messenger RNA. Andrea Caporali and Costanza Emanueli are at the Laboratory of Vascular Pathology and Regeneration, School of Clinical Sciences, University of Bristol, Bristol BS2 8HW, UK.

Emerging evidence suggests that miRs might contribute to the fine-tuning of the genes involved in the angiogenic process. Disrupted balance of angiogenesis contributes to the pathogenesis of numerous disease states (Carmeliet and Jain 2011). For example, uncontrolled angiogenesis favors tumor growth and metastasis. Although it is desirable to block the growth of new blood vessels in these circumstances, the controlled stimulation of angiogenesis is beneficial in ischemic conditions, characterized by impaired local blood supply. Considering the potential clinical benefits of therapeutically manipulating blood vessel growth and blood flow, the mechanisms regulating the angiogenic process have formed a major focus for vascular research during the past two decades.

© 2011 Elsevier Inc. All rights reserved.

PII S1050-1738(12)00113-2

TCM
miR-503 Belongs to the miR-16 Family

The 5′-end portion (5′ UTR) of miRs, also known as the “seed region,” is a particularly important determinant of miR function (Lewis et al. 2005). miRs with the sequence AGCAGC (AGC2x), starting at the second nucleotide from the 5′ end of their mature form, belong to the “canonical” miR-16 family. Members of this family are miR-15a/b, miR-16, miR-195, miR-424, and miR-497. Moreover, based on the presence of AGC2x motif starting at the first nucleotide in seed sequence, miR-103, miR-107, and miR-646 could be also included in an “extended” miR-16 family (Finnerty et al. 2010). The seed region of miR-503 differs only at the second nucleotide from the 5′ end of mature form, belong to the “canonical” miR-16 family: This nucleotide is an adenosine in miR-503 and an adenine in the miR-16 family. This leads to an overlap between target genes because 8-mer sites (positions 2-8 of the mature miR followed by an “A”) for miR-503 are recognized as 7-mer-A1 sites by canonical miR-16 family. This nucleotide is a guanosine in miR-503 and an adenine in the miR-16 family. This leads to an overlap between target genes because 8-mer sites (positions 2-8 of the mature miR followed by an “A”) for miR-503 are recognized as 7-mer-A1 sites by canonical miR-16 family members and vice versa (Rissland et al. 2011) (Table 1). In agreement with the knowledge that the AGC2x motif near the miRs’ 5′ end controls the miR target specificity, there is evidence that different members of the miR-16 family, including miR-503, regulate overlapping miR targets (Forrest et al. 2010, Joglekar et al. 2007).

miR-503 is an intragenic miRNA clustered with miR-424 on chromosomal location Xq26.3 (Griffiths-Jones et al. 2008). miR-424 has been implicated in regulation of angiogenesis (Ghosh et al. 2010, Chamorro-Jorganes et al. 2011). miR-503 and miR-424 are separated by 383 bases on the genome and are likely derived from the same primary transcript as a pair of hairpins and to have related seed sequences. A further five miRs (miR-542-5p, miR-542-3p, miR-450a, miR-450b-5p, and miR-450b-3p) are within 7 kb of the miR-424–miR-503 cluster and can be transcribed from the same primary transcript. It is not known if these additional five miRs are expressed in vascular cells or involved in angiogenesis. Members of the miR-16 family are also intragenic miRNA and expressed from different loci as pairs of hairpins, closely spaced with linker regions of 150-200 nucleotides.

Considering the seed sequence similarities and the genomic organization, it is possible to classify miR-503 as part of the extended miR-16 family (Table 2).

### Table 1. Sequences of the mature forms of miRs belonging to the extended miR-16 family

| miRs        | First nucleotide of the mature miR sequence | Seed common region | Last nucleotide of the seed sequence | End of the mature miR sequence |
|-------------|---------------------------------------------|--------------------|--------------------------------------|-------------------------------|
| hsa-miR-15a | U                                           | AGCAGC             | A                                    | CAUAAUGGUUUGUG                |
| hsa-miR-15b | U                                           | AGCAGC             | A                                    | CAUAAUGGUUACACA               |
| hsa-miR-16  | U                                           | AGCAGC             | A                                    | CGUAAAUUGGGCG                 |
| hsa-miR-195 | U                                           | AGCAGC             | A                                    | CAGAAAUUGGGCC                 |
| hsa-miR-503 | U                                           | AGCAGC             | G                                    | GGAACAGUUGUGCAG               |
| hsa-miR-424 | U                                           | AGCAGC             | A                                    | AUCAUGGUUUUGAA                |
| hsa-miR-497 | C                                           | AGCAGC             | A                                    | C ACUGUGGUUUGU                |

* Note that the last nucleotide of the seed region is a G only for miR-503.

### Table 2. Extended miR-16 family in vascular biology and diabetes

| miRNAs | Identified target genes | Stimuli inducing miR expression upregulation | References |
|--------|-------------------------|---------------------------------------------|------------|
| miR-15b | Pdk4, Sgk1              | Myocardial infarction                       | Hullinger et al. (2012) |
| miR-16  | VEGFR2, FGFR1, VEGF     | VEGF-A, bFGF                                | Chamorro-Jorganes et al. (2011) |
| miR-103/107 | Caveolin-1            | Type 2 diabetes                            | Trajkovski et al. (2011) |
| miR-195/497 | ELN, Col1a1, Col1a2 | Aortic development                         | Ott et al. (2011) |
| miR-195  | Unknown                 | Cardiac hypertrophy                        | Van Rooij et al. (2006) |
| miR-424  | Check1                  | Heart development                          | Porrello et al. (2011) |
| miR-424  | CUL2                    | Ischemia                                   | Ghosh et al. (2010) |
| miR-503  | VEGFR2, FGFR-R1, VEGF   | VEGF-A, bFGF                                | Chamorro-Jorganes et al. (2011) |
| miR-503  | MEK, CCNE1              | Hemangioma                                 | Nakashima et al. (2010) |
| miR-503  | CCNE1, cdc25A           | Diabetes + critical limb ischemia           | Caporali et al. (2011) |
503 expression is subjected to the same transcriptional regulation of miR-424. The mechanisms of miR-503 transcription and maturation have not yet been demonstrated, and more “wet” experiments are needed to verify this hypothesis.

miR-503 was first identified in human retinoblastoma tissues using the miR microarray technique (Zhao et al. 2009). Moreover, miR-503 expression was found upregulated in human parathyroid carcinomas (Corbetta et al. 2010) and in adenocortical carcinomas (Tombó et al. 2009)). In monocytes differentiating toward macrophages, miR-424 and miR-503 are produced as a polycistronic message and induce G1 arrest by targeting an overlapping set of cell cycle regulators (Forrest et al. 2010). In line with this, induction of G1 phase cell cycle arrest is critical for the differentiation of myoblasts into myotubes. Moreover, miR-424 and miR-503 are induced during myogenesis and promote cell cycle arrest through cdc25A degradation (Sarkar et al. 2010). Hence, these two miRs appear to be part of the differentiation processes. In addition, miR-503 expression increases in response to serum starvation in mesenchymal stem cells (Nie et al. 2011), NIH-3T3 cells (Rissland et al. 2011), and ECs cultured in high glucose (Caporali et al. 2011). Interestingly, in NIH-3T3 G1 arrested by serum starvation, miR-503 returns to normal level upon cell cycle reentry (Rissland et al. 2011). miR-503 expression is also upregulated in response to growth arrest by cell contact inhibition. Rapid degradation during cell cycle reentry of miR-503 is dependent on its constitutive instability. Thus, miR-503 modulates the cell cycle and is itself dynamically regulated by the cell cycle.

Increased miR-503 in human umbilical vein ECs and human microvascular ECs results in cell cycle delay in G1, reduced cell proliferative and migratory capacity, and impaired EC networking capacities, suggesting an anti-angiogenic role for this miR (Caporali et al. 2011).

In vivo, miR-503 is upregulated in myocardial ECs from diabetic GK rats (Wang et al. 2009) and in ECs resident in ischemic limb muscles of diabetic mice (Caporali et al. 2011). It is also upregulated in ischemic limb skeletal muscles and plasma of diabetic patients with end-stage critical limb ischemia (Caporali et al. 2011).

- **miR-503 Validated Target Genes**
  Recent studies have proposed that miR-503 might be a master regulator of the cell cycle (Rissland et al. 2011). Gene ontology analysis of genes affected by miR-503 identified those annotated as cell cycle regulators or involved in cell adhesion, migration, and angiogenesis processes (Caporali et al. 2011). Using prediction software, four genes were consistently identified as targets of miR-503: cell division cycle 25A (CDC25A), cyclin D1 (CCND1), cyclin D2 (CCND2), and cyclin E1 (CCNE1). By 3’UTR-luciferase assays, we validated CDC25A and CCNE1 as direct target genes of miR-503 (Caporali et al. 2011). CCND1 and CCND2 were validated as miR-503 targets by others (Jiang et al. 2009). The concept of miR-503 being involved in the cell cycle process has been further enforced by the finding of Forrest et al. (2010) that miR-503 inhibits the expression of additional genes involved in cell division, the cell cycle, and mitosis, such as CCNE, CCNF, CDC14A, anillin, activating transcription factor 6 (ATF6), Aragonate 1 (Aog1), checkpoint kinase 1 (CHEK1), mitosis inhibitor protein kinase WEE1, and CDKNA1 (p21). Seven of these targets are in common with miR-424 (Forrest et al. 2010). Interestingly, cell cycle genes involved in G1-S transition, such as CCND1, CCNE1, and cdc25A, are targeted by most of the members of the extended miR-16 family (Liu et al. 2008; Rissland et al. 2011). Forrest et al. also showed that an increase in miR-503 downregulates miR-9-3, thus demonstrating reciprocal expression regulatory between miRs (Forrest et al. 2010). Because miRs can regulate other miRs, these interactions increase the complexity of gene regulation and are likely to be involved in regulatory processes that are currently unexplored.

- **Extended miR-16 Family in Vascular Biology and Diabetes**
  Ghosh et al. (2010) reported that miR-424 is induced by hypoxia in ECs and promotes angiogenesis in vivo and in vitro through PU.1-dependent transactivation and direct inhibition of cullin 2 (CUL2), which increase HIF-1α levels. In contrast, Chamorro-Jorganes et al. (2011) demonstrated that overexpression of miR-424 or miR-16 reduces proliferation, migration, and angiogenic capacity of ECs in vitro and in a Matrigel plug implanted in mice. In agreement with the findings of Chamorro-Jorganes et al., miR-424 expression inhibition using oligos anti-miR in human dermal microvascular ECs abnormally increases cell proliferation and promotes angiogenesis, possibly via increased levels of MEK1 or CCNE1 (Nakashima et al. 2010). Moreover, miR-424 and miR-16 reduce the expression of VEGF-A, VEGF receptor 2 (VEGFR2), and fibroblast growth factor receptor 1 (FGF-R1) by binding at 3’UTR sequences (Chamorro-Jorganes et al. 2011, Musumeci et al. 2011). Interestingly, either VEGF-A or basic FGF (bFGF) treatment upregulates expression of mature miR-16 and miR-424; thus, a regulatory loop between miR-16, miR-424, and the major proangiogenic growth factors exists. miR-16 is transcribed together with either miR-15a (miR-15a/-16-1 cluster) or miR-15b (miR-15b/-16-2 cluster). In line with data from Chamorro-Jorganes et al., we found that miR-16 and miR-15a are upregulated in proangiogenic circulating cells (PACs; previously known as endothelial progenitor cells) of patients with critical limb ischemia and that increased miR-15/16 impairs PAC functional capacities (Fortunato et al. 2012). Moreover, miR-15a, miR-15b, miR-16-1, miR-16-2, miR-424, and miR-497 were consistently found to be upregulated in cardiomyocytes during cardiac ischemia and heart failure (reviewed in Small et al. 2010). Hullinger et al. (2012) demonstrated that systemic inhibition of miR-15 by locked nucleic acid-modified anti-miRs reduces infarct size and cardiac remodeling and enhances cardiac function in response to myocardial infarction in mice. Cardiac expression and function are also described for another miR-16 family member, miR-195, which is upregulated during cardiac hypertrophy. Moreover, mice overexpressing miR-195 under the β-myosin heavy chain promoter develop fatal dilated cardiomyopathy (van Rooij et al. 2006). Recently, miR-195 was shown to be the most expressed miR in the heart between postnatal days 1 and 10. In particular, miR-195 is associated with cardiomyocyte postmitotic arrest due to the inhibition of several genes involved in cell cycle pro-
gression (Porrello et al. 2011). Furthermore, miR-195 and miR-497 are upregulated during aortic development, with subsequent downregulation of their target genes, including elastin (ELN), collagen 1a1 (Col1a1), and collagen 1a2 (Col1a2) (Ott et al. 2011) (Table 2). Finally, miR-103 and miR-107 are negative regulators of insulin sensitivity and may contribute to the etiology of diabetes. Moreover, global miR-103/107 silencing causes increased insulin signaling in both liver and adipose tissue (Tajkowska et al. 2011).

• **Direction of Future Research**

Dysfunction of the blood vascular endothelium is a major factor in the pathogenesis of micro- and macroangiopathy. Restoring EC function is fundamental in reestablishing vessel integrity and maintaining tissue perfusion. In the development of novel therapies aimed at preserving the EC layer and promoting reparative angiogenesis, the first challenge is to find a combination of factors able to prevent EC apoptosis and improve EC functional capacities. To achieve this, the regulation of gene networks should play a fundamental role. In this context, regulation of specific miRs or an entire family of miRs and consequently modulation of their target genes could lead to novel therapeutic approaches. In our study on miR-503, we showed the first example of a miR-based intervention normalizing post-ischemic reparative angiogenesis in diabetes. We demonstrated that antagonizing miR-503 using adenovirus-mediated local delivery of a competitive inhibitor (an miR-503 decoy) improved postischemic reparative neovascularization and blood flow recovery and restored expression of miR-503 target genes (Caporali et al. 2011). Because miR-15a, -15b, -16, -195, -424, -497, and -503 regulate overlapping lists of miR targets due to similarity in seed sequence, inhibition of the entire family of miRs could lead to a further benefit postischemic revascularization, including in diabetic patients.

However, the most advanced approach currently used to regulate miR levels in vivo is the use of anti-miRs. Anti-miRs are modified antisense oligodeoxynucleotides harboring the full or partial complementary reverse sequence of a mature miR. These oligodeoxynucleotides are able to reduce the endogenous levels of the miR, thus increasing expression of its mRNA targets. Recently, anti-miR technology has been used for miR-15 inhibition in the setting of ischemic heart disease (Porrello et al. 2011), and preclinical studies are ongoing at miRagen Therapeutics (http://www.miragentherapeutics.com) with the aim to commercialize an miR-15 inhibitor for the treatment of cardiovascular disorders.

Although there has been a significant increase in the number of patent application filings during the past 10 years and great excitement surrounding miRs as novel drugs, only one anti-miR compound has been included in clinical trials: Anti-miR-122 has been used to treat liver disease caused by hepatitis C virus (http://www.santaris.com). Further studies are necessary to clarify any "off-target" effect of miRs inhibitors, which may need to be addressed in order to fill the bench-to-bedside gap.

Finally, the discovery of miRs in biological fluids and the hypothetical possibility use of miRs as clinical diagnostic and prognostic biomarkers is an hot topic in the miR field. Further investigations of miR-503 as a possible biomarker for peripheral artery disease (PAD) in diabetic subjects are required. We plan to investigate the predictive potential of circulating miR-503 for leg ischemia in PAD patients. Larger numbers of patients and correlations with clinical outcomes need to be analyzed. Furthermore, measuring the level of other miR-16 family members in PAD patients without diabetes could help in the prediction of limb ischemia.

• **Acknowledgments**

A.C. is a British Heart Foundation (BHF) intermediate research fellow (FS/10/001/27959) and C.E. is a BHF senior research fellow (FS/10/001/27959).

References

Armulik A Genove G, & Betsholtz C: 2011. Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. Dev Cell 21:193–215.

Caporali A, Meloni M, Vollenkle C, et al.: 2011. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. Circulation 125:282–291.

Carmeliet P & Jain RK: 2011. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov 10:417–427.

Chamorro-Jorganes A, Araldi E, Penalva LO, et al: 2011. MicroRNA-16 and microRNA-424 regulate cell-autonomous angiogenic functions in endothelial cells via targeting vascular endothelial growth factor receptor-2 and fibroblast growth factor receptor-1. Arterioscler Thromb Vasc Biol 31: 2595–2606.

Corbeta S, Vaira V, Guarnieri V, et al: 2010. Differential expression of microRNAs in human parathyroid carcinomas compared with normal parathyroid tissue. Endocr Relat Cancer 17:135–146.

Díaz-Flores L, Gutiérrez R, Madrid JF, et al: 2009. Pericytes: Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche. Histol Histopathol 24:909–969.

Finney JR, Wang WX, Hébert SS, et al: 2010. The miR-15/107 group of microRNA genes: Evolutionary biology, cellular functions, and roles in human diseases. J Mol Biol 402:491–509.

Forrest AR, Kamamori-Katayama M, Tomaru Y, et al: 2010. Induction of microRNAs, mir-155, mir-222, mir-424 and mir-503, promotes monocytic differentiation through combinatorial regulation. Leukemia 24:460–466.

Fortunato O, Caporali A, Sangalli E, et al: 2012. MicroRNA-15a and mir-16 regulate sdi-1 migration of endothelial progenitor cells in diabetic patients with limb ischemia and healthy controls. Cardiovasc Res 93(Suppl):1–15.

Ghosh G, Subramanian IV, Adhikari N, et al: 2010. Hypoxia-induced microRNA-424 expression in human endothelial cells regulates HIF-α isoforms and promotes angiogenesis. J Clin Invest 120:4141–4154.

Griffiths-Jones S, Saini HK, van Dongen S, & Enright AJ: 2008. miRBase: Tools for microRNA genomics. Nucleic Acids Res 36(Database issue):D154–D158.

Hullinger TG, Montgomery RL, Seto AG, et al: 2012. Inhibition of miR-15 protects against cardiac ischemic injury. Circ Res 110:71–81.

Jiang Q, Feng MG, & Mo YI: 2009. Systematic validation of predicted microRNAs for cyclin D1. BMC Cancer 9:194.

Joglekar MV, Parekh VS, Mehta S, et al: 2007. MicroRNA profiling of developing and regenerating pancreas reveal post-transcriptional regulation of neurogenin3. Dev Biol 311:603–612.

Lee Y, Kim M, Han J, et al: 2004. MicroRNA genes are transcribed by RNA polymerase II. Embo J 23:4051–4060.

Lewis BP, Burge CB, & Bartel DP: 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of hu
Regulatory T Cells and Pulmonary Hypertension

Rasa Tamosiuniene and Mark R. Nicolls

Pulmonary hypertension (PH) is a disease of high lethality arising from numerous causes. For a significant subset of PH patients, autoimmune biomarkers or frank autoimmune disease are simultaneously present, but the extent to which lung inflammation contributes to PH is unknown. However, emerging experimental and clinical evidence suggests that immune dysregulation may lead to the propagation of vascular injury and PH. A recent preclinical study demonstrated that regulatory T cells are important mediators normally enlisted to control inflammation and that, if absent or dysfunctional, may predispose to the development of PH. (Trends Cardiovasc Med 2011;21:166-171) Published by Elsevier Inc.

• Introduction

For more than 50 years, it has been recognized that autoimmune phenomena are associated with certain forms of pulmonary hypertension (PH), but it has never been previously demonstrated that immune dysregulation may be a root cause for PH. To address this issue, our group recently demonstrated how immune dysregulation exacerbates vascular inflammation and results in severe PH (Tamosiuniene et al. 2011). In this experimental model, athymic (T-cell-deficient) rats developed significant pulmonary vascular disease following treatment with SU5416, a vascular endothelial growth factor-2 (VEGFR-2) inhibitor. The main finding of this study was that PH may arise when the absence of normal anti-inflammatory regulatory T cell (Treg) activity results in a failure to control inflammatory endothelial injury. Restoring Tregs to these animals, prior to vascular injury, prevents the development of PH. The relevant clinical parallel is that a number of diseases associated with PH are, like the athymic rat, associated with conditions having abnormal Treg numbers or activity (reviewed in Nicolls et al. 2005). The purposes of this review are to discuss the findings of the study by Tamosiuniene et al. (2011), to put into context what is...