The Emerging Role of miR-200 Family in Cardiovascular Diseases

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Recent studies have shown that reactive oxygen species increase the expression of miR-200 family (miR-200); however, little is known about this micro-RNA family in the cardiovascular system. In this Viewpoint, we provide evidence suggesting that miR-200 may be important in conditions that affect the heart and blood vessels.

Oxidative stress plays a major role in cardiovascular physiopathology and some common conditions including aging, diabetes mellitus, atherosclerosis, and reperfusion injury induce reactive oxygen species (ROS).

In 2011, it was first published that endothelial and skeletal muscle cells exposed to oxidative stress, in vitro and in vivo, exhibit a marked increase in miR-200[1]. Subsequently, ROS ability to induce miR-200 has been confirmed in many cell types. Furthermore, hypoxia enhances miR-429, an miR-200 member, and miR-429 targets HIF-1α (hypoxia-inducible factor-1α) and decreases its expression.

Most miR-200 studies have been in the cancer field: miR-200 modulates epithelial–mesenchymal transition by targeting the transcription factors ZEB1 and ZEB2 (Zinc-finger E-box binding homeobox 1 and 2) and circulating miR-200 may represent clinically useful cancer biomarkers. In contrast, the role of miR-200 in CV diseases is still poorly investigated.

miR-200 and Its Targets

MiRNAs are short (21–22 nucleotides) noncoding RNAs: their seed sequence (nucleotides 2–8 from the 5′ end) targets specific mRNAs and via this mechanism they inhibit translation and also may modulate mRNA stability. The miR-200 family is composed of 5 members clustered and expressed as 2 separate polycistronic pri-miRNA transcripts: in humans, miR-200c and miR-141 are on chromosome 12; miR-200a, miR-200b, and miR-429 are on chromosome 1. They are also identified by different seed sequences: subgroup I comprises miR-141 and miR-200a; subgroup II includes miR-200b, miR-200c, and miR-429. Because each miRNA can target numerous mRNAs and the 2 miR-200 subgroups have different seed sequences, the functional impact of miR-200 can be profound. The Figure shows the major miR-200c–activated signaling pathways relevant to vascular dysfunction; other miR-200 targets are shown in the Online Table.

Notably, different miR-200 can have opposite effects on the same target: for instance, miR-200b downmodulates c-Jun protein, whereas miR-200a increases JUN by stabilizing its mRNA. Therefore, the effect of a member cannot be extended to the whole family.

miR-200 and Epigenetics

A complex epigenetic circuitry modulates miR-200 expression level and contributes to its effects on gene expression.

Methylation of miR-200 promoters by DNA methyltransferase 3a, inhibits miR-200 expression. In turn, miR-200c represses directly DNA methyltransferase 3a.

Moreover, a strict link exists between miR-200 and histone H3 lysine 27 trimethylation, a repressive chromatin state catalyzed by Polycomb group proteins. The levels of H3 lysine 27 trimethylation result from the opposite activities of the Polycomb histone methyltransferase enhancer of zeste homolog 2 and of the histone demethylases JMJD3 and UTX. MiR-200b targets Suz12, a subunit of polycomb repressive complex 2,[2] and decreases H3 lysine 27 trimethylation at the promoters of target genes leading to transcriptional derepression.

JMJD3 knockdown enhances H3 lysine 27 trimethylation and downmodulates miR-200b and miR-200c expression. In keeping, enhancer of zeste homolog 2 represses miR-200b and miR-200c expression.

EED (embryonic ectoderm development), another component of PCR2 (polycomb repressive complex 2), modulates transforming growth factor β–induced transcriptional repression of miR-200 genes, through the recruitment of enhancer of zeste homolog 2 methyltrasferase on their promoter. Furthermore, miR-200c targets also BMI1, a polycomb repressive complex 2 component.[3]

In addition, miR-200 downmodulates 2 histone deacetylases: HDAC4 and Sirtuin1 (SIRT1).[4]

Taken together, these studies suggest an antagonistic interplay between miR-200 and the epigenetic mechanisms that maintain transcriptional repression, that is, a condition usually associated with young age and low prevalence of cardiovascular diseases.[5]

miR-200 in the Cardiovascular System

Some conditions of cardiovascular interest that are associated with enhanced miR-200 expression; however, to date, few

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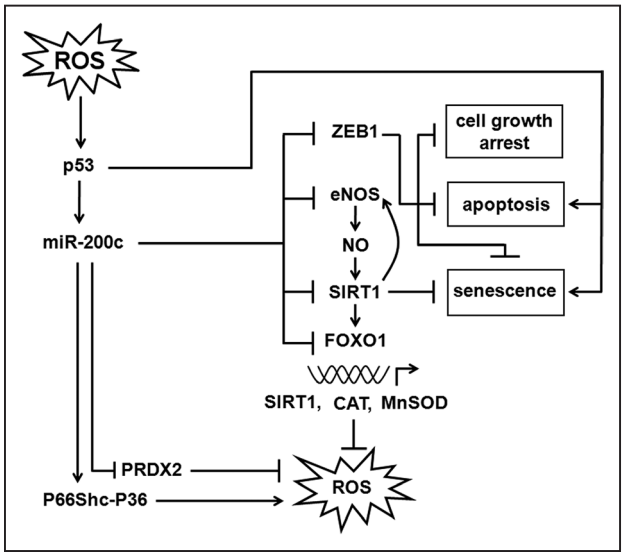

Figure. Reactive oxygen species (ROS) upregulate miR-200c via a p53-dependent mechanism.[1] MiR-200c, in turn, inhibits ZEB1 (Zinc-finger E-box binding homeobox 1) and SIRT1/endothelial nitric oxide synthase (eNOS)/forkhead box O1 (FOXO1) regulatory loop by directly targeting all of them.[7] miR-200c upregulation induces growth arrest, senescence, and apoptosis. ZEB1 downregulation is implicated in all these effects; p53 is implicated in the induction of apoptosis and senescence; diminished SIRT1 expression associates with cell senescence. Moreover, miR-200c decreases nitric oxide (NO) and increases ROS production by 2 mechanisms: (1) it decreases ROS scavengers by targeting peroxiredoxin 2 (PRDX2), and forkhead box O1 (FOXO1), a transcription factor required for catalase (CAT) and manganese superoxide dismutase (MnSOD) expression; (2) it sustains ROS production via p66Shc phosphorylation in Serine 36.

studies have examined miR-200 expression in vascular and myocardial cells.

Aging

An age-dependent increase in oxidative stress has been reported in humans and in many animal models, and increased ROS seem to be both a consequence and a cause of aging. There is evidence that human liver exhibits an age-dependent increase of miR-200c and miR-141.[6]

Furthermore, aging enhances miR-200c expression in human skin fibroblasts and mouse femoral artery.[7]

Diabetes mellitus, Obesity, and Insulin Resistance

The role of miR-200 in diabetes mellitus is complex because it acts at different levels: (1) it diminishes insulin production by inducing pancreatic β cell damage[8]; (2) it enhances insulin resistance via downmodulation of insulin receptor substrate-2; (3) it increases appetite by interfering with leptin signaling in the hypothalamus; (4) it has an arterial proinflammatory role: miR-200b, miR-429, and miR-200c increase in the aorta and vascular smooth muscle cells of diabetic mice and the downregulation of ZEB1 activates proinflammatory genes; (5) miR-200 has an antiangiogenic action (see below). Taken together, the above results suggest that miR-200 may contribute to diabetic vascular complications. However, there is also some evidence that miR-200 may facilitate insulin signaling: (1) miR-200 targets the transcription factor FOG2, a key protein that inhibits insulin signaling[9]; (2) the ablation of miR-200b/a/429 in adipocytes of mice fed a high-fat diet increases adiposity, body weight, and insulin resistance.

Atherosclerosis

There is evidence for a role of miR-200 in atherosclerosis. Our preliminary results show that circulating miR-200c is elevated in children with familial hypercholesterolemia. Furthermore, there is a positive correlation between circulating miR-200c and miR-33a and miR-33b, 2 miRNAs upregulated in familial hypercholesterolemia.[10]

Moreover, miR-200b single nucleotide polymorphism T>C may lead to atherosclerosis and stroke, possibly because of failure to downmodulate the regulatory subunit of protein kinase A, which is involved in the suppression of platelet activation and aggregation.

Ischemia

MiR-200 members increase in the ischemic skeletal muscle[1] and also in the brain, in response to ischemia and ischemia/reperfusion.[11] To date, the effect of prolonged ischemia, preconditioning, and ischemia/reperfusion injury have not been evaluated in the heart and in other tissues and organs other than the skeletal muscle and brain.

Vascular Dysfunction

Some miR-200c targets are highly relevant to vascular function. MiR-200c targets directly the 3′untranslated region of SIRT1, endothelial nitric oxide synthase, and forkhead box O1 and disrupts the regulatory loop among these factors.[7] Downmodulation of SIRT1 leads to increased acetylation of its targets, forkhead box O1, and p53; acetylated p53 exhibits enhanced transcriptional activity, induces apoptosis, and increases ROS production; acetylation inhibits forkhead box O1 transcriptional activity and via this mechanism decreases SIRT1, catalase, and manganese superoxide dismutase. Therefore, the final results are as follows: (1) an increase in ROS production, corroborated also by p66Shc phosphorylation in Serine 36 and by the downregulation of the H_2O_2 scavenger peroxiredoxin 2; (2) a decrease in NO availability. These results were validated in human skin fibroblasts from old donors, femoral arteries from old mice, and a mouse model of hindlimb ischemia.

Angiogenesis Impairment

MiR-200 inhibits angiogenesis via different mechanisms. MiR-200b directly targets ETS1 (ETS proto-oncogene 1), a transcription factor with a proangiogenic action linked to its ability to enhance vascular endothelial growth factor (VEGF)-A, VEGF receptor 1 (VEGFR1), and VEGFR2 expression, as well as the expression of hepatocyte growth factor, urokinase, and some matrix metalloproteins. In diabetic mice, miR-200b inhibits cutaneous wound angiogenesis by targeting VEGF, VEGFR1, and VEGFR2, as well as GATA2 and ETS1, transcription factors required for VEGFR2 transcription.[12] Moreover, miR-200c targets endothelial nitric oxide synthase[7] and VEGFR2.

Fibrosis

The effect of miR-200 on fibrosis has been examined in the kidney, in the lung, and in cancer.

Human and animal studies have shown that enhanced miR-200 expression has an antifibrotic effect and that miR-200 is downregulated in renal and pulmonary fibrosis; furthermore, ZEB1 induces lysyl oxidase-like 2, an enzyme that cross-links and stabilizes collagen in tumor tissue. In contrast, miR-200 can be profibrotic in mesangial cells. Notably, there is a reciprocal link between miR-200 and transforming growth factor-β, a profibrotic cytokine; miR-200 is downregulated by transforming growth factor-β1 and miR-141 targets transforming growth factor-β2. The role of miR-200 on cardiac fibrosis remains to be determined.

Arrhythmias

In vitro experiments have shown that miR-200 targets and downmodulates the expression of sodium voltage-gated channel α-subunit 5 (SCN5A/$Na_v1.5$) in mouse HL-1 myocardial cells, suggesting a role for these miRNAs in cardiac arrhythmias.[13]

Stem Cell Differentiation

Few studies have examined the role of miR-200 in stem cell differentiation toward the vascular and myocardial lineages.

In mouse embryonic stem cells miR-200 increase in NO-dependent differentiation toward mesendoderm and cardiovascular lineage.[14] Moreover, miR-200c and miR-141 increase in human embryonic stem cells differentiating toward the endothelial lineage. Finally, miR-200c* modulates hanging drop–induced cardiac differentiation of ES cells. The role of miR-200 in stem and progenitor cell differentiation and cardiovascular repair is still unknown.

miR-200 Potential Role in Cardiovascular Diseases

Both acute and chronic conditions are expected to modulate miR-200 expression in the cardiovascular system. For instance, cardiac ischemia/reperfusion should markedly and transiently enhance miR-200 expression. However, it is unknown whether this occurs and, eventually, has an effect on cell death, regeneration, fibrosis, contraction, and arrhythmias. In contrast, there is evidence of a link between miR-200 and diabetes mellitus, obesity, and atherosclerosis; furthermore, miR-200 exhibits an age-dependent increase that may parallel the prevalence and severity of diabetes mellitus and atherosclerosis in the aging population. Is the increase in miR-200 relevant in age-dependent insulin resistance, vascular dysfunction, and impaired angiogenesis? Because of its effect on the epigenetic machinery, may miR-200 be responsible for the chromatin opening and consequent expression of aging- and cardiovascular disease–related genes? Is it possible that pharmacological epigenetic repressors of miR-200 be effective in attenuating noxious miR-200 effects on the cardiovascular system? Interventions aimed at decreasing miR-200 expression, for example, anti-miRNAs, may enhance epithelial–mesenchymal transition and carry an oncogenic risk. In fact, although most studies suggest a detrimental role of miR-200 in the cardiovascular system, it is possible that the age-dependent increase in miR-200 may have a protective role and inhibit cancer development in the elderly. It will be important to establish whether miR-200 downmodulation is tumorigenic, and eventually, which tissues are more likely to form tumors. Since the heart and blood vessels, are less prone to cancer development than other tissues/organs, one way to limit the oncogenic risk of miR-200 downmodulation would be to avoid systemic exposure to the anti-miRNAs.

In conclusion, the consequences of miR-200 increase will depend on different factors: which miR-200 members are modulated, the magnitude and time course of the change, the cell type in which the change/s occur, the release of miRNA in the extracellular space, and the presence of other interacting factors, including other ROS-modulated miRNAs. It will be a complex picture to decipher. Nevertheless, miR-200 may account for some features of common diseases and also represents a potential novel therapeutic target.

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Disclosures

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