REVIEW

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The role of miR-145 in microvasculature

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Increasing evidence suggests that microRNAs(miRs) play a crucial role in the cardiovascular system, and recent studies have revealed a significant role of miRs in vascular biology and disease. miR-145 is one of the most-studied miRs, and especially in the vascular smooth muscle cell (VSMCs) proliferation, differentiation, and phenotypic switching. In cardiovascular system, miR-145 is not only important for heart and vascular development but also plays an essential role in cardiac pathological factors, such as hypertrophy, and ischemia. However, its potential role in microvasculature has not been systematically evaluated yet. We are just beginning to understand the regulation of miR in vascular biology. In particular, the miR biogenesis and regulatory pathways in the vascular system have not yet been well characterized, This review focuses on the basic biology and mechanism of action of miR-145 specifically pertaining to microvascular development, pericyte and disease, In addition it addresses the potential for miR-145 to be used therapeutically in the treatment of microvascular disease.

1. Introduction

In recent years, increasing evidence suggests that microRNAs (miRs) play a crucial role in the cardiovascular system, and recent studies have revealed a significant role of miRs in vascular biology (Ohtani et al. 2011; Yu et al. 2011; Turchinovich et al. 2011; Vickers and Remaley 2010; Cordes et al. 2009). Many miRs are highly expressed in the vasculature and dysfunctionally expressed in diseased vessels. Some miRs have been confirmed to be pivotal modulators of vascular etiological factors, such as vascular smooth muscle cell (VSMCs) differentiation, contraction migration, proliferation, arterial remodeling, angiogenesis, and neointimal hyperplasia. In the cardiovascular system, miR-145 is found to be not only important for VSMCs proliferation, differentiation, and phenotypic switching but also plays an essential role in cardiac development. However, it's potential role in microvasculature has not been systematically evaluated yet. This review focuses on the basic biology and mechanism of action of miR-145 specifically pertaining to microvascular development and addresses the potential for miR-145 to be used therapeutically in the treatment of microvascular disease.

2. miR-145 gene organization

miRs are 20–25-nt-long noncoding RNAs that negatively regulate gene expression by binding to sites in the 3' untranslated region (UTR) of target mRNAs at the posttranscriptional level (Bagga et al. 2005). In general, one miR can regulate the expression of multiple target genes. Similarly, one mRNA can be regulated by several miRs, which form complex regulatory feedback networks. It is speculated that the human genome may encode more than 1000 miRs (Bentwich et al. 2005) that are abundant in many human cell types, and these miRs may target about 30% to 60% of the mammalian genes (Friedman et al. 2009). In various developmental processes, miRs fine tune or restrict cellular identities by targeting important transcription factors or key pathways (Boucher et al. 2011). Using small RNA cloning techniques, miR-145 was first identified in mice from heart tissue (Lagos-Quintana et al. 2001) and later reported in humans (Michael et al. 2003), revealing a unique seed sequence that is conserved in Xenopus and mammals. miR-145 is located on murine chromosome 18 within a 1.4 kilobases (kb) region and human chromosome 5 (5q32–33) within a 4.09 kb region (http://microrna.sanger.ac.uk). Although the pri-microRNA structure has not been identified, it is suggested often to be co-transcribed with miR-143, called miR-143/145 gene cluster (Cordes et al. 2010; Fig.). This fact was indeed validated by RT-PCR. Human miR-145 is enriched in germline and meso-derm-derived tissues, such as uterus, ovary, testis, prostate, spleen, vessel and heart (Lakshmipathy et al. 2007).

3. Role of miR-145 in microvascular development, angiogenesis, and microvascular disease

Recently, evidence suggests that miRs are involved in microvascular development, angiogenesis, and microvascular disease. Studies are applied to elucidate the role of miRs in microvasculature and find that let-7 g, miR-16, miR-20a, miR-21 and miR-29c up- regulated while miR-18a, miR-125a, miR-127, miR-148b, miR-189 and miR-503 were down-regulated after ionizing radiation in primary human microvascular endothelial cells (HMEC), and overexpression or inhibition of let-7 g, miR-189, and miR-20a markedly influenced HMEC proliferation (Wagner-Ecker et al. 2010). Similar finding observed by Shen et aln (2011). They explored to the mechanisms of the regulation of angiogenesis by heparin and thrombin. Heparins have been validated as antithrombotic agents and inhibits angiogenesis, while thrombin

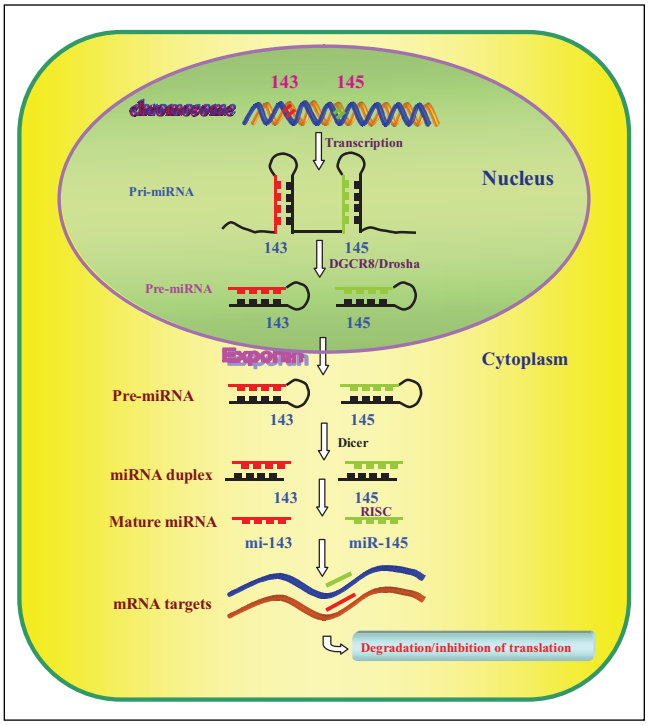

Fig.: Schematic representation outlining miR-143/145 biogenesis including transcription, maturation, and miRNA/mRNA targeting. The specific details included that miR-143 and miR-145 are cotranscribed as a single primary-miRNA transcript, which is further processed by DGCR8 (DiGeorge Syndrome Critical Region 8) and drosha to form the respective pre-miRNAs of miR-143 and miR-145 at first, Pre-miRNAs are then exported to the cytosol of VSMCs by a nucleocytoplasmic shuttle protein, exportin. Dicer cleaves the hair-pin of pre-miR-143 and pre-miR-145 in the cytoplasm, yielding their respective imperfect miRNA:miRNA-duplexes, which is finally unwinded to yield a mature miR-143/145, and finally mature miR-143/145 gets incorporated into the RNA-induced silencing complex (RISC) and acts on its mRNA targets

induces angiogenesis. In their experiments, they found that miR-10b is down-regulated by heparin and up-regulated by thrombin in HMEC, Overexpression of miR-10b induces HMEC cell migration, tube formation and angiogenesis. Further study they identified that homeobox D10(Hoxd10) is a functional target of miR-10b in HMEC. Heparin attenuates miR-10b expression and induces Hoxd10 expression *in vivo* to inhibit angiogenesis, whereas they also found that thrombin treatment increases Twist expression levels and led to increased expression of miR-10b, meanwhile decreased expression of HoxD10. Another study showed that miR-320 play a big role in impaired angiogenesis of type 2 diabetic Goto-Kakizaki rats in myocardial microvascular endothelial cells and transfection of an miR-320 inhibitor may be a therapeutic approach for the treatment of microvascular disease in diabetes (Wang et al. 2009).

However, the latest research by Truettner et al. (2011) explored to the role of miRs in the microvasculature of central nervous system, and found that miR-145 is selectively expressed in pericytes and is involved with cell migration. They investigated the changes in miR expression following exposure of cortical pericyte cultures to hypoxic conditions by microarray analysis, and found 27 miRs increased and 31 decreased in primary rat cortical pericytes after 48 h of hypoxia. miR-145 was the highest increased by up to 345% of control after hypoxia. Interestingly, Larsson et al. (2009) also identified that miR-145 is microvascular-enriched miR, and showed that overexpression of miR-145 leads to reduced VSMCs migration. miR-145 has previously been shown to be selectively expressed in smooth muscle cells (Cordes et al. 2009). Further investigations confirmed that miR-145 is expressed by pericytes of the microvasculature. Larsson et al. (2009) observed that expression of miR-145 is reduced in defective pericyte mice microvasculature, whereas expression of the other miRs were no notable differences. This suggests that miR-145 is selectively expressed in microvascular pericytes. The Ets transcription factor Friend leukemia integration 1 (Fli1) is an early marker of hemangioblast differentiation that has an important role in blood/vascular development and angiogenesis (Liu et al. 2008; Brown et al. 2000). Finally, they verified that miR-145 targets the hematopoietic transcription factor Fli1 and blocks microvascular cell migration in response to growth factor gradients.

Conditional deletion of Dicer has proved to be of great value to demonstrate a requirement for miRs in the development and/or function of several tissues and organs, including microvascular development (Stefani and Slack 2008; Damiani et al. 2008). Those experiments highlight the importance of miRs in tissue development and/or function. To define miRs role in renal glomerular function, screening several candidates, miR-145, miR-126 and miR-30a were shown to be expressed in mesangial cells, endothelium and podocytes (Harvey et al. 2008). Podocyte-specific Dicer knock-out mice develop proteinuria by 3 weeks and progress rapidly to end-stage kidney disease. These findings demonstrate miRs are required for maintenance of podocyte structure and function. Knockout of Dicer in podocytes leads to cytoskeletal disorganization and dedifferentiation, causing progressive glomerulonephritis and death by approximately 6 weeks. Thus a critical role for miR in glomerular function can be seen. In parallel, research by Sequeira-Lopez et al. (2010) indicated that the primary role of Dicer is to generate mature miRs, which is crucial for the maintenance of the juxtaglomerular(JG) cell, and miR-145 plays a key role in this process. JG cells are highly specialized myoepithelioid granulated cells located in the glomerular afferent arterioles. These cells synthesize and release renin. In this study they generated mice with a conditional deletion of Dicer specifically and found that expression of miR-145 in Dicer conditional knock-out mice is completely abolished in JG cells, while miR-145 is expressed in the JG cells and in arterioles in control mice. It indicated that miR-145 serves as a marker for the presence of Dicer. Its absence confirms loss of Dicer activity. Lack of JG cells was accompanied by decreased renin gene expression and circulating rennin. Deletion of Dicer in cells from the renin lineage resulted lead to renal abnormalities, and include interstitial fibrosis involving both cortical and medullary regions of the kidney. Within the fibrotic bands were noticeable alterations in the microvasculature ranging from near replacement of the arterioles by interstitial cells to distorted arterioles affected by fibroplasia. The study reveals a potential new role about miR-145 in renal microvasculature.

Growing evidence suggests that miRs play an important role in insulin production, secretion beta-cell differentiation, and indirectly control glucose and lipid metabolism (Correa-Medina et al. 2009; Joglekar et al. 2007, 2009; Poy et al. 2004). Diabetes leads to changes in miR expression profiles in many tissues. miRs open a new window for better understanding of diabetes pathogenesis and its complications (Poy et al. 2007; Tavintharan et al. 2009). Manipulation of the miR-221-c-kit pathway may offer a novel strategy for treatment of vascular dysfunction in diabetic patients (Li et al. 2009). High levels of miR-29 led to insulin resistance and overexpression of miR-29 caused a decrease in the levels of insulin-induced gene 1 and Cav2 proteins. Dysregulations of various miRs have been identified to be critical in diverse metabolic processes leading to diabetes and its microvascular complications. Furthermore, miR-145 has been identified to target and downregulate the Insulin Receptor Substrate 1 (IRS-1) protein in human colon cancer cells (Shi et al. 2007). IRS-1is a major mediator of insulin signaling and its mutation or dysfunction has been associated with diabetes (Baroni et al. 2001; Marini et al. 2003).

4. Mechanism of action of miR-145 in microvasculature

As mentioned above, miR-145 plays central biological roles by regulating the pricytes of microvasculature (Larsson et al. 2009). Pricytes were once known as "Rouget" cells, after the initial description Rouget of a perivascular cell adjacent to capillaries. Pericytes are regulatory cells that communicate with the components of the microvasculature, and mature pericyte plays a big role in maturation of the microvasculature. Pericytes are also multipotent cells, it has been established that they can differentiate into VSMCs and myofibroblasts/fibroblasts (Nehls and Drenckhahn 1993; Sundberg et al. 1996). A number of factors, such as: PDGF-B, angiopoietins (Hawighorst et al. 2002), gap junctions and TGF-β (Nishishita et al. 2004), and endothelial nitric oxide synthase (eNOS, Yu et al. 2005) regulate pericyte differentiation and maturation.

The phenotypic switch is thought to be regulated by the transcription factor Tal1 (Ema et al. 2003). Pericytes play a leading role in angiogenesis. In this respect, pericyte derived VEGF act to promote endothelial survival and guide migration. Meanwhile, endothelial tube formation is followed by pericyte coverage and pericytes use the developing sprouts as migration guidance cues during microvascular development.

It is now becoming clear that pericytes are directly involved in the pathogenesis of microvascular diseases. Pericytes play a crucial role in the development of diabetic retinopathy (Hammes 2005). In tumors, pericytes may stabilise blood vessels, inhibit endothelial proliferation, maintain capillary diameter, regulate blood flow, and provide endothelial survival signals via heterotypic contacts and soluble factors. Tumor vessels are patently abnormal, and tend to be dilated, tortuous, also with continuous microvascular growth and remodelling. Evidence suggests that these abnormalities result from defects in both compartments of tumor vasculature-endothelial and pericytes (Baluk et al. 2005).

Mechanisms of action of miR-145 in microvasculature have partly been elucidated by Larsson et al. (2009). Firstly, they found that miR-145, miR-126, miR-24, and miR-23a were selectively expressed in microvascular fragments. Microvascular fragments were isolated from mouse tissues and embryonic stem cell cultures using mechanical andenzymatic digestion followed by incubation with magnetic Dynabeads coated with anti-CD31 (anti-platelet endothelial cell adhesion molecule (PECAM)). In particular, miR-145 showed consistent and high differential expression in microvasculature (24-, 7-, 75- and 18-fold for brain, muscle, skin and kidney, respectively). To test the hypothesis that miR-145 is expressed by pericytes, two experiments have been done. The first experiment is to evaluate miR expression in immature blood vessels, CD31 + microvascular fragments were isolated from mouse kidneys at embryonic day 14, as well as from VEGFA-induced angiogenic sprouts formed in EB cultures. miR-126 showed strong enrichment in CD31 + fractions from both tissues. miR-23a and miR-24 were enriched in sprouts from EBs but not in fragments from embryonic day 14 kidneys. miR-145, in contrast, was predominantly expressed in the leftover fractions. The pericyte marker Pdgfrb showed a similar pattern with strong enrichment in CD31 + fragments from adult tissues but not in embryonic vascular fragments, which suggests that miR-145 could be expressed by pericytes. In the second experiment, CD31 + fragments were purified from the brains of Pdgfb retention-motif mutant mice (Pdgfbret/ret) that lack a stretch of basic amino acids in the carboxyl terminus of PDGF-B. These mice display defective pericyte investment of microvessels (Lindblom et al. 2003). As expected, Pdgfrb mRNA levels were reduced in Pdgfb ret/ret vascular fragments compared to wild-type mice. Expression of miR-145 was also reduced in mutant microvessels, whereas no notable differences were observed for the other miRNAs. These results gave further support to the idea that microvascular miR-145 expression is derived primarily from pericytes.

Secondly, they used miRNA target prediction software to identify possible targets for miR-145 and found that the highest-scoring predicted target is Fli1, then they confirm that Fli1 is a target gene of miR-145 with 3'UTR luciferase reporter assays. As is well-known that Fli1 is an early marker of hemangioblast differentiation and plays an important role in blood/vascular development and angiogenesis (Spyropoulos et al. 2000; Landry et al. 2005; Pimanda et al. 2007; Liu et al. 2008).

Finally, to further explore the role of miR-145 in cell migration, functional assays were performed in human foreskin fibroblasts. Because PDGF-BB is known to stimulate cell migration and is also a key regulator of pericytes *in vivo* (Seppa et al. 1982; Ferns et al. 1991; Lindahl et al. 1997), they investigated cell migration in response to a stable gradient of PDGF-BB using a microfluidic chemotaxis chamber. The results of this experiment have shown that miR-145 reduce migration of human foreskin fibroblasts, and the same results was gotten in VEGFA-165-induced migration in HUVECs.

In conclusion, from what the experiments have demonstrated above, the mechanism of miR-145 in microvasculature is to regulate the pricytes of microvasculature and to target Fli1. Elevated levels of miR-145 reduce migration of microvascular cells in response to growth factor gradients *in vitro*.

5. miR-145 in clinical applications and therapeutic strategies

Studies revealed a significant role of miR-145 in microvascular biology and disease. miR-145 has been reported to be easily detected in serum samples (Zhu et al. 2009). Hence, miR-145 may act as a useful biomarker, as it is one of the miRs upregulated significantly in mouse microvasculature and relevant to microvascular disease. Dysfunction of miR-145 expression has been found in renal microvasculature, and is associated with renal glomerular disease. In conclusion, miR-145 may become a helpful and reliable tool for the diagnosis and prognosis of patients with microvascular disease. However, there is no evidence that miR-145 is abnormally expressed in serum of patients with microvascular disease. Hence, we must soberly recognize the limitations of present knowledge, although studies are promising. We must still soberly recognize that miR-145 is just one of the miRs which regulate microvascular development and diseases. More studies are needed to further evaluate miR-145 as a novel biomarker for microvasculature. However, multicentric investigations, multiethnic patient populations, and standardized procedures for sample processing are needed to confirm whether miR-145 will fulfill the criteria for microvascular disease biomarkers.

The main difficulty with the use of miRs for therapeutic purposes is the delivery of the drug to target cells. Recently, many efficient techniques to manipulate miR levels *in vivo* have been developed (Sehm et al. 2009; Zeng et al. 2009). Presently, chemically synthesized antagomir and miR mimics have been widely used in investigating the biological functions of miR genes. Adenoviral and lentiviral vectors have been used to efficiently over-express or knockdown miRs *in vitro* and *in vivo*. Antagomirs can diminish miR bioavailability by binding stably to the targeted miRs, suggesting their great potential for pharmaceutical development. Treating various diseases with these methods is being explored for a wide range of diseases (Sehm et al. 2009; Zeng et al. 2009). miR-145 plays a significant role in microvascular development and disease, as well as for normal microvascular structural integrity. Identification of miR-145 target genes that contribute

to various microvascular diseases and understanding the complex network involving miR-145 in microvascular development undoubtedly will provide important tools to develop novel therapeutic strategies not only to enhance neovascularization of ischaemic tissue but also to offer help for remedying microvascular diseases. In short, miR-145 hold promise for treatment of microvascular diseases.

6. Perspectives

The microvasculature is a complex organ, in which many components, such as VSMCs, pericytes, and endotheliocytes, play important roles in microvascular function and disease, and have their own miRs that contribute to miR profiles. miR-145 is a characteristic miR of the microvascular expression profile. Vascular cell movement plays a pivotal role in the development of various diseases. miR-145 has been found to regulate microvascular cell movement. Therefore, how to make use of this feature for regulating microvascular development and remedying microvascular diseases is worth further studying.

The miR-145 expression signature, selectively expressed in pericytes, and targeting Fli1 reveal an essential role of miR-145 in microvascular formation and development. miR-145 may be a new therapeutic target for microvascular diseases such as atherosclerosis, diabetic retinopathy, diabetic nephropathy, and ischemic cardiomyopathy. But currently we are only touching the tip of the iceberg. It is urgent demand that all researchers in the microvasculature field pay close attention to these advances, identify new relevant miRs and find new therapeutic strategies for microvasculature development and diseases.

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