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Invited review

MicroRNAs as pharmacological targets in endothelial cell function and dysfunction

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ABSTRACT

Endothelial cell dysfunction is a term which implies the dysregulation of normal endothelial cell functions, including impairment of the barrier functions, control of vascular tone, disturbance of proliferative, migratory and morphogenic capacities of endothelial cells, as well as control of leukocyte trafficking. MicroRNAs are short non-coding RNAs that have emerged as critical regulators of gene expression acting predominantly at the post-transcriptional level. This review summarizes the latest insights in the identification of endothelial-specific microRNAs and their targets, as well as their roles in controlling endothelial cell functions in both autocrine and paracrine manner. In addition, we discuss the therapeutic potential for the treatment of endothelial cell dysfunction and associated vascular pathophysiological conditions.

Contents

1. Introduction

The endothelium is the monolayer of endothelial cells (ECs) lining the lumen of blood vessels in every organ system. These cells function as a protective biocompatible barrier between all tissues and the circulating blood [1]. ECs facilitate the bidirectional passage of nutrient substances and active molecules from blood to tissues, but also play a major role in controlling the passage of blood cells themselves. ECs are specially designed and spatially located to detect changes in hemodynamic forces and blood-borne signals.

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In addition, ECs regulate the release of a number of autocrine and paracrine factors in response to these signals to favor the maintenance of vascular homeostasis [2–9]. Therefore, normal EC function is critical for all aspects of vascular homeostasis (*i.e.* control of blood vessel development, growth and differentiation; control of leukocyte trafficking; control of vascular tone; control of vascular barrier; control of platelet function, coagulation and fibrinolysis). These have been reviewed in depth elsewhere [2–4,6–8,10].

EC dysfunction disrupts the balance between vasoconstriction and vasodilation and initiates a number of events that trigger EC activation and predispose the vessel wall to increased endothelial permeability, leukocyte adherence, endothelial proliferation, pro-oxidation and thrombosis [5,7,11–18]. Perturbations in EC functions have been implicated in several diseases including atherosclerosis, diabetes, tumor metastasis, inflammatory diseases (*e.g.* rheumatoid arthritis) and hypertension [5,12,16,19–21]. Importantly, the processes involved in EC activation require integration of the molecular and cellular events induced by both stimulatory and inhibitory signals. These include signal transduction pathways leading to transcriptional regulation of gene expression programs, as well as post-transcriptional and post-translational modifications that fine-tune this response. In this regard, microRNAs (miRNAs) have emerged as critical regulators of gene expression, acting predominantly at the post-transcriptional level [22–25].

In the present review we summarize the latest insights in the identification of endothelial-specific miRNAs and their targets, as well as their role in regulating EC functions both in an autocrine and paracrine fashion. In addition, we discuss their therapeutic potential in the treatment of EC dysfunction and associated vascular pathophysiological conditions.

2. MicroRNA biogenesis and function

MiRNAs are short noncoding RNAs that have emerged as critical regulators of gene expression acting predominantly at the post-transcriptional level [23,25]. According to the miRNA.org data resource, as of April 2013, 1100 miRNAs have been annotated in *Homo sapiens* and 717 in *Mus musculus* [26], while MirBase reports 2042 human and 1281 murine miRNAs [27,28]. Canonically these small RNAs are transcribed by RNA polymerase II from individual miRNA genes, introns of protein coding genes, or polycistronic transcripts as capped and polyadenylated primary miRNA transcripts (pri-miRNA) [29,30]. Then the pri-miRNA is processed in the nucleus into a ~70-nucleotide precursor hairpin (pre-miRNA) by ribonuclease III (RNase III), called Drosha in cooperation with a dsRNA binding protein, DiGeorge syndrome critical region gene 8 (DGCR8) [31,32]. Additionally, there is a group of intronic miRNAs (miRtrons) that bypass the Drosha pathway and are produced by splicing and debranching [33]. The transport receptor Exportin-5, RanGTP-dependent dsRNA-binding protein, exports the pre-miRNAs to the cytoplasm [34], where they are further processed by the RNaseIII enzyme, Dicer, into an approximately 22 nt miRNA/miRNA* duplex [35,36]. Although the miRNA/miRNA* duplex are produced in equal amounts by transcription, the abundance of mature strands is asymmetric at the steady state and it depends on the thermodynamic stability of the 5′ end of the miRNA strand [37,38]. Usually the miRNA* strand (or passenger strand) is the least thermodynamically stable and is rapidly degraded, however recent reports have shown that it has well-conserved target recognition sites that are also functional [39,40].

Following processing, the selected miRNAs are incorporated into the RNA-induced silencing complex (RISC) [41,42] which mediates the miRNA binding to the 3′ untranslated regions (3′UTR) of target messenger mRNA (mRNA) and negatively regulates gene expression by translational inhibition or target mRNA degradation or a combination of both [41,43,44]. In order to repress the transcript, it is crucial that the nucleotides in position 2–8 of the miRNA (called the seed sequence) are almost perfectly complimentary to regions at the 3′UTR of their target genes [45].

Computational predictions have revealed that 60% of protein-coding genes harbor miRNA target sites in their 3′UTR [46] and that a single miRNA can modulate the expression of hundreds genes. Not only are certain miRNA genes highly conserved in animals, but their target sites in the 3′UTR of genes are also under positive evolutionary selection. MiRNAs are powerful modulators of genetic networks and they do so by acting both in a coherent and incoherent fashion on their target genes [47,48]. MiRNAs, rather than functioning as regulatory on-off switches, often function to modulate or fine-tune cellular phenotypes [24,49,50]. The deregulation of miRNA expression could affect multiple cellular processes. Therefore it is not surprising that miRNAs have been implicated in various processes, from development to aging [24,49,50]. Additionally, many miRNAs exhibit striking tissue specific expression patterns [51–56], suggesting a cell type-specific function [51].

MiRNAs are necessary for development and organogenesis. Loss of Dicer in mice results in embryonic lethality at E7.5 and embryonic stem cells cannot be obtained from Dicer null mutants, suggesting that Dicer is especially important in maintaining the pluripotent status [57,58]. Also Ago loss gives rise to severe developmental defects by E10.5 [59]. At E11.5–E14.5 the developing mouse embryo expresses Dicer1 and the Ago proteins only in certain anatomical compartments and in a dynamic fashion, suggesting a further role for miRNAs in organogenesis as well [60]. Given the essential role of the miRNA processing machinery in development, it is not surprising that miRNAs have been shown to be relevant in vascular development [51] and vascular functions [61–67].

3. Control of blood vessel development, growth and differentiation by endothelial microRNAs

The *de novo* generation and remodeling of blood vessels is essential for embryonic growth and throughout postnatal life. During adulthood, the endothelium remains essentially quiescent to fulfill its main function in conducting nutritive blood flow to organs, with turnover rates on the orders of months to years. Rapid changes in EC proliferation rates occur following activation of the endothelium by angiogenic cytokines [68–71]. In fact, in the healthy adult, angiogenesis occurs only in select phases of the female reproductive cycle and as a protection mechanism in wound healing/tissue repair and is almost exclusively associated with pathology when angiogenesis is induced by micro-environmental factors such as hypoxia or inflammation [68,70,72–75]. ECs play a key role in angiogenesis which is dependent on the proliferation, migration and differentiation of these cells [76].

The pathological processes associated with angiogenesis include diseases as diverse as cancer, macular degeneration, psoriasis, diabetic retinopathy, thrombosis, and inflammatory disorders, including arthritis and atherosclerosis. Moreover, insufficient angiogenesis is characteristic of ischemic heart disease, peripheral vascular disease and pre-clampsia [68,69]. The above examples represent the broad array of diseases that are associated with dysfunction of the angiogenic activated EC phenotype.

The first evidence of the importance of miRNAs in vascular development was shown by Yang et al. who generated a Dicer $ex^{1/2}$ knockout mice, where Dicer was hypomorphic because of the deletion of its first and second exons. However, homozygous embryos