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Review Article

The role of miR-126 in embryonic angiogenesis, adult vascular homeostasis, and vascular repair and its alterations in atherosclerotic disease

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ABSTRACT

Expression of microRNA (miR)-126 is enriched in endothelial cells (ECs) and endothelial progenitor cells (EPCs). MiR-126 is considered a master regulator of physiological angiogenesis. In embryonic vasculogenesis, this miRNA is involved in induction of angiogenic signaling, supports differentiation of embryonic stem cells to EPCs and ECs, and promotes EC maturation. However, in mature ECs and adult EPCs, miR-126 contributes to vascular homeostasis by inhibiting angiogenesis and maintaining the quiescent endothelial phenotype associated with increased vascular integrity and inhibited proliferation and motility. In a case of vessel injury and/or hypoxia, miR-126 up-regulation activates EPCs and ECs and contributes to vascular healing and neovessel formation. Indeed, miR-126 exhibits vasculoprotective and atheroprotective properties. The promising regenerative and proangiogenic potential of this miRNA will be helpful for development of cardioprotective strategies and cardiovascular repair therapies of myocardial infarction, heart failure, and other cardiovascular pathology.

Contents

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1. Introduction

Vascular endothelium is the integral part of the circulatory system. The endothelial layer forms the inner envelope of the vascular wall that separates intraluminal blood components from the vessel intima media. Endothelial cells (ECs) play a unique role in the vascular function. They are responsible for the barrier function, vascular integrity and permeability. ECs are involved in the regulation of vascular tone and hemostasis/coagulation. ECs modulate leukocyte trafficking, immune responses, and inflammation [1,2]. In vascular injury, endothelial progenitor cells (EPCs) and other vascular progenitors resided in the adventitial stem cell-like niches of postnatal vessels up-regulate vessel repair, wall tissue remodeling, and angiogenesis [3]. In vascular pathology such as atherosclerosis, the arterial endothelium is functionally impaired or dysfunctional due to the deleterious effects of cardiometabolic risk factors. The atherogenic risk factors induce numerous alterations in endothelial function and disturb or prevent vascular repair that cause irreversible changes in the vessel wall and promote plaque progression [4]. Loss of the proper regulation of endothelial function critically contributes to these pathophysiological changes.

MicroRNAs (miRNAs), a class of small non-coding RNAs, form a regulatory network that targets 18,500 mRNAs [5]. MiRNAs are involved in the control of all cellular functions, cell fate decisions, and the development of cellular phenotypes. By binding to a partially complementary seed sequence on the 3′ untranslated region (UTR) of an mRNA target, miRNAs typically inhibit but may sometimes stimulate gene expression [6]. The targeted mRNA is then subjected to the degradation or silencing in special cytoplasmic compartments called P-bodies [7].

Expression of miRNAs is strictly controlled to prevent adverse functional effects. However, in vascular diseases, miRNA expression is frequently altered leading to pathologic consequences. Expression and levels of some miRNAs are increased in certain tissues. The enrichment in so called tissue-specific miRNAs is due to their special role in these tissues [8]. MiR-126 belongs to endothelial-specific miRNAs. Compared with other tissues, expression of miR-126 is up-regulated in ECs and EPCs [9]. This miRNA plays a significant role in angiogenesis, EC function, and vascular repair. In vascular tissue, altered expression of miR-126 could contribute to the development of cardiovascular pathology and vessel wound healing. In this paper, we will consider endothelial-specific properties of miR-126 in physiological and pathological conditions.

2. MiR-126 biogenesis and tissue expression

Human miR-126 is encoded by a single gene located in intron of the EGFL7 gene (encodes EGF-like domain-containing protein 7) at chromosome 9q34.3 (Fig. 1). The murine Egfl7 gene also contains the intronic mmu-miR-126 gene (intron 7) [10], suggesting for the conservation of the EGFL7/miR-126 locus in mammals [11]. By contrast to many other miRNAs, the passenger strand of premiR-126 is not completely destructed meaning the guide strand, miR-126 (*i.e.* miR-126-3p) and miR-126* (*i.e.* miR-126-3p) are both highly expressed [12]. Sequences of both mature miR-126 isoforms differ only by 1 nucleotide. Both miR-126 isoforms were found in zebrafish thereby indicating the conservation of this miRNA in vertebrates [13].

In embryonic tissues, miR-126 is the only miRNA that is specifically expressed in the EC lineage, hematopoietic progenitor cells, and EC lines [12,14]. In adult body, expression of miR-126 is increased in well-vascularized tissues such as heart, lung, and liver [15].

3. miR-126 conservation in vertebrates

Like a human orthologue, the murine Egfl7 gene also contains the intronic mmu-miR-126 gene (intron 7) [10] suggesting the conservation of the EGFL7/miR-126 locus in mammals [11]. Furthermore, both miR-126 isoforms (*i.e.* miR-126-5p and miR-126-3p) were found in zebrafish thereby indicating the conservation of this miRNA in vertebrates [13]. EGFL7, a host gene for miR-126, is also evolutionarily preserved since its orthologue (CG7447) was found in *Drosophila melanogaster* [16].

4. miR-126 deficiency in experimental models: effects on angiogenesis

In zebrafish, miR-126 inhibition results in vascular developmental defects such as loss of vascular integrity and hemorrhage during embryogenesis [12]. Similarly, targeted deletion of miR-126 in mice leads to the formation of fragile and leaky vessels, lumen collapse, aberrant endothelial tube hierarchy, hemorrhages, and impaired EC proliferation and migration [10,11]. In summary, these findings indicate a key role of miR-126 in embryonic and postnatal angiogenesis, post-traumatic vascular regeneration, and endothelial function. miR-126 also regulates expression and angiogenic function of its host gene EGFL7.

5. miR-126 and its host gene, EGFL7

Intragenic MiRNAs located in intronic sequences are usually involved in the regulation of expression of host genes. This regulatory mechanism is evolutionarily conserved. For example, in *Drosophila*, the jing-interacting gene regulatory 1 (jigr1) gene contains coding sequences for miR-92a and miR-92b, which are embedded in the intron and 3′ untranslated region (3′UTR) of jigr1 [17]. Jigr1 is a potential transcription factor containing DNA-binding domain that can be involved in differentiation of neuroblasts to neuronal and glial cells during neurogenesis [18]. In embryogenesis, it is important to maintain the balance between self-renewal and differentiation. MiR-92a and miR-92b target jigr1 and therefore maintain neuroblast self-renewal by suppressing premature differentiation [17].

A miR-126-harboring EGFL7 is highly expressed in early embryonic stages involving primordial germ cell differentiation [19]. In adult organism, expression of this protein factor found in the germ line of ovaries and testes but down-regulated in vasculature [19] except for some vessels of highly vascularized organs such as lungs, heart, uterus, and kidney [16] but could be up-regulated in vascular injury [20] suggesting for the role in vessel healing. EGFL7 is a secreted endothelial-specific angiogenic factor whose expression is the highest in proliferating endothelium. The factor is involved in tubulogenesis and regulation of vascular patterning and integrity [20].

Generally, EGFL7 and miR-126 are expressed in parallel [12] since they share a common primary transcript. In humans, three EGFL7 isoforms utilizing alternative exons that encompass the 5′UTR are expressed (Fig.1). The isoforms contain the same open reading frame but are transcribed from separate promotors [12]. In ECs, GATA-2 and Erg, a member of the ETS transcription factor family, prime common expression of EGFL7 and miR-126. The 5.4-kb long EGFL7 promoter contains two conserved regulatory motifs, the enhancer and the −252/+38 region encompassing the exon-1b transcription start site [21]. This region contains GATA-2- and ETS-binding sites recognized by transcription factors GATA-2 and Erg [21].

However, expression of miR-126 can be negatively regulated by Crk, an adapter protein, independently on EGFL7 expression [22]. Monteys et al. [23] reported finding the intronic promoter sequence upstream the miR-126 gene suggesting for an option of independent transcription. In ECs, transcription factors Krüppel-like factor 2 (KLF2) and Ets1/Ets2 were shown to prime miR-126 expression from this independent promoter [24,25]. Vascular endothelial growth factor (Fig. 1) (VEGF) stimulates miR-126 expression [26].

The 3'UTR of EGFL7 contains a binding site for miR-126-3p (5′-ggucg-3′) (Fig. 1). Indeed, miR-126 is able to target the EGFL7 mRNA if no expression from the common EGFL7/miR-126 promoter is observed. EGFL7 plays a crucial role in early embryonic vasculogenesis by regulating the local migration and proper orientation of angioblasts