miR-125a-5p Modulates Phenotypic Switch of Vascular Smooth Muscle Cells by Targeting ETS-1

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Abstract

MicroRNAs are key regulators of vascular smooth muscle cells (VSMCs) phenotypic switch, one of the main events responsible for bare metal in-stent restenosis after percutaneous coronary intervention. miR-125a-5p is an important modulator of differentiation, proliferation, and migration in different cell types; however, its role in VSMCs is still unknown. The aim of this study was to evaluate the role of miR-125a-5p in VSMCs phenotypic switch. Our results suggest that miR-125a-5p is highly expressed in VSMCs, but it is down-regulated after vascular injury *in vivo*. Its overexpression is sufficient to reduce VSMCs proliferation and migration, and it is able to promote the expression of selective VSMCs markers such as alpha smooth muscle actin, myosin heavy chain 11, and smooth muscle 22 alpha. Interestingly, miR-125a-5p directly targets ETS-1, a transcription factor implicated in cell proliferation and migration and is crucial in PDGF-BB pathway in VSMCs. Thus, miR-125a-5p in this context inhibits PDGF-BB pathway and is therefore a potential regulator of VSMCs phenotypic switch.

Introduction

The most used strategy to treat coronary stenosis is percutaneous coronary intervention (PCI). In a variable percentage of patients within months after the PCI, the development of luminal narrowing at the site of the previous angioplasty or bare metal stenting might occur, a phenomenon known as restenosis [1].

The introduction of bare metal stents (BMSs) into clinical practice led to a substantial improvement of the clinical outcome after PCI, propelling the widespread use of percutaneous coronary revascularization [2]. However, the use of BMS is limited by a high rate of in-stent restenosis (ISR), mainly caused by uncontrolled neointimal proliferation with progressively lumen narrowing within the treated vessel segment [3,4]. The risk for ISR was substantially reduced by the introduction of drug-eluting stents (DESs) into the clinical practice, which significantly contributed to reduce the incidence of ISR. However, DESs present some dark sides, such as the inflammatory response and an increased risk of late and very-late stent thrombosis [5] related to the use of non-selective antiproliferative drugs acting on vascular smooth muscle cells (VSMCs) and on endothelial cells (ECs).

It has been shown that the mechanism responsible for restenosis after BMS implantation is represented by the proliferation of VSMCs of the media wall that should be selectively inhibited. Indeed, an abnormal proliferation of VSMCs causes the development of neointimal hyperplasia and consequent luminal narrowing [6,7]. Moreover, VSMCs proliferation has been reported in several diseases, including atherosclerosis and aortic aneurysm [8,9].

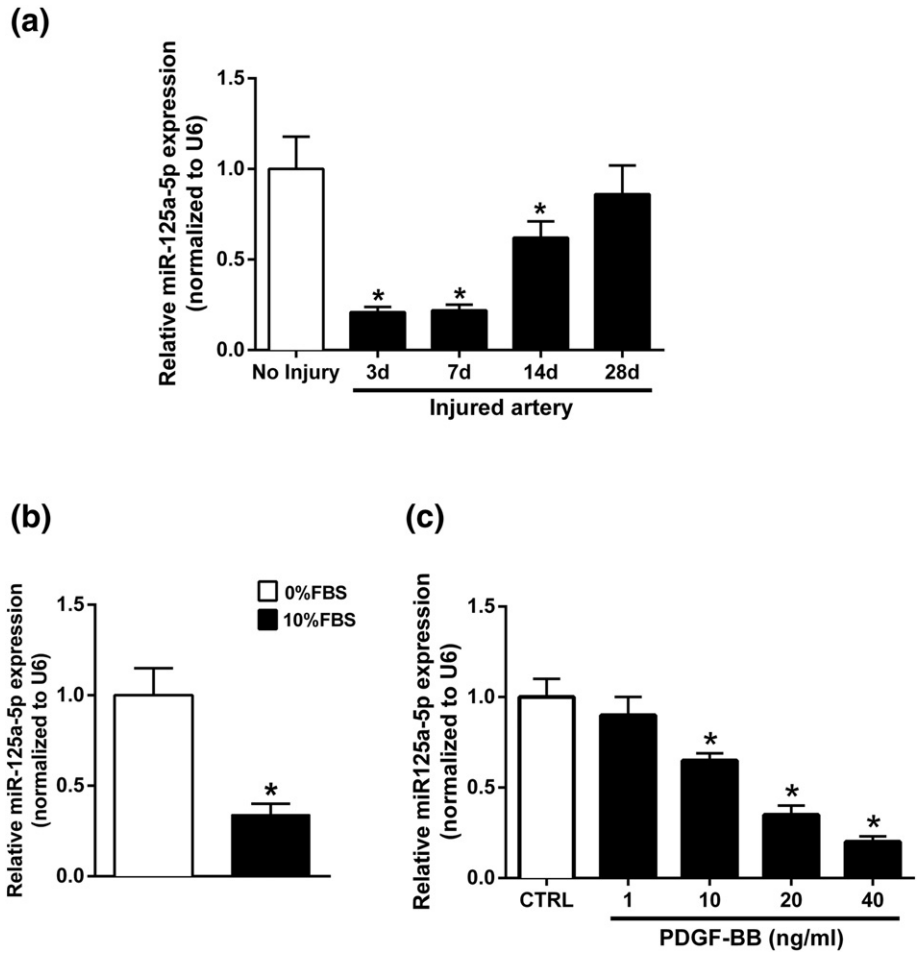

Fig. 1. miR-125a-5p expression levels *in vitro* and after injury *in vivo*. (a) miR-125a-5p expression analyzed by TaqMan MicroRNA Assay in rat carotid arteries at 3, 7, 14, and 28 days after balloon injury. *$p < 0.05$ *versus* miR-125a-5p levels in rat carotid arteries with no injury; $n = 4$. (b) miR-125a-5p expression level in VSMCs A10 in response to 10% FBS. *$p < 0.05$ *versus* miR-125a-5p levels in starved VSMCs; $n = 5$. (c) miR-125a-5p expression level in VSMCs A10 treated with different concentrations of PDGF-BB (ng/ml). *$p < 0.01$ *versus* miR-125a-5p levels in VSMC A10 untreated; $n = 5$.

VSMCs are the main cell type in adult blood vessel; they have a very low proliferation rate and synthetic activity and express a specific pattern of contractile proteins, ion channels, and signaling molecules [10]. Unlike skeletal muscle and cardiac muscle cells, which are terminally differentiated cells, adult VSMCs retain a fair plasticity and can undergo a phenotypic switch in response to different stimuli [11]. The switch of VSMCs from a contractile to a synthetic phenotype is characterized by an increased proliferation and migration rate and by a decreased expression of selective VSMCs markers, such as smooth muscle α-actin (ACTA2) and myosin heavy chain (MYH11) [12,13].

This process is well studied and has led to the introduction of DESs; these stents are characterized by the ability to release an antiproliferative drug within a given time window, usually set within the timeframe when neointimal proliferation is most probable. The establishment of DESs significantly contributed to reduce the incidence of ISR [14–18]. However, the inflammatory properties of the polymeric coating applied on stent struts, and the increased risk of late and very late stent thrombosis associated with DESs [5] remove them from consideration as a decisive solution to ISR. For this reason, the molecular mechanisms responsible for VSMCs response to injury are still being studied, in order to discover novel therapeutic targets.

The discovery of microRNAs (miRNAs) and their role in gene silencing aroused great interest [19], considering their potential role as biomarkers or therapeutic targets in cardiovascular diseases [20]. miRNAs are small non-coding RNAs of 20–22 nt, which regulate gene expression through post-transcriptional silencing [21]. Increasing evidence indicate that miRNAs are key regulators in several processes in cardiovascular biology, physiology, and disease [22–26]. miR-143 and -145 show the highest expression in VSMCs, and they are