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Research paper

MicroRNA-24 regulates vascular remodeling via inhibiting PDGF-BB pathway in diabetic rat model

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ABSTRACT

Purpose: Hyperglycemia is the high risk factor of vascular remodeling induced by angioplasty, and neointimal hyperplasia is strongly implicated in the pathogenesis of vascular remodeling caused by carotid artery balloon injury. Studies have shown that MicroRNA 24 (miR-24) plays an important role in angiocardiopathy, However, the role of miR-24 is far from thorough research. In this study, we investigate whether up-regulation of miR-24 by using miR-24 recombinant adenovirus (Ad-miR-24-GFP) can inhibit PDGF-BB signaling pathway and attenuate vascular remodeling in the diabetic rat model.

Methods: Male Sprague-Dawley rats (n = 60) were randomly divided into 5 groups and fed with high sugar and high fat diet (Sham, Saline, Scramble, Ad-miR-24 groups), or ordinary diet (Control group). The front four groups were treated with streptozotocin (STZ) four weeks later and the blood glucose level was closely monitored. After the successful establishment of diabetic rats, the external carotid artery was injured by pressuring balloon 1.5 after internal carotid artery ligation, then the blood vessels were harvested 14 days later and indexes were detected including the following: HE staining for the level of vascular intima thickness, immunohistochemical detection for PCNA and P27 to test the proliferative degree of vascular smooth muscle cells (VSMCs), qRT-PCR for the level of miR-24, RAS,PDGF-R, western blot for the protein levels of JNK1/2, p- JNK1/2, ERK1/2, p-ERK1/2, RAS, PDGF-R, AP-1,P27 and PCNA. Serological detection was conducted for TNF-α, IL-6, IL-8.

Results: The delivery of Ad-miR-24 into balloon injury site has significantly increased the level of miR-24. Up-regulation of miR-24 could regulate vascular remodeling effectively, lower the level of inflammatory factors, inhibit the expression of mRNA and protein levels of JNK1/2, ERK1/2, RAS, PDGF-R, AP-1, P27, PCNA.

Conclusion: miR-24 can inhibit the expression of AP-1 via the inhibition of PDGF-BB signaling pathway, thus inhibit VSMCs proliferation and vascular remodeling.

1. Introduction

Diabetes mellitus (DM) cannot be treated effectively in clinical due to its complex complications occurred in different physiological systems in human body, DM may cause cardiovascular complication which is the severest factor leading to the high mortality (Low Wang et al., 2016). Evidences have shown that vascular remodeling is more pronounced in DM patients than non-diabetic patients due to the poor blood sugar state (Willfort-Ehringer et al., 2004). Several studies have demonstrated that the incidence of vascular remodeling mainly related to the following factors: vascular smooth muscle cells (VSMCs) proliferation, inflammatory reaction, platelet activation and apoptosis disorder. Vascular remodeling could shorten vessel diameter and impedes the efficacy of percutaneous coronary intervention (PCI). Both animal experiments and clinical studies have proved that neointimal hyperplasia is an inevitable stage and actually crucial to the process of

Abbreviations: Diabetes mellitus, DM; vascular smooth muscle cells, VSMCs; percutaneous coronary intervention, PCI; MicroRNA, miR; platelet-derived growth factor, PDGF; proliferating cell nuclear antigen, PCNA; Sprague-Dawley, SD; green fluorescent protein, GFP; 4′,6-diamidino-2-phenylindole, DAPI; hematoxylin and eosin, H&E; Quantitative Real-Time PCR, qRT-PCR; polyvinylidene difluoride, PVDF; enzyme-linked immunosorbent assay, ELISA

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vascular remodeling (Mitchell and Libby, 2007). The formation of vascular remodeling is attributed to numerous pathophysiological processes and especially multiple molecular signaling cascades that control the proliferation, migration and differentiation of VSMCs (Tsaousi et al., 2011). However, the most effective method to inhibit vascular remodeling is still unclear (Jukema et al., 2011)

MicroRNA (miR) is normally transcribed in the nucleus by the RNA polymerase II and has the function of regulating cell growth and tissue differentiation (Lee et al., 2004). The discovery of miRs can be divided into two parts, including miRs which are upregulated (miR-21, miR-146a, miR-221/222, miR-424, et al.) and downregulated (miR-143, miR-145, miR-195, et al.) after vascular injury, all of them are important in the fine regulation of cardiovascular pathophysiologic processes (Gareri et al., 2016). Evidence proved that miR-24 can function as an inhibitory factor of human tumor cell lines that can suppress tumor growth (Michael et al., 2017). Our previous research showed that miR-24 can participate in the regulation of pathological neovascularization and attenuate restenosis after carotid artery injury (Yang et al., 2016). Therefore, miR-24 may play an important role in the process of vascular remodeling.

Platelet-derived growth factor (PDGF) is mainly produced by mononuclear macrophages, and it exists as homodimer or heterodimer dimerized by four simple chains, polypeptide-A, -B, -C, -D (Farooqi and Siddik, 2015). PDGF initiates a range of intracellular signal transduction that contributes to the synthetic VSMC phynotype and migration into the neointimal after vessel injury and accelerate VSMC proliferation (Tallquist and Kazlauskas, 2004). Evidence proved that PDGF-BB can stimulate VSMC proliferation and lead to injury-induced neointimal hyperplasia (Dong et al., 2010). PDGF-BB/PDGF-Rβ interactions play a crucial role in vascular maturation (Betsholtz et al., 2001), as an important signaling molecule, PDGF-Rβ can activate intracellular signal transduction that includes RAS/RAF/MEK/ERK1/2 and RAS/JNK1/2 pathways (Chen et al., 2014a; Roovers and Assoian, 2000). JNK and ERK translocate to the nucleus and activate c-Jun and c-Fos, which dimerize to form the activator protein-1 (AP-1) complex that mediate inflammation and VSMC proliferation (Ahn et al., 2002). Additionally, there was an association between PDGF-BB and the gene of miR-24 (Chan et al., 2010).

Evidence from experimental and clinical studies revealed that inflammatory cytokines are also important factors in vascular remodeling. It was known that inflammatory cytokines participate in the pathogenesis of atherosclerosis, as TNF-α, IL-6, IL-8. Other studies have indicated that inflammatory cells play important roles in the process of vascular remodeling, as vascular balloon injury was followed by neutrophil infiltration and inflammatory cytokines promotion that could prolong macrophage accumulation and lead to the formation of vascular remodeling (Toutouzas et al., 2004). Besides, proliferating cell nuclear antigen (PCNA) can be used as an index to evaluate the state of cell proliferation.

In this study, we are skilled in establishing diabetic rats and carotid artery balloon injury model. With the successfully recombinant of miR-24 and adenovirus, we deliver Ad-miR-24-GFP to the vascular injury site and conduct a series of researches on its effect of VSMC proliferation, inflammatory and neointimal hyperplasia. Thus, reveal the underlying mechanism of miR-24 on vascular remodeling after carotid artery balloon injury.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats (n = 60; about 170 g) came from China Three Gorges University (Certificate No. 42010200000969). All mice were given high glucose and high fat diet and unlimited to drinking water. The experimental process and environment were accord with the Animal Care and Use Committee of China Three Gorges University, and also in line with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, Publication No. 85–23.

2.2. Construction of adenoviral vector targeting miR-24

The recombinant adenovirus of miR-24 was synthesized by Genechem (Shanghai, China). The precursor of Rno-miR-24 (MI0000854) was generated and the two miR viruses were constructed using the adenoviral vectors, miR-24 and Ad-Scramble-GFP were designed by the AdMax adenovirus system (Microbix Biosystems, Toronto, ON, Canada) and amplified in human embryonic kidney 293 (HEK 293) cells. Finally adjust the concentration of viral titer to 1×10^9 PFU/mL using plaque assays.

2.3. Establishment of diabetic rat model

Sixty male rats were divided into five groups according to different feeding diets (diabetic group, n = 48; control group, n = 12) and all of them were free to drink. The rats in diabetic group were fed by high-sugar-fat diet (20% kcal protein, 20% kcal Carbohydrate, 60% kcal Fat) purchased by Huafukang in Beijing, China, and the rats in control group were fed with standard feed. Four weeks later, the pancreatic islet of rats in diabetic group were destroyed with a single intraperitoneal injection of streptozotocin (STZ, 30 mg/kg) and rats in control group were injected with same dose of citrate buffer. Then the rats' blood glucose were tested and the diabetic rat model was established successfully once the level of fasting blood glucose was higher than 16.7 mmol/L (Yang et al., 2016). The whole process of the experiment was carried out at SPF level of animal laboratory.

STZ administration: First, A solution and B solution were prepared according to the following methods: citric acid 2.1 g added double distilled water 100 mL was prepared into A solution, citrate trisodium citrate 2.94 g added double distilled water 100 mL was prepared into B solution, 28 ml A liquid and 22 ml B solution were taken and double distilled water was added to 100 ml, this liquid was AB mixture, also called STZ configuration liquid.

2.4. Experimental rats grouping and diabetic rat carotid artery balloon injury model

All of the diabetic rats were divided into four groups randomly (n = 12 in each group): diabetic rats without damaging the carotid artery (Sham group); diabetic rats receiving balloon injury with saline injection (Saline group); diabetic rats receiving balloon injury with Ad-Scramble-GFP injection (Scramble group); diabetic rats receiving balloon injury with Ad-miR-24-GFP injection (miR-24 group). The healthy non-diabetic rats without damaging the carotid artery (Control group).

After feeding for five weeks totally, rats were anesthetized with chloral hydrate (10%, 0.3 ml/100 g) through intra peritoneal injection. The rats' skin was disinfected with alcohol and cut open along the front center line after they were fixed on the platform, then the fascia and anterior cervical muscles were separated layer by layer. The left common, external and internal carotid artery was further exposed. The external carotid artery was cut partially with eye scissors after clipping the common and internal carotid artery with two artery clamps and ligating the distal end of the external carotid artery using 6–0 silk suture. Then, send the 1.5F balloon catheter from the breach into common carotid artery until about 2.5 cm from the bifurcation of the external carotid artery, pressurize the balloon to the instrument panel with a scale of 6, pull the balloon back and forth three times in the blood vessel in order to strip the intima. Inject with insulin needles for adenovirus transduction, 100 μL Ad-miR-24, Ad-Scramble or normal saline, after 30 min incubation. Ligate the proximal end of the external carotid artery and loose the artery clamps, the carotid artery was filled and pulsatile. Then the skin incision was closed. 14 days later, the rats