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Expression of hypoxia-inducible factor 1 alpha ameliorate myocardial ischemia in rat

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ABSTRACT

Hypoxia-inducible factor 1 alpha (HIF-1a), the upstream regulator of Vascular endothelial growth factor (VEGF), is the vital hypoxia related gene expression control factor. To evaluate HIF-1a therapeutic efficacy to acute myocardial infarction, the HIF-1a expressing recombinant Adeno-associated virus (rAAV) was constructed. The Wistar rat ischemic heart animal model was established with left anterior descending coronary artery ligation. The ischemic rats were treated with HIF-1a expressing and GFP expressing rAAVs respectively. Four weeks post the injection, the cardiac function of treated rat was compared by TM_WAVE system; size of infracted area was calculated by Evan's blue stain and capillary density was determined by CD31 immunohistochemical staining. Compare to the control group, the rats received HIF-1a expressing rAAV have smaller infracted heart size, the better heart function and higher capillary density than vehicle control group. The results show that the injection of HIF1a expressing rAAV can improve cardiac function and ameliorate acute myocardial ischemia (AMI) in rats.

1. Introduction

Coronary artery heart disease (CHD) is one of the most common heart diseases with high mortality [1]. Myocardial ischemia (MI), the major pathological mechanism of CHD, refers to the heart's blood perfusion reduction of oxygen which causes the heart to reduce myocardial energy metabolism impair hear function. As we know, myocardial cell belongs to permanent cell which lack of regenerative capability. Once the lesion happens, it is permanent damage to myocardial cell, which will be replaced by scar tissue. The damage will result in myocardial cell number reduction and the heart structure and function disorder; eventually induce congestive heart failure (CHF) [2]. Therefore, revascularization after AMI is the key to the cardiac function recovery. The classical surgery revascularization methods include Coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI), which are effective in most of patients [3]. However, the gene therapy method, 'molecular bypass', which is a promising advantage provide a new revascularization possibility. With gene transfer promoting angiogenesis and collateral circulation, it help to restore blood flow for the treatment of occlusive vascular induced MI. Compare to classical method, gene therapy have some specific advantages, such as, waive the surgery risk, decrease operation difficulty and prevent the Coronary In-Stent Restenosis [4,5]. As one option, it seems possibility that the gene therapy is used as a combination with classical methods to improve MI patience prognosis [6].

Hypoxia, a critical component of myocardial ischemia, triggers a wide range of cellular responses, including regulation of gene expression and post-translational modification of proteins [7]. In response to hypoxia, the gene regulations followed by a series of signal transduction mechanisms. In multiple cell culture and animal models, it has been demonstrated that the stability of hypoxia inducible factor 1 alpha (HIF-1α) subunit are directly controlled by intracellular oxygen concentration [8].

HIF-1a was first found as a kind of transcription factors from the Hypoxia induced cell Nuclear extracts in 1992 [9]. Importantly, as the rheostat for oxygen sensing in the cell, HIF-1a controls several pathways critical for cellular response to hypoxia [10–12]. These include: i). The transcriptional activation of angiogenesis genes such as VEGF, angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), placental growth factor, and platelet-derived growth factor beta; ii). The recruitment of endothelial progenitor cells to areas of tissue ischemia through an SDF-1-CXCR4 pathway; iii). the

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preconditioning of tissue to tolerate ischemic insult; iv). The stimulation of solid organ tumor growth; v). The activation of proinflammatory chemokine production by endothelium through transcriptional modulation of heme oxygenase 1. Indeed, it is reported that more than 2% of all human genes are regulated by HIF-1 in human pulmonary artery endothelial cells, demonstrating the broad range of effects this transcriptional activator has on diverse classes of genes [12].

Under ischemia condition, HIF-1a was first induced and activates the downstream gene, which worked as a control of a series of hypoxia response to enhance vascular growth, ameliorate MI and improve the patient survival chance from acute myocardial ischemia [13,14]. It is reported that the directly injection of HIF-1a to acute ischemia rat model can increase the vascular density in both infracted area and normal cardiac tissue [15]. Therefore, we suppose the HIF-1a is necessary for the recovery of MI, and it can play therapeutic role to the myocardial tissue.

The viral vectors have many merits compare to non-viral vectors, such as more efficient transfection *in vivo*, higher and relative longer term expression [4,16]. As one of the most common vectors, AAV has good safety record in clinical trials [17]. In current study, two designs were made to achieve specific high expression in cardiac system. First, the AAV9 serotype was selected for its specific high expression in the heart. Second, cardiac troponin T promoter (cTnT) was cloned to improve the expression specificity.

Actually, VEGF is the first gene that has been employed to treat MI. However, some researcher found that the some regenerated-blood-vessels that enhanced by the VEGF is immature and lack of blood transport function [18]. Therefore, in current research, the HIF-1a, upstream of the VEGF, was studied and demonstrate widely protection against myocardial ischemia and reperfusion injury, promote Angiogenesis.

2. Materials and methods

2.1. Construction of plasmids and adeno-associate virus generation

The Rat HIF-1a cDNA (GenBank accession No. NM_001530) was generated by the thermoscript RT-PCR system (Invitrogen, Grand Island, NY). The HIF-1a cDNA was cloned into an AAV vector plasmid under the transcriptional control of major histocompatibility complex (MHC) enhancer myocardial specific troponin T promoter (TnT). GFP cDNA was cloned into same AAV vector backbone, which was used as a control treatment.

Recombinant AAV viruses expressing Rat HIF 1a (AAV-MHC-TnT-HIF1α) or green fluorescence protein (AAV-GFP) were produced and purified with standard method after triple plasmid transfection of 293 cells [19]. The viral particles were purified by polyethylene glycol precipitation followed by CsCl centrifugation. Electron microscope (EM) was performed to confirm the virus purity. DNA dot blot was used to determine the titers of the purified viral stocks as vg/mL. Intravenous (i.v.) injection is for the systemic delivery, AAV serotype 9 was chosen as our delivery vector, and the final vector was named AAV9-HIF1α and AAV9-GFP.

2.2. Primary rat myocardial cell and western blot

For western blot, infected HEK 293 cell and primary rat myocardial cell were homogenized and run on an 8–12% sodium dodecyl sulfate-polyacrylamide gel. The anti- HIF-1a antibody was purchased from santacruz biotechnology (Dallas, Texas). The anti-CD31 antibody was purchased from Abcam (Cambridge, MA).

2.3. Rat ischemic model and groups

Male Wistar rat (12–14 weeks old, average weight: 316.7 ± 10.5) were purchased and from Changsha Dongchuang Experimental Animal Branch (Hunan, China) and kept in the animal facility of the Hunan normal University. All animal procedures were performed according to approved protocols (SCXK2009-0012) and in accordance with recommendations for the proper use and care of laboratory animals.

The ischemic heart rat model was established utilizing the ligation of left anterior descending (LAD) coronary artery. Briefly, animal were anesthetized with a cocktail of ketamine and xylazine. Animals were intubated and positive pressure ventilation was provided by a ventilator. Tidal volume (30 ml/kg) and rate (70/min) were determined by animal weight. A 2.5 cm transverse incision was made between the third and fourth intercostals spaces. The LAD coronary artery was ligated between the pulmonary cone and the left auricle using 7–0 silk and the ligation depth is 1~2 mm, the center of ligation is great cardiac vein. Both LAD coronary artery and great cardiac vein were ligated in such model.

48 rats were divided into 4 groups (12/group). Group 1: The ligated rat receiving rAAV9-MHC-TnT-HIF1a with tail vein injection (AAV9-HIF1a group); Group 2: The ligated rat receiving rAAV9-MHC-TnT-GFP with tail vein injection (AAV9-GFP control); Group 3: The ligated rat without treatment (Model control); Group 4: the rats without ligated were used as a sham operation group.

2.4. Evaluation of cardiac function

The cardiac function was evaluated at 2 weeks (6/group) and 4 weeks (6/group) post LAD ligation using a TM_WAVE system (Taimeng, Chengdu). Each rat was anesthetized with 1.5% isoflurane and placed on a heating table in a supine position, and its extremities were fixed to 4 electrocardiography leads on the table. The chest was shaved and cleaned. Heart apex was exposed. The probe was left in left ventricle, and the left ventricular pressure (LVP) was recorded. Four parameter was recorded and analyzed: left ventricular systolic pressure (LVSP); left ventricular end diastolic pressure (LVED); +dp/dtmax is the maximal rate of rise of left ventricular pressure and −dp/dtmax is the maximal rate of decrease of left ventricular pressure.

2.5. Preparation of heart sections

After cardiac function evaluation, the heart was excised under deep anesthesia with inhalation of 5% isoflurane. In some cases described below, 0.1 ml saturated KCl was injected into the LV chamber before the hearts were harvested to arrest them in diastole. The left and right atria and large vessels were reseted, the heart was washed with saline, embedded in O.C.T. compound (Sakura Finetechnical USA, Inc., Torrance, CA), and frozen in a bath of 2-methylbutane with dry ice. The hearts were stored at −80 °C and sliced transversely from the apex to the basal part of the LV with the use of a cryostat at 6 μm thickness with the interval of 300 μm between each section. All sections were mounted on glass slides and stained with Masson trichrome stain for quantitative analysis of infarct size.

2.6. Measurement of myocardial infarct size

The Evans blue staining was used to determine the areas of infarction. Following occlusion of a coronary artery, the perfused myocardium may be stained by injecting a dye into the circulation of the animal, either *in vivo* or postmortem, through retrograde perfusion after cannulation of the aorta. The normal area will be left