Original Paper

Effects of Triple-Mutated Hypoxia-Inducible Factor-1 α on Angiogenesis and Cardiac Function Improvement in Rats with Myocardial Infarction

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Key Words

Hypoxia-inducible factor-1 α • Angiogenesis • Cardiac function

Abstract

Background/Aims: The effects of hypoxia-inducible factor-1 α (HIF-1 α) on angiogenesis and cardiac function improvement in rats with myocardial infarction (MI) is unknown and our current study was to evaluate whether HIF-1 α would be beneficial for angiogenesis and cardiac function improvement in MI rats. **Methods:** A mutant of adenovirus HIF-1 α (Ad-HIF-1 α -Trip) was constructed by three sites mutation (Pro402, Pro564 and Asn803) in HIF-1 α . The rat MI model was produced by permanent ligation of left anterior descending artery and 1×10^9 PFU adenovirus (Ad) vector particles of Ad-Null, Ad-HIF-1 α -564/402, Ad-HIF-1 α -Trip, 250ng vascular endothelial growth factor (VEGF) in 0.5ml saline or only 0.5 ml saline were injected intramuscularly around the infarct border zone. Real-time PCR, ELISA and western blotting were used to evaluate angiogenesis factors expression. Capillary density and necrotic areas were detected by immunohistochemistry staining and TTC staining, respectively. Cardiac function assessment was done by echocardiography before operation and on day 7, 14 and 28 after MI. Blood samples were drawn for the measurement of cardiac biomarkers, liver function and kidney function. **Results:** On day 7, compared to the other groups, the expressions of HIF-1 α and angiogenesis factors, and the capillary density were all significantly higher in the Ad-HIF-1 α -Trip group. However, on day 28, no significant between-group differences were observed. After 72 hours of MI, serum level of cardiac biomarkers and the necrotic areas were significantly lower in the Ad-HIF-1 α -Trip group compared to the other groups. Echocardiography showed that on day 7, cardiac functions were significantly reduced in all groups compared to the baseline. Cardiac function in the Ad-HIF-1 α -Trip group was decreased less profoundly through day 7 to day 28 compared to the other groups. Importantly, no significant differences in liver and renal function were observed. **Conclusion:** Mutation of

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Pro402, Pro564 and Asn803 are beneficial for enhancement of the efficacy of HIF-1 α . Ad-HIF-1 α -Trip is able to improve angiogenesis and cardiac function, which may be a promising avenue for treatment of ischemic heart disease in the future.

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Introduction

Endothelial dysfunction is significantly associated with atherosclerosis and ischemic heart diseases [1]. In the past decades, experimental studies have showed that administration of vascular endothelium growth factor (VEGF) and/or fibroblast growth factor (FGF) is beneficial for improvement of endothelial function and angiogenesis in the ischemic tissues, thereby reducing tissue necrosis [2-5], which represents a promising approach for ischemic heart disease. Nevertheless, in the preliminary clinical researches, no prominent improvement of tissue perfusion was observed after administration of VEGF and FGF. The underlying mechanisms were speculated due to tissue edema and inflammation [6-8]. Compared to VEGF and FGF, hypoxia inducible factor-1 (HIF-1) might be more powerful to stimulate angiogenesis because vessel formation requires different cell type's interplay and interaction [9, 10].

HIF-1 has been demonstrated to be a key regulator for gene expression in response to hypoxia and ischemia [11-13]. As a transcription factor, HIF-1 comprises 2 basic proteins namely HIF-1 α and HIF-1 β [14]. Notably, HIF-1 α regulates numerous genes expressions, including those encoding angiogenesis cytokines such as VEGF, platelet growth factor (PLGF), angiopoietin 1 and 2 and stromal-derived factor [15-18]. However, HIF-1 α is extremely unstable and its expression level is very low during normal oxygen stress [19, 20]. The oxygen sensitive signal is generated by a series of iron and 2-oxoglutarate dependent dioxygenase, which catalyze post-translational hydroxylation of specific prolyl (Pro402 and Pro564) and asparaginyl residues (Asn803) in HIF-1 α subunit. Therefore, theoretically, mutation of Pro402, Pro564 and Asn803 in HIF-1 α may enhance its stability and transcriptional activity.

Prior studies have showed that transgenic expression of a constitutively stable HIF-1 α mutant, namely Ad-HIF-1 α -Trip, not only resulted in increased expression of angiogenesis cytokines *in vitro* but also increased tissue perfusion in acute ischemic limb of rabbits [21]. Although Ad-HIF-1 α -Trip improved the prognosis of acute ischemic limb, it is unclear whether it would also promote tissue perfusion and protect cardiac function from ischemic insult.

We thus produced rat myocardial infarction (MI) model via permanent ligation of left anterior descending artery (LAD) [22-24] and our study showed that Ad-HIF-1 α -Trip treatment significantly increased the expressions of angiogenesis cytokines including VEGF, FLGF and PDGF. Furthermore, a prominent improvement of angiogenesis, reduction of infarct size and improvement of cardiac function were also observed.

Materials and Methods

Ethics

The present study was approved by the Ethics Review Board of Guangzhou Medical University. All the procedures were performed according to the Institutional Guidelines for Animal Research and the investigators conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Construction of the HIF-1 α mutants

More than one mutant model (Ad-HIF-1 α -564, Ad-HIF-1 α -402 and Ad-HIF-1 α -564/402, Ad-HIF-1 α -Trip) has been successfully tested in previous studies [21, 25-27]. In addition, in our pilot studies, we compared the stability and effects on some downstream angiogenesis genes among different mutants and we found that Ad-HIF-1 α -Trip was the best which was used in our current study.

Construction of recombinant adenovirus (Ad) vectors was based on prior reports with mild modification [21, 25-27] as follows: Human wild-type HIF-1 α cDNA was cloned into plasmid pcDNA3.1 (+) and then cloned into multiple cloning sites (MCS) between human cytomegalovirus promoter and SV40 polyadenylation sequences of plasmid pShuttle2 (BD, Clontech) namely pShuttle2-HIF-1 α -native. Then the codons of Pro402 (CCA), Pro564 (CCC) and Asn803 (AAT) in pShuttle2-HIF-1 α -native were mutated to the codons of alanine (GCA, GCC and GCT, respectively), namely pShuttle2-HIF-1 α -Trip. The expression of cassette of HIF-1 α -Trip was ligated with Adeno-X Viral DNA (BD, Clontech) so as to construct the recombinant adenoviral plasmids pAdeno-HIF-1 α -Trip. The linear Pac I-digested pAdeno-HIF-1 α -Trip and lipofectamine2000 (Invitrogen) were co-transfected into the low passage HEK293 cells (ATCC) to generate recombinant adenoviruses, namely Ad-HIF-1 α -Trip. Ad-HIF-1 α -564/402 and Ad-Null (the empty adenovirus vector) were constructed in a similar way.

MI model production and experimental protocol

Sprague-Dawley (SD) rats weighing 250-300 g were randomly divided into five groups. Each rat was anaesthetized with 10 % chloral hydrate solution by intra-peritoneal injection (0.3 ml/100 g) and then underwent oral intubation with rodent ventilator connection. Disappearance of righting reflex or pedal withdrawal reflex indicated sufficient anesthesia [27]. Left thoracotomy was performed and LAD was ligated as previously described [28]. Anterior ST segment elevated after successful ligation of LAD. Five minutes later, myocardial tissue around ligation area turned to be pale, indicating this area was the pre-infarcted area. 1×10^9 Ad vector particles of Ad-Null, Ad-HIF-1 α -564/402, Ad-HIF-1 α -Trip, 250 ng VEGF (R&D system, America) in 0.5 ml saline, or only 0.5 ml saline were injected intramuscularly at five areas around the pre-infarct area with a Hamilton syringe.

Serial transthoracic echocardiography was performed to evaluate cardiac function using echocardiographic system (Vevo2100, Visual Sonics, Canada) as previously described [28, 29]. Transthoracic echocardiography was performed on day 0 before operation, and on day 7, 14 and 28 after LAD ligation (n = 10 per group). Blood samples (n = 3 per group) were drawn before operation and at different time points (24h, 48h, 72h and 28d) after operation. RNA and protein expressions of HIF-1 α and angiogenesis factors including VEGF, PDGF and PLGF were assessed using real-time PCR, ELISA and western blotting. The capillary density was determined by immunohistochemistry, and the area of myocardial infarction was measured using 2, 3,5-triphenyltetrazolium chloride (TTC) staining on day 28 after LAD ligation.

Real-time PCR

Total RNA was extracted from homogenized ischemic myocardium using Trizol (Invitrogen, USA). The mRNA levels of specific genes at different time points were measured by ABI Prism 7500 Sequence Detection System (Applied Biosystems, Inc, USA) and normalized to ACTB mRNA. Primers were designed according to ABI-Perkin Elmer guidelines. Sequences of PCR primer are shown as follows:

R-HIF-1 α -F: AGCAATTCTCCAAGCCCTCC,
R-HIF-1 α -R: TTCATCAGTGGTGGCAGTTG,
R-VEGF-F: GGGAGCAGAAAGCCCATGAA,
R-VEGF-R: AGATGTCCACCAGGTCTCA,
R-PDGF-F: CCGCTCCTTTGATGACCTTC,
R-PDGF-R: GCTCAGCCCCATCTTCGTC,
R-PLGF-F: GACTTCTGCTCACCACGAG,
R-PLGF-R: CCCGGTGAGTTGGAGAGATG.

ELISA

7 days after gene transfection, the concentrations of HIF-1 α , VEGF, PDGF and PLGF in ischemic myocardia were determined using the corresponding Immunoassay Kit (R&D systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Western blotting

Immunoblotting for HIF-1 α , VEGF, PDGF and PLGF was performed on day 7 after gene transfection. The immune-reactive band of different kDa was visualized in all protein preparations with an enzyme-linked chemiluminescence detection system (Amersham Pharmacia Biotech) and a mouse monoclonal anti-human

HIF-1 α antibody (1:1000; R&D), rabbit anti-VEGF antibody (1:2000, Abcam, Uk), rabbit anti-PDGF antibody (1:1000, Santacruz, USA) or rabbit anti-PLGF antibody (1:1000, Abcam, Uk) which shows cross-reaction with the above angiogenic genes respectively and normalized to anti-GAPDH antibody (1:10000, Abcam, UK).

Immunohistochemistry staining

Transverse tissue specimens obtained from the ischemic myocardium were immersion-fixed in formalin and embedded in paraffin. Each of the sections was cut at a thickness of 5 μ m with the muscle fibers oriented transversely from paraffin-embedded sections of the muscle. Specimens were stained with CD31 antibodies (sc-1506, Santa Cruz biotechnology) to assess the capillary density. The slides were examined under microscope. The number of capillary was determined by vessels positive staining for CD31 and counted in five randomly selected fields from one muscle section. The number of capillaries/mm² represents the capillary density. The investigator was blinded to the animals' treatment.

Cardiac function assessment by echocardiography

Cardiac function was quantified by echocardiography. Transthoracic echocardiography was performed when rats were anesthetized using intra-peritoneal injection of 10 % chloral hydrate solution (0.3ml/100g) with an echocardiographic system equipped with a 10-MHz linear-array transducer as previously reported [28, 29]. The values of cardiac output (CO), left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) were measured and calculated.

TTC staining

On day 28 after LAD ligation, the rats were killed using overdose sodium pentobarbital and the hearts were resected immediately. The ventricles were frozen, sliced into 4-mm-thick blocks and stained using a standard TTC staining method [30].

Blood parameter examination

Blood samples were drawn from the heart of rats using overdose anesthesia (10 % chloral hydrate solution by intra-peritoneal injection, 0.4 ml/100 g) on different time points. An automatic detector measured cardiac biomarkers (creatine kinase-Mb [CK-MB]) and cardiac troponin-I [cTNI]), liver function panels (aspartate transaminase [AST] and alanine transaminase [ALT]) and kidney function panels (blood urea nitrogen [BUN] and creatinine [Cr]).

Statistical analysis

Continuous variables were presented as mean and standard deviation (SD) and comparisons between these groups were conducted using one-way ANOVA using SPSS 18.0 software (SPSS, Chicago, IL, USA), and then was analyzed by Least-Significant Difference (LSD) post hoc comparison test. Statistical significance was considered as two-sided P value < 0.05.

Results

Effects of mutant on HIF-1 α and angiogenesis factors expression at different time points in ischemic myocardium

As shown in Fig. 1A, in ischemic myocardium, Ad-Null had no obvious effect on the expressions of HIF-1 α , VEGF, PLGF and PDGF at mRNA level on day 7, 14, 21 and 28 after LAD ligation, which was comparable to the saline group. However, on day 7, in the Ad-HIF-1 α -Trip group, the expressions of HIF-1 α and the other angiogenesis factors at mRNA level were significantly higher compared to the other groups. The expressions of VEGF induced by Ad-HIF-1 α -Trip increased 1.22-, 2.36- and 2.40-fold compared to the Ad-HIF-1 α -564/402, Ad-Null and saline groups, respectively. No difference in VEGF expression was observed between the Ad-HIF-1 α -Trip and VEGF groups. While the expressions of these angiogenesis factors were all markedly decreased on day14 and 21. On day 28, the expressions of these angiogenesis factors were comparable between all groups. Notably, VEGF injection had no

Fig. 1. Effects of HIF-1 α mutation on HIF-1 α , VEGF, PLGF and PDGF expressions. The ischemic heart of rat was injected with Saline, Ad-Null, VEGF, Ad-HIF-1 α -564/402 or Ad-HIF-1 α -Trip. The mRNA levels of HIF-1 α , VEGF, PLGF and PDGF at different time points were measured by ABI Prism 7300 Sequence Detection System (Applied Biosystems, Inc., USA) and normalized to ACTB RNA. (mean $\pm$ SD, n = 3). *P<0.05 vs Saline or Ad-Null; #P<0.05 vs VEGF; &P<0.05 vs Ad-HIF-1 α -564/402 (Fig. 1A). The protein levels of HIF-1 α , VEGF, PLGF and PDGF at day 7 after gene transfection were measured by ELISA and Western blotting (Fig. 1B and Fig. 1C).

Fig. 2. Effects of HIF-1 α mutation on angiogenesis in rat with MI at different time points after gene transfection. CD31 staining of ischemic heart tissues showed that treated with Ad-HIF-1 α -Trip had greatest vessel density compared to the groups on day 7 after gene transfection. Brown staining-stained dots indicated vessel. (mean $\pm$ SD, n = 5) *P<0.05 vs Saline or Ad-Null; #P<0.05 vs VEGF or Ad-HIF-1 α -564/402 (Bar = 100 μ m).

Fig. 3. Effects of HIF-1 α mutant on cardiac function in rat with MI at different time points after gene transfection. Cardiac function of rat with MI treated with Ad-HIF-1 α -Trip recovered more quickly than the other groups at three different time points after gene transfection. (mean $\pm$ SD, n = 10) *P<0.05 vs Saline or Ad-Null; #P<0.05 vs VEGF or Ad-HIF-1 α -564/402.

Fig. 4. Effects of HIF-1 α mutation on plasma concentration of CK-MB and cTnI of rat with MI at different time points after gene transfection. After gene transfection at 24h, 48h, 72h and on 28 day after gene transfection, plasma concentration of CK-MB and cTnI among the five groups were in the order as follows: Ad-HIF-1 α -Trip < VEGF/ Ad-HIF-1 α -564/402 < Ad-Null/Saline. The value of CK-Mb and cTnI concentration decreased over time in all groups. (mean $\pm$ SD, n = 5) *P<0.05 vs Saline or Ad-Null; # P<0.05 vs VEGF or Ad-HIF-1 α -564/402.

Fig. 5. Effects of HIF-1 α mutation on infarct size. On day 28, cardiac infarct size of each group was displayed (Fig. 5A). Results of TTC showed that Ad-HIF-1 α -Trip decreased cardiac infarct size more significantly than other groups (Fig. 5B) (mean $\pm$ SD, n = 5) *P<0.05 vs Saline or Ad-Null; #P<0.05 vs VEGF or Ad-HIF-1 α -564/402.

effects on PLGF and PDGF expressions. Ad-HIF-1 α -564/402 increased the expressions of VEGF, PLGF and PDGF at mRNA level, which was less than that in Ad-HIF-1 α -Trip group.

Ad-HIF-1 α -Trip increased protein expression of HIF-1 α , VEGF, PLGF and PDGF as measured by western blotting than the other groups 7 days after gene delivery. Expression of VEGF increased by Ad-HIF-1 α -Trip was comparable to the VEGF group (Fig. 1B, 1C).

Effects of HIF-1 α mutant on angiogenesis in ischemic heart

Immunohistochemistry staining for CD31 on day 7, 14, 21 and 28 after LAD ligation was used to estimate the capillary density. On day 7, Ad-HIF-1 α -Trip increased capillary densities

Table 1. Liver and renal function panels. Unit: ALT(U/L); AST (U/L); BUN (mmol/L); Cr (μ mol/L)

Parameter	Saline	Ad-Null	VEGF	Ad-HIF-1 α -564/402	Ad-HIF-1 α -Trip	Saline	Ad-Null	VEGF	Ad-HIF-1 α -564/402	Ad-HIF-1 α -Trip
			Baseline					14d		
AST	29.2 $\pm$ 6.2	34.0 $\pm$ 4.4	28.1 $\pm$ 5.3	31.6 $\pm$ 4.7	28.8 $\pm$ 3.7	35.9 $\pm$ 6.6	41.2 $\pm$ 7.1	39.5 $\pm$ 7.2	40.6 $\pm$ 3.1	38.5 $\pm$ 5.3
ALT	30.0 $\pm$ 2.3	28.7 $\pm$ 4.8	32.2 $\pm$ 5.9	26.6 $\pm$ 3.8	31.2 $\pm$ 4.2	30.2 $\pm$ 7.8	27.5 $\pm$ 5.3	31.1 $\pm$ 6.5	29.4 $\pm$ 5.7	32.2 $\pm$ 6.1
BUN	5.4 $\pm$ 0.5	5.2 $\pm$ 0.7	4.9 $\pm$ 0.6	5.1 $\pm$ 0.4	5.3 $\pm$ 0.5	5.2 $\pm$ 0.4	4.7 $\pm$ 0.6	4.8 $\pm$ 0.7	5.2 $\pm$ 0.5	5.1 $\pm$ 0.4
Cr	68.5 $\pm$ 7.4	63.0 $\pm$ 4.7	66.1 $\pm$ 5.5	59.9 $\pm$ 6.3	64.3 $\pm$ 3.8	63.1 $\pm$ 4.9	60.5 $\pm$ 5.7	63.4 $\pm$ 7.0	61.2 $\pm$ 6.5	65.5 $\pm$ 5.9
Parameter	Saline	Ad-Null	VEGF	Ad-HIF-1 α -564/402	Ad-HIF-1 α -Trip	Saline	Ad-Null	VEGF	Ad-HIF-1 α -564/402	Ad-HIF-1 α -Trip
			28d					14d		
AST	31.2 $\pm$ 6.3	29.0 $\pm$ 3.8	27.1 $\pm$ 2.7	28.8 $\pm$ 3.5	28.1 $\pm$ 3.5					
ALT	29.5 $\pm$ 2.7	27.8 $\pm$ 3.5	30.6 $\pm$ 4.6	28.6 $\pm$ 4.2	31.4 $\pm$ 4.0					
BUN	5.0 $\pm$ 0.7	5.2 $\pm$ 0.3	4.9 $\pm$ 0.5	5.4 $\pm$ 0.6	5.3 $\pm$ 0.4					
Cr	64.3 $\pm$ 4.8	61.2 $\pm$ 3.9	59.7 $\pm$ 4.8	60.7 $\pm$ 5.8	62.3 $\pm$ 2.2					

more prominently compared to the other groups (Fig. 2). Numbers of capillaries per mm² was 33.00 in the saline group, 35.42 in the Ad-Null group, 92.92 in the VEGF group, 91.50 in the Ad-HIF-1 α -564/402 group and 142 in the Ad-HIF-1 α -Trip group ($P < 0.05$). Of note, capillary density on day 7 reached the peak level and decreased on day 14. On day 28, no significant differences in capillary density were observed.

Effects of HIF-1 α mutant on cardiac function

Echocardiography was performed to determine the effect of Ad-HIF-1 α -Trip on cardiac function. On day 0, CO, LVEF and LVFS of the five groups were comparable. On day 7, cardiac functions were significantly reduced in all groups compared to the baseline. Cardiac function in the Ad-HIF-1 α -Trip group was decreased less significantly through day 7 to day 28 compared to the other groups, indicating that Ad-HIF-1 α -Trip was beneficial for improvement of cardiac function (Fig. 3).

Effects of HIF-1 α mutant on myocardial damages

Plasma concentrations of CK-MB and cTnI were comparable among the five groups before LAD ligation. After 24 hour of LAD ligation, plasma concentrations of CK-MB and cTnI were increased significantly in the five groups and then declined gradually which reached to the baseline level on day 28. Notably, at 24h, 48h and 72h after LAD ligation, the plasma concentrations of CK-MB and cTnI were in the order as follows: Ad-HIF-1 α -Trip < VEGF/ Ad-HIF-1 α -564/402 < Ad-Null/Saline (Fig. 4). The results of TTC staining on day 28 after LAD ligation showed that Ad-HIF-1 α -Trip significantly reduced myocardial infarction compared to the other groups. The standardized infarct size in the Ad-HIF-1 α -Trip, VEGF, Ad-HIF-1 α -564/402, Ad-Null and saline groups were 11.69 %, 15.43 %, 15.50 %, 24.09 %, and 23.73 % of the total left ventricle, respectively (Fig. 5).

Toxicity of HIF-1 α mutant on liver and kidney

Serum liver and renal function panels were all within normal range at baseline. No significantly differences in liver and renal function panels were observed among the five groups on day14 and 28 after MI (Table 1). Furthermore, no serious pathological abnormalities or evidence of malignant tumours were detected.

Discussion

To the best of our knowledge, this should be one of the first few studies to compare angiogenesis and cardiac function in MI rats with HIF-1 α mutant treatment. Prior studies have suggested that VEGF might play an important role in ischemic diseases. However, results from other studies were conflicting [31-35], which requires further investigation. Our current study evaluated the effects of Ad-HIF-1 α -Trip, which could enhance VEGF, PLGF and PDGF expression *in vitro* [21], on angiogenesis and cardiac function of rat with MI. Our results showed that Ad-HIF-1 α -Trip administration increased the expression of

angiogenesis-related genes and capillary density and cardiac function improvement was also observed.

Angiogenesis is a complicated process consisting of endothelial cell proliferation, directional migration, extracellular matrix remodelling, and vessel maturation [36]. Previous study has showed that VEGF was incapable to achieve sufficient and durable neovascularisation [37]. HIF-1 is an essential regulator for angiogenesis gene expression especially in ischemic conditioning. HIF-1 consists of O₂-regulated HIF-1 α and constitutively-expressed HIF-1 β subunits [38]. The oxygen sensitive signal is generated by a series of iron and 2-oxoglutarate dependent dioxygenase that catalyze post-translational hydroxylation of specific prolyl and asparaginyl residues in HIF-1 α subunit, which was inactivated in the presence of oxygen. While in hypoxic condition, these processes are suppressed which allow HIF-1 to activate massive transcriptional cascade [39]. Under normoxic condition, rapid degradation and inactivation of transcription hampers the application of HIF-1 α for angiogenesis. In specific, hydroxylation of proline residues 402 and/or 564 by prolyl 4-hydroxylases usually leads to undetectable level in the cell nuclei [40]. Hydroxylation of the asparagine residue Asn803 results in lower transcriptional activity of HIF-1 α in normoxia condition. According to the oxygen-dependent regulation mechanisms, mutation of Pro402, Pro564 and Asn803 in HIF-1 α could be useful to stabilize HIF-1 α and increase its transcriptional activity [21]. Vincent et al. reported that increased transcriptional activity of HIF-1 α was achieved by trans-activation domain from herpes simplex virus VP16 [41, 42]. However, this approach may affect genes expression. Therefore, we adopted the method of gene mutant to achieve optimization with the greatest degree of HIF-1 α retention [21].

Preclinical data have indicated that VEGF was beneficial for improvement of blood flow and cardiac function in animal MI models [43, 44]. The actions of VEGF were mediated partially by activating PI3K/Akt pathway, which in turn leads to vascular permeability, endothelial cell proliferation and vascular tubular structures formation [45]. However, tissue edema due to hypo-perfusion and arteriolar vasodilation resulted in disappointed outcome, which was speculated due to insufficient production of downstream angiogenesis factors [46]. We compared the expression of downstream angiogenesis factors in ischemic myocardium tissues. Our results indicated that Ad-HIF-1 α -Trip was the optimal mutant in terms of possessing full constitutive activity and effective transcriptional activity. The expression of HIF-1 α , VEGF, PLGF, and PDGF increased most by Ad-HIF-1 α -Trip on day 7 after gene delivery, which was consistent to prior reports [21]. Of note, through day 7 to day 28, the expressions of these angiogenesis genes at mRNA level were blunted, which was also consistent to previous reports [10, 47]. Similar results were also observed for protein levels of HIF-1 α , VEGF, PLGF and PDGF in ischemic myocardial tissues on day 7 after gene delivery.

One of the principal functions of the cardiovascular system is to deliver oxygen to other tissues and organs [39]. HIF-1 α is widely expressed in the developing heart tube [48, 49]. Interestingly, Yamashita T et al. reported that multiple HIF-1 α isoforms with distinct cellular patterns were expressed in the developing heart tissue [50]. One of our key findings was that the reduced myocardial damage and improved cardiac function were more prominently in the Ad-HIF-1 α -Trip group compared to other groups, and these benefits sustained 28 days. Immunohistochemistry examination was chosen to assess angiogenesis. On day 7, higher capillary density was observed in the Ad-HIF-1 α -Trip group compared to the other groups. Echocardiography also showed cardiac function was better with Ad-HIF-1 α -Trip administration, suggesting that angiogenesis might play essential roles in improving cardiac function.

In our current study, intramuscular injection was used to ensure that the transfected gene and its downstream genes expressed maximally in the local ischemic tissues. No adverse effects occurred in the liver and kidney. Gross pathological abnormalities, tumors or angioma formation was also not observed in heart and other organs, indicating that Ad-HIF-1 α -Trip administration was a safe strategy.

Conclusion

Our present study shows that mutation of Pro402, Pro564 and Asn803 are beneficial for enhancing the efficacy of HIF-1 α administration. Ad-HIF-1 α -Trip is capable to improve angiogenesis and cardiac function, which may be a promising avenue for ischemic heart disease treatment in the future.

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Disclosure Statement

The authors declare that they have no conflicts of interest.

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