J Appl Biomed
DOI: 10.32725/jab.2023.005
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

hKLK alleviates myocardial fibrosis in mice with viral myocarditis

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Abstract
Myocardial fibrosis is the most serious complication of viral myocarditis (VMC). This study aimed to investigate the therapeutic benefits and underlying mechanisms of lentivirus-mediated human tissue kallikrein gene transfer in myocardial fibrosis in VMC mice. We established VMC mouse model via intraperitoneal injection with Coxsackie B3 virus. The effect was then assessed after treatment with vehicle, the empty lentiviral vectors (EZ.null), and the vectors expressing hKLK1 (EZ.hKLK1) via tail vein injection for 30 days, respectively. The results showed that administering EZ.hKLK1 successfully induced hKLK1 overexpression in mouse heart. Compared with EZ.null treatment, EZ.hKLK1 administration significantly reduced the heart/weight ratio, improved cardiac function, and ameliorated myocardial inflammation in VMC mice, suggesting that hKLK1 overexpression alleviates VMC in mice. EZ.hKLK1 administration also significantly abrogated the increased myocardial collagen content, type I/III collagen ratio, TGF-β1 mRNA and protein expression in VMC mice, suggesting that hKLK1 overexpression reduces collagen accumulation and blunts TGF-β1 signaling in the hearts of VMC mice. In conclusion, our results suggest that hKLK1 alleviates myocardial fibrosis in VMC mice, possibly by downregulating TGF-β1 expression.

Keywords: Human tissue kallikrein; Myocardial fibrosis; Myocardial inflammation; Transforming growth factor β1; Viral myocarditis

Highlights:
- The lentivirus-mediated hKLK1 gene transfer ameliorated myocardial fibrosis in VMC mice.
- hKLK1 alleviated myocardial fibrosis in VMC mice, possibly by downregulating TGF-β1 expression.
- This work showed lentivirus-mediated hKLK1 gene transfer to be a promising therapeutic approach for VMC therapy.

Introduction

Viral myocarditis (VMC) is an inflammatory process of the myocardium resulting from infections with viruses, such as coxsackievirus B3 (CVB3) (Kang and An, 2020). With the progression of VMC, different degrees of myocardial fibrosis may be present at all stages of the disease. This represents the main pathologic basis for multiple complications of VMC (Li et al., 2019), leading to dilated cardiomyopathy with a two-year mortality of 50% (Sagar et al., 2012). Suppressing myocardial fibrosis is important for maintaining the cardiac structure and improving cardiac function in the treatment of VMC.

The mammalian myocardium comprises cardiomyocytes and a large amount of interstitial noncardiomyocytes enmeshed within the extracellular matrix (ECM) network. Myocardial fibrosis is characterized by an excessive accumulation of collagen (mainly type I), the major component of myocardial ECM, in the myocardium, leading to increased ventricular stiffness and impaired diastolic filling (Pinto et al., 2016). The degradation and synthesis of the ECM components are controlled by the balance between matrix metalloproteinases

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http://doi.org/10.32725/jab.2023.005
Submitted: 2022-04-21 • Accepted: 2023-03-24 • Prepublished online: 2023-04-03
J Appl Biomed 21/1: 15–22 • EISSN 1214-0287 • ISSN 1214-021X

(MMPs) and their tissue inhibitors (TIMPs). In VMC progression, inflammatory stimuli, such as interleukins, transforming growth factor (TGF)-β, and tumor necrosis factor α, induce the imbalance between MMPs and TIMPs, leading to an excessive accumulation of collagen and collagen-producing fibroblasts and finally cardiac fibrosis (Frangogiannis, 2017). To date, TGF-β is the most well-known profibrotic cytokine during cardiac fibrosis. TGF-β1-overexpressing mice could exhibit significant interstitial fibrosis (Rosenkranz et al., 2002). Conversely, TGF-β1-deficient mice demonstrated decreased age-associated myocardial fibrosis and improved cardiac compliance (Brooks and Conrad, 2000). Importantly, TGF-β receptor antagonist attenuated myocardial fibrosis in mice (Sakata et al., 2008). Administration of anti-TGF-β antibody could prevent collagen accumulation and diastolic dysfunction induced by pressure overload (Kuwahara et al., 2002). Thus, targeting TGF-β signaling is reasonable and promising to prevent and alleviate myocardial fibrosis in the treatment of VMC.

Human tissue kallikrein (hKLK1) belongs to the serine protein kinase family, serving as an active component of the kinin-kallikrein system. hKLK1 mainly exists in the pancreas, kidney, and heart. It was studied that the overexpression of hKLKl could inhibit the extracellular matrix remodeling in diabetic cardiomyopathy (Tschöpe et al., 2004). It was reported that hKLK1 overexpression could reduce cardiac fibrosis and improve aging related cardia dysfunction (Xu et al., 2018). It was also reported that hKLK1 inhibited myocardial apoptosis and mitigated myocardial fibrosis after ischemic injury (Yao et al., 2007; Yin et al., 2008). However, the effects of hKLK1 on myocardial fibrosis and TGF-β signaling in VMC remain unknown.

In this study, we investigated the therapeutic effects of lentivirus-mediated hKLK1 overexpression on VMC in mice. We also examined the effects of hKLK1 overexpression on myocardial fibrosis by measuring the collagen content, type I/III collagen ratio, and TGF-β1 expression in the heart tissue samples of mice. Our results suggest that lentivirus-mediated hKLK1 overexpression is a promising therapeutic approach for the prevention and treatment of myocardial fibrosis in VMC.

Materials and methods

Animals

All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Twenty-four 4-week-old BALb/c male mice weighing 17.0 ± 0.74 g were purchased from Beijing Weitonglihua Laboratory Animal Technology [Beijing, China, Laboratory Animal License No. SCXK (Beijing) 2016-0006].

VMC modeling and treatment

The lentiviral pEZ-Lv105 vectors overexpressing hKLK1 (EZ.hKLK1) were packaged and synthesized by Guangzhou Funeng Gene (Guangzhou, China). The empty pEZ-Lv105 vectors (EZ.null) were used as a negative control. The mice were randomly divided into normal control, VMC + vehicle, VMC + EZ.null, and VMC + EZ.hKLK1 groups (n = 6/group). VMC was induced on day 0 in the VMC + vehicle, VMC + EZ.null, and VMC + EZ.hKLK1 groups by intraperitoneal injection with 0.2 ml CVB3 virus solution (10^9 $TCID_{50}$/ml; Guangzhou Institute of Respiratory Health, Guangzhou, China). The normal control group was intraperitoneally injected with 0.2 ml MEM without virus. One mouse in the VMC + vehicle group died on day 0 after virus injection and was replaced with another mouse with the same condition. On day 1 after virus injection, the VMC + EZ.null and VMC + EZ.hKLK1 groups were administered 125 μl EZ.null (25 μl 1 × 10^{13} IU/ml EZ.null + 100 μl PBS) or EZ.hKLK1 (25 μl 1 × 10^{13} IU/ml EZ.hKLK1 + 100 μl PBS) solution via tail vein injection. The normal control and VMC + vehicle groups were administered 125 μl normal saline.

The mice were sacrificed on day 30 by cervical dislocation. Simultaneously, when the weight of a mouse dropped to 10 g, or it didn't eat for more than 5 days, we would kill it to relieve its pain or distress. The heart of each mouse was removed and divided into three parts along the left ventricular long axis. One part of the heart tissue was fixed with 4% polyformaldehyde for pathological examination; the other two parts were stored at –80 °C for collagen content measurement and Western blot analysis.

Cardiac function examination by ultrasound

On day 30, mice were anesthetized with 3% chloral hydrate (0.3 g/kg) by intraperitoneal injection, followed by removal of mouse hair with 8% sodium sulphide. The left ventricular end-diastolic dimension (LVEDd), left ventricular end-systolic dimension (LVEDs), and left ventricular fractional shortening (FS) and ejection fraction (EF) were measured by an echocardiologist, using the system of echocardiographic imaging (Version: cSound) and I13L small animal ultrasound probe (14 mHz; Version: Vidid7) from General Electric (Little Chalfont, UK).

Hematoxylin and eosin (H&E) staining

The myocardial tissue samples were fixed in 4% paraformaldehyde, dehydrated in a graded ethanol series, paraffin-embedded, and sectioned continuously (5-μm thick). The sections were deparaffinized with xylene and subjected to H&E staining. The pathological manifestations, such as inflammatory cell infiltration, necrosis, interstitial hyperemia, and edema of myocardial cells, were assessed using a polarized light microscope (BX53-p; Olympus, Tokyo, Japan). The histological changes were scored as follows: 0, no lesion; 1, lesion area < 25%; 2, 25–50%; 3, 50–75%; 4, >75%. The final score for each sample was expressed as the average score observed in five randomly selected fields in each section (Zhou et al., 2022).

UV spectrophotometry for collagen content measurement

The myocardial tissue samples were ground on ice, dried, and digested with hydrochloric acid. The standard curve was generated using hydroxyproline with analytical purity. The hydroxyproline content was measured at 550 nm using an UV spectrophotometer (LambdaBio20; PerkinElmer, Waltham, MA, USA). The collagen content in the myocardium was calculated as follows: myocardial collagen content = hydroxyproline content × 7.46 (mg/g) (Zhang et al., 2016).

Picrosirius red staining

The paraffin-embedded myocardial tissue sections (5-μm-thick) were stained with 0.1% picrosirius red solution (Wuhan Servicebio Technology, Wuhan, China), following the manufacturer's instructions. Type I collagen (yellow-red) and type III collagen (yellow-green) were observed using an inverted fluorescence microscope (Eclipse TI-SR; Nikon, Tokyo, Japan) at magnification ×200. The type I/III collagen ratios were calculated in randomly selected five visual fields using the Image Pro Plus Software 6.0 (Media Cybernetics, Silver Spring, MD, USA) (Miao et al., 2020).

Real-time quantitative PCR (qPCR)

Total RNA was isolated from the heart tissue samples using TRIzol Reagent (Invitrogen, Shanghai, China). The extracted RNA was then transformed into complementary DNA (cDNA) with the reverse transcriptase kit (TakaRa, Dalian, China). qPCR was performed with SYBR Green Master Mix (Thermo Fisher Scientific). The primer sequences used for qPCR are displayed in Table 1. The relative gene expression in the myocardial tissue samples was analyzed using the RQ Manager (Applied Biosystems).

Table 1. PCR primer sequences

Gene	Primer sequences (5'-3')
hKLK1	Forward: GTCCAGAACAATCCGCCTCA Reverse: CAGACCACCATGCCCAGTTAGA
TGF-β1	Forward: TGACAGACCCATTCAAAGT Reverse: CACCAAGCTCCTCCATAC
β-actin	Forward: TGGCACCCAGCACAATGAA Reverse: CTAAGTCATAGTCCGCCTAGAAGCA

Western blot analysis

The ground myocardial tissue samples (approximately 50 mg) were lysed on ice using RIPA buffer (1 ml), followed by centrifugation at 4 °C for 15 min at 10,000 × g. The supernatant was collected, and the proteins were quantified using a BCA kit (Beijing Solebo Technology, Beijing, China). The protein samples (70 μg) were separated on a 5% SDS-PAGE gel and transferred to a polyvinylidene fluoride membrane. After blocking with 5% skimmed milk at room temperature for 2 h, the membrane was incubated with anti-hKLK1 (1 : 1000; Santa Cruz Biotechnology, Dallas, TX, USA), anti-TGF-β1 (1 : 1000; Shanghai Bioengineering, Shanghai, China), or anti-β-actin (1 : 1000; Wuhan Servicebio Technology, Wuhan, China) antibody overnight at 4 °C. The membrane was washed with Tris-buffered saline containing Tween-20 (TBST) 3 times and incubated with the corresponding horseradish peroxidase (HRP)-conjugated secondary antibody (1 : 3000; Wuhan Servicebio Technology, Wuhan, China) at 37 °C for 1 h. After washing with TBST 4 times, the membrane was subjected to enhanced chemiluminescence solution (Millipore) for 1 min at room temperature. The images were acquired using the Bio-Rad XRS chemiluminescence detection system and analyzed using the Image J software 1.48 (NIH, Bethesda, MD, USA). The relative protein level was calculated as the ratio of the intensity of the corresponding protein band to the intensity of β-actin band. The experiment was repeated six times.

Statistical analysis

All statistical analyses were performed using SPSS 20.0 (SPSS, Chicago, IL, USA). Continuous data were expressed as the mean ± standard deviation. Multigroup comparison was performed using one-way analysis of variance. Comparisons between two groups were conducted using Tukey-test for homogenous variance or Dunnett's T3-test for heterogeneous variance. A P value < 0.05 was considered statistically significant.

Results

EZ.hKLK1 administration express exogenous hKLK1 protein in mouse myocardium

After CVB3 virus injection, the VMC mice showed increased irritability, arched backs, reduced stimulation response, loss of appetite and body mass, and mental depression, compared with the normal control mice. These symptoms were ameliorated after EZ.hKLK1 treatment. The body weights started to increase at 10 days after EZ.hKLK1 treatment, and at 20 days after vehicle or EZ.null treatment (Fig. 1).

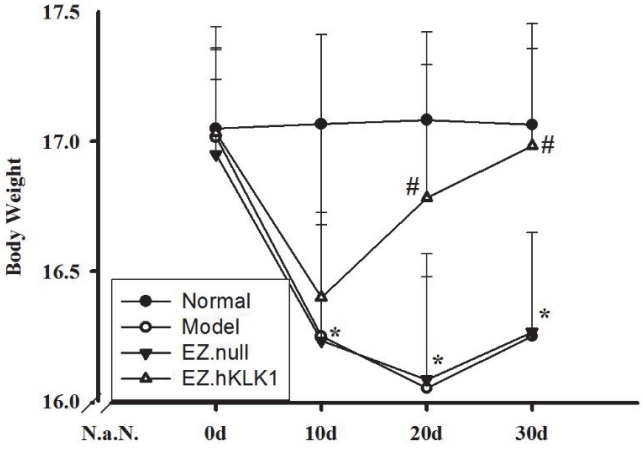

Fig. 1. The body weights of mice. Data are expressed as the mean ± SD. * P < 0.05 vs. normal group; # P < 0.01 vs. model group; n = 6.

To evaluate whether EZ.hKLK1 vectors can sustainably express hKLK1 protein *in vivo*, we detected hKLK1 protein expression in mouse myocardium on day 30. As shown in Fig. 2A, compared with the model group, EZ.hKLK1 administration remarkably upregulated hKLK1 protein expression in mouse myocardium (0.60-fold vs. 0.20-fold; P < 0.01). No significant difference was observed in hKLK1 protein expression between EZ.null-treated and vehicle-treated mice. Moreover, the EZ.hKLK1 group obviously increased the mRNA expression of hKLK1 gene in comparison with the model group (displayed in Fig. 2B). This finding indicates that EZ.hKLK1 vectors effectively induce hKLK1 overexpression in mouse myocardium and that hKLK1 overexpression is sustained for at least 30 days.

EZ.hKLK1 administration reduces the heart/body weight ratio in VMC mice

To assess the effect of EZ.hKLK1 administration on VMC, we measured the heart/body weight ratios of mice. As shown in Fig. 3A, the heart/body weight ratios of the vehicle-treated VMC mice were significantly increased compared with the control mice (P < 0.05). Compared with the model group, EZ.hKLK1 administration significantly reduced the heart/body weight ratio in VMC mice (P < 0.05). This finding suggests that EZ.hKLK1 administration might alleviate VMC in mice.

EZ.hKLK1 administration improves cardiac function in VMC mice

Next, we measured cardiac function parameters in the mice to evaluate the therapeutic effects of EZ.hKLK1 on VMC. As shown in Fig. 3B–E, vehicle-treated VMC mice exhibited significantly increased LVEDd and LVEDs along with remarkably decreased FS and EF (P < 0.05) compared with the normal control mice, indicating the impaired cardiac functions in VMC mice. Compared with the model group, EZ.hKLK1 administration effectively reduced the LVEDd and LVEDs while elevating FS and EF in VMC mice, suggesting that EZ.hKLK1 administration improves the cardiac functions of VMC mice.

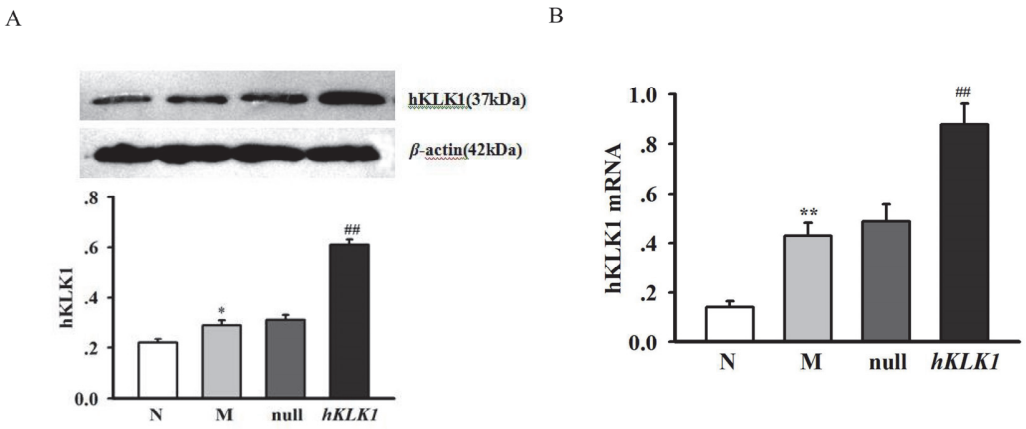

Fig. 2. The expression of human tissue kallikrein 1 (hKLK1) protein and gene. (**A**) The Western blots and relative densitometric quantification of the levels of hKLK1 protein in the heart tissue samples. β-actin was used as an internal control. (**B**) qPCR experiment was performed to determine hKLK1 gene expression in the heart tissue samples. β-actin was used as an internal control. Data are expressed as the mean ± SD. * $P < 0.05$ vs. normal group; ** $P < 0.01$ vs. normal group; ## $P < 0.01$ vs. model group; $n = 6$. N, normal group; M, model group; null, VMC + EZ.null group; hKLK1, VMC + EZ.hKLK1 group.

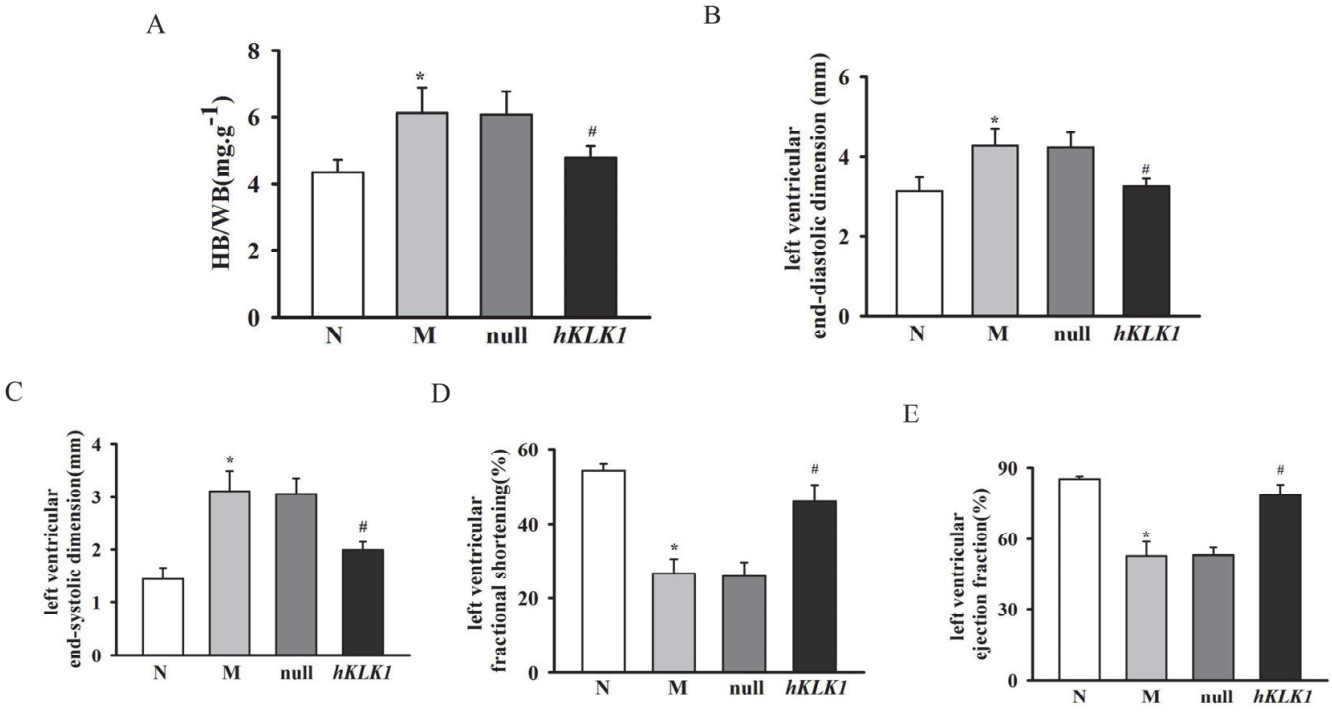

Fig. 3. The effects of lentivirus-mediated human tissue kallikrein 1 (hKLK1) overexpression on cardiac function parameters in mice with viral myocarditis (VMC). The VMC mice were administered vehicle (normal saline), the empty lentiviral vetors (EZ.null), or the vectors overexpressing hKLK1 (EZ.hKLK1). The mice were sacrificed at 30 days after treatment. The heart/body weight ratio (**A**), On day 30 before sacrificing, the left ventricular end-diastolic dimension (**B**), left ventricular end-systolic dimension (**C**), and left ventricular fractional shortening (**D**), and ejection fraction (**E**) were measured. Data are expressed as the mean ± standard deviation (SD). * $P < 0.05$ vs. the normal group; # $P < 0.05$ vs. the model group; $n = 6$. LVEDd, left ventricular end-diastolic dimension; LVEDs, left ventricular end-systolic dimension; FS, fractional shortening; EF, ejection fraction; VMC, viral myocarditis; HW, heart weight; BW, body weight. N, normal group; M, model group; null, VMC + EZ.null group; hKLK1, VMC + EZ.hKLK1 group.

EZ.hKLK1 administration reduces myocardial collagen content and type I/III collagen ratio in VMC mice

Considering that myocardial fibrosis is characterized by abnormal myocardial collagen content and proportions, we investigated the effects of EZ.hKLK1 administration on myocardial collagen content and type I/III collagen ratio in mice. We found that compared with the model group, EZ.hKLK1 administration markedly abrogated the elevation of myocardial collagen contents in VMC mice ($P < 0.05$; Fig. 4A). Consistently, the picrosirius red staining (Fig. 4B) revealed an increased accumulation of type I (yellow-red) and type III collagen (yellow-green) in the heart tissue samples of vehicle- and EZ.null-treated VMC mice compared with that in the normal mice. EZ.hKLK1 administration substantially abolished the collagen I and III deposition in VMC mice ($P < 0.05$). Furthermore, compared with the model group, EZ.hKLK1 administration effectively countered the elevated collagen I/III ratio in VMC mice ($P < 0.05$). Taken together, these data suggest that hKLK1 overexpression reduces collagen deposition and the collagen I/III ratio in mouse myocardium, thereby suppressing myocardial fibrosis in VMC mice.

EZ.hKLK1 administration alleviates histopathological damage in the myocardium of VMC mice

Then, we examined the effect of EZ.hKLK1 administration on the histopathological damage in mouse myocardial tissue samples. As shown in Fig. 4C, compared with the model group, EZ.hKLK1 treatment significantly alleviated the histopathological changes, including myocardial fibrosis, hyaline degeneration, fibrotic foci, and lymphocyte infiltration, in the myocardial tissue of VMC mice. In addition, the histopathological scores in EZ.hKLK1-treated mice were lower than those in the model group. These results indicate that EZ.hKLK1 administration alleviates VMC-induced histopathological damage in mouse myocardium.

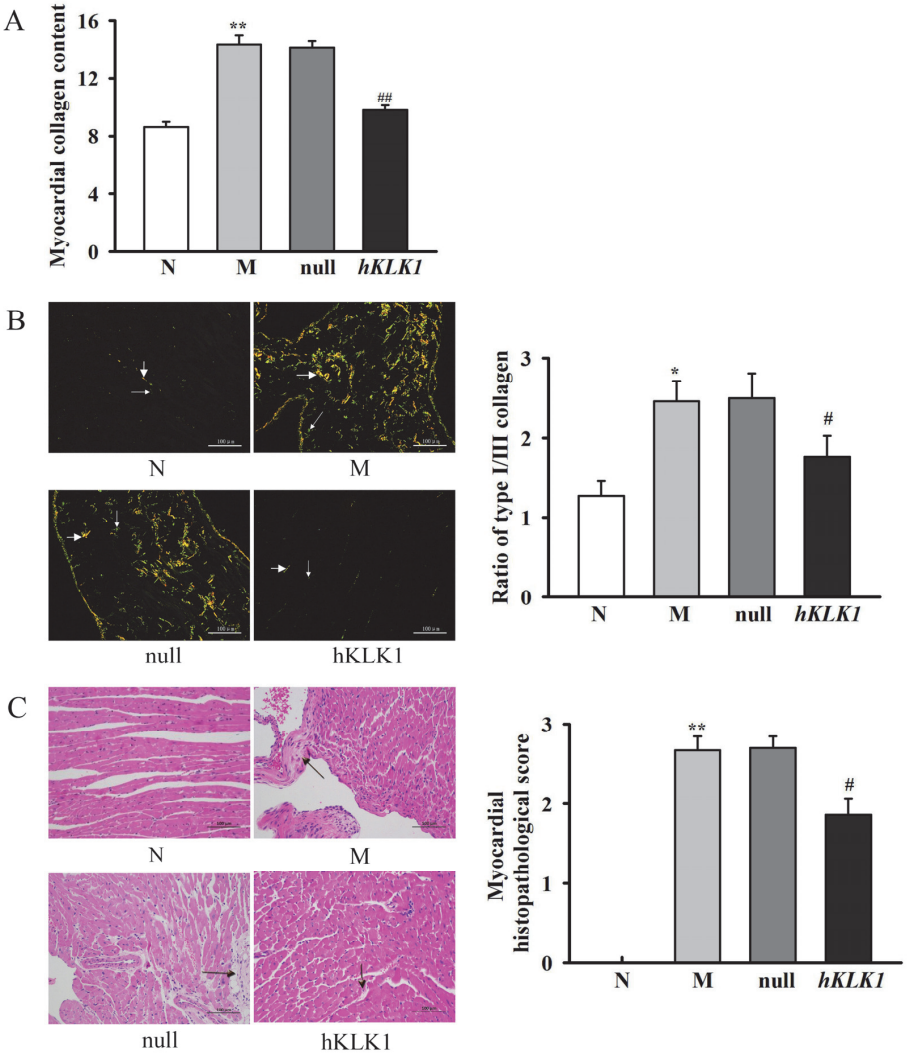

Fig. 4. The effects of hKLK1 overexpression on collagen content, type I/III collagen ratio, and histopathologic changes in the hearts of VMC mice. (**A**) UV spectrophotometry was used to measure myocardial collagen content. (**B**) A picrosirius red staining was performed to detect type I (yellow-red) and III (yellow-green) collagens and measure the type I/III collagen ratio in mouse heart. Representative images and the quantification of type I/III collagen ratio are shown. Big white arrows indicate type I collagen; small white arrows indicate type III collagen. Data are expressed as the mean ± SD. ** $P < 0.01$ vs. normal group; # $P < 0.05$ vs. model group; $n = 6$. (**C**) The histopathologic damage in mouse heart tissue samples were examined and scored using hematoxylin and eosin (H&E) staining. Representative images and the quantification of myocardial histopathologic changes are shown. Data are expressed as the mean ± SD. * $P < 0.05$ vs. the normal group; # $P < 0.05$. vs. the model group; $n = 6$. N, normal group; M, model group; null, VMC + EZ.null group; hKLK1, VMC + EZ.hKLK1 group.

EZ.hKLK1 administration downregulates TGF-β1 expression in the heart of VMC mice

To explore the involvement of TGF-β1 in the therapeutic benefits of hKLK1 in VMC, we detected the protein expression and the mRNA expression of TGF-β1 in VMC mouse hearts. Western blotting showed that compared with the model group, EZ.hKLK1 administration significantly reversed the upregulation of TGF-β1 protein expression in the hearts of VMC mice (0.29-fold vs. 0.49-fold; $P < 0.05$; Fig. 5A). Furthermore, there was an apparent downregulation of the TGF-β1 expression in the EZ.hKLK1 group, compared with that of the model group (Fig. 5B). The results suggest that suppression of TGF-β1 signaling might be involved in the therapeutic benefits of hKLK1 in VMC.

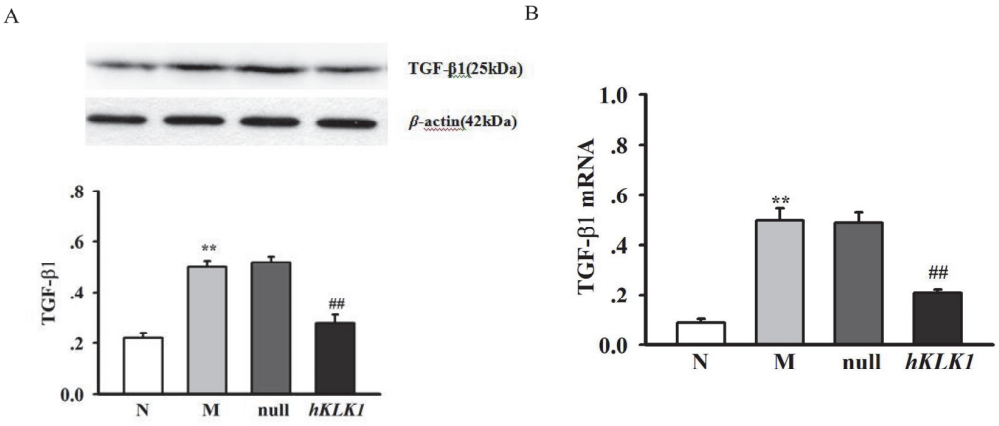

Fig. 5. hKLK1 overexpression downregulated transforming growth factor (TGF)-β1 protein and gene expression in the hearts of VMC mice. (**A**) The western blots and the relative densitometric quantification of TGF-β1 protein expression in the heart tissue samples of mice on day 30 after treatment. β-actin were used as an internal control. (**B**) qPCR experiment was performed to determine TGF-β1 gene expression in the heart tissue samples of mice on day 30 after treatment. β-actin was used as an internal control. Data are expressed as the mean ± SD. ** $P < 0.01$ vs. normal group; ## $P < 0.01$ vs. model group; $n = 6$. N, normal group; M, model group; null, VMC + EZ.null group; hKLK1, VMC + EZ.hKLK1 group.

Discussion

Myocardial fibrosis in VMC is characterized by excessive collagen deposition and an increased type I/III collagen ratio in myocardial ECM. Our study showed that lentivirus-mediated hKLK1 overexpression could alleviate the elevated heart/body weight ratio, myocardial collagen content, and type I/III collagen ratio in VMC mice. hKLK1 overexpression also attenuated the cardiac inflammation and improved the cardiac functions in VMC mice. The downregulation of TGF-β1 expression is involved in the beneficial roles of hKLK1 in VMC. Our results suggest that lentivirus-mediated hKLK1 gene transfer may alleviate myocardial fibrosis in VMC, possibly by suppressing TGF-β1 signaling.

Currently, drugs such as carvedilol (Ge et al., 2013) and captopril (Guo et al., 2010) have been shown to inhibit myocardial fibrosis in VMC mice, but their clinical efficacy has not been confirmed. Lentivirus-mediated gene therapy has emerged as a promising therapeutic strategy in human diseases. Recombinant lentivirus-mediated hKLK gene transfer has shown positive effects in the treatment of diabetes (Maneva-Radicheva et al., 2014), hypertension (Yan et al., 2008; 2009), and chronic renal insufficiency (Tu et al., 2008). Studies have demonstrated that hKLK1 can inhibit myocardial fibrosis induced by various factors. For example, Silva et al. have observed that hKLK1 inhibits isoproterenol-induced myocardial hypertrophy in genetically modified rats (Silva et al., 2000). Intracaudal vein injection with lentivirus-carried hKLK1 gene significantly ameliorates myocardial hypertrophy and fiber deposition in rats with spontaneous hypertension (Bledsoe et al., 2003) or myocardial infarction (Agata et al., 2002). However, the effects of hKLK1-based gene therapy in myocardial fibrosis in VMC remains unexplored. In this study, the gene and protein expression of KLK1 was upregulated in the myocardium of mice with VMC, suggesting that KLK1 is involved in myocardial inflammation and fibrosis. Lentivirus-mediated hKLK1 gene transfer via tail vein injection attenuated the inflammation, inhibited myocardial collagen biosynthesis, and reduced the type I/III collagen ratio in the myocardium of VMC mice, thereby improving cardiac function in the mice.

The imbalance of MMPs/TIMPs is the main mechanism of collagen remodeling in the VMC myocardium (Cheung et al., 2006). TGF-β1 signaling contributes to collagen remodeling induced by viruses and other stimuli. TGF-β1 prevents collagen degradation and induces excessive collagen deposits in myocardial ECM by reducing the MMPs/TIMPs ratio (Heymans et al., 2006; Lauer et al., 2014; Rosenkranz et al., 2002). It has been reported that in the myocardium of CVB3-infected mice, TGF-β1 upregulation occurs at 3 days after infection, peaks at 7–21 days, and persists for 56 to 98 days (Glück et al., 2001). Our results showed that hKLK1 overexpression significantly reversed the upregulation of TGF-β1 expression at protein and mRNA levels in the myocardium of VMC mice. hKLK1 cleaves low-molecular weight kininogen into vasodilator kinin peptides, such as bradykinin and kallidin. By binding to bradykinin B2 receptor, the bradykinin peptide initiates antihypertensive, antiproliferative, and antihypertrophic effects on the cardiovascular system (Maurer et al., 2011). Xu et al. have demonstrated that bradykinin B2 receptor is essential for the suppression of TGF-β1 expression in mouse heart (Xu et al., 2013). Thus, we speculate that hKLK1 overexpression blunts TGF-β1 protein expression in the hearts of VMC mice, possibly via bradykinin/bradykinin B2 receptor signaling. However, the precise molecular mechanism requires further investigation.

Conclusions

This study showed that lentivirus-mediated hKLK1 gene transfer ameliorated myocardial fibrosis in VMC mice, as evidenced by decreased heart/body weight ratio, myocardial collagen accumulation, and type I/III collagen ratio in response to EZ.hKLK1 administration. hKLK1 overexpression also attenuated histopathological changes of the myocardium and improved cardiac function in VMC mice. The downregulation of TGF-β1 expression was involved in the beneficial roles of hKLK1 in VMC. Our results showed lentivirus-mediated hKLK1 gene transfer to be a promising therapeutic approach for VMC therapy. However, the mechanism under hKLK1 overexpression in the prevention of cardiac fibrosis is still undefined, and more studies need to be conducted to make inroads into the treatment of cardiac fibrosis.

Funding
This work was supported by Scientific Research Project of Traditional Chinese Medicine Bureau of Guangdong Province, China (No. 20171273; No. 20201368); Medical Scientific Research Foundation of Guangdong Province, China (No. B2017033); and Dongguan Science and Technology Bureau, China (No. 202071715024129); Medical Scientific Research Foundation of Guangdong Province, China (No. B2022026).

Conflict of interests
The authors have no conflict of interests to declare.

Ethical approval and consent to participate
The study was approved by the Ethics Committee of the SSL Central Hospital of Dongguan City. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press).

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