Improving myocardial fractional flow reserve in coronary atherosclerosis via CX37 gene silence: a preclinical validation study in pigs

Suxia Guo, Ying Yang, Zhenyu Yang, Huayan You, Yunke Shi, Zhao Hu, Zhaohui Meng and Jianming Xiaoa,*

OBJECTIVES: The purpose of this study was to evaluate the effect of CX37 gene silence on myocardial fractional flow reserve (FFR).

METHODS: A total of 90 male pigs were randomly divided into saline, mock and 3 different doses (5, 10 and 20 μl) of CX37 viral suspension groups that could induce coronary plaque formation with high-fat diet. After performing myocardial FFR by intravascular ultrasound, different doses of CX37 viral suspension, saline and mock small interfering RNA (siRNA) were transfected into the related coronary. The FFR, the myocardial enzymes and the cardiac structures and functions of the pigs were detected at baseline, 4th, 8th and 12th week after transfection, respectively.

RESULTS: Repeated measures analysis of variance comparison showed that the difference in the FFR among the 5 groups was statistically significant (F = 27.0, P < 0.01). Post hoc analysis showed that FFR were highest in the siRNA CX37 group (20 μl), followed by the siRNA CX37 group (5 μl), and lowest in the mock and saline groups. Left ventricular end-diastolic diameter was significantly smaller and ejection fraction was obviously higher in the 3 siRNA CX37 groups compared with the untreated groups.

CONCLUSIONS: Our study showed that FFR levels increased along with decreased doses of siRNA CX37 lentivirus, indicating that siRNA CX37 lentivirus may reduce the risk of coronary atherosclerosis and provide a potential approach to treat coronary heart disease.

Keywords: Atherosclerosis · CX37 · Fractional flow reserve · FFR · Gene therapy · SiRNA

INTRODUCTION

Coronary artery disease has become the main cause of mortality and morbidity globally [1–3], and its initial and key factor is atherosclerosis [4]. Atherosclerosis is a complicated and chronic inflammatory disease of large- and medium-sized arteries, characterized by leucocyte accumulation in the vessel wall [5–7]. The association between genes and atherosclerosis has been widely explored by scientists and clinicians in the past decade. Gap junctions were comprised of connexions that could form transmembrane channels connecting cytoplasm of neighbouring cells, which would allow intercellular communication and the transfer of ions and small molecules [8]. The protein Connexin 37 (CX37) has been suggested as one of the main participants in the intercell communications. Its C allele is reported to be associated with susceptibility to atherosclerosis and cardiovascular disease [9–14]. Small interfering RNAs (siRNAs) have proven to be efficacious in mediating gene-specific silencing primarily via recognizing and inducing degradation of the messenger RNAs (mRNAs) in targeted genes [15]. This mechanism is conserved in nature from yeast to humans. Since Lentivirus stably transduces both dividing and non-dividing cells, it is an ideal vehicle to efficiently deliver siRNA [16]. Gene knockout studies have shown that CX37 forms gap junction channels between endothelial cells. Through intravascular ultrasound, CX37 gene silencing was found to effectively inhibit the expression of CX37 gene in the porcine aortic atherosclerotic plaque, which may affect the stabilization and reduction of plaque [17]. Although these results are encouraging, the relative contribution effects of CX37 on arterial blood flow remain unknown.

A previous study has shown that atherosclerosis in pigs and non-human primates are similar to the human model [17]. Myocardial fractional flow reserve (FFR), the ratio of maximal blood flow in a stenotic artery to the normal maximal flow, is now a gold standard for invasive assessment of coronary artery stenosis [18, 19]. In this study, one lentiviral vector was
constructed to knockdown CX37 and to assess its role on FFR in pigs, which was measured by intravascular ultrasound.

**MATERIALS AND METHODS**

**Modelling of pigs**

A total number of 90 male pigs were obtained from Wuzhishan area, China, and were provided with normal diet and water. After 1 week, all pigs received high-fat diet with 5% lard, 1% sugar, 3% cholesterol and 0.2% propylthiouracil; they were fed 3 times daily as long as 8 months. Then, FFR was assessed in coronary arteries including left anterior descending, left circumflex artery and right coronary artery. The vessels with FFR under 90% were set as target vessels. If many vessels of coronary of a pig were fit to be target vessels, the one with the lowest FFR was set as target vessel. All pigs were divided into 5 groups and provided with high-fat diet until the 11th month.

The animal use procedure was reviewed and approved by the Institutional Animal Care and Use Committee of The Affiliated Hospital of Nanjing Medical University, Wuxi People’s Hospital (Wuxi, China). This study was conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health strictly.

**Fractional flow reserve analysis**

A Volcano SSi imaging system and Prime Wire™ pressure guide wire were used for FFR measurements at 8th month. The pressure guide wire was calibrated and introduced into the guiding catheter. The pressure transducer was advanced just outside the tip of the guiding catheter, and the pressure measured by the sensor was then equalized to that of the guiding catheter. The wire was then advanced distally to the target coronary stenosis. Special attention was paid to avoid arterial pressure wave damping, unselective catheterization of coronary ostia and variation in the position of the pressure wire. The targeted vessels were identified by FFR, then the pigs were divided into 5 groups, including the saline group, the mock group and the 3 CX37 siRNA groups (5, 10 and 20 μl). The preparation of lentiviral vectors and target screening for RNA interference have been described previously[17]. Coronary dilatation catheter (Abbott Vascular, 3200 Lakeside Drive, Santa Clara, CA, USA) was used to block the distal blood flow, then a microcatheter (William Cook Europe ApS, Denmark) was reached to the target area. The end of the microcatheter was connected to the syringes. Virus suspension (titre 2 × 10⁹ transducing units/l), mock siRNA and saline were slowly injected to the target blood vessels for 10 min. Afterwards, the microcatheter and the balloon exited. Coronary angiography and FFR measurement were performed for all pigs. Each vessel throughout the entire length of the coronary artery underwent FFR analysis at 4th, 8th and 12th weeks after transfection, respectively. At study termination, all pigs were euthanized with an overdose of pentobarbital (150 mg/kg intravenously), and tissue samples of coronary arteries were carefully excised and stored.

**Polymerase chain reaction**

DNA was purified from samples of pigs, which had been stored frozen at -20°C. Purification was performed using a DNA Purification Kit (Promega Inc., Madison, WI, USA) according to the manufacturer’s instructions. The polymerase chain reaction was conducted with the upstream primer, 5’- CTTCCGTACAGGCTTACCGG-3’, and downstream primer, 5’- CATCCAGGCAGCCACT-3’. A 20-μl reaction volume was used for polymerase chain reaction containing 10 μl 2× mix (including Mg²⁺, deoxyribonucleotide triphosphates and Taq DNA polymerase), 1.0 μl upstream primer (10 pmol), 1.0 μl downstream primer (10 pmol), 2 μl genomic DNA template (up to 4.0 μl, according to the concentration) and 6 μl double-distilled water. The reaction began with denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 95°C (30 s), annealing at 60°C (30 s), extension at 72°C (30 s) and a final extension at 72°C (7 min). Genotype analysis was performed using DNA sequencing at the 4th, 8th and 12th week after transfection.

**Western blot analysis**

The total cellular protein of coronary plaque tissue of pigs was extracted at the 4th, 8th and 12th week after transfection. Ten percent sodium dodecyl sulphate–polyacrylamide gel electrophoresis of the sample was performed. The protein was transferred to the collodion membrane, followed by blocking. The membrane was incubated with primary antibody (anti-Cx37 antibody, 1:200, Abcam, Cambridge, MA, USA) at 4°C overnight. Following washing for 3 times, the membrane was incubated with secondary antibody (1:2000) for 1 h, followed by washing for 3 times. Protein expression was quantified using an enhanced chemiluminescence detection kit (Beijing Kang Century Biotech Co., Ltd, Beijing, China).

**Green fluorescent protein analysis**

One pig from each CX37 siRNA group (5, 10 and 20 μl) was, respectively, euthanized at the 1st week, the 4th week, the 8th week and the 12th week after transfection. Formalin-fixed, paraffin-embedded tissue sections (4-μm thick) were mounted on positively charged microscope slides. Antigen retrieval was performed with microwave heating in ethylenediaminetetraacetic acid buffer (1 mmol/l ethylenediaminetetraacetic acid, 0.05% Tween 20, pH 9.0). Endogenous peroxidase and biotin activities were blocked, respectively, using 3% hydrogen peroxide and avidin/biotin blocking kit (Lab Vision). After blocking for 15 min with 10% normal horse serum diluted in casein solution (Dako), polyclonal rabbit anti-green fluorescent protein (GFP) primary antibody (Ab290, Abcam) was applied at 1:1000 dilution and incubated at room temperature for 30 min. An anti-rabbit antibody conjugated with Alexa Fluor 488 was then applied at 1:200 dilution and incubated at room temperature for 60 min. Slides were then washed and mounted with Vectashield mounting medium with 4’,6-diamidino-2-phenylindole and immediately imaged on a Nikon 80i microscope, and images were recorded using an attached charge-coupled device camera using ACT-4 U software.

**Weight, serum lipid level and hepatic function and inflammation profile measurement**

All pigs were weighed with an electronic scale (METTLER TOLEDO XK3141 IND131/331, Switzerland). Serum lipid was detected by
orbital venous sinus blood. The plasma levels of total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were measured by the oxidase method. Hepatic (aspartate aminotransferase) function and inflammation profile (high-sensitivity C-reactive protein) were measured by HITACH 7170s automatic biochemical analyser after fasting for 12 h (Center Laboratory of Wuxi People’s Hospital, Wuxi, China). Weight, serum lipid level, hepatic function and high-sensitivity C-reactive protein were measured before pigs were fed with high-fat food, before transfection and 12 weeks after transfection, respectively.

Effects of CX37 small interfering RNA silence on the myocardial enzymes and on cardiac structures and functions

Twenty-four hours prior to the echocardiography, the pigs were washed and fasted for 16 ± 2 h, and afterwards, they were anaesthetized with intramuscular 10 mg/kg ketamine. Myocardial enzymes including lactate dehydrogenase, creatine kinase and MB fragment of creatine kinase were evaluated with Synchron CX4 PRO (Beckman Coulter Inc., Brea, CA, USA). Echocardiography was performed with a Vivid E9 instrument (GE Healthcare, Pittsburgh, PA, USA) to evaluate the cardiac structures including left ventricular end-diastolic diameter (LVEDD), the inter-ventricular septum thickness and the left ventricular posterior wall thickness as well as the cardiac functions including stroke volume and ejection fraction (EF).

Statistical analysis

Continuous variables with normal distribution are presented as means ± standard deviation and compared with the use of a Student’s t-test or analysis of variances (ANOVA). Continuous variables with non-normal distributions are presented as medians and interquartile ranges and are compared with the use of the Mann–Whitney test. Dichotomous or nominal categorical variables are compared with the use of the χ² test with normal approximation or Fisher’s exact test, when appropriate. Two-way repeated measures ANOVA was performed to examine the main effect of group, time and Group × Time interaction on outcome measures. Sphericity-assumed estimates were applied, if the Mauchly’s test indicated no violation of assumptions; otherwise, Greenhouse-Geisser estimates were used. When necessary, Fisher’s least significant difference tests were used for post hoc comparison.

All statistical analyses were performed with the SPSS statistical software program package (SPSS version 20.0 for Windows, SPSS Inc., Chicago, IL, USA). All tests were 2-tailed, and the significance level was defined as P < 0.05.

RESULTS

Efficient transfection of lentivirus

GFP expression provides an efficient and convenient means to assess the transfection efficiency of Lentivirus. Four weeks after transfection, GFP fluorescence in coronary atherosclerosis was detected (Fig. 1A). siRNA transfection and greater fluorescence were observed in the 8th week and the 12th week after transfection (Fig. 1B and C), and GFP was still visible when the study was terminated. However, it has already weakened (Fig. 1C) at the 12th week after transfection.

Gene silencing in vivo

To evaluate the efficacy of lentivirus-mediated gene silencing in vivo, coronary atherosclerosis for CX37 mRNA and protein expression were detected using real-time polymerase chain reaction and western blot analysis. Table 1 lists that there is a significant difference in CX37 mRNA levels among the 5 groups at 4th week (F = 168.7, P < 0.01), 8th week (F = 2576.5, P < 0.01) and 12th week (F = 4006.2, P < 0.01). The differences in the CX37 protein were also significant among the 5 groups at 4th week (F = 372.1, P < 0.01), 8th week (F = 545.8, P < 0.01) and 12th week (F = 598.3, P < 0.01). Post hoc analysis showed that at each period, CX37 mRNA and protein levels were lowest in the siRNA CX37 group (20 μl) (P < 0.01 compared with other groups), followed by the siRNA CX37 group (10 μl) and the siRNA CX37 (5 μl) (P < 0.01 compared with 20 μl group and untreated groups; P > 0.05 between 10 μl and 5 μl groups), and highest in the mock-siRNA group and saline group (P < 0.01 compared with 3 siRNA CX37 groups; P > 0.05 between mock-siRNA and saline groups). Our results indicate that the intracoronary infusion of siRNA-lentivirus could be efficiently silenced and that there is an obvious dose-effect relationship.

Body weight, serum lipid and hepatic function and inflammation profile

The repeated measures ANOVA comparison showed that there is no significant group effect in the body weight (Fig. 2A; F = 0.48, P = 0.75), serum cholesterol (Fig. 2B; F = 0.59, P = 0.68), low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were measured by the oxidase method. Hepatic (aspartate aminotransferase) function and inflammation profile (high-sensitivity C-reactive protein) were measured by HITACH 7170s automatic biochemical analyser after fasting for 12 h (Center Laboratory of Wuxi People’s Hospital, Wuxi, China). Weight, serum lipid level, hepatic function and high-sensitivity C-reactive protein were measured before pigs were fed with high-fat food, before transfection and 12 weeks after transfection, respectively.

Effects of CX37 small interfering RNA silence on the myocardial enzymes and on cardiac structures and functions

Twenty-four hours prior to the echocardiography, the pigs were washed and fasted for 16 ± 2 h, and afterwards, they were anaesthetized with intramuscular 10 mg/kg ketamine. Myocardial enzymes including lactate dehydrogenase, creatine kinase and MB fragment of creatine kinase were evaluated with Synchron CX4 PRO (Beckman Coulter Inc., Brea, CA, USA). Echocardiography was performed with a Vivid E9 instrument (GE Healthcare, Pittsburgh, PA, USA) to evaluate the cardiac structures including left ventricular end-diastolic diameter (LVEDD), the inter-ventricular septum thickness and the left ventricular posterior wall thickness as well as the cardiac functions including stroke volume and ejection fraction (EF).

Statistical analysis

Continuous variables with normal distribution are presented as means ± standard deviation and compared with the use of a Student’s t-test or analysis of variances (ANOVA). Continuous variables with non-normal distributions are presented as medians and interquartile ranges and are compared with the use of the Mann–Whitney test. Dichotomous or nominal categorical variables are compared with the use of the χ² test with normal approximation or Fisher’s exact test, when appropriate. Two-way repeated measures ANOVA was performed to examine the main effect of group, time and Group × Time interaction on outcome measures. Sphericity-assumed estimates were applied, if the Mauchly’s test indicated no violation of assumptions; otherwise, Greenhouse-Geisser estimates were used. When necessary, Fisher’s least significant difference tests were used for post hoc comparison.

All statistical analyses were performed with the SPSS statistical software program package (SPSS version 20.0 for Windows, SPSS Inc., Chicago, IL, USA). All tests were 2-tailed, and the significance level was defined as P < 0.05.
Table 1: CX37 mRNA and protein expression of pigs in the CX37 siRNAs (5, 10 and 20 μl), mock and saline groups

<table>
<thead>
<tr>
<th>siRNA CX37</th>
<th>n</th>
<th>20 μl</th>
<th>10 μl</th>
<th>5 μl</th>
<th>n</th>
<th>Mock siRNA</th>
<th>n</th>
<th>Saline</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX37 mRNA</td>
<td>4th week</td>
<td>17</td>
<td>45.8 ± 2.7a</td>
<td>61.0 ± 3.3b</td>
<td>61.9 ± 4.0c</td>
<td>18</td>
<td>96.5 ± 0.7c</td>
<td>18</td>
<td>97.5 ± 0.7c</td>
<td>168.7</td>
</tr>
<tr>
<td></td>
<td>8th week</td>
<td>16</td>
<td>42.6 ± 0.9b</td>
<td>58.4 ± 0.7b</td>
<td>59.3 ± 1.3b</td>
<td>18</td>
<td>97.5 ± 0.7c</td>
<td>18</td>
<td>98.3 ± 0.6c</td>
<td>2576.5</td>
</tr>
<tr>
<td></td>
<td>12th week</td>
<td>15</td>
<td>41.9 ± 1.0b</td>
<td>58.6 ± 0.5b</td>
<td>59.6 ± 1.4b</td>
<td>18</td>
<td>97.8 ± 0.4c</td>
<td>18</td>
<td>97.5 ± 1.0c</td>
<td>4006.2</td>
</tr>
<tr>
<td>CX37 protein</td>
<td>4th week</td>
<td>17</td>
<td>0.22 ± 0.01a</td>
<td>0.41 ± 0.03b</td>
<td>0.42 ± 0.02b</td>
<td>18</td>
<td>0.76 ± 0.03c</td>
<td>18</td>
<td>0.75 ± 0.03c</td>
<td>372.1</td>
</tr>
<tr>
<td></td>
<td>8th week</td>
<td>16</td>
<td>0.22 ± 0.01a</td>
<td>0.41 ± 0.03b</td>
<td>0.43 ± 0.02b</td>
<td>18</td>
<td>0.77 ± 0.03c</td>
<td>18</td>
<td>0.76 ± 0.03c</td>
<td>545.8</td>
</tr>
<tr>
<td></td>
<td>12th week</td>
<td>15</td>
<td>0.23 ± 0.02a</td>
<td>0.41 ± 0.03b</td>
<td>0.43 ± 0.02b</td>
<td>18</td>
<td>0.76 ± 0.02c</td>
<td>18</td>
<td>0.77 ± 0.03c</td>
<td>598.3</td>
</tr>
</tbody>
</table>

mRNA: messenger RNA; siRNA: small interfering RNA.

aCX37 mRNA and CX37 protein levels were significantly lowest in the siRNA CX37 (20 μl) group.
bCX37 mRNA and CX37 protein levels in the siRNA CX37 (10 μl) group and the siRNA CX37 (50 μl) group were significantly lower than those in the siRNA CX37 (20 μl) group and were significantly higher than those in the mock siRNA group and saline group.
cCX37 mRNA and CX37 protein levels were significantly highest in the mock siRNA group and the saline group.

Figure 2: Body weight, serum lipid and hepatic function and inflammation profile: (A) body weight; (B) total cholesterol; (C) low-density lipoprotein cholesterol; (D) high-density lipoprotein cholesterol; (E) aspartate aminotransferase and (F) high-sensitivity C-reactive protein. siRNA: small interfering RNA.
density lipoprotein (Fig. 2C; \(F = 1.08, P = 0.39\)), high-density lipoprotein (Fig. 2D; \(F = 2.57, P = 0.06\)), aspartate aminotransferase (Fig. 2E; \(F = 0.82, P = 0.52\)) and high-sensitivity C-reactive protein (Fig. 2F; \(F = 0.90, P = 0.48\)), indicating that the transfection of virus did not influence the body growth, serum lipid and hepatic function and inflammation.

Effects of CX37 small interfering RNA silence on fractional flow reserve

Repeated measures ANOVA results (Fig. 3) indicated a non-significant time effect (\(F = 1.37, P = 0.27\)), which suggests that the levels of FFR across the study time points were dependent on the type of treatment groups.

The main effect of group on FFP showed that the difference in the FFR among the 5 groups was significant (\(F = 26.99, P < 0.01\)). Post hoc analysis showed that the FFR were substantially higher in the 3 siRNA CX37 groups compared with that in the untreated saline and mock-siRNA groups (siRNA CX37 group 20 \(\mu\)l: 75.1 ± 7.4; 10 \(\mu\)l: 73.3 ± 7.7, 5 \(\mu\)l: 72.8 ± 7.1; mock-siRNA group: 64.7 ± 5.5; saline group: 65.5 ± 7.8; \(P < 0.01\), for each siRNA CX37 group compared with mock-siRNA and saline groups). Among the 3 siRNA CX37 groups, FFR seemed to be highest in the siRNA CX37 group (20 \(\mu\)l) (\(P = 0.02\) compared with 10 \(\mu\)l and \(P = 0.03\) compared with 5 \(\mu\)l, respectively). The difference in the FFR between siRNA CX37 group (10 \(\mu\)l) and siRNA CX37 group (5 \(\mu\)l) (\(P = 0.81\)) was not statistically significant.

Effects of CX37 small interfering RNA silence on myocardial enzymes and cardiac structures and functions

ANOVA with repeated measures test of myocardial enzymes showed that there is no significant differences in the lactate dehydrogenase (Fig. 4A; \(F = 0.76, P = 0.47\)), creatine kinase (Fig. 4B; \(F = 1.12, P = 0.41\)) and MB fragment of creatine kinase (Fig. 4C; \(F = 1.08, P = 0.37\)) among these 5 groups.

In terms of cardiac structures, there is a significant group effect in the LVEDD (Fig. 4D; \(F = 46.92, P < 0.01\)). Post hoc analysis showed that the LVEDD of 3 siRNA CX37 groups were obviously smaller than untreated groups (siRNA CX37 group 20 \(\mu\)l: 46.8 ± 4.1, 10 \(\mu\)l: 50.4 ± 4.9, 5 \(\mu\)l: 51.2 ± 5.0; mock-siRNA group: 54.0 ± 6.4; saline group: 54.7 ± 6.4; \(P < 0.01\), for each siRNA CX37 group compared with mock-siRNA and saline groups), and the differences among the 3 siRNA CX37 groups were not statistically significant (\(P > 0.05\), for each comparison). The differences in the inter-ventricular septum thickness (Fig. 4E; \(F = 1.25, P = 0.30\)) and the left ventricular posterior wall thickness (Fig. 4F; \(F = 0.57, P = 0.68\)) among these 5 groups were not substantial.

There is no significant difference in the stroke volume (Fig. 4G; \(F = 0.65, P = 0.52\)) among these 5 groups. The repeated measures ANOVA of cardiology functions showed that the differences in the EF (Fig. 4H; \(F = 76.52, P < 0.001\)) were significant among 5 groups. Post hoc analysis showed that the EF was substantially higher in the 3 siRNA CX37 groups compared with untreated groups (siRNA CX37 group 20 \(\mu\)l: 58.9 ± 4.3, 10 \(\mu\)l: 55.5 ± 4.1, 5 \(\mu\)l: 54.0 ± 3.4; mock-siRNA group: 48.7 ± 8.0; saline group: 48.1 ± 7.8; \(P < 0.01\), for each siRNA CX37 group compared with mock-siRNA and saline groups), and the differences among the 3 siRNA CX37 groups were not significant (\(P > 0.05\), for each comparison).

DISCUSSION

The frequency of the C allele at base pair 1019 of CX37 gene in patients with coronary heart disease and restenosis following coronary stenting was significantly higher than that in healthy controls [9]. CX37 gene interference in pigs showed stabilization and reduction of plaque [17]. It had been demonstrated in in vivo study that CX37 gene interference in pigs resulted in improved FFR. As far as we know, our study is the first one to report the effect of CX37 siRNA silence on FFR.

Atherosclerosis is a progressive inflammatory condition that underlies coronary artery disease [20], which is related to genetic factors [21]. Previous studies have demonstrated that CX37 gene polymorphism was a risk factor for coronary heart disease [9]. Since CX37 represents significant role in recognizing evolutionary highly conserved molecular motifs in pathogens, it could be regarded as an attractive therapeutic target in atherosclerosis.

As a class of short RNAs, microRNAs play vital roles in various biological processes and in the development of human disease through specific post-transcriptional down-regulation of gene expression [22]. RNA interference has been applied in several diseases for gene as an effective method to selectively silence mRNA for a wide range of proteins. Although many delivering approaches are available, significant challenges remain, such as success rate, safety and off-target effects of RNA in vivo. Recently, lentivirus expression cassette is reported to have the potential to overcome these limitations and to increase transfection efficiency. Lentiviral vector-delivered siRNAs were successfully manipulated to silence gene function in primary mammalian cells, stem cells and rabbits [23].

In this study, we used site-specific delivery to derive high-titre lentivirus-siRNA in the targeted plaque site. The efficacy of this method was confirmed by the observation of GFP fluorescence in the coronary plaque during the 1st, 4th, 8th and the 12th week after transfection. Stronger fluorescence was observed at the 12th week after transfection. There was no hepatic function damage and inflammation in this study. In addition, no significant difference in body weight and serum lipid among the 5 groups was found, indicating that the safety of virus transfection in these pigs and the therapeutic effects among the various CX37 siRNA groups were not caused by the serum lipid stimulation.
FFR assesses the reduction in flow resulting from a coronary artery stenosis. It is believed that lower FFR is associated with a higher rate of adverse cardiac events for lesions deferred revascularization among patients with acute coronary syndrome [24]. In this study, repeated measures of ANOVA showed that FFR is highest in the siRNA CX37 group (20 μl), followed by the siRNA CX37 group (10 μl) and the siRNA CX37 group (5 μl), and lowest in the untreated mock and saline groups, indicating that lentivirus-mediated siRNA may reduce the risk of coronary atherosclerosis.

The mechanisms underlying the therapeutic effects of CX37 are not completely known, but they may be related to its recognition patterns. If a single-nucleotide polymorphism in the CX37 gene is a cytosine-to-thymine replacement at Position 1019 (C1019T), the result is a non-conservative amino acid change in the regulatory C-terminus of the connexin37 protein, a proline-to-serine substitution (P319S). This shift from proline to serine may lead to functional changes and different responses to regulatory mechanisms, such as phosphorylation. The creation of a new phosphorylation site may provide greater capacity for modulating the function from the gap junctions made of this protein, which may modify endothelial cell functions and lead to different susceptibilities to cardiovascular diseases. Thus, the effects of CX37 on plaque can be attributed to its recognition of position. Recent studies demonstrated that CX37 was found in endothelial...
cells, monocytes/macrophages and platelets [4]. In this study, multiple conductance levels and pronounced voltage dependence were shown in the expressed CX37 channels.

Left ventricular dilation, defined as a high LVEDD, was reported to be associated with a high risk of cardiovascular events in the multi-ethnic study of atherosclerosis [25]. Such left ventricular dilation precedes clinical symptoms of heart failure and has been the target for therapies such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and aldosterone antagonists [26, 27]. In addition, a study conducted by Jia et al. [28] indicated that the left ventricular EF was significantly negatively associated with the severity of coronary atherosclerosis, after adjusting other major risk factors including age, body mass index, blood pressure, fasting blood glucose, blood lipid and leucocyte count. In this study, LVEDD was significantly smaller and EF was obviously higher in the 3 CX37 siRNA groups compared with the untreated groups, indicating that CX37 gene silencing has the potential to reduce the risk of atherosclerosis and other cardiovascular events.

CONCLUSION

In conclusion, lentivirus-mediated siRNA could be used to efficiently knockdown CX37 genes to increase the FFR of pigs with high-fat diet, which might provide an approach to the treatment of coronary heart disease.

Conflict of interest: none declared.

REFERENCES