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ORIGINAL ARTICLE

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Different angiogenesis effect of mini-TyrRS/mini-TrpRS by systemic administration of modified siRNAs in rats with acute myocardial infarction

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Abstract We aimed to clarify the different angiogenesis effects of mini-tyrosyl-tRNA synthetase (TyrRS)/mini-tryptophanyl-tRNA synthetase (TrpRS) in rodent primates with acute myocardial infarction, by delivering small interfering RNAs (siRNAs) systemically in a liposomal formulation. Left coronary artery ligation was used to establish the model of acute myocardial infarction in rats; mini-TyrRS/mini-TrpRS-specific siRNAs were encapsulated in stable nucleic acid lipid particles (SNALP), and administered by intravenous injection to rats. Rats were divided into four experiment groups: sham operated group (no left anterior descending artery [LAD] occlusion); negative control group (LAD occlusion + saline injection); mock transfection group (LAD occlusion + mock transfected injection); experiment group (LAD occlusion + mini-TyrRS/mini-TrpRS-specific siRNAs injection). Silencing efficiency was assayed by Western blotting. To determine whether mini-TyrRS/mini-TrpRS affected the angiogenesis activity of rats with myocardial infarction, we measured the myocardial infarction size by TTC staining, and the capillary density using immunohistochemistry staining, to investigate the expression of factor VIII. The myocardial infarction size and the capillary density of mini-TyrRS-siRNA group were respectively 18.89% and 8.64/0.1 mm^2 1 month after ligation, while in the mini-TrpRS-siRNA group these values were 7.33% and 17.32/0.1 mm^2, significantly different compared with the mock transfection group (14.19%; 13.56/0.1 mm^2) and negative control group (14.28%; 13.89/0.1 mm^2), $P < 0.05$. There were no significant changes between the mock transfection group and the negative control group, $P > 0.05$. These results indicated that angiogenesis is either stimulated by mini-TyrRS or inhibited by mini-TrpRS in rat models with acute myocardial infarction.

Key words Mini-TyrRS · Mini-TrpRS · Angiogenesis · Acute myocardial infarction · SiRNA

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Introduction

Myocardial ischemia is one of the leading causes of morbidity and mortality in humans in Western world. In China, the incidence and prevalence of this disease is also increasing year by year. Existing treatment, such as percutaneous transluminal coronary angioplasty (PTCA)[1] and coronary artery bypass grafting (CABG), are often insufficient, particularly in patients with multiple small vessel disease.[2] Thus, there is clear need for the development of novel treatment approaches, such as therapeutic angiogenesis using protein or gene therapy.[3–6] With the increasing range of potential therapeutic molecules and delivery vectors, animal models of ischemic heart disease are therefore necessary to support further advancement in this filed.

A rodent model of acute myocardial infarction (MI) was first developed in the rat.[7] More recently a murine equivalent has been described,[8] providing the means to exploit the increasing availability of many useful transgenic and knockout mouse strains. Complete occlusion of the left anterior descending (LAD) coronary artery induces an acute MI, and many circulating factors are involved in the formation of coronary artery thrombi.[9] The resulting ischemia in the left ventricular wall has been visualized with Evans blue/TTC perfusion assays,[10–12] which allows quantification of the infarct area. Further work by Guo et al.[13] has demonstrated ischemic preconditioning after short-term LAD occlusion in mouse, thus validating the physiologic relevance of this infarct size.

Aminoacyl-tRNA synthetases catalyze the first step of protein synthesis that consists of the aminoacylation of tRNAs. However, they have a broad repertoire of functions

beyond protein synthesis, including transcriptional and translational regulation as well as cell signaling.[14] Recently, it has been demonstrated that two of the tRNA synthetases, human tyrosyl-tRNA synthetase (TyrRS) and human tryptophanyl-tRNA synthetase (TrpRS), have novel cytokine functions.[15] This demonstration established a link between protein synthesis and signal transduction. At the same time, mammalian TyrRS and TrpRS have also been shown to regulate angiogenesis.[16–21]

Under apoptotic conditions in culture, full-length TyrRS is secreted, and two distinct cytokines can then be generated by an extracellular protease such as leukocyte elastase.[15] The NH_2-terminal catalytic fragment, mini-TyrRS, binds strongly to the CXC-chemokine receptor CXCR1 and, like interleukin-8, functions as a chemoattractant for polymorphonuclear leukocytes (PMNs) to promote angiogenesis,[15] whereas the full-length enzyme lacks cytokine activity. The catalytic core domain of TrpRS is a close homologue of the catalytic domain of TyrRS.[22–24] In normal cells, human TrpRS exists as two forms. The major form is the full-length protein and the other is a truncated TrpRS (mini-TrpRS) in which most of the extra NH_2-terminal domain is deleted because of alternative splicing of the pre-mRNA,[25,26] with Met-48 being deduced as the NH_2-terminal residue of mini-TrpRS.[25] Polymorphonuclear leukocyte elastase digestion of recombinant full-length TrpRS produced two fragments designated T_1-TrpRS and T_2-TrpRS, respectively. These fragments were similar in size to mini-TrpRS. In addition, mini-TrpRS and T_2-TrpRS blocked vascular endothelial growth factor-stimulated angiogenesis in both chick cell adhesion molecule and mouse Matrigel assays in vivo.[18,19] The full-length enzyme lacks cytokine activities (Fig. 1).

The opportunity to harness the RNA interference (RNAi) pathway to silence disease-causing genes holds great promise for the development of therapeutics directed against targets that are otherwise not addressable with current medicines.[27,28] Although there are numerous examples of in vivo silencing of target genes after local delivery of small interfering RNAs (siRNAs),[29–31] there remain only a few reports of RNAi-mediated silencing in response to systemic delivery of siRNA.[32–34] Here we show that siRNAs, when delivered systemically in a liposomal formulation, can silence the disease target mini-TyrRS/mini-TrpRS in rodent primates with acute myocardial infarction in order to prove whether mini-TyrRS and mini-TrpRS could be expressed in myocardial cells of rats, and to begin to comprehend their angiogenesis mechanism. All of this may be helpful for healing ischemia diseases, such as coronary atherosclerotic heart disease.

Materials and methods

Materials

Sprague-Dawley (SD) male rats (250–300 g, 2–3 months old) were provided by the experimental animal center of Sichuan University.(Sichuan, China). PGPU6/GFP/Neo siRNA expression vector kit and RNAi-Mate transfection reagent were purchased from Genepharma (Shanghai, China). The ECL chemiluminescence kit and BCA protein quantitative kit were from Pierce (Rockford, IL, USA). The plasmid extraction kit was purchased from Promega (Madison, WI, USA). Goat monoclonal antirat mini-TyrRS or mini-TrpRS IgG antibodies, and horseradish peroxidase (HRP) conjugated rabbit antigoat IgG were all from Santa Cruz Biotechnology (Santa Cruz, CA, USA). HRP-conjugated glucaraldehyde-3-phosphate dehydrogenase (GAPDH) was from Kang Chen Biotechnology (Nanjing, China). Protein Marker was purchased from MBI Company (Vilnius, Lithuania). Cytoplasm proteins extraction kit was from Keygen Biotechnology (Nanjing, China). Factor VIII related antigen and antibody was from Boster (Wuhan, China). Immunohistochemistry staining kit was purchased from Zhongshan Goldenbridge (Beijing, China)

SiRNA target site selection

Beginning with the AUG start codon, the transcript was scanned for AA dinucleotide sequences. Each AA and the 3′ adjacent 19 nucleotides were considered as potential siRNA target sites when the G/C ratio was 30%–50% as measured with Ambion siRNA analysis software (http://www.ambion.com/techlib/misc/siRNA_finder.html). Since

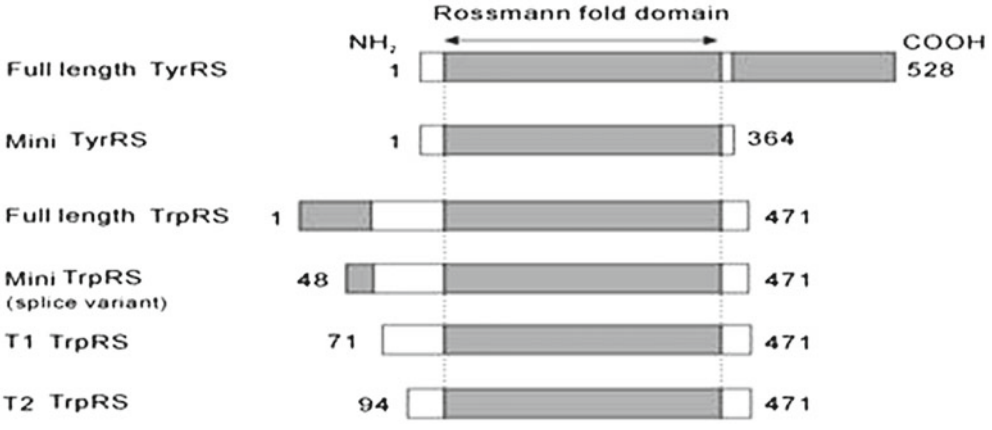

Fig. 1. Schematic representation of human TyrRS, TrpRS, and their variant constructs used in this study. *Shaded regions* of full-length TyrRS and TrpRS represent COOH- and NH_2-terminal appended domains, respectively. *Numbers* on the left and right correspond to the NH_2- and COOH-terminal residues relative to the human full-length enzymes, respectively

some regions of mRNA may be either highly structured or bound by regulatory proteins, we selected each of the three siRNA target sites at different positions along the length of the gene sequence; the procedure is described in detail as follows. We did not see a correlation between the position of target sites on the mRNA and siRNA potency. Then we compared the potential target sites to the appropriate genome database and eliminated from consideration any target sequences with more than 16–17 contiguous base pairs of homology to other coding sequences. We used BLAST, which can be found on the NCBI server at: http://www.ncbi.nlm.nih.gov/BLAST. A complete siRNA experiment should include a negative control siRNA with the same nucleotide composition as siRNA but which lacks significant sequence homology to the genome. To design a negative control siRNA, we scrambled the nucleotide sequence of each of our prospective siRNAs and conducted a search to make sure that it lacked homology to any other gene.

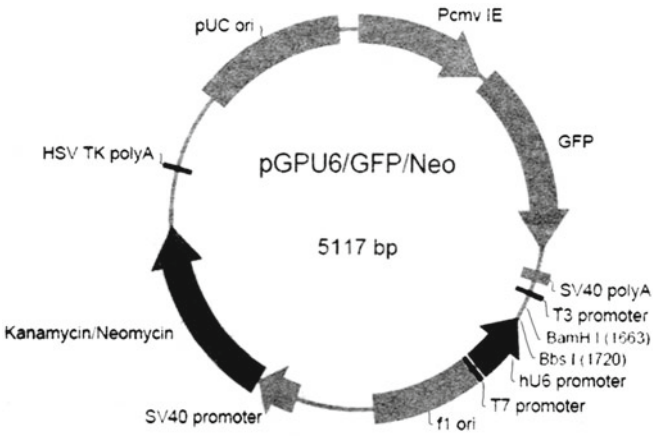

Fig. 2. pGPU6/GFP/Neo expression vector containing a RNA polymerase III expression element. Silencing efficiency was assayed by real-time fluorescent quantitation polymerase chain reaction by expression of green fluorescence protein (*GFP*)

Hairpin siRNA template oligonucleotide design and preparation

Two complementary oligonucleotide strands were designed with the following sequences: mini-TyrRS (Top strand: 5′-CAC CGC CTG CAC TTG GCT ATT CAA TTT CAA GAG AAA TTG AAT AGC CAA GTG CAG GTT TTT TG-3′; and bottom strand: 5′-GAT CCA AAA AAC CTG CAC TTG GCT ATT CAA TTT CTC TTG AAA TTG AAT AGC CAA GTG CAG GC-3′); mini-TrpRS (Top strand: 5′-CAC CGA GAT GTT GGT GTC ATT AAA TTT CAA GAG AAA TTT AAT GAC ACC AAC ATC TTT TTT TG-3′; and bottom strand: 5′-GAT CCA AAA AAA GAT GTT GGT GTC ATT AAA TTT CTC TTG AAA TTT AAT GAC ACC AAC ATC TC-3′). Five microliters of sense siRNA template oligonucleotide strand (100 μM), 5 μl of antisense siRNA template oligonucleotide strand (100 μM), and 5 μl of 10× siRNA annealing buffer solution were mixed, and 35 μl sterilized deionized H_2O was added to a final volume 50 μl. The reaction mixture was denatured at 95°C for 3 min, then annealed at room temperature for 20 min until it formed double-stranded oligonucleotides (ds-oligo).

Ligation of annealed siRNA template insert into pGPU6/GFP/Neo expression vector

pGPU6/GFP/Neo expression vector containing an RNA polymerase III expression element (Fig. 2) was used as the vector in this research. The annealed siRNA template insert was diluted with nuclease-free water to a final concentration of 100 nM. Two 10-μl ligation reactions were set up. The first was a plus-insert ligation: 1 μl of diluted annealed siRNA insert, 6.5 μl of nuclease-free water, 1 μl of 10× T4 DNA ligase buffer, 1 μl of pGPU6/GFP/Neo expression vector, and 0.5 μl of T4 DNA ligase (5 U/μl). The second was the minus-insert negative control: 1 μl of 1× siDNA annealing solution, 6.5 μl of nuclease-free water, 1 μl of 10× T4 DNA ligase buffer, 1 μl of pGPU6/GFP/Neo expression vector, and 0.5 μl of T4 DNA ligase (5 U/μl). T4 DNA ligase was used to clone the ds-oligo into the linear pGPU6/GFP/Neo expression vector. The ligation reactions were incubated overnight at 16°C for high ligation efficiency. TOPO 10 competent cells of *Escherichia coli* were transformed with the plus-ligation products and the minus-ligation products at room temperature for 10 min, on ice for 35 min, then spread on Luria Broth (LB) solid medium with kanamycin (100 μg/ml) and grown overnight at 37°C. Monoclonal colonies were selected 1 day later, and the plasmid DNA was isolated and digested with *Bam*HI and *Eco*RI. The plasmids were confirmed by sequencing by Genepharma.

siRNA formulation

We have previously demonstrated silencing of mini-TyrRS/mini-TrpRS in vitro using pGPU6/GFP/Neo expression vector.[35] In the current in vivo study, we used a liposomal formulation of SNALP to evaluate systemic delivery of siRNA directed towards mini-TyrRS/mini-TrpRS. The SNALP formulation contained the lipids *3-N*-[(ω-methoxypoly(ethyleneglycol)$_{2000}$)carbamoyl]-1,2-dimyristyloxy-propylamine(PEG-C-DMA),1,2-dilinoleyloxy-*N,N*-dimethyl-3-aminopropane(DLinDMA),1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), and cholesterol, in a 2:40:10:48 molar percent ratio.

Ethics

All animal procedures were conducted with prior institutional ethical approval under the requirements of the Chinese Prevention of Cruelty to Animals Act and the Code of Practice for the Care and Use of Animals for Scientific Purposes. Prior clearance was obtained from the Animal Experimentation Ethics Committees of West China Medical

Center and Institutes of Animal Science. The animals used in this study were inspected by members of the West China Medical Centre Animal Ethics Committee.

Left anterior descending (LAD) artery ligation

Animals were anesthetized with 100 g/l chloral hydrate (0.2 ml per 100 g body weight injected intraperitoneally). The analgesic buprenorphine was given preoperatively (10–20 μg/kg body weight). Occlusion of the left anterior descending coronary artery (LAD) was performed as previously reported,[11] under sterile conditions, with minor alterations. Briefly, the anesthetized animal was incubated endotracheally in a supine position, and ventilated with a Harvard Mouse Mini-Vent (Harvard apparatus, Marchhugstetten, Germany), which supplied 0.2–0.25 ml room air 120 times per minute. The animal was moved onto its right side, and a left thoracotomy in the third intercostal space provided access to the beating heart. After removing the pericardium, the LAD was visualized with a stereomicroscope (Leica MZ6, Heerbrugg, Switzerland), and occluded with 8-0 Prolene suture. The suture position of the LAD coronary artery was 0.3 mm distal to the atrioventricular junction. Occlusion was confirmed by observation of left ventricular pallor immediately post ligation and an electrocardiogram was used to observe changes such as widening of QRS and ST-T segment elevation. The chest was closed, the lungs reinflated, and the animal moved to a prone position until spontaneous breathing occurred. Animals were monitored closely for signs of infection at the surgical site; none were observed in any animals.

Experimental groups

A total of 80 rats (200–250 g) were divided into four different groups (n = 20 per group). (1) Sham group: animals underwent a thoracotomy with removal of the pericardium, but no LAD occlusion. No suture was placed in the sham animals' heart, in order to avoid unintended vessel damage or occlusion; (2) negative control group: LAD occlusion, but no mini-TyrRS/mini-TrpRS-siRNA transfected (saline injection); (3) mock transfection group: LAD occlusion, and mock transfected; (4) experiment group: LAD occlusion, and mini-TyrRS/mini-TrpRS-transfected.

In vivo silencing experiments

On three consecutive days, tail vein injections of saline or different siRNAs were given. All siRNAs were administered at dose of 3 μg/g in approximately 2 ml per injection.

Histologic expression

All of the rats were killed 1 month after ligation, then the heart was removed and fixed in fresh 4% paraformaldehyde, pH 7.4. The tissue was processed and embedded in paraffin using routine histological procedures. Five-micrometer transverse step sections were collected every 200 μm through the entire ventricle (approximately 10–12 sections per animal), and stained with hematoxylin–eosin (H&E).The sections were observed with an inverted phase-contrast microscope (Olympus, Tokyo, Japan) and photographed.

Measurement of irreversible ischemic injury

One month after coronary ligation, the heart was excised and cross-sectioned from the apex to the atrioventricular groove into four specimens of 0.8 mm in thickness with the use of a stereoscope. The two middle heart slices were incubated in 2,3,5-triphenyltetrazolium chloride (TTC) solution (1%) for 30 min in phosphate buffer at 37°C. Sections were fixed overnight in 4% paraformaldehyde for contrast enhancement between stained and unstained tissue. TTC stained the viable tissue with red, while the necrotic tissue remained discolored. The sections were then placed between two coverslips and digitally photographed using a Nikon Coolpix S10 camera, and quantified with the weight respectively. The area of irreversible injury (TTC negative) is presented as a percentage of the area (irreversible injury area/total weight of ventricles).

Measuring capillary density

The streptavidin peroxidase (SP) immunohistochemical method was used to detect the expression of factor VIII in myocardial infarction margin areas. The dilution of factor VIII rat monoclonal antibody (Santa Cruz, CA, USA) was 1:100. The procedure was performed according to the manufacturer's instructions. The positive cells were identified, counted, and analyzed under the inverted phase-contrast microscope (Olympus, Japan) in three different fields (0.1 mm^2) at 400× magnification using Image-proplus 6.0 software.

Western blot analysis

Myocardial cytoplasmic protein extracts were prepared according to the instructions of the cytoplasm proteins extraction kit. The extracted proteins (35 μg) were subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred onto PVDF membranes (Millipore, Billerica, MA, USA). The membranes were then placed into blocking buffer containing nonfat dry milk. Goat monoclonal antirat mini-TyrRS or mini-TrpRS IgG antibodies (final dilution 1:500) were used as the primary antibody, and HRP conjugated rabbit antigoat IgG (final dilution 1:10 000) was used as the secondary reagent. An internal control, HRP-conjugated GAPDH, and protein marker were also used in this experiment. Detection was performed using an ECL chemiluminescence kit. The data were scanned and ana-

lyzed by the Gel Doc 1000 gel imaging system (Bio-Rad, Hercules, CA, USA). The same test was repeated three times.

Statistical analysis

Results are expressed as mean ± standard deviation. Comparison of means was performed by means of the analysis of variance procedure (Student–Newman–Keuls test, SPSS 13.0 for Windows). $P < 0.05$ was considered statistically significant.

Results

H&E stain 1 month after myocardial infarction

In the sham group, cardiac muscle fibers were tightly aligned, and no new capillaries were found, while in other groups, obvious myocardial fibers rupture, inflammatory cell infiltration, granulation tissue, and new capillaries formation around the infarction zone were present. Compared with other groups, in the mini-TrpRS group cardiac muscle fibers were rarefaction aligned in spatium intermusculare, but the situation in the mini-TyrRS group was totally the opposite (Fig. 3).

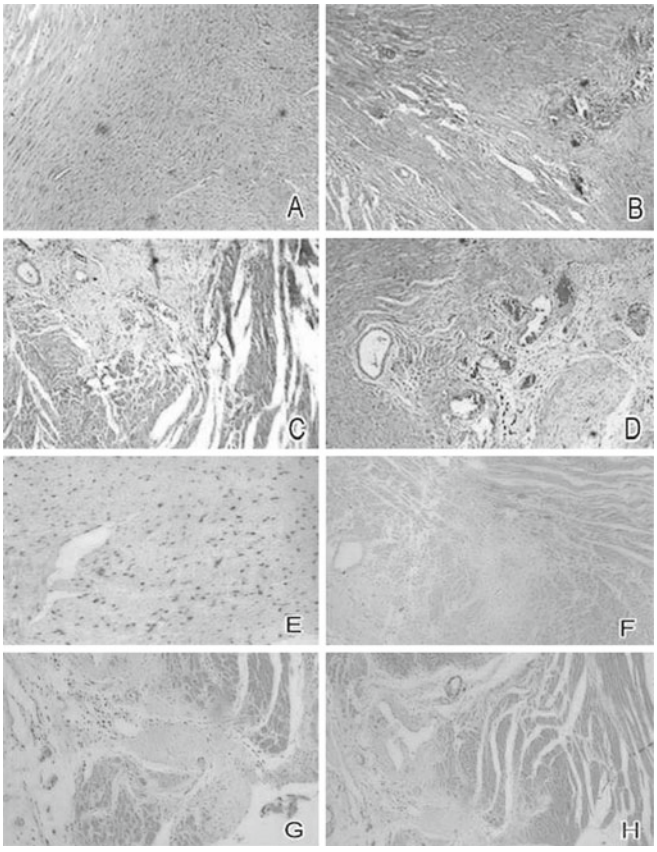

Fig. 3A–H. Mini-TyrRS-siRNA/mini-TrpRS-siRNA transfected H&E stain 1 month after myocardial infarction (×200). **A** and **E** (sham group); **B** and **F** (mock transfection group); **C** and **G** (negative control group); **D** (mini-TrpRS-siRNA group); **H** (mini-TyrRS-siRNA group). In the sham group, cardiac muscle fibers were tightly aligned, and no new capillaries were found; but in other groups myocardial fiber rupture, inflammatory cell infiltrates, granulation tissue, and new capillaries formation around the infarction zone were obviously present. Compared with other groups, in the mini-TrpRS-siRNA-transfected group cardiac muscle fibers were observed to be rarefaction aligned in spatium intermusculare and the amount of capillaries increased evidently, but the situation in the mini-TyrRS-siRNA-transfected group was totally the opposite

Measurement of irreversible ischemic injury (TTC staining)

No myocardial infarction was found in the sham group, but obvious infarction areas were seen in other groups. Compared with the negative control group (14.28 ± 1.36) and mock transfection group (14.19 ± 0.77), the myocardial infarction area of the mini-TyrRS-siRNA-transfected group (18.89 ± 0.92) was increased to a statistically significant degree ($P < 0.05$), and in the mini-TrpRS-siRNA-transfected group to a lesser degree (7.33 ± 1.55), $P < 0.05$. There were no significant changes between the mock transfection group and negative control group (Figs. 4 and 5).

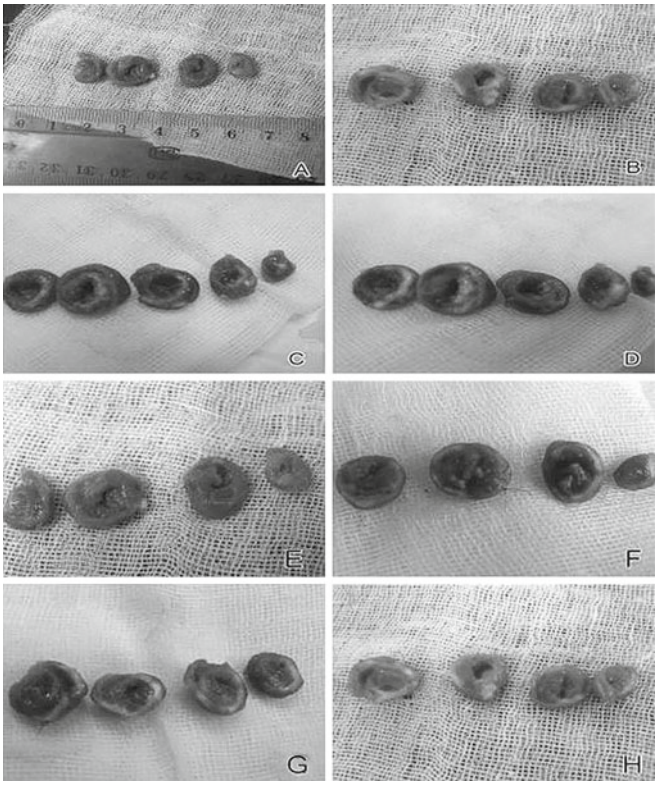

Fig. 4A–H. Mini-TyrRS-siRNA/mini-TrpRS-siRNA transfected TTC stain 1 month after myocardial infarction. **A** and **E** (sham group); **B** and **F** (mock transfection group); **C** and **G** (negative control group); **D** (mini-TyrRS-siRNA group); **H** (mini-TrpRS-siRNA group). In the sham group, no myocardial infarction was found, but more obvious infarction areas were seen in other groups. Mini-TyrRS-siRNA-transfected group had the most myocardial infarction areas, while the least were present in the mini-TrpRS-siRNA-transfected group

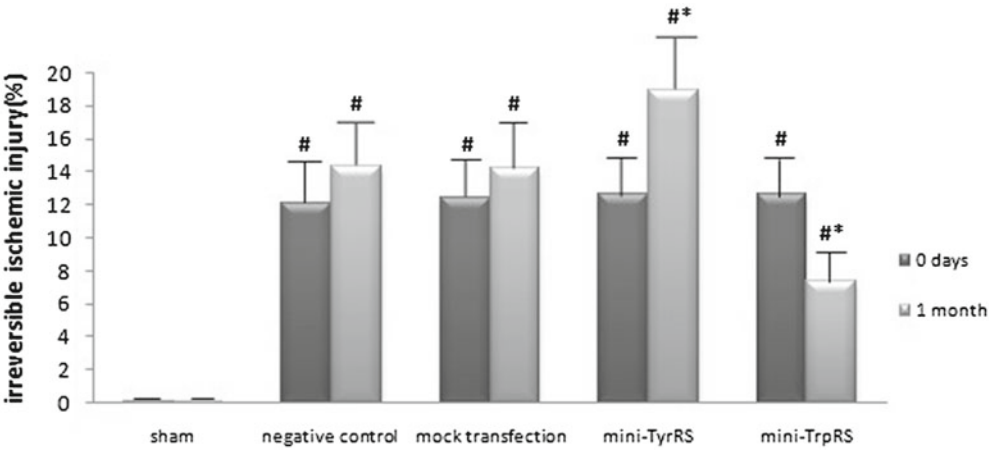

Fig. 5. Measurement of irreversible ischemic injury area after pGPU6/GFP/Neo-mini-TyrRS/mini-TrpRS-siRNA expression vector transfection. Compared with negative control group and mock transfection group, the myocardial infarction area increased in the mini-TyrRS- siRNA group, while it decreased in the mini-TrpRS-siRNA group. $^{\#}P < 0.01$ vs sham operation group; $^{*}P < 0.05$ vs mock transfection and negative control group

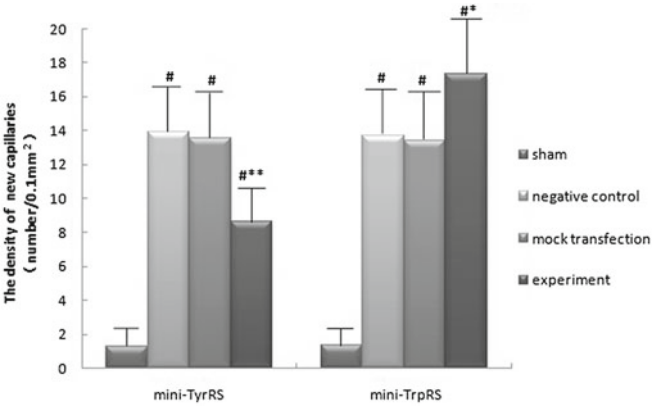

Fig. 6. Measurement of the density of new capillaries after pGPU6/GFP/Neo-mini-TyrRS-siRNA/mini-TrpRS-siRNA expression vector transfection. $^{\#}P < 0.01$ vs sham operation group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs mock transfection and negative control group

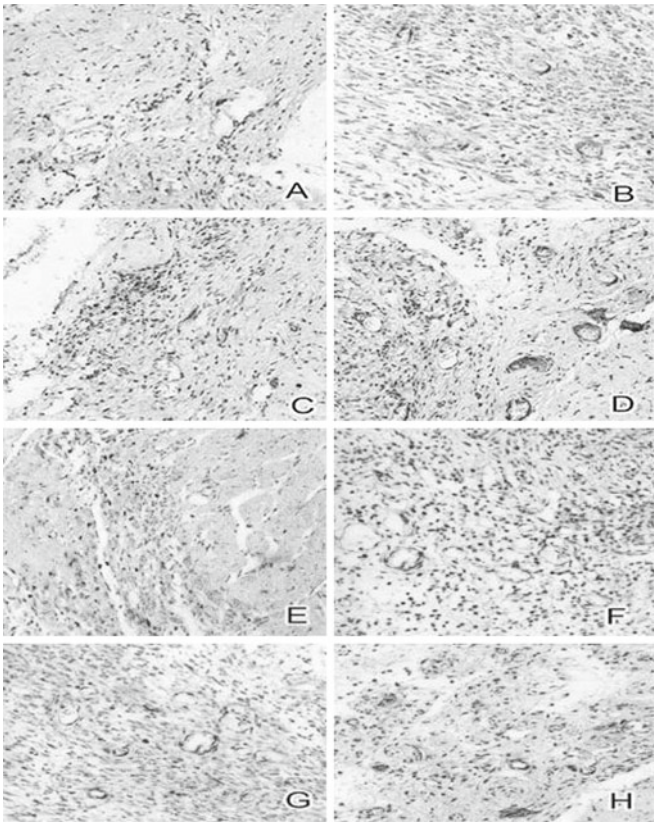

Fig. 7A–H. Mini-TyrRS-siRNA/mini-TrpRS-siRNA transfected streptavidin peroxidase (SP) immunohistochemical method 1 month after myocardial infarction (×400). **A** and **E** (sham group); **B** and **F** (mock transfection group); **C** and **G** (negative control group); **D** (mini-TrpRS-siRNA group); **H** (mini-TyrRS-siRNA group). In the sham group, no new capillaries were found, but were seen in other groups. Mini-TyrRS-siRNA-transfected group had the most new capillaries, while the least amount was present in the mini-TrpRS-siRNA-transfected group

Measurement of the capillary density (SP method)

The streptavidin peroxidase (SP) immunohistochemical method was used to detect the expression of factor VIII in myocardial infarction areas. The capillary density of mini-TyrRS-siRNA group (8.64 ± 2.34) was decreased compared with the negative control group (13.89 ± 1.47) and mock transfection group (13.56 ± 3.21), $P < 0.05$; but was increased in the mini-TrpRS-siRNA group (17.32 ± 3.76), negative control group (13.66 ± 2.51), and mock transfection group (13.38 ± 4.45), respectively, $P < 0.05$. There were also no significant changes between the mock transfection group and the negative control group (Figs. 6 and 7).

Protein expression of mini-TyrRS/mini-TrpRS at infarction marginal zone

Western blotting was used to detect the protein expression of mini-TyrRS/mini-TrpRS at the infarction zone. The results indicated that: the gradation value of mini-TyrRS/GAPDH (Fig. 8, 0.273) was considered statistically significant ($P < 0.05$) compared to the sham group (0.382), mock transfection group (0.724), and negative control group (0.775); while in the mini-TrpRS group (Fig. 9), values of 0.116, 0.411, 0.238, and 0.212, respectively, also showed a statistically significant difference ($P < 0.05$).

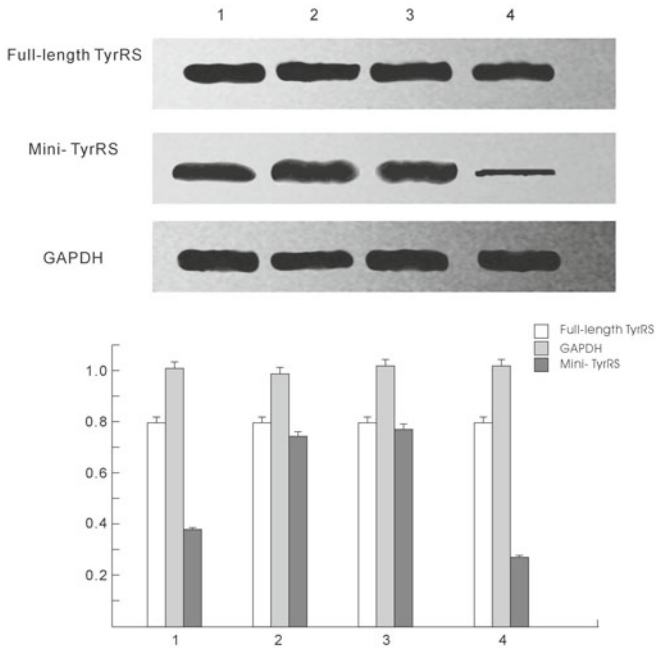

Fig. 8. Protein expression of mini-TyrRS at infarction marginal zone 1 month after transfection. *1*, sham group; *2*, mock transfection group; *3*, negative control group; *4*, experiment group. After transfection, the protein expression of mini-TyrRS was significantly decreased in the experiment group compared with the other three groups ($P < 0.05$).

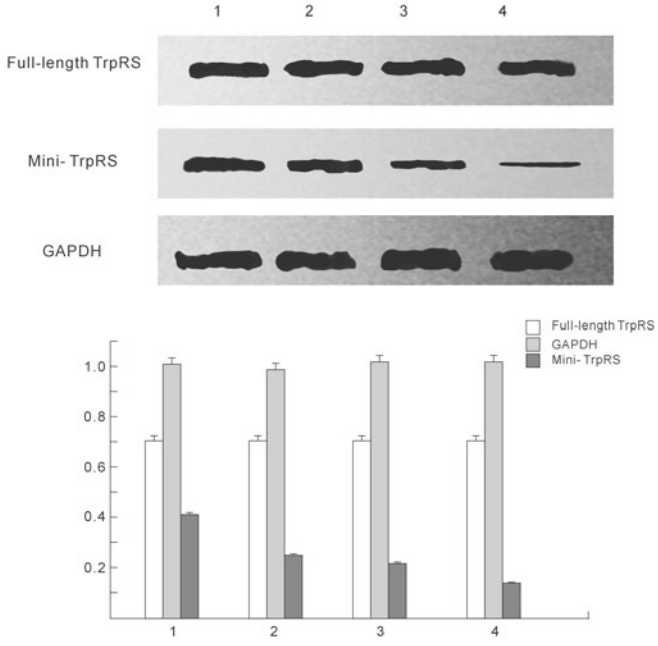

Fig. 9. Protein expression of mini-TrpRS at infarction marginal zone 1 month after transfection. *1*, sham group; *2*, mock transfection group; *3*, negative control group; *4*, experiment group. After transfection, the protein expression of mini-TrpRS was significantly decreased in the experiment group compared with the other three groups ($P < 0.05$)

Discussion

Preclinical models of myocardial ischemia have been reported in several large animal species, including dogs[36,37] and goats.[38] The model that most closely resembles the response seen in humans is the pig ameroid model,[39] which has been used in a variety of therapeutic studies.[40–48] However, the expense and practical demands of porcine surgical facilities severely limit the extent of such studies, precluding the use of this model for large-scale screening studies of novel therapeutic approaches.

RNA interference (RNAi) by double-stranded RNA (dsRNAs) molecules of approximately 20–25 nucleotides, termed short interfering RNAs (siRNAs) is a powerful method for preventing the expression of a particular gene. The dsRNA molecules can target mRNAs with complementary sequence for degradation via a cellular process.[49] This technique was first developed in *Caenorhabditis elegans*, and was rapidly applied to a wide range of organisms. Methods for expressing siRNAs in cells in culture and in vivo using viral vectors, and for transfecting cells with synthetic siRNAs, have been developed and are being used to establish the functions of specific proteins in various cell types and organisms.[50–52] For example, chemically synthesized or in vitro transcribed siRNAs can be transfected into cells, injected into mice, or introduced into plants.[53] siRNAs can also be expressed endogenously from siRNA expression vectors or PCR products in cells or in transgenic animals.[54] Using siRNA expression vectors has the advantage that the expression of a target gene can be reduced for weeks or even months,[55] eclipsing the 6–10 days typically observed with in vitro prepared siRNA used for transient transfection.[55]

In normal cells, human TrpRS exists as a full-length form and as a truncated form designated mini-TrpRS, which is produced by alternative splicing.[56] Expression of mini-TrpRS is highly stimulated in human cells by the addition of interferon-γ.[57–59] Although both human full-length TrpRS and mini-TrpRS are enzymatically active in aminoacylation, they differ in angiostatic activity.[23,60] The same phenomenon is also present between full-length TyrRS and mini-TyrRS. Research has found a kind of angiogenesis regulatory factor, CXC chemotactic factor, whose function is dependent on whether or not it has the Glu-Leu-Arg (ELR) motif.[61] The CXC chemotactic factors that have the ELR motif could promote angiogenesis, while CXC chemotactic factors that do not have the ELR motif would be the antagonistic factors of angiogenesis. The situation is analogous to human mini-TyrRS and mini-TrpRS. Mini-TyrRS has an ELR motif and promotes angiogenesis, but mini-TrpRS does not have the ELR motif. Therefore, their functions are totally contrasting.[62]

We were aware that angiogenic and angiostatic factors may work together to regulate angiogenesis. To test our hypothesis that mini-TyrRS (containing a natural ELR sequence) is an angiogenic factor as well as a PMN cell chemoattractant, and that mini-TrpRS (containing a natural ELQ sequence) is an angiostatic factor, we evaluated whether mini-TyrRS/mini-TrpRS induced myocardial cells to form new capillaries, using a rat myocardial infarction model. We found that irreversible ischemic injury areas and new capillary formation was increased after mini-TyrRS-siRNA transfected by using TTC staining (Figs. 4 and 5) and

the SP immunohistochemical method (Figs. 6 and 7), while being decreased in transfected mini-TrpRS-siRNA. Silencing efficiency was assayed by Western blotting. In contrast, angiogenesis was not observed with full-length TyrRS and TrpRS. Interestingly, angiogenesis is stimulated by either mini-TyrRS or is inhibited by mini-TrpRS in rat myocardial infarction models, raising the possibility that mini-TyrRS/mini-TrpRS stimulates a common downstream signaling event. Thus, naturally occurring fragments of the two proteins involved in translation, TyrRS and TrpRS, have opposing activity on angiogenesis in the rat model. The opposing activities of the two tRNA synthetases suggest tight regulation of the balance between pro- and antiangiogenic stimuli, but the exact mechanism and relationship between them has not been clearly demonstrated. More research should be done to further explore the mechanism.

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