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MicroRNA-221 sponge therapy attenuates neointimal hyperplasia and improves blood flows in vein grafts

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ABSTRACT

Background: Vein graft failure due to neointimal hyperplasia remains an important and unresolved problem of cardiovascular surgery. MicroRNA-221 (miR-221) has been shown to play a major role in regulating vascular smooth muscle cell (VSMC) proliferation and phenotype transformation. Thus, the purpose of this study is to determine whether adenovirus mediated miR-221 sponge gene therapy could inhibit vein graft neointimal hyperplasia.

Methods: Adenovirus encoding miR-221 sponge (Ad-miR-221-SP) was used to inhibit VSMC proliferation in vitro and neointimal formation in vivo. Expression of miRNA-221 was evaluated in cultured VSMC and in rat vein graft models following transduction with Ad-miR-221-SP, Ad-Control-SP (without miR-221 antisense binding sites), or Ad-GFP (control). To accelerate the transfer of miR-221 sponge gene to the vein grafts, 20% poloxamer F-127 gel was used to extend virus contact time and 0.25% trypsin to increase virus penetration.

Results: miR-221 sponges can significantly decrease the expression of miR-221 and proliferation in cultured VSMC. Cellular proliferation rates were significantly reduced in miR-221 sponge treated grafts as compared with controls at 6 weeks after bypass surgery (19.8% versus 43.6%, $P = 0.0028$). miR-221 sponge gene transfer reduced the neointimal area (210.75 ± 24.13 versus 67.01 ± 12.02, $P < 0.0001$), neointimal thickness (171.86 ± 27.87 versus 64.13 ± 16.23, $P < 0.0001$) and neointima/media ratio (0.74 ± 0.21 versus 1.95 ± 0.25, $P < 0.0001$) in vein grafts versus controls. miR-21 sponge treatment was also improved hemodynamics in vein grafts. We have further identified that p27 (Kip1) is a potential target gene of miR-221 in vein grafts.

Conclusion: miR-221 sponge therapy can significantly reduce miR-221 activity and inhibit neointimal hyperplasia in vein grafts. Locally adventitial delivery of adenoviruses mediated miRNA sponges may be promising gene therapies to prevent vein graft failure.

1. Introduction

Today, vein graft failure occurs as a result of neointimal hyperplasia remains an important and unresolved problem of cardiovascular surgery. Vascular smooth muscle cell (VSMC) migration from the media into the intima and proliferation in response to the release of a variety of mediators, growth factors, and cytokines is the key event in the development of neointimal hyperplasia [1–3]. Recent studies have pointed out the importance of microRNAs (miRNAs) regulate key genetic programs in cardiovascular biology, physiology, and disease [4–7]. Several miRNAs have been shown to play important roles in regulating VSMC functions in vitro and in vivo [8–12]. Both miR-221 and miR-222 have been demonstrated to be implicated in VSMC proliferation and neointimal lesion formation via their target genes p27 (Kip1) and p57 (Kip2) [13,14]. miR-221 and 222 have effects of pro-proliferation, pro-migration, and anti-apoptosis in VSMC. Furthermore, knockdown of miR-221 decreased VSMC proliferation, migration and neointimal formation in rat carotid artery [13]. These novel findings indicate that miR-221 may have extensive implications for the treatment of vein graft failure.

The present study is to determine whether adenovirus (Ad) mediated gene transfer miR-221 sponge therapy as a useful novel therapeutic approach could inhibit vein graft neointimal formation in rat models. Furthermore, to improve efficiency of Ad-mediated gene transfer of miR-221 sponges to the vein graft surface, we use poloxamer F-127

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gel to extend virus contact time and low concentration of trypsin to increase virus penetration.

2. Materials and methods

An expanded and detail Methods section is available in the online data supplement (see Supplementary material online, Methods).

2.1. Designing of MicroRNA-221 sponges

The basic approach for miRNA sponge design was developed by Kluiver [15]. In the current study we have modified this basic approach (Fig. 1A and B), for the generation of sponges that target miRNA, the oligonucleotide duplex approach can be used. Briefly, oligonucleotides are designed with two identical MBSs, but each MBS mismatches in the middle portion to create a bulge at positions 9–12 of the miR-221 sequence (Figs. 1B, 2A). The bulge was created to prevent RNA interference-type cleavage and degradation of the sponge RNA through endonucleolytic cleavage [15].

The two MBSs were separated by a short 4 nt sequence (AATT). The 5′ and 3′ ends of the oligonucleotide duplex consist of overhangs that were compatible with the PpuMI restriction endonuclease. Furthermore, PpuMI restriction endonuclease on the left of MBS was modified (5′-GG to 5′-CC) to allow flexibility of potential further directional subcloning (Fig. 2A).

The Ad vector containing the enhanced green fluorescent protein (Ad-GFP) reporter gene was kindly provided by the Key Laboratory for Regenerative Medicine of Chinese University of Hong Kong. To enable directional cloning of the oligo duplexes, the linker designed to introduce a PpuMI restriction enzyme recognition site in the Ad. A 5′PspXI site and a 3′BglII site were added to the ends of the linker to efficient directional subcloning (Fig. 2B).

By ligating oligo duplexes with PpuMI compatible ends with PpuMI digested Ad-GFP vector, sponge constructs with a variable number of MBS were generated by a single ligation reaction (Fig. 1B).

2.2. Construction of the adenoviruses

The miR-221 sponge oligonucleotide and the reverse complement were synthesized at BGI (Shenzhen, China). The sequences were as follows: (1): sense: 5′-GTCGGGAAACCCAGCCCTTAATGTAGCT AATTGA AACCCAGCCCTTAATGTAGCT GG-3′ and (2): antisense: 5′-GACCCAGC TACATTCAGGGCTGGGTTTC AATTAGCTACATTAGGGCTGGGTTTCCC-3′. The two oligonucleotides were annealed to make double stranded DNA fragment with PpuMI overhangs. The sponge linker oligonucleotide and the reverse complement were synthesized at BGI (Shenzhen, China). The sequences were as follows: (1): 5′-TCGAGCCTGGATATCG ACGGGTCCCGACTCTAGAGACA-3′ and (2): 5′-GATCTGTCTCTAGAGTCG GGACCCGTCGATATCCAGGC-3′ for sponge linker and complementary oligos, respectively. Polynucleotide Kinase (PNK) (Roche Applied Science, Indianapolis, USA) was used to add a phosphate to the 5′ end of a DNA fragment. Mix equal volumes of both sense and antisense oligonucleotides in a PCR tube. Place the tubes in a thermal cycler and set up a program to perform the following profile: (a) heat to 95 °C and remain at 95 °C for 5 min, (b) ramp cool to 65 °C for 10 min, (c) ramp cool to 22 °C for 60 min, and (d) proceed to a storage temperature of 4 °C. The control sponge was described previously [16].

2.3. VSMC isolation and culture

Primary rat VSMCs were isolated from aortas of male Sprague–Dawley rats (150 g) by explant method as described [17]. VSMC grew out from the arterial tissues and formed a confluent layer after several weeks. The purity of VSMC was verified by smooth muscle α-actin immunofluorescence, and cells at passages 3 to 6 were used in all experiments.

2.4. Surgical procedures and vein graft treatment

Male Sprague–Dawley rats (6–8 weeks old, 200–250 g) underwent interposition bypass grafting from the autologous jugular vein to the carotid artery. The vein grafting procedures were similar to what was described previously [18].

To locally deliver Ad vectors to the vein graft walls and avoid any potential systemic side effects, we applied an established local delivery model via F-127 pluronic gel as previously described [19–21]. All protocols involving experimental animals were approved by the Institutional Animal Care and Use Committee of Chinese University of Hong Kong, and all experimental procedures were complied with the guide for the care and use of laboratory animals.

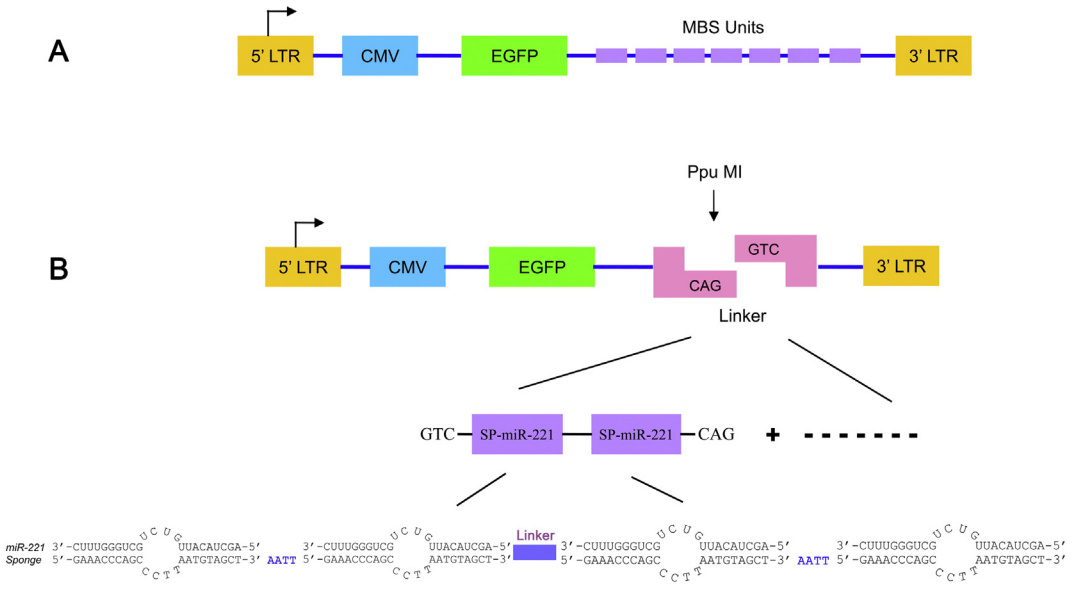

Fig. 1. Design of miR-221 sponges and directional cloning strategy. (A) Schematic overview of construction of adenoviruses mediated miRNA sponges. (B) The method to ligate miR-221 sponge oligonucleotide duplexes into the Ad. Each oligonucleotide duplex contains two MBSs and phosphorylated PpuMI restriction enzyme compatible overhangs to generate miR-221 sponge with varying sizes using a single ligation reaction.