Use of small interfering RNA to target matrix metalloproteinase-9 to suppress stent-induced tissue hyperplasia in rats

Ji Hoon Shin,MD Dept. of Radiology, University of Ulsan Asan Medical Center, Korea



Introduction

Benign esophageal stricture is a common complication in cases of caustic ingestion, esophageal surgery, and radiotherapy. Stenting technique is a valid alternative when other local therapies are not effective. Although the short-term effect of stenting is good, the long-term effect is limited by complications like tissue hyperplasia (TH) secondary to stent placement.

Recently, RNA interference (RNAi) technique has been popular to down-regulate protein expression at the level of RNA transcription. RNAi is an RNA-dependent gene silencing process that is controlled by the RNA-induced silencing complex and is initiated by short double-stranded RNA molecule. Combination of RNAi technique to prevent TH is limited.

Purpose

To establish character, level, and expression of MMP-9 in TH and to evaluate the efficacy of RNAi therapy using Chol-R9 delivered siRNA targeting MMP-9 to prevent TH after bare stent insertion in a rat esophageal model

Materials and Methods

After 3 weeks following esophageal stent placement (5㎜ diameter & 15㎜ length) in 10 rats, esophageal samples were subjected to histological examination and quantification of MMP-9 by zymography, western blotting and quantitative real-time polymerase chain reaction (qRT-PCR). Chol-R9 was used to deliver MMP-9 targeted siRNA into rat esophagus. Ten rats underwent Chol-R9 delivered siRNA targeting MMP-9 at 1 week and 2 weeks after stenting and were sacrificed at 3 weeks. To assess the relationship between the therapeutic effect of Chol-R9/siRNA complex and MMP-9 over-expression in tissue hyperplasia, degree of TH and change of MMP-9 level were evaluated in esophagus samples (rat's esophagus of Chol-R9/siRNA treatment group).

In vitro selection of most potent siRNA

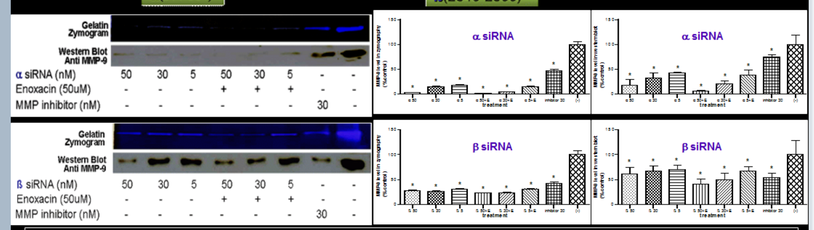


Fig 1. MMP-9 down-regulation effect of siRNA in 9L cells. 9L cells were transfected with various conc. of siRNA (α siRNA, ß siRNA) against rat MMP-9 in the absence or presence of enoxacin. 9L cells were treated with 30 nM MMP-9 inhibitor as control. Enoxacin potentiates additional synthetic siRNA-induced gene knockdown. MMP-9 down-regulation was assessed by zymography and western blot analysis after 48 hr incubation. * : p<0.05 vs. control group (-).

Synthesis of Chol-R9

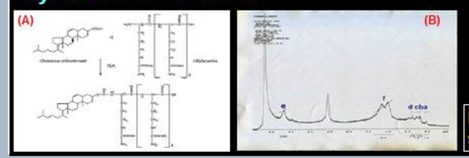


Fig 2. Synthesis scheme (A) and ^{1}H NMR spectrum (B) of Chol-R9 conjugate.

Gene transfer by instillation using balloon dilation

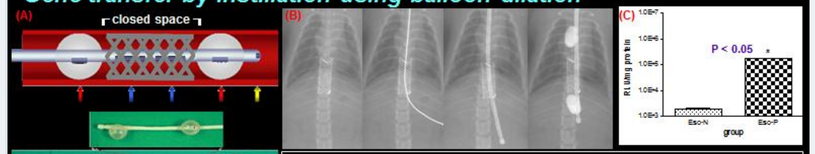


Fig 3. (A) Micro double balloon catheter & schematic figure showing closed space for gene therapy. (B) Insertion of double balloon catheter into the stent in the rat esophagus. Inflation of two balloons created a protected space where solution containing siRNA would be injected. (C) Transfection efficiency of Chol-R9 was measured by luciferase assay. Note. Eso-P : rat's esophagus tissue of Chol-R9/pCMV-luc treatment group

Results

MMP-9 expression in rat esophagus stenting model

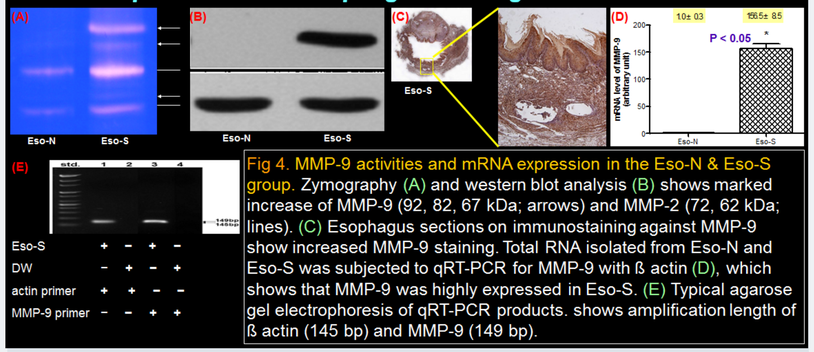


Fig 4. MMP-9 activities and mRNA expression in the Eso-N & Eso-S group. Zymography (A) and western blot analysis (B) shows marked increase of MMP-9 (92, 82, 67 kDa; arrows) and MMP-2 (72, 62 kDa; lines). (C) Esophagus sections on immunostaining against MMP-9 show increased MMP-9 staining. Total RNA isolated from Eso-N and Eso-S was subjected to qRT-PCR for MMP-9 with ß actin (D), which shows that MMP-9 was highly expressed in Eso-S. (E) Typical agarose gel electrophoresis of qRT-PCR products. shows amplification length of ß actin (145 bp) and MMP-9 (149 bp).

Complex formation of Chol-R9 with pCMV-luc or siRNA

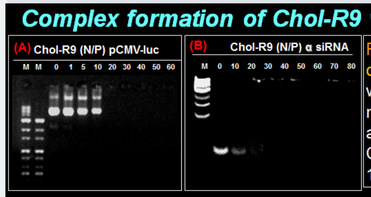


Fig 5. Electrophoretic patterns of Chol-R9/pCMV-luc or α siRNA complexes. 1.25 ㎍ pCMV-luc (or α siRNA) was incubated with various amounts of Chol-R9 for 30 min. The Chol-R9/pCMV-luc complex formations were assessed by 1% agarose gel retardation assay (A) and Chol-R9/α siRNA complex formations were assessed by 13% polyacrylamide gel assay (B).

Note. (A)-M : 1 kb plus step DNA Ladder, 100 bp DNA Ladder, (B)-M : 100 bp DNA Ladder, 0 ~ 90 : N/P ratio of complex, G : 5% glucose solution

Chol-R9 influence on cell growth

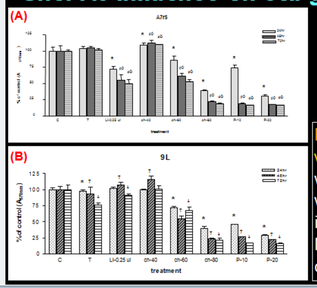


Fig 6. Time- and dose -response effect of complexes on viability of rat A7r5 (A) and 9L (B) cells. Cells were cultured with different N/P ratios for 24, 48 and 72 hr, and cell viability was determined by the MTT assay. Results from three independent experiments were shown as mean ± SD. Note. C : untreated cell, T : telebrix, Li : lipofectamineTM 2000, ch : Chol-R9, P : PEI, *, †, ‡ : p<0.05 vs. control group.

Transfection efficiency of Chol-R9

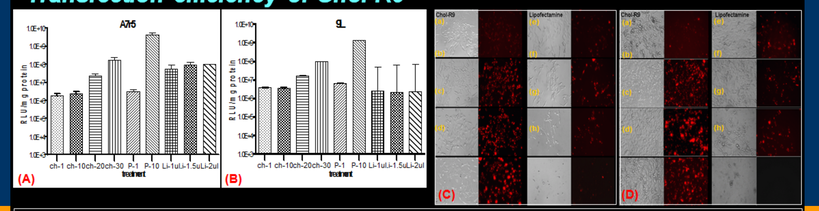


Fig 7. Gene expression after the transfection at various N/P ratios of Chol-R9/pCMV-luc complex in A7r5 and 9L cells. (A,B) Transfection efficiency of Chol-R9 was measured by luciferase assay and compared with PEI and lipofectamine. The graph represents mean values ± SD of experiments run in triplicate. Transfection efficiencies of Chol-R9/Alexa at 24 hr. 9L cells were transfected with Chol-R9 of various N/P ratios (a-d :1, 10, 20, 30). Transfection efficiencies of Chol-R9 conjugates compared to lipofectamine control (e-h : 1, 1.5, 2, 2.5 ㎕). The picture was taken 24hr (C) and 72hr (D) after transfection. Transfection by Chol-R9 is more efficient than lipofectamine.

Inhibition of tissue hyperplasia with Chol-R9/siRNA complex

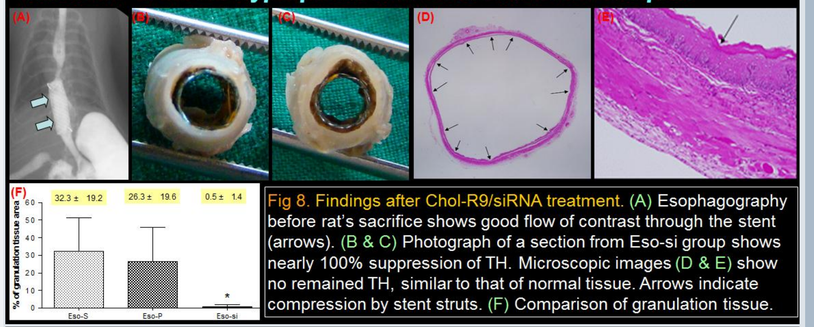


Fig 8. Findings after Chol-R9/siRNA treatment. (A) Esophagography before rat's sacrifice shows good flow of contrast through the stent (arrows). (B & C) Photograph of a section from Eso-si group shows nearly 100% suppression of TH. Microscopic images (D & E) show no remained TH, similar to that of normal tissue. Arrows indicate compression by stent struts. (F) Comparison of granulation tissue.

Inhibition of MMP-9 with Chol-R9/siRNA complex

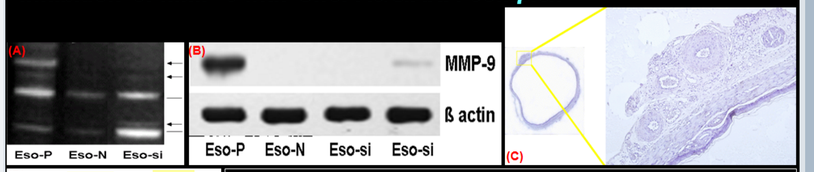
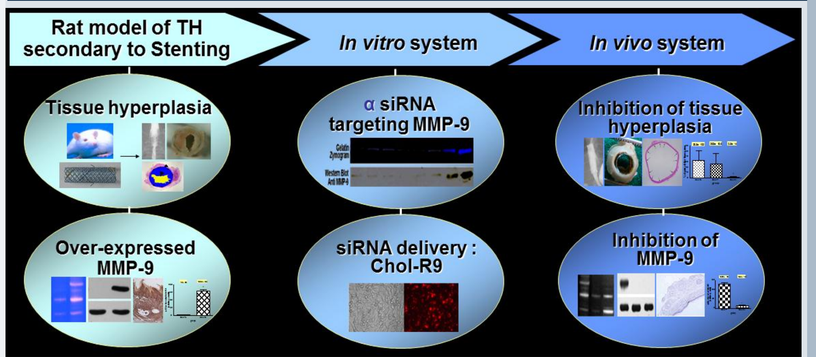


Fig 9. Inhibitory effect of Chol-R9/siRNA on MMP-9 activities. 10 rats of each group underwent a Chol-R9/siRNA or Chol-R9/pCMV-luc (control) complex 1 and 2 weeks after esophageal stenting. Zymography (A) and western blot analysis (B) showed suppression of MMP-9 (arrows) (C) Immunostaining against MMP-9 in Eso-si group shows disappeared or significantly decreased MMP-9 staining. (D) Relative comparison value of MMP-9 mRNA expression in Eso-P or Eso-si and Eso-N, shows that MMP-9 was greatly reduced in Eso-si. * : p<0.05 vs. Eso-P group.

Summary



- MMP-9 may play a key prognostic factor in TH secondary to stent placement.
- RNAi has great potential to prevent the expression of disease-associated proteins in the first place at the RNA level, unlike most other drug classes today that act at the protein level. RNAi technique can be applied to various vascular and non-vascular luminal structures.

www.PosterPresentations.com