Survivin-targeted siRNA loaded polysaccharide nanoparticles for anti-tumor therapy in mice

Zhong-gao Gao1, Fei-fei Yang1, Wei Huang1, Ming-ji Jin1

1Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100050, China. zggao@imm.ac.cn

ABSTRACT SUMMARY

The purpose of this study was to use polysaccharide vector TAT-g-CS we synthesized to deliver functional siRNA and evaluate its in vivo anti-tumor activity. We use siRNASur targeting survivin gene to assess the in vitro and in vivo delivery capacity of TAT-g-CS and its anti-tumor effects. The results demonstrated that TAT-g-CS/siRNASur nanoparticles not only strongly inhibited the in vitro proliferation of 4T1-Luc tumor cells via inducing cell apoptosis, but also effectively inhibited the in vivo growth and metastasis of malignant breast tumor, which suggested that TAT-g-CS/siRNA nanoparticle was a highly efficient non-viral system for siRNA delivery, especially for anti-tumor therapy based on siRNA therapeutics.

INTRODUCTION

It is well known that chitosan has been used as non-viral vector to deliver plasmid DNA and siRNA [1,2]. However, the delivery potential of TAT surface-modified chitosan nanoparticles has not been investigated. In the present study, we directly grafted the TAT peptides at the site of the primary amino groups of chitosan (CS) molecules to synthesize the copolymer TAT-g-CS as non-viral vector for siRNA delivery. The structure composition and cytotoxicity of copolymer TAT-g-CS were well characterized. Importantly, we used TAT-g-CS to load survivin-targeted siRNA to prepare nanoparticles for anti-tumor therapy, and the anti-tumor effects of such nanoparticles in vitro and in vivo were investigated in detail.

EXPERIMENTAL METHODS

The synthesis route of copolymer TAT-g-CS is shown in Fig. 1. TAT-g-CS was synthesized through a coupling reaction between primary amine groups in CS molecules and carboxyl groups of TAT peptide using EDC and NHS as coupling reagents. The structure of synthetic copolymers was characterized with nuclear magnetic resonance (NMR) (Fig. 1).

RESULTS AND DISCUSSION

The results of 1H NMR are showed in Fig. 1. The δ = 3.5-4.1 ppm was attributed to the protons of -CH2- in CS, while the δ = 2.5-3.0 ppm and δ = 0.5-2.0 ppm were attributed to -NH2-CH2- of spermine protons in the TAT peptide. Also, the δ = 4.0 ppm and δ = 6.5-7.5 ppm were respectively attributed to the protons of glycine and the methine proton of the tyrosine in TAT. Based on the results of 1H NMR, it was also concluded that TAT peptides were conjugated with the primary amino groups of CS. According to the characteristics peaks integral values of the tyrosine and CS, the substitution degree of TAT peptides was calculated as being 12.9%.

![Fig. 1. The synthesis route and NMR. TAT was used as a Cell penetrating peptide (CPP), and TAT peptide with the amino acid sequence of GCAGGGYGRKKRRQRRR was synthesized by GL Biochem Ltd. 1H NMR spectra of TAT-g-CS (a) and TAT (b).](image)

Although part of the amino groups (12.9%) was replaced with TAT peptide fragments, there were still enough free amino groups at the C-2 position of CS backbone in the synthesized TAT-g-CS molecules. Also, these amino groups can still contribute a certain density of positive charge to TAT-g-CS molecules. Therefore, it was inferred that TAT-g-CS could also sufficiently condense siRNA to form nanoparticles by electrostatic interaction. In our study, we firstly performed the gel retardation assay to explore the TAT-g-CS vector’s ability to load siRNA. The assay result is shown in Fig. 2a, and particle size
is presented in Fig. 2b. It was found that the characteristic band of siRNA disappeared when the weight ratio of TAT-g-CS to siRNA reached to 90:1 as shown in Fig. 2a (a to h means the weight ratio of TAT-g-CS to siRNA are 0:1, 30:1, 60:1, 90:1, 120:1, 150:1, 200:1, 250:1 ratio, respectively). It was hence not only confirmed that TATg-CS could condense siRNA by electrostatic interaction, but also suggested that siRNA could be completely loaded to TAT-g-CS molecules at a suitable weight ratio.

![Fig. 2](image)

Fig. 2. The gel retardation assay to explore the TAT-g-CS vector’s ability to load siRNA (a); Transmission electron microscopy (TEM) image of TAT-g-CS/siRNA nanoparticles. The scale bar represents 200 nm (b). Anti-tumor effect of TAT-g-CS/siRNAsur nanoparticles on tumor-bearing mice after i.v. administration (c and d).

In this study, we tried to employ TAT-g-CS/ siRNAsur nanoparticles to inhibit the tumor growth in vivo. Firstly, 4T1-Luc cells were inoculated into female BALB/c mice to establish a malignant breast tumor model. When tumor volume reached to about 150 mm3 (on the 13th day after inoculation), TAT-g-CS/siRNAsur nanoparticles and naked siRNAsur were administrated to tumor-bearing mice for anti-tumor therapy, respectively. The monitor results of tumor volume showed that the tumors of mice in naked siRNAsur and NS control groups both grew quickly (Fig. 2c and d). There was no remarkable difference between the tumor growth curves of these two groups, which suggested that the naked siRNAsur could not inhibit the tumor growth. However, the tumors of mice in TAT-g-CS group grew very slowly (Fig.3), which confirmed that TAT-g-CS/siRNAsur nanoparticles showed the strong inhibitory effect on tumor growth in vivo.

Bioluminescent images of mice treated with TAT-g-CS/siRNAsur nanoparticles and naked siRNAsur to tumor-bearing mice for anti-tumor therapy are shown in Fig. 3. At the end of treatment (day 48 after inoculation), TAT-g-CS/siRNAsur nanoparticles achieved a better control of tumor metastasis, and the fewest luciferase activities of tumor metastasis compared with the naked siRNAsur and control groups, (Fig. 3).

![Fig. 3](image)

Fig. 3. Representative tumor tissue image of tumor-bearing mice respectively treated with normal saline, naked siRNAsur and TAT-g-CS/siRNAsur nanoparticles on the final i.v. administration treatment. (siRNALuc: 5’-CUUACGUGAGAGUGAUCATT-3’).

CONCLUSION

TAT-g-CS vectors were used to load the siRNAsur targeting surviving gene to prepare nanoparticles for anti-tumor therapy. These TAT-g-CS/siRNAsur nanoparticles could not only effectively inhibit the proliferation of 4T1-Luc tumor cells in vitro, but also show the strong inhibitory effect on the growth and metastasis of malignant breast tumor in vivo, which suggested that TAT-g-CS/siRNA nanoparticles could serve as a highly efficient non-viral system for siRNA delivery, especially for anti-tumor therapy based on siRNA therapeutics. In the future study, the cellular uptake mechanism of TATg-CS/siRNA nanoparticles will be explored for better understanding of its siRNA delivery potential.

REFERENCES