

siRNA Nanomedicine for Cancer Therapy

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ABSTRACT

A polyelectrolyte complex (PEC) micelle-based siRNA delivery system was developed for treatment of cancer. A PEGylated siRNA targeting human vascular endothelial growth factor (hVEGF) was synthesized. The hVEGF expression was almost completely inhibited in vitro as well as in vivo by treating prostate cancer cells with the PEC micelles formed from the interaction between the conjugate and polyethylenimine (PEI). The efficient hVEGF gene silencing by hVEGF siRNA-PEG/PEI complexes dramatically suppressed tumor neovascularization and tumor growth in animals with PC-3 tumor xenograft.

Keywords: VEGF, siRNA, polyelectrolyte complex micelle, cancer gene therapy

1 INTRODUCTION

RNA interference (RNAi), a post-transcriptional gene silencing phenomenon initiated by small interfering RNAs (siRNAs), has recently been emerged as a powerful tool for treatment of various gene-related diseases (1). However, the therapeutic use of siRNA in clinical applications is still problematic due to the inherent instability in the presence of serum components in vivo and poor cellular uptake efficiency at a target site. The siRNA should be delivered into the target cells efficiently and stably to attain sufficient therapeutic effect. In our previous studies, we introduced a new delivery system for antisense ODNs, based on polyelectrolyte complex (PEC) micelles (2,3). The PEC micelles generated by interacting PEG-conjugated ODN with a polycation, such as PEI, showed enhanced cellular uptake and increased intracellular activity of the nucleic acid drugs. In this study, siRNA was attached to PEG via a disulfide linkage to overcome the obstacles of siRNA delivery problems. siRNA-s-s-PEG conjugates were used to produce the siRNA PEC micelles by interacting with PEI. The reducible disulfide linkage between PEG and siRNA was designed to be cleaved in the reductive intracellular environment. The inhibition of VEGF expression and tumor growth by VEGF siRNA-s-s-PEG/PEI formulation were evaluated in vitro and in vivo.

2 EXPERIMENTAL METHODS

2.1 Preparation of siRNA-PEG conjugate.

To synthesize VEGF siRNA-s-s-PEG conjugate, 3'-amine-siRNA was activated with SPDP. After 1 h incubation, un-reacted reagent free SPDP was removed from the mixture. The SPDP-siRNA was reacted with mPEG5,000-SH overnight. After further incubation for 1 day at 4°C, the reaction solution was dialyzed against water to remove un-reacted mPEG-SH. The siRNA-PEG conjugate solution was concentrated to the level of siRNA working solution (0.5 mg/ml).

2.2 Cell culture and transfection.

Human prostate carcinoma cells (PC-3) were maintained in 10 % serum RPMI1640 medium. Twenty hours after cell plating, various siRNA formulations were transfected to the cells. After the transfection, VEGF proteins secreted from the siRNA transfected cells were collected for 18 h and analyzed by using human VEGF ELISA kit.

2.3 Tumor model and treatment.

PC-3 cells were inoculated subcutaneously in the right flank of nude mice with 5×10^6 cancer cells. When the tumor volume attained 50 mm³, various siRNA formulations (7.5 µg siRNA/tumor, injection volume = 50 µl) were intratumorally injected into tumor regions. For the systemic delivery of siRNA, 20 µg siRNA (injection volume = 100 µl) was regularly injected via tail vein. Tumor sizes were measured at regular intervals with a caliper. The extent of angiogenesis in tumor region was evaluated by immunohistochemistry using Von Willibrand factor (factor VIII) antibody.

3 RESULTS

3.1 Formation of PEC micelles.

PEG was conjugated to the 3'-end of the sense strand of VEGF siRNA via a reducible disulfide linkage (siRNA-PEG). The molecular design of the siRNA-PEG conjugate maintained the structural integrity of the siRNA-PEG/PEI PEC micelles prior to cellular uptake. Within cells, un-PEGylated and intact siRNA could be liberated into the

intracellular space. The PEC micelles had a size of 50 - 80 nm as observed by atomic force microscopy (AFM) (Figure 1).

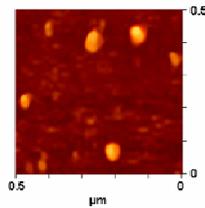


Figure 1. AFM image of siRNA-PEG/PEI polyelectrolyte complex (PEC) micelles.

3.2 In vitro characterization of PEC micelles.

In vitro gene silencing efficiency of the PEC micelles was evaluated in human prostate carcinoma cells (PC-3) (Fig. 2). The VEGF siRNA-PEG/PEI PEC micelles exhibited much higher silencing of VEGF expression than the siRNA/PEI complexes, suggesting that VEGF siRNA can suppress VEGF expression in a highly sequence specific manner.

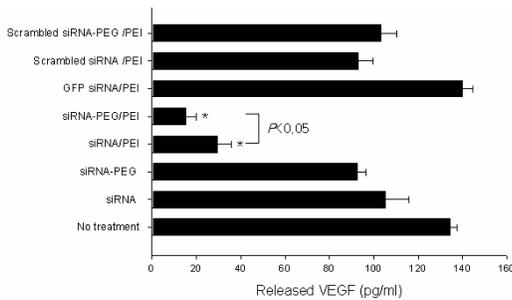


Figure 2. VEGF siRNA-PEG/PEI PEC micelles delivered into prostate cancer cells. VEGF sequence-specific gene silencing by VEGF siRNA-PEG/PEI PEC micelles in prostate cancer cells (PC-3).

3.3 Anti-tumor effect after intratumoral injection of PEC micelles

The therapeutic efficacy of VEGF siRNA-PEG/PEI PEC micelles was evaluated by monitoring tumor growth in an animal model after intratumoral injection. The tumor suppression effect was also highly dependent on the siRNA formulation. The VEGF siRNA-PEG/PEI PEC micelles exhibited the most pronounced effect on tumor suppression (Fig. 3A). At day 36 after injection, the relative tumor volumes for groups injected with the siRNA-PEG/PEI PEC micelle, siRNA/PEI complex, and naked siRNA were reduced to 13.3 %, 32.8 %, and 55.4 %, respectively, compared to the mock-treated group.

3.4 Anti-tumor effect after intravenous injection of PEC micelles.

By systemic injection, tumor growth was significantly suppressed for the siRNA/PEI complex or siRNA-PEG/PEI PEC micelle injection group. Naked siRNA and scrambled siRNA had a minor inhibitory effect on tumor growth (Fig. 3B). PEC micelles exhibited the most dramatic effect on inhibiting intratumoral neovascularization and tumor growth, suggesting the feasibility of using VEGF siRNA-PEG/PEI PEC micelles for systemic anti-angiogenic cancer therapy.

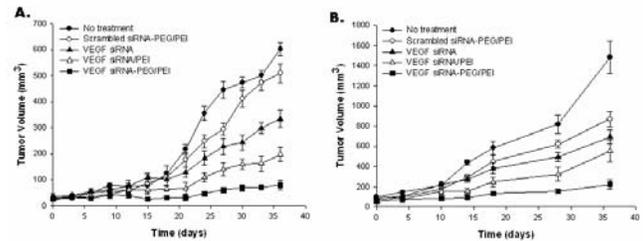


Figure 3. Tumor suppression effect by intratumoral (A) and intravenous (B) injection of VEGF siRNA-PEG/PEI PEC micelles.

4 CONCLUSIONS

In conclusion, we demonstrated a novel approach to siRNA-based gene therapy for cancer treatment. The PEC micelles provide not only a protective core-shell structure against the harsh biological environment, but an opportunity for passive targeting to the tumor site owing to their stable nano-scale colloidal feature. The unique physico-chemical characteristics of the PEC micelles maximize the silencing effect of the anti-angiogenic siRNA, leading to successful suppression of tumor growth in an animal tumor model. The current PEC micelle formulation, different from other previously reported polymer and lipid-based nano-complexes, holds great potential for clinical applications of siRNA and its use in cancer gene therapy.

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