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Octaarginine-modified multifunctional envelope-type nano device for siRNA

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Abstract

The multifunctional envelope-type nano device (MEND) is a novel non-viral gene delivery system for plasmid DNA (pDNA) and oligodeoxynucleotides (ODN). We showed previously that octaarginine-modified MEND (R8-MEND) produces a high transfection activity without cytotoxicity via macropinocytosis and efficient release of a condensed DNA core to the cytosol. In the present study, we succeeded in developing an efficient method for packaging siRNA into the R8-MEND, and its silencing effect was compared with that of transfection reagent TransIT-TKO. A polycation able to condense siRNA was screened for by measuring the size and zeta-potential of complexes formed between siRNA and the polycations poly-l-lysine (PLL), stearyl octaarginine (STR-R8) and protamine. Only STR-R8 was able to condense siRNA to form nano particles (<100 nm), whereas all three polycations were able to condense pDNA or ODN. The siRNA packaged in R8-MEND inhibited luciferase activity by more than 80% in HeLa cells stably expressing luciferase. Much amount of siRNA was internalized into the cells as R8-MEND, and siRNA was effectively released from lipid envelope of MEND to cytoplasm near the nucleus. Consequently, R8-MEND can deliver condensed siRNA into cells to produce an efficient and persistent silencing effect with minimum cytotoxicity.
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Keywords: siRNA; Non-viral delivery system; Liposomes; Octaarginine; Multifunctional envelope-type nano device

1. Introduction

Synthetic siRNA can silence specific genes by RNA interference (RNAi), which is considered a more powerful tool for gene silencing than anti-sense oligodeoxynucleotide (ODN) technology [1–4]. Delivery of sufficient siRNA into the cell is required to induce an efficient RNAi effect, although siRNA is known to be recycled by the RNA-induced silencing complex (RISC) [5,6]. In general, synthetic siRNA is transfected into cells in vitro by various commercially available cationic liposomal reagents as a cationic liposome/siRNA complex [7–12]. However, cytotoxicity of the cationic lipids is widely recognized as a serious problem, especially in the case of vulnerable cells like neurons. Moreover, control of the intracellular fate of the cationic liposome/siRNA complexes also needs to be improved because endocytosis, which is the dominant cellular uptake mechanism of LipofectAMINE PLUS/plasmid DNA (pDNA) complex, often directs the complex into the lysosome [7,13]. Thus, reducing toxicity and controlling intracellular trafficking are required for efficient introduction of siRNA into cells.

Many groups are currently in the process of developing various carrier systems for siRNA delivery, such as polyplex-type and lipoplex-type, to improve those drawbacks by incorporating some functional devices [14–16]. Several polyplex-type vectors have been reported. Grzelinski et al., used a well known cationic polymer polyethylenimine to deliver siRNA for silencing of secreted growth factor pleiotrophin (PTN), which has been over-expressed and functionally relevant in glioblastoma. The polyethylenimine/siRNA polyplex showed significant silencing effect on PTN gene expression in vitro, and inhibition of tumor growth in vivo [15]. Leng et al., synthesized highly branched HK peptides H^3K8b, which would be expected to efficiently escape from the endosome by buffering with histidine residues, and the H^3K8b/siRNA complex showed a high RNAi effect on β-gal

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expression [14]. On the other hand, Li and Huang developed a tumor-targeted siRNA delivery system liposome-polycation-DNA lipoplex (LPD) formulation, which modified with polyethyleneglycol and anisamide, and the LPD showed a highly efficient apoptosis induction [16]. Furthermore, various other vectors also have been developed. Hanai et al., applied atelocollagen as carrier of siRNA, and the atelocollagen/siRNA complex delivered siRNA to metastasized tumors in bone tissue and inhibited their growth [17]. Matsui et al., synthesized a macrocyclic octaamine having a covalently liked lipid-bundle structure, and the siRNA complex silenced exogenous luciferase gene and endogenous DsRed2 gene [18]. Veldhoen et al., adopted cell-penetrating peptide MPGα, which spontaneously forms complexes with nucleic acids, as a carrier of siRNA [19]. However, simple mixing of nucleotides with multiple devices does not guarantee that each device is functional and effective in reducing the barriers to efficient delivery.

Therefore, we recently proposed a novel packaging approach for assembling multiple devices in a single delivery system, so that each device can function at the correct time and correct site according to a program, which reflects a rational strategy for controlling intracellular trafficking to efficient nucleotide delivery and considers the topology of the functional devices to achieve maximum activities [20]. Based on the packaging approach, we developed a multifunctional envelope-type nano device (MEND) as a novel delivery system for pDNA and ODN [20–25]. MEND has an envelope-type-virus-like structure, i.e., it consists of a DNA core condensed by polycation and covered with lipid membranes. The advantages of condensed DNA are a high efficiency of encapsulation, protection from nucleases, control of particle size, and controlled release of DNA in cells. Moreover, the surface of the lipid envelope and core can be modified with various functional devices, such as polyethyleneglycol for long circulation, specific ligand for targeting, and fusogenic peptide for endosomal escape [26]. When we introduced membrane-permeable octaarginine peptide (R8) [27–29] to the surface of MEND, the DNA transfection activity was increased 100-fold comparable to adenovirus [20,21]. In addition, we succeeded in efficient inhibition of specific target genes using R8-MEND-encapsulated siRNA-expression pDNA [23] or anti-sense ODN [25]. We recently determined that MEND modified with a high density of R8 is taken up via a non-classical endocytic pathway, macropinocytosis, which can circumvent lysosomal degradation, whereas low density R8-modified MEND is internalized by clathrin-mediated endocytosis [30]. Thus, it appears that the macropinocytic uptake mechanism is responsible for the high transfection activity of R8-MEND, and the condensed core of R8-MEND is efficiently released from macropinosomes into the cytoplasm. Moreover, no cytotoxicity was observed in cells treated with R8-MEND [20,21,23]. Therefore, R8-MEND is expected to be an efficient and safe delivery system of siRNA in vitro.

However, it was unclear whether short double-stranded siRNA could be condensed with polycations as small size nano particles and encapsulated into a liposome such as MEND. In the present study, we developed a new method for packaging synthetic siRNA into R8-MEND. We first evaluated the condensation of siRNA achieved by different types of polycations, namely poly-l-lysine (PLL), stearylated R8 and protamine, which are known to condense pDNA and ODN [23,25,31]. Then, the siRNA condensed core with a diameter of less than 100 nm was covered with a lipid membrane by a lipid hydration method, producing the R8-MEND carrier. The silencing effect of R8-MEND was evaluated by comparison with commercially available transfection reagent in HeLa cells stably expressing luciferase protein, and intracellular trafficking of R8-MEND was observed by confocal laser scanning microscope.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 4-nitrobenzo-2-oxa-1,3-diazolyl-DOPE (NBD-DOPE) were purchased from AVANTI Polar Lipids Inc (Alabaster, AL). Cholesteryl hemisuccinate (5-Cholesten-3β-ol 3-hemisuccinate; CHEMS) and poly-l-lysine (PLL, M.W. 27400) were obtained from Sigma-Aldrich Co. (St. Louis, MO). Stearyl octaarginine (STR-R8) was synthesized as described previously [27]. Protamine sulfate was purchased from Merck KGaA (Darmstadt, Germany). TransIT-TKO was obtained from Mirus Bio Corporation (Madison, WI). The anti-luciferase siRNA (21mer, 5′-GCGCUGCUGGUGCCAACCCTT-3′, 5′-GGGUUGGCACCAGCAGCGCTT-3′) and the anti-green fluorescent protein (GFP) siRNA (5′-GCUGACCCUGAAGUUCAUCTT-3′, 5′-GAUGAACUUCAGGGUCAGCTT-3′) were obtained from Thermo Electron GmbH (Ulm, Germany).

2.2. Preparation of R8-MEND

The siRNA-encapsulated R8-MEND was prepared by a lipid hydration method as reported previously [20–25]. siRNA and the polycation were dissolved with RNase-free water. To condense the siRNA, the siRNA solution (0.1 mg/ml) was added to the polycation solution (0.1 mg/ml) under vortexing at room temperature. The siRNA content of a suspension of the siRNA/STR-R8 complex prepared at a nitrogen/phosphate (N/P) ratio of 2.9 was 0.033 mg/ml. The siRNA content of siRNA/PLL or siRNA/protamine complex prepared at an N/P ratio of 2.4 was 0.05 mg/ml. After the condensation of siRNA, 0.25 ml of the condensed siRNA suspension was added to the lipid film, formed by the evaporation of a chloroform solution of 137.5 nmol lipids, [DOPE/CHEMS=9:2 (molar ratio)] on the bottom of a glass tube, followed by an incubation for 10 min to hydrate the lipids. The final concentration of lipid was 0.55 mM. To coat the condensed siRNA with lipids, the glass tube was then sonicated or about 1 min in a bath-type sonicator (125 W, Branson Ultrasonics, Danbury, CT). An STR-R8 solution (5 mol% of lipids) was added to the suspension to attach the membrane-permeable R8 peptide to the envelope of the lipid-coated particle, and the mixture was then incubated for 30 min at room temperature.

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