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A gene delivery system containing nuclear localization signal: Increased nucleus import and transfection efficiency with the assistance of RanGAP1

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ABSTRACT

In the present report, a degradable gene delivery system (PAMS/DNA/10NLS) containing nucleus location signal peptide (NLS) was prepared. The agarose gel electrophoresis, particle size and zeta potential of PAMS/DNA/10NLS were similar to those of PAMS/DNA, which proved that NLS did not affect the interaction between PAMS and DNA. PAMS/DNA/10NLS exhibited marked extracellular and intracellular degradation under acidic conditions. The degradation was believed to allow NLS to come into contact with importins easily, which was able to mediate the nucleus import. With the help of NLS, PAMS/DNA/10NLS exhibited a higher transfection capability than PAMS/DNA. Moreover, the transfection of PAMS/DNA/10NLS was less dependent on the breakdown of the nucleus envelope than PAMS/DNA. Considering that GTPase-activating protein 1 (RanGAP1) was able to activate the endogenous GTPase, which was necessary for NLS-mediated nucleus import, RanGAP1 overexpressed cells (RanGAP1 cells) were produced. This result showed that RanGAP1 cells had higher GTPase activities than normal cells. Both the nucleus import and transfection efficiency of PAMS/DNA/10NLS were markedly higher in RanGAP1 cells than that in normal cells. The in vivo transfection results also showed that the transfection efficiency of PAMS/DNA/10NLS was higher in RanGAP1 pre-treated mice than that in normal mice. These findings showed that PAMS/DNA/10NLS is a promising gene delivery system with the assistance of RanGAP1.

Statement of Significance

The present report describes the increased transfection efficiency of a degradable gene delivery system (PAMS/DNA/10NLS) containing nuclear location signal (NLS) with the assistance of GTPase-activating protein 1 (RanGAP1). The physicochemical properties of PAMS/DNA/10NLS were similar to those of PAMS/DNA. PAMS/DNA/10NLS exhibited great extracellular and intracellular degradations, which might allow NLS to contact with importins easily. With the help of NLS, PAMS/DNA/10NLS exhibited a higher transfection capability than PAMS/DNA. The transfection of PAMS/DNA/10NLS had less dependence on the breakdown of nuclear envelope. Both the nuclear import and transfection efficiency of PAMS/DNA/10NLS were higher in RanGAP1 overexpressed cells than that in normal cells. Moreover, the transfection efficiency of PAMS/DNA/10NLS was higher in RanGAP1 pre-treated mice than that in normal mice.

Abbreviations: PAMAM-OH, hydroxyl terminal PAMAM dendrimer (G4); PAMAM, amino terminal PAMAM dendrimer (G4); PAMS, SMLC-modified PAMAM-OH; PAMN, L-Norvaline-modified PAMAM-OH. RanGAP1, GTPase-activating protein 1; RanGAP1 cells, KB cells overexpressing RanGAP1; G_1 cells, KB cells in G_1 phase; G_2/M cells, KB cells in G_2/M phase.

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1. Introduction

Since cancer is the top leading cause of mortality worldwide, gene therapy has emerged as one of the most common methods to combat this disease [1,2]. In particular, it attracts more public concerns because of the successful sequencing of the human genome in the 1990s [3]. Although many important advances have been made, the development of gene delivery systems poses many challenges to successful therapeutics. It is well known that the transfection of exogenous DNA is dependent on three main steps: uptake by the cells, escape from the endosomes and the nucleus import [4]. Among these, the nucleus import is crucial for a successful gene delivery.

The nucleus compartment is the cellular compartment that contains the necessary chromatin and machinery for gene transcription. One of the main limitations in gene delivery is the inefficient nucleus delivery of DNA into the cells [5], which is particularly essential for the transfection of non-dividing cells [6]. Some reports have shown that in rapidly dividing cells macromolecules are able to accumulate in the perinucleus area along with mitosis because the nucleus envelope is broken down during the G_2/M phase period [7]. In resting cells, such as non-dividing cells or in vivo cells, the main route by which macromolecules can enter the nucleus is through the nucleus pore complex (NPC) [8]. The NPC is built of a diverse set of nucleoporins and associated nucleus and cytoplasmic filaments surrounding a central channel structure [9]. Studies have shown that the upper size limit for a cargo that can diffuse through the NPC is 9 nm in diameter or 50 kDa in molecular weight. This size is markedly smaller than general exogenous genes such as plasmid [10]. Thus, the passive diffusion of exogenous DNA into the nucleus through NPC seems to be impossible and an active approach is expected to solve the problem. In fact, although the nucleus uptake is a significantly restricted process, a multitude of macromolecules have the capability to enter the nucleus freely, such as nucleoproteins, for the maintenance of basic cellular metabolism or responding to changes in environmental conditions. Most nucleoproteins usually carry one or more nucleus targeting signal peptides called nucleus localization sequences (NLS). The best characterized NLS is derived from Simian Virus 40 (SV40) T antigen, which consists of one or two clusters of basic amino acid residues or two clusters of basic residues [11,12]. NLS can interact with the nucleus transport system and thereby induce the nucleus import [13,14].

NLS is able to initiate the binding of cargoes (e.g. PAMS/DNA in the present report) to importins and form an import ligand complex (Fig. 1b). And the nucleus import of the complex nuclear import depends on cytoplasmic RanGDP, which can dock at the NPC [15,16]. The main source of RanGDP depends on the GTPase. In the cytoplasm, Ran is a kind of GTPase, which is able to catalyze the conversion of RanGTP to RanGDP. However, the endogenous GTPase activity of Ran is very low.

Some reports have shown that Ran can be greatly stimulated by a protein named GTPase-activating protein1 (RanGAP1), which is the activating protein for the Ran. GTPase is localized in the cytoplasm and on the cytoplasmic side of the NPC [17,18]. The intrinsic rate of GTP hydrolysis by Ran is very low ($k_{cat} = 1.8 \times 10^{-5}\ s^{-1}$) [19]. RanGAP stimulates the GTPase activity of Ran by several orders of magnitude to approximately $2^{-10}\ s^{-1}$. RanGAP1 causes a change in Ran conformation, increasing the rate of intrinsic hydrolysis of RanGTP to RanGDP state (Fig. 1b). Another interaction partner of Ran, Ran binding protein RanBP1, can converse RanGDP to RanGTP. There is no doubt that RanBP1 can induce the nucleus export. In order to increase the nucleus transport, RanGAP1 level must be evaluated.

So far, many investigators have demonstrated that NLS does increase the nucleus uptake of plasmid DNA and the ultimate transfection [20,21]. Although RanGAP1 is believed to be necessary for NLS-mediated nucleus import, as far as we know, no study has been carried out to study whether the high RanGAP1 level can facilitate the transfection efficiency of a polyplex containing NLS.

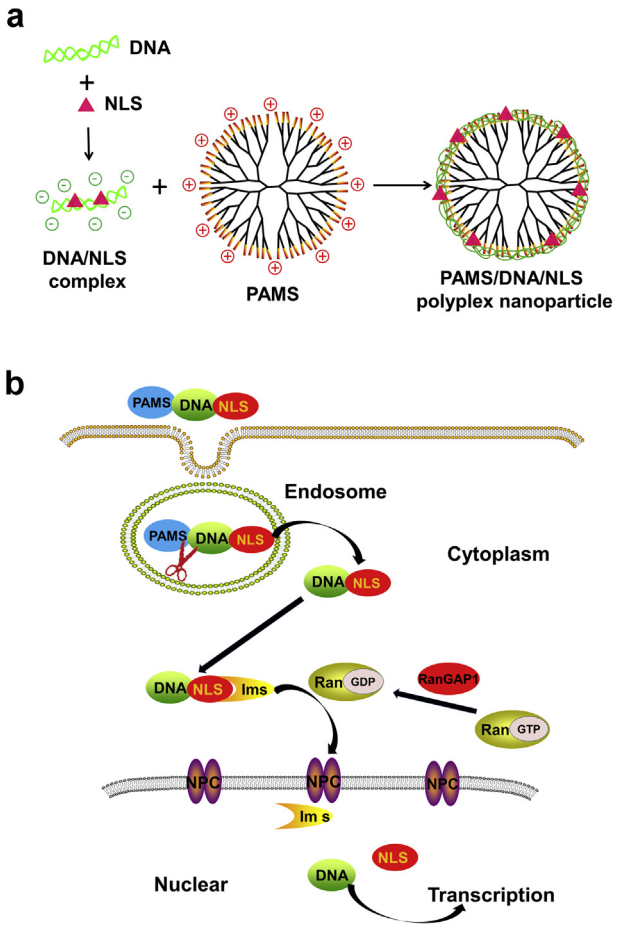

Fig. 1. (a) The formation of PAMS/DNA/NLS polyplex (PAMS/DNA/NLS). NLS binds to DNA to form DNA/NLS complex (DNA/NLS). PAMS/DNA/NLS is prepared with positive PAMS and negative DNA/NLS. (b) The nucleus import of DNA payloads with the assistance of GTPase-activating protein1 (RanGAP1). PAMS/DNA/NLS degrades in endosome organelles. After escape from endosomes, DNA/NLS binds to importins (Ims) to form an import ligand complex. The nucleus import of the complex requires RanGDP, which can be promoted by RanGAP1. As a result, more therapeutic DNA accumulated in the nucleus region and was transcribed.

In a previous study, we synthesized a hydroxyl terminal PAMAM dendrimer derivative (PAMS) that contained a labile ester bond linkage, the β-thiopropionate bond, which is degradable under acidic conditions [22]. A luciferase assay showed that PAMS/DNA polyplex (PAMS/DNA) was an excellent system for effective gene delivery. In this article, NLS was incorporated into PAMS/DNA to prepare PAMS/DNA/NLS polyplex (PAMS/DNA/NLS, Fig. 1a). When the polyplex degraded, NLS interacted easily with importins [23]. Thus, a high nucleus importing ability was achieved. The present study aimed to investigate the following issues.

Firstly, the extracellular and intracellular degradations of PAMS/DNA/NLS were studied. Secondly, the transfection efficiencies of PAMS/DNA and PAMS/DNA/NLS were compared. In addition, cell circle-synchronized cells at the G_1 or G_2/M phase were transfected with PAMS/DNA or PAMS/DNA/NLS to confirm the role of NLS in gene delivery. Thirdly, our interest also focused on the study of the nucleus import and transfection efficiency of PAMS/DNA/NLS in RanGAP1 overexpressed cells. A schematic diagram depicting the cellular degradation of PAMS/DNA/NLS and the nucleus