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A Multifunctional Envelope-type Nanodevice for Use in Nanomedicine: Concept and Applications

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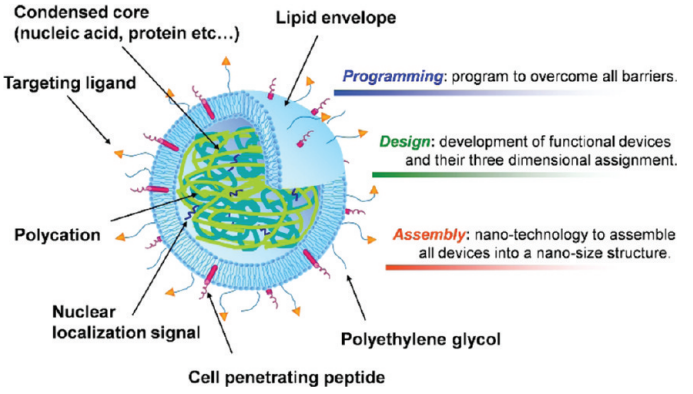
CONSPECTUS

In the 21st century, drug development has shifted toward larger molecules such as proteins and nucleic acids, which require the use of new chemical strategies. In this process, the drug delivery system plays a central role and intracellular targeting using nanotechnology has become a key technology for the development of successful new medicines.

We have developed a new delivery system, a multifunctional envelope-type nanodevice (MEND) based on "Programmed Packaging." In this new concept of packaging, multifunctional nanodevices are integrated into a nanocarrier system



according to a program designed to overcome all barriers during the course of biodistribution and intracellular trafficking. In this Account, we introduce our method for delivering nucleic acids or proteins to intracellular sites of action such as the cytosol, nucleus, and mitochondria and for targeting selective tissues in vivo via systemic administration of the nanodevices.

First, we introduce an octaarginine-modified MEND (R8-MEND) as an efficient intracellular delivery system, designed especially for vaccinations and transgene expression. Many types of cells can internalize the R8-MEND, mainly by inducing macropinocytosis, and the MEND escapes from macropinosomes via membrane fusion, which leads to efficient antigen presentation via the major histocompatibility complex I pathway in antigen-presenting cells. In addition, the transfection activities of the R8-MEND in dividing cells, such as HeLa or A549 cells, are as high as those for adenovirus. However, because the R8-MEND cannot induce sufficient transgene activity in primary cultured dendritic cells, which are critical regulators of the immune response, we converted the R8-MEND into a tetralamellar MEND (T-MEND). The T-MEND uses a new packaging method and delivers condensed pDNA into the nucleus via fusion between the envelopes and the nuclear membrane.

To achieve efficient transfection activity, we also optimized the decondensation of nucleic acids within the nucleus. To optimize mitochondrial drug delivery, we introduced the MITOPorter. Many types of materials can be packaged into this liposome-based nanocarrier and then delivered to mitochondria via membrane fusion mechanisms. Finally, we describe an integrated strategy for in vivo tumor delivery and optimization of intracellular trafficking. Successful tumor delivery typically requires coating the surfaces of nanoparticles with PEG, but PEG can also limit uptake by the reticuloendothelial system and reduce the efficiency of intracellular trafficking. Here we integrate the optimum biodistribution and intracellular trafficking of the MEND with an innovative strategy such as enzymatically cleavable PEG and a short membrane peptide, GALA. Some of these strategies will soon be tested in the clinic.

Introduction

In the 21st century, a paradigm shift occurred in the field of drug discovery and the development of drugs with an extended molecular size such as proteins and nucleic acids. The change clearly requires new strategies for the creation of new drugs for unmet medical needs. In this process, the drug delivery system plays a central role and intracellular targeting using nanotechnology becomes a key technology for successful nanomedicines. To achieve this goal, we developed a new delivery system, a multifunctional envelope-type nanodevice (MEND) based on a new packaging concept "Programmed Packaging".[1,2] In this Account, we

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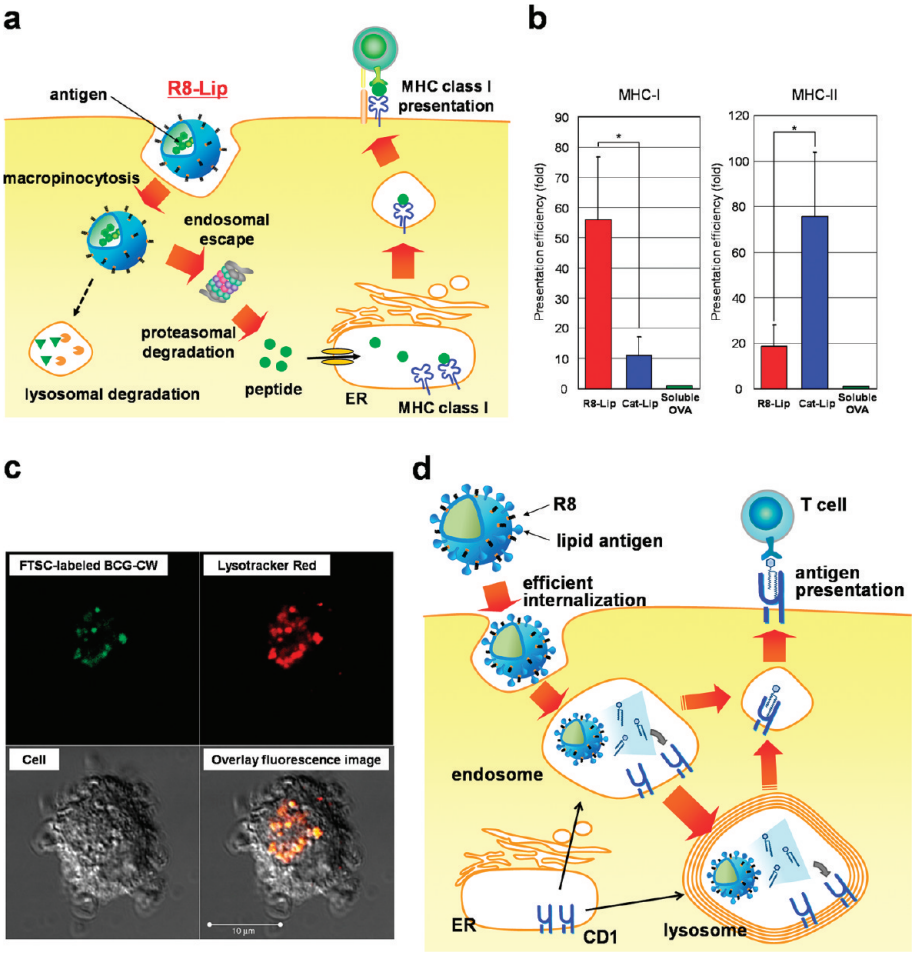

FIGURE 1. Applications of R8-Lip to the study of vaccines. (a) Strategy used for antigen delivery with R8-Lip into MHC-I. (b) Specific antigen presentation via MHC-I by R8-Lip.[7] The vertical axis shows relative value per OVA 1 μg. Values are the mean $\pm$ SD of at least three different experiments (*$P < 0.01$). Reproduced from ref 7. Copyright 2008 American Society of Gene & Cell Therapy. (c) Intracellular localization of R8-Lip/BCG-CW in DCs. Green and red show BCG-CW and endosome/lysosome, respectively. (d) Efficient internalization and antigen presentation by lipid antigen loaded R8-Lip in antigen-presenting cells.

introduce our concept to target nucleic acids or proteins to intracellular sites of action such as the cytosol, nucleus, and mitochondria as well as to target selective tissues in vivo via systemic administration.

Unique Abilities of Octaarginine (R8) as a Functional Device

First, we focused the unique abilities of R8, which is a synthetic peptide that mimics the trans-activating transcriptional activator of the human immunodeficiency virus,[3] as a useful device for a delivery system. A high density of stearylated R8 (STR-R8) modified liposomes (R8-Lip) were found to be internalized to cells via macropinocytosis.[4] Moreover, our findings showed that the R8-Lip avoided lysosomal degradation, suggesting that the R8-Lip might have the ability of escape from the endosome. Macropinocytosis provides some advantages, such as an increased uptake of macromolecules and the avoidance of lysosomal degradation.[5] Since macropinosomes have a relatively leaky nature, it is advantageous for nanocarriers to escape from macropinosomes. In other uptake pathways, clathrin- or caveolae-mediated endocytosis, the size of molecules taken up is limited to 50−150 nm.[5] Moreover, the clathrin-mediated endosomes fuse with lysosomes. Therefore, it is suggested that macropinocytosis substantially increase the efficient internalization into cells and endosomal escape of R8-Lip. In addition, we consider another reason. We previously compared endosomal escape between R8-Lip and octalysine-modified liposome (K8-Lip) using fusogenic lipids.[6] R8-Lip was found to be able to escape from the endosome by membrane fusion at both acidic pH and neutral pH, especially acidic conditions. Briefly, R8 achieves efficient endosomal escape by macropinocytosis-mediated cellular uptake and effective membrane fusion with endosomal

membranes. Thus, modification of the liposomal surface with R8 is one of the attractive strategies for the production of an intelligent delivery system.

Applications of R8-Lip to the Study of Vaccines

The properties of R8-Lip suggest that it would be suitable for use as a vaccine carrier. To induce immune responses against a tumor, an induction of the cellular immunity is indispensable. The tumor antigen specific cellular immunity is led by presenting antigens derived peptide on the major histocompatibility complex I (MHC-I) in antigen-presenting cells (APCs). APCs take up pathogens or tumor cells, degrade them, and transfer information of antigens via MHC molecules to T cells. Then, CD8-positive T cells recognize the MHC-I/peptide complex on APCs and differentiate into cytotoxic T cells, which attack tumor cells. Because MHC-I presents peptides derived from cytosolic antigens, it is necessary to deliver antigens to the cytosol in APCs. Therefore, we hypothesized that antigens could be efficiently delivered to the cytosol in APCs by encapsulating antigens into the R8-Lip, and we evaluated the functions of R8-Lip encapsulated antigen preparations (Figure 1a).[7] As evidence by confocal laser scanning microscopy (CLSM), encapsulated substances were efficiently delivered to the cytosol of APCs by the R8-Lip. To investigate the efficiency of antigen presentation, ovalbumin (OVA), a model antigen, encapsulated by R8-Lip (R8-Lip/OVA) was pulsed to APCs. The R8-Lip/OVA showed a specifically higher presentation efficiency for MHC-I compared with OVA encapsulated in general cationic liposomes (Cat-Lip/OVA) (Figure 1b). Moreover, significant antitumor effects were detected in mice immunized with R8-Lip/OVA in vivo. Consequently, the R8-Lip appears to be a promising device for use in cancer immunotherapy.

The components of *Mycobacterium* are thought to have immunostimulatory characteristics and to contain lipid antigens. The components of *Mycobacterium* are hardly taken up by cells because of poor solubility due to lipid structure. Also, they are recognized by receptors in the endosome/lysosome after degradation. Therefore, we used the high cellular affinity of R8 in the vaccine studies using the components of *Mycobacterium*. It is well-known that the *Mycobacterium bovis* bacillus Calmette–Guerin cell wall (BCG-CW) functions as a potent immunotherapeutic agent. However, this application is hampered by unfavorable physicochemical characteristics of BCG-CW, such as low cellular association and poor solubility. To overcome these problems, we loaded BCG-CW into R8-Lip.[8] The R8-Lip loaded with BCG-CW (R8-Lip/BCG-CW) was efficiently internalized into APCs and was localized in acidic compartments (endosome/lysosome) (Figure 1c). Moreover, R8-Lip/BCG-CW completely inhibited the growth of mouse bladder cancer cell line (MBT-2) tumors, while BCG-CW alone had no effect.[9]

Construction of MEND for a Nonviral Gene Delivery System

We developed the R8-MEND, the first generation of MENDs, as a delivery system for nucleic acids.[10−12] The R8-MEND is a condensed core of nucleic acids covered with a lipid envelope, like a DNA virus, and STR-R8 is modified on the lipid surface. The transfection activity of R8-MEND loaded with plasmid DNA (pDNA) was compared with that of an adenovirus, a potent viral vector, in HeLa cells and in A549 cells.[11] The activity of the R8-MEND loaded with pDNA for gene expression in cell lines was comparable to that for adenovirus. The R8-MEND would also be expected to be an efficient delivery system for small interference RNA (siRNA).[12] The silencing effect of R8-MEND loaded with antiluciferase siRNA was measured in HeLa cells stably expressing luciferase. The R8-MEND silenced the luciferase activity to a significant extent, and no cell toxicity was observed. Furthermore, siRNA was effectively released from the lipid envelope of the R8-MEND to the cytoplasm, as evidenced by CLSM observations. Therefore, the R8-MEND appears to be a viable and safe delivery system for delivering nucleic acids in cell lines.

Cytoplasmic Transport of R8-MEND

Both adenovirus and artificial vectors (i.e., lipoplex and MEND) are considered to be taken up via the endocytosis pathway. Subsequently, the carriers escape from the endosomes, followed by nuclear delivery. The control of cytoplasmic transport is an important issue for the nuclear targeting of pDNA, since restricted diffusion is also a crucial barrier for macromolecules. Viruses such as adenoviruses[13,14] have developed sophisticated machinery for overcoming these biological barriers and are able to successfully deliver their genomes to the nucleus via microtubule-dependent transport. Meanwhile, our findings also revealed that R8-MEND gradually accumulates on the nuclear fraction in a time-dependent manner.[4] This observation prompted us to analyze the mechanism responsible for the cytoplasmic transport of R8-MEND.[15] The transfection activity of R8-MEND was drastically decreased in the presence of nocodazole, a microtubule-disrupting reagent, in a concentration-dependent manner. The tracking of R8-MEND and adenovirus particles by multicolor

real-time imaging revealed that R8-MEND as well as adenovirus was subject to directional transport along with the microtubules. We then investigated whether the directional transport of R8-MEND occurred before or after endosomal release. A portion of the R8-MEND showed directional transport colocalizing with endosomes, while the colocalization of adenovirus was negligible. Moreover, directional motion was observed without any colocalization in endosomes. Collectively, these data show that the mechanism for the directional transport of R8-MEND is completely different from that for adenovirus; vesicular trafficking is involved in the directional transport of R8-MEND, whereas the direct association with motor proteins occurred in the case of adenovirus.

Evolution of MENDs

Tetralamellar MEND (T-MEND) for Nuclear Delivery. In mitotic cells, it is generally assumed that pDNA primarily enters the nucleus when the nuclear membrane structure breaks down during the M-phase. However, in nondividing cells, the nuclear membrane structure remains intact. Therefore, the nuclear membrane represents the ultimate barrier to be overcome for successful nuclear delivery in nondividing cells.

Meanwhile, we proposed a nuclear pore complex independent nuclear transport approach, in which intracellular membrane barriers are overcome using stepwise membrane fusion by means of T-MEND. The critical structural elements of the T-MEND are a DNA−polycation condensed core coated with two nuclear membrane fusogenic inner envelopes (composed of cardiolipin and 1, 2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)) and endosome-fusogenic outer envelopes (composed of phosphatidic acid and DOPE), which are shed in a stepwise fashion as the core is trafficked through the cell.[16] The membrane fusion occurs in response to the acidic pH environment in the macropinosome. One of the targets of this system is dendritic cells (DCs), which play a crucial role in the initiation of an immunoresponse and the regulation of cell-mediated immunoreactions. While the R8-MEND shows a high gene expression in dividing HeLa cells, comparable to adenovirus, as mentioned above, transfection activity in JAWS II cells, a DC-derived cell line, was negligible. When a T-MEND was prepared using a single endosome-fusogenic lipid or nuclear-membrane-fusogenic lipids, stimulation of transfection activity was rarely observed compared with the conventional MEND. In contrast, when JAWS II cells were transfected with a T-MEND prepared with both double nuclear (inner) and endosomal membranes (outer), transfection activity was dramatically increased by several hundred-fold. Further upgrading of the T-MEND system was successfully demonstrated by (i) modification with a KALA peptide, a pH-independent fusogenic peptide, and (ii) replacing core particles with more highly condensed ones ($\pm$ ratio of 5) to negatively charged ones ($\pm$ ratio of 0.5).[17]

Control of the Intranuclear Decondensation of pDNA. Recent quantitative comparison of intracellular trafficking of pDNA and adenovirus collectively revealed that postnuclear delivery processes (i.e., transcription and translation) are dominant rate-limiting processes for the 2 or 3 order of magnitude decrease in transfection efficiency of nonviral vectors.[18,19] Further mechanism-based studies clarified that pDNA transfected with a lipoplex is mainly present in a condensed form in the nucleus, which prevents it from gaining access to transcription factors.[18] In contrast, adenovirus genomic DNA is efficiently released into the nucleus. Moreover, adenoviral genome DNA was specifically localized in the euchromatin region,[18] where transcription activity is relatively active.

We established an imaging technology for visualizing and quantifying nuclear decondensation in specified nuclear subdomains by means of fluorescence energy transfer (FRET) between quantum dot (QD)-labeled pDNA as a donor and a rhodamine-labeled polycation as an acceptor (Figure 2).[20] Nuclear subdomains (i.e., heterochromatin and euchromatin) were distinguished by Hoechst33342 staining. Thereafter, a Z-series of confocal images were captured by CLSM. pDNA in condensation/decondensation status in heterochromatin or euchromatin was quantified based on the pixel area of the signals derived from the QD and rhodamine. Using this method, we successfully obtained a condensation/decondensation profile in the intranuclear subdomain using a synthetic polycation, polyrotaxane (PRX), which possessed a necklace-like structure composed of dimethylaminoethyl-modified α-cyclodextrin.[21] To render it biocleavable, the end of the poly(ethylene glycol) (PEG) chain was capped with benzyloxycarbonyl tyrosine (Z-L-Tyr) via disulfide bonding. This molecule is designed to facilitate the docondensation of pDNA in the nucleus in response to the reductive environment in the cells. Thus, the method is useful in investigating the mechanism of extensive gene expression and in understanding the rate-limiting processes that must be overcome for further gene expression from the point of view of intranuclear disposition.

Mitochondrial Delivery

Mitochondrial dysfunction has been implicated in a variety of human diseases (Figure 3a). It is now well accepted that

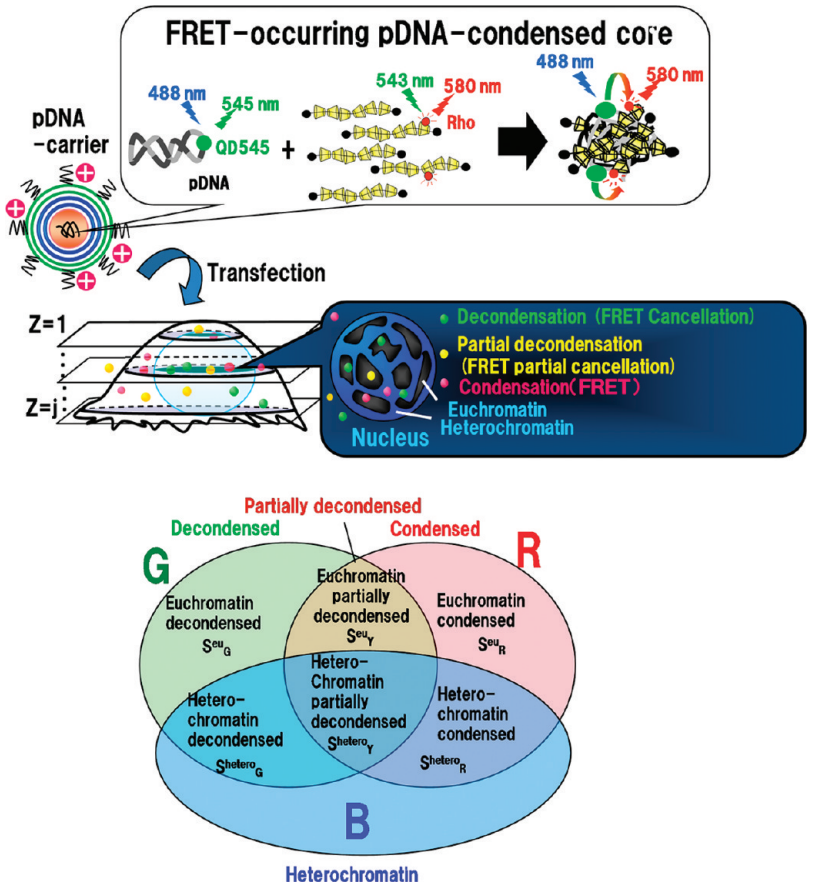

FIGURE 2. Strategy used to analyze an intranuclear condensation/decondensation profile with a FRET-occurring pDNA core particle. QD-labeled pDNA was condensed with polycation labeled with donor fluorophores. The core was encapsulated in the T-MENDs for nuclear delivery. Intracellular decondensation was monitored by the cancellation of FRET signals.

mutations and defects in the mitochondrial genome (mtDNA) form the basis of these diseases.[22,23] Therefore, mitochondrial gene therapy and diagnosis would be expected to have great medical benefits. To achieve such an innovative strategy, therapeutic agents need to be delivered into the mitochondria.

Current Reports of Mitochondrial Delivery System and the Approach of Mitochondrial-Targeted DNA Delivery. A number of mitochondrial delivery systems for various cargoes have been reported;[24] however, reports of mitochondrial-targeted DNA delivery are limited. It was expected that mtDNA, pDNA coding therapeutic protein, and therapeutic oligo-DNA could be therapeutic candidates. The use of a mitochondrial targeting signal peptide (MTS) makes it possible to selectively deliver oligo-DNA to isolated mitochondria.[25] However, the use of MTS is required for the cellular uptake of the cargo, because the MTS conjugate itself cannot be internalized through the cellular membrane. Weissig and co-workers reported on the development of DQAsomes, which are mitochondriotropic and cationic vesicles designed for mitochondrial-targeted DNA delivery.[26] They showed that DQAsomes specifically release pDNA, circular DNA, proximal to mitochondria in living cells,[26] although the delivery of cargoes into the interior of the mitochondria has not been validated. In the future, the effective delivery of circular DNA to the mitochondrial matrix using such novel approaches is expected to be used in mitochondrial gene therapy.

MITO-Porter. To achieve efficient mitochondrial drug delivery, two independent processes, cytoplasmic delivery through the cell membrane and mitochondrial delivery through the mitochondrial membrane, are required. In previous studies, we reported on the development of MITO-Porter, a liposome-based nanocarrier that delivers cargoes to mitochondria via membrane fusion.[27] Using the green fluorescence protein as a model macromolecule and analysis by CLSM, we were able to confirm mitochondrial macromolecule delivery by the MITO-Porter (Figure 3b). Additionally, transmission electron microscopy (TEM) analysis showed that Gold Colloid as a model macromolecule

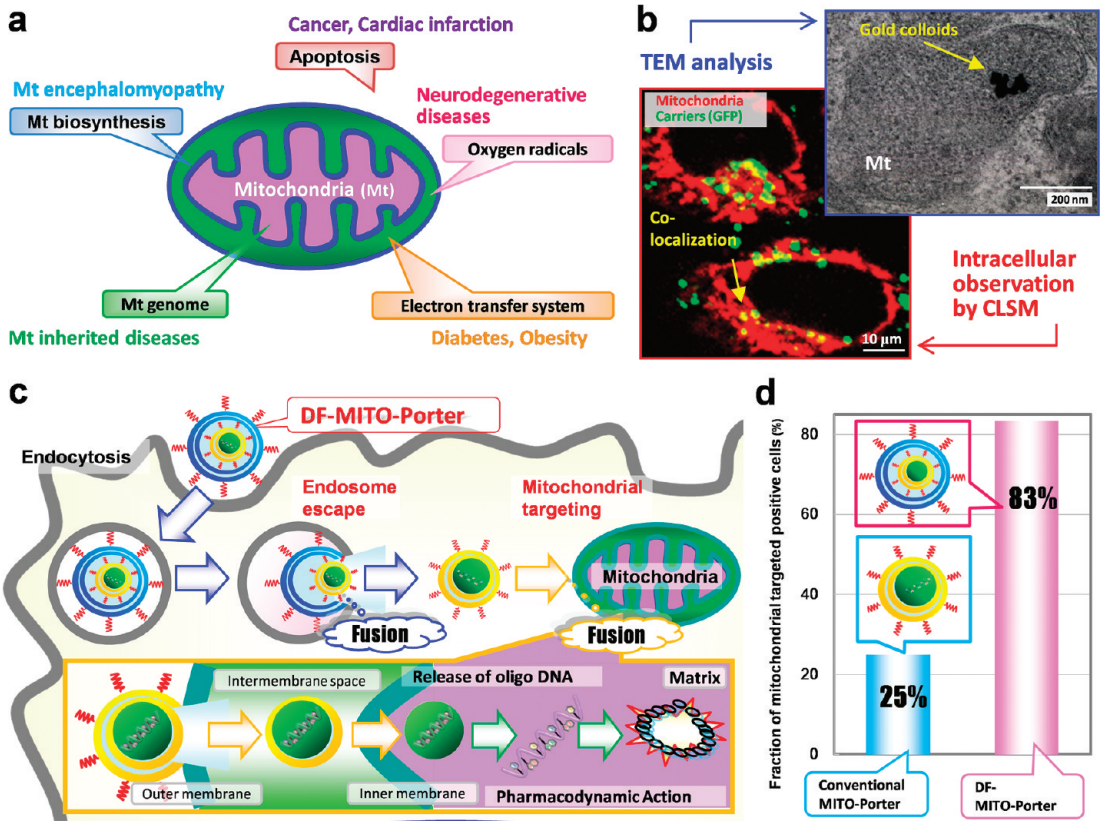

FIGURE 3. Mitochondrial gene therapy strategy and mitochondrial delivery by DF-MITO-Porter. (a) Relationship between mitochondria and diseases. (b) Intracellular observation of MITO-Porter (green) after staining mitochondria (red) using CLSM and TEM analysis indicating that gold colloids encapsulated in MITO-Porter were delivered to mitochondria. Reproduced from ref 27. Copyright 2008 Elsevier. (c) Schematic image illustrating mitochondrial macromolecule delivery via a series of membrane fusions using DF-MITO-Porter. (d) The fraction of mitochondrial targeted positive cells was calculated using a confocal image-assisted integrated quantification method. Reproduced from ref 29. Copyright 2011 American Society of Gene & Cell Therapy.

could be delivered by MITO-Porter to the interior of mitochondria (Figure 3b). We were also able to verify that the MITO-Porter delivered cargoes to the mitochondrial matrix, which pools mtDNA, using the novel imaging method.[28]

We recently reported on the development of a Dual Function (DF)-MITO-Porter,[29] based on the concept in which both high-density R8-modified liposomes and the conventional MITO-Porter were integrated (Figure 3c). The critical structural elements of the DF-MITO-Porter include a complexed particle of cargoes that are coated with a mitochondria-fusogenic inner envelope and an endosome-fusogenic outer envelope. Modification of the outer envelope surface with a high density of R8 greatly assists in the efficient internalization of the carriers into cells. Inside the cell, the carrier escapes from the endosome into the cytosol via membrane fusion, a process that is mediated by the outer endosome-fusogenic lipid membranes. The carrier then binds to mitochondria via electrostatic interaction with R8 and fuses with the mitochondrial membrane to deliver cargoes into mitochondria. Intracellular observations using CLSM permitted us to compare mitochondrial targeting activity between the DF-MITO-Porter and the conventional MITO-Porter, and the results indicated that the DF-MITO-Porter effectively delivered exogenous bioactive macromolecules into the mitochondria in living cells (Figure 3d).[29] Moreover, we confirmed that mitochondrial delivery of DNase I protein by the DF-MITO-Porter drastically decreased mtDNA-levels via mitochondrial specific fusion.[30] The results provided a demonstration of its potential use in therapies aimed at mtDNA.[29,30] We believe that mitochondrial-targeted delivery via MITO-Porter has considerable potential for use as a novel therapeutic strategy. Studies related to mitochondrial gene therapy using MITO-Porter are currently in progress.

Tumor Delivery with MEND

PEG Dilemma. For systemic use to deliver cargoes to tumors, a MEND must meet several criteria, including long-circulating

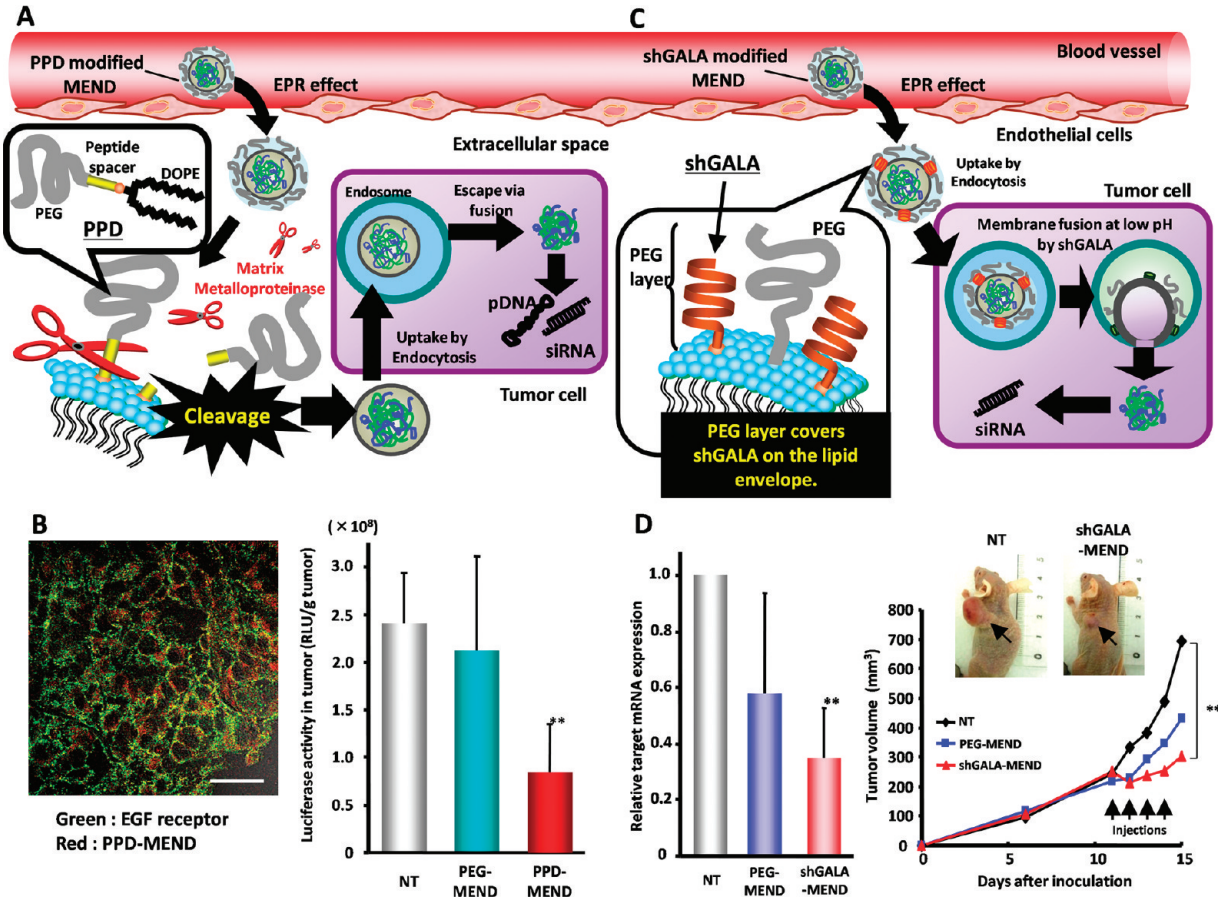

FIGURE 4. The strategy for delivering MEND to tumors. (a) PPD-modified MEND accumulates in tumors via the EPR effect, followed by the cleavage of PEG in response to MMP. The "bare" MEND could associate with tumor cells and exert better effect. (b) PPD-MEND distributed in tumor tissue after iv injection and effected knockdown of luciferase activity. (c) shGALA modification does not disturb the systemic stability of PEGylated MEND due to the mask of shGALA by the PEG layer, which results in the accumulation in tumor via the EPR effect. shGALA accelerates endosomal escape of MEND in response to low pH in endosomes. (d) shGALA-MEND showed efficient silencing of the target gene and exerted antitumor effects compared with unmodified PEG-MEND.

properties and a sufficiently small size to permit them to pass through the neovasculature via the enhanced permeability and retention (EPR) effect.[2,31] It is known that PEGylation allows liposomes to circulate for long periods of time in the blood after intravenous administration because they can escape uptake by the reticuloendothelial system (RES).[32] Therefore, we installed PEG–1,2-distearoyl-*sn*-gycero-3-phosphoethanolamine (PEG–DSPE) in the lipid envelope of a MEND. The addition of PEG–DSPE to the lipid envelope resulted in a smaller diameter (around 100–200 nm) to the PEG-unmodified version and reduced the positive charge due to the presence of an aqueous layer on the surface of the MEND and the formation of a stable lamellar structure with a larger curvature. After intravenous administration, the PEGylated MEND exhibited a longer systemic circulation than the unmodified material. These results suggest that a PEGylated MEND would be expected to accumulate in a tumor after an intravenous injection through the EPR effect. However, the PEGylated MEND showed a significantly lower transgene expression in tumor cells than the unmodified MEND. These results suggest that PEGylation can be advantageous in terms of controlling the biodistribution and tumor accumulation of nanoparticles, while it has a disadvantage for controlling the intracellular trafficking.[33] The crucial issue associated with the use of PEG is referred to as the "PEG dilemma".[2]

New Strategies To Overcome the "PEG Dilemma": Cleavable PEG and Endosomal Escape. One of the major problems associated with PEGylation is the low binding and uptake of the resulting carriers to the target cells. The PEG moiety and the aqueous phase on the surface of MEND inhibit the interaction of the positively charged lipid envelope with the cell surface, which results in less binding and cellular uptake via endocytosis. To solve this, cleavable PEG systems that respond to an intracellular environment, such as a low pH in endosomes/lysosomes, a reduced environment, and

an enzyme-environment, have been developed.[2] We focused on matrix metalloproteinases (MMPs), which are abundantly secreted from tumor cells. A PEG−peptide−DOPE (PPD) ternary conjugate was synthesized, in which the peptide sequence is sensitive to MMPs (Figure 4a).[34] A MEND modified with PPD (PPD-MEND) showed increased efficiency for delivering both pDNA and siRNA compared with a conventional PEG-modified MEND (PEG-MEND) due to its enhanced cellular uptake in response to MMP expression and subsequent endosomal escape (Figure 4b).[34,35] These findings suggest that a cleavable PEG in response to a tumor-specific environment is a potential strategy for overcoming the PEG dilemma.

The endosomal escape of liposomes is mainly caused by membrane fusion.[33,36] PEGylation confers steric stability to the lipid envelop of a MEND and inhibits fusion with the endosomal membrane, leading to poor endosomal escape. It is known that a pH-sensitive fusogenic membrane peptide, GALA (WEAALAEALAEALAEHLAEALAEALEALAA), undergoes a structural change to an α-helix under acidic conditions like those found in endosomes/lysosomes.[37] Our group recently demonstrated that a combination of GALA with a PEG-MEND encapsulating pDNA or siRNA facilitated the endosomal release of the cargo, resulting in efficient knockdown upon in vitro and in vivo local administration.[38,39] However, GALA modification decreased the circulation time of the PEGylated MEND in blood, which is disadvantageous for tumor targeting via the EPR effect. We hypothesized that GALA is partially covered by the PEG layer, which is recognized by RES. Therefore, we designed a shorter version GALA (shGALA, WEAALAEALAEALAEHLAEALA) (Figure 4c).[40] The systemic stability of the shGALA-modified PEG-MEND (shGALA-MEND) was comparable to that for the PEG-MEND. Finally, shGALA-MEND was found to enhance the activity of target gene silencing in tumors and exerted anti-tumor effects compared with the PEG-MEND after systemic administration (Figure 4d).

As described above, the crucial issue associated with the use of PEG, that is, the "PEG dilemma" is expected to be solved by the appropriate manipulation of cellular uptake and endosomal escape of gene carriers based on strategies using cleavable PEG systems or fusogenic devices.

Perspective

The R8-MEND has been successfully applied to the treatment of bladder cancer by delivering BCG components to tumor cells. The next challenge is how to establish large scale production under GMP levels before clinical tests with pharmaceutical companies. We are excited to have established a platform where clinically applicable samples of MENDs can be produced under GMP conditions within Hokkaido University and then proceed to the clinical test phase by linking with Pharmaceutical Companies. In the near future, we believe that the principles established as described above will be effective nanomedicines that deserve to be tested in clinical trials and finally to reach patients.

BIOGRAPHICAL INFORMATION

Takashi Nakamura is an Assistant Professor in the Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University. He received B.S., M.S., and Ph.D. degrees from Hokkaido University in 2004, 2007 and 2010, respectively. After a period of postdoctoral training with the Faculty of Pharmaceutical Sciences at Hokkaido University, he was appointed an Assistant Professor in the Faculty of Pharmaceutical Sciences at Hokkaido University in 2011. His main research interest is the development of delivery system for vaccines using DNA, siRNA, antigens, and adjuvants.

Hidetaka Akita is an Associate Professor of Pharmaceutics in the Laboratory of Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University. He received the B. S., M.S., and Ph.D. degrees from The University of Tokyo in 1997, 1999, and 2002, respectively. After a Research Fellowship for Young Scientists from the Japan Society for Promotion of Science (JSPS), he was appointed to the Faculty of Pharmaceutical Sciences, Hokkaido University. He was promoted to the rank of Associate Professor at Hokkaido University in 2010. His main research interest is development of gene delivery systems for functional nucleic acids by controlling intracellular trafficking.

Yuma Yamada is an Assistant Professor in the Faculty of Pharmaceutical Sciences, Hokkaido University, and a Pharmacist in the Department of Pharmacy, Hokkaido University Hospital. He received B.S., M.S., and Ph.D. degrees from Hokkaido University in 2003, 2005 and 2008, respectively. After completing a Research Fellowship for Young Scientists from the Japan Society for Promotion of Science, he was appointed an Instructor at Faculty of Pharmaceutical Sciences, Hokkaido University, in 2007. He became an Assistant Professor in the Faculty of Pharmaceutical Sciences, Hokkaido University in 2009. His main research interest is the development of mitochondrial drug delivery systems and nanoparticle packaging for various cargoes (proteins, oligo DNA, pDNA, etc.).

Hiroto Hatakeyama is an Assistant Professor in the Faculty of Pharmaceutical Sciences, Hokkaido University. He received B.S., M.S., and Ph.D. degrees from Hokkaido University in 2003, 2005 and 2008, respectively. After completing a Research Fellowship for Young Scientists from Japan Society for Promotion of Science, he was appointed an Assistant Professor in the Faculty of Pharmaceutical Sciences, Hokkaido University, in 2008. He has participated in the newly developed Laboratory of Future Nanomedicine since April 2009. His main research interest is the development of drug (small molecules as well as nucleic acids, pDNA, siRNA, miRNA) delivery systems for both tumor cells and tumor endothelial cells for cancer therapy.

Hideyoshi Harashima is a Professor of Pharmaceutics and chair of the Laboratory of Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University. He received B.S., M.S., and Ph.D. degrees from The University of Tokyo in 1981, 1983, and 1987, respectively. After a period of postdoctoral training in the School of Medicine (anesthesiology) at Stanford University, he was appointed an Associate Professor at Faculty of Pharmaceutical Sciences, The University of Tokushima. He accepted the position of Full Professor at Hokkaido University in 1999. He currently serves as an Associate Editor of the Journal of Controlled Release and Cancer Science. He is a member of the editorial boards of Molecular Therapy, International Journal of Pharmaceutics. He is a director of Japanese Society of Drug Delivery System and served as vice president of the Association of Pharmaceutical Science and Technology of Japan (2008−2010). He also serves as a Co-Chair of SIG (Pharmaceutical Biotechnology) of FIP.

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