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Overcoming the polyethylene glycol dilemma via pathological environment-sensitive change of the surface property of nanoparticles for cellular entry

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ABSTRACT

Modification with polyethylene glycol (PEG) is currently considered an important strategy for anti-cancer drug delivery, because PEGylated-nanoparticles would be effectively delivered to tumor tissue by enhanced permeation and retention effects. However, PEGylation suppresses the cellular uptake of nanoparticles (NPs) to target cells (known as the PEG dilemma). Here, we propose a novel strategy, namely conferring a pathological environment-sensitive property of nanoparticles for overcoming the PEG dilemma. Specifically, although nanoparticles have an overall negative surface charge to avoid interactions with biogenic substances in blood circulation, inversion of surface charge (to positive) at the pH of the tumor microenvironment may allow the nanoparticles to be taken up by cancer cells. To prove this concept, charge-invertible nanoparticles modified with novel slightly acidic pH-sensitive peptide (SAPSP-NPs) were developed. The negatively-charged SAPSP-NPs were delivered to tumor tissue, and were successfully taken up by cancer cells upon inversion of the surface charge to positive at intratumoral pH. SAPSP-NPs may serve as an alternative carrier to the PEGylated NP for anti-cancer drug delivery.

1. Introduction

Drug carriers that can deliver anti-cancer drugs specifically to cancer cells in tumor tissue may achieve ideal cancer therapy without side effects. Thus, it is necessary to control both the biodistribution and cellular uptake of drug carriers. To achieve this, physicochemical properties of the drug carriers, especially surface charge, are important. Cationic carriers are currently used for in vitro experiments, as these show high binding affinity to cells via electrostatic interactions between the carrier and cellular membranes [1,2]. However, biodistribution of cationic carriers is not effective due to their strong interactions with circulating blood proteins, blood cells, cells of mononuclear phagocyte system, and the luminal surface of blood vessels, which is rich in negatively-charged substances [3]. Therefore, PEGylated carriers have been developed to avoid these undesired interactions [4]. Although PEGylated carriers are effectively delivered to tumor tissue via an enhanced permeation and retention (EPR) effect [5], the cellular uptake efficiency of PEGylated carriers is known to be low [6,7]. This problem is recognized as the PEG dilemma [6,7]. For enhancement of cellular uptake of the PEGylated carriers, targeting ligands against surface proteins on cancer cells, such as peptides and antibodies, have been attached to the tip of PEG [8,9]. However, as cellular uptake of the ligand-modified carriers depends on the quantity of surface protein expressed [10], cellular uptake efficiency is typically not very high. Therefore, it is difficult to control both the biodistribution and cellular uptake of currently available carriers under pathological conditions.

Thus, to overcome the PEG dilemma, alternative carriers are required. The aim of our research was to develop a novel carrier that can effectively deliver a drug to tumor tissue and be efficiently taken up by cancer cells. We focused on the surface charge of the carriers, as a positive surface charge is required for effective cellular uptake [11,12], whereas a negative surface charge is necessary to avoid interactions with biogenic substances [13,14]. Therefore, the strategy proposed in this study is one of surface charge conversion of DDSs from negative to positive in response to pathological conditions. The pH value within tumor tissue is generally recognized as slightly acidic (pH 6.5–7.2) [15,16]. Thus, we focused our attention on the specific acidic pH environment within tumor tissue as a trigger for reversing the surface charge of the carriers. However, charge-invertible devices responsive to such slightly acidic pH media have not yet been developed. Here, to develop novel charge-invertible nanoparticles responsive to intratumoral pH, we designed a novel slightly acidic pH-sensitive peptide (SAPSP) comprised of histidine (His) and glutamic acid (Glu) residues, the sequence of which is shown in Fig. 1a. Various peptides containing His have

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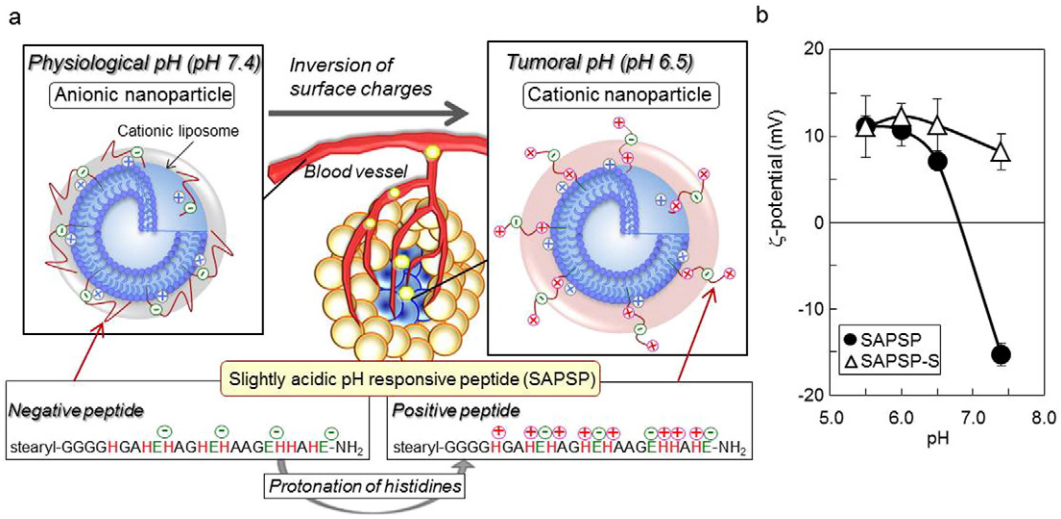

Fig. 1. Conceptual diagram and characteristics of slightly acidic pH responsive peptide modified nanoparticles (SAPSP-NPs). a) Conceptual diagram of SAPSP-NPs. At physiological pH, the SAPSP-NPs are negatively charged and are effectively delivered to tumor tissue due to the weak interactions with biogenic substances. In contrast, at the intratumoral pH (slightly acidic pH), the surface charge of the SAPSP-NPs is inverted from negative to positive, and the cellular uptake of the SAPSP-NPs is accelerated. b) Surface charge of SAPSP- or its scramble peptide (SAPSP-S)-modified nanoparticles (SAPSP-NPs or SAPSP-S-NPs, respectively) at various pH values. The surface charge of these nanoparticles was determined using a Zetasizer Nano (Malvern Ins. Ltd.). Data indicate mean ± SD at from three individual experiments.

previously been used in the escape of nanoparticles from acidic organelles resulting from protonation responsive to acidic pH (<6.0) [17]. However, since the pKa value of His is around 6.0, these peptides often exhibit difficulties in responding to the slightly acidic pH of the tumor milieu (around pH 6.5). Therefore, to design charge-invertible peptides comprised of His, whose pKa is >6.5, Glu residues were introduced as neighbors to His in the peptide sequence, as a means to increase the pKa value of the basic amino acid via stabilization of the protonated form [18–20].

Here, we show that SAPSP-modified nanoparticles (SAPSP-NPs) comprised of cationic and neutral lipids exhibit high sensitivity to a slightly acidic pH (around 6.5), and that the overall charge inverts from negative to positive. Furthermore, the SAPSP-NPs show efficient biodistribution at physiological pH, while efficient cellular uptake of the cationic nanoparticles modified with charge-inverted SAPSPs was observed under slightly acidic pH conditions.

2. Materials and methods

2.1. Materials

B16-F1 cells, a mouse melanoma cell line, were obtained from Dainippon Sumitomo Pharma Biomedical Co., Ltd (Osaka, Japan). Amiloride hydrochloride hydrate and Hoechst 33342 were purchased from Sigma Aldrich (St. Louis, MO, U.S.A.). Egg phosphatidylcholine (EPC) and *N*-(carbonyl-methoxypolyethyleneglycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (PEG_{2000}-DSPE) were obtained from NOF Corporation (Tokyo, Japan). 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (NBD-PE) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rh-PE) were purchased from Avanti Polar Lipid (Alabaster, AL, U.S.A.). Stearylated octaarginine (stearyl-R8), stearylated slightly acidic pH sensitive peptide (SAPSP) and slightly acidic pH sensitive peptide scramble, stearyl-GGGGHGEAHHAEGHHAEAHHGEAH-NH_2 (SAPSP-S), were synthesized by Scrum Inc. (Tokyo, Japan). LysoTracker Green DND-26 and 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) were obtained from Invitrogen (Carlsbad, CA, U.S.A.). Anti-luciferase siRNA (21-mer, 5′-GCGCUGCUGGUGCCAACCCTT-3′, 5′-GGGUUGGCACCAGCAG CGCTT-3′) and negative control siRNA (21-mer, 5′-UAUUGCGUCU GUACACUCATT-3′, 5′-UGAGUGUACAGACGCAAUATT-3′) and luciferase primer (forward: 5′-GAGGACCAGGTTGTCTCCTG-3′, reverse: 5′-ATGTAGGCCATGAGGTCCAC-3′) were synthesized by Invitrogen (Carlsbad, CA, U.S.A.). The plasmid pcDNA3.1(+)Luc encoding the luciferase gene was purified with a Qiagen Endofree Plasmid Giga Kit (Qiagen GmbH, Hilden, Germany).

2.2. Preparation of SAPSP-NPs, cationic liposomes and PEG-liposomes

Liposomes were prepared by a simple hydration method. Briefly, EPC and DOTAP dissolved in ethanol were mixed at a molecular ratio of 7.6:1 in a glass test tube. To prepare the film, the lipid mixture was dried using nitrogen gas. Next, the lipid film was hydrated by addition of PBS(−) (total lipid concentration: 10 mM), followed by sonication in a bath-type sonicator (ULTRASONIK 14B, NEY, CA). To modify the liposomes with SAPSP, stearyl SAPSP (5 mol%) was incubated with the liposomes for 30 min. The lipid compositions of cationic liposomes and PEG-liposomes used in this study were EPC/DOTAP (7.6/1 mol/mol) and EPC/cholesterol/DSPE-PEG (1.85/1/0.15 mol/mol), respectively. pDNA or siRNA-encapsulated SAPSP-NPs were prepared according to previous reports with a minor modification [21,22]. To prepare the core for siRNA-encapsulated SAPSP-NPs, siRNA and stearyl-R8 were mixed at a ratio of 1.05:1.0, as determined by the nitrogen/phosphate ratio. On the other hand, to prepare the core for pDNA-encapsulated SAPSP-NPs, pDNA and protamine were mixed at a ratio of 1.0:1.0, as determined by the nitrogen/phosphate ratio. The core was added to the lipid film and then siRNA- or pDNA-encapsulated SAPSP-NPs were prepared by sonication. The lipid composition of PEG-modified nanoparticles containing pDNA was EPC/DOTAP/cholesterol/DSPE-PEG (4/3/3/0.5 mol/mol). The particle size and surface charge of the nanoparticles were examined using a Zetasizer Nano (Malvern Ins. Ltd.).

Table 1
Particle size of SAPSP-NPs and SAPSP-S-NPs.

pH	7.4	6.5	6.0	5.5
SAPSP-NPs	173 ± 7	265 ± 43	198 ± 14	158 ± 5
SAPSP-S-NPs	1471 ± 718	723 ± 523	684 ± 47	946 ± 550

The data are expressed as the mean ± SD values from at least three different preparations.