Journal of Controlled Release 267 (2017) 181–190

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

A PEGylated hyaluronic acid conjugate for targeted cancer immunotherapy

Jung Min Shin[a,1], Se Jin Oh[b,c,d,1], Seunglee Kwon[a], V.G. Deepagan[a], Minchang Lee[a], Seok Ho Song[a], Hyo-Jung Lee[b,c,d], Suyeon Kim[b,c,d], Kwon-Ho Song[b,c,d], Tae Woo Kim[b,c,d,e,*], Jae Hyung Park[a,**]

[a] School of Chemical Engineering, College of Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea
[b] Laboratory of Tumor Immunology, Department of Biomedical Sciences, Graduate School of Medicine, Korea University, Seoul 02841, Republic of Korea
[c] Department of Biochemistry and Molecular Biology, College of Medicine, Korea University, Seoul 02841, Republic of Korea
[d] Department of Biomedical Science, College of Medicine, Korea University, Seoul 02841, Republic of Korea
[e] Translational Research Institute for Incurable Diseases, College of Medicine, Korea University, Seoul 02841, Republic of Korea

ARTICLE INFO

Keywords:
Cancer immunotherapy
Foreignization
Antigen delivery
Hyaluronic acid
PEGylation
Matrix metalloproteinase 9 (MMP9)

ABSTRACT

The cell-free approach to foreignizing tumor cells with non-self antigens has received increasing attention as a method to induce cytotoxic T lymphocyte (CTL)-mediated immunological rejection of tumors, because the clinical translation of the conventional CTL-based cancer immunotherapies has been limited by a complicated manufacturing process and autotransplantation. In this study, we prepared matrix metalloproteinase 9 (MMP9)-responsive polymeric conjugates consisting of PEGylated hyaluronic acid (HA) as the targeting moiety and ovalbumin (OVA) as the model foreign antigen. The MMP9-cleavable linker was introduced between PEG and the HA backbone to facilitate the detachment of the PEG corona from the conjugate at the tumor site. From the *in vitro* cellular uptake study, it was revealed that the conjugate was effectively taken up by the CD44-expressing TC-1 cancer cells in the presence of MMP9 *via* receptor-mediated endocytosis. When the conjugate was systemically administered into the tumor-bearing mice with endogenous OVA-specific CTLs, the tumor growth was markedly inhibited, which was attributed to the significant antigen presentation on the tumor cells. Overall, the MMP9-responsive conjugates bearing foreign antigens might have the potential as an alternative to CTL-based cancer immunotherapeutics.

1. Introduction

$CD8^+$ cytotoxic T lymphocytes (CTLs) have been explored as a tool to eliminate target cells with antigenic peptides presented by surface major histocompatibility complex (MHC) class I molecules [1–3]. Therefore, CTL-based cancer immunotherapies such as adoptive cell therapy (ACT) and engineered chimeric antigen receptor (CAR) T cell therapy have shown the potential to treat cancer without side effects [4–7]. In the clinic, these therapies have exhibited statistically significant improvement over conventional chemotherapy and radiotherapy by avoiding non-specific cell death [8–10]. Although ACT and CAR T cell therapies are effective strategies to overwhelm cancer, they require a high cost, a complicated manufacturing process and autografting, limiting their extended applications to cancer patients [11–13]. Besides, most existing approaches for CTL activation focus on delivering tumor antigen to antigen-presenting cells not tumors. In this regard, the innovative cell-free approach to foreignizing tumor cells *via* site-specific non-self antigen delivery can be an option to induce the CTL-mediated immunological rejection of tumors. Recently, we reported on a hyaluronic acid (HA)-based polymeric conjugate as a potential immunotherapeutic agent to foreignize cancer cells by delivering the antigen *via* receptor-mediated endocytosis [14]. Owing to its specific binding affinity to the HA receptor (CD44), the conjugate was selectively taken up by the cancer cells, resulting in high antitumor efficacy after its systemic administration into the tumor-bearing mice [15]. The conjugate, however, exhibited significant accumulation in the liver. This might be due to its uptake by phagocytic cells in the reticuloendothelial system and by liver sinusoidal endothelial cells expressing the other HA receptor (stabilin-2), and thus requires a strategy to minimize the liver accumulation of the conjugate [16,17].

In recent years, stimuli-sensitive drug delivery systems have emerged to treat intractable diseases, since they release the drug at the

* Correspondence to: Tae Woo Kim, Laboratory of Tumor Immunology, Department of Biomedical Sciences, Graduate School of Medicine, Korea University, Seoul 02841, Republic of Korea.
** Corresponding author.
E-mail addresses: twkim0421@korea.ac.kr (T.W. Kim), jhpark1@skku.edu (J.H. Park).
[1] These authors contributed equally to this paper.

http://dx.doi.org/10.1016/j.jconrel.2017.08.032
Received 3 May 2017; Received in revised form 14 August 2017; Accepted 23 August 2017
Available online 26 August 2017
0168-3659/

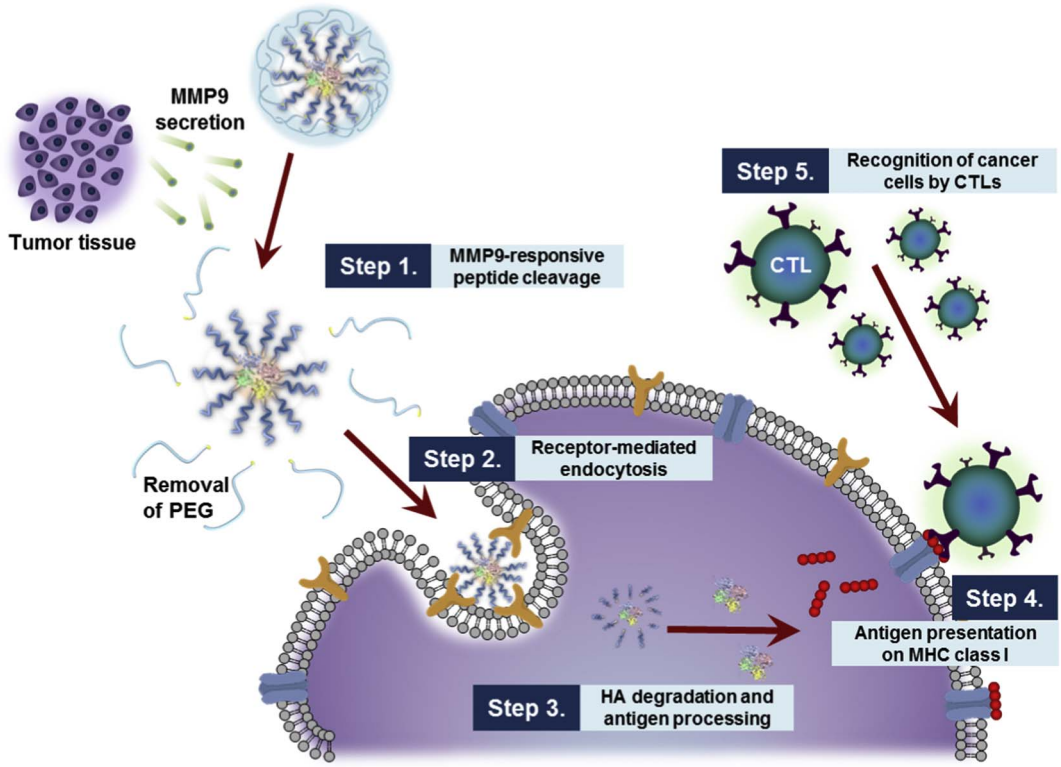

Fig. 1. Schematic illustration depicting working mechanism of MMP9-responsive polymeric conjugate.

target site by changing their physicochemical properties in response to external (changes in temperature, magnetic force or light) or internal (variations in pH, enzyme concentration or redox potential) stimuli [18–25]. The relevant microenvironmental characteristics to differentiate malignancy from the normal tissues include acidic pH, high levels of specific enzymes such as matrix metalloproteinase (MMP) and hypoxia [26–29]. In particular, MMPs play important roles in tumor initiation, progression and metastasis while their levels are proportional to the malignancy of the tumor [30,31]. Therefore, significant efforts have been made to harness MMPs as the keys to developing nanomedicines for cancer imaging and therapy [32–34].

In an attempt to foreignize the cancer cells effectively, we herein prepared an HA-based polymeric conjugate grafted with poly(ethylene glycol) (PEG) through an MMP9-cleavable linker (Fig. 1). The PEGylated HA derivative was then chemically attached to ovalbumin (OVA), which was chosen as the model foreign antigen. Since the resulting conjugate had a PEG corona that could be detached in the presence of MMP9, the HA of the conjugate could be exposed only at the tumor site, facilitating its cellular uptake *via* receptor-mediated endocytosis. Consequently, foreignized cancer cells may present antigenic epitopes on the MHC class I molecules, resulting in the immunological rejection by CTLs. To test this hypothesis, we investigated the antigen presentation capability, tumor-homing ability and therapeutic efficacy of the conjugate *in vivo*.

2. Materials and methods

2.1. Materials

HA (MW = 3.5×10^4 g/mol) was purchased from Lifecore Biomedical Inc. (Chaska, MN, USA). Monomethoxy polyethylene glycol amine (PEG-NH_2, MW = 5×10^3 g/mol) was bought from Laysan Bio Inc. (Arab, AL, USA). The $OVA_{257–264}$ peptide (purity > 95%) and 9-fluorenylmethoxycarbonyl (Fmoc)-PLGLWADR peptide (pep; purity > 95%) were obtained by custom synthesis from Peptron (Daejeon, Korea); their purity was verified by high performance liquid chromatography. Sodium cyanoborohydride ($NaBH_3CN$), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide·hydrochloride (EDC·HCl), *N*-hydroxysuccinimide (NHS), 1-hyroxybenzotriazole (HOBt), fluorescein isothiocyanate (FITC), and OVA were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). MMP9 was purchased from R & D Systems, Inc. (Minneapolis, MN, USA), and incubated with 2.5 mM of *p*-aminophenyl mercuric acid in 0.1% NaOH buffer for 1 h at 37 °C to activate it before use. Biotin-conjugated anti-mouse MHC class I $OVA_{257–264}$ (pMHC-$OVA_{257–264}$) antibody was obtained from eBioscience (San Diego, CA, USA). FITC-conjugated CD8 antibody, phycoerythrin (PE)-conjugated active caspase-3 antibody, PE-conjugated interferon (IFN)-γ antibody, and Cytofix/Cytoperm™ kit were purchased from BD Biosciences (San Diego, CA, USA). Active caspase-3 antibody was used to detect apoptotic cells through its specific recognition of enzymatically active p17 and p12 fragments of caspase-3. IFN-γ antibody was applied to the detection of activated CTLs. Cell culture products including RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Hyclone (Logan, Utah, USA). Athymic nude mice and C57BL/6 mice were purchased from Orient-bio Inc. (Seongnam, Korea). All animal experiments were performed in compliance with the relevant laws and guidelines of Korea University Institutional Animal Care and Use Committee (KUIACUC-2015-282) under its approval for the experimental procedures. All other chemicals were of reagent grade and were used without further purification.

2.2. Preparation and characterization of MMP9-responsive polymeric conjugate

2.2.1. Synthesis of the MMP9-responsive PEG (PEG-pep-NH_2)

The PEG-pep-NH_2 was synthesized *via* EDC/NHS chemistry. Briefly, the MMP9-cleavable Fmoc-protected RDAWLGLP (20 mg) was dissolved in dimethyl sulfoxide (1 ml), and this was followed by the addition of phosphate buffer (1 ml, pH 6.8) containing EDC (10.1 mg) and NHS (10.1 mg). The PEG-NH_2 (87 mg) was then dissolved in phosphate