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A polymeric conjugate foreignizing tumor cells for targeted immunotherapy in vivo

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ABSTRACT

Antigen-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) are key elements of immunological rejection in transplantation as well as cancer immunotherapy. Most tumors, however, are not immunologically rejected because they have self antigens, which are not recognized as the foreigner by CTLs. In this study, we hypothesized that "foreignizing" tumor cells by delivering non-self foreign antigens into the tumors would result in rejection by foreign antigen-reactive CTLs. As the model system to foreignize the tumors, we prepared a polymeric conjugate consisting of hyaluronic acid as the $CD44^+$ tumor-targeting ligand and ovalbumin (OVA) as a foreign antigen. When the conjugate was treated with $CD44^{high}$ TC-1 tumor cells, it was effectively taken up and allowed for displaying of antigenic $OVA_{257–264}$ peptide at MHC class I on the surface of the cells. In addition, the conjugate was effectively accumulated into tumor tissue after its systemic administration to mice which are immunized with a vaccine for a vaccinia virus expressing OVA to generate $OVA_{257–264}$ specific CTLs, resulting in substantial inhibition of tumor growth. Overall, these results suggest that the polymeric conjugates bearing foreign antigens may be innovative and promising cancer immunotherapeutic agents by foreignizing tumor cells, leading to immunological rejection.

1. Introduction

Cytotoxic T lymphocytes (CTLs), differentiated from antigen-specific $CD8^+$ lymphocytes, have an ability to destroy target cells by recognizing antigenic peptides which are presented by surface major histocompatibility complex (MHC) class I molecules [1–4]. Owing to this unique function, CTLs are key players in cancer immunotherapy and transplant rejection [5–10]. In particular, the CTLs can be used as an alternative to overcome non-specific cell death caused by chemotherapy and radiotherapy. Therefore, cancer vaccine which can generate tumor antigen-specific CTLs is expected as a promising therapeutic agent with high tumor specificity and low side effects. Transplant rejection is a similar process in which a transplant recipient's immune system rejects the grafted organ or tissue [11,12]. This rejection also occurs in the presence of CTLs which can recognize foreign antigens in the transplanted organ or tissue. In general, immunological tolerance to a foreign graft is readily broken, since the graft contains immunologically reactive foreign antigens. Most tumors, however, are not immunologically rejected because they have "self antigens" which are not recognized by the immune system. This phenomenon is referred to as self-tolerance, which prevents self-destructive autoimmunity [4,13,14]. Owing to the self-tolerance as a defensive measure in the immune system, the potency of immunotherapeutic agents targeting endogenous tumor antigens has been often hindered. On the basis of those facts, it is expected that foreign antigen-specific CTLs of the host immune system can recognize and destroy tumor cells if they present foreign "non-self" antigens. For effective foreignization of tumor cells, the foreign antigens need to be internalized via the receptor-mediated endocytosis because extracellular antigens, taken up by fluid-phase endocytosis, are rarely presented by MHC class I molecules on tumor cells [15]. It should be emphasized that, to date, no significant effort has been devoted to the development of the tumor-specific, intracellular delivery system for the foreign antigen.

Polymeric conjugates or nanoparticles bearing targeting ligands have been extensively used as the drug carriers for cancer therapy

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because they accumulate passively at the tumor site through the enhanced permeation and retention effect, followed by receptor-mediated endocytosis by tumor cells [16–22]. Of polymeric materials, considerable effort has focused on using hyaluronic acid (HA) as a drug carrier for cancer therapy and imaging, because tumor cells over-express HA receptors, such as CD44 and a receptor for hyaluronan-mediated motility (RHAMM) [23–25]. In recent years, we also developed HA-based nanocarriers for targeted delivery of therapeutic and imaging agents to tumors, demonstrating that the HA surface allowed for their preferential accumulation at tumor tissue and effective uptake by tumor cells through passive and active mechanisms [26–31].

In this study, we hypothesized that targeted delivery of foreign antigens to tumor cells could facilitate presentation of foreign antigen fragments on the cellular surface, thus allowing antigen-specific CTLs to eradicate a tumor (Fig. 1a). In an attempt to verify this hypothesis, we prepared a polymeric conjugate consisting of HA as the tumor-targeting ligand and ovalbumin (OVA) as a model foreign antigen (Fig. 1b). The antigen presentation behaviors after treatments of the conjugate were quantitatively evaluated both in vitro and in vivo using a flow cytometer. The in vivo biodistribution and anti-tumor efficacy of the conjugate were also investigated after its systemic administration into the tumor-bearing mice.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (MW = 3.5×10^4 Da) was purchased from Lifecore Biomedical LLC (Chaska, USA). Ovalbumin (OVA), sodium cyanoborohydride ($NaBH_3CN$), fluorescein isothiocyanate (FITC), triton X-100, and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Biotin-conjugated anti-mouse MHC CI OVA peptide (pMHC-$OVA_{257-264}$) antibody was purchased from eBioscience (San Diego, CA, USA). FITC-conjugated CD8 antibody, phycoerythrin-conjugated active caspase-3 antibody, and Cytofix/Cytoperm™ kit were purchased from BD Bioscience (San Diego, CA, USA). Active caspase 3 antibody which can specifically recognize enzymatically-active p17 and p12 fragments of caspase 3 was used to detect the cells undergoing apoptosis. RPMI-1640 media and fetal bovine serum (FBS) were obtained from Welgene Inc. (Daegu, Korea).

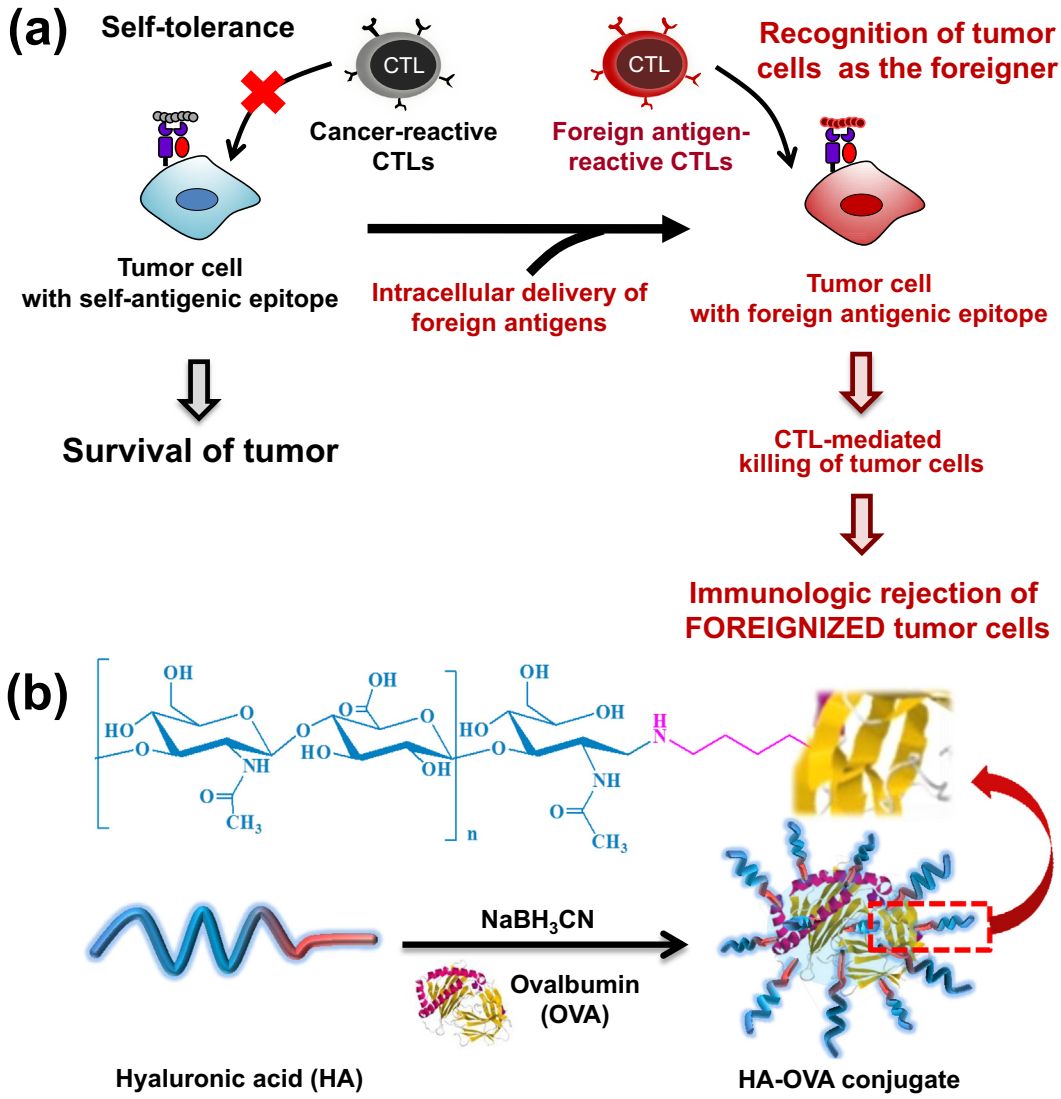

Fig. 1. Schematic illustration for immunological rejection of foreignized tumor cells. (a) Targeted delivery of foreign antigens into tumor cells prevents self-tolerance of tumors by foreignizing tumor cells. Consequently, the foreignized tumor cells are recognized and rejected by antigen-specific CTLs without harmful systemic side effects. (b) Synthesis of a polymer conjugate for targeted delivery of foreign antigen (OVA). The HA-OVA conjugate was prepared by reductive amination between the reducing end of HA to the amino group of OVA.