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Programmed nanoparticles for combined immunomodulation, antigen presentation and tracking of immunotherapeutic cells

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ABSTRACT

We report programmed nanoparticles (pNPs) that can tailor the immunotherapeutic function of primary bone marrow-derived dendritic cells (BMDCs) by *ex vivo* combined immunomodulation and track the *in vivo* migration of them after injection into body. Because DCs are the most effective antigen-presenting cells (APCs) that are able to present the antigens to T cells that contribute to tumor rejection, the maturation and monitoring of therapeutic DCs are essential for the efficient cancer immunotherapy. For combined immunomodulation of DCs, poly (lactic-co-glycolic acid) (PLGA) NPs containing both small interfering RNA (siRNA) for the knock-down of immune-suppressor gene (signal transducer and activator of transcription-3, STAT3) of DCs and an immune response modifier (imiquimod, R837) for the activation of DCs through the toll-like receptor 7 (TLR7) were developed. To deliver tumor antigen-specific information to DCs *ex vivo* and track the migration of DCs *in vivo*, another type of PLGA NPs containing tumor model antigen (ovalbumin, OVA) and near-infrared (NIR) fluorophores (indocyanine green, ICG) were also fabricated. These pNPs were taken up efficiently by DCs and various cytokines were expressed in matured DCs. DCs treated with pNPs also efficiently presented antigen-peptide to CD8 OVA 1.3 T cells through cross-presentation. Immunization of mice with these pNPs-treated DCs induced OVA-specific cytotoxic T lymphocytes (CTL) activity against the EG7-OVA tumor model and inhibited tumor growth efficiently. In addition, the migration of PLGA NPs-treated DCs to lymph nodes was monitored by NIR imaging technique. These multifunctional pNPs represent a promising technology for the combined immunomodulation and antigen-specific tumor therapy.

1. Introduction

The development of multifunctional nanomaterials has attracted interest in various biomedical fields for both disease prevention and therapy [1–4]. Various types of nanoparticles (NPs) have been used for the delivery of antigens to immune cells, which can exert strong immunotherapeutic effects in cancer and infectious disease [5–12]. Dendritic cells (DCs) are the most effective antigen-presenting cells (APCs) that are able to present the antigens to T cells and secrete the pro-inflammatory cytokines, resulting in tumor antigen specific activation of T cells that contribute to tumor rejection [13,14]. To present the antigens to cytotoxic T cells, DCs progress the cross-presentation that indicate extracellular antigens with histocompatibility complex (MHC)-I molecules. In this regard, the programmed maturation of DCs is essential for the efficient immune cells-based cancer therapy. Recent studies have shown that antigen-loaded PLGA particle enhances and prolongs antigen cross-presentation in DCs that induce cytotoxic T cell responses [15,16]. Two different approaches are issues of interest in DC-based cancer therapy. *Ex vivo* manipulation of the functioning of DCs by using nanoparticles is undergoing clinical trial and has been reported as more safe [17]. *Ex vivo* generated DCs secrete cytokines and express MHC as well as T-cell co-stimulatory molecules. In contrast, direct *in vivo* injection of NPs for DC programming can be less efficient as it shall be non-specifically endocytosed by other cells.

Activation of toll-like receptors (TLRs) in DCs leads to the expression of pro-inflammatory cytokines such as IL-12, IL-6, and TNF-α, resulting in enhanced innate and adaptive responses [18]. Imiquimod (R837), one of the most promising DCs activators, is a TLR7 agonist with immunomodulatory effects in mice [19]. R837 has been already used clinically against a wide range of infections and neoplasms, such as genital warts and actinic keratosis. Although activation of DCs by R837 leads to the expression of various pro-inflammatory cytokines [20], immunosuppressive genes in DCs, such as STAT3 (signal transducer and activator of

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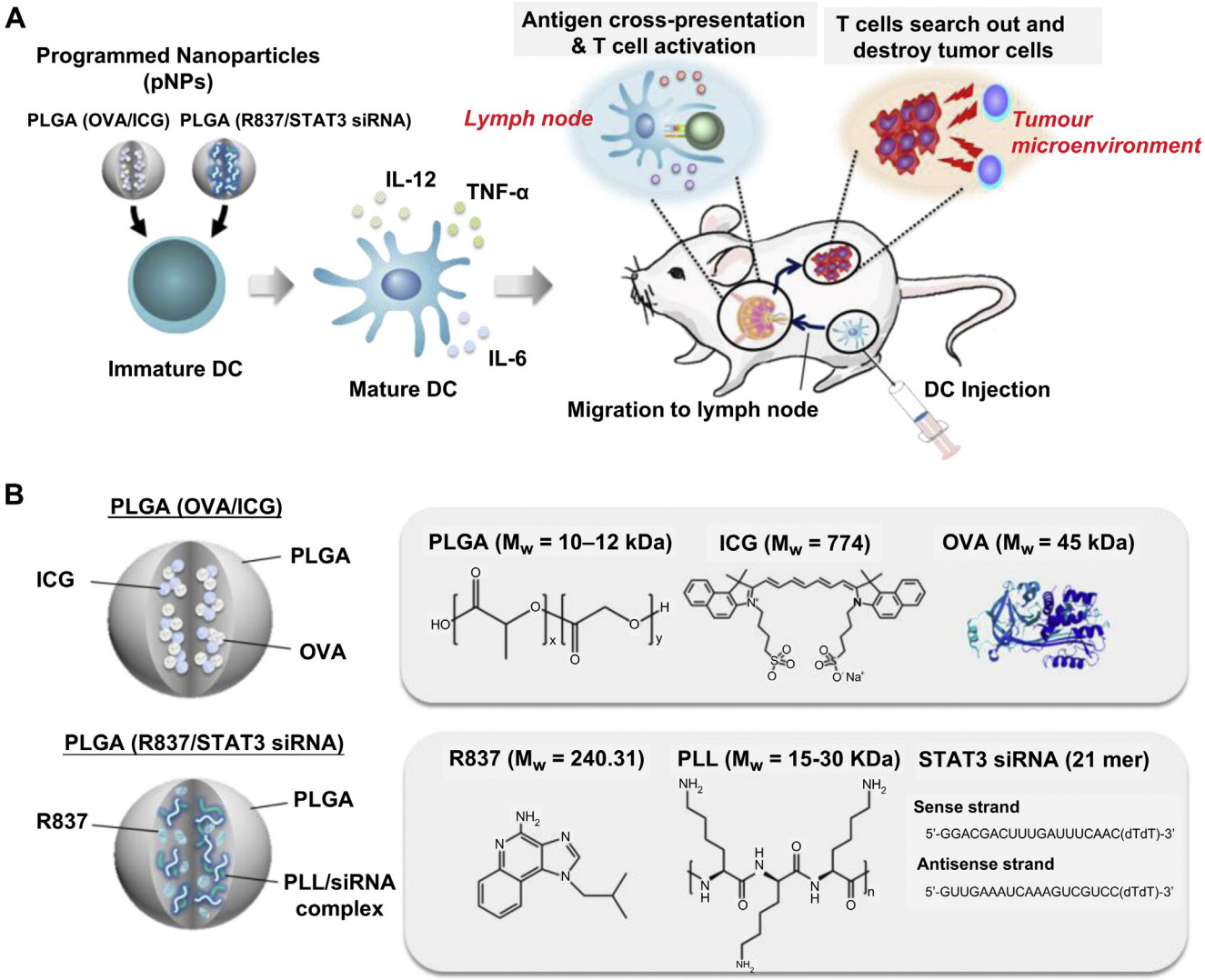

Scheme 1. Programmed nanoparticles (pNPs) help immune cell-based cancer therapy. (A) Activated dendritic cells (DCs) by pNPs migrate to lymph node, induce the antigen-specific immune responses, and lead to activation of cytotoxic T cells, which can kill tumor cells. (B) Synthesis of pNPs: PLGA(OVA/ICG); antigen presentation (ovalbumin; OVA) and monitoring DCs (indocyanine green; ICG), PLGA(R837/STAT3 siRNA); combined immunomodulation with R837 (for activation of TLR7) and STAT3 siRNA (for silencing of immunosuppressive genes, STAT3).

transcription-3), inhibit the immunostimulatory effects of R837 [21]. Because STAT3 influences the DCs maturation process mediated by R837, we reasoned that the simultaneous silencing of STAT3 by small interfering RNAs (siRNAs) and activation of TLR7 by R837 might effectively induce anti-tumor immunity in the tumor microenvironment. For the effective induction of a DCs-based immune response, the activated DCs must migrate into lymphoid tissues and interact with antigen-specific T cells. Therefore, the effective tracking of DCs to the lymph nodes of the patient is important for DCs-based cancer immunotherapy.

In this study, we have developed programmed nanoparticles (pNPs) that can tailor the immunomodulatory function of DCs *ex vivo* and track the *in vivo* migration of them after injection into body (Scheme 1). We adopted the powerful technique of RNA interference (RNAi) for the silencing of specific immune-suppressing mRNAs in therapeutic DCs *ex vivo*. In fact, most efforts to develop siRNA-based cancer therapies have focused on siRNA delivery systems that directly target and silence specific genes in cancer cells. In spite of their potential, siRNA-based cancer therapeutic strategies still have seen limited clinical application, because of the instability and very low *in vivo* targeting efficiency of siRNA [22–24]. In this study, because the STAT3 siRNA was introduced into activate DCs *ex vivo* condition before injection, the low targeting efficiency that previously hindered *in vivo* cancer therapy could be overcome. Two immunomodulatory materials (i.e. R837 and STAT3 siRNA) were encapsulated into a single poly (lactic-co-glycolic acid) (PLGA) NPs using the emulsion evaporation method [25]. To improve the encapsulation efficiency of both hydrophobic TLR7 ligand (R837) and small-sized hydrophilic STAT3 siRNA into hydrophobic PLGA matrix simultaneously, R837 was dispersed in oil phase while STAT3 siRNA incorporated with a cationic polymer was dispersed in water phase during the fabrication process [26]. Because efficient delivery of tumor-specific antigen into DCs *ex vivo* and tracking of matured DCs after injection, another type of PLGA NPs containing tumor model antigen (ovalbumin, OVA) and near-infrared (NIR) fluorophores (indocyanine green, ICG) were also fabricated.

2. Materials and methods

2.1. Materials

PLGA (D,L-lactide-co-glycolide) (Resomer® RG502H, monomer ratio 50:50, M_W 10–12 kDa) was purchased from Boehringer Ingelheim (Ingelheim, Germany).