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Polymer nanoparticles for enhanced immune response: Combined delivery of tumor antigen and small interference RNA for immunosuppressive gene to dendritic cells

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ABSTRACT

In this study, we report on polymer nanoparticles (NPs) that can induce an enhanced immune response in dendritic cell (DC)-based cancer immunotherapy by the combined delivery of tumor antigen and small interference RNA (siRNA) for the immunosuppressive gene to DCs. DCs are specialized antigen-presenting cells (APCs) that capture, process and present antigens and induce an antigen-specific cytotoxic T lymphocyte response. Because the suppressor of cytokine signaling 1 (SOCS1) is a negative regulator of the APC-based immune response, the inhibition of SOCS1 gene expression is essential for DCs to enhance antigen-specific anti-tumor immunity. Multifunctional poly(lactide-co-glycolic acid) (PLGA) NPs that can deliver tumor antigen and siRNA for immunosuppressive SOCS1 genes to DCs simultaneously were fabricated by the emulsion solvent evaporation method. We have found that the encapsulation efficiency of small-sized and hydrophilic SOCS1 siRNA into hydrophobic PLGA matrix is drastically enhanced by the help of a tumor model antigen such as ovalbumin (OVA), and the encapsulation efficiency of siRNA in PLGA (SOCS1 siRNA only) NPs and PLGA (OVA/SOCS1 siRNA) NPs was ~2% and 57.6%, respectively. PLGA (OVA/SOCS1 siRNA) NPs were efficiently taken up by bone-marrow-derived dendritic cells (BMDCs) and showed no detectable toxic effect. The knockdown of SOCS1 in BMDCs by PLGA (OVA/SOCS1 siRNA) NPs enhanced pro-inflammatory cytokine (tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-12 and IL-2) expression. Additionally, PLGA (OVA/SOCS1 siRNA) NP-treated BMDCs could elicit an immune response through cross-presentation in OVA-specific CD8 T cells that express IL-2 cytokine. Taken together, the combined delivery of NPs that can deliver both tumor antigen and immunosuppressive gene siRNA to BMDCs simultaneously could be a potent strategy to enhance immunotherapeutic effects in BMDC-based cancer therapy.

1. Introduction

Nanostructured materials have been used as delivery systems for antigens and adjuvant molecules in immune cell-based cancer therapies [1–8]. Polymer nanoparticles fabricated from biodegradable and biocompatible polymers, such as poly(lactide-co-glycolic acid) (PLGA), have been attractive as delivery carriers of small molecules, peptides and proteins, due to their efficient controlled release properties. In cancer immunotherapy, these copolymers are attractive from a clinical point of view because they show minimal systemic toxicity and have been approved for human use by the US Food and Drug Administration (FDA) [9,10]. Dendritic cells (DCs) are specialized antigen-presenting cells that capture, process and present antigens and induce an antigen-specific cytotoxic $CD8^+$ T lymphocyte response. Immunization of DCs loaded with antigen such as ovalbumin (OVA) protein can induce an antigen-specific immune response by efficiently presenting to T cells [11]. In an antigen presentation pathway, a process known as cross-presentation allows DCs to load extracellular antigenic peptides on major histocompatibility complex (MHC)-I molecules, which is important for activating cytotoxic T cells. Many studies have reported that antigen-loaded PLGA particles elicit cytotoxic T cell responses through antigen cross-presentation pathways [12,13]. Moreover, many research scientists have focused on the promotion of DC maturation and activation for enhanced anti-tumor immunity [14]. However, immunosuppressive factors, such as suppression of cytokine signaling 1 (SOCS1), represent a major

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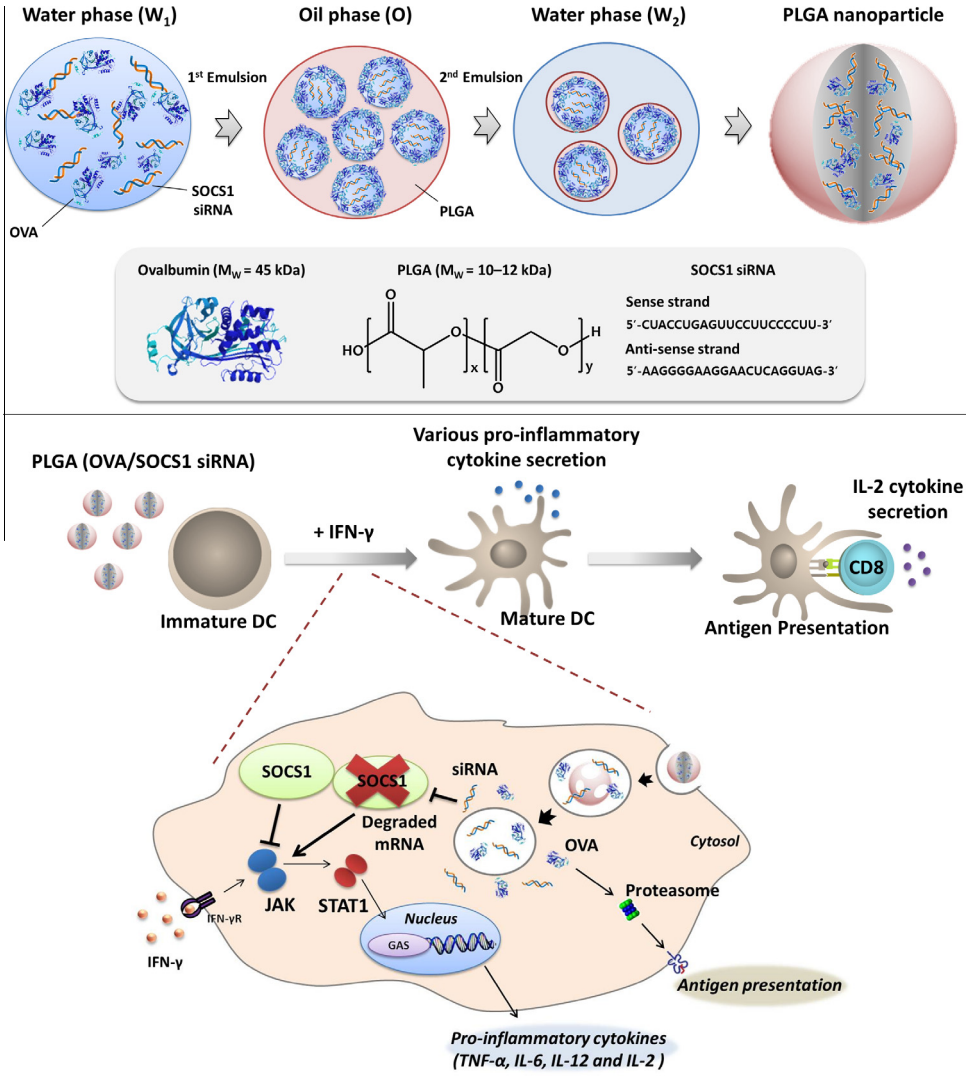
limitation for DC-based cancer therapies [15,16]. Thus, the silencing of SOCS1 in antigen-presenting DCs could enhance activation and maturation of DCs, resulting in antigen-specific high anti-tumor immunity. In this regard, small interfering RNA (siRNA) technology can be applied to DC-based cancer therapy for the silencing of specific immune-suppressing mRNAs in DCs and enhancement of the immune response and therapeutic effects [17]. Up to now, most efforts to develop siRNA-based cancer therapies have focused on siRNA delivery systems that directly target and silence specific genes in cancer cells. However, siRNA-based cancer therapeutic strategies have still seen only limited clinical application, due to the instability and very low *in vivo* targeting efficiency of siRNA [18–20]. In contrast, if we introduce SOCS1 siRNA to activate DCs *ex vivo* before *in vivo* injection, the critical problems that are induced by *in vivo* conditions, such as low targeting efficiency and stability of siRNA, could be overcome.

In this study, we developed multifunctional PLGA nanoparticles (PLGA NPs) containing immunosuppressive gene silencing siRNA (SOCS1 siRNA) and tumor model antigen (OVA) to increase the bone-marrow-derived dendritic cell (BMDC)-based antitumor therapeutic effect (Scheme 1). Interestingly, when the small-sized and hydrophilic SOCS1 siRNA was encapsulated into hydrophobic PLGA matrix in the presence of the tumor antigen OVA protein, the encapsulation efficiency of SOCS1 siRNA was dramatically increased (Scheme 1). Using PLGA (OVA/SOCS1 siRNA) NPs, we confirmed that those nanoparticles were efficiently taken up by BMDCs and the knockdown of SOCS1 enhanced pro-inflammatory cytokine expression such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-12 and IL-2. Additionally, PLGA (OVA/SOCS1 siRNA) NPs treated BMDCs could induce activation of CD8 OVA 1.3 T cells through antigen cross-presentation pathway.

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). Polyvinyl alcohol (PVA, 80% hydrolyzed, M_W 9–10 kDa) was purchased from Sigma–Aldrich (St



Scheme 1. Schematic illustration of the fabrication of PLGA (OVA/SOCS1 siRNA) NPs and their application for the immunomodulation of DCs. Because OVA protein could act as a stabilizer for the primary emulsion (W_1/O) as well as tumor model antigens, siRNA encapsulation efficiency was exceedingly increased. PLGA (OVA/SOCS1 siRNA) NP-treated BMDCs with activation factor (IFN-γ) enhance pro-inflammatory cytokine (TNF-α, IL-6, IL-12 and IL-2) expression by SOCS1 knockdown effect, and also present antigen efficiently to antigen-specific CD8 T cells via a cross-presentation pathway.