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Carrier-free nanoassembly of doxorubicin prodrug and siRNA for combinationally inducing immunogenic cell death and reversing immunosuppression

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ABSTRACT

Doxorubicin (DOX) can elicit antitumor immunity responses by inducing immunogenic cell death (ICD) but also triggers upregulated expression of various immunosuppressive genes to counteract the ICD effect. To resolve this conflict, a carrier-free nanoassembly of acid-activatable DOX prodrug and small interfering RNA (siRNA) was developed to combinationally induce ICD and reverse immunosuppression. The carrier-free nanoassembly with rather high drug contents (4.13 % for siRNA and 21.67 % for DOX) was formed *via* cooperative π–π stacking and electrostatic interactions. The formed nanoassembly, termed as PEG@D:siRNA, possessed a well-defined nanostructure: a core consisting of DOX plus siRNA and a shell consisting of polyethylene glycol (PEG). It has been demonstrated that this carrier-free nanoassembly carrying siRNA targeting PD-L1 can significantly increase tumor-infiltrating T lymphocytes, improve interferon-γ (IFN-γ) expression, and ultimately strengthen the ICD effect of the DOX prodrug, resulting in a significantly enhanced anticancer immune response and superior tumor growth inhibition. In addition, carrier-free nanoassembly PEG@D:siRNA can also be conveniently extended as a general strategy to combine chemotherapy and immunotherapy, providing a facile avenue for improving cancer chemoimmunotherapy.

Introduction

Immunotherapy is a type of cancer treatment that harnesses patients' own immune systems to detect and destroy tumor cells and has become a promising way to improve cancer treatment outcomes [1,2]. Current representative immunotherapies include T-cell transfer therapies [3], immune checkpoint blockade therapies [4–6], cancer vaccines [7,8], and cytokine therapies [9,10]. Recent accumulating evidence suggests that the traditional anticancer drug doxorubicin (DOX), an anthracycline antibiotic, not only triggers tumor cell death by apoptosis but also evokes effective antitumor immunity responses by inducing immunogenic cell death (ICD) [11,12]. DOX-treated tumor cells undergo ICD to stimulate anticancer immunity by the surface expression of calreticulin (CRT), releasing high-mobility group box 1 (HMGB-1) to improve DC maturation and antigen presentation, and secreting adenosine triphosphate (ATP) to facilitate the infiltration of cytotoxic T lymphocytes (CTLs) into tumor tissue [13,14]. Generally speaking, DOX-induced ICD could potentially turn these dying cancer cells into "vaccines" to stimulate anticancer immunity by improving the activation and infiltration of CTLs, which could enhance the antitumor immune response *in vivo* [15]. Unfortunately, accumulating preclinical and clinical evidence also indicated that the elicited antitumor immune response was inefficient to inhibit tumor growth due to various accompanying negative feedback mechanisms during DOX treatment that upregulate the expression of immunosuppression-related genes, such as programmed death ligand-1 (PD-L1) [16], cluster of differentiation 47 (CD47) [17], and indoleamine 2,3-dioxygenase-1 (IDO) [18].

To resolve the aforementioned issue, blockade of these negative regulatory pathways represents a promising strategy to reacti-

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