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Lanthanide-integrated supramolecular polymeric nanoassembly with multiple regulation characteristics for multidrug-resistant cancer therapy

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ABSTRACT

Cancer treatment can in principle be enhanced by the synergistic effects of chemo- and nucleic acid-based combination therapies but the lack of efficient drug nanocarriers and occurrence of multidrug resistance (MDR) are major obstacles adversely affecting the effectiveness. Herein, a lanthanide-integrated supramolecular polymeric nanoassembly that delivers anticancer drugs and siRNA for more effective cancer therapy is described. This nanotherapeutic system is prepared by loading adamantane-modified doxorubicin (Dox) into polyethylenimine-crosslinked-γ-cyclodextrin (PC) through the supramolecular assembly to form the interior Dox-loaded PC (PCD) followed by electrostatically driven self-assembly of siRNA and PCD to produce the PCD/siRNA nanocomplexes. The PCD/siRNA nanocomplex is further decorated with the exterior neodymium (Nd)-integrated PC (Nd-PC) layer to obtain the PCD/siRNA/Nd-PC nanoassembly in which the interior PC serves as an efficient carrier for simultaneous delivery of Dox and siRNA to the human breast cancer cell line, Dox-resistant MCF-7 (MCF-7/ADR) both *in vitro* and *in vivo*. The exterior Nd-PC layer improves the drug sensitivity to the MCF-7/ADR cells as a result of the improved nanoassembly uptake, reduced drug efflux, and enhanced apoptosis, as evidenced by multiple regulation of a series of intracellular proteins related to MDR. Furthermore, *in vivo* delivery of the PCD/siRNA/Nd-PC nanoassembly is demonstrated to inhibit tumor growth in the mouse model with MCF-7/ADR tumor xenografts as a result of reduced angiogenesis and increased necrosis at the tumor site. This study reveals a simple and universal strategy to transform polymer-based nanoassemblies into advanced organic-inorganic nanotherapeutics suitable for cancer MDR therapy.

1. Introduction

Cancer is a complex disorder resulting from multiple genetic changes as well as cellular abnormalities and treatment can be difficult. In chemotherapies, cancer cells often develop resistance to multiple drugs with different chemical structures and mechanisms. The phenomenon, known as multidrug resistance (MDR), represents one of the major hurdles in cancer chemotherapy and contributes to the failure in the treatment of metastatic cancer [1]. Owing to the complexity of MDR, combination therapies involving two or more therapeutic drugs with different mechanisms have gained more attention recently [2]. Several drug delivery strategies have been proposed to overcome MDR and one of the promising approaches is to incorporate both chemotherapeutics and MDR modulators into nanoparticles to produce synergetic effects. For example, liposomes loaded with the cytotoxic drug and efflux pump are more cytotoxic to cancer cells than the free drug or nanoparticles loaded only with drugs [3,4]. Therefore, co-delivery of chemotherapeutics and MDR inhibitors can effectively suppress tumor cell growth and invasion by reversing the

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chemotherapeutic sensitivity [5,6]. Recently, RNA interference (RNAi) strategies to inhibit efflux pumps have been explored by exploiting siRNA as an alternative MDR modulator [7]. Despite the prospect of combination therapies, the therapeutic effect of co-delivery systems is restricted due to the lack of promising delivery vectors and hence, development of a functional delivery vehicle to maximize the effectiveness of combination therapies is needed.

Metal-incorporated organic assemblies combine the virtues of organic and inorganic materials to produce synergistic effects in biological applications [8–10]. For instance, surface functionalization of polymeric nanomaterials by plasma-based metal ion implantation has been applied to artificial blood vessels and antibacterial materials [11–13]. Metals such lanthanides, which are essential elements in the body, maintain the functionalities of proteins and enzymes and are involved in many physiological processes such as metabolism regulation, cell function promotion, and immune system maintenance [14,15]. Lanthanides can be transported into cells through the calcium ion channels or sodium-calcium exchange pathway [16–18] and the ability of lanthanides to activate endocytosis in plant cells has been demonstrated recently [19]. Lanthanides can facilitate cellular uptake of certain drugs by increasing the cell permeability [20,21] and suppress proliferation of cancer cells, as manifested by the decreased calmodulin (CaM) expression as well as up-regulation of other gene expressions in cancer cells [22]. Last but not least, lanthanides can induce significant morphological changes, arrest the transition from G0/G1 to S state [23], and trigger the receptor-mediated extrinsic pathway of apoptosis [24]. Our recent study on the neodymium (Nd)-functionalized gene delivery vector reveals higher gene transfection activity by stimulation of the cellular energy metabolism and enhancement of cellular uptake ability [25]. We have also demonstrated that supramolecular cationic polyethyleneimine (PEI)-cyclodextrin conjugates are efficient carriers to encapsulate hydrophilic drugs *via* host-guest interactions and induce nucleic acid complexation by electrostatic interaction [26,27].

In this work, we design and prepare a lanthanide-integrated supramolecular nanoassembly serving as an efficient carrier for simultaneous delivery of chemotherapeutic drugs and siRNA to combat cancer MDR (Fig. 1). Doxorubicin (Dox) is first conjugated with 1-adamantanecarboxylic acid (Ada-COOH) to form adamantane-modified doxorubicin (Ada-Dox). Ada-Dox is then incorporated into the hydrophobic cavities of (2-hydroxypropyl)-γ-cyclodextrins (HP-CD) on polyethylenimine-crosslinked γ-cyclodextrins (PC) *via* host-guest interactions between the adamantyl group and cyclodextrin to form Dox-loaded PC (PCD) as a core with positive surface charges. Negatively charged siRNA is then wrapped onto the exofacial surface of the PCD core by interacting with cationic polyethyleneimine arms to form the PCD/siRNA complexes. Finally, lanthanide integration is achieved by decorating the PCD/siRNA complexes with Nd-doped PC (Nd-PC) to form the PCD/siRNA/Nd-PC tertiary nanoassembly. The Nd-PC in the outer layer of the PCD/siRNA/Nd-PC nanoassembly provides a protective layer to prevent the siRNA from degradation in the bloodstream and interacts with the cell membrane to enhance penetration of the PCD/siRNA/Nd-PC nanoassembly through either the clathrin-mediated or caveolin-mediated endocytosis. Since the outer Nd-PC layer contains a large number of secondary and tertiary amines, protonation of the amino groups after cellular endocytosis causes accumulation of protons leading to influx of counter ions causing swelling and disruption of the endosome that traps the PCD/siRNA/Nd-PC nanoassembly. In the meantime, the release of siRNA is induced by the competitive interaction of the negatively charged substances in the cytoplasm [28,29] and Ada-Dox may be detached from the cavity of CD in PCD followed by the release of Dox under acidic and enzymatic conditions. The released siRNA into the cytoplasm down-regulates the P-gp protein expression, turns off the P-gp pump, and retards the efflux of the released Dox. This greatly facilitates the release Dox preserved in the cells and allows Dox to diffuse passively into nuclei to produce high cancer cell killing efficacy. The *in vitro* and *in vivo* therapeutic effects are investigated in terms of cellular uptake, cell apoptosis, tumor weight and volume, tumor angiogenesis, as well as immunohistology of tumor slices. Regulation of a series of proteins is also

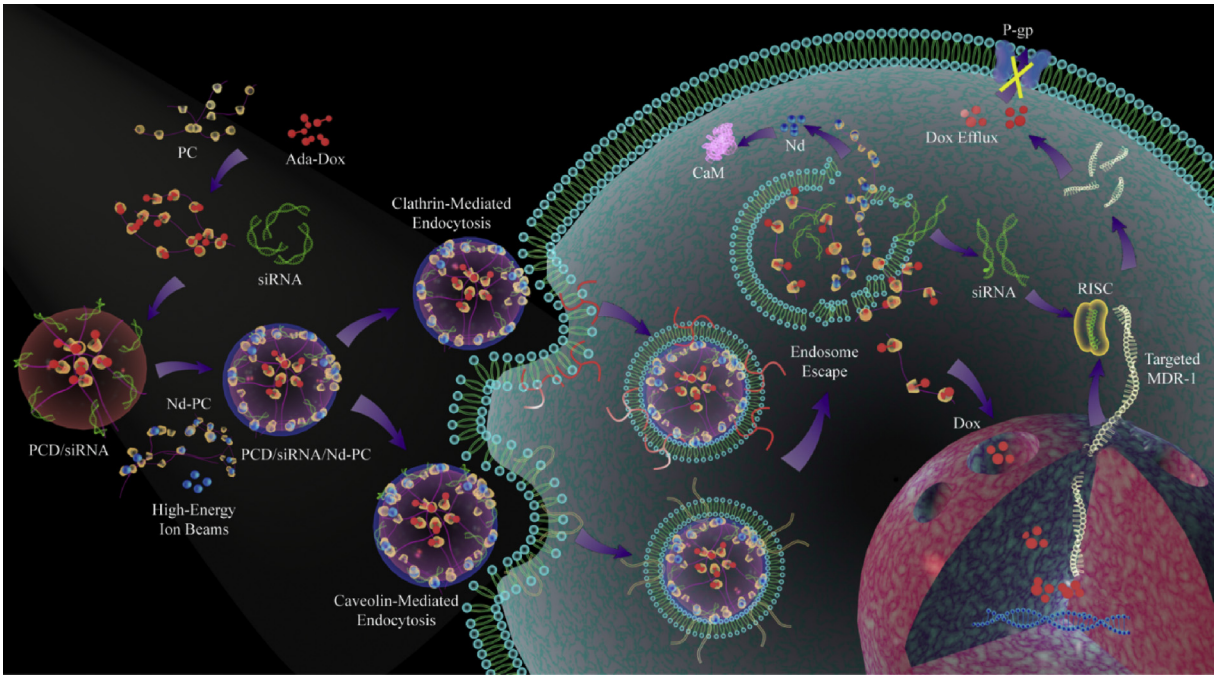

Fig. 1. Schematic illustration of the fabrication of the PCD/siRNA/Nd-PC nanoassembly and intracellular delivery mediated by the PCD/siRNA/Nd-PC nanoassembly.