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A self-assembled polymeric micellar immunomodulator for cancer treatment based on cationic amphiphilic polymers

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ABSTRACT

Here, we report a self-assembled polymeric micellar immunomodulator (SPI) for enhanced cancer treatment based on cationic amphiphilic polymers. To obtain the cationic amphiphilic polymer, the hydrophobic all-*trans*-retinoic acid (ATRA) was conjugated with a hydrophilic low-molecular-weight PEI ($_{Low}$PEI, M_n = 1.8 kDa). The ATRA–$_{Low}$PEI conjugates could self-assemble in aqueous media, forming micelles with a strong positive charge (~+40 mV) and particle sizes of ~70 nm. Compared to conventional therapeutic agents (*e.g.,* cisplatin), the SPI exhibited enhanced anti-cancer activity regardless of drug resistance. After mechanistic *in vitro* cell death studies, we revealed that the mechanical disruptive force generated by the cationic charge of SPI primarily induced necrotic cell death. Furthermore, the organelle fragments induced by the necrotic cell death triggered antitumoral immune responses. Therefore, SPI induced synergistic effects of the cationic charge-induced necrosis and antitumoral immune responses could produce an effective cancer treatment. In addition, the SPI was shielded by hyaluronic acid (HA/SPI complex) to enhance its tumor selectivity *in vivo*. Finally, the HA/SPI complex accumulated selectively into tumor sites after systemic administration into tumor-bearing mice, exhibiting effective antitumoral effects without systemic toxicity. Therefore, this technology holds great potential for translation into a clinical cancer treatment.

1. Introduction

Despite the great efforts to develop cancer treatments, including new chemotherapeutic drugs, the clinical therapeutic efficacies of conventional chemotherapy are not as high as expected due to their lack of selectivity and their susceptibility to resistance [1]. Moreover, because tumors consist of a heterogeneous population of malignant cancer cells carrying multiple genetic mutations, drug resistance arises from additional genetic and epigenetic alterations [2,3]. Consequently, treating cancer with a single low molecular chemotherapeutic drug is almost impossible [4].

Cancer immunotherapy holds great potential as a treatment strategy; it might enhance the natural ability of the immune system to recognize and kill cancer cells [5–8]. Modulating immune responses by administrating cytokines (*e.g.,* interleukin-12 (IL-12)) that facilitate the innate and adaptive immune systems is one of the most effective strategies used in cancer immunotherapy [6,9,10]. However, similar to many other therapeutic proteins, cytokine-based cancer immunotherapeutic strategies still have only limited clinical applications due to their instability and very low *in vivo* targeting efficiency [11]. Additionally, due to the difficulties encountered when manufacturing these cytokines, these therapeutic proteins are relatively expensive, limiting their clinical use [12].

Recently, many studies have revealed the relationship between the modes of cancer cell death (*e.g.,* apoptosis and necrosis) and the efficiency of inducing an immune response. The methods of cancer therapy that predominantly induce necrosis are significantly better than the methods that predominantly induce apoptosis when activating the immune system. During necrosis, the cytosolic constituents spill into the extracellular region through the damaged plasma membrane, promoting a powerful inflammatory response. More recently, we reported that cationic polymers, such as polyethylenimine (PEI), exhibit strong anti-cancer effects through necrosis due to cationic charge-induced cellular membrane damage [13,14].

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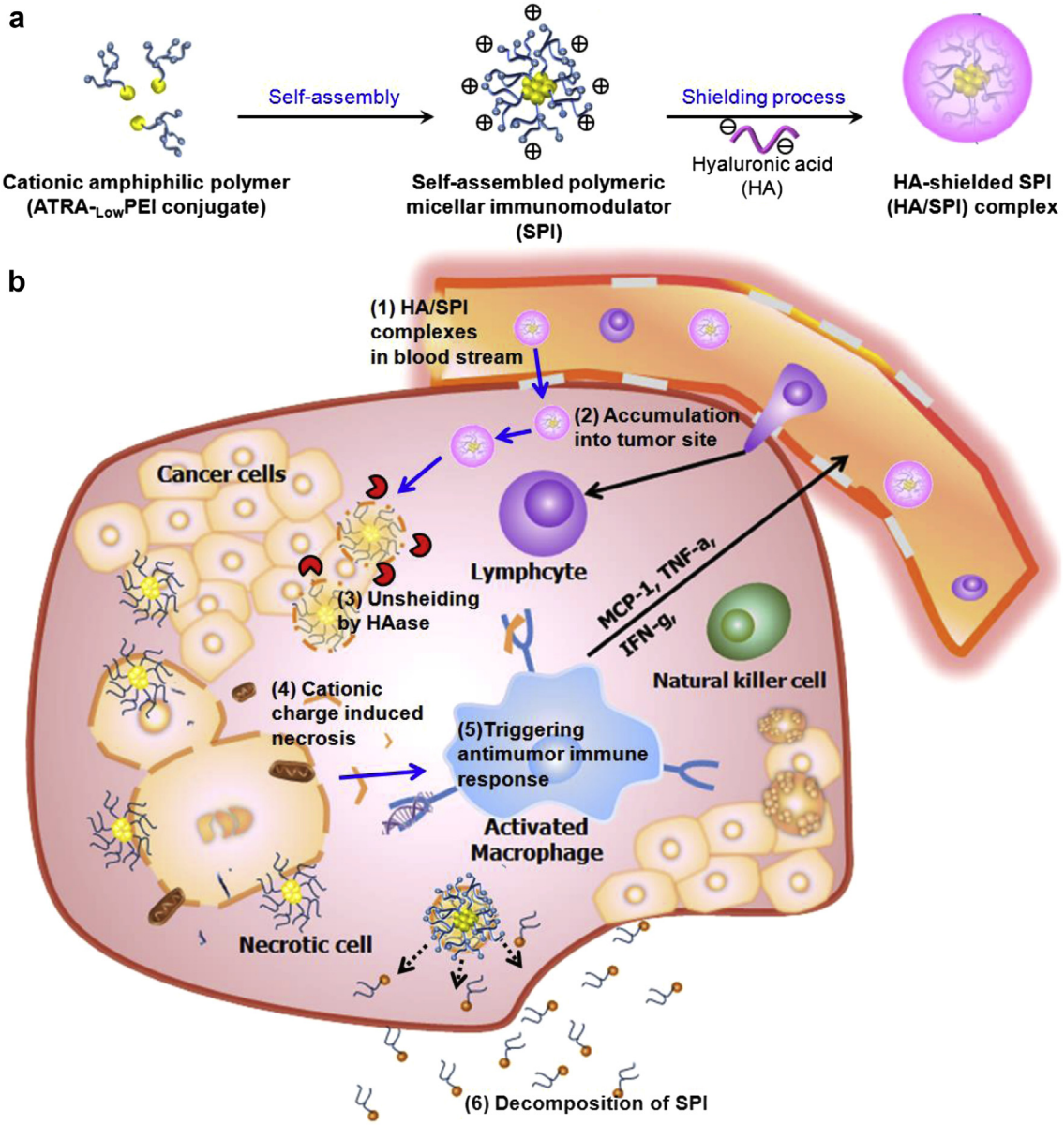
Inspired by previous studies, we designed a self-assembled polymeric micellar immunomodulator (SPI) based on cationic amphiphilic polymers for cancer treatment. The cationic amphiphilic polymer was composed of hydrophobic all-*trans*-retinoic acid (ATRA) and hydrophilic low-molecular-weight PEI ($_{Low}$PEI, M_n = 1.8 kDa), which can self-assemble into micelles in aqueous media. However, the positively charged nanomaterials exhibit severe toxicity, instability and a rapid clearance from the blood compartment, limiting their applications *in vivo* [15–17]. To overcome this problem, hyaluronic acid (HA) is used as a tumor specific shielding material (Scheme 1a); this substance is an anionic polysaccharide composed of repeating *N*-acetyl-D-glucosamine and D-glucuronic acid disaccharide units [18]. We hypothesized that shielding SPI with HA would significantly enhance the physical stability of the nano-complex *in vivo* while increasing the selectivity toward tumors. As shown in Scheme 1b, when circulating in the blood, the HA/SPI complexes would circulate for prolonged periods and leak into tumor sites through the enhanced permeability and retention (EPR) effect. At the tumor milieu, the HA/SPI unshielded, resulting in the formation of SPI when hyaluronidase (HAase) degrades HA; this enzyme is common in the tumor extracellular matrix and is critical for tumor growth, invasion and metastasis [19–21], Afterwards, the restored cationic charge of the SPI disrupted the cell membrane and induced necrosis. Furthermore, the organelle fragments generated by the necrotic cell death triggered cytokines, such as monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor α (TNF-α), thereby inhibiting cell growth. Therefore, the synergistic effects of cationic charge-



Scheme 1. Schematic illustration of a self-assembled polymeric micellar immunomodulator (SPI) for cancer treatment. (a) Preparation of SPI- and HA-shielded SPI (HA/SPI) complexes. (b) Anticancer mechanism of SPI in tumor tissue. The HA/SPI complexes circulate and passively diffuse into tumor tissue. The charge recovery (from negative to positive) occurs after the HA/SPI complexes react within the tumor tissue in response to the overexpressed HAase. The positively charged SPI forms a large cluster with the anionic plasma membrane on the outer surface, disrupting the integrity of the cell plasma membrane and inducing necrosis. The released organelles activate the macrophages at the tumor site. The activated macrophages release cytokines that recruit lymphocytes, activated natural killer cells and cytotoxic T lymphocytes. Finally, SPI decomposes into small molecule compounds for excretion without inducing systemic toxicity.