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Multifunctional "core-shell" nanoparticles-based gene delivery for treatment of aggressive melanoma

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ABSTRACT

Gene therapy may be a promising and powerful strategy for cancer treatment, but efficient targeted gene delivery *in vivo* has so far remained challenging. Here, we developed a well-tailored and versatile "core-shell" ternary system (RRPHC) of systemic gene delivery for treatment of aggressive melanoma. The capsid-like "shell" of this system was engineered to mediate depth penetration to tissues, simultaneously target the CD44 receptors and integrin $\alpha_v\beta_3$ receptors overexpressed on neovasculature and most malignant tumor cells, while the "core" was responsible for nucleus-targeting and effective transfection. The RRPHC ternary complexes enhanced cellular uptake via dual receptor-mediated endocytosis, improved the endosomal escape and significantly promoted the plasmid penetration into the nucleus. Notably, RRPHC ternary complexes exhibited ultra-high gene transfection efficiency (~100% in B16F10 cells), which surpassed that of commercial transfection agents, PEI 25K, Lipofectamine 2000 and even Lipofectamine 3000. Especially, RRPHC ternary complexes showed excellent serum resistance and remained high gene transfection efficacy (~100%) even in medium containing 30% serum. *In vivo* biodistribution imaging demonstrated RRPHC ternary complexes possessed much more accumulation and extensive distribution throughout tumor regions while minimal location in other organs. Furthermore, systemic delivery of the pro-apoptotic mTRAIL gene to tumor xenografts by RRPHC ternary complexes resulted in remarkable inhibition of melanoma, with no systemic toxicity. These results demonstrated that the designed novel RRPHC ternary complexes might be a promising gene delivery system for targeted cancer therapy *in vivo*.

1. Introduction

Gene therapy has attracted much attention in the past decades. It has great potential to treat a variety of diseases, such as cystic fibrosis, genetic diseases and cardiovascular diseases [1–3]. Currently, its potential in cancer treatment is widely recognized [4–6]. Here, we hypothesize that gene therapy may be a promising strategy for aggressive lung melanoma metastases treatment [7–9]. Since nucleic acid is prone to hydrolysis in physiological condition and its low permeability of the plasma membrane due to the polyanionic nature, gene delivery systems are inevitably needed for gene therapy [10,11]. Although viral vectors have shown high transfection efficiency, the fundamental drawbacks of severe immune/inflammatory reactions and the risk of recombination with wild-type viruses significantly limited their further clinical applications [12].

Non-viral gene vectors have attracted much attention in the past decade due to their safety, inherent low immunogenicity, easy of manufacture as well as tunable surface and structural properties [13,14]. However, the relative low transfection efficacy and cytotoxicity with conventional non-viral gene vectors largely limited their application [15]. Additionally, most of the current developed non-viral gene vectors were positively charged, which were subject to non-specific phagocytosis by the reticuloendothelium systems (RES), resulting in short blood circulation time [16]. Although PEGlation was successfully introduced to prolong the circulation,

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the cellular uptake and the releasing of gene may also be inhibited by the "PEG dilemma" [17]. On the other hand, as we know, the enhanced permeability and retention effect (EPR) is beneficial for the retention of nanoparticles from vascular systems to the solid tumors. Nevertheless, the EPR effect is a physiology-based principal mechanism subjected to many factors, including the blood pressure, the circulating duration in the blood stream, the nitric oxide level, etc [18,19]. The development of therapeutics based solely on passive targeting strategy might not capitalize on the potential efficacy of a nanoparticle-based delivery system. Indeed, active targeting is necessary for therapeutic delivery systems [20,21]. In this regard, many targeting moieties have been exploited in cancer-targeted therapy, but those directed to vasculature receptors offer a more effective strategy since the endothelial cells are the first accessible component after systemic administration [22–24]. Tumor neovasculature targeted moieties may recruit more nanocarriers to the tumor sites and result in effective accumulation into tumor tissues. Additionally, the weak tumor tissue penetration ability of the conventional nanoplexes resulted from the high oncotic pressure in solid tumors also largely limited their efficacy in *in vivo* application [25,26].

Herein, to solve the above problems with cancer gene therapy, we designed a novel "core-shell" ternary system with multi-stage and multiple tumor-targeting, nucleus-targeting and depth penetration ability for *in vivo* gene delivery (Fig. 1). The system consisted of a core of fluorinated polymers (PFs) binding with plasmid (pDNA) and a negatively charged multifunctional RRPH (RGD-R8-PEG-HA) shell. PFs could effectively condense pDNA, facilitate the endosomal escape and was especially with self nucleus-targeting ability. RRPH endowed this system with multi-stage, multiple tumor-targeting and depth penetration ability. RRPH was constructed by grafting hyaluronan polymer with PEG side chains, which were further conjugated with R8-RGD tandem peptide in the distal side. Hyaluronan (HA) is an anionic, non-toxic, biodegradable polysaccharide that specifically binds to the CD44, which is an adhesion molecule expressed on many types of tumors, such as breast cancer, ovarian cancer, colon cancer and melanoma [27–30]. Meanwhile, CD44 has been identified as one of the most established and common biomarkers associated with cancer stem cells (CSCs) in many types of tumors [31,32]. Additionally, hyaluronan can be partially degraded by hyaluronidase (HAase) over expressed in tumor stoma and tumor intracellular compartments to "turn off" the protective function of HA [33]. PEG side chains could prolong the circulation time of nanoparticles in the blood [34,35]. R8-RGD tandem peptide consisting of a specific peptide (RGD) in the front position and a cell penetrating peptide (R8) in the backend, possessed both specific targeting and high penetrating ability [36]. R8 could mediate the depth penetration to solid tumors, while RGD could specifically binds to integrin $\alpha_v\beta_3$ receptor, which was over expressed on tumor neovasculature and many types of tumor such as melanoma, breast cancer, colon cancers etc [37–39].

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising anticancer death ligand, which triggers apoptosis through interaction with the death receptors DR4 and DR5 [40]. It has been widely considered an optimal candidate for cancer gene therapy because of its tumor cell specificity and minimal effect on most normal cells [41,42]. Therefore, in the current study, we introduced our novel "core-shell" ternary system to the delivery of mTRAIL plasmid for *in vivo* cancer gene delivery. The cellular uptake, endosomal escape, multiple tumor-targeting ability and transfection efficiency *in vitro* and *in vivo* was carefully evaluated. Furthermore, the therapeutic efficacy of the system loaded with mTRAIL plasmid was also assessed in the model of B16F10 malignant melanoma.

2. Materials and methods

2.1. Materials

Sodium hyaluronate (HA) with an average molecular weight of 35 kDa was obtained from Shandong Freda Biochem Co., Ltd. (Shandong, China). R8-RGD peptides with a terminal cysteine [Cys-

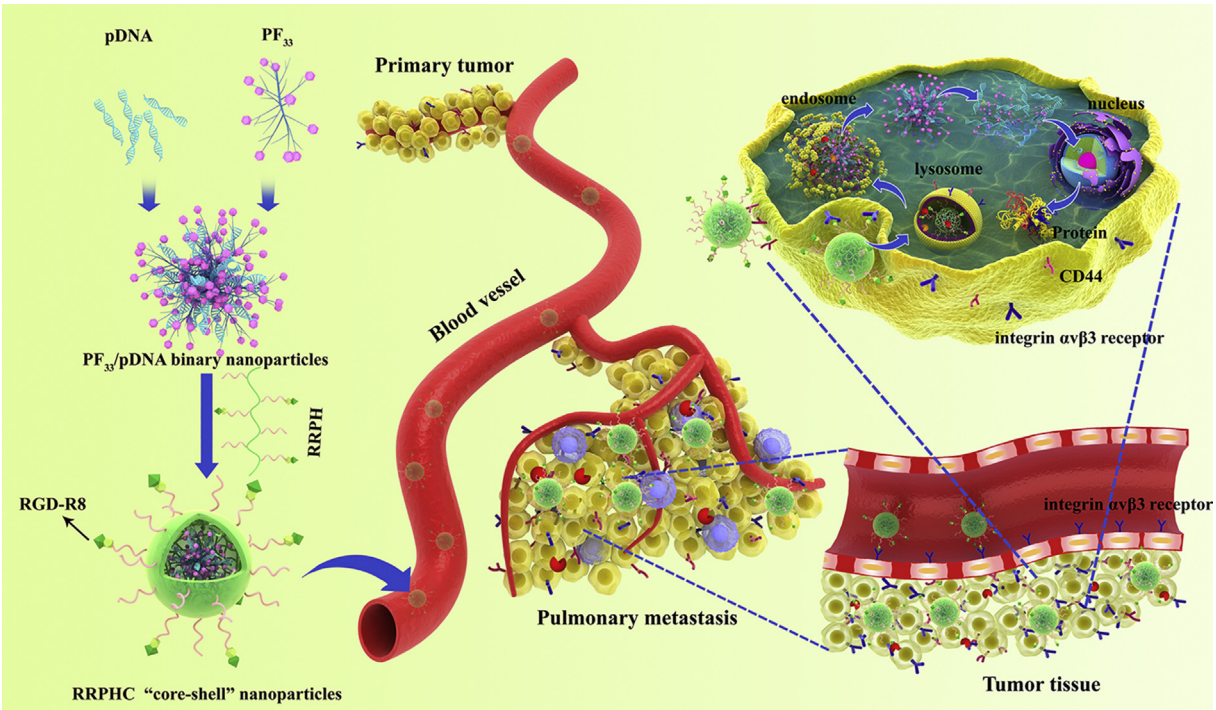

Fig. 1. Schematic illustration of the preparation, multi-stage and multiple tumor-targeting of RRPHC ternary complexes.