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Osteotropic peptide-mediated bone targeting for photothermal treatment of bone tumors

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ABSTRACT

The treatment of bone tumors is a challenging problem due to the inefficient delivery of therapeutics to bone and the bone microenvironment-associated tumor resistance to chemo- and radiotherapy. Here, we developed a bone-targeted nanoparticle, aspartate octapeptide-modified dendritic platinum-copper alloy nanoparticle (Asp-DPCN), for photothermal therapy (PTT) of bone tumors. Asp-DPCN showed much higher affinity toward hydroxyapatite and bone fragments than the non-targeted DPCN *in vitro*. Furthermore, Asp-DPCN accumulated more efficiently around bone tumors *in vivo*, and resulted in a higher temperature in bone tumors during PTT. Finally, Asp-DPCN-mediated PTT not only efficiently depressed the tumor growth but also significantly reduced the osteoclastic bone destruction. Our study developed a promising therapeutic approach for the treatment of bone tumors.

1. Introduction

Bone tumors are classified into primary tumors and metastatic ones. Primary bone tumors such as osteosarcoma, Ewing's sarcoma and fibrosarcoma start in bone or cartilage are frequently diagnosed in children and adolescents [1,2]. Metastatic bone tumors begin elsewhere in the body and spread to the bone. Bone metastases occur in 65–80% of patients with advanced breast and prostate cancers, and are frequently found in lung, liver and kidney cancers [3–6]. The extended survival in cancer patients may lead to increased incidence of bone metastases [7]. During bone metastasis, cancer cells secret cytokines to activate the osteoclasts, resulting in increased bone resorption and secretion of growth factors from the bone matrix [8]. This causes severe skeletal-related events including pain, pathological fracture, hypercalcemia, bone deformity and spinal-cord compression [9]. These complications significantly reduce patients' quality of life and increase mortality. On the other hand, bone marrow microenvironment provides a fertile soil for cancer cell recruitment, survival and outgrowth. It offers a protective niche for cancer cells to resist clinical treatments including chemotherapy and radiotherapy [10–12]. Excisional surgery may prolong the survival of patients with bone tumors, but the patients are more likely to experience a tumor recurrence due to the inadequate surgical margins. Therefore, novel and efficient therapeutic regimens are urgently needed in the clinical treatment of bone tumors.

Photothermal therapy (PTT) is an effective strategy for cancer treatment [13–15]. It has been used to successfully ablate diverse tumors and even metastatic tumors [16–19]. However, PTT of bone tumors is rarely investigated [20,21], and targeting nanoparticles to bone is still in its infancy. To date, three major kinds of bone-

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targeting moieties including bisphosphonate, oligopeptide and aptamer are developed for preferentially delivering therapeutic agents to bone [22–25]. Alendronate, a representative bisphosphonate, favorably binding to active bone remodeling sites (both bone resorption and formation surfaces) has been well used to specifically deliver drug-loaded nanoparticles to bone for tumor treatments [8,26–30]. An oligopeptide with six repetitive sequences of aspartate, serine and serine and an octapeptide with eight repeating sequences of aspartate (Asp_8) are identified with the ability that preferentially bind to bone-formation and bone resorption surface, respectively [24,31,32]. In a recent study, osteoclast-targeting aptamer modified on lipid nanoparticles efficiently facilitate RNAi-based anabolic therapy [25].

Bone tumors are commonly associated with osteoclastic bone resorption. Hence, we used the octapeptide Asp_8 as a bone-targeting ligand to deliver photothermal agents to bone tissues for targeted PTT of bone tumors (Scheme 1). Dendritic platinum-copper alloy nanoparticles (DPCN), a near-infrared (NIR) photothermal agent with high photothermal conversion efficiency, high drug loading capacity, and multimodal imaging modalities [33], were used as the model photothermal agent in this study. The bone-targeting oligopeptide Asp_8 was modified to the surface of DPCN via a cysteine linker (Asp-DPCN). DPCN modified with a non-targeting octapeptide with eight repeating sequences of glycine (Gly_8) (Gly-DPCN) were used as the control material. The *in vitro* binding affinities of Asp-DPCN and Gly-DPCN to hydroxyapatite and *ex vivo* bone fragments were quantitatively determined. In addition, the *in vivo* targeted delivery of Asp-DPCN to bone tumors and the efficiency of Asp-DPCN in photothermal ablation of bone tumors were investigated.

2. Experimental section

2.1. Materials

Cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$), hexachloplatinic (IV) acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), potassium iodide (KI), poly (vinylpyrrolidone) (PVP, molecular weight = ~29000), and ethylene glycol (EG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cysteine-Asp_8 and cysteine-Gly_8 were synthesized by Top-peptide Biotechnology (Shanghai, China).

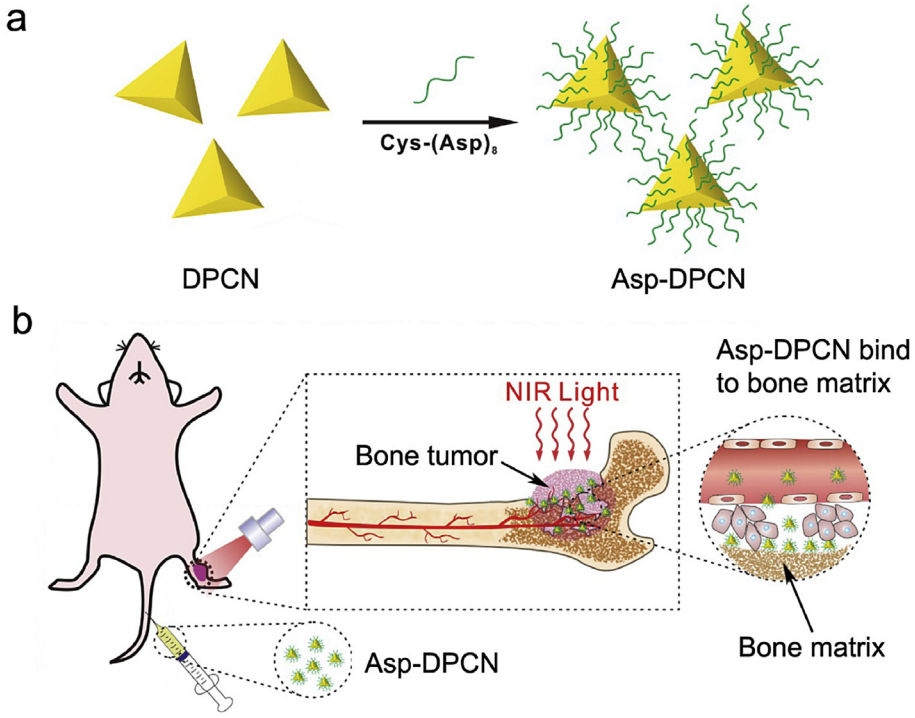
2.2. Synthesis of Asp-DPCN and Gly-DPCN

DPCN were synthesized according to the previous reported method [34,35]. Briefly, 1 mL $H_2PtCl_6 \cdot 6H_2O$ (20 mM) and 1 mL $CuCl_2 \cdot 2H_2O$ (20 mM) in aqueous solution were added in a Teflon liner under magnetic stirring. 0.025 mL aqueous KI (5 M) was added dropwise in the Teflon liner, and then 160 mg PVP (200 mg/mL) in aqueous solution was added. After that, 10 mL EG was injected in the reaction solution. The liner was sealed in a stainless vessel and heated in an oven at 140 °C for 90 min. The vessel was then cooled down to the room temperature, and the sample was washed with ethanol/acetone (volume to volume ratio = 1:1) twice and deionized (DI) water twice via centrifugation.

Asp-DPCN were prepared by simply mixing DPCN with cysteine-Asp_8 at a platinum (Pt)-to-peptide molar ratio of 10:1 in DI water under magnetic stirring. After incubation for 24 h, the Asp-DPCN were then isolated via centrifugation and washed three times with DI water. Gly-DPCN were synthesized following the same protocol for the preparation of Asp-DPCN.

2.3. Characterization

The transmission electron microscopy (TEM) images were taken using a transmission electron microscope (HT7700, Hitachi, Japan) operated at an accelerating voltage of 100 kV. The scanning electron microscopy (SEM) images were captured using a scanning electronic microscope (S-4800, Hitachi, Japan) operated at 10 kV. The ultraviolet–visible–near infrared (UV–Vis–NIR) spectra were recorded using a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, USA). Inductively coupled plasma mass spectrometer (ICP-MS) analysis was conducted by using Neptune MC-ICP-MS (Thermo, USA). The hydrodynamic diameters and zeta potentials



Scheme 1. Illustration of bone-targeted PTT of bone tumors. (a) Synthesis of bone-targeted Asp-DPCN. (b) Targeting delivery of Asp-DPCN to bone for PTT of bone tumors.