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PEGylated doxorubicin nanoparticles mediated by HN-1 peptide for targeted treatment of oral squamous cell carcinoma

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ABSTRACT

HN-1, a 12-amino acid peptide, has been reported to possess strong capabilities for targeting and penetrating head and neck squamous cell carcinoma. Here, we designed a simple but effective nanoparticle system for the delivery of doxorubicin (DOX) targeting oral squamous cell carcinoma (OSCC) through the mediation of HN-1. PEGylated DOX (PD) was firstly synthesized by the conjugation of DOX with bis-amino-terminated poly(ethylene glycol) via succinyl linkage, and then PD nanoparticles were prepared by a modified nanoprecipitation method. After that, PD nanoparticles were surface-modified with HN-1 to form HNPD nanoparticles, which had a uniform spherical shape and a small size about 150 nm. In human OSCC cells (CAL-27 and SCC-25), HNPD nanoparticles exhibited significantly higher cellular uptakes and cytotoxicities than PD nanoparticles. Furthermore, HNPD nanoparticles showed a certain degree of functional selectivity for CAL-27 and SCC-25 cells as compared to human hepatoma HepG2 cells. In SCC-25 tumor-bearing nude mice, HNPD nanoparticles showed remarkably enhanced tumor-targeting and penetrating efficiencies as compared to PD nanoparticles, and effectively inhibited the tumor growth. In conclusion, our study demonstrated for the first time that HN-1 could be used for mediating the OSCC-targeted delivery of nanoparticles.

1. Introduction

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies in the head and neck, and nearly more than 400,000 of new cases are diagnosed annually worldwide (Jemal et al., 2011). This disease has significant physical and psychological morbidity and a survival rate of approximately 50% over 5 years (Zanaruddin et al., 2013). The patients with early-stage OSCC can be successfully treated by the surgery, but unfortunately most patients are diagnosed at middle and advanced stages. Thus, systemic chemotherapy has been used as a major therapeutic modality in OSCC clinical treatment during the past decades. However, the side-effects of chemotherapeutic drugs caused from their non-targeting characters have greatly limited their clinical applications (Huang and O'Sullivan, 2013). Recently, the rapid development of nanocarrier technologies provides the possibility for overcoming the lack of tumor specificity of chemotherapeutic drugs. Nanocarriers with the size of 20–200 nm can extravasate through the leaky tumor vessels and accumulate in the tumor interstitial space via the enhanced permeability and retention (EPR) effect (Danhier et al., 2010; Nakamura et al., 2016). Furthermore, the nanocarriers can be surface-modified with the targeting ligands such as antibodies (Yu et al., 2010; Dai et al., 2015; Bazak et al., 2015), peptides (Boohaker et al., 2012; Liu et al., 2010, 2015) and aptamers (Tang et al., 2015; Zhuang et al., 2016), thus endow them the active tumor targeting capability. So chemotherapeutic drugs loaded by these nanocarriers can be efficiently delivered to and specifically accumulated in the tumors. In view of these facts, we hope to design a nanocarrier system for OSCC-targeted delivery of chemotherapeutic drugs, thus improve their therapeutic efficacy and reduce their toxic and side effects.

HN-1, a 12-amino acid peptide with ~1% of the mass of typical antibodies, was firstly discovered by screening phage-displayed peptide libraries using human head and neck squamous cell

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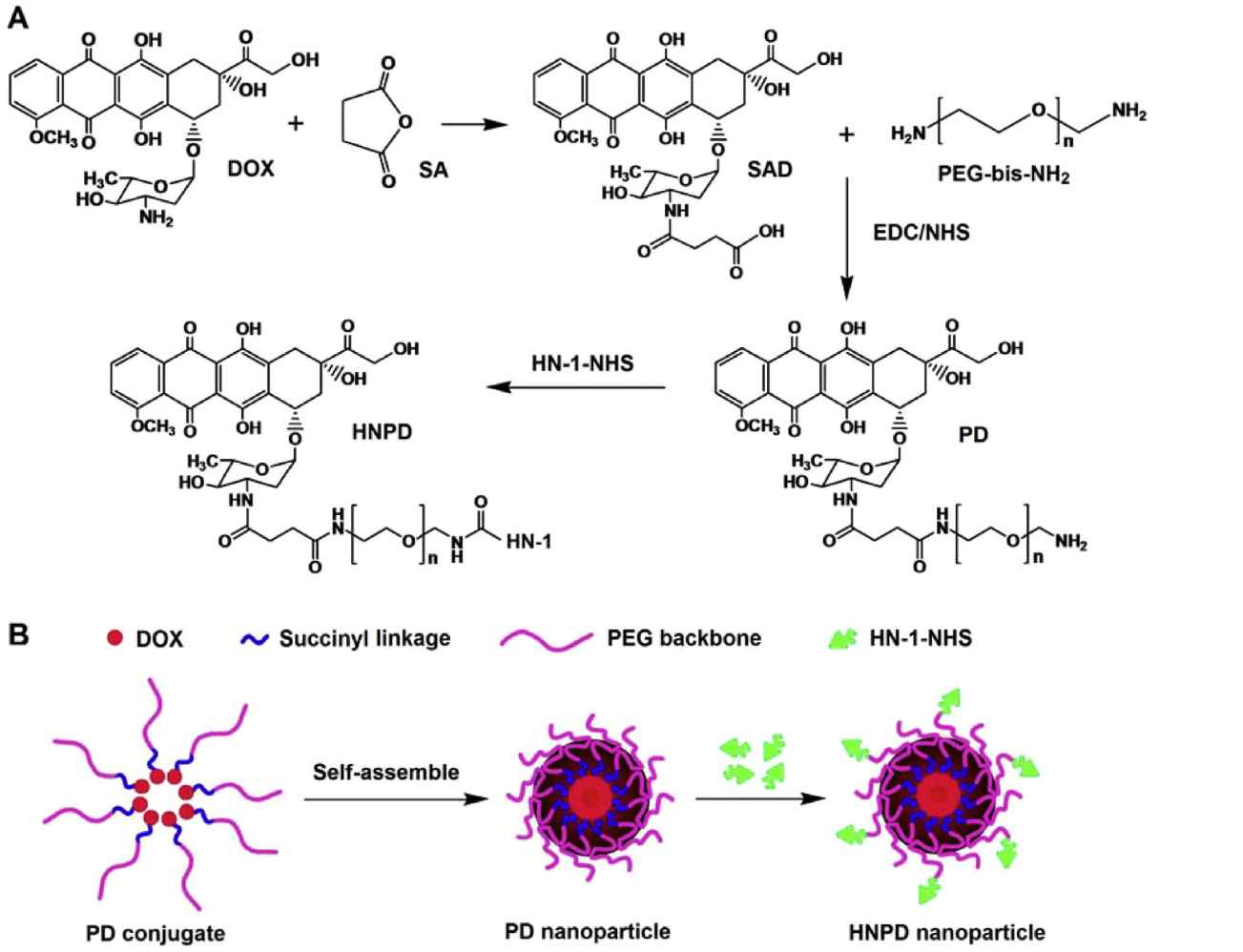
carcinoma (HNSCC) tumor cells. It can specifically bind to and be efficiently internalized in HNSCC cells (Hong and Clayman, 2000). Furthermore, HN-1 also possesses strong cell penetrating activity, and therefore it is believed as a novel cell-penetrating peptide (CPP) recently. Existing studies have shown that HN-1 can effectively mediate the HNSCC-targeted delivery of macromolecular drugs such as proteins and genes both in vitro and in vivo via chemical conjugation with these drugs. Bao et al. constructed a bifunctional peptide HN-1-PKCε by connecting HN-1 (HNSCC-homing peptide) and PKCε (specific PKCε inhibitor) with a peptide linker. The results showed that HN-1-PKCε preferentially penetrated HNSCC cells in vitro and in vivo, and successfully blocked the translocation of active PKCε in HNSCC cells. Moreover, HN-1-PKCε effectively inhibited the cell invasion, motility and proliferation in vitro, and significantly retarded the growth of HNSCC xenografts in nude mice (Bao et al., 2009). Potala and Verma designed a fusion toxin of diphtheria toxin-HN-1 and found this fusion toxin had a remarkably high degree of cytotoxicity specific to HNSCC cells (Potala and Verma, 2011). Recently, Yen and his co-workers successfully realized the targeted delivery of siRNA into human cancer cells through the mediation of HN-1 (Un et al., 2012). However, to our knowledge, there is no evidence showing that HN-1 can mediate the targeted delivery of nanoparicles.

In this study, we designed a simple but effective nanoparticle system for the OSCC-targeted delivery of doxorubicin (DOX) through the mediation of HN-1. DOX is an anthracycline antibiotic and has been widely used in clinical cancer treatment, but it may cause serious heart damage and some common side effects including hair loss, nausea and vomiting (Hortobágyi, 1997). We hope this nanoparticle system can enhance the therapeutic efficacy of DOX and simultaneously reduce its toxic and side effects. Scheme 1 illustrates the preparation of this nanoparticle system. PEGylated DOX (PD) was firstly synthesized by the conjugation of DOX with bis-amino-terminated poly(ethylene glycol) (PEG-bis-NH_2) via succinyl linkage (Scheme 1A). PD nanoparticles were prepared by a modified nanoprecipitation method, and then HN-1 was grafted onto the surfaces of PD nanoparticles via chemical bond to form HNPD nanoparticles (Scheme 1B). The morphology, size and in vitro drug releases of HNPD nanoparticles were detected in this study. Moreover, the OSCC-targeting capability and therapeutic efficacy of HNPD nanoparticles were also evaluated both in vitro and in vivo, and thus to evaluate the potential application of this novel nanosystem in OSCC treatment.

2. Materials and methods

2.1. Materials

FITC-labeled HN-1 (TSPLNIHNGQKL), NH-1 succinimidyl ester (NH-1-NHS) that was capped on the N-terminus with an acetyl group, and FITC-labeled irrelevant (Ir) peptide



Scheme 1. Illustration for tpreparation of HNPD nanoparticle system. (A) PD conjugate was synthesized by two-step reactions. (B) HNPD nanoparticle system was prepared by self-assembly of PD conjugate in aqueous medium and followed by chemically linking with HN-1 via amide bond.