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$\alpha_v\beta_3$ Integrin-targeted reduction-sensitive micellar mertansine prodrug: Superb drug loading, enhanced stability, and effective inhibition of melanoma growth in vivo

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ABSTRACT

Antibody-maytansinoid conjugates (AMCs) have emerged as one of the most promising active targeting cancer therapeutics. Their clinical use is, however, challenged by their low drug content, poor stability, high cost and potential immune response. Here, we designed and developed robust, cRGD-functionalized, reduction-sensitive polymeric micellar mertansine (DM1) prodrug (cRGD-MMP) that showed targeted treatment of B16F10 melanoma-bearing C57BL/6 mice. cRGD-MMP was obtained with a superb drug content of ~40 *wt.%* and a small size of ~45 nm from poly(ethylene glycol)-*b*-(poly(trimethylene carbonate)-*graft*-SSDM1) (PEG-P(TMC-*g*-SSDM1)) and cRGD-functionalized PEG-P(TMC-*g*-SSDM1) copolymers. cRGD-MMP exhibited excellent stability in 10% fetal bovine serum and cell culture medium while fast swelling and markedly accelerated drug release under a reductive environment. Confocal microscopy, flow cytometry and MTT assays indicated receptor-mediated uptake and high antitumor effect of cRGD-MMP in $\alpha_v\beta_3$ integrin over-expressing B16F10 melanoma cells. Notably, cRGD-MMP displayed a long elimination half-life of 5.25 h and 4-fold better maximum-tolerated dose than free DM1. The *in vivo* studies demonstrated that cRGD-MMP effectively inhibited B16F10 melanoma growth and greatly improved mice survival rate as compared to free DM1 and non-targeted MMP control. cRGD-MMP with superior stability, drug loading, and $\alpha_v\beta_3$ targetability offers an attractive alternative to AMCs for malignant tumor therapy.

1. Introduction

Mertansine (DM1) is a powerful tubulin polymerization inhibitor that can effectively treat various malignancies including breast cancer, melanoma, multiple myeloma and lung cancer [1,2]. The recent FDA approval of Kadcyla® (ado-trastuzumab emtansine) for the treatment of HER2-positive metastatic breast cancer inspired intensive development of antibody-maytansinoid conjugates (AMCs) for different malignancies [3–5]. Notably, there are nearly ten types of AMCs have entered various phases of clinical trials [6–8]. AMCs have emerged as one of the most promising active targeting cancer therapeutics. It has to be noted, however, that the clinical use of AMCs, as for other antibody-drug conjugates (ADCs), is challenged by their low drug content, poor stability, high cost, small scale production, and potential immunogenicity [9–11].

Water soluble polymer-drug conjugates, analogous to ADCs, can effectively improve drug solubility, blood circulation time, and drug toleration [12–17]. Unlike ADCs, polymer-drug conjugates usually possess a low immunogenicity and can be easily produced in a large scale. A couple of polymer-drug conjugates such as polyglutamic acid conjugated paclitaxel (PGA-PTX) and poly(N-(2-hydroxylpropyl) methacrylamide)-doxorubicin conjugates (HPMA-DOX) have been translated to the clinical trials [18–21]. The overall therapeutic effects of polymer-drug conjugates are low, partly resulting from their too small size (<10 nm), poor tumor accumulation and inefficient tumor cell uptake [22,23]. In recent years, polymeric micellar prodrugs, which combine the virtues of both polymer-drug conjugates and nano-micelles, have attracted increasing interests for cancer therapy [24–30]. For instance, Torchilin et al. developed a MMP2-sensitive paclitaxel-conjugated micellar nanoparticles with low risk of drug leakage that exhibited enhanced anticancer activity in vitro and in a A549 xenografted mouse model [29]. We reported a hyaluronic acid-shelled pH-activatable micellar PTX prodrug that showed potent inhibition of CD44-overexpressing MCF-7 human breast tumor xenografts [30].

Here, we designed and developed a robust, cRGD-functionalized, reduction-sensitive polymeric micellar mertansine prodrug (cRGD-MMP) for targeted treatment of B16F10 melanoma-bearing C57BL/6 mice (Scheme 1). cRGD-MMP is *co*-self-assembled from poly(ethylene glycol)-*b*-(poly(trimethylene carbonate)-*graft*-SSDM1) (PEG-P(TMC-*g*-SSDM1)) and cRGD-functionalized PEG-P(TMC-*g*-SSDM1) copolymers.

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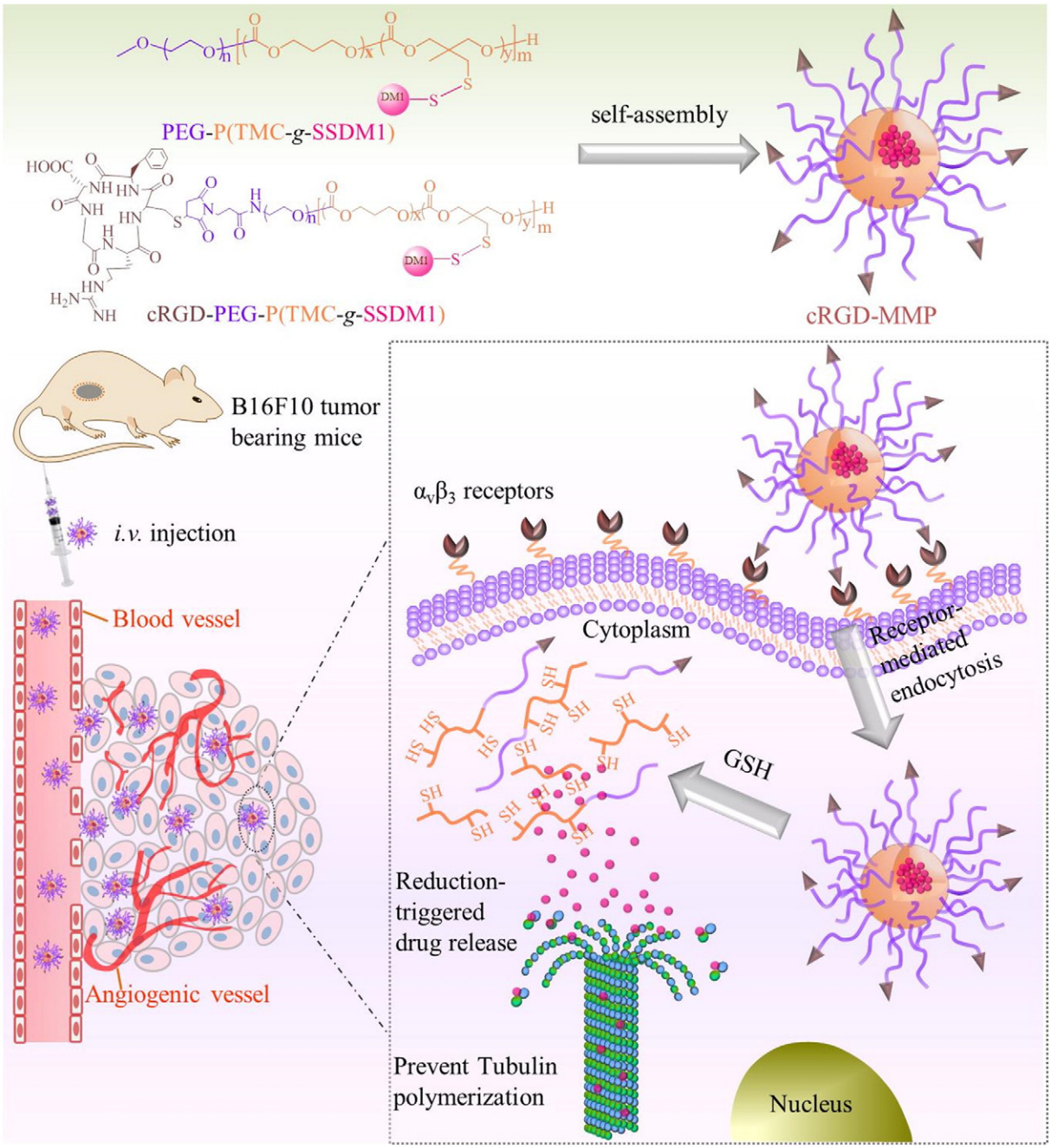

Scheme 1. Schematic illustration of cRGD-functionalized, reduction-sensitive micellar mertansine prodrug (cRGD-MMP) for targeted treatment of B16F10 melanoma-bearing C57BL/6 mice. cRGD-MMP was co-self-assembled from PEG-P(TMC-*g*-SSDM1) and cRGD-functionalized PEG-P(TMC-*g*-SSDM1) copolymers.

DM1, a thiol-containing maytansinoid [31], is grafted to polycarbonate backbone via a disulfide bond that can be cleaved under a reductive condition, leading to fast cytoplasmic release of pristine DM1. Various reduction-sensitive nanosystems have recently been explored for triggered intracellular drug release [32–38]. PTMC is a well-established synthetic biomedical materials that possess excellent biocompatibility, biodegradability and flexibility [39,40]. The cyclic arginine-glycine-aspartate (cRGD) peptide has shown a strong binding affinity toward $\alpha_v\beta_3$ integrin overexpressed on angiogenic endothelial cells and several malignant cancer cells such as glioblastoma and melanoma [41–46]. It should be noted that several antibody-maytansinoid conjugates with a disulfide linker have advanced to phase I–II clinical trials [10,47]. The disulfide linkage in these AMCs, however, turned out too labile in blood circulation. In the present work, disulfide bonds and DM1 are designed to locate in the micellar core, which is expected to afford a high stability and reduced premature drug release. To the best of our knowledge, this is a first report on smart micellar mertansine prodrug that possesses a superb drug loading, high stability, active tumor targetability and internalization, and accelerated intracellular drug release.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol) (PEG-OH M_n = 5.0 kg/mol, Fluke) was dried by azeotropic distillation from anhydrous toluene before use. Trimethylene carbonate (TMC, Jinan Daigang Biomaterial Co., Ltd.) was purified through recrystallizing from dry ethyl acetate before use. Zinc bis[bis(trimethylsilyl) amide] (97%, Aldrich), cyclo(RGD*f*C) (cRGD-SH, 98%, China Peptides Co., Ltd.), maleimide poly(ethylene