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Short communication

A cleavable self-delivery nanoparticle for tumor photo-immunotherapy



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ARTICLE INFO

Article history:

Received 12 January 2021

Revised 25 January 2021

Accepted 29 January 2021

Available online 4 February 2021

Immunotherapy of tumor attracts great attention in the past several years attributing to the potential of completely erasing primary tumor and metastasis. Therefore, pharmaceutical companies developed many antibodies that act as immune check point blockade (ICB), such as Nivolumab, Atezolizumab and Ipilimumab. However, the antitumor immune response in clinic is relatively low because of the immunosuppressive conditions in the tumor sites. To improve the immunotherapy, many strategies are developed to stimulate the immune response and relieve the immunosuppressive microenvironment [1]. Photothermal therapy, photodynamic therapy and several chemotherapeutics are demonstrated with the ability of inducing immunogenic cell death (ICD), which show promising synergic effect with ICB. For tumor photo-immunotherapy, ICD and cytotoxicity effects can only be produced after laser irradiation, which can avoid the side effects of non-target organs brought by chemotherapy. Therefore, there is a trend in the tumor immunotherapy of developing photo-immunotherapy combinational strategies.

The development of nanotechnology provides powerful tools for drugs codelivery to target site with designed behaviors, and many fancy systems have showed excellent antitumor immunotherapy effect [2]. However, the clinical translation of these fancy systems is shadowed by the low drug loading capacity, complex preparation procedure and toxicity of the carriers. Therefore, carrier-free drug delivery system is emerged as a promising direction [3].

In the paper published recently in *Advanced Functional Materials*, we designed a self-delivery nanoparticle (MA-pepA-Ce6 NPs) for tumor photo-immunotherapy [4]. In the system (Fig. 1A), metformin (MET), a small-molecule programmed cell death ligand 1 (PD-L1), was conjugated with chlorin e6 (Ce6), a photosensitizer, through a peptide linker (GPLGVRGDK, pepA). The obtained macromolecule, MA-pepA-Ce6, could self-assemble into 109 nm negative charged nanoparticles (NPs) in water due to amphiphilic property of the molecule. After entering primary tumor by enhanced permeability and retention (EPR) effect, the overexpressed matrix metalloproteinase-2 (MMP-2) could cleave the pepA

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Peer review under responsibility of Shenyang Pharmaceutical University.

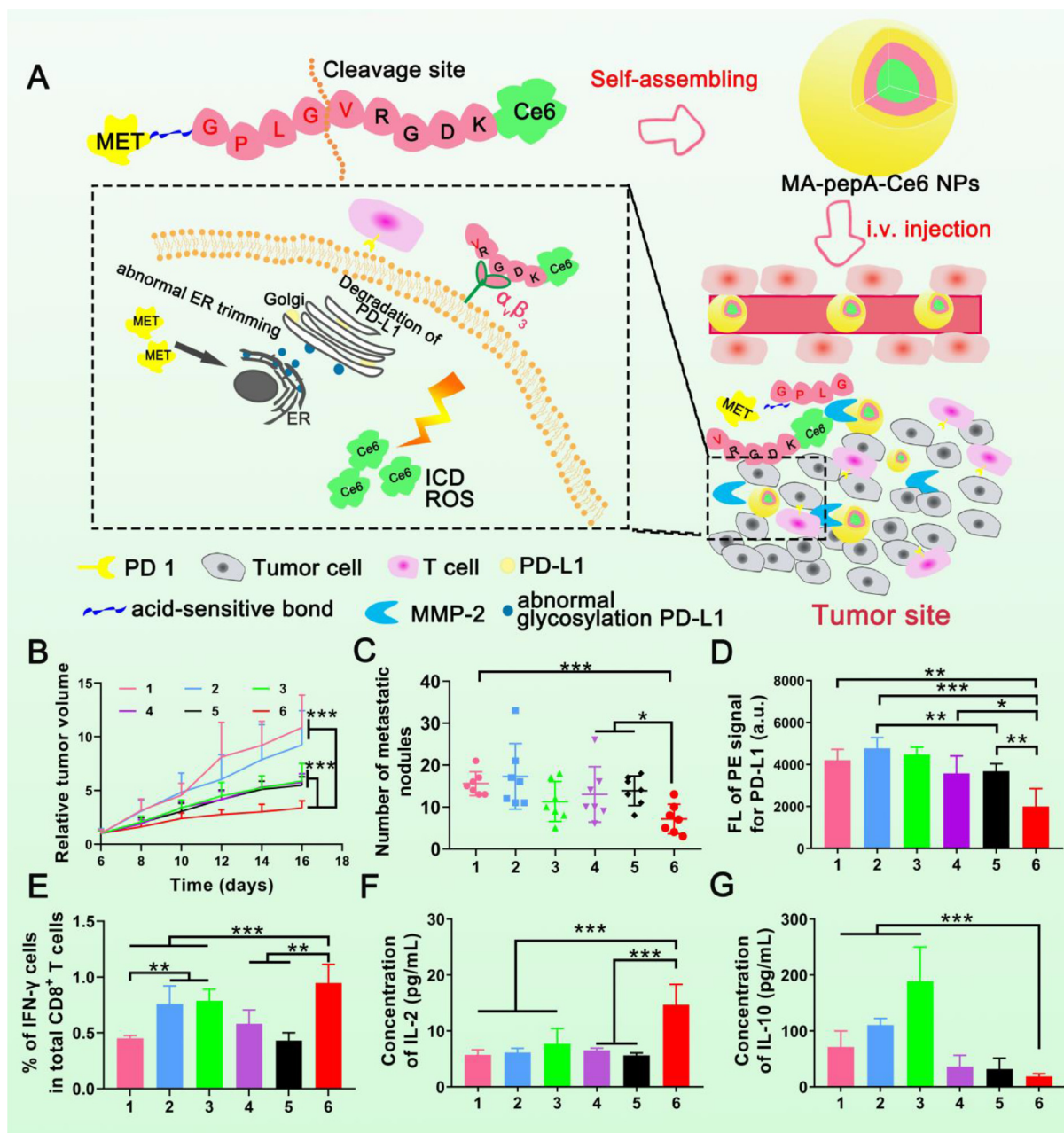


Fig. 1 – Diagram depicting the design of self-delivery NPs for tumor photo-immunotherapy (A); Change in relative tumor volume during the treatment (B); Number of metastatic nodules on the lungs (C); Flow cytometry analysis the expression of PD-L1 in tumor cells after various treatments (D); Flow cytometry analysis the frequencies of IFN- γ -secreting CD8⁺CTLs in spleens (E); Cytokine concentration of IL-2 (F) and IL-10 (G) in tumor tissue lysate. Treatment groups: 1, PBS; 2, MET; 3, MET + Ce6 + Laser; 4, MA-pepA-Ce6 NPs + Laser; 5, MA-pepA-Ce6 NPs; 6, MA-pepA-Ce6 NPs + Laser. Data are presented as means \pm s.d. ($n \geq 4$). (Reproduced with permission from [4]. Copyright WILEY-VCH GmbH.).

between glycine (G) and valine (V). The exposed VRGDK-Ce6 could specifically bind with integrin $\alpha v \beta 3$ receptor of tumor cells, enhancing tumor penetration and tumor cell internalization. After laser irradiation, Ce6 could produce high level of O_2^1 , inducing tumor cell apoptosis. What's more, the ICD caused by Ce6 sufficiently stimulate immune response and recruit high level of T cells in tumor. Consequently, MET can easily enter cells mediated by its guanidine group and downregulate the expression of PD-L1. In combination,

the MA-pepA-Ce6 NPs synergistically improve both primary tumor and metastasis treatment by photo-immunotherapy (Fig. 1B and 1C).

MMP-2 sensitive strategy has been extensively evaluated in tumor specific polyethylene glycol (PEG) de-shielding, activatable cell penetrating peptides, size-changeable nanomaterials and drug release [5]. Several peptides could be cleaved by MMP-2, such as LG-LVG, PVG-LIG, GPLG-IAGQ, and GPLG-VRGDG (“-” indicated cleavable site). Normally, these

peptides only act as linkers for MMP-2 sensitive cleavage. But the interesting point of GPLG-VRGDG peptide is the exposure of RGD sequence after MMP-2 cleavage, which could bind with integrin $\alpha v \beta 3$ receptor and improve tumor cell targeting [6]. After a small change, we designed GPLGVRGDK (pepA) as linker and Ce6 targeting ligand. Results showed the cleavable NPs has higher tumor cell uptake, tumor spheroid penetration and primary tumor accumulation compared with uncleavable control.

MET is a drug for type 2 diabetes treatment with good toleration. Recent studies showed MET also involves in immunosuppressive microenvironment modulation. Different from ICB that blocks the interaction between immune checkpoints, the MET could directly degrade PD-L1 through endoplasmic-reticulum pathway [7]. Additionally, MET is a hydrophilic molecule with good serum stability. Therefore, MET was selected to conjugate with hydrophobic pepA-Ce6 to form an amphiphilic macromolecule, which could easily form NPs in water by self-assemble. After cleavage by MMP-2 in tumor, MET could easily enter tumor cells mediated by its guanidine group. Although previous reports showed the downregulation of PD-L1 by MET was dose-dependent, and 1.25 mM is the lowest effective dose, our study showed the MET could effectively reduce PD-L1 expression at lower than 1 nM [4]. Consequently, *in vivo* experiments showed the 0.4 mg/kg MET and 3 mg/kg Ce6 could effectively boost antitumor immune response (Fig. 1D to 1G). Although the MA-pepA-Ce6 NPs showed good tumor targeting capacity and enhanced photo-immunotherapy, there are still several aspects need to further discuss in the future.

The chemical modification of drugs needs to carefully optimize. As we know, many prodrugs need to be released for performing their activity. Enzyme sensitive cleavage has good tissue or site specificity, but the release rate may be not very quick, which may reduce the initial concentration. To address the concern, it is important to develop prodrug strategies with sensitive and quick release behavior. Additionally, the residue groups on drugs should not influence drug activity. In the study, Ce6 was conjugated to VRGDG after cleavage by MMP-2. So, the researchers should evaluate the drug activity with the residue groups.

The drug ratio is another important issue for synergistic effect. Normally, researchers should optimize the drug ratio before encapsulation them into nanoparticles. In the study, MET has activity at very low concentration, but we still used 1:1 (MET: Ce6) because MET acts as not only immune modulator but also hydrophilic group of the nanoparticles. The final administration dose was determined by Ce6 dose.

In addition, many recent top-notch studies overturned EPR effects and new mechanisms have been put forward [8,9], so passive targeting based on EPR alone of MA-pepA-Ce6 NPs may face limitations. Combination with actively targeted drug delivery strategies, for example, adding targeted peptide modification to improve the efficiency of endothelial transcytosis in tumor site [10], or using the tumor tendency effects of cells *in vivo* etc. [11], to improve the initial

accumulation of NPs in the tumor site may lead to better therapeutic outcomes.

Overall, the study developed a simple but multifunctional nanoparticle by simply conjugating three parts together. Each part has dual functions. This study is a good example for researchers to design nanoparticles by fully utilizing the function and property of drugs.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgements

The authors acknowledge the financial support received from 111 Project (B18035) and the Fundamental Research Funds for the Central Universities.

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