Tumor Microenvironment-Sensitive 5-Aminolevulinic Acid Prodrug Nanocarriers for Targeted Photodynamic Cancer Therapy

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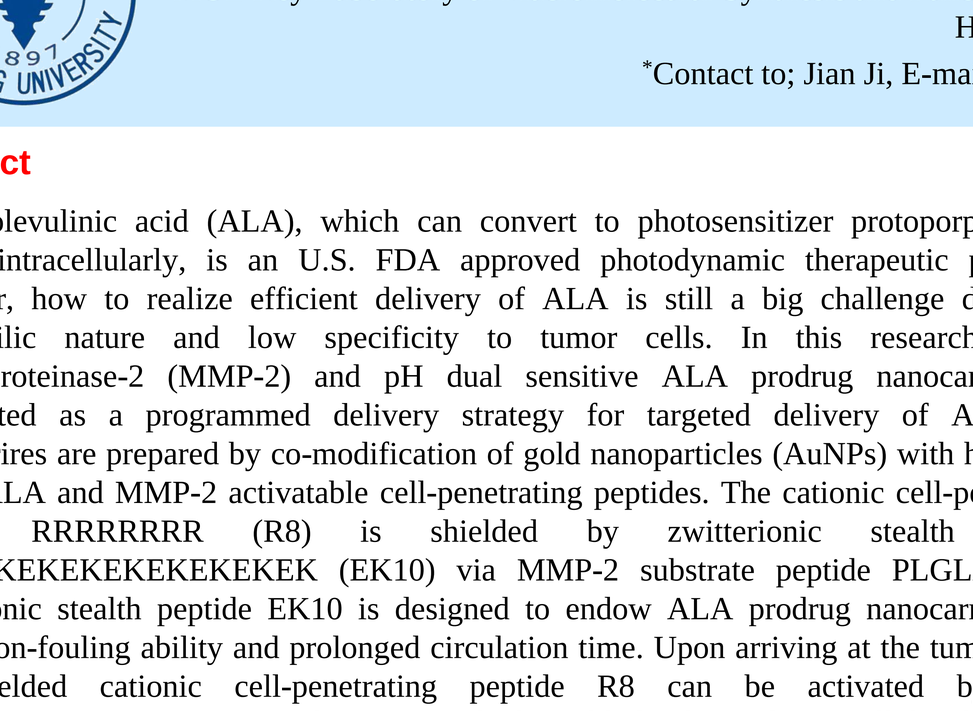
Abstract

5-Aminolevulinic acid (ALA), which can convert to photosensitizer protoporphyrin IX (PpIX) intracellularly, is an U.S. FDA approved photodynamic therapeutic precursor. However, how to realize efficient delivery of ALA is still a big challenge due to its hydrophilic nature and low specificity to tumor cells. In this research, matrix metalloproteinase-2 (MMP-2) and pH dual sensitive ALA prodrug nanocarriers are constructed as a programmed delivery strategy for targeted delivery of ALA. The naoncarrires are prepared by co-modification of gold nanoparticles (AuNPs) with hydrazone linked ALA and MMP-2 activatable cell-penetrating peptides. The cationic cell-penetrating peptide RRRRRRRR (R8) is shielded by zwitterionic stealth peptide EKEKEKEKEKEKEKEKEKEK (EK10) via MMP-2 substrate peptide PLGLAG. The zwitterionic stealth peptide EK10 is designed to endow ALA prodrug nanocarriers with strong non-fouling ability and prolonged circulation time. Upon arriving at the tumor tissue, the shielded cationic cell-penetrating peptide R8 can be activated by tumor microenvironment overexpressed MMP-2, which enabled enhanced intracellular delivery of ALA. The *in vitro* and *in vivo* experiments of drug loading and release, cellular uptake, PpIX generation and accumulation, photodynamic cytotoxicity assay and photodynamic tumor inhibition demonstrate that such tumor microenvironment sensitive ALA prodrug nanocarriers are potential candidates for targeted photodynamic cancer therapy.



Scheme.1 *Schematic illustration of enzyme and pH dual- sensitive ALA conjugated AuNPs for Targeted PDT.*

Results and Discussion

1. Enzyme MMP-2 and pH Dual-sensitivity of ALA Conjugated AuNPs

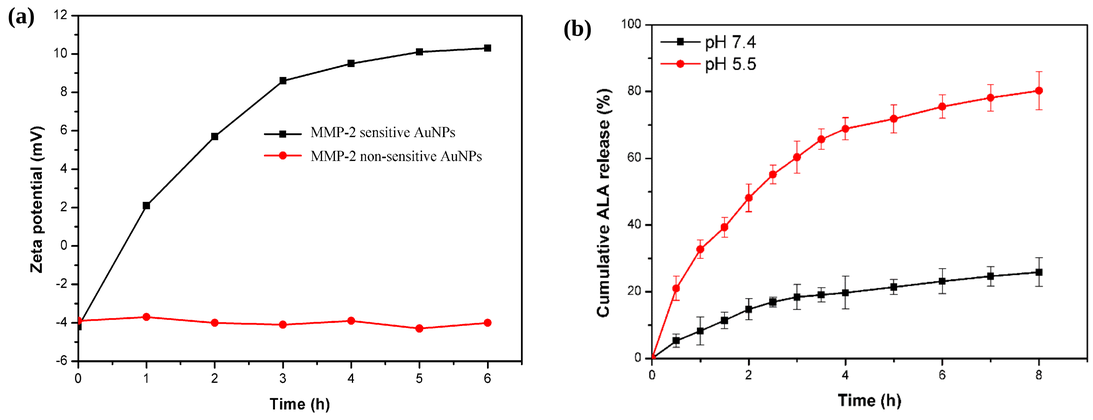


Fig. 1 *(a)Zeta potential change of the ALA conjugated AuNPs with or without MMP-2. (b) In vitro cumulative release of ALA from the ALA conjugated AuNPs.*

The ALA Conjugated AuNPs were MMP-2 and pH dual sensitive, which showed excellent potential in tumor targeted photodynamic therapy.

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2. Cellular Uptake of ALA Conjugated AuNPs and Generation of PpIX

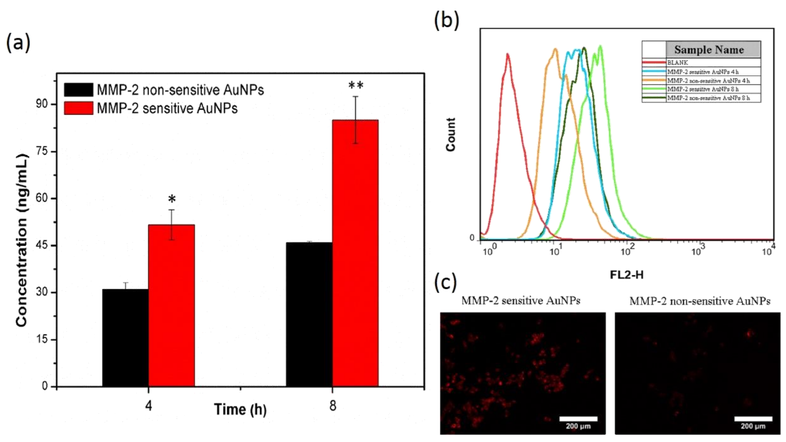

Fig. 2 *(a) Cellular uptake of the ALA conjugated AuNPs with or without MMP-2 sensitivity. (b) Flow cytometric histogram profiles and (c) Fluorescent microscopy images of PpIX generated in SCC-7.*

ALA Conjugated AuNPs demonstrated MMP-2 enhanced cellular uptake and generation of PpIX at cellular level.

3. *In Vitro* Photodynamic Cytotoxicity Assay

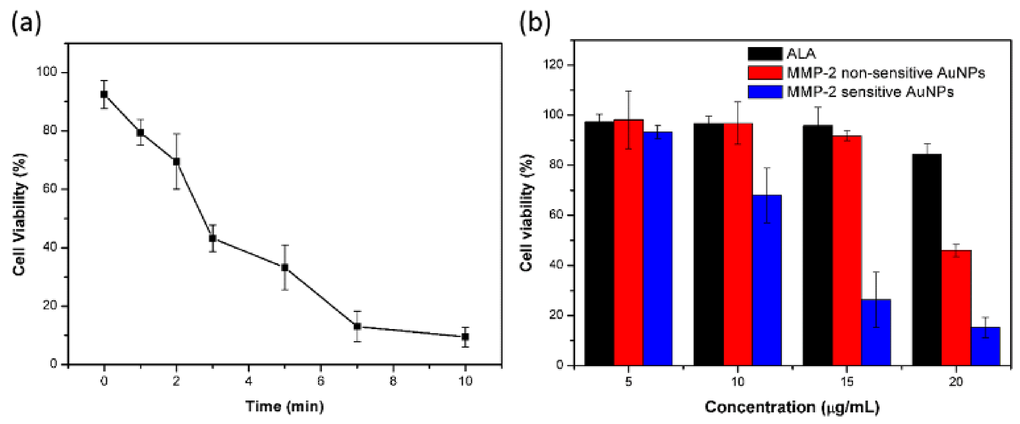

Fig.3 Time (a) and concentration (b) dependent cell viability of SCC-7 cells incubated with ALA or AuNPs with or without MMP-2 sensitivity.

ALA conjugated AuNPs demonstrated negligible dark cytotoxicity. However, strong photodynamic ablation of SCC-7 cells was observed with the irradiation time prolonged. In addition, The ALA conjugated AuNPs were more effective in PDT than free ALA and MMP-2 non-sensitive ones, owing to the more efficient delivery of ALA into cancer cells.

4. *In Vivo* Circulation Evaluation and Photodynamic Therapy Treatment

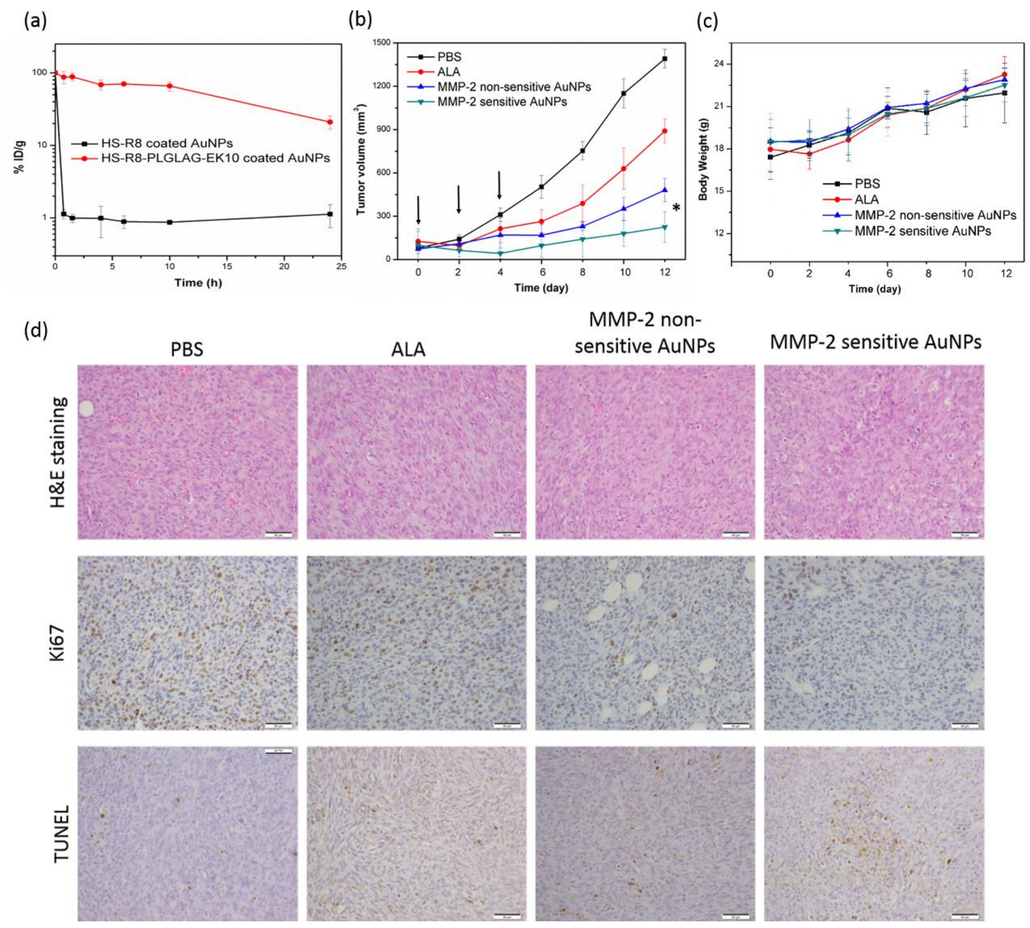

Fig. 4 *(a) Blood circulation curves of peptide HS-R8-PLGLGA-EK10 and HS-R8 coated AuNPs in female ICR mice. (b) Relative tumor volume and (c) relative body weight after post-treatment, with the arrow representing the injection. (d) H&E staining, Ki67 and TUNEL assay of tumor tissue.*

The results demonstrated that HS-R8-PLGLAG-EK10 coated AuNPs had much longer circulation time. Moreover, compared with PBS, free ALA and MMP-2 non-sensitive AuNPs groups, MMP-2 sensitive AuNPs exhibited much better PDT efficacy.

Conclusions

An efficient ALA prodrug delivery system with programmed tumor targeting ability for enhanced photodynamic therapy was developed. The zwitterionic shield peptide EK10 endow the nanoparticles with prolonged blood circulation time. Once reaching the tumor part, the peptide PLGLAG would be cleaved overexpressed MMP-2, the cationic cell-penetrating peptide R8 was exposed, thereby accelerating internalization by cancer cells. The PDT precursor, ALA was then released at endo/lysomal pH, which can further produce PpIX endogenously for PDT. The *in vitro* and *in vivo* results showed that MMP-2 sensitive ALA prodrug nanocarriers exhibited better photodynamic therapeutic efficacy. Such design of programmed enzyme and pH dual-sensitive ALA prodrug naoncarriers provides a new strategy for targeted photodynamic cancer therapy.

Acknowledgment

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