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DOCTORAL THESIS

Development of a targeted liposomal delivery system for encapsulated cantharidin to treat hepatocellular carcinoma

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ABSTRACT

Background: Despite increasing incidence and morbidity globally, hepatocellular carcinoma (HCC) remains a big challenge clinically. The difficulty to treat HCC is largely due to non-specific chemotherapy causing life-threatening toxicity and severe drug-related adverse effects. Extensive studies on targeted drug delivery systems (DDS) have revealed a great potential in specific delivery of chemotherapeutics for cancer treatment, which should be a way to overcome the limitations of conventional chemotherapy.

Cantharidin (CTD) is a natural product from Chinese medicine showing a great potency but narrow therapeutic window with high toxicity. Its therapeutic potential is proposed to be improved with nanoliposomal encapsulation. To explore the potential of this liposomal delivery system for HCC treatment, in this study we developed and characterized liposomal carriers with CTD encapsulated and liposomal surface modified for targeted delivery to the HCC models *in vitro* and *in vivo*.

Methods: In the present study, liposomal delivery system was developed with cantharidin (CTD) encapsulated as anticancer assembly for HCC treatment. Firstly, in order to demonstrate the feasibility of liposomal encapsulation for CTD, the plain liposomal CTD was prepared and the anticancer effects were evaluated *in vitro* and *in vivo* with comparison to the free CTD formulation (Chapter 2). Then, to achieve specific penetrability of the liposomal CTD for HCC, it was further modified with a cancer cell specific penetrating peptide BR2, and its superior penetrability was evaluated on both *in vitro* monolayer and 3D HepG2 cells including MTT assay, cellular uptake, internalization, tumor spheroid penetration and inhibition, and *in vivo* subcutaneous HCC mice model (Chapter 3). Finally, the dual-functionalized liposomes with BR2 and anti-carbonic anhydrase IX (CA IX) antibody were achieved for more efficient delivery with specific penetrating and targeting properties on orthotopic HCC model (Chapter 4).

Results: The key results of the study are: (1) liposomal CTD can augment the anti-proliferative effects of CTD, and enhance the anticancer efficacy on subcutaneous HepG2-bearing nude mice, which might be due to the enhanced solubility of the drug as well as intracellular delivery (Chapter 2); (2) with BR2 penetrating peptide modification, the liposomal CTD can get into cancerous cells specifically and penetrate deeper in 3D tumor models. A better tumor growth inhibition was also seen in the subcutaneous HCC mice of BR2-modified liposomes treatment than that of the other group, which could be contributed to the passive targeting of liposomes as well as the specific penetrating properties induced by BR2 peptide (Chapter 3); (3) the dual-functionalized liposomes with BR2 peptide and anti-CA IX antibody modification can enhance the drug internalization into HepG2 cells and further improve the anticancer efficacy of drugs compared to other formulations on orthotopic HCC nude mice (Chapter 4).

Conclusion: These results demonstrate 1) the liposomal delivery system as a powerful tool to improve anticancer effects of chemotherapeutic agent; 2) the usefulness of BR2 and CA IX modified-liposomal nano-delivery of CTD and their combination might be a potential modality for HCC treatment. The study paved a way for clinical translational medicine of this ligands-modified liposomal delivery system for targeted treatment of HCC.

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