Thailandepsin A-loaded Unimolecular Micelles for Targeted Carcinoid Therapy

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Statement of Purpose: Carcinoids are gastrointestinal (GI) neuroendocrine tumors (NETs) occurring throughout the GI tract which produce hormones causing the carcinoid syndrome. Although NETs are slow growing, they are frequently metastatic at the time of their discovery and no longer amenable to curative surgery. Therefore, there is a great need to develop novel therapeutic strategies both to reduce tumor burden and control symptoms in patients with carcinoid neoplasms. To address this need, we developed and optimized a family of novel unimolecular micelles capable of delivering a newly reported anticancer drug Thailandepsin A (TDP-A).These micelles specifically target the NET cancer cells with overexpressed somatostatin receptors (SSTRs) using octreotide (OCT) as the active-tumor targeting ligand. In the current study, we assessed the antitumor effects of TDP-A loaded unimolecular micelles both in *in vitro* and *in vivo*.

Methods: Stable unimolecular micelles were prepared in an aqueous solution using multi-arm star amphiphilic block copolymer (Fig.1). The hydrophobic polyester core (PLA) was used to encapsulate the TDP-A, and the polyethylene glycol (PEG) shell was used to provide water solubility and reduce opsonization. OCT was conjugated to the PEG for active tumor-targeting. A human GI carcinoid cell line (BON) was treated with a family of micelles (TDP-A-loaded targeted and non-targeted, and empty targeted and non-targeted) at a TDP-A concentration of 5 nM for 2 or 24, 48 and 72 hours. MTT assay and Western blot were used to determine the cell proliferation and assess the expression of NET markers (ASCL1 and CgA), respectively. The effect of the OCT targeting ligands on the cellular uptake of the micelles was measured by flow cytometry and confocal laser scanning microscope (CLSM). The antitumor efficacy of TDP-A loaded micelles was determined in BON xenografts after five intravenous injections performed every 5 days with a dose of 3.125mg/kg BW.

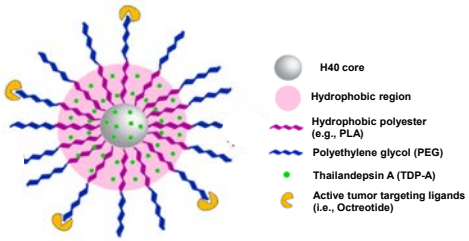

Fig. 1. A schematic illustration of the H40-PLA-PEG-OCT drug nanocarriers for tumor-targeted drug delivery

Results: A family of unimolecular micelles were developed for targeted delivery of TDP-A to carcinoids. The targeted micelles exhibited a much higher cellular uptake (4 fold increase) than non-targeted micelles based on flow cytometry and CLSM analyses (Fig.2). Moreover, the TDP-A loaded and OCT-conjugated targeted micelles had the strongest inhibitory effect on BON cell proliferation and NET markers expression (Fig.3). Additionally, TDP-A loaded targeted micelles demonstrated the best antitumor efficacy with the highest decrease of tumor volume (76%) (Fig.4).

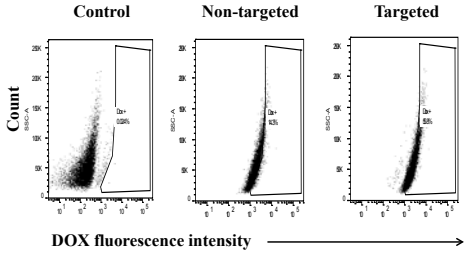

Fig. 2. Flow cytometry revealed that intracellular uptake of micelles labeled with doxorubicin (2h incubation, DOX concentration: 1 μg/mL) was 3 times higher for OCT-conjugated micelles (55.8%) in comparison to the non-targeted controls (14.3%).

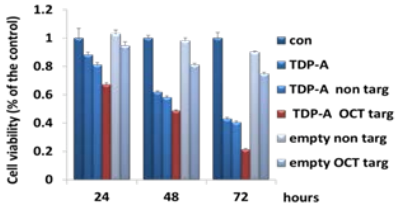

Fig. 3. MTT assay of BON cells treated with various micelles (TDP-A concentration of 5 nM). TDP-A loaded OCT-targeted micelles (4th bar) were much more effective in suppressing tumor cell proliferation than other types of micelles containing fewer components.

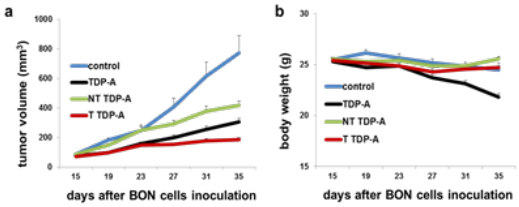

Fig. 4. Each mouse received five intravenous injections (3.125 mg/kgBW/dose) every 5 days. Results were represented by (**a**) tumor growth inhibition and (**b**) body weight change. OCT-conjugated and TDP-A loaded targeted micelles showed the best antitumor efficacy. No toxicity for these micelles was observed during the experiment.

Conclusions: The OCT targeting ligands conjugated onto the surface of the micelles substantially increased the cellular uptake of the micelles in BON cells and improved cytotoxic effect of TDP-A both *in vitro* and *in vivo*. Thus, targeted delivery of anticancer drug TDP-A, specifically to the tumor tissue could significantly improve the therapeutic outcomes in treating carcinoid disease while reducing systemic toxicity.