Theranostic Polymer Micelles for Targeted Imaging and Therapy of Lung Cancer

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Statement of Purpose: Lung cancer is the leading cause of cancer-related death for both men and women in the USA. Current chemotherapy of lung cancer does not provide adequate specificity and efficacy and has significant toxic side effects. Integrated nanoplatforms with cancer targeting, imaging and therapeutic functions are emerging to potentially revolutionize cancer diagnosis and therapy. These multifunctional nanoparticles can pinpoint and visualize the tumor location, kill the cancer cells and monitor the therapeutic response. Here we designed a multimodal polymeric micelle system loaded with MRI contrast agents and anticancer drugs (**Fig. 1A**). Lung cancer targeting peptide (LCP, with the sequence of RGDLATLRQL), which specifically binds to the $\alpha_v\beta_6$ integrin expressed in lung cancer cells,[1] was conjugated on the surface of micelles. We hypothesize that LCP-encoded polymer micelles will promote the tumor-specific delivery of imaging probes and therapeutic agents in lung cancer treatment.

Methods: Amphiphilic block copolymer methoxy-poly(ethylene glycol)-co-poly(D,L-lactic acid) (MeO-PEG-PLA) and maleimide terminated polymer Mal-PEG-PLA were synthesized by ring opening polymerization and used for micelle formation. The density of lung cancer targeting peptides on the surface of the micelles was controlled by the amount of the maleimide PEG-PLA introduced on the micelles. Doxorubicin (Doxo) and superparamagnetic iron oxide (SPIO) nanoparticles were loaded inside the core of micelles by a solvent evaporation method.[2] Targeting and cytotoxicity tests of different micelle formulations were examined in the $\alpha_v\beta_6$(+) H2009 and $\alpha_v\beta_6$(-) H460 lung cancer cells, respectively. For MR imaging, H2009 and H460 lung cancer cells were subcutaneously injected on each flank of scid mice. The images were taken before and after intravenous injection of LCP-encoded micelles with 6 mg/kg of iron concentration under 4.7 T scanner (spin echo pulse sequence, TR= 2 s and TE= 45 ms). *In vivo* antitumor efficacy at 4 mg/kg Doxo dose of micelles was examined on scid mice containing subcutaneous H2009 lung tumors.

Results: For comparison, scrambled peptide (SP) with the sequence of DALRLQGTLR was also conjugated on the surface of micelles. Peptide conjugation efficiency was confirmed by HPLC analysis. The loading content and efficiency of SPIO and Doxo were comparable in all multifunctional micelle (MFM) formluations. The resulting micelles showed a mean size of 60-70 nm with narrow size distribution. Significantly increased amount of LCP-encoded MFM was observed in $\alpha_v\beta_6$(+) H2009 lung cancer cells over the $\alpha_v\beta_6$(-) H460 cells, demonstrated by confocal laser scanning microscope. LCP-encoded MFM showed much lower IC_{50} value than SP-encoded MFM in H2009 cells. We also evaluated the *in vivo* MR imaging specificity by i.v. injection of LCP-encoded micelles to the mice bearing H2009 and H460 tumor xenografts. Only signal intensity of H2009 tumors dropped significantly 2 hours after micelle injection, while H460 tumor still kept the same signal intensity (**Fig. 1B**). *In vivo* efficacy study showed that tumors treated with LCP-encoded MFM had much slower tumor growth, at an average volume of 1200 mm^3 after 44 days of initial injection, compared to 2200 mm^3 for PBS control and 1900 mm^3 for SP-encoded micelles (**Fig. 1C**). We performed biodistribution studies of ^{3}H-labelled MFM samples 24 hours after i.v. administration (**Fig. 1D**). It is noted that LCP-encoded MFM accumulated significantly more in H2009 tumors than SP-encoded MFM. Liver and spleen are the two major organs that also take up the nanoparticles.

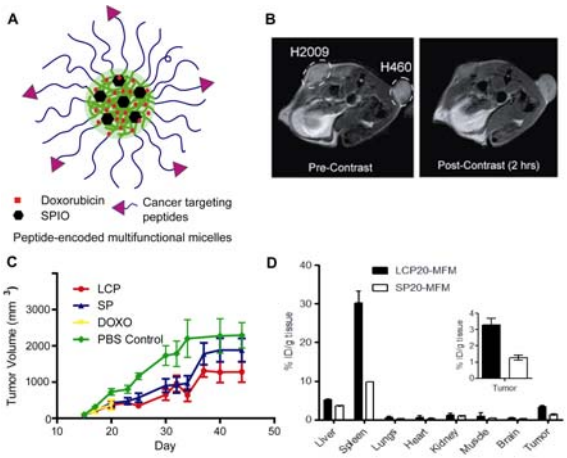

Figure 1. (**A**) Scheme of a theranostic polymer micelle. (**B**) T_2-weighted images of H2009 and H460 tumor xenografts in a mouse before and 2 hours after i.v. injection of LCP-encoded micelles. (**C**) *In vivo* antitumor efficacy of multifunctional micelles injected i.v. in scid mice bearing H2009 subcutaneous tumors. (**D**) Biodistribution profiles of LCP- and SP-encoded micelles 24 hours after i.v. administration.

Conclusions: A theranostic polymer micelle nanoplatform with effective cancer imaging and therapeutic modalities is successfully established. LCP-encoded micelles showed specific targeting efficiency and ultrasensitive MR detection towards the H2009 lung tumor model *in vitro* and *in vivo*. The LCP-encoded micelles inhibited the tumor growth better than the SP-encoded micelles and PBS control. By combining tumor targeting, imaging and therapy function in an all-in-one system, these theranostic nanoparticles can provide a promising target-specific, imaging-guided treatment of lung cancer.

References:

1. Guan, H. *et. al Bioconjugate Chem*. 2008 (**19**, 1813-1821).
2. Nasongkla, N. *et. al Nano Lett*. 2006 (**6**, 2427-2430).