**Employment of PD-L1-conjugated lipid-coated calcium phosphate**

**nanoparticles to treat metastatic cancer**

*Fatemeh MovahediA, Wenyi GuA, Zhi Ping XuA*

AAustralian Institute for bioengineering and nanotechnology, The University of Queensland, Brisbane, Australia

**Abstract**

Metastasis dissemination of tumor is the leading reason of mortality in the majority of patients with cancer (Guan, 2015). Although it is complicated to target metastatic cells, inhibition of regulatory pathways for progression of metastatic phenotypes can be an applicable approach. The T-LAK Cell-originated Protein Kinase (TOPK) pathway is highly expressed in circulating tumour cells (Sun *et al.*, 2015). OTS 964 is a novel TOPK inhibitor discovered in 2014 which is highly effective against a wide range of cancer cells (Matsuo *et al.*, 2014). On the other hand, Hypoxia Inducible Factor-1 (HIF-1) –whose over-expression is the hallmark of cancer cells- is the key regulator of cancer metabolism and cancer cell proliferation. Activation of HIF-1α can lead to angiogenesis and tumour progression (Chouaib *et al.*, 2012). The anti-parasite agent, albendazole (ABZ) is recently introduced as an anti-cancer drug which can inhibit HIF-1α (Pourgholami *et al.*, 2010). Combination of OTS-964 and ABZ can be a promising approach for treatment of metastatic and drug-resistant cancers.

In this study, we developed pH-responsive lipid-coated calcium phosphate nanoparticles (LCPs) for co-delivery of albendazole and OTS964. ABZ- and OTS-coloaded LCPs (OTS-ABZ-LCP) were examined against metastatic breast cancer (4T1) cells as well as healthy cell lines, HUVEC and HEK293T.

Encapsulating ABZ and OTS into LCPs, tiny crystals of albendazole were entrapped in the core and OTS 964 was loaded into the lipid bilayer, which provided significantly enhanced solubility and bioavailability for both drugs. The drugs were released from LCPs in an acidic environment rapidly (57% in 4 hours) while the release was considerably slow in the physiological condition (29% after 48 hours). ABZ-LCPs, OTS-LCPs and OTS-ABZ-LCPs significantly reduced the viability of 4T1 cells and OTS-ABZ-LCP caused synergistic cell toxicity with no significant cytoxicity against HUVEC and HEK293T cells. Moreover, enhanced cellular uptake and cell cytotoxicity were observed by conjugating PD-L1 antibody to the surface of ABZ-LCP and OTS-LCP.

As for the mechanism of action, synergistic apoptosis induction was observed. Moreover, OTS-ABZ-LCPs enhanced reactive oxygen spice (ROS) production which can be promising for the suppression of drug efflux pumps and overcoming drug resistance. Both ABZ-LCPs and OTS-LCPs reduced the migratory and invasive activity of the cells while OTS-ABZ-LCP was superior.

To conclude, combination of OTS and ABZ can result in a synergistic inhibition to the cancer growth and metastasis. While LCPs provide a pH-responsive platform for enhanced bioavailability of the drugs, they can also enhance the efficiency by providing the possibility of tailoring for targeted delivery. Overall, this system offers an effective treatment with minimum side effects for the metastatic cancers.

**References**

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