

## DOCTORAL THESIS

### The delivery of triptolide to non-small cell lung cancer with CA IX and CPP conjugated liposomes

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## ABSTRACT

Lung cancer accounted for 28% of all cancer related deaths in Hong Kong and has been the leading cause of cancer death worldwide. Non-small cell lung cancer (NSCLC) is the most common lung cancer (85%) and has been linked to poor prognosis with 5-year survival rates of only 15%. Low accumulation and lack of efficient penetration of therapeutic agents in the tumor site, and severe adverse effects are the main obstacles in efficient lung cancer chemotherapy.

Triptolide (TPL), a diterpenoid triepoxide, was first isolated from the Chinese medicinal plant *Tripterygium wilfordii* Hook F. It had attracted extensive attention for its anti-tumor effect. However, its therapeutic potential has been limited by the poor water solubility (0.017 mg/mL) and strong toxicity with LD<sub>50</sub> of 0.8 mg/kg. To improve the therapeutic effects and facilitate the application of TPL in lung cancer therapy, we developed different ligands-modified TPL-loaded liposomal formulations for lung cancer specific delivery.

Antibody-decorated liposomes can facilitate the precise delivery of chemotherapeutic drugs to the lung by targeting a recognition factor present on the surface of lung tumor cells. Carbonic anhydrase IX (CA IX), an enzyme overexpressed on the surface of lung cancer cells with a restricted expression in normal lungs, is used as the target for NSCLC therapy. In the present study, anti-CA IX antibody-modified TPL-loaded liposomes was developed. CA IX-directed liposomes exhibited 1.7-fold enhancement in internalization effects and 2-fold higher cytotoxicity in CA IX-positive human non-small cell lung cancer cell line A549. *In vivo*, CA IX-directed liposomes confined the delivery specifically to the lung and resided up to 96 h, which further showed enhanced therapeutic efficiency in orthotopic lung tumor bearing mice.

CPP33 is a tumor lineage-homing cell-penetrating peptide reported to be highly permeable into human lung cancer cell. Here, we utilized CPP33 for translocation of TPL-liposomal formulation into lung tumor cells. *In vitro*, CPP33-TPL-lip significantly improved apoptotic feature on A549 cells than non-modified liposomes. CPP33-lip specifically promoted the penetration ability of liposomes on A549 rather than human lung fibroblast cells (MRC-5), showing prominent cell selectivity. Furthermore, CPP33-lip showed superior penetrating ability on 3D tumor spheroids compared to non-modified liposomes.

A dual-ligand TPL-loaded liposomes (dl-TPL-lip) via conjugation of anti-CA IX antibody (targeting module) and CPP33 (trans-membrane module) was further developed to improve the therapeutic efficacy of NSCLC. The dl-TPL-lip showed superior penetrating ability and inhibiting effect on 3D tumor spheroids and significantly enhanced TPL anti-cancer efficacy following pulmonary administration in orthotopic lung cancer nude mice. The encapsulation of TPL in liposomes reduced the exposure of TPL in systemic circulation, which is demonstrated by pharmacokinetic study in rat plasma by endotracheal

administration. Further anti-cancer effect study showed that dl-TPL-lip exhibited the greatest efficacy compared to TPL solution, non-modified TPL-loaded liposomes, anti-CA IX Ab or CPP33 single ligand-modified liposomes.

In summary, the findings of this study establish promising TPL delivery systems for targeted therapy of lung cancer. Current research focusing on drug delivery systems provides an insight into targeted and safe delivery of TPL in preclinical setting.

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