

DOCTORAL THESIS

Peptide functionalized drug delivery system for an efficient lung cancer therapy

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ABSTRACT

Lung cancer has a high incidence rate globally and the leading cause of cancer related mortalities. In 2018, lung cancer has been estimated to cause 1.76 million deaths worldwide (18.33% of total cancer mortalities). In Hong Kong lung cancer has been a leading cause of cancer related deaths, and in 2016 caused 3780 deaths (26.6% of total cancer mortalities). Non-small cell lung cancer (NSCLC) is the major (~85%) lung cancer type, and five-year survival rate for lung cancer has estimated to be 18%. Thus, an efficient lung cancer treatment with lesser adverse effects is need of the hour. In this connection, active targeting of overexpressed receptors at lung tumor site with a ligand functionalized drug delivery system is the current approach, and pulmonary administration could augment chemotherapeutic effect of the drug through localized administration, minimizing the off-target effects by retention of the drug in lungs.

Quercetin (QR), a natural flavonoid present in edible fruits and vegetables possess anticancer activity i.e. inhibits lung cancer growth. However, the application of QR in lung cancer therapy has been restricted by various factors i.e. low water solubility (2.15 $\mu\text{g/ml}$ at room temperature), low bioavailability and rapid plasma clearance. To overcome the issues, we have formulated various QR-loaded liposomes surface functionalized with transferrin receptor (TFR) targeting peptides i.e. T7 (HAIYPRH) and T12 (THRPPMWSPVWP) in two research projects with active targeting ability, prolonged circulation time, and sustained release behavior for lung cancer specific QR delivery.

In first research project, T7 targeted liposomes with different peptide densities i.e. 0.5%, 1% and 2% and QR-lip (non-targeted) were formulated. TFRs are over expressed (~100 folds) in various cancers including lung cancer and have low expression in most normal cells. T7 surface-functionalized liposomes (2% T7-QR-lip) demonstrated significantly enhanced cytotoxicity (~3-folds), cellular-uptake, S-phase cell cycle arrest and apoptosis in A549 cells. However, in MRC-5 (normal-lung fibroblast) cells no significant difference was observed after treatment with T7-

QR-lip and QR-lip in cytotoxicity and cellular uptake studies. In tumor spheroid penetration and inhibition studies, T7 targeted liposomes showed deeper penetration and pronounced inhibition. *In vivo* biodistribution study via pulmonary administration of T7-DiR-lip has demonstrated liposomes accumulation in the lungs and sustained-release behavior upto 96h. Further, T7-QR-lip significantly enhanced anticancer activity of QR and life-span of orthotopic lung-tumor bearing mice (**p < 0.01, compared with control) via pulmonary administration.

In second research project, T12 surface-functionalized liposomes with 0.5%, 1% and 2% T12 peptide densities and QR-lip have been formulated with ~95 % encapsulation efficiency. *In vitro* drug release study showed sustained release of QR from T12-QR-lip and QR-lip. *In vitro* experiments showed A549 cells treatment with 2% T12-QR-lip enhanced cellular-uptake, *in vitro* cytotoxicity, induced apoptosis and S-phase cell cycle arrest due to TFR mediated endocytosis. No significant variation has been observed in cellular-uptake and cytotoxicity after MRC-5 cells were treated with T12-QR-lip and QR-lip. Further, T12-Cou6-lip showed significantly deeper penetration i.e. 120 μm in 3D lung tumor-spheroids. Biodistribution study showed retention of T12-DiR-lip and DiR-lip mainly in the lungs upto 96h after pulmonary administration, as compared to free DiR. Pulmonary administration of T12-QR-lip showed the strongest tumor growth inhibition and survival time of orthotopic lung tumor implanted mice without any systemic toxicity as compared to QR-lip and free-QR.

In summary, *in vitro* and *in vivo* results of the two research projects suggest that surface functionalization of the liposomes with TFR targeting peptides i.e. T7 and T12 is a promising approach for lung cancer therapy through active targeting and receptor mediated endocytosis of QR at lung tumor site. Moreover, T7 and T12 functionalized liposomes provides a potential drug delivery system for a range of anticancer drugs to enhance their therapeutic efficacy by localized i.e. pulmonary administration and targeted delivery.

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