SYNOPSIS

Introduction

The daunting disease of cancer owe its origin to numerous complex genetic and non-genetic factors, both intrinsic and extrinsic. Despite the century old efforts from scientists and physicians across the globe for finding cure for this deadly disease, long term cure without any relapse of tumor remains elusive. The hallmarks of most, if not all, types of cancer include: self-sufficiency in growth signals, resistance to growth suppressors, evading apoptosis, limitless proliferation, inducing angiogenesis, invasion & metastasis, reprogramming energy metabolism and lastly, evading the host's immune system. Clinical success of most of the contemporary cancer therapies are limited due to non-availability of highly tumor specific drug delivery systems leading to adverse side effects on various non-cancerous healthy body tissues, insufficient bioavailability of many potent cancer therapeutics, drug resistivity, and the poor physicochemical properties of many anti-cancer drugs. To overcome these challenges, chemotherapeutic drugs (single or combination) or genetic drugs are often delivered selectively to the diseased site with the help of ligand coated liposomal or polymeric drug delivery systems for targeting drugs to cancer cells via receptors over expressed selectively on the exo-surfaces of cancer cells. The current thesis embodies design, synthesis, physico-chemical characterizations, bio-activity evaluations of two novel ligand-coated liposomal drug delivery systems for treatment of pancreatic cancer and lung carcinoma. Furthermore, the findings of the present thesis demonstrate for the first time that liposomally targeted chemotherapy, when used in combination with in vivo dendritic cell (DC), targeted DNA vaccination elicits markedly enhanced overall survivability of orthotopic pancreatic and lung carcinoma-bearing mice.

Statement of the problem

Cancer has long been considered to be a profoundly complex disease with second highest mortality rate treatable only with blunt approaches that frequently do as much damage to the patient as to the tumor. Pancreatic cancer is often detected late, spreads rapidly, and has a poor prognosis. After diagnosis, 25% of people survive one year and 5% live for five years. In an estimated 53,070 new cases of pancreatic cancer, 41,780 death of pancreatic cancer patients have been reported in 2016. Lung cancer is

the number one cause of cancer deaths in both men and women in the U.S. and worldwide. The vast majority (85%) of cases of lung cancer are due to long-term tobacco smoking. The general prognosis of lung cancer is poor because doctors tend not to find the disease until it is at an advanced stage. Five-year survival is around 54% for early stage lung cancer that is localized to the lungs, but only around 4% in advanced, inoperable lung cancer. Lung cancer occurred in 1.8 million people and resulted in 1.6 million deaths Worldwide in 2012. Thus, there is an urgent need for developing novel and more competent approach for eradicating and enhancing the overall survival rate of both pancreatic and lung cancer patients using targeted chemotherapy and cancer immunotherapy.

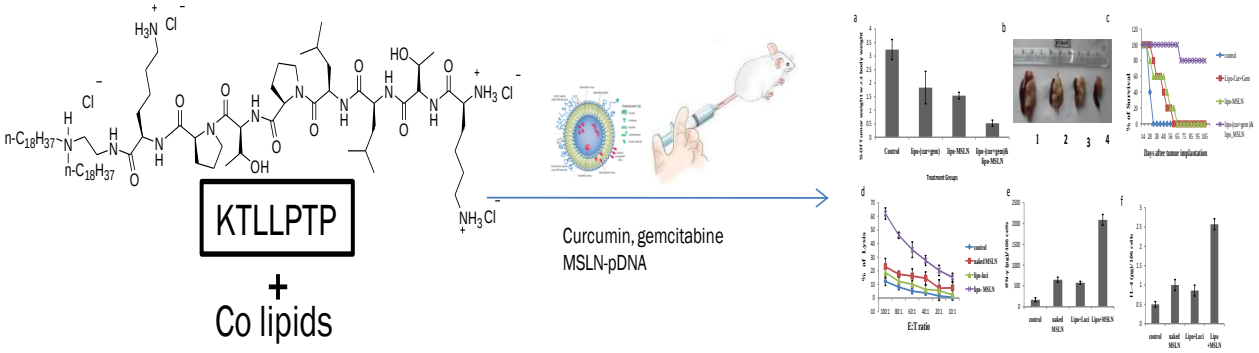
Objectives of the study

1. To develop the plectin-1 receptor (plec) targeting liposomal formulations of novel plectin-1 selective KTLLPTP-lipopeptide and to evaluate their therapeutic promises in combating orthotopic mouse pancreatic cancer using our newly developed approach of combining targeted chemotherapy and direct in vivo DC-targeted DNA vaccination.
2. To develop lung cancer targeting liposomal formulations of novel SNIDARAK-lipopeptide & its non-targeting control NSSSVDKK-lipopeptide and to evaluate their therapeutic promises in combating orthotopic mouse lung cancer using our newly developed approach of combining targeted chemotherapy and direct in vivo DC-targeted DNA vaccination.
3. Structure-activity studies on the use of liposomes of mono-, di-, and tri-guanidinylated cationic amphiphiles for systemic co-delivery of potent anti-cancer drugs paclitaxel (PTX) & CDC20 siRNA co-encapsulated within the liposomes of these guanidinylatd amphiphiles using an orthotopic mouse lung tumor model.

Methodologies Used and Sample Results

Part 1 describes development of the plectin1-targeting liposomal formulations of lipopeptide containing phase display derived pancreatic cancer targeting ligan KTLLPTP in the head group region (lipopeptide **1**) and its nontargeting control

lipopeptide with SNLHPSD in the head group region (lipopeptide **2**). The fluorescently labeled liposomal formulations of lipopeptide **1** showed higher cellular uptake in pancreatic cancer cells (Pan02, Panc-1, and Miapaca-2) compared to normal NIH3T3 Cells. Plectin-1 receptor selective cellular uptake process for these liposomal formtions were confirmed by antibody saturation. The *ex vivo* fluorescence values of isolated organs revealed that mice injected with NIR-labeled liposomal formulation of lipopeptide **1** had strong fluorescence in the orthotopic pancreatic tumor compared to other isolated organs. Importantly, combination of *i.v.* administration of the liposomal formulation of lipopeptide-**1** containing both curcumin & gemcitabine and genetic immunization with in vivo DCs targeting lipoplex of liposomes of mannose-mimicking cationic amphiphile (recently developed in our laboratory) & p-CMV-MSLN (a plasmid DNA encoding mesothelin expressed in the majority of pancreatic cancer cells) regressed and increased survivability of the established orthotopic mouse pancreatic tumor model in syngeneic C57BL/6J mice. The findings in CTL assay and cytokines assay (IFN-γ & IL-4) clearly demonstrated that the in vivo DCs targeted lipoplexes were efficient in inducing cellular and humoral response against pancreatic cancer.

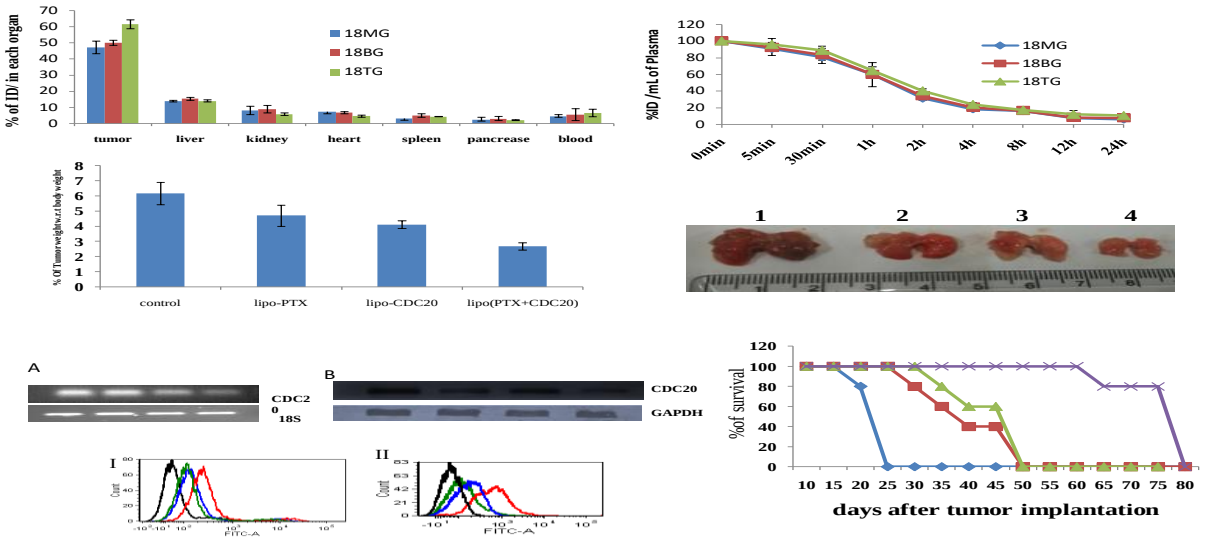


The Part 2 of the present thesis describes design of a novel lung cancer targeting liposomal formulations of SNIDARA-lipopeptide (lipopeptide **1**) and its non-targeting control lipopeptide with NSSSVDK in the head group region (lipopeptide **2**). Further, this parts embodies development of a novel liposomal delivery system prepared using the above-mentioned lipopeptides for lung cancer targeted delivery of curcumin and doxorubicin. The labeled liposomal formulation of lipopeptide **1** showed higher cellular

uptake in lung cancer cells (LLC, H460, and A549) compared to normal NIH3T3 Cells. The *ex vivo* fluorescence values of isolated organs revealed that mice injected with doxorubicin loaded liposomal formulation of lipopeptide **1** had strong fluorescence in the orthotopic pancreatic tumor compared to other isolated organs. Findings in the MTT-assay basedcCell viability studies and annexin-v binding based apoptosis studies demonstrated that co-delivery of curcumin and doxorubicin loaded targeted liposomes maximally enhanced the anti-cancer effect. The same result was also found during cell cycle study where curcumin and doxorubicin delivery *via* targeting liposome exhibited maximum G2/M population. Importantly, combination of *i.v.* administration of the liposomal formulation of lipopeptide-**1** containing both curcumin & doxorubicin and immunization with in vivo DCs targeting lipoplex of mannose-mimicking cationic amphiphile (recently developed in our lab) & p-CMV-MUC-1 (a plasmid DNA encoding MUC-1 expressed in the majority of lung cancer cells) regressed and increased survivability of the established orthotopic mouse lung tumor model in syngeneic C57BL/6J mice.

Part 3 of the present thesis embodies structure activity studies using different liposomal formulations of mono-, di- and tri- guanidinylated cationic amphiphiles containing co-encapsulated anti-cancer drugs and genes. PTX (hydrophobic drug) was solubilized within the lipid bilayer regions of the liposomes and CDC20 siRNA (hydrophilic anti-cancer siRNA) was encapsulated within the aqueous core of the liposomal formulations. Bio-activities of the liposomal formulations of these cationic guanidinylated amphiphiles were evaluated both in vitro and in vivo. Biodistribution studies using FAM-siRNA labeled liposomes revealed orthotopic mouse lung tumour selective accumulation of the liposomal formulations in syngeneic C57BL/6J mice. The plasma stability of FAM-siRNA labeled liposomes, MTT-based cytotoxicity assays, Annexin-V binding based apoptosis studies and cell cycle arrest at G2M phase were studied. Finally, *in vivo* tumor inhibition and survival studies were done with the most efficient liposomal formulation containg both PTX and CDC20 siRNA in orthotopic mouse lung tumor model. Importantly, the findings in these structure-activity studies convincingly demonstrated that presence of more positive charge (due to presence of 3

guanidine groups in the head-group region) can enhance the entrapment efficiency of drugs and genes, and increase the plasma stability of the liposomal formulations.



Conclusion

The findings described in the present thesis collectively demonstrate that use of tumor-targeted liposomal drug delivery in combination with *in vivo* dendritic cell targeted DNA vaccination leads to remarkable survivability enhancements in orthotopic pancreatic and lung tumor bearing mice. Such combination therapy holds potential for future use as a "platform technology" in the war against the dreaded disease of cancer.