SYNOPSIS

Folate Receptor Targeted Liposomal and Carbon Nanosphere-Based Drug Delivery Systems for Combating Melanoma and Glioblastoma

Chapter I

Cancer in organs generally originates from abnormal cell division, so called it is malignant disease as cells metastasize to other parts of the body through circulating blood and form new tumors. In diseases ranking, cancer now crosses infection related deaths and reached to second highest death rate after cardio vascular diseases. Therapeutic anti-cancer drugs such as doxorubicin, docetaxel etc. are toxic to cancer cells as well as normal healthy cells. To overcome this direct drug treatment strategy therapeutic modalities such as targeted chemotherapy with various drug delivery systems are frequently developed to overcome this issue.

Targeted chemotherapy: is the most widely used treatment modality for cancer. For treating several other diseases including cancer which involve selective delivery of chemotherapeutics (potent drug or gene) employ different drug delivery systems such as liposomes or material based systems. In cancers, anti-cancer drug or therapeutic siRNA or both can be selectively delivered to cancer site by using biomarkers over-expressed on tumor cells or tumor environment with minimal harm to other healthy tissue.

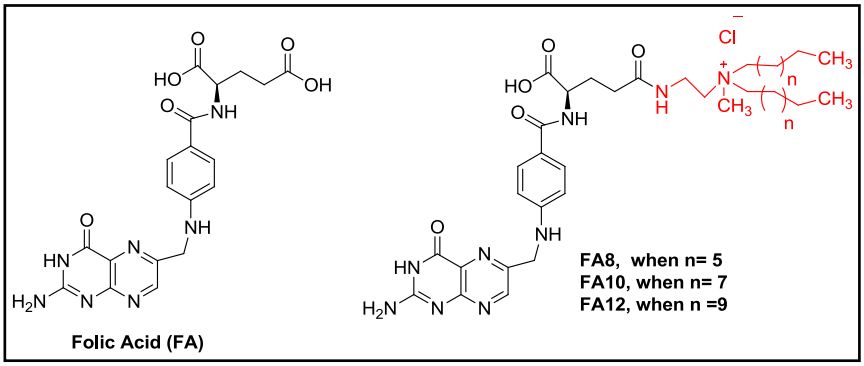
Folate receptor targeting in cancer: Folate receptor (FR) is glycosylphosphatidylinositol (GP)-anchored membrane bound cell surface receptor. It binds and transports folates inside the cell by endocytosis. Rapidly dividing cancer cells in tumors needs increased requirement of folic acid, as a result elevated FR expression is often observed in these cells.

Liposomes: Liposome is spherical vesicle of lipid bi-layers consisting of amphiphillic lipids (cationic or anionic lipids), neutral lipids enclosing in watery layer. These spherical vesicles contain a hydrophobic domain which can encapsulate anti-cancer hydrophobic drugs (example taxol in hydrophobic lipid bi-layer) and hydrophilic domain (can encapsulate hydrophilic drug or gene inside aqueous compartment) to deliver the cargo at cancer site by EPR effect without much affecting normal tissues. However, developing an efficient liposomal system to improve maximum drug delivery to cancer sites with enhanced overall survivability is challenging.

Carbon nanospheres (CSP): Glioblastma is deadly and debilitating brain tumor with abysmal prognosis. Presence of blood brain barrier (BBB) in brain renders majority of anti cancer therapeutic agents ineffective for treatment of glioblastoma (cancer of the brain). Nowadays, Glucose derived carbon nanospheres are emerging as a class of intra cellular and BBB overcoming nanocarriers. However the advantage with these CSPs is that any hydrophilic or hydrophobic drug or an imaging agent can be easily adsorbed on the surface of the CSP.

Chapter-II

Previously our lab members established that when conjugating a cationic twin chain head group molecule to Steroid molecules like Estrogen, Progesterone, Hydrocortisone or Dexamethasone, final resulted molecule have shown anti-cancer activity by kinase inhibiting property. Since FR is more expressed in cancer cells than normal cells in Ph.D work I have synthesized folic acid analogues FA8, FA10 and FA12 by conjugating corresponding cationic twin chain molecules to FA. But from *in vitro* studies, IC_{50} found in cancer cells at 20μM. So, I have used these folic acid conjugates as targeting ligands in liposomal and carbon spheres based drug delivery systems.



Chapter-III

In this study we developed Folate receptor (FR) targeting liposomal formulation carrying a hydrophobic drug called NME2 to treat aggressive melanoma. Melanoma is malignant skin cancer that originates from pigment containing cells called melanocytes. However, melanoma cells have moderate FR expression but have functional ER in their cytoplasm. A drug like molecule called bis-arylidene oxindole (NME2) is initially developed as anti-cancer drug acting via targeting Estrogen receptor(ER). It is structurally similar to tamoxifen. Since melanoma cells also have functional ER in their cytoplasm we wanted to use this potent anticancer drug in FR targeting liposomal systems to treat aggressive melanoma *in vitro* and *in vivo* models. The targeted (FA8 +NME2) liposome showed good targeting ability in FR moderately expressing B16F10 cells. We wanted to investigate the efficacy of FR targeted liposome in both FR and ER expressing B16F10 cells. Interestingly, we found NME2 carrying FA8 liposome is up-regulating caspase-8 protein, which is important in both intrinsic and extrinsic cell death pathways, where as naked NME2 or FA8 could not induce alone. This observation also confirmed by *in vivo* lysate western results in mouse melanoma model. We got similar results when we used another

hydrophobic anti cancer drug, docetaxel incorporated in the FR-targeted liposome. Since caspase-8 down regulation is linked with drug resistance in some gynecological cancers this presently described (FA8+Drug) liposomal formulation is an important potential therapeutic system in treating FR expressing (even though moderately) aggressive cancers.

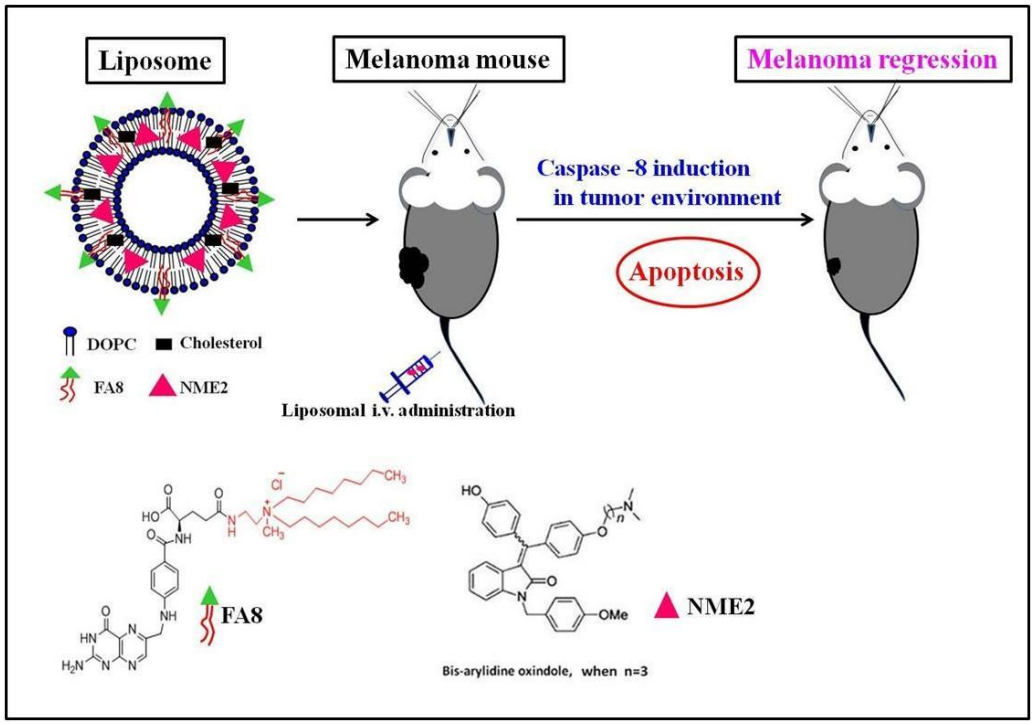


Figure 1: Schematic representation of Folate receptor targeted (FA8+NME2) liposome inhibiting melanoma tumor in mouse model.

Chapter-IV

Use of nano materials in biology has opened the door in improving therapeutic procedures especially in imaging and drug delivery in malignant diseases like cancer. Glioblastma is deadly and debilitating brain tumor with abysmal prognosis. Standard treatment for glioma starts with surgical resection followed by radio- and chemotherapy. But prognosis remains dismal with less survival. The major obstacle for chemotherapy in glioma is to deliver the anticancer drug across the blood brain barrier (BBB) which renders majority of anti cancer therapeutic agents ineffective. Nowadays, Glucose derived carbon nanospheres are emerging as a class of intra cellular and BBB overcoming nanocarriers. Expression of Folate receptor (FR) occurs in many epithelial cancers as well as tumor associated macrophages. Tumor associated macrophages (TAM), especially M2 types are tumor's most professional drivers for progression and metastasis. However, Chemotherapy and radiotherapy can have dual influence on TAM, in that an

orchestrated tissue repair response can also result in induction of chemo resistance. TAM also switches on angiogenesis by VEGF production and suppress immune response in tumor**.** Tumor associated macrophages are isolated by magnetically labeled CD-11b micro beads from subcutaneous glioma GL-261 tumor. TAMs show good expression of FR as observed by FACS analysis. In order to target tumor microenvironment bearing folate receptor expressing cells, ie., tumor epithelial cells and TAM, a dual targeting carbon nano-spheres (CSP) have been developed by conjugating FA8 to CSP. The CSP modification allows CSP to become functional and tumor site selective. As a result, when modified CSP was conjugated with doxorubicin the effective delivery of anticancer drug cargo was is shown to be on FR expressing cancer cells as well as tumor microenvironment associated macrophages (i.e., TAM) without affecting normal tissues. Findings in *in vitro* and *in vivo* experimental results convincingly demonstrated that presence of FA8 molecule on CSPs can make more selective doxorubicin delivery towards FR expressing cancer cells and tumor microenvironment macrophages. This conferred dual uptake of CSP on TAM and tumor cells via FR. Doxorubicin associated FR-targeted CSP formulation (CFD) in an orthotopic glioma model and in glioma subcutaneous model, induced maximum anticancer effect with enhanced average mice survivability twice to that of untreated mice and without any systemic liver toxicity**.**

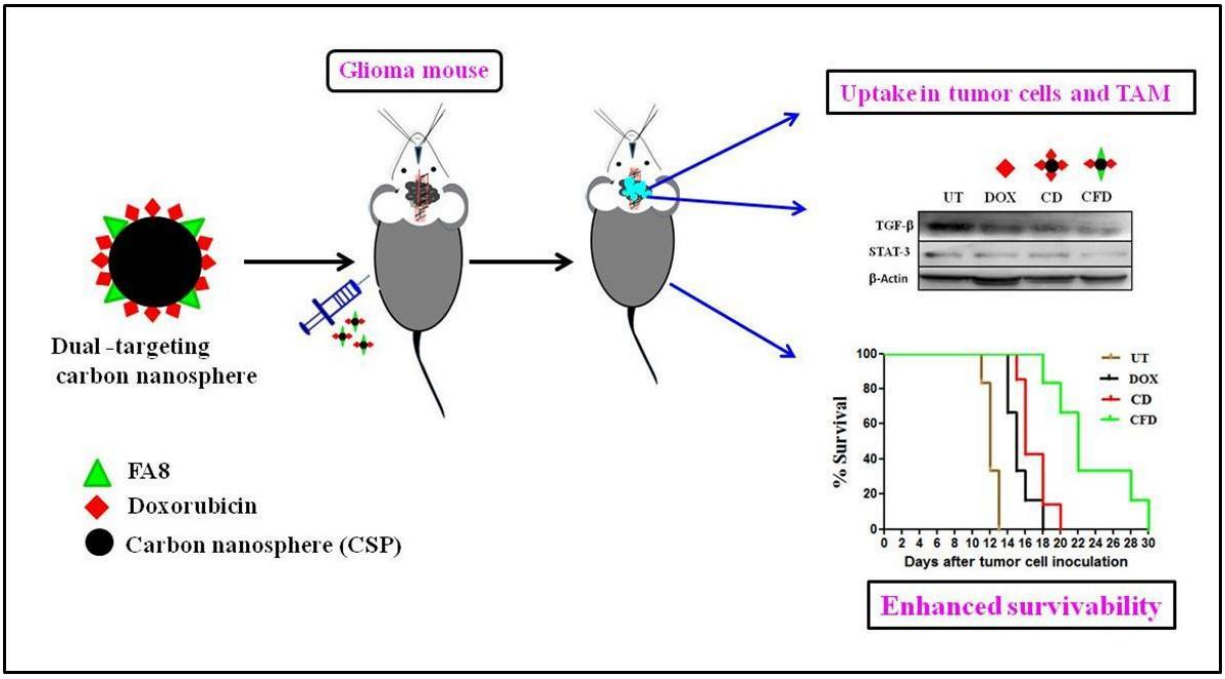


Figure 2: Schematic representation of Doxorubicin drug carrying Dual-targeting carbon nanospheres and its therapeutic application in glioma treatment with down regulation of several tumor promoting proteins in tumor environment in mouse glioma model.

Summary

Thus my thesis work focuses on developing Folate receptor (FR) targeted liposomal as well as carbon nanosphere based drug delivery systems. When anti cancer drug is incorporated or adsorbed these formulations show good targetability and efficient drug delivery to the melanoma and glioma cancer cells and their corresponding tumor microenvironment. We believe, among the delivery systems so developed, our FR targeting carbon nanosphere based formulation opens up new prospects and presents a dual targeting drug delivery system for tumor-associated macrophages (TAM) and tumor-associated epithelial cells.