Chapter 1: Introduction

Cancer, the age-old immortal illness, arises from uncontrolled division of a set of diseased cells which can be identified with a limitless replicative potential as well as the capacity of out-of-bound migration, invasion and metastasis. The loss of cellular regulation leading to cancer may often arise from mutations in two broad classes of genes, namely, oncogenes and tumor suppressor genes. Though cancer is often described as 'The wound that never heals', there have been intense efforts worldwide to bring a remedy including hormonal therapy, targeted chemotherapy, cancer immunotherapy, gene therapy, anti-angiogenic therapy etc.

Chemotherapy usually refers to use of drugs (chemotherapeutics) that kill rapidly dividing cancer cells. While chemo drugs kill cancer cells, they also harm cells that divide rapidly under normal conditions. Such side effects are partly overcome by **targeted therapy** which is a special type of chemotherapy that takes advantage of small differences between normal cells and cancer cells ("molecular targets"), thereby selectively delivering drugs to cancer cells via suitable drug delivery systems.

Drug delivery systems refer to approaches, formulations, and systems for transporting a pharmaceutical compound in the body to safely achieve its desired therapeutic effect. These include liposomes, microspheres, gels, polymers, nanoparticles, micelles, cyclodextrins etc**. Liposomes,** consisting of spherical lipid bilayers and enclosing a watery interior, have long been viewed as bio-compatible drug/gene delivery reagents owing to their structural similarity to cell membranes. In the field of targeted chemotherapy, specific targeting of liposomally encapsulated chemotherapeutics to tumor cells are often accomplished using liposomes made from lipids covalently conjugated with tumor targeting ligands. Past decade has also witnessed wide use of **Gold nanoparticles** (GNPs) in the field of nanomedicine with diverse biomedical applications such as drug delivery vehicles or theranostic agents. Due to their small size, good biocompatibility, tailor-able surface chemistry, characteristic surface plasmon absorption, and ease of synthesis, gold nanoparticles serve as promising drug nanocarriers.

Despite significant advances, **resistance to chemotherapy** and molecularly targeted therapies is a major challenge in current cancer research. The resistance to 'classical' cytotoxic chemotherapeutics and to therapies that are designed to be selective for specific molecular targets may be intrinsic or acquired. The most common reason for acquisition of resistance to a broad range of anticancer drugs is expression of one or more energy-dependent transporters that detect and eject anticancer drugs from cells, but other mechanisms of resistance including insensitivity to drug-induced apoptosis and induction of drug-detoxifying mechanisms also play an important role in acquired anticancer drug resistance. Multidrug based combination therapy sometimes helps to circumvent such resistance of cancer cells to chemotherapeutics. Rational drug combinations are often proposed on the basis of *in vitro* and *in vivo* synergy between agents; either hitting the same pathway at multiple points or targeting completely independent pathways. Thus, strategies to circumvent cancer drug resistance by targeting alternative pathways may improve cancer chemo therapy leading to efficient tumor management.

Here, in my thesis, I report on the exploitation of two targets: Glucocorticoid receptor and N-end rule pathway as a strategy to induce drug sensitization in cancer cells and inhibit tumor regression (melanoma & colon).

Chapter 2

The differentiation of epithelial cells to motile mesenchymal phenotype, a process known as epithelial-mesenchymal transition (EMT) is involved in drug resistance, cancer progression and metastasis. Therefore, inhibiting EMT could be an attractive therapeutic modality to overcome drug resistance and metastasis in cancer cells. Recent studies demonstrate that glucocorticoids (GCs), a class of steroid hormones can block EMT of mink lung epithelial cells, estrogen receptor (ER) negative breast cancer cells and human peritoneal mesothelial cells. However, elucidation of role of glucocorticoids in inducing EMT has just started and glucocorticoid receptor's (GR) functional importance in this regard is not clear yet. **Chapter 2** of my thesis delineates the development of a new glucocorticoid receptor (GR)-targeted gold nanoparticle formulation, that can stably carry anticancer drug Withaferin A (WFA) in its hydrophobic core. To achieve GR target-ability, we conjugated Dexamethasone (a synthetic Glucocorticoid) to GNP surface via thiol

bond. The primary –OH group of dexamethasone was converted into thiol functionality (**DSH**) to ensure facile binding with gold nanoparticle surface. The presently synthesized gold nanoparticle formulation (GNP-DSH-WFA) showed GR-dependent cancer cell selective cytotoxicity, inhibited growth of aggressive mouse melanoma tumor while reversing EMT in tumor-associated cells and reduced mice mortality. Same treatment also led to near-complete down-regulation of ABCG2 drug transporter in tumor cells thereby attributing it to its drug sensitization ability. The present report thus demonstrates the design of a new metallic nano-formulation that reduces aggressive tumor growth through GR-mediated reversal of EMT and induction of drug sensitivity.

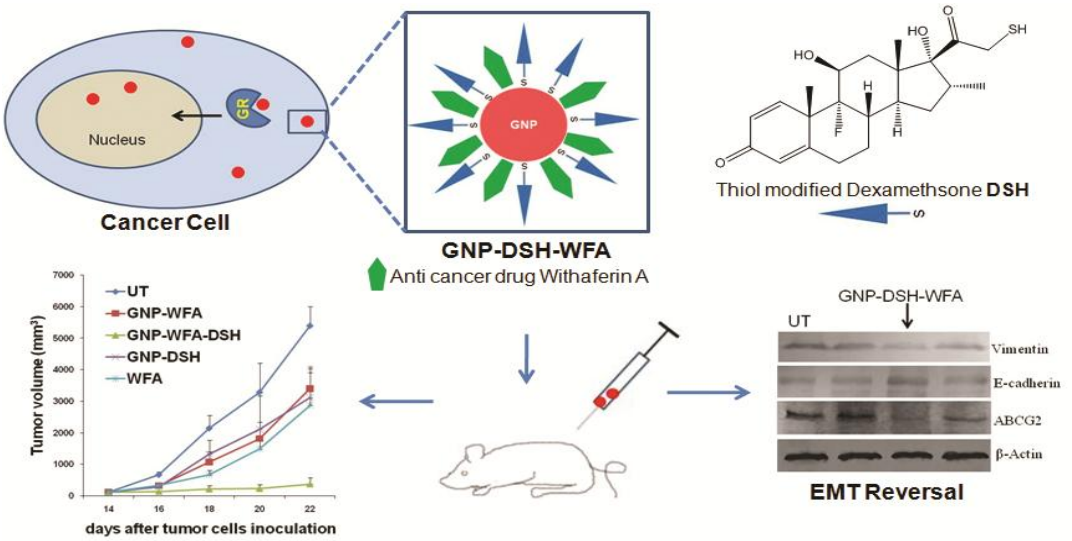

Figure 1. ***Glucocorticoid receptor targeted gold nanoconjugates inhibit tumor growth in mice via EMT reversal.*** Schematic representation of Glucocorticoid receptor targeted gold-withaferin nanoconjugate and its application in inhibiting growth of melanoma tumor in mice while reversing EMT in tumor cells.

Chapter 3A

Biotin (vitamin B7, vitamin H) is an essential micronutrient for normal cellular functions and is required in excess by various cancer cells to sustain their rapid proliferation. Biotin receptor is often found to be over expressed in a number of cancer cell lines of ovarian, colorectal etc. origins and has emerged as a promising

molecular marker for targeted drug delivery. This chapter reports on the synthesis of a biotin based amphiphilic lipid, **BIO-C18**, which along with appropriate co-lipids in particular mole ratios form stable liposome. Cellular uptake study demonstrates that Rhodamine PE labelled liposomes of **BIO-C18** lipid efficiently internalize in CT26, A549 and Hela cells via biotin receptor. This liposome can also preferentially deliver a hydrophobic fluorescent molecule in mice bearing colon tumor (biotin receptor over-expressed) under *in vivo* settings.

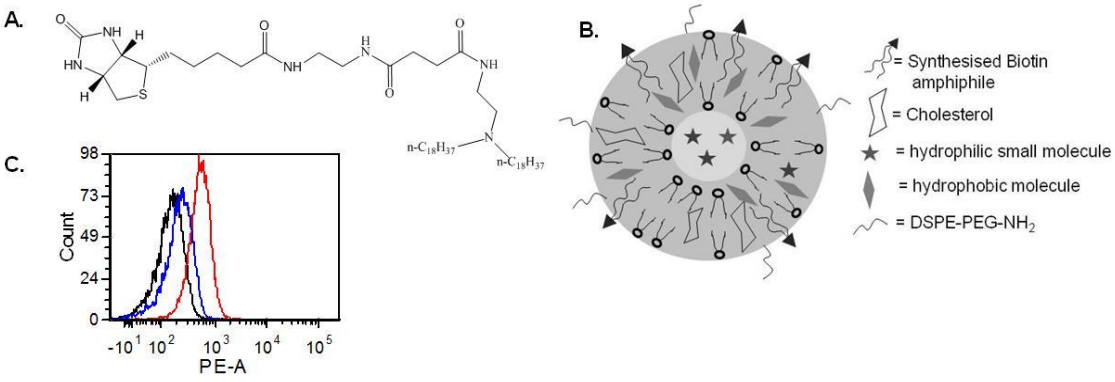

Figure 2. ***Cellular uptake of liposomes of biotinylated amphiphile are biotin receptor mediated***. Structure of biotin derived amphiphile, BIO-C18 (**A**) and schematic representation of biotin receptor targeted liposome (**B**) of BIO-C18. Flow cytometric uptake analysis (**C**) of Rh-PE liposome of BIO-C18 (red) which decreased considerably (blue) when cells were pre-saturated with biotin when compared to untreated (black).

Chapter 3B

N-end rule pathway is an ubiquitin dependent protein degradation pathway that relates the *in vivo* half-life of a protein to the identity of its N-terminal residue. Recent study has shown that N-end rule pathway counteracts cell death by degrading many anti-survival protein fragments like BCL_{xL}, BRCA1, RIPK1 etc. Inhibition of the N-end rule pathway can lead to metabolic stabilization of pro-apoptotic protein fragments like RIPK1, thereby sensitizing cells to programmed cell death. Receptor interacting serine-threonine protein kinase-1 (RIPK1) is one of the upstream regulators of programmed necrosis known as necroptosis. Necroptosis is particularly gaining attention of cancer biologists as it provides an alternate therapeutic modality to kill cancer cells, which often evolve multiple strategies to circumvent growth inhibition by apoptosis. Utilizing the over expression of biotin receptor in cancer

cells, herein, we report that co-administration of synthetic hetero-bivalent N-end rule inhibitor RFC11 & anticancer drug shikonin solubilized in a stable biotin receptor-targeted liposome (described in **Chapter 3A**) exhibited significant synergistic anti-tumor effect in both subcutaneous and orthotopic mouse colon tumor model through induction of necroptosis. Besides developing a newly targeted formulation for necroptosis induction, this report is the first *in vivo* evidence demonstrating that potent inhibition of N-end rule pathway can enhance therapeutic efficacy of conventional chemotherapeutics.

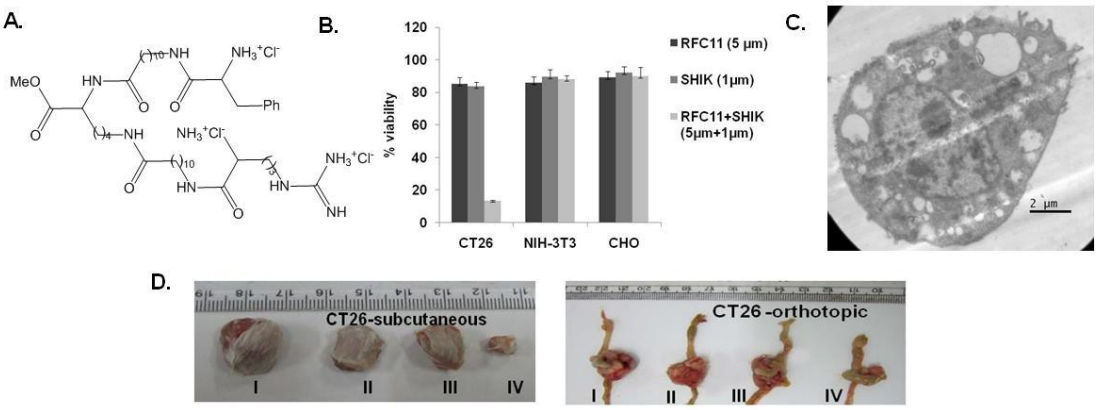

Figure 3. ***RFC11 & shikonin co-solubilised within the liposomes of BIO-C18 synergistically inhibit colon tumor growth via induction of necroptosis.*** Structure of RFC11 (**A**). Viability assay showing RFC11 could synergise anticancer effect of shikonin in colon cancer cell (**B**). Transmission electron micrograph of CT26 cell showing signs of necroptosis (**C**). Representative tumor size (**D**) in mice bearing orthotopic and subcutaneous colon tumor treated with glucose (I), biotinylated liposomes containing RFC11(II), shikonin (III), both RFC11 & shikonin (IV).

Conclusion

My thesis work thus focuses on the development of two different nano-assemblies, one liposomal and other gold nanoparticle based, both when loaded with suitable adjuvant could sensitize cancer cells to chemotherapeutics leading to efficient tumor growth inhibition in melanoma and colon carcinoma. The present thesis work also demonstrates that inhibition of N-end rule pathway can act as a platform to enhance therapeutic efficacy of conventional chemotherapeutics, thus opening new prospects in the field of cancer chemotherapy.