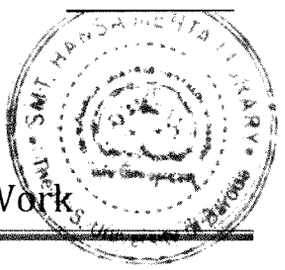
CHAPTER 1:

INTRODUCTION & OBJECTIVES OF WORK



1.1 Introduction

A genetic disorder is a disease caused by abnormalities in genes or chromosomes. While some diseases as cystic fibrosis are inherited, some others, such as cancer, are caused due to genetic abnormalities acquired in few cells during life. However, the term "genetic disease" most commonly refers to diseases present in all cells of the body and present since conception. Currently around 4,000 genetic disorders are known, with more being discovered. Most disorders are quite rare and affect one person in every several thousands or millions. In lungs, three major diseases observed by alterations in genetic makeup of the body are cystic fibrosis, lung cancer and α-1- antitrypsin deficiency. While cystic fibrosis and α-1- antitrypsin deficiency are hereditary diseases present since conception and affects the infants and newborns most; lung cancer is an acquired disease caused due to changes in genes acquired during life.

Cancer is one of the most dreadful diseases affecting mankind and is characterized by uncontrolled cell growth and spread of cells that may affect any tissue of the body. Most of the cancers are characterized by abnormalities in genetic makeup acquired during life. They involve **activation of certain oncogenes, suppression or mutation of certain controller genes like tumor suppressor *p*53 gene, activation of multiple drug resistance (MDR) genes and inhibition of antiapoptotic defense genes of BCL family** [M. Saad, et al (2008)]. Lung cancer is the second most common cancer in both men and women, and is responsible for highest death count after heart diseases [http://www.cancer.gov/cancertopics/types/lung]. It is characterized by uncontrolled cell growth in lung tissues leading to metastasis, invasion of adjacent tissue and infiltration beyond the lungs. It is responsible for over 0.15 million deaths in United States in 2010 with over 0.2 million cases registered annually [http://www.cancer.gov/cancertopics/types/lung]. Two main types of lung cancer based on the characteristics of the disease are non small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) out of which NSCLC accounts for 80% of all lung cancers. NSCLC is the very aggressive type of lung cancer and is responsible for significant mortality. Although surgery is a preferred method of cancer removal, it can not remove the tissue completely and is required to be supplemented by multidrug chemotherapy and / or radiation as preferred treatment of choice. **Etoposide (ETP)** and **Docetaxel (DTX)** are the current drugs of choice along with Doxorubicin, Carboplatin, Cisplatin, etc. for NSCLC treatment. The existing chemotherapy comprises only symptomatic management and partial cure. The major problems associated with

chemotherapeutic agents and delivery systems are inadequate tumor specificity, narrow therapeutic indices and emergence of resistant cancer cells [J. Aisner et al (1992)]. Extensive side effects due chemotherapeutic anticancer drugs on normal dividing cells as hair follicles, germ cells and hematopoietic cells are well known resulting in dose limiting toxicity [J. Aisner et al (1992)]. Although improved drug delivery by dry powder inhaler (DPI) formulation with above drugs in free form or in carrier encapsulated forms as liposomal and nanoparticulate carrier has been reported for sustained and cell specific action with reduced side-effects, but still issues related to chromosomal malfunctioning actually resulting in cancer are still to be resolved [P. Chene (2003)].

The preferred chemotherapeutic agents used in cancer therapy have shown limited therapeutic action after initiation of chemotherapy because of development of cellular multiple drug resistance mediated by membrane efflux pumps (P glycoprotein and multi drug resistance pumps), activation of anti-apoptotic cellular defense system mediated through BCL2 protein family and inactive or mutant *p*53 gene resulting in loss of apoptotic function [M. Saad, et al (2008)]. To overcome this resistance, higher doses / frequency of dosing of the toxic anticancer drugs is required to be administered, thus resulting in unwanted adverse side effects. Gene therapy is a novel and currently, most promising approach in treatment of genetic disorders including lung disorders and cancer [I. M. Verma & and N. Somia (1997)]. Currently, more than 65 % of gene therapy clinical trials have been aimed to cure cancer [M. L. Edelstein et al (2007)]. Recent developments in cancer gene therapy have shown chromosomal alterations and malfunction of cell growth controller genes as a major cause of cancer [R. Li et al (2003)]. **Mutations observed in *p*53, a tumor suppressor and apoptosis inducer gene in majority of human cancers, induction of apoptosis in cancer cells after *p*53 restoration and reported regulatory role of *p*53 in normal cell functioning, make *p*53 one of the premiere candidates in cancer gene therapy** [K. F. Pirollo et al (2000)]. Statistically, mutation in *p*53 has been associated in 15-50 % of breast cancer, 25-75 % of lung cancer, 25-70 % prostate and bladder cancer, 33-100 % of head and neck cancer and various lymphomas and leukemias [K. F. Pirollo et al (1997), H. E. Ruley (1996)]. Presence of mutant *p*53 has also been associated with an unfavorable prognosis for many human cancers including lung, colon and breast [H. E. Ruley (1996)]. In addition, *p*53 protein transcriptionally regulates genes involved in angiogenesis essential for solid tumor growth [V. Chiarugi et al (1998)]. *p*53 also plays a significant role in

diverse cellular pathways activated in response to DNA damage, such as DNA repair, regulation of the cell cycle and programmed cell death (apoptosis) [K. F. Pirollo et al (2000), D. Sidransky and M. Hollstein (1996)] which when malfunctioned results in tumorogenesis. Further, increased chemo and radiation therapy resistance of cancerous cells in absence of *p*53 or presence of mutant *p*53 also supports the regulatory role of active *p*53 in maintaining the normal cell function [L. Xu et al (2001)]. These abnormalities in *p*53 gene in a significant fraction of human cancers and its regulatory role for normal cell functioning make it one of the premiere candidates for cancer gene therapy.

In normal cells, wild type *p*53 induces expression of genes that are involved in various aspects of cellular growth regulation. Elevated levels of wt *p*53 in response to cellular stress situations such as DNA damage can lead to apoptosis or induce cell cycle arrest in G_1 or G_2 phase. In a normal cell *p*53 is inactivated by its negative regulator, mdm2. Upon DNA damage or other stress, various pathways leading to the dissociation of the *p*53 and mdm2 complex thus activates the *p*53 cascade. Once activated, *p*53 either induces a cell cycle arrest to allow repair and survival of the cell or induce apoptosis to discard the damaged cell, if the DNA damage proves to be irreparable. Restoration of normal *p*53 function in *p*53 mutant or *p*53 null cancer cells by delivering the gene by viral and nonviral methods has shown positive control of cellular proliferation by induction of apoptosis and regulation of cell cycle events [L. Xu et al (1999), Y. Zou et al (2000), S. G. Swisher et al (2003), D. Y. Logunov et al (2004)]. Furthermore, ***p*53 has also shown to restore appropriate responses and sensitize the resistant cells to DNA-damaging agents (e.g. chemotherapy and radiotherapy) by controlling the cell cycle, reducing the BCL-2 mediated cell resistance towards drug uptake and restoration of apoptotic pathway** [K. F. Pirollo et al (1997), L. Xu et al (2001)].

Delivery of therapeutic *p*53 gene to the lung for the treatment of lung cancer has been widely reported [K. F. Pirollo et al (2000)]. The various approaches as naked plasmid, viral delivery, cationic liposomal DNA delivery and polymeric complexation with DNA have all been well established [S. H. Choi et al (2008), K. G. Wiman (1999)]. Cancer treatment using non-viral gene delivery vectors as liposomes, nanoparticles, lipopolymers, etc. has been a promising approach because of their safety and non-immunogenic profile and their local and systemic targeting possibility by attachment of ligands as transferrin, folate, hyaluronic acid etc. to the vector [M. P. Deonarain (1998)]. However, local delivery of these formulations to the lungs via

pulmonary route still remains a challenge. Some researchers have tried aerosolization of these formulations by nebulization containing *p*53 *p*DNA for treatment of lung cancer [Y. Zou et al (2007)], however long term stability of these formulations is a vital concern. **Freeze dried formulations of the lipoplexes after formulating as dry powder inhaler (DPI) formulation may resolve these issues and provide an effective and stable delivery system for direct local delivery of the therapeutic *p*DNA.** It is expected that, the cancer can be managed by delivering the chemotherapeutic agents; however, permanent cure can not be achieved by chemotherapy alone. Gene delivery to remove or replace the mutated gene and insert working copy of the defective gene in defective cells can only be the way out to treat the diseases completely. Further, the combination of gene and chemotherapy has been observed in sensitizing the chemotherapy towards cancer remission and may reduce the dose required of the chemotherapeutic agent.

In the last few years, delivery of genes i.e. *p*DNA, siRNA and AS ODN using nanoparticulate and liposomal vectors encapsulating the chemotherapeutic agent as a therapeutic approach for **co-administration of drug and gene** has been successfully demonstrated [M. Saad et al (2008), Y. Wang et al (2006), R. I. Pakunlu et al (2004)]. Co-administration of drug and gene in the same vehicle not only can improve patient compliance due to the reduced number of injections, but can also achieve a synergistic therapeutic effect because both drug and gene can be delivered to the same cancer cells or tissues. These vectors have shown dual advantage of cytotoxic behavior of drug along with expression of gene for producing or blocking the desired protein for improving the cellular entry of chemotherapeutic agent and its anticancer activity though multiple mechanisms of action, thus helping to overcome the resistance.

Keeping in mind all the drawbacks of the chemotherapy and the potential of *p*53 gene delivery using varying reported lipoplexes, polyplexes and lipopolyplexes in lung cancer treatment, **the present research work was aimed to develop ligand targeted delivery system complexed with *p*53 *p*DNA for enhanced *p*53 expression in lung cancer cells and reducing cytotoxicity in other organs.** Further, **the work was also aimed towards development of multicomponent liposomal vectors for delivering anticancer agents as Etoposide and Docetaxel as cytotoxic agents along with *p*53 for sensitizing the cells towards chemotherapy during lung cancer treatment *in vitro*.** The study was performed to investigate the effect of *p53* delivery as

pretreatment and co-administration approach on sensitization and synergism towards chemotherapy.

1.2 Aims and Objectives of Work

The entire work was planned with following objectives:

- To incorporate the *p*53 (tumor suppressor) *p*DNA into cationic liposomes; optimization and characterization of developed lipoplexes.
- Attaching these lipoplexes with suitable cancer targeting ligands via covalent linkage and simple mixing to enhance cellular surface receptor mediated uptake and gene expression.
- To assess the performance of developed formulations *in vitro* on NSCLC cell lines as H 1299 (*p*53 null).
- Developing the optimized lipoplex formulations as dry powder inhaler formulations by freeze drying or formulations ready for nebulization after reconstitution and studying the *in-vitro* lung deposition pattern of lipoplexes using cascade impactor.

It was hypothesized that these formulations will enhance the cellular uptake, provide more efficient and direct delivery of the genes in lung cells & hence, will increase possibility of gene transfection and expression for better therapeutic response in patients.

- Development of Etoposide and Docetaxel loaded liposomes.
- Development of a multicomponent gene delivery system containing a) Blank cationic liposomes and *p*53 lipoplex b) Etoposide or Docetaxel encapsulated in above liposomes (same composition) and *p*53 complexed with above liposomes to form ETP-*p*53 or DTX-*p*53 lipoplex.
- Studying the effect of pretreatment and co-administration of *p*53 on cytotoxicity of liposomally encapsulated ETP and DTX in *p*53 null H 1299 and *p*53 (wt) A-549 lung adenocarcinoma cell lines.
- Development of DPI formulations of optimized lipoplexes and studying their *in vitro* lung deposition pattern.

These studied were based on the hypothesis that, *p*53 restoration in *p*53 deficient / mutated cancer cells would ameliorate the altered apoptotic pathway and also reduce the BCL-2 mediated antiapoptotic non pump resistance thus sensitizing the cells towards chemotherapeutic agent [Y. Wu et al (2001)]. The effect of pre-sensitization and co-administration on comparative cytotoxicity in two different cell lines with varying *p*53 character was determined to demonstrate effect of time of *p*53 delivery and added advantage offered by multicomponent gene delivery system. The studies were also aimed to develop DPI formulations of these lipoplexes for studying the lung deposition pattern for direct lung delivery and targeting, thus enhancing the therapeutic efficiency, reducing unwanted side effects and lowering the dose of the drug.

1.3 References

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