Introduction
Chapter 1: INTRODUCTION

Cancer, medically known as neoplasm, is one of the most fatal diseases affecting the worldwide population. It is evolved by a combination of genetic, epigenetic and pathogenic factors that endow the cancerous cells with selective advantage over the non-cancerous cells. There is a vast and divergent arena for the occurrence of this dreadful disease. It can develop in almost any organ/tissue of the body such as the lung, colon, stomach or skin. For the women worldwide, breast cancer is the most common cancer diagnosed with more than 1.3 million cases every year and has the highest death rate. Not only women, men are also affected by breast cancer (Ferlay et al., 2010). It is shocking but true that some 13,000 men are being diagnosed with breast cancer every year (Speirs, 2012). Similarly, oral cancer accounts as 6th most common cancer globally with annual estimated incidence rate of approximately 275,000. India, Sri Lanka and Pakistan report the highest incidence of disease thus making it the most common cancer among men in these countries and unlike other diseases, the number is increasing (Brocklehurst et al., 2010).

There are ten hallmarks of cancer cells that contribute to their survival against all odds. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism, evading immune destruction, genome instability and mutation and tumor promoting inflammation (Hanahan and Weinberg, 2011). There are number of pathways that are deregulated to impart these characteristics specifically to cancer cells. Targeting pathways or molecules which interfere with these one or more acquired capabilities of cancer could lead to the development of an effective cancer treatment. Among the repertoire of such pathways/molecules, the polyamines has gained special interest as it has been shown to play an indispensable role in key cellular processes such as the regulation of growth, differentiation and macromolecular functions. Polyamines (PAs) are small, ubiquitous,
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organic polycations. The natural PAs are putrescine (Put), spermidine (Spd) and spermine (Spm). Elevated levels of polyamines have been shown to be one of the major factors involved in carcinogenesis. The diamine Put, an obligatory precursor for the formation of higher polyamines, Spd and Spm, is synthesized from ornithine by the action of ornithine decarboxylase (ODC). These steps are catalyzed by aminopropyl transferases, namely Spd and Spm synthases, respectively, which add propyl amino groups to Put and Spd sequentially and such propyl amino groups are donated by the decarboxylated S-adenosylmethionine (dcSAM), which is turn synthesized from SAM by SAM decarboxylase (SAMDC). ODC and SAMDC, key enzymes in polyamines biosynthesis, are markedly overexpressed in many human cancers (Paz et al., 2011). The elevated polyamines result in increased malignant potential of cancer cells and evading immune response. In fact, a number of inhibitors like α-difluoromethylornithine (DFMO - a specific and irreversible inhibitor of ODC) have undergone clinical trials to control cancers through the selective inhibition of polyamine biosynthesis (Meyskens and Gerner, 1999). However, toxicity of DFMO in its therapeutic concentrations prevented it from commercialization. Thus, there is a need to look for more safe and robust alternatives to inhibit polyamine biosynthesis pathway to completely harness this significant target. RNAi is such a strategy which has emerged as a potential tool for gene silencing.

RNA interference (RNAi) is a process that demonstrates a sequence-specific degradation of RNA by a double-stranded RNA (dsRNA) with the intervention of RISC (RNA inducing silencing complex). RNAi has been well established for functional genomic studies to decipher the role of a particular gene in eukaryotic system (Vanhecke and Janitz, 2005). Along with loss-of-function studies, RNAi has also found application in the arena of therapeutics. Utilizing this platform, a vast number of diseases including cancer, dominant genetic disorders, viral infections, etc. have been approached with a perspective of finding a safe and specific cure (Fjose and Drivenes, 2006). Since its inception, it is widely used as a screening strategy for shortlisting cancer target. Using
this tool, a plethora of factors has been rated to have a role in tumor biogenesis. Along with this, various individual genes have been targeted using RNAi technique in different tumor cells *in vitro* and *in vivo*. These genes include oncogenes/anti-apoptotic molecules, telomerase, growth factor receptor genes, signaling molecules and some other genes (Gartel and Kandel, 2006). However, the utility of RNAi as a means of gene silencing depends on several factors. These include the amount of gene silencing, the duration for which the gene remains silenced, the degree of recovery of gene function, and the response of the silencing process on general cell functions (Lamberton and Christian, 2003). Therefore, we carried out a comparative study of suppression of ODC gene using small interfering nucleic acids (siNA) such as siRNA, LNA modified siRNA and siHybrids (RNA-DNA duplex) to control the growth of cancer cell lines and also examined the degree and duration up to which the RNAi effect persists in each case.

Despite the presence of enormous supporting preclinical data, daunting obstacles restricts its use in therapeutics. The main and foremost hurdle in the way of successful RNAi – based drug is the delivery. It is very important to deliver these RNAi drugs specifically to the target organs/tissues along with a long, consistent and active stay to bring about an effective treatment. Thus, there is a need to find a safer, effective and targeted delivery approach to curb this obstacle. Nanoparticles (NPs) have come a long way in intracellular drug delivery scenario with much success. The important characteristics of a good NP carrier polymer are excellent endocytosis, passive tumor-targeting, high encapsulation efficiency, and high stability. There has been a bulk of carrier polymers used, of which poly (D, L lactic-co-glycolic acid) (PLGA) is noteworthy in this regard (Makadia and Siegel, 2011). Being clinically validated biodegradable polyesters with the degradation products being lactic acid and glycolic acid, which are naturally occurring substances that further break down into water and carbon dioxide are apt for this purpose (Crotts and Park, 1998). For targeted delivery, the proteins overexpressed on cancer cell surface can be exploited (Attarwala, 2010). Among these, mucins, cell surface binding glycoproteins are used as tumor biomarkers (Haab *et al.*, 2010).
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2010). Aberrant glycoforms are associated with many epithelial cancer cells (breast, ovary, colon, pancreas lungs and prostate). Targeting these glycoforms is a powerful approach because they are expressed only on cancer cells and are distinct from those expressed on normal cells (Gendler, 2001). Aptamers (APTs) specific to these glycoforms have been used as targeting moieties in the present study.

The goal of the present research was to investigate the effect of silencing of polyamine biosynthesis genes in cell proliferation and apoptosis in cancer cells using RNAi and thus establishing polyamine pathway as a promising target to treat cancer through APT-targeted nanoparticle delivery of siRNAs. In designing the targeting strategy, four main steps were considered (i) the agent used to disrupt the target gene i.e. the variants of siNA to bring about most efficacious silencing of target genes, (ii) the target gene that will result in maximum inhibition of cell proliferation i.e. targeting key genes of polyamine biosynthesis, (iii) the mechanism of inhibition of cancer cell growth, and (iv) the delivery system that optimally delivers the agent to the target site.

To investigate the aforementioned hypothesis, following objectives were considered.

1. To design siNAs (siRNA, LNA modified siRNA and siHybrid) specific to polyamine biosynthesis genes, ornithine decarboxylase (ODC), S-adenosylmethionine decarboxylase (SAMDC) and Spd synthase (SPDSYN) and to evaluate the efficacy of each variant of siNA in inhibiting the growth of oral (KB) and breast (MCF7) cells in vitro in a dose- and time-dependent manner.

2. To investigate the effect of individual and combinatorial targeting of polyamine biosynthesis genes on tumor cell proliferation of oral (KB) and breast (MCF7 and MDA MB 231) cancer cell lines.

3. To study the changes in expression pattern of cell cycle - and apoptosis - related genes in siRNA- treated MCF 7 cells.
4. Preparation and characterization of PEG-PLGA nanoparticles containing siRNA and evaluating their efficacy in inhibiting MCF 7 cancer cell growth \textit{in vitro.}

5. Conjugation of MUC 1 aptamer to siRNA loaded PEG-PLGA nanoparticles and performing their biophysical characterization and evaluating their therapeutic efficacy in specifically inhibiting the growth of MCF 7 cancer cells \textit{in vitro.}