A Research Proposal

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Aptamers Mediated siRNA Delivery to Supress *TWIST* Gene Expression in Lungs Cancer

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1. Abstract

Lungs cancer treatment is a worldwide concern of current era. Unfortunately, current treatment such as chemotherapy, radiations and antiviral drugs etc. administered to lungs cancer patients are limited by patient's will and stage. Hence this awakens the need of new therapeutics to target gene or molecular pathways that are directly involved in cancer prognosis. Aptamers have emerged as powerful tool for efficient targeting of biological molecules. The aim of this proposed study is to synthesize such aptamer based therapeutics complex with siRNA to target *TWIST* gene expression. *TWIST* gene encodes transcription factor that are actively involved in embryogenesis and turns down in adult cells. Overexpression of this gene leads to tumorigenesis and reactivates pathways that result in metastasis of cancer. In this study, aptamers mediated siRNA delivery will be subjected to *TWIST* gene to evaluate the effect of supressed *TWIST* expression in tumour cells lines. This study play significant role in future medical advancements for target specific lungs cancer treatment with minimal side effects.

2. Introduction

Delivery of siRNA and other small molecules to specific cell or tissue type is a main obstacle in their development [1]. A wide variety of small molecules, lipids, peptides and proteins have been reported as efficient delivery vehicles and vectors for nucleic acids. For example, the non-specific uptake of cholesterol labeled siRNAs has been demonstrated o be effective for delivery to cells grown in culture as well to liver, heart, kidney and lung tissue in mice [2]. Several peptides are also reported for the cell-specific delivery of siRNAs [3]. However, tissue-specific delivery of siRNAs has been attained by fusions with protamine to target to tumour cells via antibodies [4]. Aptamers are reported as nucleic acid oligomers an alternative to antibodies that are used as potential targeting agents for the delivery of siRNA cargoes [5]. They possesses high affinity and specificity for their targets. Aptamers are usually synthesized chemically and become an attractive reagents for therapeutics and other applications. Aptamers targets surface antigens as well as whole cells are previously reported in cancer cells [6, 7]. However, recently it is reported that nucleic acids are selected to bind to prostate-specific membrane antigen and can be internalized [8]. Hence proposing a new dimension for advance applications of aptamers in cancer therapeutics and diagnostics.

As Cancer is caused due to abnormal production of key proteins from various genetic mutation by carcinogens such as ultraviolet radiations, chemicals, hazardous metals ultraviolet radiations, chemicals and hazardous metals[9]. Activation of oncogenes is one of the leading cause cancer that may disturbs normal cell processes such as cell growth and apoptosis leading to cancer [10]. Apart from various oncogenes, *TWIST* gene overexpression in adult cells play significant role in cancer prognosis. TWIST are transcription factor that are actively involved in embryonic development and turns down their expression after birth and are bound to precursor cells in adult tissues [11]. Invariably, TWIST proteins are imperceptible in epithelial cells and actively involved in lungs cancer prognosis. Conversely, during tumorigenesis, TWIST1 and TWIST2 gene overexpression is actively involved in multiple carcinomas such as breast, bladder, sacrcoma, kidney, colon, gastric, liver, pancreas and especially in lungs cancer [12]. Thus, cancer is associated with expression leading to poor prognosis, high grade, invasive and metastatic lesions [11].The mechanisms causing reactivation of both TWIST genes in tumors have been deeply identified. It is reported that TWIST gene reactivation leads to the deregulation of pathways that usually regulate their expression during development, highlighting a striking relationship between embryogenesis and tumorigenesis [13].

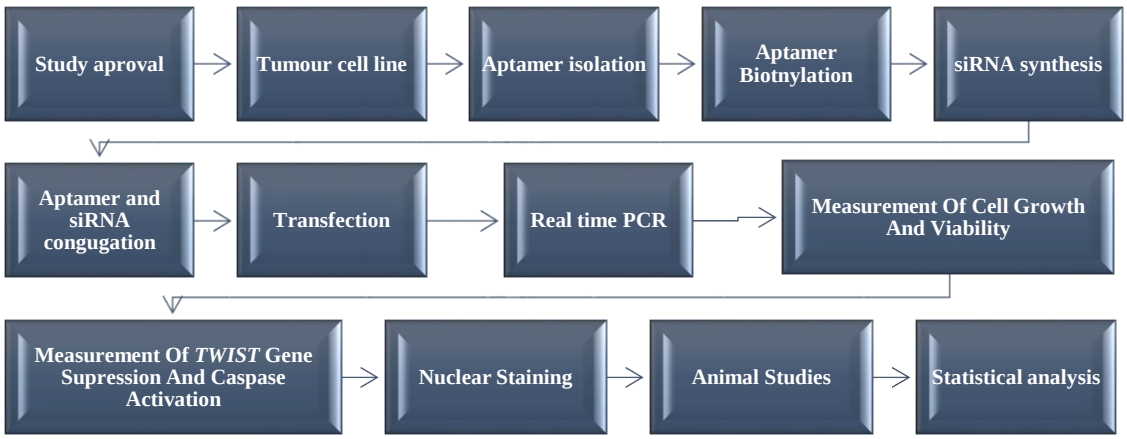
3. Objective of study

As supported by literature review, *TWIST* gene overexpression is actively involved in tumorigenesis in adult cells and various current treatment such as chemotherapy and antiviral drugs administered to cancer patients are limited to some stages and patient's will. The aim of this study is to design aptamers based therapeutics such as aptamers-siRNA complexes to target *TWIST* gene directly to supress the overexpression of TWIST protein to inhibit abnormal cell proliferation.

4. Methodology

1. The lung adenocarcinoma cell line A549, will be obtained from American Type Culture Collection and will be maintained in 37°C in a humidified atmosphere containing 5% CO2.
2. Aptamers will be synthesized and isolated according to protocol as described earlier [14] and will be biotnylated [15].
3. All *in-silico* designed siRNA with non-biotinylated antisense strands and the biotinytlated sense strands of will be purchased from IDT (Corriville, IA).
4. Aptamers-siRNA complexes will be prepared according to standard protocol as mention earlier [16].
5. Aptamers-siRNA complexes will be transfected to lung adenocarcinoma cell line A549 to supress the expression of *TWIST* gene [17].
6. Total RNA will be extracted from cells *and TWIST* gene expression will be analyzed by real time PCR [18].
7. Growth inhibition of A549 cells will be analysed by colorimetric MTT cell viability/proliferation assay according to standard protocol [19].
8. *TWIST* gene suppression activity in cells will be determined through colorimetric test system [20].
9. Detached cells harvested from the culture medium will be mounted for nuclear staining according to standard protocol [21] and will be observed by using a Leica confocal laser scan microscope equipped with SCANware software (Leitz).
10. For animal studies, mice as a model organism will be injected with aptamers treated A549 cells to evaluate evaluate tumour growth in vivo [22].
11. Statistical analysis of data will be performed with one way ANOVA (SPSS11.0, USA) to evaluate TWIST gene suppression and apoptosis in tumour cells. Differences with $P<0.05$ will be considered statistically significant.

5. Work Plan



6. Expected outcomes

1. Aptamers will effectively deliver siRNA to target site
2. siRNA will effectively bind to TWIST mRNA to supress over expression in tumorigenesis
3. siRNA mediated transformation in assistance with aptamers will inhibits metastasises of cancerous cells.
4. Safe and secure transformation is expected with no harmful side effects.

7. Limitations

1. Reduced chemical information about unmodified nucleic acids
2. Particle size control
3. Immune response

8. Future expects

Aptamer technology has emerged as potential source of lead compounds of therapeutic interventions. Site directed targeting by aptamers will not only supress *TWIST* gene expression but also control the aberrant proliferation of cancerous cells posing negligible side effects as compared to antiviral and chemotherapy. In future, little

modification in these molecules will make this technology efficient and will be helpful in the production of aptamers based pharmaceutics for lungs cancer treatment.

9. References

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