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Original Article

Study of miRNA based gene regulation involved in Squamous Lung Carcinoma by assistance of Argonaute Protein

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ABSTRACT: miRNAs are responsible for gene silencing and has functions in various diseases like cancer. miRNAs are responsible for cancer and at the same time act as tumor suppressors. The work mentioned here is based on study on differentially expressed miRNAs for squamous cell carcinoma target genes in the metabolic pathways. The study focused on combination of miRNA and their target genes for squamous cell carcinoma as well as how they are involved in metabolic pathways. miRNAs were categorized into normal, up-regulated and down-regulated genes. Prediction of target genes for miRNAs followed by developing a relationship between them was done. miRNA target genes involved in metabolic pathways which were studied for squamous lung carcinoma. miRNA target genes were docked with argonaute protein. Docking was done in order to understand how miRNA tend to bind to target gene. Results added information to the study of how miRNAs targets genes in various metabolic pathways. The expression level of miRNA is different in different conditions which can be modified as a result of defects in the miRNA biogenesis pathway.

INTRODUCTION

miRNA (microRNA)

A micro RNA functions for post-transcriptional regulation and gene silencing. It has functions in plants, viruses and animals. It is of 22 nucleotides long, [1, 2] functions to target mRNAs causing degradation or translational repression. They have functions [3] in various human diseases including cardiovascular, metabolic, immunity and liver related diseases. They bind to the target site present within the 3' UTR of targeted mRNA leading to condition of gene silencing. It is helpful in studies related to gene regulatory networks. miRNA binds to target sites of mRNA and an individual miRNA targets as many as 100 different mRNAs.

Every mRNA contains multiple binding sites for different miRNAs resulting in complex regulatory networks. The function of microRNAs [4] have been shown to be involved in a wide range of biological processes such as cell cycle control, apoptosis and several developmental and physiological processes including stem cell differentiation, hematopoiesis, hypoxia, cardiac and skeletal muscle development, neurogenesis, insulin secretion, cholesterol metabolism, aging, immune responses and viral replication.

In addition, highly tissue-specific expression and distinct temporal expression patterns during embryogenesis suggest that microRNAs play a key role in the differentiation and maintenance of tissue identity.

miRNAs and human cancer

Since the year 2000, more than 2000 studies have been conducted regarding miRNAs [5, 6] with their correlations with various diseases such as cancers, cardiovascular disease, schizophrenia, renal function disorders, psoriasis, primary muscular disorders, fragile-X mental retardation syndrome, diabetes, chronic hepatitis, AIDS, and obesity. In 2000, the first investigation was done indicating the relation between miRNA and human cancers. The study found frequent deletion /down regulation of miR-15 and miR-16-1 in the most commonly deleted region, [7] 13q14, in CLL. MiR-15 and miR-16-1 function as tumor suppressors, and their expression inversely correlates with anti-apoptotic BCL2 expression, their inhibition enhances apoptosis in leukemic cells. Many other findings indicated relationship of miRNA and cancer with the detection of over 50% of miRNA genes in regions of loss of heterozygosity, amplification, fragile sites, viral integration sites, and other cancer-associated genomic regions.

Evidences related to deregulation of miRNA expression in various forms of cancers including colorectal cancer and in leukemia, lung, and breast cancers are there. miRNAs can function both as oncogene- or tumor-suppressors. Over expression [8] of oncogenic miRNAs and under-expression of tumor-suppressive miRNAs may help in tumorigenesis affecting the pathways promoting acquisition of the hallmarks of cancer which includes sustaining proliferative signaling, resisting cell death, evading growth suppressors, inducing angiogenesis, enabling replicative immortality, and activating invasion and metastasis.

Pathway Analysis

Pathways provide information about genes and their functions including cellular, metabolic processes with genetic networks. Pathway analysis includes study of genes from the gene ontology consortium. KEGG and GenMAPP are both useful to study pathways. The Cancer Cell Map is a unique database that focuses on cancer related signaling pathways. Ten cancer related pathways each containing around 100 to 400 interactions are present here. For specific miRNAs their mRNA genes are obtained from the TargetScan. These mRNA genes are thus used to construct these pathways.

Each pathway contains information about proteins, their localization, physical interactions, molecular interactions, biochemical reactions, and catalysis, transport and post translational protein modifications. As miRNAs are related to specific mRNAs (which could be found in miRNA mRNA modules where these miRNAs having similar functions like mRNAs) we can find the relation of these miRNAs in regulatory pathway. Cytoscape is network visualization and analysis software to study gene expression data through Cancer call maps. Like cancer signaling pathways, there are immune signaling pathways which again relates the miRNA related mRNAs. They are available at NetPath and contains around ten pathways. Thus any type of pathways that are obtained from the mRNA genes that are related to miRNAs draws a gene regulatory network showing the importance of miRNAs in regulation.

Relating miRNA mRNA modules to gene regulatory network is done to study the interconnectivity between them on basis of their function and biological processes.

Squamous cell lung cancer

Squamous cell lung cancer [9, 10] or squamous cell carcinoma of the lung is one type of non-small cell lung cancer (NSCLC). It is also called epidermoid carcinoma. This type of lung cancer begins in the squamous cells thin, flat cells that look like fish scales when seen under a microscope. They line the inside of the airways in the lungs. Squamous cell lung tumors usually occur in the central part of the lung or in one of the main airways (left or right bronchus). The tumor's location is responsible for symptoms such as cough, trouble breathing, chest pain, and blood in the sputum. If the tumor grows to a large size, a chest X-ray or computed tomography (CT or CAT) scan may detect a cavity in the lung.

A cavity is a gas- or fluid-filled space within a tumor mass or nodule and is a classic sign of squamous cell lung cancer which can spread to multiple sites, including the brain, spine and other bones, adrenal glands, and liver. The signs and identification symptoms of this disease are generally considered to be smoking by far the leading risk factor for lung cancer. Cigarette smoke contains more than 6,000 components, many of which lead to DNA damage. Other causes include radon, non-industrial air-pollution, industrial exposure to some chemicals, and asbestos. In general, DNA damage appears to be the primary underlying cause of cancer. Though most DNA damages are repairable, leftover un-repaired DNA damages from cigarette smoke are the likely cause of NSCLC. DNA replication past an un-repaired damage can give rise to a mutation because of inaccurate translation synthesis. In addition, during repair of DNA double-strand breaks, or repair of other DNA damages, incompletely cleared sites of repair can lead to epigenetic gene silencing. The diagnostic approaches can be done by many different tests which are used to diagnose lung cancer and determine whether it has spread to other parts of the body. Some can also help to decide which treatments might work best. The steps and tests used in diagnosing squamous cell lung cancer include: Imaging tests, Laboratory tests, Biopsies.

Argonaute Protein

The Argonaute protein family plays a central role in RNA silencing processes, as essential component of the RNA induced silencing complex (RISC). RISC is responsible for the gene silencing phenomenon known as RNA interference (RNAi). Argonaute proteins bind different classes of small non-coding RNAs including miRNA, siRNA and piRNAs. Argonaute proteins are guided by small RNAs to their specific target through sequence complementarity (base-pairing) which then leads to mRNAs cleavage & translation inhibition.

Objective

The main objective of this work is to study microRNAs involved in squamous lung carcinoma by assistance of Argonaute protein. The work is done in two modules.

The first module includes developing relations between miRNAs, squamous cell carcinoma target genes and metabolic pathways. The second module is to dock 3D structure of miRNA-mRNA duplex and Argonaute Protein.

MATERIALS AND METHODS

Data Collected

The data was collected from Broad Institute database. Broad Institute of MIT and Harvard was launched in 2004 to improve human health by using genomics to advance our understanding of the biology and treatment of human disease, and to help lay the groundwork for a new generation of therapies. 20 Diseased and 17 Normal miRNA expression datasets of patients were extracted from Broad Institute.

Data Pre-Processing

Preprocessing of data was done to convert the diseased data into normalized data. The process of data mining is used for knowledge discovery. The process includes discovering interesting knowledge such as patterns, associations, changes, anomalies and significant structures from large amounts of data stored in databases, data warehouses or other information repositories. In general, a knowledge discovery process consists of an iterative sequence of the following steps:

1. Data Cleaning, which removes noisy, enormous, missing or irrelevant data.
2. Data Integration, where multiple heterogeneous data sources may be integrated into one.
3. Data selection, where data relevant to the analysis task are retrieved from the databases.
4. Data transformation, where data are transformed or consolidated into forms appropriate for mining by performing summary or aggregation operations.

Gene set enrichment approach (also functional enrichment analysis) is a method to identify classes of genes or proteins that are over-represented in a large set of genes or proteins and may have an association with disease phenotypes. The method uses statistical approaches to identify significantly enriched or depleted groups of genes. Microarray and proteomics results often identify thousands of genes which are used for the analysis.

Data Mining

Data Mining is an essential process where intelligence methods are applied to extract data patterns. Out of the six common classes of data mining tasks Clustering was done. Clustering, is the task of discovering groups and structures in the data that are in some way or another "similar", without using known structures in the data. The miRNA datasets were categorized into normal, up regulated and down regulated based on threshold expression values.

Prediction of miRNA and targets genes

The prediction was done based on threshold expression values and correlation. The relationship developed helps to understand how miRNA is related to target genes [11, 12]. miRNAs can be related to target genes in many ways: one to one, one to many and many to one [13].

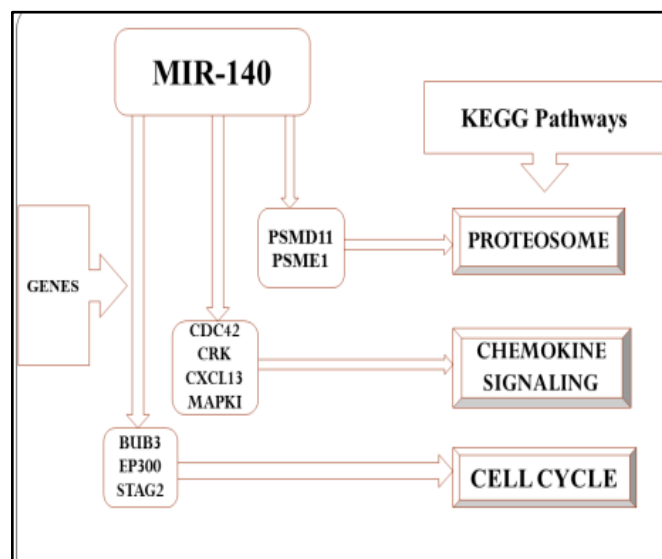
Prediction of structural model in miRNA- mRNA complex and AGO protein

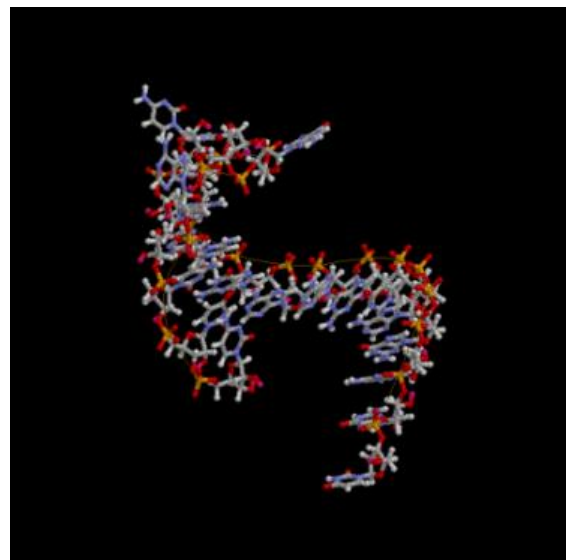
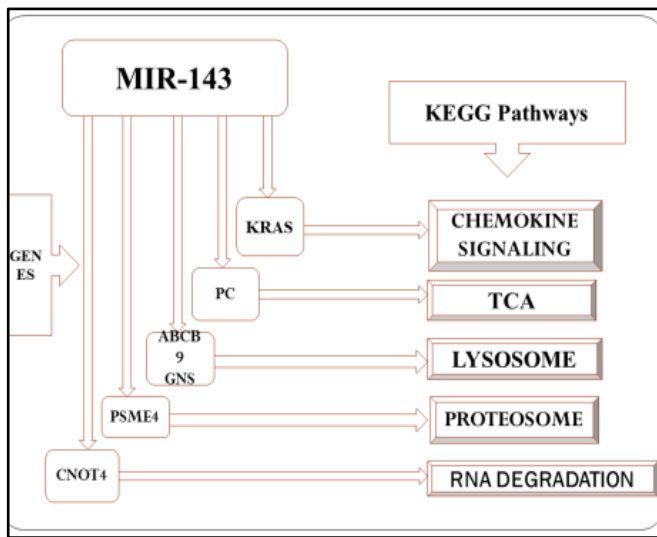
After predicting the miRNA and target genes the folding affinity between the miRNA and target genes complex was verified through RNA fold which gives the secondary structure of RNA showing dot bracketed results of the duplex as well as the MFE score. RNA composer was used to produce the three-dimensional structure. Docking was performed between AGO protein and miRNA-mRNA duplexes. In the process of miRNAs mediated gene regulation, the Argonaute (AGO) [14, 15] class of proteins play a significant role in the hybrid formation between miRNA-mRNA.

RESULTS

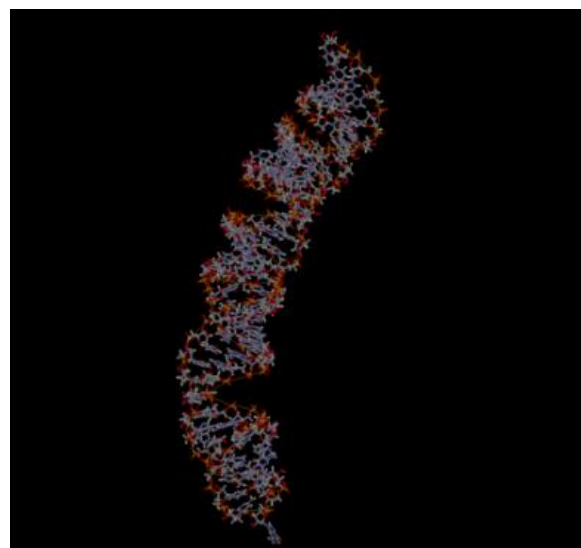
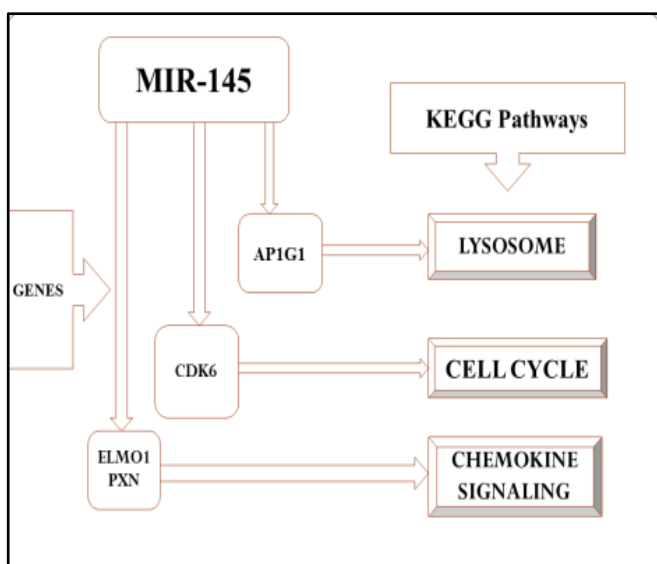
Table 1: miRNA - down regulated, normal, up regulated

S. No.	Down regulated	Normal	Up regulated
1	hsa-let-7g	hsa-miR-152	hsa-let-7f-2
2	hsa-let-7g	hsa-miR-125b	hsa-let-7g
3	hsa-let-7g	hsa-miR-16-1	hsa-let-7g
4	hsa-let-7g	hsa-miR-485-3p	hsa-let-7g
5	hsa-let-7g	hsa-miR-509	hsa-let-7g
6	hsa-let-136	hsa-miR-765	hsa-let-7g
7	hsa-let-140	hsa-miR-128	hsa-miR-136
8	hsa-let-200a	hsa-miR-148a	hsa-miR-140
9	hsa-miR-635	hsa-miR-155	hsa-miR-143
10	hsa-miR-1	hsa-let-7a	hsa-miR-200a
11	hsa-miR-10a	hsa-let-7a	hsa-miR-203
12	hsa-miR-10a	hsa-let-7a	hsa-miR-145





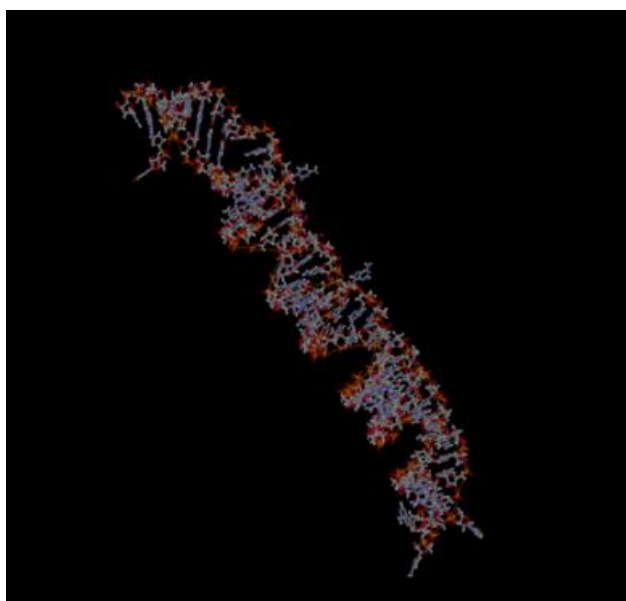
b) miRNA 143



c) miRNA 145

Figure 1: miRNA – target gene – KEGG pathway

Figure 2: Results of RNA composer



a) miRNA 140

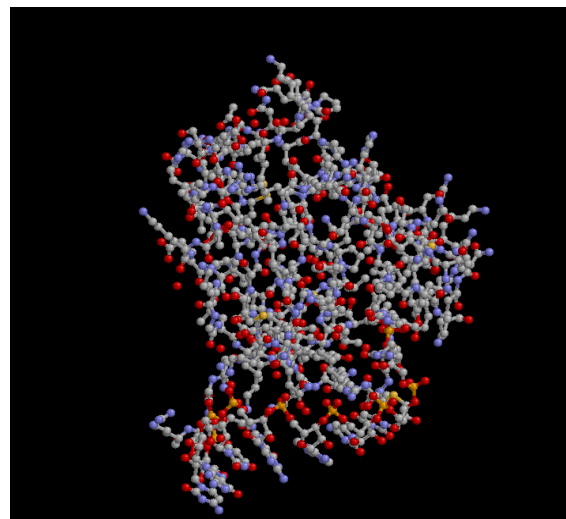


Figure 3: Result of Docking

DISCUSSION

The expression level of miRNA can also be modified as a result of defects in the miRNA biogenesis pathway. Deregulation of enzymes and cofactors involved in these pathways [16, 17] can affect the levels of mature miRNAs and have important biological ramifications. Hence, the study of interaction mechanism between miRNAs and their target genes is essential. In the process of miRNAs mediated genes regulation, the Argonaute (AGO) class of protein plays a significant role in the hybrid formation between miRNA and mRNA. The recent study emphasizes on how the involvement of Argonaute and their relationship with the miRNAs can be used in building the PPI (protein-protein interaction) Networks through depiction of the metabolic pathways that have been hindered by the miRNA& its target genes.

Several studies have demonstrated a role of miRNAs in other transcription factor networks in cancer, like that of the activated Ras oncogene. Ras activation leads to repression of the miR-143/145 cluster in KRAS mutant pancreatic cancers through Ras-responsive element-binding protein (RREB1) binding to the miR-143/145 promoter (Kent *et al.*, 2010). Furthermore, KRAS and RREB1 are themselves targets of miR-143/miR-145, representing a feed-forward loop that further amplifies Ras signaling. The expression level of miRNA can also be modified as a result of defects in the miRNA biogenesis pathway. Deregulation of enzymes and cofactors involved in these pathways can affect the levels of mature miRNAs and have important biological ramifications. Therefore, at the end we can conclude that our own study has shown response towards RAS oncogene and several other genes, further process will be done to study their significance involved in specific pathways and the site involved.

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