Computer Aided miRNA Target Prediction in Four Frequently Amplified and Mutated Genes in Lung Cancer

By

Maimoona Ali

20-MSBI/FBAS/F08

Supervisor:

Dr Sumbul Khalid

Assistant Professor, DES

Department of Environmental Sciences

Faculty of Basic and Applied Sciences

International Islamic University

Islamabad

(2010)

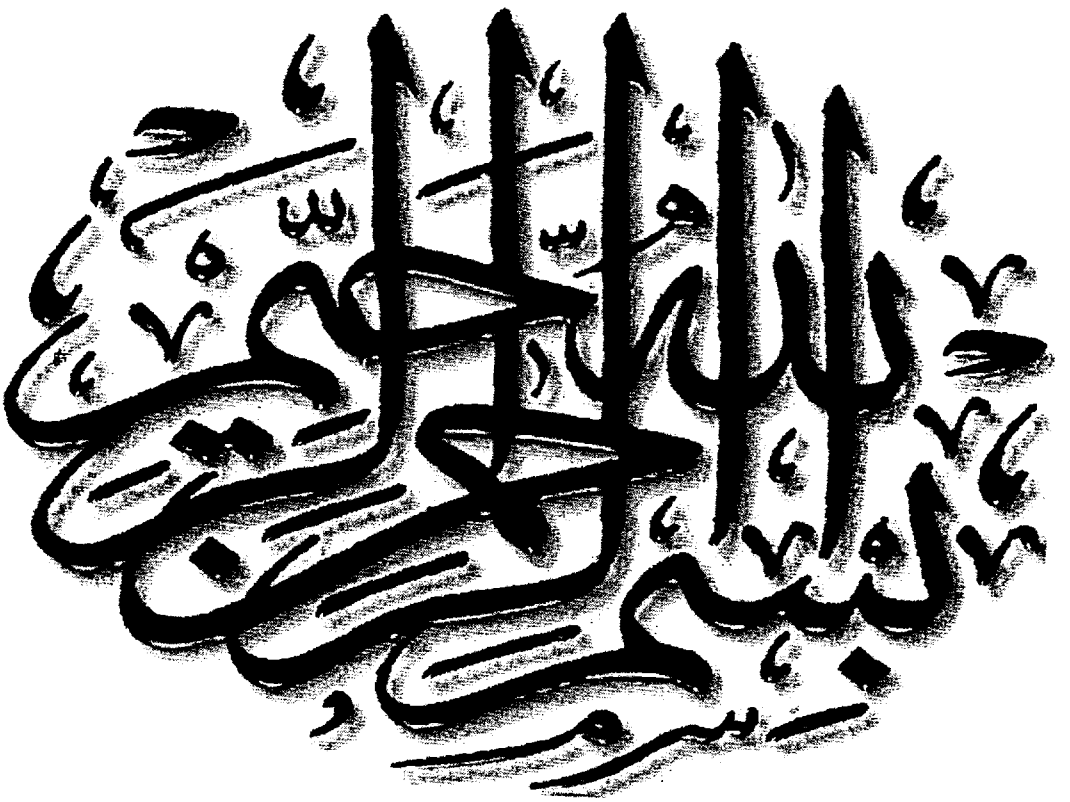
Computer Aided miRNA Target Prediction in Four Frequently Amplified and Mutated Genes in Lung Cancer

Researcher

Maimoona Ali

12-FBAS/MSBI/F08

Department of Environmental Sciences
Faculty of Basic & Applied Sciences,
International Islamic University Islamabad
(2010)



In the name of Allah Most Gracious and Most Beneficial

Department of Bioinformatics and Biotechnology

International Islamic University Islamabad

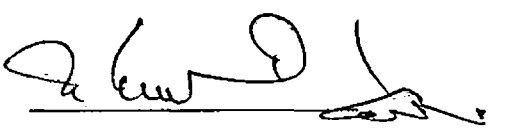
Dated: 15-12-2011

FINAL APPROVAL

It is to certify that we have read the thesis submitted by Ms. Maimoona Ali and it is our judgment that this research is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the award of M.S Degree in Bioinformatics

COMMITTEE

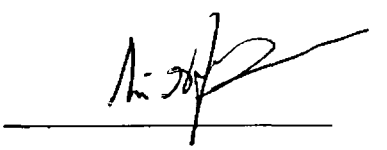
External Examiner
Dr. Ghazala Kaukab Raja
Associate Professor
Department of Biochemistry
PMAS Arid Agriculture University, Islamabad.



Internal Examiner
Dr. Shaheen Shahzad
Chairperson
Department of Environmental Sciences,
International Islamic University, Islamabad

Supervisor
Dr. Sumbul Khalid
Assistant professor
Department of Environmental Sciences,
International Islamic University, Islamabad

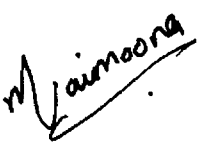
Dean, FBAS
Dr. Irfan Khan
International Islamic University, Islamabad



A Thesis Submitted To Department of Environmental Sciences,
International Islamic University, Islamabad as a Partial
Fulfillment of Requirement for the Award Of The
Degree of M.S in Bioinformatics

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort; all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.



Maimoona Ali.

Dedicated to those who believe in Al-Quran and the endless healing powers of Al-Quran for the spiritual and physical sufferings of mankind as Allah (SWT) says in Al-Quran: "We sent down (reveled) of the Qur'an that which is a healing and mercy to those who believes"

TABLE of CONTENTS

ACKNOWLEDGMENTS

The foremost and heartiest thanks to Allah (Subhana-o-Tahalla); the creator who created me a human being and the Sovereign and the All Knowing, who showered such a knowledge upon me; the Guider who guided me well and broaden my understanding to differentiate between right and wrong; the Source of Peace and Provider due to Whom I was able to perform my task even in the time of severe depression and hopelessness.

I pay my paramount and doubtless gratitude for my parents, who always sacrificed their own needs for mine and tried their level best to provide me full convenience at the cost of their own rest since my birth. Countless words of thanks to my sisters and the only brother Ahmed Saad they provided a very friendly and peaceful environment that ensured the accomplishment of my goal.

I extend my heartiest thanks to my supervisor Dr. Sumbul Khalid, Assistant Professor, International Islamic University Islamabad who guided me with patience and kindness and helped me all the way along.

I shall be failing my duty if I do not put forth heartiest gratitude to my whole class who cooperated with me and showed complete friendly attitude during this study period in International Islamic University Islamabad particularly Attia Mehmood for answering my endless queries and guiding me at each and every step.

I pray to Allah (Subhana-o-Tahallah) that may He bestow me with true success in all fields in both worlds and shower His blessed knowledge upon me for the betterment of all Muslims and whole Mankind. Ameen

Maimoona Ali

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

A: Adenine

AAT: Anterior Axillary Thoracotomy

ALT: Anterior Limited Thoracotomy

BRAF: Serine/threonine-protein kinase B-Raf

BAT3 and MSH5: HLA-B associated transcript 3 and mutS homolog 5

C: Cytosine

C-MET: MNNG HOS Transforming gene

CHRNA3 and CHRNA5: Cholinergic Receptor, Neuronal Nicotinic, Alpha 3 and Cholinergic Receptor, Neuronal Nicotinic, Alpha 5

CLPTM1L: Cleft Lip and Palate Transmembrane Protein 1-Like Protein

DLEC1: Deleted in Lung and Esophageal Cancer 1

EGFR: Epidermal Growth Factor Receptor

EML4-ALK: Echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase

EPHA3: Eph-like tyrosine kinase

ERBB: Erythroblastic Leukemia Viral Oncogene Homolog

ERCC6: Excision repair cross-complementing rodent repair deficiency, complementation group 6.

G: Guanine

GSTM1: Glutathione S-transferase M1

HMOX1: Heme Oxygenase (Decycling) 1

ITGA9: Iintegrin, Alpha 9

KDR: Kinase insert Domain Receptor (a type III receptor tyrosine kinase)

KLC1: Kinesin Light Chain 1

KRAS: v-Ki-ras2 Kirsten rat sarcoma viral Oncogene Homolog

LINUX: Linus' UNIX

LKB1: Serine/threonine kinase 11

miRNAs: Micro RNAs

mRNA: Messenger RNA

MALAT1: Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)

MAP3K8: Mitogen-Activated Protein Kinase 8

MET: Met Proto-Oncogene (Hepatocyte Growth Factor Receptor)

MYCL1: v-myc Myelocytomatosis viral Oncogene homolog 1, lung carcinoma derived (avian)

MPO: Myeloper Oxidase

NKX2-1: Homeobox Protein Nkx-2.1

NTRK: SLIT and NTRK-like family, member 6

NSCLC: Non Small Cell Lung Cancer

PARK2: Parkinson Protein 2, E3 Ubiquitin Protein Ligase (Parkin)

PIK3CA: Phosphoinositide-3-kinase, Catalytic Alpha Polypeptide

PLT: Posterolateral Thoracotomy

RNA: Ribose Nucleic Acid

RNase III: Ribonuclease III

SCLC: Small Cell Lung Cancer

SOX2: SRY (sex determining region Y)-box 2, also known as SOX2

STK11: Serine/Threonine kinase 11

T: Thymine

TERT: Telomerase Reverse Transcriptase

Tp53: Tumor protein 53

TSG11: Tumor suppressor gene on chromosome 11

U: Uracil

ABSTRACT

The silent epidemic of lung cancer that has been claiming innumerable precious human lives across the globe persistently for the last many decades needs to be addressed seriously and unconventionally. The complexity of disease, involvement of multigenic factors and complicated signaling and molecular pathways, lack of affectivity of screening techniques and limitations of available treatment options and consistent high death tolls and low survival rates are harsh realities pertaining to lung cancer. The advent of miRNAs and their acknowledged role in post transcriptional gene expression and their requirement of partial complementarity to bind to their targets make them highly probable to be used as therapeutic agents to control gene expression in cancer. In this research work targets for human miRNAs have been identified in four frequently mutated genes i.e. EGFR, ERBB, Kras and Tp53 which lead to the development of lung cancer by using computer aided tools that predict targets on the basis of sequence complementarity and minimum free energy of the hybridized complex. Out of 721 human miRNAs six were identified to have targets in the normal and mutated forms of these genes. The target miRNA strand can be designed by analyzing the conserved sequences of these six identified miRNAs, namely: miR-939, miR-93, miR-765, miR-1273, miR-887 and miR-1285 and may be applied as a therapeutic agent to address the four commonly mutated genes involved in lung cancer.

INTRODUCTION

Tobacco smoking is the only form of drug abuse that is socially acceptable among all classes of the society worldwide. It is rather accepted as a norm in society and for long we have watched adventurous and dramatic advertisements for various tobacco companies being openly advertised followed by a public service message that consumption of tobacco smoke is hazardous to human health. Throughout the late 19th century the tobacco industry targeted young people around the world through heavy advertisement campaigns on T.V, radio, billboards, etc. There was a rapid increase in consumption of tobacco smoke worldwide followed by distinct rise in incidence and mortality rates of lung cancer (Youlden *et al.*, 2008). A direct relationship existed between tobacco smoking trends and lung cancer prevalence but the exact nature of relation was far from clear. For nearly five decades tobacco smoking was considered as

the sole cause of lung cancer and it has not been very long that other environmental and genetic factors associated with lung cancer have been revealed. It has also been found that most people who develop cancer today have either stopped smoking years earlier or have never smoked. Furthermore not all people who consume tobacco smoke develop lung cancer (Michael *et al.*, 2002).

Like most forms of cancer, lung cancer normally occurs when two general classes of genes i.e. oncogenes and tumor suppressor genes become defected and are not able to perform their normal functions. These normal genes become cancerous as a result of exposure to environmental carcinogens or as a result of inherited DNA mutations (Peter *et al.*, 2000).

The inheritance of defected genes is a primary disposition of developing cancer and the risk increases many folds when an individual with such defected genes is exposed to environmental carcinogens. In case of lung cancer, the risk of developing cancer increases enormously when a person with defected genes is exposed to first and second hand tobacco smoke, asbestos or radon gas and has a history of lung diseases like asthma, emphysema and chronic bronchitis (Biesalski *et al.*, 1998).

Like the world, lung cancer is a major contributor of cancer related deaths in Pakistan as well. According to IARC (International Agency for Research on Cancer) fact sheet for the year 2008 the age standardized incident rate was 12.3 and mortality rate was 11.5 for men and for women 2.7 and 2.5 accordingly (http://globocan.iarc.fr). These high incidence and mortality rates for lung cancer show a grieve situation for a country like Pakistan whose more than 17.2% of the total population is living below the poverty line (https://www.cia.gov).

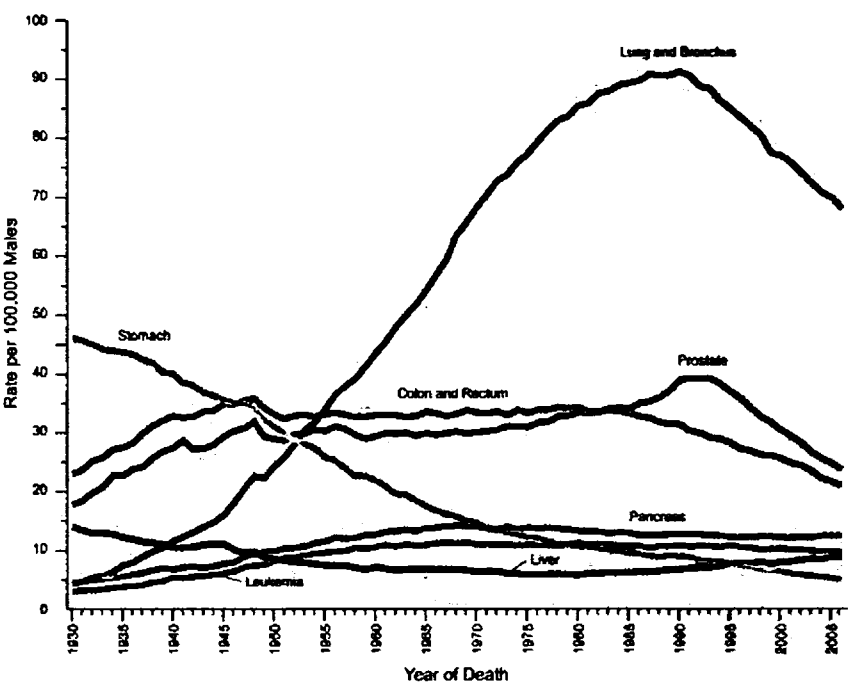


Figure1.1: High rates for lung cancer compared with other common types of cancer.

The graph shows a sharp difference between death toll by lung cancer and other types of common human cancer (http://i52.tinypic.com/r9euci.jpg).These high death rates for lung cancer are attributed to tobacco consumption (Ezzati *et al.*, 2003). The rates of tobacco consumptions in Pakistan are relatively higher than other under developed countries of the same rank. About 40% of men and 8% of women in Pakistan consume tobacco smoke in different forms (Nighat *et al.*, 2007). Although tobacco smoke does not directly or solely causes lung cancer but this tobacco consuming population is at higher stake of developing lung cancer throughout their life span. Facts and figures developed so far show that nearly 90% of all lung cancer cases are attributed to tobacco smoking. (Biesalski *et al.*, 1998).

The incident rates for lung cancer across the world are devastating and show a persistent increase despite sanctions on tobacco cigarettes advertisements and promotion of awareness programs around the world. It remains to be the most common of all cancers and there were nearly 1.61 million new cases reported, that make approximately 12.7% of all the new cancer cases for the year 2008 (http://globocan.iarc.fr/). Another horrifying fact about lung cancer is that there is a minute difference between the incidence and mortality rates as is clearly shown by figure 1.2 which also shows high fatality rates for lung cancer as compared to other cancer types.

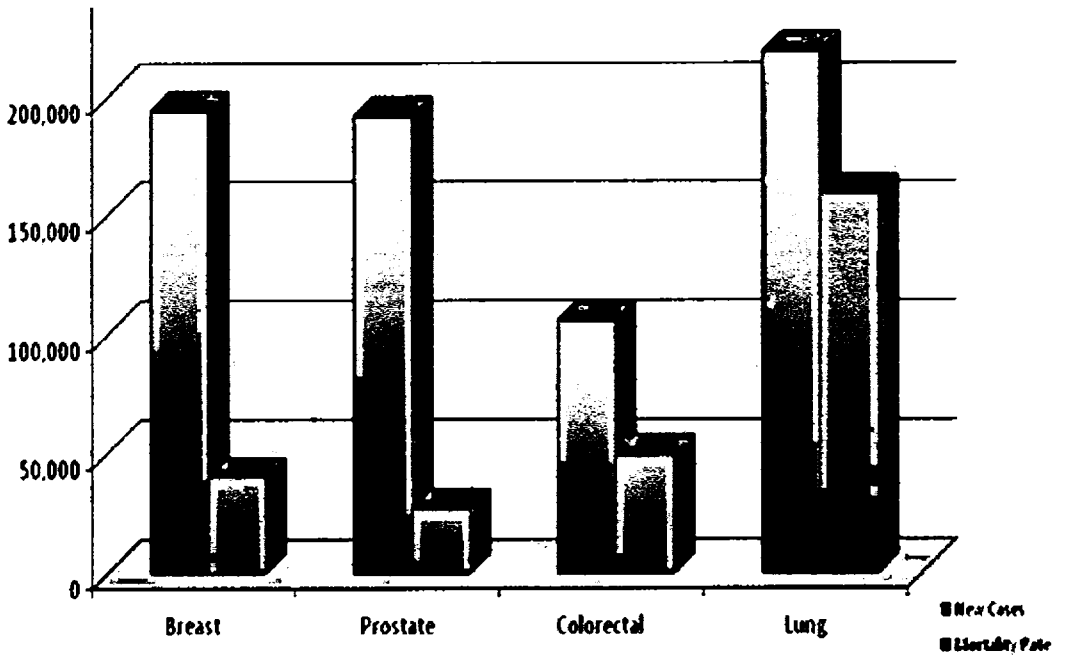


Figure1.2: Comparison of Incidence and Mortality Rates of Lung and other Cancers.

These findings make us look into the treatments available for lung cancer. Like other types of cancer, treatment depends on type and stage of lung cancer (Schiller *et al.*, 2007). The traditional treatments available for lung cancer include surgery, chemotherapy, radiation therapy and target therapy. Like most of the other cancers lung cancer is usually treated with a combination of therapies (Sandler *et al.*, 2006).

Of all lung cancer cases, 88% are of non small cell type (Govindan *et al.*, 2006), and 75% of these cases are diagnosed at advance stages where they cannot be operated or addressed by other treatment options (Weiss *et al.*, 2006). Chemotherapy is pain-relieving and moderately effective for lung cancer (Weiss *et al.*, 2008). Surgery is a treatment of

choice but may not always result in cure of lung cancer. Although chemotherapy has shown adequate improvement for treatment of metastatic non small cell lung cancer there is still a dire need for better treatment options (Sandler *et al.*, 2006).

Understanding the genetic factor that attribute to lung cancer provides new horizons for the development of more effective treatment. There are a variety of genes that are directly or indirectly involved in the development of lung cancer (Herbst et al., 2008). Some of these genes play a vital role in the development of lung cancer for example *BRAF,ERBB2, EGFR, Kras,Tp53 and STK11.* (Ji *et al.*, 2007).

RNA-interference is a technique that is highly rated for its outstanding results for controlling gene expression in laboratory and provides new dimensions to the therapeutic application of miRNA. (Matthias *et al.*, 2005). miRNA are regulatory RNAs found in multicellular eukaryotes, including humans where they are involved in cancer (Johnson *et al.*, 2005). miRNAs either repress the translation of mRNA or enhance the instability of mRNA. The advent of miRNAs provides a new ray of hope for highly incurable lung cancer.

The prime focus of this study was:

- To identify all genes that have been reported to be involved in lung cancer through comprehensive literature review.
- To identify key genes and their mutated forms that are critical for prognosis and development of lung cancer through literature review.

- To identify targets for all known human miRNAs in these genes using computer aided tools and algorithms.
- Based on these identified targets designing a miRNA based therapy that will target the mutated genes and will silence them thus helping in supression and control of lung cancer.
- Purpose is successful designing and implementation of a miRNA based therapy that can target more than one mutated genes.

LITERATURE REVIEW

2.1 Cancer

Cancer, a leading cause of death, occurs when fundamental processes in building blocks of body i.e. cells go unchecked. Body loses control over cell growth and apoptosis (Croce, 2008). Cells grow uncontrolled, multiply to form tumors, break surrounding barriers to enter blood or lymph stream then invade other tissues and organs via metastasis. This whole phenomenon leads to cancer that is a common occurrence but hard to cure. In 2007, cancer claimed the lives of about 7.6 million people in the world (Seffrin *et al* ., 2009) and the death toll persists to rise in the subsequent years (Ahmedin *et al.*, 2008). There are more than hundred different types of cancer and they are usually named after the type of cell they initially affect. There are multiple causes of cancer like exposure to chemical and environmental carcinogens, errors in DNA replication or inherited mutations (Anand *et al.*, 2008). All these factors can cause abnormalities in genetic material, transforming normal cells to cancerous forms.

The end of the first decade of this century shows little change in cancer statistics around the word. Lung cancer stands persistently on its prime position as the most deadly cancer. Its low survival rates have not climbed and high incident and death rates worldwide continues to be more or less the same.

2.1.1 Lung Cancer

Lung cancer contributes heavily to worldwide cancer death toll for both of the genders (Weiss *et al.*, 2008). In 2010 lung cancer claimed lives of 1.38 million people around the world. Each year more women become victims of lung cancer than breast cancer and lose their lives (Minami *et al.*, 2000). Nearly 55% of the cases occur in the developing world . The death ratio to occurence is that 0.86 which is clearly a very high fatility rate and there is little or no variability in survival rates for both developing and underdeveloped countries (http://globocan.iarc.fr) as is clearly shown in figure 2.1.that lung cancer causes highest mortality rate.

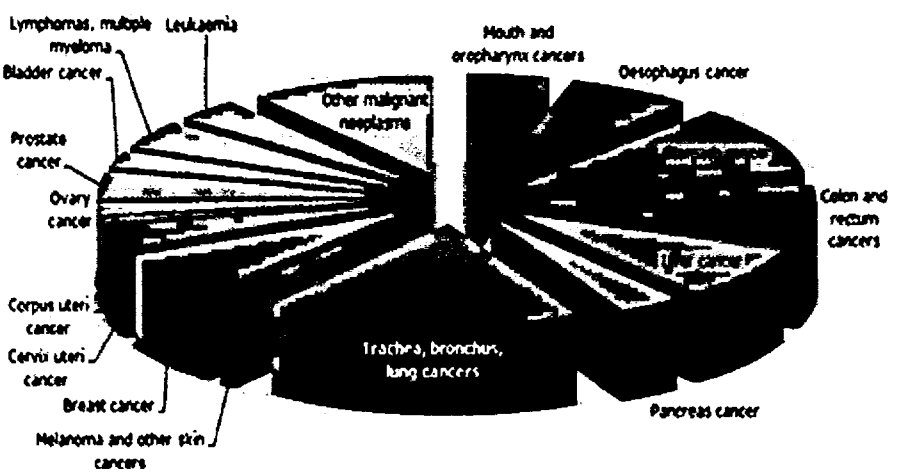


Figure 2.1: Contribution of Lung Cancer to Worldwide mortality rates for 2010

2.1.2 Clinical Features Of Lung Cancer

Multiple analysis have been conducted to clearly identify the symptoms that are unique to lung cancer and help medical personnel in its diagnosis. Symptoms that are distinct characteristic of lung cancer include haemoptysis i.e. coughing of blood, cachexia i.e. loss of weight , dyspnoea i.e. shortness of breath, thoracic pain, fatigue, loss of appetite and cough (Hamilton *et al.*, 2005). The most common and severe symptoms are pain , dyspnoea and anorexia. There is no differences in symptoms between males and females (Krech *et al.*, 1992). Lung cancer that has metasatized to the bones may produce unbearable pain at the sites where bones are involved. Psychological symptoms like depression and mood swings are also frequently observed (Hopwood *et al.*, 2000).

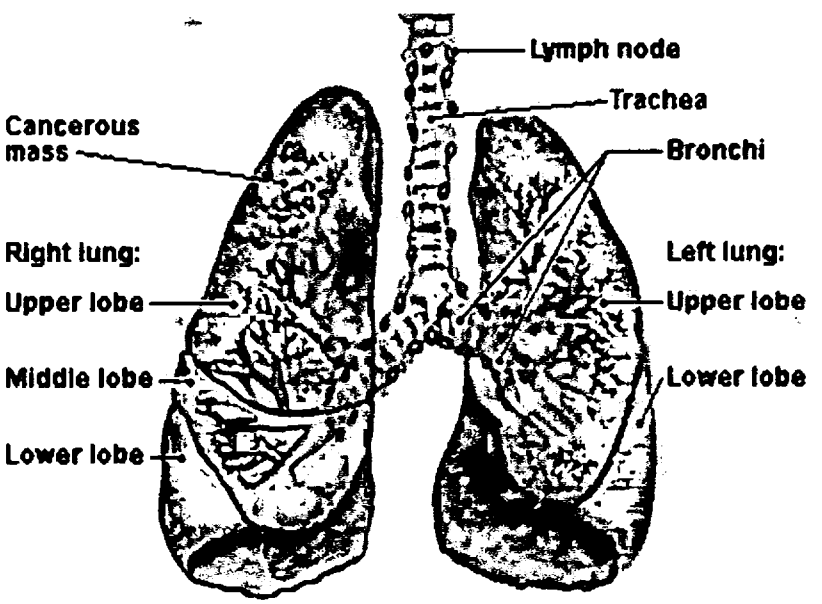


Figure 2.2: General appearance/ morphology of a cancerous lung

2.1.3 Types of Lung Cancer

Broadly lung cancer is divided into two classes, nonsmall cell lung cancer (NSCLC) and small cell lung cancer (SCLC). These account for 85% and 15% of all lung cancers, respectively (Birrer *et al.*, 1988) and are classified on the bases of size of tumor cells when viewed under a microscope.

2.1.3.1 Non-Small Cell Lung Cancer

Nearly 88% of all the lung cancer cases are of NSCLC subtype (Weiss *et al.*, 2006). NSCLC is further divided into three subtypes "squamous cell lung carcinoma, adenocarcinoma, and large cell lung carcinoma". The non-small-cell lung carcinomas (NSCLC) are grouped together because their diagnosis and treatment are quite related (Roth *et al.*, 1994). Each type of non-small cell lung cancer affects different kinds of cancer cells. The cancer cells of each type grow and spread in different ways. The types of non-small cell lung cancer are named for the kinds of cells found in the cancer and how the cells look under a microscope (Philippe *et al.*, 2000).

One fourth of total lung cancers is Squamous cell lung carcinoma (Travis et al., 2000). Tumor usually develops near centre of bronchi and usually contains a hollow cavity. As compared to other types of lung cancer growth of Squamous cell lung carcinoma is slow (Vaporciyan *et al.*, 2004).

Adenocarcinoma contributes to 40% of NSCLC (Travis *et al.*, 2002). It is mostly found in peripheral lung tissues and is usually associated with smoking, however its occurrence is not infrequent in people who have never smoked. (Subramanian *et al.*, 2007).

Third type of NSCLC is large cell lung cancer and is relatively rare as compared to other types of NSCLC. Tumors are normally large when they are diagnosed. Extensive bleeding and tissue damage are characteristics of large cell lung cancer. Unlike other two types, tumors are undifferentiated, grow more quickly and metastasize to other parts of body (Pedersen *et al.*, 1994).

2.1.3.2 Small Cell Lung Cancer

Occurrence of small cell lung cancer is less common as compared to NSCLC and accounts for nearly 15% of total lung cancer cases, but it is strongly associated with smoking (Barbone *et al.*, 1997). Tumors are very hostile and spread rapidly to other parts of the body. Tumors are mostly found in primary part of bronchi and quickly grow to become large in size (Collins *et al.*, 2007). The small cell lung cancer have vesicles containing neuroendocrine hormones (Rosti *et al.*, 2006). Lung cancer is very diversified and single case may have more than one types of tumors.

2.1.4 Enviromental Factors

Tobacco smoking is the major contributor of lung cancer cases worldwide (Biesalski *et al.*, 1998). Figure 2.3 depicts 30 year lagtime between smoking and development of lung cancer. Cigarette smoke is known to contain more than 60

carcinogens. Cigarette smoking not only amplifies the risk of lung cancer development but also put other people at risk who move in the close proximity and inhale the tobacco smoke via passive smoking (Field *et al.*, 2000). In this way cigarette smoke accounts for lung cancer in non smokers as well (Schick *et al.*, 2005).

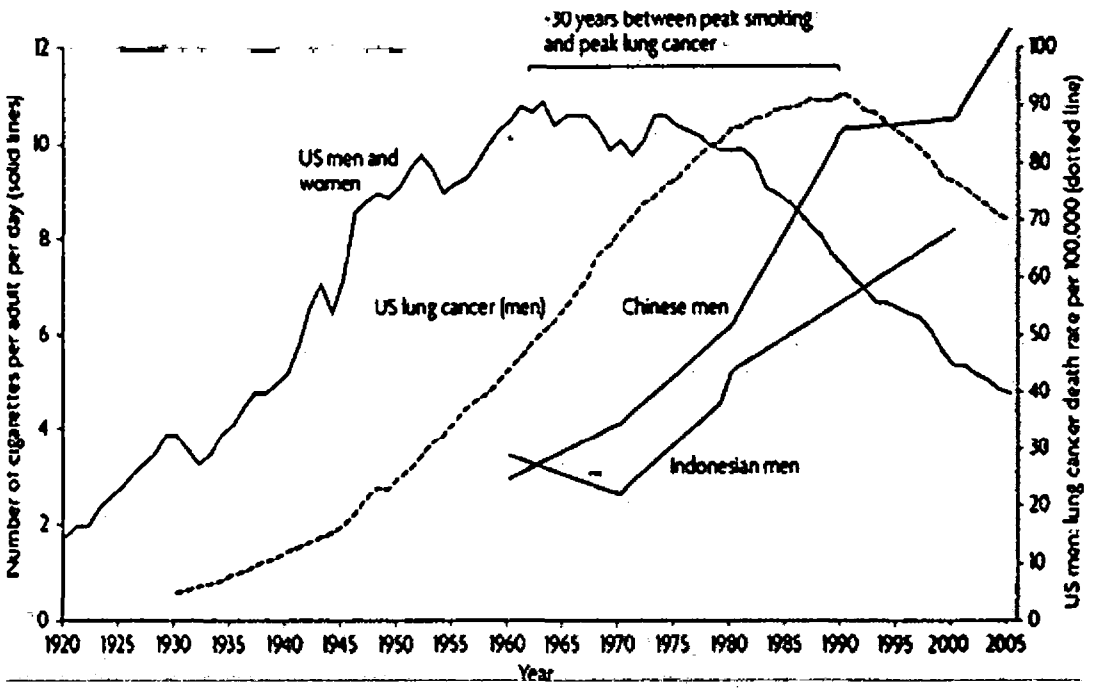


Figure 2.3: 30 Years Lag Time between Peak Smoking and Peak Lung Cancer from 1920-2005.

Besides smoking another environmental factor strongly associated with lung cancer is exposure to radon gas (Catelinois *et al.*, 2006). Meta-analysis suggests a significantly increased risk of lung cancer in people exposed to radon gas in their homes (Pavia *et al.*, 2003). Research indicate substantial danger from residential radon, predominantly for smokers and latest ex-smokers, and specify that it is accountable for about 2% of all deaths from cancer in Europe (Darby *et al.*, 2004).

Asbestos, another important factor is a ubiquitous, naturally occurring fiber that has been linked to the development of malignant and fibrotic diseases of the lung and pleura which may lead to the development of lung cancer (Christopher *et al.*, 2002). 2-3% of lung cancer cases and deaths are attributed to asbestos (Darnton *et al.*, 2006). Asbestos exposure and silica exposure are each related with inflammation of the lung and hence may contribute to the development of lung carcinoma (Karin *et al.*, 2007).

2.1.5 Genetics of Lung Cancer

There are many genes with known or potential relationship with human lung cancer. Most commonly associated is *EGFR*. It is a member of the Erbb family and is mostly expressed in many cases of NSCLC, and its expression leads to the poor diagnosis of lung cancer (Lin *et al.*, 2010). Recently *EML4-ALK* fusion gene has been identified to be playing crutial role in the development of NSCLC (Wong *et al.*, 2009).

P53 is a tumor supressor gene and its mutations are identified in many types of cancer. The risk of developing smoking induced lung cancer increases 1.7 folds with the presence of polymorphic *p53* gene that has proline instead of arginine at codon 72 (Kawajiri *et al.*, 1993). An amplified risk of a variety of histologic types of lung cancer was observed in people who carried p53 mutations. The risk was 3.16 folds higher in smokers than non smokers (Hwang *et al.*, 2003). *Kras* mutations in codon 12 particularly conversion of glycine 12 to cystine is also observed in many cases of lung cancer (Ahrendt *et al.*, 2001).

Mutated *BRAF* gene particularly a serine replaced by threonine at codon 338 is a common occurrence in many cases of lung cancer (Naoki *et al.*, 2002). *STK11* and *PIK3CA* mutations are also related with human lung cancer (Samuels *et al.*, 2004). *NKX2-1* gene that encodes a transcription factor, acts as a proto oncogene and its expression is amplified in noteworthy cases of lung cancer (Weir *et al.*, 2007). Some of many other genes that are often mutated or show amplified expression in lung cancer are *c-MET,GSTM1,SOX2, HMOX1, LKB1* (Herbst *et al.*, 2008).

A complex pathway leading to the development of NSCLC shown in figure 2.4 below clearly shows the multigenic factors involved in the mutagenesis that results in carcinoma of lung.

2.1.6 Treatment For Lung Cancer

Like other cancers there are multiple treatments available for lung cancer such as surgery, chemotherapy, radiotherapy and target therapy. The oncologists have to make most suitable choice after analyzing the stage of disease and types of tissues affected for every individual patient. Surgical resection of the tumor is mostly opted for cancer that is restricted inside the lung and there is general consensus that patients with NSCLC have best chance of cure with surgery and chances increases sufficiently with early diagnosis of disease (Fountain *et al.*, 1998).

The two standered methods used for lung cancer surgery are Thoractomy and median sternotomy . Thoractomy is performed via chest wall where as median sternotomy is performed by cutting the breast bone. Other approaches comprise of anterior limited thoractomy (ALT) performed via front of chest through a small

cut, anterioraxillary thoracotomy (AAT) performed on the front of chest near the underarm and posterolateral thoracotomy (PLT) performed on the back or side area of the chest (www.oncologychannel.com: 15-05-2010).

The effectiveness of surgery for NSCLC is inadequate if the disease is diagnosed at later stages. The median period of survival increases from 8 months to 26 months if patients with NSCLC are treated with chemotherapy along with surgery (Rosell *et al.*, 1994). Chemotherapy is modestly effective and is used along with radiotherapy for inoperable but curative NSCLC patients (Arriagada *et al.*, 2002).

Latest therapeutic advances use gefitinib and erlotinib for NSCLC patients which act as tyrosine kinase inhibitors and these belong to epidermal growth factor receptor family (Weiss *et al.*, 2008). So far use of these small target molecules have little significance to improve the survival rates of lung cancer (Bencardino *et al.*, 2007). Of all lung cancer subtypes small cell lung cancer is different because of its rapid growth and metastases. It also shows response to both radiotherapy and chemotherapy. Chemotherapy and radiotherapy are used in combination to patients who have limited SCLC (Perry *et al.*, 1989). If left untreated SCLC grows very rapidly, generally two to four months from the time of diagnosis, resulting in the shortest survival of any pulmonary neoplasm (Hinson *et al.*, 1993). Surgery is not a good option for SCLC patients, though some researchers have suggested that surgery adjunct to chemotherapy may improve local tumor control (Hinson *et al.*, 1993).

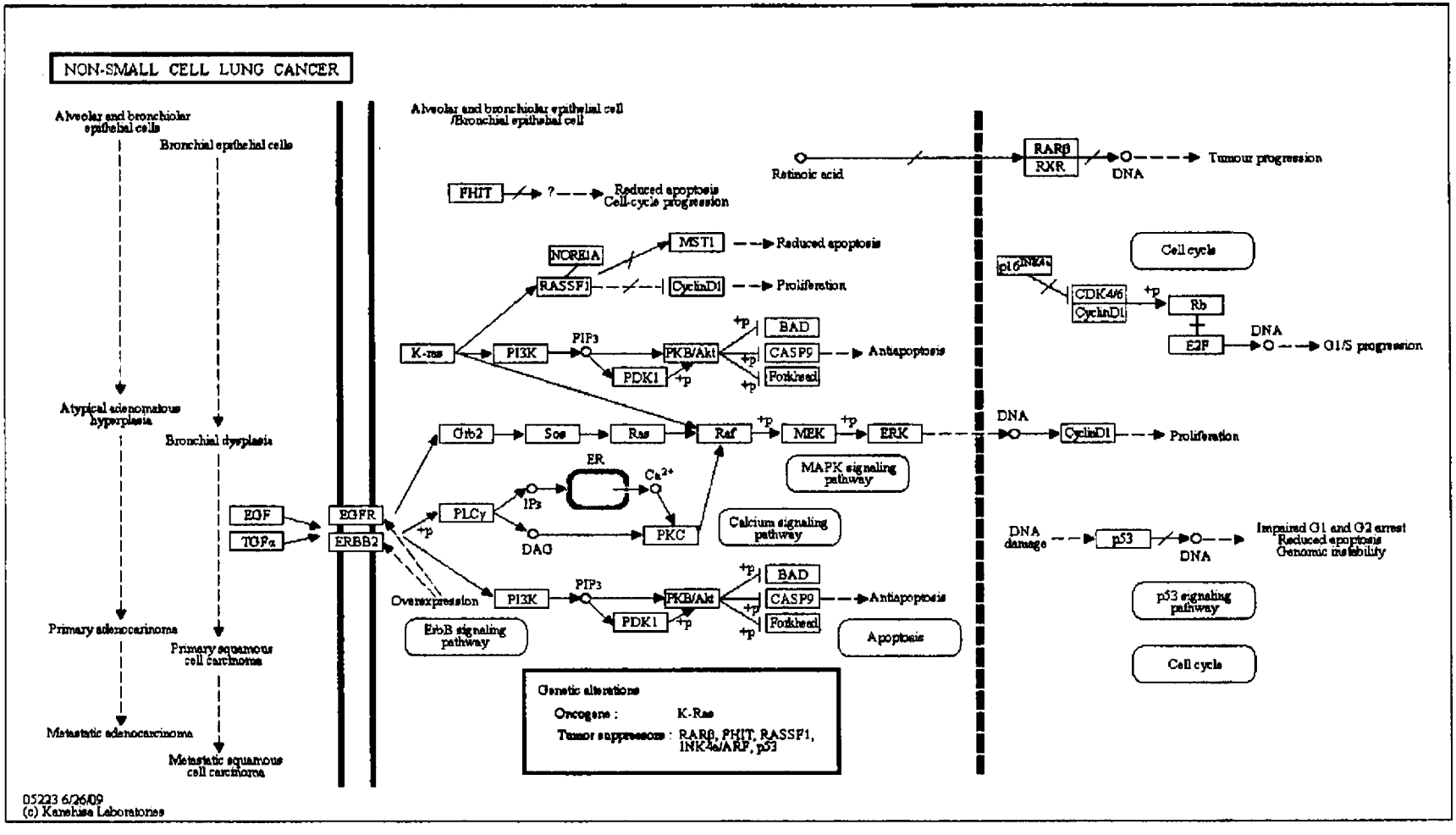


Figure 2.4: A complex pathway that leads to the development of non small cell lung cancer.

2.1.7 Stages of Lung Cancer

On the basis of number system lung cancer can be divided into four main groups

- Stage I– the cancer is small and only in one area of the lung (localised).
- Stage II and III – the cancer is larger and may have grown into the surrounding tissues and there may be cancer cells in the lymph nodes (locally advanced).
- Stage IIIa– cancer has not affected the lymph nodes yet where as in IIIb lymph nodes are also affected.
- Stage IV – the cancer has spread to another part of the body (secondary or metastatic cancer). (Mountain, 1997)

2.1.8 Complexity of Lung Cancer

Lung cancer continues to be the most challenging disease for the medical professionals because of its high occurrence and low survival rates. Majority of NSCLC cases are diagnosed at advance inoperable stages (Weiss *et al.*, 2008). Chemotherapy and radiotherapy are not very effective options for NSCLC because of the involvement of multiple and complex genetic pathways and very late diagnosis (Eddy *et al.*, 1989). Despite its sensitivity to chemotherapy and radiotherapy, SCLC shows poor prognosis and many people die of the disease (Hinson *et al.*, 1993).

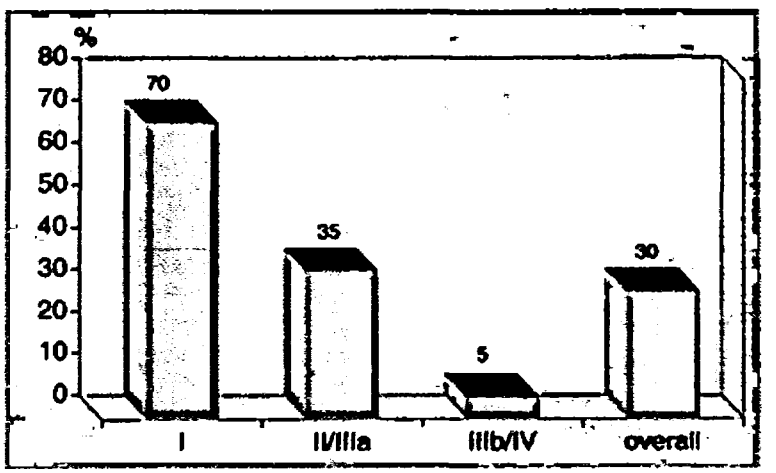


Figure 2.5: Five year survival rates for different classes of lung cancer.

The average survival rate of people that are not treated and have advanced non small cell lung cancer is 6 months. Even the treatment shows little or no improvement. Figure 2.5 shows five year survival rate for different classes of lung cancer patients which is less than 1% (Travis *et al.*, 2002). These persistant low survival rates for lung cancer urges the researchers to investigate new dimensions for early diagnosis and effective therapies against lung cancer. It is therefore, necessary to develop a better and comprehensive understanding of the molecular basis of lung cancer and also the development of additional effective therapeutic agents (Hoffman *et al.*, 1984). Even though much improvement has been made like incidence rates have been stabilized, mortality rates have been reduced and survival rates have been improved, but lung cancer still accounts for more deaths than any other cancer type each year (Wingo *et al.*, 1995). Further development can be achieved by sustaining

new developments and applying already known techniques to control cancer on all classes of the general population (Jemal *et al.*, 2008).

2.2.1 Micro RNA

MicroRNAs (miRNAs) are classified as a unique class of RNA as they do not code protein and are nearly 22nt in length (Yoon *et al.*, 2010) They were first identified by Ambros and his colleagues in 1993 while they were studying the mode of functioning of heterochronic gene *lin 14* in *Caenorhabditis elegans* (Harris *et al.*, 2007) . They are involved in important biological functions such as cell maturity, cell propagation, differentiation, and cell death (Caldas *et al.*, 2007). Nearly 700 miRNAs that regutulate protein coding genes in humans have been cloned (Griffiths *et al.*, 2008). Of all human genes 3% encode miRNAs and they in return regulate nearly 30% of human protein encoding genes (Filipowicz *et al.*, 2008).

2.2.2 Locations of miRNAs

miRNAs are located at various locations in genome as shown in figure 2.6. They may be located either in introns of genes that code proteins or they may be found in introns and exons of noncoding RNAs (Zamore *et al.*, 2005) .

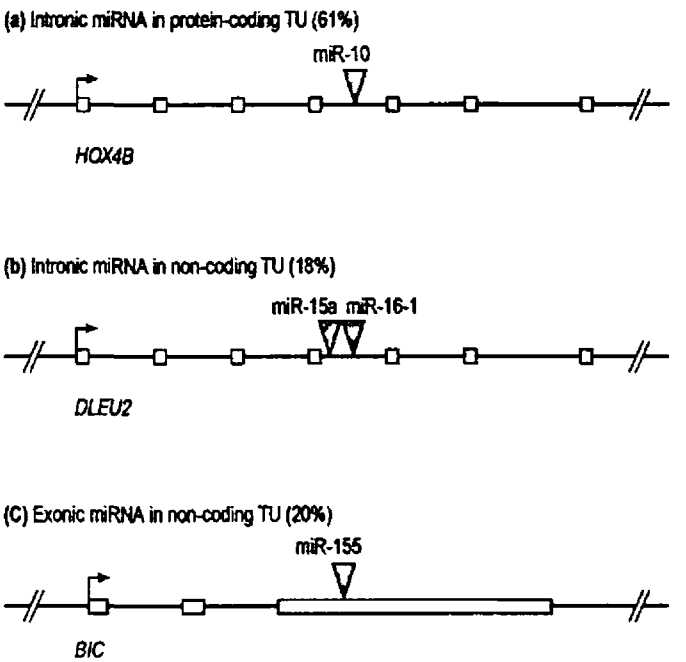


Figure 2.6: Different locations of miRNAs in genome.

2.2.3 Processing of miRNAs

As illustrated in figure 2.7 RNA polymerase II transcribes miRNA into primary miRNAs (pri-miRNAs) inside the nucleus (Provost *et al.*, 2010) Pri-miRNAs are long primary transcripts that may have length of thousand nucleotides (Bartel, 2004). Inside the nucleus, Drosha, an RNase III, cleaves the two strands of the pri-miRNA (Serge *et al.*, 2009). This results in the formation of a stem loop which is 70 to 100 neucleotides in length known as the precursor miRNA (pre-miRNA)(Carmell *et al.*, 2004).

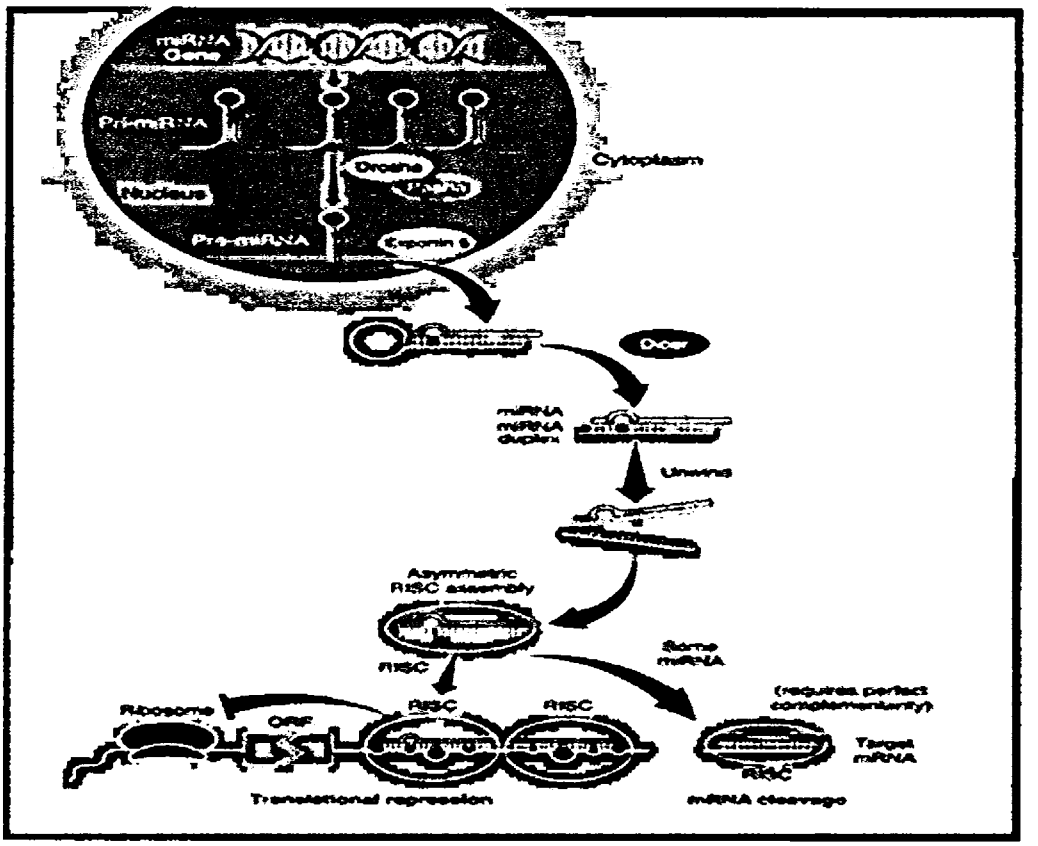


Figure 2.7: Multiple steps involved in the maturation of miRNA inside the nucleus and cytoplasm.

Cleavage by Drosha is important as it determines the structure of final miRNA product. Exportin 5 exports the pre-miRNA to cytoplasm from the nucleus (Kanellopoulou *et al.*, 2005). Inside the cytoplasm a second RNase III called Dicer cuts the pre-miRNA to generate nearly 22-nucleotide long RNA duplex of mature miRNA and its complement (Garcia *et al.*, 2005). Just one strand of the duplex enters the protein complex where as the other strand is destoyed in the cytoplasm (Murchison *et al.*, 2005).

2.2.4 Functions of miRNAs

The mRNA transcript that is being targetted by miRNA dictates the biology of function for that particular miRNA (Srivastava *et al.,* 2007). miRNAs bind to the complementary sequence of their mRNAs targets and this binding of miRNAs to their target mRNAs results either in repression of translation of target mRNA or induction of degradation of mRNA (Xiuying *et al.,* 2009). The mature miRNA combines with proteins and forms a complex known as the RNA-induced silencing complex. This miRNA is now active and pairs up with its target mRNA via sequence complementarity (Caldas et al., 2007).

The two modes by which miRNA regulate gene expression in mammals i.e. suppression of translation and degradation of mRNA, require less complementarity (Soifer *et al.,* 2007). The base pairing between miRNAs and their mRNA targets in case of humans entertain some exemptions i.e. they do not require perfect complementarity (Xiuying *et al.,* 2009) The six nucleotides 2-8 in the 5′ end of miRNA binds in the 3'UTR of mRNA targets (Doench *et al.,* 2004). This results in the repression of target mRNA. This 2-8 nucleotide sequence at the 5'end of miRNA is known as the "seed sequence". In case of plants miRNAs bind in the coding region of mRNAs and result in the degradation of their targets (Chuck *et al.,* 2009). Along with repression miRNAs are observed to activate transcription by binding to the 5 'UTR of mRNAs target (Henke *et al.,* 2008).

2.2.5 miRNA target prediction

miRNA target site prediction is complicated in humans as complemetary sites of miRNA in 3' UTR regions of their target mRNA may not be functional in all cases (Vella *et al.*, 2004). mRNA sites with imperfect complementarity of miRNA seed region may also prove to be can very good targets for miRNA binding (Harris *et al.*, 2007). However in majority of cases complementarity of seed sequence is necessary for binding of miRNA to its mRNA target (Gaidatzis *et al.*, 2007). Other factors like sequence context of the target site, it's position in the 3' UTR and its distance from other neighbours are also crucial for the proper function of the target site (Saetrom *et al.*, 2007). Computer aided miRNA target prediction is progressing day by day and different steps involved in designing a short siRNA/miRNA that regulate target mRNA are shown in figure 2.8.

Many computational programs use bioinformatics tools to predicts the targets for miRNA but accuracy of these programs is low as they generate large number of targets making result validation hard (Srivastava *et al.*, 2007). Rapid progress is being made as many bioinformatics programs and softwares have been designed for the putative target prediction of miRNAs. These approaches take into account the fact that miRNAs increase or decrease the expression of their target mRNAs .Microarray techniques are used to record changes in level of gene expression.

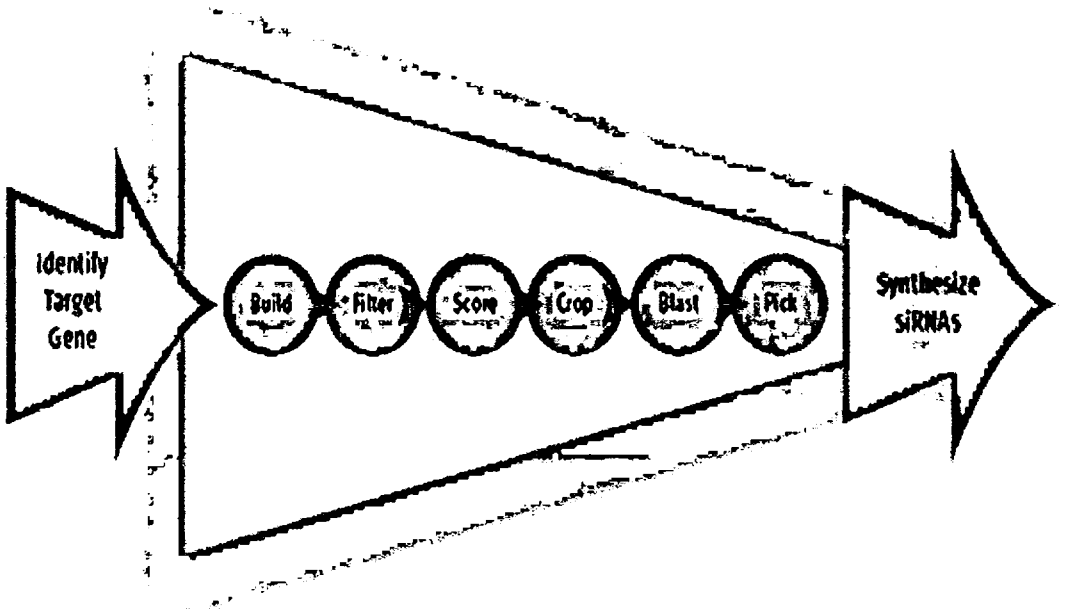


Figure 2.8: Multiple steps involved in RNA interference therapy.

2.2.6 miRNA Implications for Cancer

Some important interpretations in the beginning of miRNA discovery suggested their potential role in the development of cancer. First miRNAs discovered were thought to be controlling the profileration and apoptosis of cells (Brennecke *et al.*, 2003). However first noteworthy proof of role of miRNAs in cancer progression was identified in year 1999 (Calin *et al.*, 2004). Deregulation of these miRNAs may therefore cause cancer. It was also observed that miRNA genes were mostly found in the fragile regions of the genome which were amplified or deleted in many cases of cancers in humans (Calin *et al.*, 2004).Further more a widespread deregulation of miRNA expression was observed in tumourous cell lines (Croce *et al.*, 2006) . It is yet not well understood that if the changed expression of miRNAs seen in cancer is the cause or outcome of malignant transformation (Gaur *et al.*, 2007).

2.2.7 Computer Aided miRNA Target Prediction

The advent of miRNAs greatly influenced computational biological approaches along with traditional ones (Marc *et al.*, 2010). miRNA based computational programs are used for the prediction of genes encoding miRNAs and their mRNA targets (Hyeyoung *et al.*, 2010). Target prediction and biological validation of the results is major hinderence in miRNA research. Animal miRNAs show partial complementarity to their target sequences, therefore prediction of animal miRNA targets with high accuracy is a tough job (Bentwich, 2005). Computational algorithms used for the identification of miRNA target genes are designed on bases of rules miRNA take into account for recognizing their targets derived from experimental evidences (Brennecke *et al.*, 2005).

The programs for the identification of human miRNA targets are based on:

1) Pattern of base pairing between miRNA and their target mRNA

2) Thermodynamic stability of miRNA-mRNA hybrid

3) Comparative analysis for sequence conservation

4) Identification of multiple possible sites for the binding of miRNA in genome

2.2.7 Therapeutic Application of miRNAs

It is remarkable that in such short time period since miRNA's role was first identified in post transcriptional gene regulation such progress has been made and the

potential role of miRNAs as analytic markers for disease identification is well on its way (Takamizawa *et al.*, 2004).

Abnormal miRNA expression appears to be the characteristic of a wide variety of diseases. There is a graet application of expression profiles of miRNAs in diagnosis of cancer and other diseases (Sethupathy *et al.*, 2006). miRNAs have therapeutic application as well. Unusually expressed miRNAs may prove to be significant targets for cancer therapies (Caldas *et al.*, 2007). miRNAs have improved our understanding of carcinogenesis. Along with oncogenes and tumor suppressor genes, we must now consider miRNAs and the networks that regulate them if we wish to understand the complicated phenomenon mistifying malignant transformation and address poorly curable and complex life threatening cancers such as lung cancer (Caldas *et al.*, 2007 and Harris *et al.*, 2007).

METHODOLOGY

This study was aimed to prupose a miRNA based therapy to regulate most commonly and frequently mutated genes in lung cancer. In order to achieve this goal a multistep approach was applied and following steps were taken during this study:

3.1 Genetic Analysis of Lung Cancer

The prime step in this research work was to identify all genes reported till date, that are related to lung cancer. To achieve this purpose a comprehensive research was done utilizing all resources including published and printed research papers and review articles, books and online data bases. All genes that are directly or indirectly related to lung cancer were identified, listed and considered for further study and analysis. The prime focus was to study all genes that have been reported to mediate signaling pathways which result in the progression of lung cancer one way or another.

3.2 Target Gene Selection

After the identification of all the genes involved in lung cancer the genes that were most crucial to the development of lung cancer were short listed since it is not possible to cover all genes under the scope of this study. The cell signaling pathways and cascades were studied in detail to short list these genes.

The four genes selected were *EGFR, ERBB, p53* and *Kras*. According to the published resources available online on various databases, published research papers and articles, there was clear evidence that there are multiple mutated forms of these genes which play critical role in the development and progression of lung cancer.

3.3 Incorporating Mutations in Target Genes

The sequences for the normal forms of *EGFR, ERBB2, Kras* and *p53* genes were downloaded from *Entrez Gene*, an online database available on the site of *NCBI*.

Three mutated forms of *EGFR* genes have been reported to be involved in the development of lung cancer. These mutations were identified and incorporated manually in the normal sequence to get three mutated forms of *EGFR* gene. Similarly there were three mutated forms of *p53* and *ERBB* and a single mutated form of *Kras* which resulted in the progression of lung cancer. All these mutations were manually incorporated in the reference sequences to get the three mutated forms of *p53* and *ERBB* each and one mutated form of *Kras*.

3.4 Downloading Human miRNA Sequences

There were 721 mature human miRNA sequences downloaded from www.mirbase.org in unaligned fasta format. The *"miRBase"* database incorporates interfaces that provide inclusive miRNA sequence data, their annotation and gene targets predicted for them. It not only provides sequences for human miRNAs but covers more than 36 species as well (Sam *et al.,* 2005). This database facilitates with both, sequences of miRNA precursors and sequences of mature miRNA. miRNAs can be searched using their names, keywords, annotations or references and a complete set of miRNAs for any organism can be downloaded in a matter of few clicks for free. The miRBase takes its attributes from *"miRNA Registry"* and provides a primary online source for all published mature miRNA sequences along with their hairpin precursors and annotations relating to their structures and functions (Sam *et al.*, 2005).

3.5 Target Prediction Via miRanda

A main step of this study was to find targets for the 721 human miRNAs in commonly mutated genes of lung cancer but miRNA target prediction is complicated in case of humans and poses many hurdles. miRNA sequences in humans are short and show partial sequence complementarity to their targets. The programs used for identification of target sites for human miRNAs take many features into account like pattern of complementarity between mRNA target sites and miRNA, thermodynamic stability of miRNA-mRNA hybrid, and identification of multiple possible sites for the

binding of miRNA in genome (Yoon *et al.*, 2010). There are a variety of computational methods for miRNA target prediction such as *DIANA-microT*, *PicTar*, *miRanda*, *Target Scan, e.t.c.* Out of many options *miRanda* was selected for miRNA target prediction. *miRanda* identifies target sites by considering complementarity of sequences , free energies of mRNA and miRNA duplexes, and conservation analysis of related genomes. *miRanda* uses a local alignment algorithm that assigns weights to positions and is based on a strict model that checks that binding site has almost complete complementarity in the seed region and allows only G:U wobble (Yoon *et al.*, 2010). After predicting the target sites it uses Vieena library to calculate the thermodynamic energy of miRNA and target duplex (Anton *et al.*, 2003).

miRanda is a *LINUX* based algorithm that is configured and then installed by using the shell commands. Two input files, one containing the 721 miRNA sequences and other having the target gene sequence were made and given as input to the algorithm and ran with standard parameters i.e."Gap open penalty = -8.0, Gap extend = -2.0, Score threshold = 50.0, Energy threshold = -20.0 Kcal / mol, and the scaling parameter = 2.0". The output file contained alignment, alignment score, binding energy of the duplex, position of the target site and length of the duplex along with some other parameters. Algorithm was run for all target genes and their mutated forms.

3.6 Shuffled Vs Nonshuffled Sequences

miRanda generated large bulk of data, which showed all possible target sites against a single miRNA across the entire length of gene. A filter was needed to separate probable false positive hits and separate most suitable targets with high scores. To design the filter a cut off score was needed which filters the miRNA/mRNA pair. This cut off value was calculated as follows:

3.6.1 Calculation of Cut off Value

In order to find cut off score, scores of shuffled and non shuffled sequences were plotted. miRanda had already been run against non shuffled gene sequences. To get shuffled sequences an online application DNA shuffle provided by the Sequence Manipulation Suit and maintained by Bioinformatics organization was used. The gene sequences were submitted in fasta format in segments of 10,000 base pairs only. This software shuffled the sequences by keeping their base composition constant. miRanda was run against these shuffled sequences of genes by keeping the parameters default.

3.6.2 Data Parsing

The output files generated by miRanda contained many parameters but only alignment scores of shuffled and non shuffled sequences were needed to get the cut off value. In order to extract alignment scores of shuffled and non shuffled sequences from output files a data parsing application "*gawk*" was used. *Gawk* is the GNU implementation of *AWK*, a data driven programming language designed for processing

text based data (Siever *et al.*, 2009). Gawk extracts the particular parameter that had been specified in a text file format.

3.6.3 Data Plotting

To plot the alignment scores for shuffled and non shuffled sequences two tools *MS Excel* and *Mat lab* were used. *MS Excel* was unable to plot this huge bulk of data. No significant graph was generated by *Mat lab* as alignment scores for a large number of shuffled and non shuffled sequences had repetition of values thus the graph overlapped and therefore did not give a clear cut off value.

3.6.4 Data Analysis

miRanda outputs were analyzed and an average of shuffled and non shuffled alignment scores was used as a cut off value to filter the results. The miRNA pairs above the cut off score were selected for further analysis. A comparative analysis was done to find all the miRNAs that had targets in more than one gene. All these miRNAs were separated and selected for further processing.

3.7 Filtering Results via RNAhybrid

RNAhybrid finds the minimum free energy of hybridization for an elongated target sequence and a short miRNA sequence. *RNAhybrid* can take a sequence up to 1800 nucleotides long so the target sequence was cut into fragments of 1800 nucleotides. Since there was wide difference in length of miRNA sequence and its target sequence even in

fragments *RNAcalibrate* was used to calculate length normalized free energy values for ε and θ. These extreme value distribution parameters "ε and θ." were used as input parameters by RNAhybrid to calculate p-values of minimum free energy.

RNAhybrid functions in domain manner, i.e. the short miRNA sequence is hybridized to the part of the target sequence where it fits best therefore this software is primarily meant for miRNA target prediction. "The program calculates p-values based on extreme value distributions of length normalised energies along with minimum free energies".

3.8 Gene Mapping

miRNAs were further short listed on the basis of minimum free energy values generated by RNAhybrid. The target regions were mapped on genome and target sites for selected miRNAs were aligned using *ClustalW*. *ClustalW* is a multiple sequence alignment program used for DNA or protein sequence alignment. It is available on EBI server of EMBL (www.ebi.ac.uk).

3.9 miRNA Designing

The conserved sequences in target sites of these four genes were analyzed and on the basis of conservation of base pairs in all mutated forms of these genes a miRNA strand was designed.

RESULTS AND DISCUSSION

4.1 Genes Involved in Lung Cancer

The table 4.1 shows a list of genes that are directly and indirectly involved in lung cancer and play multivariate roles like some are involved in the mutagenesis and progression while others are associated with poor diagnosis, risk assessment and treatment of lung cancer.

This table presents just a small picture of molecular diversity that contributes to the development and progression of lung cancer. In 2008, Dinge and his colleagues sequenced 623 cancer causing genes that have evident or possible relationship to carcinogenesis in 188 human lung cancer cases. For this present study 30 genes were recognized to be mutated at high frequencies and potentially playing key role in lung carcinogenesis. Since it was not possible to detect miRNA targets in such wide variety of genes, genes that are most critical to the development and progression of lung cancer were selected.

The prime focus was on genes that are tumor suppressors and oncogenes that regulate cell signaling pathways as they are the most crucial ones. The mutations in these genes result in the malfunctioning of entire signaling cascades.

Table 4.1: Some Important Genes Involved in Lung Cancer

S.NO	Genes	Significance in Lung Cancer	References
1	ALK/EML4 fusion gene	Non-small cell lung cancer	(Manabu *et al.*, 2007)
2	BAT3 and MSH5	Increases risk/reported in NSCLC	(Zhang *et al.*, 2009)
3	BRAF	All lung cancer types	(Davies *et al.*, 2002)
4	CHRNA3 and CHRNA5	Increases nicotine dependent risk	(Saccone *et al.*, 2009)
5	CLPTM1L and TRET	Influence the risk of lung cancer	(Wang *et al.*, 2010)
6	DLEC1	Poor prognosis of lung cancer	(Hidefumi *et al.*, 2010)
7	EGFR	Mutated and over expressed in all lung cancer types	(Suda *et al.*, 2009)
8	EPHA3	Adenocarcinoma of lung	(Li *et al.*, 2008)
9	ERBB2	Adenocarcinom a subtype of NSCLC	(Stephens *et al.*, 2004)
10	ERCC6	Genetic variant of ERCC6 increases smoking related risk of lung cancer	(Ma *et al.*, 2009)
11	HMOX1	Drug induced toxicity in NSCLC	(Fer *et al.*, 2010)
12	ITGA9	Squamous cell lung cancer and lung adenocarcinoma	(Anedchenko *et al*, 2008)
13	KDR	Non small cell lung cancer	(Koukourakis *et al.*, 2000)
14	KLC1	Poor prognosis of lung cancer	(Hidefumi *et al.*, 2010)
15	KRAS	Mutations in NSCLC	(Bae *et al.*, 2007)
16	MALAT1	Associated with metastasis in NSCLC	(Ping *et al.*, 2003)

17	MAP3K8	Lung adenocarcinoma	(Clark *et al.*, 2004)
18	MET	*MET* Amplification Leads to Gefitinib Resistance in Lung Cancer	(Engelman *et al.*, 2007)
19	MYCL1	Genome wide allele typing of lung cancer	(Dacic *et al.*,2004)
20	MPO	The variant genotype of MPO increases abestos related lung cancer risk	(Schabath *et al.*, 2002)
21	NKX2-1	Associated with poor survival of lung cancer patients	(Hsua *et al.*, 2008)
22	NTRK	Lung adenocarcinoma	(Li *et al.*, 2008)
23	PARK2	Intragenic deletions of PARK2 abrogate the growth-suppressive effects in lung cancer	(Veeriah *et al.*, 2010)
24	PIK3CA	Plays role in metastasis of tumor	(Okudela *et al.*, 2007)
25	PPP2R1B	Altered in 15% of lung tumors	(Wang *et al.*, 1998)
26	SLC22A18	A SNP found in lung cancer	(Sung *et al.*, 2006)
27	STK11	Loss of DNA repair	(Adina *et al.*, 2010)
28	TERT	Increases the risk of lung cancer	(Rafnar *et al.*, 2009)
29	TP53	Non small cell lung cancer	(Cespedes *et al.* , 1999)
30	TSG11	Non small cell lung cancer	(Varinderpal *et al.*, 2003)

4.2 Target Genes.

Germ line and somatic mutations have been recognized and reported in the *BRAF*, *EGFR*, *ERBB*, *Kras*, *MET*, *PIK3CA* and *p53* genes more frequently in lung cancer patients. Four genes were selected for this research work of target prediction and these are:

- *EGFR*
- *ERBB*
- *Kras*
- *P53*

4.2.1 EGFR Gene

EGFR gene encodes a glycol protein that is trans membrane and acts as a receptor for *EGF* (Epidermal Growth Factor) family members. When a ligand i.e. member of *EGF* family binds to this protein autophosphorylation of tyrosine takes place and receptors are dimerized. Mutations in this gene are a common incidence in lung cancer cases. There are multiple isoforms of *EGFR* gene like 60-kDa and 110-kDa isoforms in humans (Jill *et al.*, 2006).

A variety of mutated forms of *EGFR* gene have been reported out of which clearly associated with lung cancer are:

- Deletion of 18 base pairs i.e. deletion of six codons (747-753) at nucleotide position 2240 and insertion of a single Serine residue (Lynch *et al.*, 2004).
- Transversion of Guanine with Thymine at nucleotide position 2155 that results in the conversion of Glycine at codon 719 to Cystine (Paez *et al.*, 2004).
- Transversion of Thymine with Guanine at nucleotide position 2573 that result in the conversion of Leucine at codon 858 to Argenine (Paez *et al.*, 2004).

Mutations of *EGFR* are more common in NSCLC and particularly in adenocarcinoma subtype. *EGFR* mutations are relatively common in patients who have no background of smoking than those who have history of smoking.

4.2.2 P53 Gene

The protein encoded by this gene is tumor protein 53 which is expressed when a cell goes under stress. This protein regulates multiple genes to act under stressful conditions by taking steps like arresting cell cycle, repairing DNA, inducing apoptosis, *etc.* Expression of this gene is low in normal cells as compared to cancerous cells. It acts as a tumor suppressor gene by inhibiting uncontrolled cell growth and binds to *p53* binding site of DNA (Patrick *et al.*, 1993).

There are many mutant forms of *p53* gene that are unable to bind to the binding site in DNA and hence cannot induce tumor suppression. *P53* mutations are a common occurrence in a variety of human cancers. Mostly related to lung cancer are following mutant types of *p53*:

- Transversion of GC to TA at codon number 245 resulting in missense mutation (Takahashi *et al.*, 1989).
- A point mutation, resulting in conversion of Argenine to Histidine at codon number 175 of mRNA (Hwang *et al.*, 2003).
- A point mutation resulting in conversion of Argenine to Histidine at codon number 273 of mRNA (Hwang *et al.*, 2003).

4.2.3 Kras Gene

This gene belongs to *ras* gene family of mammals and encodes a small *GTPase* protein that is a member of *GTPase* super family. The *Ras* superfamily of GTP-binding proteins has more then 50 members and controls a varied range of intracellular processes. These comprise cellular proliferation and differentiation, intracellular vesicular trafficking, cytoskeletal control and NADPH oxidase function (Der *et al*,, 1993). Single substitution of an amino acid results in active mutation which is a common cause of many malagnincies specialy adenocarcinoma of lung in human (Galen *et al.*, 2001).

- Guanine at nucleotide position 34 is substituted by Thymine or Cytosine resulting in amino acid Cysteine or Arginine respectively (Ahrendt *et al.*, 2001).

4.2.4 ERBB Gene

This gene encodes a tyrosine kinase protein that is member of epidermal growth factor receptor family. This protein lacks a ligand binding site and can only bind to already ligand bound members of EGF family. Its binding results in the formation of

hetrodimer which stabilizes the binding of ligand and triggers signaling pathways that are involved in cell growth and cell division . This gene is amplified and overexpressed in many human cancers like lung and breast cancer. In 2004 " Cancer Genome project and Collaborative Group" identified following three mutant forms of ERBB gene in various lung cancer cell lines of humans:

- Insertion/ duplication of GCATACGTGATG at position 2322 resulting in insertion of 4 amino acids Alanine, Tyrosine,Valine and Methionine at codon number 774.
- Insertion of CTGTGGGCT at 2335 resulting in insertion of 3 amino acids Valine, Glycine and Serine at codon number 779.
- TT-CC base pair substitution at position 2263 and 2264 converting Leucine at codon number 755 to Proline.

On the basis of the clear evidence of involvement and importance of these genes in the development and progression of cancer of lungs in human, they are selected for target prediction of human encoded miRNAs.

4.3 Target Prediction of miRNAs

Since the discovery of miRNAs a variety of tools and algorithms have been designed to predict their targets in different species. There are a variety of target prediction tools available like *TargetScan, TargetScanS, Pictar, DIANA-MicroT, miRanda, Support Vector machine* method and *statistical methods* (Praveen *et al.*,

2006) Of so many options available *miRanda* was selected because it selects targets taking into account multiple properties of miRNA and mRNA binding. Like *TargetScan* and *TargetScanS miRanda* checks sequence allignment using Vienna package but unlike them it does not seek perfect complementarity in seed region and allows G:U wobble which makes it more flexible and more suitable (Benjamin *et al.*, 2005). *Pictar* alligns sequences using *HMM* where as *DIANA-MicroT* only calculates minimum free energies of miRNA- mRNA duplexes. Statistical methods are also not very suitable as there is a large scale of sequence comparison (Praveen *et al.*, 2006).

The best choice in this scenario was *miRanda* that not only checks sequence complementarity but also calculates the thermodynamic stability of miRNA mRNA duplexes and assigns a score and weighted average to the target site on the basis of these features. The sites with the highest scores are the most suitable targets (Rajewsky, 2006).

4.4 Results of miRanda

miRanda generates large bulks of data i.e all possible target sites for a single miRNA in the entire target sequence and the target sequence in this case were human genes commonly mutated in lung cancer. The number of miRNAs used in this research work were 721. Since miRanda predicted multiple target sites for a single miRNA the number of target sites for 721 miRNAs were enormous. In order to separate false positive results and select the most probable target sites a cut off value of 163 was selected. In most cases cut off value is predicted by plotting the alignment

scores generated by *miRanda* for shuffled and non shuffled sequences of target genes. The graphs plotted for scores of shuffled and non shuffled sequences for this study did not show any clear cut off point on the graph due to many repititions for values of scores generated by *miRanda*, the graphs over lapped instead of intersecting or cutting at a clear point. Therefore cut off was calculated by taking the weighted average of top 50 scores of *miRanda* outputs as the targets with highest scores had highest weighted averages and showed more thermodynamic stability and hence had more chances of being the most suitable target. *miRanda* was run for the second time by changing the score threshold from 50 to 163 and the results were filtered and probable targets were subjected to further processing and analysis. The results for each of the selected four genes are discussed in coming sections.

4.5 miRanda Predicted targets for EGFR Gene

miRanda was run seperately for normal and three mutated forms of *EGFR* gene to predict targets. The filtered results of miRanda for *EGFR* gene are shown in table 4.2. Several interesting conclusions can be drawn from these results. Since the goal was to design a miRNA that can single handedly regulate multiple genes which are most commonly mutated in lung cancer therefore, only those miRNAs were selected that targeted normal and atleast one mutated form of *EGFR* gene and their score was above the threshold of 163.

S.No	miRNA	EGFR -N	EGFR-M1	EGFR-M2	EGFR-M3
1	miR-17	✓	×	×	×
2	miR-18b	✓	✓	✓	✓
3	miR-20a	✓	×	×	×
4	miR-20b	✓	×	×	×
5	miR-24	✓	✓	✓	✓
6	miR-30b*	✓	×	×	×
7	miR-34c-5p	✓	✓	✓	✓
8	miR-93	✓	×	×	×
9	miR-106a	✓	×	×	×
10	miR-106b	✓	×	×	×
11	miR-130b*	✓	✓	✓	✓
12	miR-329	✓	✓	✓	✓
13	miR-372	✓	×	×	×
14	miR-433	✓	✓	✓	✓
15	miR-490-3p	✓	×	×	×
16	miR-492	✓	×	×	×
17	miR-501-5p	✓	✓	✓	✓
18	miR-505*	✓	✓	✓	✓
19	miR-574-5p	✓	✓	✓	✓
20	miR-593	✓	✓	×	✓
21	miR-593*	×	×	✓	×
22	miR-598	✓	×	×	×
23	miR-615-5p	✓	✓	✓	✓
24	miR-634	✓	✓	✓	✓
25	miR-658	✓	✓	✓	✓
26	miR-661	✓	✓	✓	✓
27	miR-887	×	✓	✓	✓
28	miR-877*	✓	×	×	✓
29	miR-914	✓	×	×	×
30	miR-922	✓	✓	✓	✓
31	miR-939	✓	✓	✓	✓
32	miR-1226	✓	✓	✓	✓
33	miR-127-5p	✓	✓	✓	✓
34	miR-1228*	✓	✓	✓	✓
35	miR-1276	✓	✓	✓	×
36	miR-1471	✓	✓	✓	✓
37	miR-1469	✓	✓	✓	✓
38	miR-1976	✓	✓	✓	✓

miRNAs with nearly identical sequences are annotated with an additional lower case letter in this table miR-20a and miR-20b are two closely related miRNAs. Pre-miRNAs that lead to 100% identical mature miRNAs but that are located at different places in the genome are indicated with an additional dash-number suffix. Species of origin is designated with a three-letter prefix, e.g., hsa-miR-123 is a human miRNA. When two mature miRNAs originate from opposite arms of the same pre-miRNA, they are denoted with a -3p or -5p suffix. When relative expression levels are known, an asteriek following the name indicates a miRNA expressed at low levels relative to the miRNA in the opposite arm of a hairpin. For example in this case miR-887 and miR-887* share a pre-miRNA hairpin, but more miR-887 are found in the cell.

4.6 miRanda Predicted Targets for P53 Gene

Table 4.3 shows targets predicted by *miRanda* for normal and two mutated forms of *p53* gene. There were 21 miRNAs that lie above the threshold score of 163 which targeted the normal and two mutated forms of *p53* gene. This shows that mutations did not alter binding of miRNAs to their target sites (as both mutations are single point mutations) and thus had no effect on binding of miRNAs with their target sites.

This table also has two pairs of closely related miRNAs 20a and 20b, and 106a and 106 b. miR-141*, miR-143*. 150* and 225* are expressed in lower level in cells with mutated genes as compared to normal cells.

Table 4.3 miRNA Targets for P53 Gene

	MiRNAs	p53 Normal	P53-M1	P53-M2
1	miR-17	✓	✓	✓
2	miR-20a	✓	✓	✓
3	miR-20b	✓	✓	✓
4	miR-93	✓	✓	✓
5	miR-106a	✓	✓	✓
6	miR-106b	✓	✓	✓
7	miR-122	✓	✓	✓
8	miR-141*	✓	✓	✓
9	miR-143*	✓	✓	✓
10	miR-146-5p	✓	✓	✓
11	miR-150*	✓	✓	✓
12	miR-224*	✓	✓	✓
13	miR-372	✓	✓	✓
14	miR-542-5p	✓	✓	✓
15	miR-543	✓	✓	✓
16	miR-708	✓	✓	✓
17	miR-885-3p	✓	✓	✓
18	miR-936	✓	✓	✓
19	miR-1285	✓	✓	✓
20	miR-1304	✓	✓	✓
21	miR-2276	✓	✓	✓

4.7 miRanda Predicted Targets for Kras Gene

The targets predicted by *miRanda* for normal and two mutated forms of Kras gene are given in table 4.4. Seventy eight miRNAs lied above the threshold score of 163 and out of those only 30 bound to normal gene while failed to bind to either of the mutated forms of Kras gene. The miR-1207 was the vice versa case as it failed to bind to the normal Kras gene but targeted both mutated forms of Kras and if only these specific mutations were to be addressed in lung cancer patients than this miRNA would have played a significant role. The miRNAs that targeted both the normal and mutated forms of Kras were selected and their target sites were further analyzed. The remaining miRNAs were considered because the aim was to design a miRNA strand with a broad spectrum that can target both normal and all three mutated forms of *Kras* gene simultaneously for lung carcinoma.

Table 4.4 miRNA Targets for Kras Gene

S.NO	miRNAs	KRAS NORMAL	KRAS- M1	KRAS- M2
1	let-7a	✓	✓	✓
2	let-7i	✓	✓	✓
3	miR-9*	✓	✓	✓
4	miR-16	✓	×	×
4	miR-16-2*	✓	×	×
6	miR-17	✓	✓	✓
7	miR-19b	✓	×	×
8	miR-20a	✓	✓	✓
9	miR-20b	✓	✓	✓
10	miR-20b*	✓	×	×
11	miR-27b*	✓	×	×
12	miR-34a*	✓	×	×

13	miR-92a*	✓	×	×
14	miR-93	✓	✓	✓
15	miR-106a	✓	✓	✓
16	miR-106b	✓	✓	✓
17	miR-127-5p	✓	×	×
18	miR-128	✓	×	×
19	miR-140-3p	✓	✓	✓
20	miR-141	✓	✓	✓
21	miR-141*	✓	×	×
22	miR-143	✓	×	×
23	miR-146a	✓	✓	✓
24	miR-146b-5p	✓	✓	✓
25	miR-148a*	✓	×	×
26	miR-183	✓	✓	✓
27	miR-185	✓	✓	✓
28	miR-198	✓	✓	✓
29	miR-217	✓	✓	✓
30	miR-320a	✓	✓	✓
31	miR-320b	✓	✓	✓
32	miR-347a*	✓	×	×
33	miR-363	✓	✓	✓
34	miR-367	✓	✓	✓
35	miR-373	✓	×	×
36	miR-378	✓	✓	✓
37	miR-453	✓	×	×
38	miR-454	✓	✓	✓
39	miR-484	✓	×	×
40	miR-485-3p	✓	✓	✓
41	miR-491-5p	✓	×	×
42	miR-499*c	✓	✓	✓
43	miR-507	✓	×	×
44	miR-509-3-5p	✓	✓	✓
45	miR-548d-3p	✓	×	×
46	miR-548m	✓	✓	✓
47	miR-566	✓	✓	✓
48	miR-576-3p	✓	×	×
49	miR-580	✓	×	×
50	miR-603	✓	✓	✓
51	miR-613	✓	✓	✓

52	miR-619	✓	×	×
53	miR-630	✓	×	×
54	miR-637	✓	✓	✓
55	miR-641	✓	×	×
56	miR-652	✓	×	×
57	miR-665	✓	✓	✓
58	miR-764	✓	✓	✓
59	miR-765	✓	✓	✓
60	miR-766	✓	✓	✓
61	miR-937	✓	✓	✓
62	miR-939	✓	✓	✓
63	miR-1178	✓	×	×
64	miR-1183	✓	✓	✓
65	miR-1207	×	✓	✓
66	miR-1229	✓	✓	✓
67	miR-1268	✓	✓	✓
68	miR-1273	✓	✓	✓
69	miR-1285	✓	✓	✓
70	miR-1290	✓	×	×
71	miR-1303	✓	×	×
72	miR-1304	✓	×	×
73	miR-1909	✓	✓	✓
74	miR-1974	✓	×	×
75	miR-1976	✓	✓	✓
76	miR-12866	✓	×	×
77	miR-1207-5p	✓	✓	✓
78	miR-1228*	✓	×	×

4.8 miRanda Predicted Targets for ERBB Gene

In table 4.5 miRNA targets predicted by miRanda for normal and three mutated forms of *ERBB* genes are shown. There were 53 miRNAs whose targets had scores above the threshold of 163. There was a single miRNA (miR-320a) that bound to the normal sequence of *ERBB* gene but failed to bind to any of the three mutated forms of

gene. This failure of miRNA to *bind* to mutant *ERBB* sequences may indicate a contribution of these mutations in the development of malignancy. The 32 miRNAs that bound to the normal and the three mutated forms of *ERBB* were the most considerable ones.

Some of the miRNAs bound to the normal *ERBB* gene and either one of the three mutated forms while there were a few which did not bind to the normal gene but targeted one or any two of the three mutated forms of *ERBB*. miR-302a is unique that it only binds to the normal sequence of *ERBB* and fail to bind to any one of the mutated forms hence may be crucial for normal expression of *ERBB* gene. On the other hand 302a* that is closely related to 302a binds to all three mutated forms but fails to bind the normal *ERBB* sequence. Hence in case of cells with normal *ERBB* gene 302a expression is high and in case of cancerous cell expression of 302a* is high.

The table 4.5 shows that 13 mi-RNAs only bind to the normal and first mutated form of *ERBB* gene and hence are of little significance. Two miRNAs, miR-143* and miR-216a bind to the normal and second and third mutated form of ERBB gene. miR-126 and miR216 only bind to the second and third mutated forms.

Table 4.5 miRNA Targets for ERBB Gene

	miRNAs	ERBB-NORMAL	ERBB-M1	ERBB-M2	ERBB-M3
1	17	✓	✓	✓	✓
2	20a	✓	✓	✓	✓
3	20b	✓	✓	✓	✓
4	29b	✓	✓	✓	✓
5	30b	✓	✓	✓	✓
6	30b*	✓	✓	×	×
7	30c	✓	✓	✓	✓
8	34c-5p	✓	✓	×	×
9	93	✓	✓	✓	✓
10	106a	✓	✓	✓	✓
11	106b	✓	✓	✓	✓
12	126	×	×	✓	✓
13	138	✓	✓	×	×
14	143	×	✓	×	×
15	143*	✓	×	✓	✓
16	188-3p	✓	✓	✓	✓
17	214*	✓	✓	✓	✓
18	216	×	✓	×	×
19	216a	✓	×	✓	✓
20	220b	✓	✓	✓	✓
21	302a*	×	✓	✓	✓
22	320a	✓	×	×	×
23	367	✓	✓	✓	✓
24	372	✓	✓	✓	✓
25	373	✓	✓	✓	✓

26	378*	✓	✓	✓	✓
27	484	✓	✓	✓	✓
28	505*	✓	✓	✓	✓
29	508-5p	✓	✓	✓	✓
30	548a-3p	✓	✓	×	×
31	566	✓	✓	×	×
32	574-5p	✓	✓	✓	✓
33	608	✓	✓	✓	✓
34	631	✓	✓	×	×
35	642	✓	✓	×	×
36	643	✓	✓	✓	✓
37	649	✓	✓	✓	✓
38	650	✓	✓	×	×
39	744	✓	✓	✓	✓
40	765	✓	✓	✓	✓
41	942	✓	✓	✓	✓
42	1207-5p	✓	✓	✓	✓
43	1226	✓	✓	×	×
44	1226*	✓	✓	✓	✓
45	1236	✓	✓	×	×
46	1258	✓	✓	×	×
47	1260	✓	✓	✓	✓
48	1273	✓	✓	✓	✓
49	1285	✓	✓	✓	✓
50	1302	✓	✓	×	×
51	1910	✓	✓	×	×
52	1972	✓	✓	✓	✓
53	1978	✓	✓	×	×

4.9 miRNAs Targeting Multiple Genes

Since the fact has been established that mutations in more than one gene lead to the development of lung cancer. This research work was primarily aimed to design a single miRNA that can target multiple genes most frequently mutated in lung cancer.

It has already been established that multigenic factors contribute to the development of lung cancer. Therefore, in this study only four important genes were considered and effort was made that only those miRNAs, which targeted the normal and all mutated forms of these four genes, were selected. All of these miRNAs lied above the threshold score of 163 and hence were selected as the potential miRNAs strands. As is shown in Table 4.6 that out of 721 miRNAs used, 16 had targets in normal and mutated forms of more than one gene while of these 16, only five miRNAs targeted any three of the selected genes simultaneously. No one miRNA binds to all four of the genes.

The diversity and capacity to bind to target sites in multiple genes made these miRNAs highly potential therapeutic agents to be used against lung cancer. The alignment scores predicted by *miRanda* for these 16 miRNAs are given in table 4.7. *miRanda* also calculated minimum free energy values for miRNA-mRNA duplexes. miRanda also generated the positions of target sites, that is the number of nucleotide from which the target site starts and the number of nucleotide at which it ends.

The table 4.7 also shows miRNAs that target genes in different combinations. For instance miR-93, miR-20a, miR-20b, miR-106a and miR-106b targets normal and all

mutated forms of *ERBB*, *kras* and *p53*. miR-939 and miR-1976 have targets in normal and all mutated forms of *EGFR* and *kras* genes. miR-505* targets normal and mutants of *EGFR* and *ERBB*. miR-373, miR-765 and miR-1273 targets *ERBB* and *kras* genes. miR-17 and miR-1285 targets normal and mutated forms of *kras* and *p53*. miR-372 and miR-302a* target normal and mutated *ERBB* and *p53*. Only miR-887 targets the normal and mutated forms of *EGFR* along with *ERBB* and *p53*.

Table 4.7 shows scores, energy values of miRNA-mRNA duplex and position of their target sites within the gene sequences. In order to find out where these target sites were located within the sequence of genes, gene mapping was done. The sequence maps of the genes available on the *NCBI* data bases were used for this purpose. These maps gave details of all exons, introns, 3` UTR and 5`UTR regions that make up a gene. By mapping genes it was found that where the target sites of these miRNAs were exactly located.

It is clear from the table that most of the target sites of these 16 miRNAs out of the original 721, lies in the 3`UTR regions of these genes i.e. *EGFR*, *Kras*, *p53* and *ERBB*. Others lie within different exons of these genes. Whereas none of the target sites lie in the intron regions of these genes. As the duplex considered was with functional mRNA which does not have intron sequences therefore none of the miRNAs binds in the intron region of a gene.

Table 4.6 miRNAs Targeting Multiple Genes

NO	MiRNAs	EGFR	ERBB	Kras	P53
1	miR-17	✓	×	✓	×
2	miR-20a	×	✓	✓	✓
3	miR-20b	×	✓	✓	✓
4	miR-93	×	✓	✓	✓
5	miR-106a	×	✓	✓	✓
6	miR-106b	×	✓	✓	✓
7	miR-302a*	×	✓	×	×
8	miR-372	×	✓	×	✓
9	miR-373	×	✓	✓	×
10	miR-505*	✓	✓	×	×
11	miR-765	×	✓	✓	×
12	miR-887	✓	×	×	×
13	miR-939	✓	×	✓	×
14	miR-1273	×	✓	✓	×
15	miR-1285	×	×	✓	✓
16	miR-1976	✓	×	✓	×

Table 4.7 Scores and Positions of miRNA Targets

NO	MiRNAs		EGFR	ERBB	KRAS	TP53
1	miR-939	Score	166	-	165	-
		e-value	-39.26	-	-31.87	-
		position	1186-1210	-	824-851	-
			exon-8	-	exon-9	-
2	miR-505*	Score	171	167	-	-
		e-value	-26.7	-22.48	-	-
		position	6660-6681	367-391	-	-
			3'UTR	exon6	-	-
3	miR-1976	Score	166	-	165	-
		e-value	-31.7	-	-24.07	-
		position	6447-6468	-	7691-7710	-
			3'UTR	-	3'UTR	-
4	miR-373	Score	-	171	168	-
		e-value	-	-22.29	-21.35	-
		position	-	5792-5814	1214-1233	-
			-	3'UTR	3'UTR	-
5	miR-765	Score	-	171	174	-
		e-value	-	-27.98	-28.48	-
		position	-	918-936	441-462	-
			-	exon-8	exon-6	-
6	miR-93	Score	-	165	165	169
		e-value	-	-29.07	-28.48	-30.3
		position	-	2671-2783	441-462	3555-3577
			-	exon-24	exon-6	3'UTR
7	miR-1273	Score	-	171	203	-
		e-value	-	-28.25	-44.23	-
		position	-	2132-2156	28774-28798	-
			-	exon-20	3'UTR	-
8		Score	-	165	173	177
		e-value	-	-25.69	-25.72	-26.2

	miR-20b	position	-	2764-2780	10784-10806	3555-3577
			-	exon-25	3'UTR	3'UTR
9	miR-106a	Score	-	165	173	177
		e-value	-	-21.45	-22.15	-21.96
		position	-	2764-2786	10784-10806	3555-3577
			-	exon-25	3'UTR	3'UTR
10	miR-1285	Score	-	-	188	187
		e-value	-	-	-35.89	-34.84
		position	-	-	1337-1358	3321-3342
			-	-	exon-13	3'UTR
11	miR-17	Score	-	-	165	177
		e-value	-	-	-25.69	-26.2
		position	-	-	20866-20891	3555-3577
			-	-	3'UTR	3'UTR
12	miR-20a	Score		165	173	177
		e-value	-	-23.58	-24.28	-24.09
		position	-	2764-2786	10784-10806	3555-3577
			-	exon-25	3'UTR	3'UTR
13	miR-106b	Score	-	167	167	175
		e-value	-	-20.7	-22.89	-22.67
		position	-	2764-2786	2782-2802	3557-3577
			-	exon-25	3'UTR	3'UTR
14	miR-372	Score	-	163	-	167
		e-value	-	-21.14	-	-21.2
		position	-	2758-2780	-	3549-3576
			-	exon-25	-	3'UTR
15	miR-302a*	Score	-	169	-	-
		e-value	-	-22.02	-	-
		position	-	3532-3554	-	-
			-	exon-30	-	-
16	miR-887	Score	175	-	-	-
		e-value	-35.64	-	-	-
		position	6437-6457	-	-	-
			3`UTR	-	-	-

4.10 Verification of Results using RNAhybrid

RNAhybrid was also used for target prediction of miRNAs. *RNAhybrid* finds the most suitable and favorable hybridization site between the two, miRNA and its target sequence. The number of false positive results generated by *RNAhybrid* is relatively low and it is efficient and flexible and offers a large choice of options and features that can be selected by user according to requirement (Rehmsmeier *et al.*, 2006).

The table 4.8 presents a comparison of minimum free energy values generated by *miRanda* and *RNAhybrid* for the miRNAs and their duplexes with their target mRNAs. The results of *RNAhybrid* differ from those of *miRanda* the reason could be that both the softwares consider different parameters for calculating the minimum free energy. The energy values calculated by *RNAhybrid* are considered more significant than those calculated by *miRanda*. RNAhyrbid finds the energetically most favourable hybridization sites between miRNAs and their target mRNAs using integrated powerful statistical model (Verbeek *et al.*, 2010).

Lower the minimum free energy of miRNA and mRNA duplex stronger the binding and interaction between the two and most suitable the target site was. The energy value of -30 was considered as a good energy value for the hybridized duplex. A closer look at the table shows that the energy values generated by RNAhybrid for six miRNAs (that are highlighted intable) were below -30. The energy values calculated by *miRanda* and *RNAhybrid* for two miRNAs i.e. miR-505* and miR-1976 differ widely. The values of minimum free energies calculated by *RNAhybrid* were consistent with the values

calculated by *miRanda* for rest of the miRNAs and were not significant as they were quite high and were not even close to -30.

Table 4.8 Comparison of miRanda and RNAhybrid Results for mfe Values

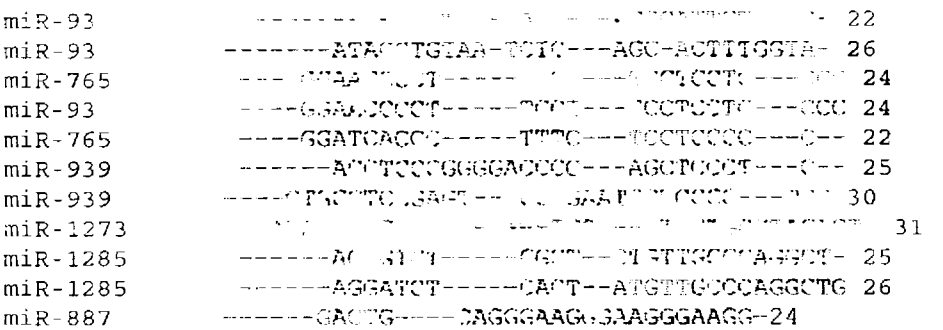
S.NO	miRNAs	mfe values miRanda	mfe values RNAhybrid
1	miR-939	-37.92	-44.7
2	miR-505*	-30.1	-23.7
3	miR-1976	-31.7	-21.97
4	miR-373	-22.25	-21.35
5	miR-765	-28.48	-31.2
6	miR-93	-30.3	-32.9
7	miR-1273	-28.55	-32.8
8	miR-20b	-24.32	-25.01
9	miR-106a	-20.08	-22.5
10	miR-1285	-35.89	-37.9
11	miR-17	-26.2	-24.22
12	miR-20a	-22.21	-25.6
13	miR-106b	-20.77	-22.67
14	miR-302a*	-22.02	-19.6
15	miR-372	-21.2	-22.65
16	miR-887	-35.64	-31.3

On the basis of the results produced by the two softwares six miRNAs were selected for designing the miRNA strand that can target the mutated *EGFR, ERBB, kras* and *p53* in lung cancer. Both of the software programs generated results using different parameters and giving results in the form of alignment scores and minimum free energy of hybridization. The six most appropriate miRNAs selected on the basis of these results are:

- hsa-miR-939
- hsa-miR-765
- hsa-miR-93
- hsa-miR-1273
- hsa-miR-1285
- hsa-miR-887

4.11 Multiple Sequence Alignment

Multiple sequence alignment, MSA, of the target sites for these 6 miRNAs was done to check for conserved sequences. The binding of miRNA with incomplete complementarity increases their flexibility to bind to multiple target sites that do not show sequence complementarity. Some miRNAs like miR-93, miR-939 and miR-1285 varied in their length according to their target sites.



The MSA of the miRNAs that target these sites was also done to look for conserved sequences.

- miR-939 GUGGGGGUCUCGGGUCGAGGGG
- miR-93 UGGACGUGCGUCGUGAAAC
- miR-765 UAGUGGGGAAGAGGAGG
- miR-1273 UCUUUUCAGAACGAACAGCGGG
- miR-887 GAGCCCUACCGCGGGGUG
- miR-1285 UCCAGAGUGACCGGGUG

All these miRNAs have more than 50% purine bases and have purine rich 3' ends. miR-939 has 22 nucleotides out of which 9 are purine and 7 are pyramidine. miR-93 has 19 nucleotides out of which 11 are purine and 8 are pyramidine. miR-765 has 17 nucleotides out of which 15 are purine and 2 are pyramidine. miR-1273 has 22 nucleotides out of which 12 are purine and 10 are pyramidine. miR-887 has 18

nucleotides out of which 10 are purine and 8 are pyramidine. miR-1285 has 17 nucleotides out of which 10 are purine and 7 are pyramidine.

Most of these have a purine to pyramidine ratio between 54% to 68% except miR-765 where 88% nucleotides are purine. For primer binding in PCR 40-60% GC content ensures stable binding and presence of G or C base at the 3' end helps to promote correct binding at the 3' end due to stronger H bonding of G and C bases.

4.12 Designing miRNA

The miRNA that can target multiple genes most frequently mutated in lung cancer should have:

Purine to pyramidine ratio between 55-70%.

The GC to AU ratio should be around 60%.

The last base should be G or C.

Preferably the 3' end should be purine rich with two of the last three bases definitely be purine.

CONCLUSION

An attempt had been made to design a miRNA that is diverse in its nature and can target and regulate multiple genes. As lung cancer in most of the cases is diagnosed at very late stages and involvement of multigenic factors make its treatment complicated and limited. This research work mainly focused on the designing of a miRNA, which may be applied for the gene knockdown mechanism like siRNA or may be used to design the RNA based therapy that may address four most commonly and frequently mutated genes which lead to the mutagenesis and progression of lung cancer. Out of 721 human miRNAs six were identified to have targets in the normal and mutated forms of the selected four genes. The target RNA strand was designed by analyzing the sequences of these six miRNAs, namely:

- miR-939
- miR-93
- miR-765
- miR-1273
- miR-887
- miR-1285

The scope of this study was limited and covered only four genes out of a large variety of genes associated with the development and progression of lung cancer. Similar work can be extended to other genes related to lung cancer. Furthermore this study only

suggests miRNA based therapy for lung cancer but it needs to be experimentally validated and therapeutically tested before it can be applied to any practical use.

REFFERENCES

1. Alberg A. J. and J. M. Samet. 2003. Epidemiology of Lung Cancer. Chest; 123:21-49.
2. Alvarez-Garcia I. and E. A. Miska. MicroRNA functions in animal development and human disease. Development; 132: 4653-4662.
3. Anand P., A. B. Kunnumakara, C. Sundaram, K. B. Harikumar, S.T. Tharakan, O. S. Lai, B. Sung and B. B. Aggarwa. 2008. Cancer is a Preventable Disease that Requires Major Lifestyle Changes. Pharmaceutical Research; 25(9): 2097-2116.
4. Anedchenko A. E., A. A. Dmitriev, G. S. Krasnov, O. O. Kondrat'eva, E. P. Kopantsev, T. V. Vinogradova, M. V. Zinov'eva, I. B. Zborovskaya, B. E. Polotsky and O. V. Sacharova. 2008. Downregulation of *RBSP3/CTDSPL, NPRL2/G21, RASSF1A, ITGA9, HYAL1* , and *HYAL2* in non-small cell lung cancer. Molecular Biology; 42(6): 859-869.
5. Arriagada R. and G. Straw. 2002. Oxford Textbook of Oncology. Oxford University Press.
6. Bae N.C., M. H. Chae, M. H. Lee, K. M. Kim, E. B. Lee, C. H. Kim, T. Park, S. B. Han, S. Jheon, T. H. Jung and J. Y. Park. 006. EGFR, ERBB2, and KRAS mutations in Korean non-small cell lung cancer patients. Cancer Genetics and Cytogenetics; 173 (2): 107-113.
9. Barbone F., M. Bovenzi, F. Cavallieri and G. Stanta. 1997. Cigarette Smoking and Histologic Type of Lung Cancer in Men. Chest; 112 (6): 1474-1479.
10. Bartel D .P. 2004. MicroRNAs: Genomics, biogenesis, mechanism, and function . Cell; 116(2): 281-297.

11. Bencardino K., M. Manzoni, S. Delfanti, A. Riccardi, M. Danova and G. R. Corazza. 2007. Epidermal growth factor receptor tyrosine kinase inhibitors for the treatment of non-small-cell lung cancer: results and open issues. Internal and Emergency Medicine; 2 (1): 3-12.

12. Bentwich I. 2005. Prediction and validation of microRNAs and their targets. FEBS Letters; 579 (26): 5904-5910.

13. Biesalski K. H., B. B. D. Mesquita , A. Chesson , F. Chytil , R. Grimble , R. J. J. Hermus, J. Köhrle , R. Lotan , K. Norpoth , U. Pastorino and D. Thurnham. 2008. European Consensus Statement on Lung Cancer: risk factors and prevention. CA Cancer Journal for Clinicians; 48(3): 167-176.

14. Boyle P. 2009. World Cancer Report 2009. Lyon, France: World Health Organization. International Agency for Research on Cancer.

15. Brennecke J., D. R. Hipfner, A. Stark, R. B. Russell and S. M. Cohen. 2003. bantam Encodes a Developmentally Regulated microRNA that Controls Cell Proliferation and Regulates the Proapoptotic Gene hid in Drosophila. Cell; 113(1): 25-36.

16. Brennecke J., A. Stark, R. B. Russell and S. M. Cohen. 2005. Principles of MicroRNA–Target Recognition. Plos Biology; 3(3): 404-418.

17. Calin A. G. and C. M. Croce. 1997. MicroRNA signatures in human cancers. Nature Reviews Cancer; 6: 857-866.

18. Calin A. G., C. Sevignani, C. D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri , M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, and C. M. Croce. 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers.

Proceedings of the National Academy of Sciences of the United States of America; 101(9): 2999-3004.

19. Carmell A. M. and G. J. Hannon. 2004. RNase III enzymes and the initiation of gene silencing.Nature Structural & Molecular Biology; 11: 214-218.
20. Catelinois O., A. Rogel, D. Laurier, S. Billon, D. Hemon, P. Verger and M. Tirmarche. 2006. Lung Cancer Attributable to Indoor Radon Exposure in France: Impact of the Risk Models and Uncertainty Analysis. Environ Health Perspect; 114(9): 1361–1366.
21. Clark M. A., S. H. Reynolds, M. Anderson and J. S. Wiest. 2004. Mutational activation of the *MAP3K8* protooncogene in lung cancer. Genes, Chromosomes and Cancer; 41(2): 99-108.
22. Collins L.G., C. Haines, R. Perkel and R.E Enck. Lung cancer: diagnosis and management. American Family Physician; 75(1): 56-63.
23. Croce M. C.2008. Oncogenes and cancer. The New England Journal of Medicine; 358(5): 502-511.
24. Dacic S., E. Sasatomi, P. A. Swalsky, D. W. Kim, S. D. Finkelstein and S. A. Yousem. 2004. Loss of Heterozygosity Patterns of Sclerosing Hemangioma of the Lung and Bronchioloalveolar Carcinoma Indicate a Similar Molecular Pathogene. Archives of Pathology & Laboratory Medicine; 128(8): 880-884.
25. Daniel A. Haber. 2004. Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib. The New England Journal of Medicine; 350(12): 2129-2139.

26. Darton J.A., D. M. Mcelvenny and J. T. Hodgson. 2006. Estimating the Number of Asbestos-related Lung Cancer Deaths in Great Britain from 1980 to 2000. The Annals of Occupational Hygiene; 50(1): 29-38.

27. Davies H., G. R. Bignell, C. Cox, P.Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing nd Y. Floyd. 2002. Mutations of the *BRAF* gene in human cancer. Nature; 417: 949-954.

28. Ding L., G.Getz, D. A. Wheeler, E. R. Mardis, M. D. McLellan, K. Cibulskis, C. Sougnez, H. Greulich, D. M. Muzny, M. B. Morgan, L. Fulton, R. S. Fulton, Q. Zhang, M. C. Wendl, M. S. Lawrence, D. E. Larson, K. Chen, D. J. Dooling, A.Sabo, A. C. Hawes and H. Shen. 2008. Somatic mutations affect key pathways in lung adenocarcinoma. Nature; 455: 1069-1075.

29. Hamilton W., T. J. Peters, A. Round and D. Sharp. 2005. What are the clinical features of lung cancer before the diagnosis is made? A population based case-control study. Thorax; 60: 1059-1065.

30. Herbst S. R., J. V. Heymach, and S. M. Lippman. 2008. **Molecular Origins of Cancer:** Lung Cancer. Th e new england journal o f medicine; 359: 1367-1380.

31. Hoffman C. P., K. S. Albain, J. D. Bitran and H. M. Golomb. 1984. Current Concepts in Small Cell Carcinoma of the Lung. CA Cancer Journal for Clinicians; 34: 269-281.

32. Hsua S. D., C. R. Acharyaa, B. S. Balakumarana, R. F. Riedela, M. K. Kima, M. Stevensona. S. Tuchmana. S. Mukherjeea. W. Barrya, H. K. Dressmana, J. R. Nevinsa, S. Powersd, D. Muf and Anil Pottia. 2009. Characterizing the developmental pathways TTF-1, NKX2–8, and PAX9 in lung cancer. Proceedings of the National Academy of Sciences of the United States of America; 106(13): 5312-5318

33. Hwang S., L. S. Cheng, G. Lozano, C. I. Amos, X. Gu and L. C. Strong. 2003. Lung cancer risk in germline p53 mutation carriers: association between an inherited cancer predisposition, cigarette smoking, and cancer risk. Human Genetics; 113(3): 238-243.

34. Engelman A. J., K. Zejnullahu, T. Mitsudomi, Y. Song, C. Hyland, J. O. Park, N. Lindeman, C. Gale, X. Zhao, J. Christensen, T. Kosaka, A. J. Holmes, A. M. Rogers, F. Cappuzzo, T. Mok, C. Lee, B. E. Johnson, L.C. Cantley and P. A. Janne. 2007. MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling. Science; 316(5827): 1039-1043.

35. Field W. R, D. J. Steck, B. J. Smith, C. P. Brus1, E. L. Fisher, J. S. Neuberger, C. E. Platz, R. A. Robinson, R. F. Woolson and C. F. Lynch. 2000. Residential Radon Gas Exposure and Lung Cancer, the Iowa Radon Lung Cancer Study. American Journal of Epidemiology; 151 (11): 1091-1102.

36. Figl A, S. Dominique, N. Eduardo, B. J. Lorenzo, B. Rafael, G. Andreas, T. Ranjit , P.Dolores, H. Kari, S. Dirk and K. Rajiv. 2010. Single-nucleotide polymorphisms in DNA-repair genes and cutaneous melanoma. Mutation research; 702(1):8-16

37. Filipowicz W., S. N. Bhattacharyya and N. Sonenberg. 2008. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nature Reviews Genetics; 9:102-114.

38. Fountain S. W. 1998. Surgery for lung cancer. Thorax; 53: 441.

39. Gaidatzis D., E. Nimwegen, J. Hausser and M. Zavolan. 2007. Inference of miRNA targets using evolutionary conservation and pathway analysis. BMC Bioinformatics; 8: 69-75.

40. Gaur A., D. A. Jewell, Y. Liang, D. Ridzon, J. H. Moore, C. Chen, V. R. Ambros and M. A. Israel. 2007. Characterization of MicroRNA Expression Levels and Their Biological Correlates in Human Cancer Cell Lines. Cancer Research; 67: 2458.

41. Griffiths-Jones S., H. K. Saini, S. Dongen and A. J. Enright. 2007. miRBase: tools for microRNA genomics. Nucleic Acid Research; 36(1): 54-58.

42. Govindan R., N. Page, D. Morgensztern, W. Read, R. Tierney, A. Vlahiotis, E. L. Spitznagel and J. Piccirillo. 2006. Changing Epidemiology of Small-Cell Lung Cancer in the United States Over the Last 30 Years: Analysis of the Surveillance, Epidemiologic, and End Results Database. Journal of Clinical Oncology; 24(28): 4539-4544.

43. Jackman M. D. and B. E. Johnson. 2005. Small-cell lung cancer. The Lancet; 366(9494): 1385-1396.

44. Jemal A., R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray and M. J.Thun, 2008. Cancer Statistics. CA Cancer Journal for Clinicians; 58(2): 71-96.

45. Ji H., Matthew R. Ramsey, D. N. Hayes, C. Fan, K. McNamara, P. Kozlowski, C. Torrice, M. C. Wu, T. Shimamura, S. A. Perera, M. Liang, D. Cai, G. N. Meyerson, D. J. Kwiatkowski, D. H. Castrillon, N. Bardeesy, N. E. Sharpless and K. Wong 2007. LKB1 modulates lung cancer differentiation and metastasis. Nature; 448: 807-810.

46. Ji P., S. Diederichs, W. Wang, S. Boing, R.Metzger, P.M. Schneider, N. Tidow, B. Brandt, H. Buerger, E. Bulk, M. Thomas, W. E Berdel, H. Serve and C. Müller-Tidow 2003. MALAT-1, a novel noncoding RNA, and thymosin β_4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene; 22: 8031-8041.

47. Johnson M. S., H. Grosshans, J. Shingara, M. Byrom, R. Jarvis, A. Cheng, E.Labourier, K. L. Reinert, D. Brown and F. J. Slack.2005. RAS Is Regulated by the let-7 MicroRNA Family. Cell; 120(5): 635-647.

48. Kanellopoulou C., S. A. Muljo, A. L. Kung, S. Ganesan, R. Drapkin, T. Jenuwein, D. M. Livingston and K. Rajewsky. 2005. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. Genes and Development; 19: 498-501.

49. Kawajiri K., K. Nakachi, K. Imai, J. Watanabe and S. Hayashi. 1993. Germ line polymorphisms of p53 and CYP1A1 genes involved in human lung cancer. Carcinogenesis; 14 (6): 1085-1089.

50. Kenichi S., O., Ryoichi, Y.Yasushi and M. Tetsuya. 2009. EGFR T790M Mutation: A Double Role in Lung Cancer Cell Survival? Journal of Thoracic Oncology; (1): 1-4.

51. Kim N. V. 2005. MicroRNA biogenesis: coordinated cropping and dicing. Molecular Cell Biology; 6: 376-385.

52. Krüger J. and M. Rehmsmeier. 2006. RNAhybrid: microRNA target prediction easy, fast and flexible. Nucleic Acid Research; 34(2): 451- 454.

53. Lewis P. B., C. B. Burge and D.P. Bartel. 2005. Conserved Seed Pairing, Often Flanked by Adenosines. Indicates that Thousands of Human Genes are MicroRNA Targets. Cell; 120(1): 15-20.

54. Lin C., K. Yeh, Y. Chang, N. C. Hsu and J. Chang. 2010. Resaerch detection of epidermal growth factor receptor mutations with multiplex PCR and primer extension in lung cancer. Journal of Biomedical Science; 17(1): 37-42.

55. Lynch J. T., D. W. Bell, R. Sordella, S. Gurubhagavatula,R. A. Okimoto, B.W. Brannigan. P. L. Harris, S. M. Haserlat,J. G. Supko, F.G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman and Patz F. E., P. C. Goodman and G.Bepler. 2000. Screening for Lung Cancer. The New England Journal of Medicine: 343(22): 1627-1633.

56. Maa H., Z. Hua, H. Wangb, G. Jina, Y. Wangb, W. Sunb, D. Chenc, T. Tiana, L. Jinbc, Q. Weid, D. Luc, W. Huangb and H. Shen. 2009. ERCC6/CSB gene polymorphisms and lung cancer risk. Cancer Letters; 273(1): 172-176.

57. M. B. Schabath , M. R. Spitz , G. L. Delclos, G. B. Gunn, L. W. Whitehead and X. Wu.2002. Association between asbestos exposure, cigarette smoking, myeloperoxidase (MPO) genotypes. and lung cancer risk. American Journal of Industrial Medicine; 42(1): 29--37.

58. Minami H, M. Yoshimura, Y. Miyamoto, H. Matsuoka, N. Tsubota. 2000. Lung cancer in women: sex-associated differences in survival of patients undergoing resection for lung cancer. Chest: 118(6):1603-1609.

59. Mountain F. C., 1986. A New International Staging System for Lung Cancer. Chest; 89(4): 225-233.

60. Murchison P. E., J. F. Partridge, O. H. Tam, S. Cheloufi and G. J. Hannon.2005.Characterization of Dicer-deficient murine embryonic stem cells. Proceedings of theNational Academy of Science of the United States of America; 102(34): 12135-12140.

61. Naoki K., T. Chen, W. G. Richards, D. J. Sugarbaker and Matthew Meyerson. 2002. Missense Mutations of the BRAF Gene in Human Lung Adenocarcinoma. Cancer Research: 62: 7001-7003.

62. Nicole F., S. Robert and M. Anne. 2010. Adaphostin toxicity in a sensitive non-small cell lung cancer model is mediated through Nrf2 signaling and heme oxygenase 1. Journal of Experimental & Clinical Cancer Research; 29(1): 29- 91.

63. Okudela K., M. Suzuki, S. Kageyama, T. Bunai, K. Nagura, H.Igarashi, K.Takamochi, K. Suzuki, T. Yamada, H. Niwa, R. Ohashi, H. Ogawa, H. Mori, H.Kitamura, T. Kaneko, T. Tsuneyoshi, H. Sugimura1. 2007. PIK3CA mutation and amplification in human lung cancer. Pathology International; 57(10): 664-671.

64. Ouellet L. D. and P. Provost. 2010. Current Knowledge of MicroRNAs and Noncoding RNAs in Virus-Infected Cells. Methods in Molecular Biology; 623(1): 35-65.

65. Paez J. G., P. A. Janne, J. C. Lee1, Sean Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson,and M. Meyerson. 2004. EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy. Science; 304(5676): 1497-1500.

66. Pedersen H., N. Brunner, D. Francis, K. Osterlind, E. Ronne, H. H. Hansen, K. Dano and J. Grondahl-Hansen.1997. Prognostic Impact of Urokinase, Urokinase Receptor, and Type 1 Plasminogen Activator Inhibitor in Squamous and Large Cell Lung Cancer Tissues. Cancer Research; 54: 4671-4675.

67. Perry M.C.,W. L. Eaton K. J., Propert , J. H. Ware , B. Zimmer, P Chahinian , A.Skarin, R.W. Carey , H.Kreisman and C. Faulkner. 1987. Chemotherapy with or without radiation therapy in limited small-cell carcinoma of the lung. The New England Journal of Medicine: 316(15):912-918.

68. Rajewsky N. 2006. microRNA target predictions in animals. Nature Genetics; 38: 8-13.

69. Roth A. J., F.Fossella, R. Komaki, M. B. Ryan, J. B. Putnam., J. S. Lee, H. Dhingra, L. D- Caro, M. Chasen, M. McGavran, E. N. Atkinson and W. K. Hong. 1994. A Randomized Trial Comparing Perioperative Chemotherapy and Surgery With Surgery Alone in Resectable Stage IIIA Non-Small-Cell Lung Cancer. Journal of the National Cancer Institute: 86(9): 673-680.

70. Saccone L. N., J. C. Wang, N. Breslau, E. O. Johnson, D. Hatsukami, S. F. Saccone, R. A. Grucza, L. Sun, W. Duan, J. Budde, R. C. Culverhouse, L. Fox, A. L. Hinrichs, J. H. Steinbach, M. Wu, J. P. Rice, A. M. Goate, and L. J. Bierut. 2009. The CHRNA5-CHRNA3-CHRNB4 Nicotinic Receptor Subunit Gene Cluster Affects Risk for Nicotine Dependence in African-Americans and in European-Americans. Cancer Research; 69(17): 6848-6856.

71. Sanchez-Cespedes M., A. L. Reed , M. Buta, L.Wu , W. H. Westra , J. G. Herman, S.C. Yang, J. Jen and D. Sidransky. 1999. Inactivation of the INK4A/ARF locus frequently coexists with TP53 mutations in non-small cell lung cancer. Oncogene; 18(43): 5843-5849.

72. Sandler A., R. Gray, M. C. Perry, J. Brahmer, J. H. Schiller, A. Dowlati, R. Lilenbaum, and D. H. Johnson. 2006. Carboplatin Alone or with Bevacizumab for Non– Small-Cell Lung Cancer. The New England Journal o f Medicine; 355:2542-50.

73. Sasaki H., Y. Hikosaka , O. Kawano , S. Moriyama , M. Yano and Y. Fujii. 2010. Methylation of the DLEC1 gene correlates with poor prognosis in Japanese lung cancer patients. Oncology Letters; 1(2): 283-287.

74. Sassen S., E. A. Miska and C. Caldas. 2007. MicroRNA—implications for cancer. Virchows Archiv; 452(1): 1-10.

75. Satrom P., B. S.E. Healel. O. Snove. L. Aagaard. J. Alluin and J. J. Rossi. 2007. Distance constraints between microRNA target sites dictate efficacy and cooperativity. Nucleic Acid Research; 35(7): 2333-2342.

76. Schick S. and S. Glantz. 2005. Philip Morris toxicological experiments with fresh sidestream smoke: more toxic than mainstream smoke. Tobacco Control; 14: 396-404

77. Seffrin R. J, D. Hill , W. Burkart, I. Magrath, R. A. Badwe , T. Ngoma , A. Mohar and N. Grey. 2009. It Is Time to Include Cancer and Other Noncommunicable Diseases in the Millennium Development Goals. CA Cancer Journal for Clinicians; 59(5): 282-284.

78. Sekido Y., K. M. Fong and J. D. Minna. 2003. Molecular Genetics of Lung Cancer. Annual Review of Medicine; 54: 73-87.

79. Sethupathy P., M. Megraw and A. G. Hatzigeorgiou. 2006. A guide through present computational approaches for the identification of mammalian microRNA targets. Nature Methods; 3: 881-886.

80. Simon G., R. J.Ginsberg and J. C. Ruckdeschel. 2001. Small-cell lung cancer. Chest; 11(1):165-188.

81. Shih T. Y and M. T. Halpern. 2008. Economic Evaluations of Medical Care Interventions for Cancer Patients: How, Why, and What Does it Mean? CA: A Cancer Journal for Clinicians; 58(4): 231-244.

82. Soda M., Y. Lim Choi, M. Enomoto, S. Takada, Y. Yamashita, S. Ishikawa, S. Fujiwara, H. Watanabe, K. Kurashina, H. Hatanaka, M. Bando, S. Ohno, Y. Ishikawa, H. Aburatani, T. Niki, Y. Sohara, Y. Sugiyama and H. Mano. 2007. Identification of the transforming *EML4–ALK* fusion gene in non-small-cell lung cancer. Nature; 448: 561-566.

83. Soifer S.H., J. J. Rossi and P. Satrom. 2007. MicroRNAs in Disease and Potential Therapeutic Applications. Molecular Therapy: 15(12): 2070-2079.

84. Steven A. A., D. P. Anthony, E. A. Alawi, Y. Zhu, M. Sanchez-Cespedes, S. C. Yang, G. B. Haasler, A. Kajdacsy-Balla, M. J. Demeure and D. Sidransky. 2001. Cigarette smoking is strongly associated with mutation of the K*ras* gene in patients with primary adenocarcinoma of the lung. Cancer; 92(6): 1525–1530.

85. Stephens P., C. Hunter, G.Bignel, S. Edkins, H. Davies, J. Teague, C. Stevens, S. O'Meara, R. Smith, A.Parker, A.Barthorpe, M. Blow, L. Brackenbury, A. Butler, O. Clarke, J. Cole, E. Dicks, A. Dike and A.Drozd. 2004. Lung cancer: Intragenic ERBB2 kinase mutations in tumours. Nature; 431: 525-526.

86. Subramanian J.,and R.Govindan. 2007. Lung Cancer in Never Smokers: A Review. Journal of Clinical Oncology; 25(5): 561-570.

87. Sung S. J., Y. M. Whang, W. B. You, J. S. Jung, H. D. Shin, B. L. Park, L. H. Kim, H. S. Cheong, I. K. Choi, S. C. Oh, H. J. Sung, J. S. Kim and Y. H. Kim. 2006. SNPs study of lung cancer candidate genes in Korean lung cancer patients and normal population. Cancer Research; 47: 6315-6321.

88. Yong Zhao Y. and D. Srivastava.2007. A developmental view of microRNA function. Trends in Biochemical Sciences: 32(4) : 189-197.

89. Veeriah S., B. S Taylor, S. Meng, F. Fang, E. Yilmaz, I. Vivanco, M. Janakiraman, N. Schultz, A. J. Hanrahan, W. Pao, M. Ladanyi, C. Sander, A. Heguy, E.C. Holland, P.B .Paty, P. S. Mischel, L. Liau, T. F Cloughesy, I. K Mellinghoff, D. B Solit and T. A. Chan. 2010. Somatic mutations of the Parkinson's disease-associated gene *PARK2* in glioblastoma and other human malignancies. Nature Genetics; 42:77-82.

90. Vella C. M., E. Choi, S. Lin, K. Reinert and F. J. Slack. 2004. The C. elegans microRNA let-7 binds to imperfect let-7 complementary sites from the lin-41 3'UTR. Genes and Development: 18: 132-137.

91. Vella C. M., K. Reinert and F. J. Slack.2004. Architecture of a Validated MicroRNA::Target Interaction. Chemistry & Biology: 11(12): 1619-1623.

92. Wang S. S., E. D. Esplin, J. L. Li, L. Huang, A. Gazdar, J. Minna and G. A. Evans. 1998. Alterations of the PPP2R1B Gene in Human Lung and Colon Cancer. Science; 282(5387): 284-287.

93. Weisse J. G., L. T. Bemis, E. Nakajima, M. Sugita, D. K. Birks, W. A. Robinson, M. Varella-Garcia, P. A. Bunn Jr, J. Haney, B. A. Helfrich, H. Kato, F. R. Hirsch and W. A. Franklin. 2008. EGFR regulation by microRNA in lung cancer: correlation with clinical response and survival to gefitinib and EGFR expression in cell lines. Annals of Oncology; 19 (6): 1053-1059.

94. W.H.O. 2007:12. Future health: projected deaths for selected causes to 2030. World Health Organization: In: World Health Statistics 2007.

95. Wingo A. P., T. Tong and S. Bolden. 1995. Cancer statistics. 1995. CA Cancer Journal for Clinicians; 45(1): 8-30.

No.	ID	Accession.	Cromosome	Start	End	Strand
1	hsa-let-7a-1	MI0000060	9	96938239	96938318	+
2	hsa-let-7a-2	MI0000061	11	122017230	122017301	-
3	hsa-let-7a-3	MI0000062	22	46508629	46508702	+
4	hsa-let-7b	MI0000063	22	46509566	46509648	+
5	hsa-let-7c	MI0000064	21	17912148	17912231	+
6	hsa-let-7d	MI0000065	9	96941116	96941202	+
7	hsa-let-7e	MI0000066	19	52196039	52196117	+
8	hsa-let-7f-1	MI0000067	9	96938629	96938715	+
9	hsa-let-7f-2	MI0000068	X	53584153	53584235	-
10	hsa-let-7g	MI0000433	3	52302294	52302377	-
11	hsa-let-7i	MI0000434	12	62997466	62997549	+
12	hsa-mir-1-1	MI0000651	20	61151513	61151583	+
13	hsa-mir-1-2	MI0000437	18	19408965	19409049	-
14	hsa-mir-7-1	MI0000263	9	86584663	86584772	-
15	hsa-mir-7-2	MI0000264	15	89155056	89155165	+
16	hsa-mir-7-3	MI0000265	19	4770682	4770791	+
17	hsa-mir-9-1	MI0000466	1	156390133	156390221	-
18	hsa-mir-9-2	MI0000467	5	87962671	87962757	-
19	hsa-mir-9-3	MI0000468	15	89911248	89911337	+
20	hsa-mir-10a	MI0000266	17	46657200	46657309	-
21	hsa-mir-10b	MI0000267	2	177015031	177015140	+
22	hsa-mir-15a	MI0000069	13	50623255	50623337	-
23	hsa-mir-15b	MI0000438	3	160122376	160122473	+
24	hsa-mir-16-1	MI0000070	13	50623109	50623197	-
25	hsa-mir-16-2	MI0000115	3	160122533	160122613	+
26	hsa-mir-17	MI0000071	13	92002859	92002942	+
27	hsa-mir-18a	MI0000072	13	92003005	92003075	+
28	hsa-mir-18b	MI0001518	X	133304071	133304141	-
29	hsa-mir-19a	MI0000073	13	92003145	92003226	+
30	hsa-mir-19b-1	MI0000074	13	92003446	92003532	+
31	hsa-mir-19b-2	MI0000075	X	133303701	133303796	-
32	hsa-mir-20a	MI0000076	13	92003319	92003389	+
33	hsa-mir-20b	MI0001519	X	133303839	133303907	-
34	hsa-mir-21	MI0000077	17	57918627	57918698	+
35	hsa-mir-22	MI0000078	17	1617197	1617281	-
36	hsa-mir-23a	MI0000079	19	13947401	13947473	-
37	hsa-mir-23b	MI0000439	9	97847490	97847586	+
38	hsa-mir-24-1	MI0000080	9	97848303	97848370	+
39	hsa-mir-24-2	MI0000081	19	13947101	13947173	-
40	hsa-mir-25	MI0000082	7	99691183	99691266	-
41	hsa-mir-26a-1	MI0000083	3	38010895	38010971	+
42	hsa-mir-26a-2	MI0000750	12	58218392	58218475	-

Contd....

43	hsa-mir-26b	MI0000084	2	219267369	219267445	+
44	hsa-mir-27a	MI0000085	19	13947254	13947331	-
45	hsa-mir-27b	MI0000440	9	97847727	97847823	+
46	hsa-mir-28	MI0000086	3	188406569	188406654	+
47	hsa-mir-29a	MI0000087	7	130561506	130561569	-
48	hsa-mir-29b-1	MI0000105	7	130562218	130562298	-
49	hsa-mir-29b-2	MI0000107	1	207975788	207975868	-
50	hsa-mir-29c	MI0000735	1	207975197	207975284	-
51	hsa-mir-30a	MI0000088	6	72113254	72113324	-
52	hsa-mir-30b	MI0000441	8	135812763	135812850	-
53	hsa-mir-30c-1	MI0000736	1	41222956	41223044	+
54	hsa-mir-30c-2	MI0000254	6	72086663	72086734	-
55	hsa-mir-30d	MI0000255	8	135817119	135817188	-
56	hsa-mir-30e	MI0000749	1	41220027	41220118	+
57	hsa-mir-31	MI0000089	9	21512114	21512184	-
58	hsa-mir-32	MI0000090	9	111808509	111808578	-
59	hsa-mir-33a	MI0000091	22	42296948	42297016	+
60	hsa-mir-33b	MI0003646	17	17717150	17717245	-
61	hsa-mir-34a	MI0000268	1	9211727	9211836	-
62	hsa-mir-34b	MI0000742	11	111383663	111383746	+
63	hsa-mir-34c	MI0000743	11	111384164	111384240	+
64	hsa-mir-92a-1	MI0000093	13	92003568	92003645	+
65	hsa-mir-92a-2	MI0000094	X	133303568	133303642	-
66	hsa-mir-92b	MI0003560	1	155164968	155165063	+
67	hsa-mir-93	MI0000095	7	99691391	99691470	-
68	hsa-mir-95	MI0000097	4	8007028	8007108	-
69	hsa-mir-96	MI0000098	7	129414532	129414609	-
70	hsa-mir-98	MI0000100	X	53583184	53583302	-
71	hsa-mir-99a	MI0000101	21	17911409	17911489	+
72	hsa-mir-99b	MI0000746	19	52195865	52195934	+
73	hsa-mir-100	MI0000102	11	122022937	122023016	-
74	hsa-mir-101-1	MI0000103	1	65524117	65524191	-
75	hsa-mir-101-2	MI0000739	9	4850297	4850375	+
76	hsa-mir-103-1	MI0000109	5	167987901	167987978	-
77	hsa-mir-103-1-as	MI0007261	5	167987909	167987970	+
78	hsa-mir-103-2	MI0000108	20	3898141	3898218	+
79	hsa-mir-103-2-as	MI0007262	20	3898149	3898210	-
80	hsa-mir-105-1	MI0000111	X	151560691	151560771	-
81	hsa-mir-105-2	MI0000112	X	151562884	151562964	-
82	hsa-mir-106a	MI0000113	X	133304228	133304308	-
83	hsa-mir-106b	MI0000734	7	99691616	99691697	-
84	hsa-mir-107	MI0000114	10	91352504	91352584	-
85	hsa-mir-122	MI0000442	18	56118306	56118390	+
86	hsa-mir-124-1	MI0000443	8	9760898	9760982	-

Contd....

87	hsa-mir-124-2	MI0000444	8	65291706	65291814	+
88	hsa-mir-124-3	MI0000445	20	61809852	61809938	+
89	hsa-mir-125a	MI0000469	19	52196507	52196592	+
90	hsa-mir-125b-1	MI0000446	11	121970465	121970552	-
91	hsa-mir-125b-2	MI0000470	21	17962557	17962645	+
92	hsa-mir-126	MI0000471	9	139565054	139565138	+
93	hsa-mir-127	MI0000472	14	101349316	101349412	+
94	hsa-mir-128-1	MI0000447	2	136422967	136423048	+
95	hsa-mir-128-2	MI0000727	3	35785968	35786051	+
96	hsa-mir-129-1	MI0000252	7	127847925	127847996	+
97	hsa-mir-129-2	MI0000473	11	43602944	43603033	+
98	hsa-mir-130a	MI0000448	11	57408671	57408759	+
99	hsa-mir-130b	MI0000748	22	22007593	22007674	+
100	hsa-mir-132	MI0000449	17	1953202	1953302	-
101	hsa-mir-133a-1	MI0000450	18	19405659	19405746	-
102	hsa-mir-133a-2	MI0000451	20	61162119	61162220	+
103	hsa-mir-133b	MI0000822	6	52013721	52013839	+
104	hsa-mir-134	MI0000474	14	101521024	101521096	+
105	hsa-mir-135a-1	MI0000452	3	52328235	52328324	-
106	hsa-mir-135a-2	MI0000453	12	97957590	97957689	+
107	hsa-mir-135b	MI0000810	1	205417430	205417526	-
108	hsa-mir-136	MI0000475	14	101351039	101351120	+
109	hsa-mir-137	MI0000454	1	98511626	98511727	-
110	hsa-mir-138-1	MI0000476	3	44155704	44155802	+
111	hsa-mir-138-2	MI0000455	16	56892430	56892513	+
112	hsa-mir-139	MI0000261	11	72326107	72326174	-
113	hsa-mir-140	MI0000456	16	69966984	69967083	+
114	hsa-mir-141	MI0000457	12	7073260	7073354	+
115	hsa-mir-142	MI0000458	17	56408593	56408679	-
116	hsa-mir-143	MI0000459	5	148808481	148808586	+
117	hsa-mir-144	MI0000460	17	27188551	27188636	-
118	hsa-mir-145	MI0000461	5	148810209	148810296	+
119	hsa-mir-146a	MI0000477	5	159912359	159912457	+
120	hsa-mir-146b	MI0003129	10	104196269	104196341	+
121	hsa-mir-147	MI0000262	9	123007257	123007328	-
122	hsa-mir-147b	MI0005544	15	45725248	45725327	+
123	hsa-mir-148a	MI0000253	7	25989539	25989606	-
124	hsa-mir-148b	MI0000811	12	54731000	54731098	+
125	hsa-mir-149	MI0000478	2	241395418	241395506	+
126	hsa-mir-150	MI0000479	19	50004042	50004125	-
127	hsa-mir-151	MI0000809	8	141742663	141742752	-
128	hsa-mir-152	MI0000462	17	46114527	46114613	-
129	hsa-mir-153-1	MI0000463	2	220158833	220158922	-
130	hsa-mir-153-2	MI0000464	7	157367028	157367114	-

Contd....

131	hsa-mir-154	MI0000480	14	101526092	101526175	+
132	hsa-mir-155	MI0000681	21	26946292	26946356	+
133	hsa-mir-181a-1	MI0000289	1	198828173	198828282	-
134	hsa-mir-181a-2	MI0000269	9	127454721	127454830	+
135	hsa-mir-181b-1	MI0000270	1	198828002	198828111	-
136	hsa-mir-181b-2	MI0000683	9	127455989	127456077	+
137	hsa-mir-181c	MI0000271	19	13985513	13985622	+
138	hsa-mir-181d	MI0003139	19	13985689	13985825	+
139	hsa-mir-182	MI0000272	7	129410223	129410332	-
140	hsa-mir-183	MI0000273	7	129414745	129414854	-
141	hsa-mir-184	MI0000481	15	79502130	79502213	+
142	hsa-mir-185	MI0000482	22	20020662	20020743	+
143	hsa-mir-186	MI0000483	1	71533314	71533399	-
144	hsa-mir-187	MI0000274	18	33484781	33484889	-
145	hsa-mir-188	MI0000484	X	49768109	49768194	+
146	hsa-mir-190	MI0000486	15	63116156	63116240	+
147	hsa-mir-190b	MI0005545	1	154166141	154166219	-
148	hsa-mir-191	MI0000465	3	49058051	49058142	-
149	hsa-mir-192	MI0000234	11	64658609	64658718	-
150	hsa-mir-193a	MI0000487	17	29887015	29887102	+
151	hsa-mir-193b	MI0003137	16	14397824	14397906	+
152	hsa-mir-194-1	MI0000488	1	220291499	220291583	-
153	hsa-mir-194-2	MI0000732	11	64658827	64658911	-
154	hsa-mir-195	MI0000489	17	6920934	6921020	-
155	hsa-mir-196a-1	MI0000238	17	46709852	46709921	-
156	hsa-mir-196a-2	MI0000279	12	54385522	54385631	+
157	hsa-mir-196b	MI0001150	7	27209099	27209182	-
158	hsa-mir-197	MI0000239	1	110141515	110141589	+
159	hsa-mir-198	MI0000240	3	120114515	120114576	-
160	hsa-mir-199a-1	MI0000242	19	10928102	10928172	-
161	hsa-mir-199a-2	MI0000281	1	172113675	172113784	-
162	hsa-mir-199b	MI0000282	9	131007000	131007109	-
163	hsa-mir-200a	MI0000737	1	1103243	1103332	+
164	hsa-mir-200b	MI0000342	1	1102484	1102578	+
165	hsa-mir-200c	MI0000650	12	7072862	7072929	+
166	hsa-mir-202	MI0003130	10	135061015	135061124	-
167	hsa-mir-203	MI0000283	14	104583742	104583851	+
168	hsa-mir-204	MI0000284	9	73424891	73425000	-
169	hsa-mir-205	MI0000285	1	209605478	209605587	+
170	hsa-mir-206	MI0000490	6	52009147	52009232	+
171	hsa-mir-208a	MI0000251	14	23857805	23857875	-
172	hsa-mir-208b	MI0005570	14	23887196	23887272	-
173	hsa-mir-210	MI0000286	11	568089	568198	-
174	hsa-mir-211	MI0000287	15	31357235	31357344	-

Contd....

175	hsa-mir-212	MI0000288	17	1953565	1953674	-
176	hsa-mir-214	MI0000290	1	172107938	172108047	-
177	hsa-mir-215	MI0000291	1	220291195	220291304	-
178	hsa-mir-216a	MI0000292	2	56216085	56216194	-
179	hsa-mir-216b	MI0005569	2	56227849	56227930	-
180	hsa-mir-217	MI0000293	2	56210102	56210211	-
181	hsa-mir-218-1	MI0000294	4	20529898	20530007	+
182	hsa-mir-218-2	MI0000295	5	168195151	168195260	-
183	hsa-mir-219-1	MI0000296	6	33175612	33175721	+
			HSCHR6_MHC_COX	33097238	33097347	+
			HSCHR6_MHC_DBB	33153522	33153631	+
			HSCHR6_MHC_MANN	33329535	33329644	+
			HSCHR6_MHC_MCF	33345976	33346085	+
			HSCHR6_MHC_QBL	33104442	33104551	+
184	hsa-mir-219-2	MI0000740	9	131154897	131154993	-
185	hsa-mir-220a	MI0000297	X	122695946	122696055	-
186	hsa-mir-220b	MI0005529	19	6495959	6496045	+
187	hsa-mir-220c	MI0005536	19	49063529	49063611	-
188	hsa-mir-221	MI0000298	X	45605585	45605694	-
189	hsa-mir-222	MI0000299	X	45606421	45606530	-
190	hsa-mir-223	MI0000300	X	65238712	65238821	+
191	hsa-mir-224	MI0000301	X	151127050	151127130	-
192	hsa-mir-296	MI0000747	20	57392670	57392749	-
193	hsa-mir-297	MI0005775	4	111781738	111781803	-
194	hsa-mir-298	MI0005523	20	57393281	57393368	-
195	hsa-mir-299	MI0000744	14	101490131	101490193	+
196	hsa-mir-300	MI0005525	14	101507700	101507782	+
197	hsa-mir-301a	MI0000745	17	57228497	57228582	-
198	hsa-mir-301b	MI0005568	22	22007270	22007347	+
199	hsa-mir-302a	MI0000738	4	113569339	113569407	-
200	hsa-mir-302b	MI0000772	4	113569641	113569713	-
201	hsa-mir-302c	MI0000773	4	113569519	113569586	-
202	hsa-mir-302d	MI0000774	4	113569160	113569227	-
203	hsa-mir-302e	MI0006417	11	7255997	7256068	+
204	hsa-mir-302f	MI0006418	18	27878876	27878926	+
205	hsa-mir-320a	MI0000542	8	22102475	22102556	-
206	hsa-mir-320b-1	MI0003776	1	117214371	117214449	+
207	hsa-mir-320b-2	MI0003839	1	224444706	224444843	-
208	hsa-mir-320c-1	MI0003778	18	19263471	19263558	+

Contd....

209	hsa-mir-320c-2	MI0008191	18	21901650	21901699	+
210	hsa-mir-320d-1	MI0008190	13	41301964	41302011	-
211	hsa-mir-320d-2	MI0008192	X	140008337	140008384	-
212	hsa-mir-323	MI0000807	14	101492069	101492154	+
213	hsa-mir-324	MI0000813	17	7126616	7126698	-
214	hsa-mir-325	MI0000824	X	76225829	76225926	-
215	hsa-mir-326	MI0000808	11	75046136	75046230	-
216	hsa-mir-328	MI0000804	16	67236224	67236298	-
217	hsa-mir-329-1	MI0001725	14	101493122	101493201	+
218	hsa-mir-329-2	MI0001726	14	101493437	101493520	+
219	hsa-mir-330	MI0000803	19	46142252	46142345	-
220	hsa-mir-331	MI0000812	12	95702196	95702289	+
221	hsa-mir-335	MI0000816	7	130135952	130136045	+
222	hsa-mir-337	MI0000806	14	101340830	101340922	+
223	hsa-mir-338	MI0000814	17	79099683	79099749	-
224	hsa-mir-339	MI0000815	7	1062569	1062662	-
225	hsa-mir-340	MI0000802	5	179442303	179442397	-
226	hsa-mir-342	MI0000805	14	100575992	100576090	+
227	hsa-mir-345	MI0000825	14	100774196	100774293	+
228	hsa-mir-346	MI0000826	10	88024451	88024545	-
229	hsa-mir-361	MI0000760	X	85158641	85158712	-
230	hsa-mir-362	MI0000762	X	49773572	49773636	+
231	hsa-mir-363	MI0000764	X	133303408	133303482	-
232	hsa-mir-365-1	MI0000767	16	14403142	14403228	+
233	hsa-mir-365-2	MI0000769	17	29902430	29902540	+
234	hsa-mir-367	MI0000775	4	113569030	113569097	-
235	hsa-mir-369	MI0000777	14	101531935	101532004	+
236	hsa-mir-370	MI0000778	14	101377476	101377550	+
237	hsa-mir-371	MI0000779	19	54290929	54290995	+
238	hsa-mir-372	MI0000780	19	54291144	54291210	+
239	hsa-mir-373	MI0000781	19	54291959	54292027	+
240	hsa-mir-374a	MI0000782	X	73507121	73507192	-
241	hsa-mir-374b	MI0005566	X	73438382	73438453	-
242	hsa-mir-375	MI0000783	2	219866367	219866430	-
243	hsa-mir-376a-1	MI0000784	14	101507119	101507186	+
244	hsa-mir-376a-2	MI0003529	14	101506406	101506485	+
245	hsa-mir-376b	MI0002466	14	101506773	101506872	+
246	hsa-mir-376c	MI0000776	14	101506027	101506092	+
247	hsa-mir-377	MI0000785	14	101528387	101528455	+
248	hsa-mir-378	MI0000786	5	149112388	149112453	+
249	hsa-mir-379	MI0000787	14	101488403	101488469	+
250	hsa-mir-380	MI0000788	14	101491354	101491414	+
251	hsa-mir-381	MI0000789	14	101512257	101512331	+
252	hsa-mir-382	MI0000790	14	101520643	101520718	+

Contd....

253	hsa-mir-383	MI0000791	8	14710947	14711019	-
254	hsa-mir-384	MI0001145	X	76139698	76139785	-
255	hsa-mir-409	MI0001735	14	101531637	101531715	+
256	hsa-mir-410	MI0002465	14	101532249	101532328	+
257	hsa-mir-411	MI0003675	14	101489662	101489757	+
258	hsa-mir-412	MI0002464	14	101531784	101531874	+
259	hsa-mir-421	MI0003685	X	73438212	73438296	-
260	hsa-mir-422a	MI0001444	15	64163129	64163218	-
261	hsa-mir-423	MI0001445	17	28444097	28444190	+
262	hsa-mir-424	MI0001446	X	133680644	133680741	-
263	hsa-mir-425	MI0001448	3	49057581	49057667	-
264	hsa-mir-429	MI0001641	1	1104385	1104467	+
265	hsa-mir-431	MI0001721	14	101347344	101347457	+
266	hsa-mir-432	MI0003133	14	101350820	101350913	+
267	hsa-mir-433	MI0001723	14	101348223	101348315	+
268	hsa-mir-448	MI0001637	X	114058017	114058127	+
269	hsa-mir-449a	MI0001648	5	54466360	54466450	-
270	hsa-mir-449b	MI0003673	5	54466474	54466570	-
271	hsa-mir-449c	MI0003823	5	54468090	54468181	-
272	hsa-mir-450a-1	MI0001652	X	133674371	133674461	-
273	hsa-mir-450a-2	MI0003187	X	133674538	133674637	-
274	hsa-mir-450b	MI0005531	X	133674215	133674292	-
275	hsa-mir-451	MI0001729	17	27188387	27188458	-
276	hsa-mir-452	MI0001733	X	151128100	151128184	-
277	hsa-mir-453	MI0001727	14	101522527	101522606	+
278	hsa-mir-454	MI0003820	17	57215119	57215233	-
279	hsa-mir-455	MI0003513	9	116971714	116971809	+
280	hsa-mir-483	MI0002467	11	2155364	2155439	-
281	hsa-mir-484	MI0002468	16	15737151	15737229	+
282	hsa-mir-485	MI0002469	14	101521756	101521828	+
283	hsa-mir-486	MI0002470	8	41517959	41518026	-
284	hsa-mir-487a	MI0002471	14	101518783	101518862	+
285	hsa-mir-487b	MI0003530	14	101512792	101512875	+
286	hsa-mir-488	MI0003123	1	176998499	176998581	-
287	hsa-mir-489	MI0003124	7	93113248	93113331	-
288	hsa-mir-490	MI0003125	7	136587914	136588041	+
289	hsa-mir-491	MI0003126	9	20716104	20716187	+
290	hsa-mir-492	MI0003131	12	95228174	95228289	+
291	hsa-mir-493	MI0003132	14	101335397	101335485	+
292	hsa-mir-494	MI0003134	14	101495971	101496051	+
293	hsa-mir-495	MI0003135	14	101500092	101500173	+
294	hsa-mir-496	MI0003136	14	101526910	101527011	+
295	hsa-mir-497	MI0003138	17	6921230	6921341	-
296	hsa-mir-498	MI0003142	19	54177451	54177574	+

Contd....

297	hsa-mir-499	MI0003183	20	33578179	33578300	+
298	hsa-mir-500	MI0003184	X	49773039	49773122	+
299	hsa-mir-501	MI0003185	X	49774330	49774413	+
300	hsa-mir-502	MI0003186	X	49779206	49779291	+
301	hsa-mir-503	MI0003188	X	133680358	133680428	-
302	hsa-mir-504	MI0003189	X	137749872	137749954	-
303	hsa-mir-505	MI0003190	X	139006307	139006390	-
304	hsa-mir-506	MI0003193	X	146312238	146312361	-
305	hsa-mir-507	MI0003194	X	146312502	146312595	-
306	hsa-mir-508	MI0003195	X	146318431	146318545	-
307	hsa-mir-509-1	MI0003196	X	146342050	146342143	-
308	hsa-mir-509-2	MI0005530	X	146340278	146340368	-
309	hsa-mir-509-3	MI0005717	X	146341170	146341244	-
310	hsa-mir-510	MI0003197	X	146353853	146353926	-
311	hsa-mir-511-1	MI0003127	10	17887107	17887193	+
312	hsa-mir-511-2	MI0003128	10	18134036	18134122	+
313	hsa-mir-512-1	MI0003140	19	54169933	54170016	+
314	hsa-mir-512-2	MI0003141	19	54172411	54172508	+
315	hsa-mir-513a-1	MI0003191	X	146294981	146295109	-
316	hsa-mir-513a-2	MI0003192	X	146307344	146307470	-
317	hsa-mir-513b	MI0006648	X	146280562	146280645	-
318	hsa-mir-513c	MI0006649	X	146271222	146271305	-
319	hsa-mir-514-1	MI0003198	X	146360765	146360862	-
320	hsa-mir-514-2	MI0003199	X	146363461	146363548	-
321	hsa-mir-514-3	MI0003200	X	146366159	146366246	-
322	hsa-mir-515-1	MI0003144	19	54182257	54182339	+
323	hsa-mir-515-2	MI0003147	19	54188263	54188345	+
324	hsa-mir-516a-1	MI0003180	19	54259995	54260084	+
325	hsa-mir-516a-2	MI0003181	19	54264387	54264476	+
326	hsa-mir-516b-1	MI0003172	19	54240099	54240188	+
327	hsa-mir-516b-2	MI0003167	19	54228696	54228780	+
328	hsa-mir-517a	MI0003161	19	54215522	54215608	+
329	hsa-mir-517b	MI0003165	19	54224330	54224396	+
330	hsa-mir-517c	MI0003174	19	54244567	54244661	+
331	hsa-mir-518a-1	MI0003170	19	54234260	54234344	+
332	hsa-mir-518a-2	MI0003173	19	54242587	54242673	+
333	hsa-mir-518b	MI0003156	19	54205991	54206073	+
334	hsa-mir-518c	MI0003159	19	54211989	54212089	+
335	hsa-mir-518d	MI0003171	19	54238131	54238217	+
336	hsa-mir-518e	MI0003169	19	54233092	54233179	+
337	hsa-mir-518f	MI0003154	19	54203269	54203355	+
338	hsa-mir-519a-1	MI0003178	19	54255651	54255735	+
339	hsa-mir-519a-2	MI0003182	19	54265598	54265684	+
340	hsa-mir-519b	MI0003151	19	54198467	54198547	+

Contd....

341	hsa-mir-519c	MI0003148	19	54189723	54189809	+
342	hsa-mir-519d	MI0003162	19	54216601	54216688	+
343	hsa-mir-519e	MI0003145	19	54183194	54183277	+
344	hsa-mir-520a	MI0003149	19	54194135	54194219	+
345	hsa-mir-520b	MI0003155	19	54204481	54204541	+
346	hsa-mir-520c	MI0003158	19	54210707	54210793	+
347	hsa-mir-520d	MI0003164	19	54223350	54223436	+
348	hsa-mir-520e	MI0003143	19	54178965	54179051	+
349	hsa-mir-520f	MI0003146	19	54185413	54185499	+
350	hsa-mir-520g	MI0003166	19	54225420	54225509	+
351	hsa-mir-520h	MI0003175	19	54245766	54245853	+
352	hsa-mir-521-1	MI0003176	19	54251890	54251976	+
353	hsa-mir-521-2	MI0003163	19	54219848	54219934	+
354	hsa-mir-522	MI0003177	19	54254465	54254551	+
355	hsa-mir-523	MI0003153	19	54201639	54201725	+
356	hsa-mir-524	MI0003160	19	54214256	54214342	+
357	hsa-mir-525	MI0003152	19	54200787	54200871	+
358	hsa-mir-526a-1	MI0003157	19	54209506	54209590	+
359	hsa-mir-526a-2	MI0003168	19	54230176	54230240	+
360	hsa-mir-526b	MI0003150	19	54197647	54197729	+
361	hsa-mir-527	MI0003179	19	54257272	54257356	+
362	hsa-mir-532	MI0003205	X	49767754	49767844	+
363	hsa-mir-539	MI0003514	14	101513658	101513735	+
364	hsa-mir-541	MI0005539	14	101530832	101530915	+
365	hsa-mir-542	MI0003686	X	133675371	133675467	-
366	hsa-mir-543	MI0005565	14	101498324	101498401	+
367	hsa-mir-544	MI0003515	14	101514995	101515085	+
368	hsa-mir-545	MI0003516	X	73506939	73507044	-
369	hsa-mir-548a-1	MI0003593	6	18572015	18572111	+
370	hsa-mir-548a-2	MI0003598	6	135560298	135560394	+
371	hsa-mir-548a-3	MI0003612	8	105496597	105496693	-
372	hsa-mir-548b	MI0003596	6	119390212	119390308	-
373	hsa-mir-548c	MI0003630	12	65016289	65016385	+
374	hsa-mir-548d-1	MI0003668	8	124360274	124360370	-
375	hsa-mir-548d-2	MI0003671	17	65467605	65467701	-
376	hsa-mir-548e	MI0006344	10	112748684	112748771	+
377	hsa-mir-548f-1	MI0006374	10	56367634	56367717	-
378	hsa-mir-548f-2	MI0006375	2	213290987	213291084	-
379	hsa-mir-548f-3	MI0006376	5	109849530	109849616	-
380	hsa-mir-548f-4	MI0006377	7	147075109	147075213	-
381	hsa-mir-548f-5	MI0006378	X	32659591	32659676	-
382	hsa-mir-548g	MI0006395	4	148265781	148265869	-
383	hsa-mir-548h-1	MI0006411	14	64561742	64561843	-
384	hsa-mir-548h-2	MI0006412	16	11400297	11400384	-

Contd....

385	hsa-mir-548h-3	MI0006413	17	13446846	13446963	-
386	hsa-mir-548h-4	MI0006414	8	26906370	26906480	-
387	hsa-mir-548i-1	MI0006421	3	125509247	125509395	-
388	hsa-mir-548i-2	MI0006422	4	9557789	9557937	-
389	hsa-mir-548i-3	MI0006423	8	7946463	7946611	-
390	hsa-mir-548i-4	MI0006424	X	83480760	83480836	-
391	hsa-mir-548j	MI0006345	22	26951178	26951289	-
392	hsa-mir-548k	MI0006354	11	70130061	70130176	+
393	hsa-mir-548l	MI0006361	11	94199661	94199746	-
394	hsa-mir-548m	MI0006400	X	94318140	94318225	-
395	hsa-mir-548n	MI0006399	7	34980372	34980446	-
396	hsa-mir-548o	MI0006402	7	102046189	102046302	-
397	hsa-mir-548p	MI0006420	5	100152186	100152269	-
398	hsa-mir-548q	MI0010637	10	12767253	12767352	-
399	hsa-mir-549	MI0003679	15	81134319	81134414	-
400	hsa-mir-550-1	MI0003600	7	30329410	30329506	+
401	hsa-mir-550-2	MI0003601	7	32772593	32772689	+
402	hsa-mir-551a	MI0003556	1	3477259	3477354	-
403	hsa-mir-551b	MI0003575	3	168269642	168269737	+
404	hsa-mir-552	MI0003557	1	35135200	35135295	-
405	hsa-mir-553	MI0003558	1	100746797	100746864	+
406	hsa-mir-554	MI0003559	1	151518272	151518367	+
407	hsa-mir-555	MI0003561	1	155316141	155316236	-
408	hsa-mir-556	MI0003562	1	162312336	162312430	+
409	hsa-mir-557	MI0003563	1	168344762	168344859	+
410	hsa-mir-558	MI0003564	2	32757220	32757313	+
411	hsa-mir-559	MI0003565	2	47604814	47604909	+
412	hsa-mir-561	MI0003567	2	189162219	189162315	+
413	hsa-mir-562	MI0003568	2	233037363	233037457	+
414	hsa-mir-563	MI0003569	3	15915278	15915356	+
415	hsa-mir-564	MI0003570	3	44903380	44903473	+
416	hsa-mir-566	MI0003572	3	50210759	50210852	+
417	hsa-mir-567	MI0003573	3	111831648	111831745	+
418	hsa-mir-568	MI0003574	3	114035322	114035416	-
419	hsa-mir-569	MI0003576	3	170824453	170824548	-
420	hsa-mir-570	MI0003577	3	195426272	195426368	+
421	hsa-mir-571	MI0003578	4	343946	344041	+
422	hsa-mir-572	MI0003579	4	11370451	11370545	+
423	hsa-mir-573	MI0003580	4	24521815	24521913	-
424	hsa-mir-574	MI0003581	4	38869653	38869748	+
425	hsa-mir-575	MI0003582	4	83674490	83674583	-
426	hsa-mir-576	MI0003583	4	110409854	110409951	+
427	hsa-mir-577	MI0003584	4	115577915	115578010	+
428	hsa-mir-578	MI0003585	4	166307394	166307489	+

Contd....

429	hsa-mir-579	MI0003586	5	32394484	32394581	-
430	hsa-mir-580	MI0003587	5	36147994	36148090	-
431	hsa-mir-581	MI0003588	5	53247334	53247429	-
432	hsa-mir-582	MI0003589	5	58999432	58999529	-
433	hsa-mir-583	MI0003590	5	95414842	95414916	+
434	hsa-mir-584	MI0003591	5	148441876	148441972	-
435	hsa-mir-585	MI0003592	5	168690605	168690698	-
436	hsa-mir-586	MI0003594	6	45165411	45165507	-
437	hsa-mir-587	MI0003595	6	107232000	107232095	+
438	hsa-mir-588	MI0003597	6	126805777	126805859	+
439	hsa-mir-589	MI0003599	7	5535450	5535548	-
440	hsa-mir-590	MI0003602	7	73605528	73605624	+
441	hsa-mir-591	MI0003603	7	95848974	95849068	-
442	hsa-mir-592	MI0003604	7	126698142	126698238	-
443	hsa-mir-593	MI0003605	7	127721913	127722012	+
444	hsa-mir-595	MI0003607	7	158325410	158325505	-
445	hsa-mir-596	MI0003608	8	1765397	1765473	+
446	hsa-mir-597	MI0003609	8	9599182	9599278	+
447	hsa-mir-598	MI0003610	8	10892716	10892812	-
448	hsa-mir-599	MI0003611	8	100548864	100548958	-
449	hsa-mir-600	MI0003613	9	125873825	125873922	-
450	hsa-mir-601	MI0003614	9	126164804	126164882	-
451	hsa-mir-602	MI0003615	9	140732871	140732968	+
452	hsa-mir-603	MI0003616	10	24564614	24564710	+
453	hsa-mir-604	MI0003617	10	29833933	29834026	-
454	hsa-mir-605	MI0003618	10	53059333	53059415	+
455	hsa-mir-606	MI0003619	10	77312216	77312311	+
456	hsa-mir-607	MI0003620	10	98588426	98588521	-
457	hsa-mir-608	MI0003621	10	102734742	102734841	+
458	hsa-mir-609	MI0003622	10	105978547	105978641	-
459	hsa-mir-610	MI0003623	11	28078362	28078457	+
460	hsa-mir-611	MI0003624	11	61559967	61560033	-
461	hsa-mir-612	MI0003625	11	65211929	65212028	+
462	hsa-mir-613	MI0003626	12	12917583	12917677	+
463	hsa-mir-614	MI0003627	12	13068763	13068852	+
464	hsa-mir-615	MI0003628	12	54427734	54427829	+
465	hsa-mir-616	MI0003629	12	57912946	57913042	-
466	hsa-mir-617	MI0003631	12	81226312	81226408	-
467	hsa-mir-618	MI0003632	12	81329515	81329612	-
468	hsa-mir-619	MI0003633	12	109230684	109230782	-
469	hsa-mir-620	MI0003634	12	116586365	116586459	-
470	hsa-mir-621	MI0003635	13	41384902	41384997	+
471	hsa-mir-622	MI0003636	13	90883436	90883531	+
472	hsa-mir-623	MI0003637	13	100008385	100008482	+

Contd....

473	hsa-mir-624	MI0003638	14	31483852	31483948	-
474	hsa-mir-625	MI0003639	14	65937820	65937904	+
475	hsa-mir-626	MI0003640	15	41983783	41983876	+
476	hsa-mir-627	MI0003641	15	42491768	42491864	-
477	hsa-mir-628	MI0003642	15	55665138	55665232	-
478	hsa-mir-629	MI0003643	15	70371711	70371807	-
479	hsa-mir-630	MI0003644	15	72879558	72879654	+
480	hsa-mir-631	MI0003645	15	75645952	75646026	-
481	hsa-mir-632	MI0003647	17	30677128	30677221	+
482	hsa-mir-633	MI0003648	17	61021576	61021673	+
483	hsa-mir-634	MI0003649	17	64783190	64783286	+
484	hsa-mir-635	MI0003650	17	66420592	66420689	-
485	hsa-mir-636	MI0003651	17	74732532	74732630	-
486	hsa-mir-637	MI0003652	19	3961412	3961510	-
487	hsa-mir-638	MI0003653	19	10829080	10829179	+
489	hsa-mir-639	MI0003654	19	14640355	14640452	+
490	hsa-mir-640	MI0003655	19	19545872	19545967	+
491	hsa-mir-641	MI0003656	19	40788450	40788548	-
492	hsa-mir-642	MI0003657	19	46178186	46178282	+
493	hsa-mir-643	MI0003658	19	52785050	52785146	+
494	hsa-mir-644	MI0003659	20	33054130	33054223	+
495	hsa-mir-645	MI0003660	20	49202323	49202416	+
496	hsa-mir-646	MI0003661	20	58883532	58883625	+
497	hsa-mir-647	MI0003662	20	62573984	62574079	-
498	hsa-mir-648	MI0003663	22	18463634	18463727	-
499	hsa-mir-649	MI0003664	22	21388465	21388561	-
500	hsa-mir-650	MI0003665	22	23165270	23165365	+
501	hsa-mir-651	MI0003666	X	8095006	8095102	+
502	hsa-mir-652	MI0003667	X	109298557	109298654	+
503	hsa-mir-653	MI0003674	7	93112072	93112167	-
504	hsa-mir-654	MI0003676	14	101506556	101506636	+
505	hsa-mir-655	MI0003677	14	101515887	101515983	+
506	hsa-mir-656	MI0003678	14	101533061	101533138	+
507	hsa-mir-657	MI0003681	17	79099076	79099173	-
508	hsa-mir-658	MI0003682	22	38240279	38240378	-
509	hsa-mir-659	MI0003683	22	38243685	38243781	-
510	hsa-mir-660	MI0003684	X	49777849	49777945	+
511	hsa-mir-661	MI0003669	8	145019359	145019447	-
512	hsa-mir-662	MI0003670	16	820183	820277	+
513	hsa-mir-663	MI0003672	20	26188822	26188914	-
514	hsa-mir-663b	MI0006336	2	133014539	133014653	-
515	hsa-mir-664	MI0006442	1	220373880	220373961	-
516	hsa-mir-665	MI0005563	14	101341370	101341441	+
517	hsa-mir-668	MI0003761	14	101521595	101521660	+

Contd....

518	hsa-mir-670	MI0003933	11	43581206	43581303	+
519	hsa-mir-671	MI0003760	7	150935507	150935624	+
520	hsa-mir-675	MI0005416	11	2017989	2018061	-
521	hsa-mir-708	MI0005543	11	79113066	79113153	-
522	hsa-mir-711	MI0012488	3	48616335	48616410	-
523	hsa-mir-718	MI0012489	X	153285371	153285440	-
524	hsa-mir-720	MI0006654	3	164059129	164059238	+
525	hsa-mir-744	MI0005559	17	11985216	11985313	+
526	hsa-mir-758	MI0003757	14	101492357	101492444	+
527	hsa-mir-759	MI0004065	13	53384185	53384275	+
528	hsa-mir-760	MI0005567	1	94312388	94312467	+
529	hsa-mir-761	MI0003941	1	52302016	52302074	-
530	hsa-mir-762	MI0003892	16	30905224	30905306	+
531	hsa-mir-764	MI0003944	X	113873918	113874002	+
532	hsa-mir-765	MI0005116	1	156905923	156906036	-
533	hsa-mir-766	MI0003836	X	118780701	118780811	-
534	hsa-mir-767	MI0003763	X	151561893	151562001	-
535	hsa-mir-769	MI0003834	19	46522190	46522307	+
536	hsa-mir-770	MI0005118	14	101318727	101318824	+
537	hsa-mir-802	MI0003906	21	37093013	37093106	+
538	hsa-mir-873	MI0005564	9	28888877	28888953	-
539	hsa-mir-874	MI0005532	5	136983261	136983338	-
540	hsa-mir-875	MI0005541	8	100549014	100549089	-
541	hsa-mir-876	MI0005542	9	28863624	28863704	-
542	hsa-mir-877	MI0005561	6	30552109	30552194	+
			HSCHR6_MHC_COX	30541958	30542043	+
			HSCHR6_MHC_DBB	30542353	30542438	+
			HSCHR6_MHC_MANN	30596784	30596869	+
			HSCHR6_MHC_MCF	30630566	30630651	+
			HSCHR6_MHC_QBL	30541620	30541705	+
			HSCHR6_MHC_SSTO	30543533	30543618	+
543	hsa-mir-885	MI0005560	3	10436173	10436246	-
544	hsa-mir-886	MI0005527	5	135416177	135416297	-
545	hsa-mir-887	MI0005562	5	15935291	15935369	+
546	hsa-mir-888	MI0005537	X	145076302	145076378	-
547	hsa-mir-889	MI0005540	14	101514238	101514316	+
548	hsa-mir-890	MI0005533	X	145075793	145075869	-
549	hsa-mir-891a	MI0005524	X	145109312	145109390	-

Contd....

550	hsa-mir-891b	MI0005534	X	145082571	145082649	-
551	hsa-mir-892a	MI0005528	X	145078187	145078261	-
552	hsa-mir-892b	MI0005538	X	145078716	145078792	-
553	hsa-mir-920	MI0005712	12	24365355	24365429	+
554	hsa-mir-921	MI0005713	1	166123980	166124035	-
555	hsa-mir-922	MI0005714	3	197401367	197401447	-
556	hsa-mir-924	MI0005716	18	37202087	37202139	-
557	hsa-mir-933	MI0005755	2	176032361	176032437	-
558	hsa-mir-934	MI0005756	X	135633037	135633119	+
559	hsa-mir-935	MI0005757	19	54485561	54485651	+
560	hsa-mir-936	MI0005758	10	105807847	105807944	-
561	hsa-mir-937	MI0005759	8	144895127	144895212	-
563	hsa-mir-938	MI0005760	10	29891193	29891275	-
563	hsa-mir-939	MI0005761	8	145619364	145619445	-
564	hsa-mir-940	MI0005762	16	2321748	2321841	+
565	hsa-mlr-941-1	MI0005763	20	62550794	62550882	+
566	hsa-mir-941-2	MI0005764	20	62551101	62551189	+
567	hsa-mir-941-3	MI0005765	20	62551213	62551301	+
568	hsa-mir-941-4	MI0005766				
569	hsa-mir-942	MI0005767	1	117637265	117637350	+
570	hsa-mir-943	MI0005768	4	1988111	1988204	-
571	hsa-mir-944	MI0005769	3	189547711	189547798	+
572	hsa-mir-1178	MI0006271	12	120151439	120151529	-
573	hsa-mir-1179	MI0006272	15	89151338	89151428	+
574	hsa-mir-1180	MI0006273	17	19247819	19247887	-
575	hsa-mir-1181	MI0006274	19	10514134	10514214	-
576	hsa-mir-1182	MI0006275	1	231155574	231155670	-
577	hsa-mir-1183	MI0006276	7	21510676	21510764	+
578	hsa-mir-1184	MI0006277	X	154115635	154115733	-
			X	154612749	154612847	-
			X	154687178	154687276	+
579	hsa-mir-1185-1	MI0003844	14	101509314	101509399	+
580	hsa-mir-1185-2	MI0003821	14	101510535	101510620	+
581	hsa-mir-1197	MI0006656	14	101491901	101491988	+
582	hsa-mir-1200	MI0006332	7	36958962	36959037	-
583	hsa-mir-1201	MI0006333	14	20794606	20794690	-
584	hsa-mir-1202	MI0006334	6	156267931	156268013	+
585	hsa-mir-1203	MI0006335	17	46233789	46233873	-
586	hsa-mir-1204	MI0006337	8	128808208	128808274	+
587	hsa-mir-1205	MI0006338	8	128972879	128972941	+
588	hsa-mir-1206	MI0006339	8	129021144	129021202	+
589	hsa-mir-1207	MI0006340	8	129061398	129061484	+
590	hsa-mir-1208	MI0006341	8	129162362	129162434	+
591	hsa-mir-1224	MI0003764	3	183959193	183959277	+

Contd....

592	hsa-mir-1225	MI0006311	16	2140196	2140285	-
593	hsa-mir-1226	MI0006313	3	47891045	47891119	+
594	hsa-mir-1227	MI0006316	19	2234061	2234148	-
595	hsa-mir-1228	MI0006318	12	57588287	57588359	+
596	hsa-mir-1229	MI0006319	5	179225278	179225346	-
597	hsa-mir-1231	MI0006321	1	201777739	201777830	+
598	hsa-mir-1233	MI0006323	15	34674270	34674351	-
			15	34820491	34820572	-
599	hsa-mir-1234	MI0006324	8	145625476	145625559	-
600	hsa-mir-1236	MI0006326	6	31924616	31924717	-
			HSCHR6_MHC_COX	31912168	31912269	-
			HSCHR6_MHC_DBB	31906802	31906903	-
			HSCHR6_MHC_MCF	32001063	32001164	-
			HSCHR6_MHC_QBL	31915008	31915109	-
			HSCHR6_MHC_SSTO	31916468	31916569	-
601	hsa-mir-1237	MI0006327	11	64136074	64136175	+
602	hsa-mir-1238	MI0006328	19	10662798	10662880	+
603	hsa-mir-1243	MI0006373	4	114028019	114028111	+
604	hsa-mir-1244	MI0006379	2	232578024	232578108	+
			5	118310281	118310365	+
			12	9392063	9392147	-
			12	12264886	12264970	+
605	hsa-mir-1245	MI0006380	2	189842818	189842887	+
606	hsa-mir-1246	MI0006381	2	177465708	177465780	-
607	hsa-mir-1247	MI0006382	14	102026624	102026759	-
608	hsa-mir-1248	MI0006383	3	186504461	186504566	+
609	hsa-mir-1249	MI0006384	22	45596835	45596900	-
610	hsa-mir-1250	MI0006385	17	79106996	79107108	-
611	hsa-mir-1251	MI0006386	12	97885687	97885756	+
612	hsa-mir-1252	MI0006434	12	79813037	79813101	+
613	hsa-mir-1253	MI0006387	17	2651372	2651476	-
614	hsa-mir-1254	MI0006388	10	70519075	70519171	+
615	hsa-mir-1255a	MI0006389	4	102251459	102251571	-
616	hsa-mir-1255b-1	MI0006435	4	36427988	36428050	-
617	hsa-mir-1255b-2	MI0006436	1	167967898	167967964	+
618	hsa-mir-1256	MI0006390	1	21314807	21314925	-
619	hsa-mir-1257	MI0006391	20	60528602	60528718	-
620	hsa-mir-1258	MI0006392	2	180725563	180725635	-
621	hsa-mir-1259	MI0006393	20	47896847	47896957	+

Contd....

622	hsa-mir-1260	MI0006394	14	77732561	77732633	+
623	hsa-mir-1261	MI0006396	11	90602289	90602370	-
624	hsa-mir-1262	MI0006397	1	68649201	68649293	-
625	hsa-mir-1263	MI0006398	3	163889259	163889344	-
626	hsa-mir-1264	MI0003758	X	113887130	113887198	+
627	hsa-mir-1265	MI0006401	10	14478575	14478660	+
628	hsa-mir-1266	MI0006403	15	52569314	52569397	-
629	hsa-mir-1267	MI0006404	13	108183519	108183596	-
630	hsa-mir-1268	MI0006405	15	22513229	22513280	-
631	hsa-mir-1269	MI0006406	4	67142542	67142646	+
632	hsa-mir-1270	MI0006407	19	20510081	20510163	-
			19	20579240	20579322	-
633	hsa-mir-1271	MI0003814	5	175794949	175795034	+
634	hsa-mir-1272	MI0006408	15	65054586	65054714	-
635	hsa-mir-1273	MI0006409	8	101036210	101036312	-
636	hsa-mir-1274a	MI0006410	5	41475734	41475804	+
637	hsa-mir-1274b	MI0006427	19	58024375	58024441	-
638	hsa-mir-1275	MI0006415	6	33967749	33967828	-
639	hsa-mir-1276	MI0006416	15	86313727	86313809	-
640	hsa-mir-1277	MI0006419	X	117520357	117520434	+
641	hsa-mir-1278	MI0006425	1	193105633	193105713	+
642	hsa-mir-1279	MI0006426	12	69666937	69666998	-
643	hsa-mir-1280	MI0006437	3	128081008	128081101	+
644	hsa-mir-1281	MI0006428	22	41488517	41488570	+
645	hsa-mir-1282	MI0006429	15	44085857	44085957	-
646	hsa-mir-1283-1	MI0003832	19	54191735	54191821	+
647	hsa-mir-1283-2	MI0006430	19	54261486	54261572	+
648	hsa-mir-1284	MI0006431	3	71591121	71591240	-
649	hsa-mir-1285-1	MI0006346	7	91833329	91833412	-
650	hsa-mir-1285-2	MI0006347	2	70480050	70480137	-
651	hsa-mir-1286	MI0006348	22	20236657	20236734	-
652	hsa-mir-1287	MI0006349	10	100154975	100155064	-
653	hsa-mir-1288	MI0006432	17	16185328	16185402	+
654	hsa-mir-1289-1	MI0006350	20	34041776	34041919	-
655	hsa-mir-1289-2	MI0006351	5	132763288	132763398	-
656	hsa-mir-1290	MI0006352	1	19223565	19223642	-
657	hsa-mir-1291	MI0006353	12	49048227	49048313	-
658	hsa-mir-1292	MI0006433	20	2633423	2633488	+
659	hsa-mir-1293	MI0006355	12	50627925	50627995	-
660	hsa-mir-1294	MI0006356	5	153726666	153726807	+
661	hsa-mir-1295	MI0006357	1	171070869	171070947	-
662	hsa-mir-1296	MI0003780	10	65132717	65132808	-
663	hsa-mir-1297	MI0006358	13	54886107	54886183	-
664	hsa-mir-1298	MI0003938	X	113949650	113949761	+

Contd....

665	hsa-mir-1299	MI0006359	9	69002239	69002321	-
666	hsa-mir-1301	MI0003815	2	25551509	25551590	-
667	hsa-mir-1302-1	MI0006362	12	113132839	113132981	-
668	hsa-mir-1302-2	MI0006363	1	30366	30503	+
			9	30144	30281	+
			15	102500662	102500799	-
			19	71973	72110	+
669	hsa-mir-1302-3	MI0006364	2	114340536	114340673	-
670	hsa-mir-1302-4	MI0006365	2	208133999	208134148	-
671	hsa-mir-1302-5	MI0006366	20	49231173	49231322	-
672	hsa-mir-1302-6	MI0006367	7	18166843	18166932	-
673	hsa-mir-1302-7	MI0006368	8	142867603	142867674	-
674	hsa-mir-1302-8	MI0006369	9	100125836	100125963	-
675	hsa-mir-1303	MI0006370	5	154065336	154065421	+
676	hsa-mir-1304	MI0006371	11	93466840	93466930	-
677	hsa-mir-1305	MI0006372	4	183090446	183090531	+
678	hsa-mir-1306	MI0006443	22	20073581	20073665	+
679	hsa-mir-1307	MI0006444	10	105154010	105154158	-
680	hsa-mir-1308	MI0006441	X	22080259	22080312	-
681	hsa-mir-1321	MI0006652	X	85090785	85090863	+
682	hsa-mir-1322	MI0006653	8	10682883	10682953	-
683	hsa-mir-1323	MI0003786	19	54175222	54175294	+
684	hsa-mir-1324	MI0006657	3	75679914	75680009	+
685	hsa-mir-1468	MI0003782	X	63005882	63005967	-
686	hsa-mir-1469	MI0007074	15	96876490	96876536	+
687	hsa-mir-1470	MI0007075	19	15560359	15560419	+
688	hsa-mir-1471	MI0007076	2	232756952	232757008	-
689	hsa-mir-1537	MI0007258	1	236016300	236016360	-
690	hsa-mir-1538	MI0007259	16	69599711	69599771	-
691	hsa-mir-1539	MI0007260	18	47013743	47013792	+
692	hsa-mir-1825	MI0008193	20	30825598	30825650	+
693	hsa-mir-1826	MI0008194	16	33965508	33965592	+
694	hsa-mir-1827	MI0008195	12	100583662	100583727	+
695	hsa-mir-1908	MI0008329	11	61582633	61582712	-
696	hsa-mir-1909	MI0008330	19	1816158	1816237	-
697	hsa-mir-1910	MI0008331	16	85775227	85775306	-
698	hsa-mir-1911	MI0008332	X	113997744	113997823	+
699	hsa-mir-1912	MI0008333	X	113886019	113886098	+
700	hsa-mir-1913	MI0008334	6	166922842	166922921	-
701	hsa-mir-1914	MI0008335	20	62572818	62572897	-
702	hsa-mir-1915	MI0008336	10	21785491	21785570	-
703	hsa-mir-1972	MI0009982	16	15104178	15104254	-
			16	70064249	70064325	+
704	hsa-mir-1973	MI0009983	4	117220881	117220924	+

Contd....

705	hsa-mir-1974	MI0009984	5	93905172	93905241	-
			MT	14675	14744	-
706	hsa-mir-1975	MI0009985	7	148638580	148638654	+
707	hsa-mir-1976	MI0009986	1	26881033	26881084	+
708	hsa-mir-1977	MI0009987	1	566187	566265	-
			MT	5638	5716	-
709	hsa-mir-1978	MI0009988	2	149639365	149639417	-
			MT	622	674	+
710	hsa-mir-1979	MI0009989	4	166321814	166321889	-
711	hsa-mir-2052	MI0010486	8	75617928	75617982	+
712	hsa-mir-2053	MI0010487	8	113655722	113655812	+
713	hsa-mir-2054	MI0010488	4	126428414	126428462	+
714	hsa-mir-2110	MI0010629	10	115933864	115933938	-
715	hsa-mir-2113	MI0003939	6	98472407	98472497	+
716	hsa-mir-2114	MI0010633	X	149396239	149396318	+
717	hsa-mir-2115	MI0010634	3	48357850	48357949	-
718	hsa-mir-2116	MI0010635	15	59463382	59463461	-
719	hsa-mir-2117	MI0010636	17	41522174	41522253	+
720	hsa-mir-2276	MI0011282	13	24736555	24736643	+
721	hsa-mir-2277	MI0011284	5	92956402	92956494	-

Contd....