# An *In-Silico* Analysis of Non-coding RNA-mRNA Interactions across Diverse Carcinomas and Elucidation of its Functional Implications

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By

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## CERTIFICATE

This is to certify that the thesis entitled "**An *In-Silico* Analysis of Non-coding RNA-mRNA Interactions Across Diverse Carcinomas and Elucidation of its Functional Implications**" submitted by Ms. **Harshita Sharma**, ID No. **2017PHXF0409P** for the award of Ph.D. Degree of the Institute embodies the original work done by her under supervision.

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*Dedicated to the loving memories of my mother*

*Mrs. Anita Sharma*

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(Harshita Sharma)

# Abstract

Cancer is a disease characterized by the uncontrolled growth and spread of tumor cells. If left untreated, these cells can spread to other parts of the body and interfere with the normal functioning of healthy cells, tissues, and organs. Importantly, genetic and epigenetic changes can both contribute to the development of cancer. Genetic changes, for example, mutation can cause disruption of gene function and can cause the development of cancer by disrupting the normal regulation of cell growth and division. Epigenetic changes, on the other hand, refer to modifications to the way that genes are expressed. Epigenetic changes can cause the development of cancer by non-genetic modifications leading to the silencing of tumor suppressor genes or activating oncogenes. One such epigenetic change that is majorly responsible for cancer is non-coding RNAs. Non-coding RNA (ncRNA) refers to RNA molecules that do not encode proteins. These molecules play important regulatory roles in various biological processes, including cancer. One interesting aspect of ncRNAs in cancer is how these molecules can alter the expression of specific genes. In addition to their roles in gene regulation, ncRNAs have also been implicated in other processes that are important in cancer, such as angiogenesis (the formation of new blood vessels) and immune evasion. One type of ncRNA that has been studied extensively in cancer is microRNA (miRNA). miRNAs are small, single-stranded RNA molecules that bind to complementary sequences in messenger RNA (mRNA) and inhibit their translation into protein. For example, miRNAs can bind to the mRNA of oncogenes and inhibit their translation, leading to the suppression of tumor growth. On the other hand, miRNAs can also bind to the mRNA of tumor suppressor genes and inhibit their translation, leading to the activation of oncogenes and the promotion of tumor growth. Other types of ncRNA that have been studied in the context of cancer include the long non-coding RNAs (lncRNA). These molecules also play important regulatory roles in cancer, including the regulation of gene expression, cell proliferation, and apoptosis (programmed cell death).

Overall, the study of ncRNA in cancer has contributed significantly to our understanding of the molecular mechanisms underlying this disease and has the potential to lead to the development of new diagnostic tools and therapeutic strategies. Although miRNA and lncRNAs alone have been identified as the master regulators of cancers, many studies have been conducted in the last five to seven years that have identified that these two non-coding RNA types (miRNA and lncRNA) together can work to regulate the gene expression in cancer progression. The significance of our studies is that we tried to look for the important transcripts of mRNA, miRNA, and lncRNAs that could play an important role in different cancer types. To understand the regulation of different transcripts in a disease condition, we analyzed their expression in multiple carcinomas from the GDC cancer portal. We identified specific signature transcripts for each mRNA, miRNA, and lncRNA dysregulated across sixteen cancer types [bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC)] and simultaneously identified unique/common transcripts and pathways for each cancer type as well. Our analysis showed that three important genes, FAP, CTHRC1, and COL11A1 are present universally across all the sixteen cancer types and have an important role in cancer progression. These genes give us the first indication of genes that are universally de-regulated in multiple cancer types. Furthermore, with miRNAs, we found that miRNA hsa-miR-1-3p was present in thirteen out of sixteen carcinomas. This miRNA has been found as an important biomarker in most cancer types.

Moreover, we found that the lncRNA named PVT1 is upregulated in thirteen cancer types. This lncRNA is identified as an oncogene and an important biomarker associated with multiple cancer malignancies. We also looked at important miRNA-mRNA and lncRNA- miRNA-mRNA regulatory axis across the pan-carcinomas that could probably be important biomarkers. Our analysis showed the correlated expression of hsa-miR-1-3p-CENPF, hsa-miR- 1-3p-KIF2C, and hsa-miR-1-3p-KIF4A in eleven cancer types (BLCA, BRCA, ESCA, HNSC,KICH, KIRC, KIRP, LUAD, LUSC, STAD, UCEC). miRNA hsa-miR-1-3p was found to be commonly present in all three pairs indicating that this miRNA is important in multiple carcinomas studies. While analyzing the important lncRNA-miRNA-mRNA axis, we have found four important axes PVT1-hsa-miR-195-5p-BIRC5, PVT1-hsa-miR-195-5p-CEP55, PVT1-hsa-miR-195-5p-CLSPN and PVT1-hsa-miR-195-5p-E2F7 which were present in eight cancer types (BLCA, BRCA, KICH, KIRP, LUAD, LUSC, STAD, and UCEC). Here we observed PVT1 and hsa-miR-195-5p to be a common link that was able to regulate four different kinds of genes. The potential of targeting the lncRNA-miRNA axis in cancer treatment is an active area of research, and we will likely see significant progress in this field in the coming years.

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# List of Abbreviations

| | |
|---|---|
| NCI | National Cancer Institute |
| NHI | National Health Institute |
| TCGA | The Cancer Genome Atlas |
| ICGC | International Cancer Genome Consortium |
| DNA | Deoxyribonucleic |
| ACS | American Cancer Society |
| HVV | Human herpesvirus |
| MPNST | Malignant peripheral nerve sheath tumors |
| CSF | Cerebro spinal fluid |
| GloboCan | Global Cancer Observatory |
| MDM2 | Murine double minute 2 |
| UPS | ubiquitin proteasomal system |
| DNMTs | DNA methyltransferases |
| GSTP1 | glutathione S-transferase |
| HATs | Histone acetyltransferases |
| HDACs | histone deacetylases |
| ncRNAs | non-coding RNAs |
| miRNA | microRNA |
| lncRNA | long non-coding RNA |
| ceRNAs | competing endogenous RNA |
| MREs | miRNA response elements |
| MAML1 | mastermind-like 1 |
| MEF2C | Myocyte Enhancer Factor 2C |
| BMLNs | Basic miRNA-lncRNA networks |

| | |
|---|---|
| ISMLN | Individual miRNA-lncRNA network |
| GDC | Genomic Data Common |
| BLCA | Bladder Urothelial carcinoma |
| BRCA | Breast invasive carcinoma |
| COAD | Colon adenocarcinoma |
| ESCA | Esophageal carcinoma |
| HNSC | Head and Neck squamous cell carcinoma |
| KICH | Kidney Chromophobe |
| KIRC | Kidney renal clear cell carcinoma |
| KIRP | Kidney renal papillary cell carcinoma |
| LIHC | Liver hepatocellular carcinoma |
| LUAD | Lung adenocarcinoma |
| LUSC | Lung squamous cell carcinoma |
| PRAD | Prostate adenocarcinoma |
| READ | Rectum adenocarcinoma |
| STAD | Stomach adenocarcinoma |
| THCA | Thyroid carcinoma |
| UCEC | Uterine corpus endometrial carcinoma |
| DAVID | Database for Annotation, Visualization, and Integrated Discovery |
| GO | Gene Ontology |
| BP | Biological Process |
| CC | Cellular Component |
| MF | Molecular Function |
| MTIs | miRNA target interaction database |
| OSCC | oral squamous cell carcinoma |
| NSCLC | non-small cell lung cancer |

| | |
|---|---|
| CCA | Cholangiocarcinoma |
| OS | Osteosarcoma |
| GBC | gallbladder cancer |
| NPC | nasopharyngeal carcinoma |
| HCC | hepatocellular carcinoma |
| MM | multiple myeloma |
| OC | ovarian cancer |
| PCA | Principal Component Analysis |

# CHAPTER-I

## Introduction

## 1.1. Cancer

Cancer describes a class of diseases where a population of aberrant cells proliferates uncontrollably without adhering to the laws of normal cell division (Momna Hejmadi. 2009). These cancer cells differ significantly from typical cancer cells in several ways. For example, normal cells can only divide, differentiate, or perish when certain signals are received, whereas cancerous cells can continue to grow and proliferate even when those same signals instruct them to do so. Cancer also termed malignant cells, in contrast to normal cells, do not commit suicide via apoptosis or programmed cell death pathways. On contrary, cancer cells frequently invade neighboring organs and spread to new locations in the body, whereas normal cells remain in place and cease dividing once they come in contact with another cell. By luring new blood vessels to the tumor site, cancer cells may alter the local behavior of molecules, healthy tissue, and blood vessels, allowing the tumor to receive nutrients and oxygen while ridding itself of waste. Normal cells have these functions that limit their ability to proliferate, whereas cancer cells do not. Specialization, maturity, and the ability to fulfill a variety of roles are hallmarks of healthy cells. As a result of losing these abilities, cancer cells become less functional and more proliferative (NCI/NIH 2022). Cancers and tumors have distinct characteristics. It is common practice to use both terms interchangeably. A tumor is a mass of aberrant cells that forms a lump or lesion in the body. Cancer is characterized by unchecked cellular proliferation that metastasizes throughout the body. A tumor is an uncontrolled development of cells that can either be beneficial or damaging to the body. Benign tumors are those that do not include cancer cells, while malignant tumors do. If we consider their source, we can classify tumors as either solid or liquid. Cysts and liquid areas are rarely found in solid tumors, although they are common in liquid tumors. Liquid tumors include cancers of the blood and lymphatic systems, such as leukemia and lymphoma, while solid tumors include all other types of cancer. There have always been cases of cancer in both humans and animals. It should

come as no surprise, then, that cancer has been the subject of written material ever since humankind first put pen to paper.

### 1.2. A Brief Timeline of Cancer's History

It was first described in writing about 3000 BC in the Edwin Smith Papyrus, which featured a breast cancer case. The stomach, skin, rectum, and uterus are just a few of the organs that have been the subject of tumor descriptions in the 1500 BC Ebers Papyrus. Egyptian records refer to cancer as "the curse of Gods" and describe it as a severe, incurable illness (Hajdu 2011a, 2011b). The term "carcinos" or "carcinomas" was coined by Hippocrates the Greek physician (460–370 BC), who is recognized as the "Father of Medicine," and he related cancer to a crab-like pattern in both ulcer-forming and non-ulcer-forming tumors (Mitrus et al. n.d.). According to Hippocrates, the body is made up of four fluids: blood, phlegm, black bile, and yellow bile. He also believed that an abundance of black bile in any one location in the body can lead to cancer. One of the first cases of cancer known to exist dates back to Egypt around 1500 BC. The case was described on an Edwin Smith papyrus or papyrus and involved eight cases of breast cancer that were treated by cauterization, which involves burning tissue with a heated device known as "the fire drill." Additionally, it was reported that palliative care was the only available treatment for the illness. The ancient Egyptians may have been able to tell the difference between malignant and benign cancers, as some evidence suggests they did. These Inscriptions suggest that surgical methods for treating or removing cancer were remarkably similar to those utilized today. The theories of Hippocrates laid the groundwork for the experimental methods used by the entire scientific community to study cancer. Greek physician Claudius Galen (130-200), who studied medicine in Rome, expanded on Hippocrates' theory by claiming that cancer caused by black bile was incurable, while cancer caused by yellow bile was treatable. And he described tumors with the Greek word for swelling, "oncos". Italian anatomist Giovanni Morgagni performed the first autopsy in 1762, which set the groundwork

for "oncology." The first person to advise operating on a tumor was John Hunter in the 18th century, but he was unable to do so since anesthetic had not yet been invented. After the invention of the anesthetic, the surgery took off. Environmental malignancies were recorded at this time, and cancer-focused hospitals were established. The earliest documented cases of X-ray radiation therapy successfully treating basal cell carcinoma and squamous cell carcinoma of the skin were published in 1899 by Swedish physicians Tor Stenbeck and Tage Sjogren. The development of the microscope throughout the 19th century made it possible for researchers to study cancer with their unaided eyes, giving rise to the contemporary pathologic study of cancer. Studies on cancer tissues and tumors have shown that cancer cells differ noticeably from surrounding normal tissue cells or the cells they originated from in terms of appearance. Despite these developments in the 18th century, it was not until the late 19th century that Rudolf Virchow understood that all cells, including cancer cells, originated from preexisting cells. However, despite these early successes in oncology, beliefs about the origins of cancer were still in their infancy. Parasites and physical trauma were cited as possible causes of cancer, and the disease was thought to spread like a liquid. Surgeon William Stewart Halsted of Johns Hopkins Hospital in Baltimore, Maryland, effectively healed breast cancer in the latter part of the 19th century and around the turn of the 20th century. His radical mastectomy procedure became the gold standard until the 1970s when better options became available. Currently, surgeons can extract the tissue and send it to pathologists, who can determine whether an operation was effective in removing the tumor. Cancer research made great strides in the 20th century, with the discovery of carcinogens and the subsequent development of chemotherapy, immunotherapy, radiation therapy, and improved diagnostic techniques. Scientists worked very hard to comprehend the chemistry, structure, and processes of living things. Oncology has been firmly established as a field thanks to studies into cancer in cell culture, chemical carcinogens, diagnostic methods, and chemotherapy.

Numerous origin theories for cancer were put forth and put through a rigorous testing process. The National Cancer Institute Act, whichwas approved by the U.S. Congress in 1937, aims to coordinate individual and institutional cancer research efforts while also promoting cancer research at other institutions (Lonardo, Nasi, and Pulciani 2015). The last 80 years have seen a dramatic increase in our understanding of cancer thanks to the rapid progress of cutting-edge technologies some of the major findings include completely curing the human solid tumor by chemotherapy which was performed by Roy Hertz and Min Chiu Li in 1953 to treat a patient suffering from choriocarcinoma using the drug methotrexate. In 1971 President Richard M. Nixon of the U.S. signed the National CancerAct on December 23, which empowers the NCI Director to manage the National Cancer Program, establish national cancer research centers, and develop national cancer control programs. TP53 the most commonly mutated gene in human cancer was discovered in 1973. The following years witnessed that knowledge of the human genome has progressed, and the discovery of mutations that result in oncogenes gaining dominant functions and the loss of functions of recessive tumor suppressor genes has revealed the involvement of dynamic changes in the genome, in causing cancer. Human and animal cancer cell lines used as experimental models have been found to exhibit this functional gain or loss. The role of gene cloning, medicines, antibodies, and vaccines in cancer was investigated. In 2018, NIH-funded researchers with TCGA PanCancer Atlas completed an in-depth genomic investigation of 33 cancer types. This analysis of molecular and clinical data from more than 10,000 tumors gives cancer researchers a unique insight into how, where, and why malignancies form in humans. In 2020, the International Pan-Cancer Analysis of Whole Genomes analyzed more than 2,600 genomes from 38 types of cancer and normal tissues to find molecular alterations. The Pan-Cancer Analysis of the Whole Genomes study, that employed TCGA and ICGC data, revealedthe intricate role genomic modifications play in cancer formation, growth, and spread. The study extends cancer genomic analysis beyond protein-coding areas to cell genetics.

Since then, the concept has grown in scope and depth thanks to new insights, with an increased focus on the incredibly varied roles that behaviors, genes, and pathways play in the development of cancer. These ideas were summarised by Hanahan and Weinberg in their widely-read article "The Hallmarks of Cancer," which is credited with developing the current understanding of cancer (Milestones in Cancer Research and Discovery n.d.).

### 1.3. Hallmarks or Characteristics of Cancer

Cancer is a genetic disease caused by the alteration in the functioning of genes that control cell growth, division, and death. These alterations to the DNA affect how a person's cells develop and operate. Internal factors, such as errors that occur during cell division and alteration inherited from parents, and external factors, such as DNA damage done by ultraviolet radiation from the sun and contaminant chemicals present in the environment, such as cigarette smoke, can both contribute to alterations in the functioning of the body's cells. With growing age, cells become less efficient in eliminating damaged DNA-containing cells before they can become malignant, increasing our susceptibility to the disease. Each individual's genetic makeup is different, and their cancer develops its distinct characteristics over time. Different cells within the same tumor may have undergone unique genetic changes. Tumor suppressor genes, DNA repair genes, and proto-oncogenes are frequently the targets of these genetic alterations, which are collectively referred to as "drivers of cancer." Regulating cell growth and multiplication is a function of tumor suppressor genes. A mutation that inactivates a tumor suppressor gene can lead to unchecked cell division, which can play a role in the emergence of cancer. In most cases, proto-oncogenes contribute to regular cell growth and division. In some cases, mutations in a proto-oncogene might convert it into an oncogene, which promotes unchecked cell growth and survival. Genes called DNA repair factors play a role in correcting faulty DNA. When these genes are mutated, chromosomal aberrations like duplications and deletions occur, which contribute to the development of cancer (NCI/NIH 2022). The development of cancer is

divided into various distinct stages which are termed hyperplasia, dysplasia, anaplastic, and metastasis as shown schematically in figure 1.1.

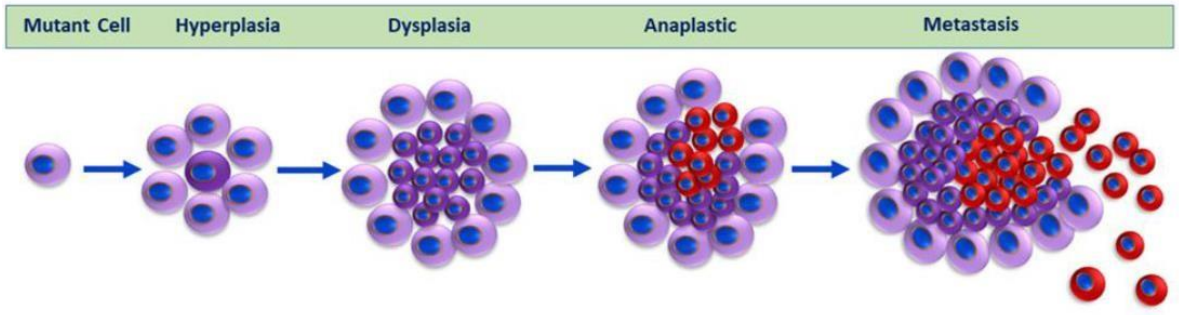



Figure 1.1 A schematic diagram describing different developmental stages of cancer cells.

During hyperplasia, cells proliferate more rapidly than normal, resulting in unregulated cellular proliferation. However, when examined under a microscope, the cells appear healthy. Dysplasia is an advanced stage of hyperplasia characterized by deferred differentiation at the microscopic scale. In general, cancer risk increases in proportion to the degree of cellular and tissue abnormality. In an anaplastic stage, the nucleus of a cell is bigger, more densely packed with chromatin (hyperchromatic), and has more nucleoli. Cells at this stage have inadequate cellular differentiation, lose cellular shape permanently, and have a new cellular orientation. Finally, a metastatic spread occurs when cancer cells break free from their original location, such as the tumor site, and make their way into the circulatory or lymphatic systems. The name of the initial tumor is used to identify these tumors. For example, the spread of breast cancer to the liver is referred to as metastatic breast cancer (Cancer Development n.d.).

The American Cancer Society (ACS) defines cancer as a collection of diseases characterized by the unchecked proliferation of abnormal cells. Although it is referred to as a single disease, it consists of more than 100 different diseases. Generally speaking, hallmarks are a collection of functional abilities that human cells acquire as they transition from normal to neoplastic growth. Cancer biology is extremely complex, but in 2000, Hanahan and Weinberg published

a review titled "The Hallmarks of Cancer," in which they attempted to categorize these features into six broad categories: insensitivity to anti-growth signals; unlimited replicative potential; self-sufficiency in growth signals; avoidance of apoptosis; sustained angiogenesis; metastasis and invasion of surrounding tissues (Hallmark I) (Hanahan and Weinberg 2000). In 2011, they published a new review updating their previous work and highlighting four characteristics shared by cancers, including two emerging hallmarks, immune system evasion, and metabolic reprogramming, as well as genome instability and mutation and tumor-promoting inflammation in a new review: Hallmarks of Cancer: The Next Generation (Hallmark II) (Hanahan and Weinberg 2011). In his 2022 review, Hallmarks of Cancer: New Dimensions, Douglas Hanahan confirmed that cellular energetics (reprogramming cellular metabolism) and"avoiding immune are part of the core set of hallmarks of cancer. Currently, the eight hallmarksinclude the ability to maintain proliferation signaling, resist cell death, evading growth suppressors, activate invasion and metastasis, enabling replicative immortality, induce vasculature access, reprogram cellular metabolism, and avoid immune destruction (Douglas Hanahan 2022).

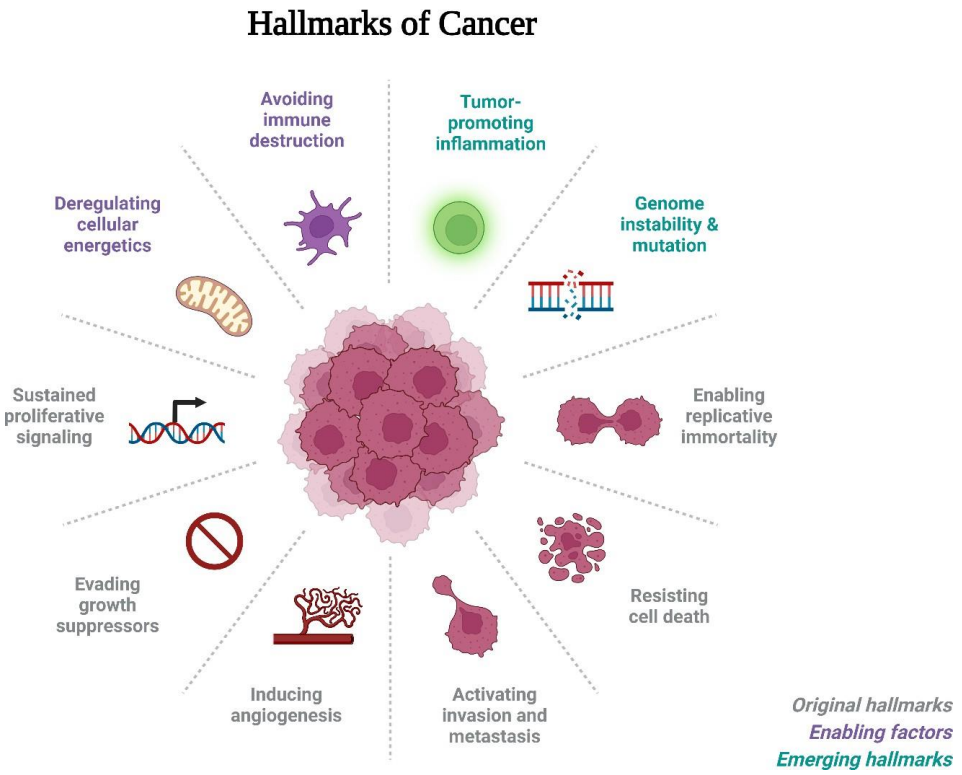



Figure 1.2 The Hallmarks of Cancer. Figure adapted from Hallmarks of Cancer: New Dimensions shows all cancer hallmarks with the inclusion of new emerging hallmarks and enabling characteristics.

The Mary Crowley Cancer Research Center which offers access to new investigational therapies through the administration of Phase I and II clinical trials has categorized the following eight hallmarks of cancer (1) sustained proliferation; (2) evasion of growth suppressors; (3) death resistance; (4) replicate immortality; (5) angiogenesis; (6) invasion; (7) reprogrammed energy metabolism; and (8) immune evasion into three core processes that are cell survival, cell fate, and genomic maintenance which are facilitated by one of 12 signaling pathways shown in figure 1.3. Defeating cancer will require interfering with the core processes that fuel its persistence and growth, which can be done by blocking the relevant pathway[s] for that cancer.

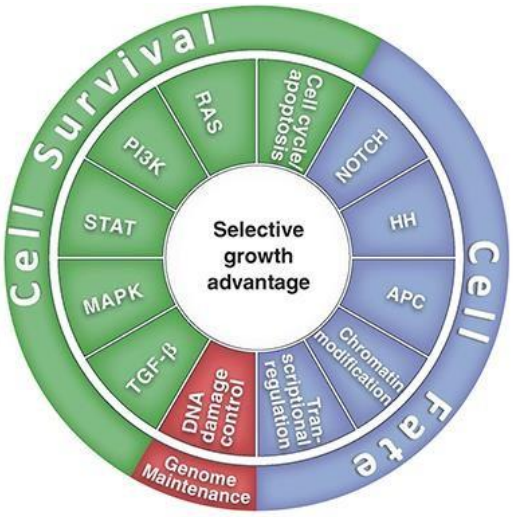



Figure 1.3 The figure depicts the twelve signaling pathways, which are responsible for carrying out the three cancer core functions involving cell survival, fate, and genome maintenance.

### 1.4. Types of Cancer

There are currently over a hundred recognized subtypes of cancer. Primarily, cancers are categorized based on the tissue type wherein cancer originates (histological type) and all cancers are classified into carcinoma, sarcoma, hematologic cancer, and brain and spinal cord cancer as shown in figure 1.4.

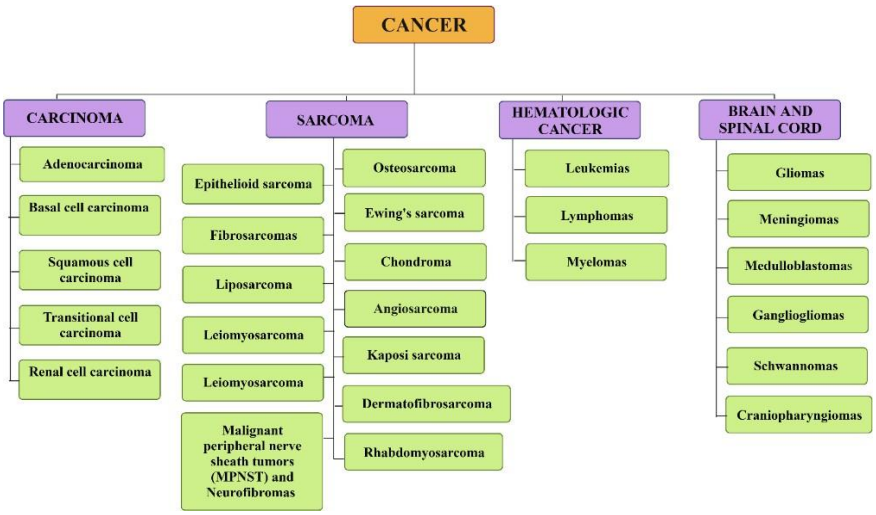



Figure 1.4 Histological classification of different Cancers.

### 1.4.1. Carcinomas

Carcinomas are the most common type of human cancer. It develops from skin epithelial tissue or the tissue that lines internal organs. In the case of carcinoma, the most commonly affected organs are the lung, breast, prostate, colon and rectum, and pancreas. Carcinoma cells can spread throughout the body or remain confined to the primary site. Based on their static nature, carcinomas are classified as carcinoma in situ which is a type of advanced cancer that remains confined to the tissue from which it originated and does not spread to other areas. Invasive carcinoma protrudes from the primary tissue layer to the adjacent tissue, whereas metastatic carcinoma spreads from one location to another throughout the body. Different types of epithelial cells can develop into five types of carcinomas. Adenocarcinoma is a type of cancer that develops in glandular tissues, where epithelial cells secrete fluids or mucus. It covers malignancies of the breast, prostate, and colon cancers (Bernstein 2022). Cancer of the basal cell known as basal cell carcinoma begins in the outermost layer of the skin (the epidermis) (Mayo Clinic 2022a). Squamous cell carcinoma develops in the skin's deeper layers. Squamous cells line many organs, including the lung, bladder, stomach, intestine, and kidneys It is also known as epidermoid carcinomas (Foundation 2022). Transitional cell carcinoma is a disease of the transitional epithelium, also known as the urothelium, which is composed of a stratified epithelial lining that can grow and shrink (WebMD 2022b). Renal cell carcinoma, the most common type of kidney cancer, is a serious public health issue. It is found in the proximal convoluted tubules, which filter or transport primary urine (WebMD 2022a) (UK 2022).

### 1.4.2. Sarcomas

After carcinomas, sarcomas are the second most common malignancy in terms of recurrence. Sarcoma develops in the bones, cartilage, tendons, fat, and muscle that surround and support the body's skeletal structure (Mayo clinic 2022e). There are several types, including osteosarcoma, which is a type of cancer that develops in juvenile bone cells, which are most

common in the bone's periphery when it grows too quickly (Ottaviani and Jaffe 2009). It typically appears in the areas of the knee, thigh, shoulder, and upper arm bones (humerus). Ewing's sarcoma is caused by bone neuroendocrine cells. This cancer most commonly manifests itself in the long bones of the legs, thighs, or arms, as well as the pelvis (Mayo clinic 2022c). Chondrosarcoma starts in the tough, resilient cartilage cells that line the bone surfaces around joints. It can appear near limb articulations, the trunk, or the entire body (Mayo clinic 2022b). Angiosarcoma is cancer that develops in the lining of blood or lymph vessels or both. This is most common near or on the skin, but it can happen anywhere (National Cancer Institute (NIH) 2022a). Kaposi sarcoma is a sarcoma that affects the lymphatic and blood vessel linings. It is caused due to infection by a virus called human herpesvirus 8 (HHV-8) that produces purple color plaques on the skin (Mayo clinic 2022d). Dermatofibrosarcoma is a rare type of skin cancer. It appears as subcutaneous tissue in the limbs, trunk, head, and neck and spreads slowly and rarely to other organs (dermatofibrosarcoma-protuberans n.d.). Epithelioid sarcoma is an extremely rare slow-growing soft tissue cancer and reappears frequently. Metastasis is possible, and it is mostly seen in the hands and feet of adults (epithelioid-sarcoma n.d.) (Fisher 1988). Fibrosarcomas are extremely rare and dangerous soft tissue sarcomas that arise from fibrocytes or fibroblasts. It begins to form in the mesenchymal tissue. (Augsburger et al. 2017). Liposarcoma is a sarcoma that develops in fat cells of the body's fatty tissues. Muscle tissue that has been affected is usually found in the limbs or the abdomen (Johns Hopkins medicine 2022a). Malignant peripheral nerve sheath tumors (MPNST) and neurofibromas aretumors that develop in the soft tissue that surrounds the peripheral nerves. These nerves transmit brain signals and control voluntary movements in the body. It usually only affects thelimbs and does not spread to other organs (malignant-peripheral-nerve-sheath-tumors n.d.) (Johns Hopkins medicine 2022b). Smooth muscle cancer, also known as leiomyosarcoma,is extremely rare.

These cancers most commonly occur in the stomach, colon, and blood vessels, all of which contain involuntary smooth muscles (National Cancer Institute (NIH) 2022c). Rhabdomyosarcoma is a malignant sarcoma that develops in the body's skeletal muscletissue. The head and neck are common locations, but the abdomen can also be a problem. Themajority of pediatric soft tissue sarcomas are rhabdomyosarcomas (American Cancer society 2022). Synovial sarcoma is a type of sarcoma that develops from cells at the proximal end of joints and tendons and can affect soft tissues such as muscles and ligaments. Although it can occur anywhere on the body, it is most common in the knees (National Cancer Institute (NIH)2022f) (NCI/NIH 2022).

### 1.4.3. Hematologic cancers

Hematologic cancers include leukemias, lymphomas, and myelomas, which develop in blood-forming tissue such as bone marrow or immune system cells (National Cancer Institute (NIH) 2022b). Errors in the genetic code of an immature blood cell cause the cell to stop developing normally and instead undergo rapid replication, resulting in an overabundance of abnormal blood cells (Klausner2001). In advanced cancer cases, malignant white blood cells, or leukemia, can be found in the patient's blood and bone marrow. Bone marrow cancers are uncommon (also called leukemias, "liquid cancers," or "blood cancers"). Leukemia is characterized by the proliferation and accumulation of abnormal white blood cells (leukemic blast cells) in the blood and bone marrow, preventing healthy blood cells from receiving oxygen (America 2022). A low normal blood cell count can make it difficult to transport oxygen to tissues, clot blood, and fight infections. This condition is also referred to as solid cancer (lymphoma). Lymphomas form in the lymphatic system's channels, nodes, and organs (such as the spleen, tonsils, and thymus), which work together to filter blood and other bodilyfluids and produce white blood cells (lymphocytes) to fight infections (T cells or B cells).

Hodgkin lymphoma and non-Hodgkin lymphoma are the two most common types of lymphoma (Ansell 2015). Myeloma is a type of bone marrow cancer that attacks a particular type of white blood cell that produces an unusual protein. This causes an increase in the production and release of new blood cells into the circulatory system. They are immature and do not function properly.

### 1.4.4. Brain and Spinal Cord

Tumors of the nervous system can manifest themselves in a variety of ways. These tumors are named after the cell type that gave rise to them and the area of the central nervous system where they first appeared. For example, the cells of origin for an astrocytic tumor are astrocytes, the star-shaped brain cells that aid in the health of nerve cells (NCI/NIH 2022). Gliomas can develop in any part of the brain but begin in the glial cells. Astrocytomas (including glioblastomas), Oligodendrogliomas, and Ependymomas are all types of tumors classified as "gliomas" (Cancer Research UK 2022). Meningiomas are the most common primary brain and spinal cord tumors which are more common in the elderly. The cerebellum is the origin of neuroectodermal cells (the first forms of nerve cells), which can develop into medulloblastomas. While these tumors grow rapidly and have the potential to spread to other parts of the central nervous system via CSF pathways (Society 2022). Medulloblastomas are typically diagnosed in children. Gangliogliomas are a mix of neuronal and glial cells on the cellular level. These tumors, which are uncommon in adults, remain a medical mystery. Most slow-growing (grade I) tumors can be successfully treated with surgery alone or surgery combined with radiation therapy (National Cancer Institute (NIH) 2022d). The schwannomas precursor is Schwann cells, which insulate and cushion nerves throughout the body. This type of tumor accounts for approximately 8% of all central nervous system tumors. The majority of schwannomas are low-grade tumors. They can arise from any nerve inthe brain. They can begin to affect spinal nerves after leaving the spinal cord.

The pressure theyplace on the spinal cord can result in bowel and bladder function issues as well as weakness (National Cancer Institute (NIH) 2022e). Craniopharyngiomas are low-grade tumors that develop at the base of the skull, above the pituitary gland. By putting pressure on thehypothalamus and pituitary gland, they may cause hormonal imbalances. They can also impairvision due to their proximity to the optic nerves of the eyes. Because of their tenacity, they arenot always easy to completely remove without causing damage to the eyes or disrupting the hormonal balance. Even though children are more likely to be diagnosed with craniopharyngioma, adults are not immune to the tumor (National Cancer Institute (NIH) n.d.) (Society n.d.).

## 1.5. Occurrence and statistics of cancers

As per the GloboCan (Global Cancer Observatory) report, 19,292,789 instances of cancer were diagnosed in people of all ages and genders worldwide in 2020. This number includes 8.8 million cases in females and 9.3 million in males. Worldwide, 12.5% of all new cancer cases in 2020 were attributable to breast cancer, and 12.2% to lung cancer. At 1.9 million cases in 2020, colorectal cancer ranked third and accounted for 10.7 percent of all cancer diagnoses that year. Figures 1.5a and 1.5b represent the estimated number of new cases and the number of deaths that were recorded in the year 2020 in the world.

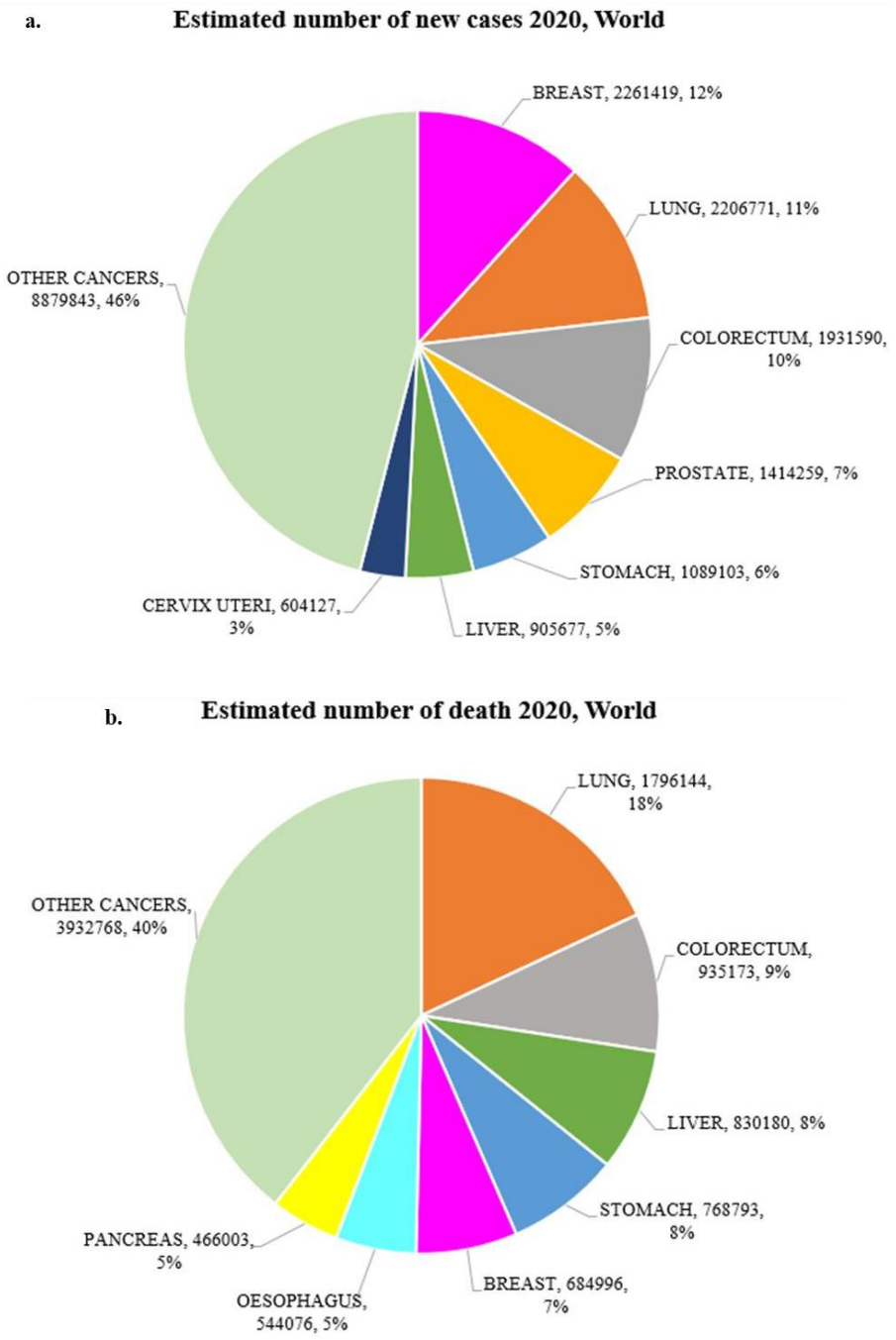



Figure 1.5 Cancer Statistics by GloboCan for the world. (a). Estimated new cases in 2020, the world (b). Estimated death in 2020.

In India, we observed that breast cancer was the most prevailing cancer contributing to 13% of the total occurrence of cancer. It is also observed that 10% of total cancer-related death is due

to breast cancer. Figures 1.6a and 1.6b represent the estimated number of new cases of cancer and the number of deaths for different cancers in India.

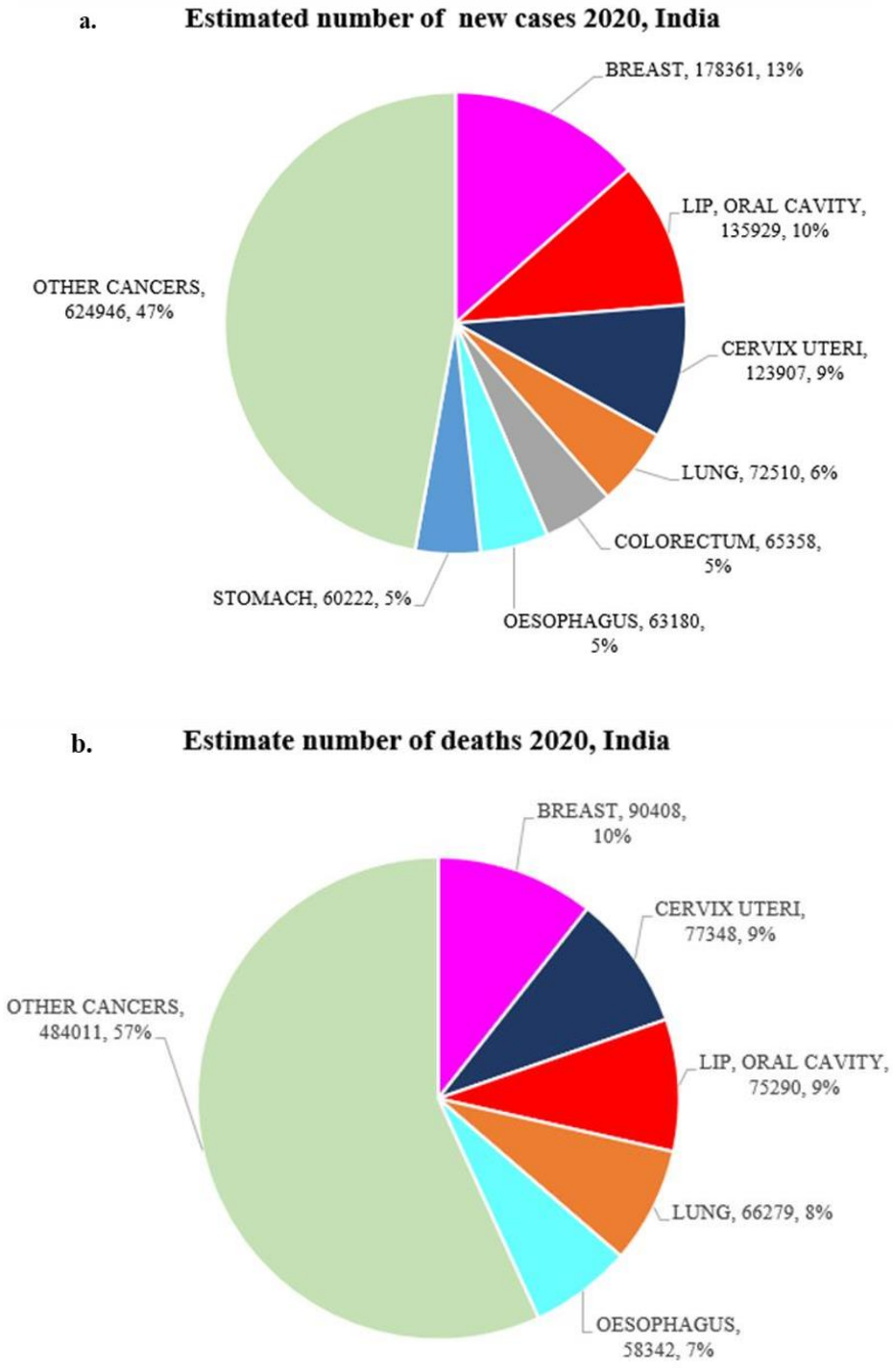



Figure 1.6 Cancer Statistics by GloboCan for India. (a). Estimated new cases in 2020 (b). Estimated death in 2020.

Furthermore, the global cancer burden and mortality in 2040 were projected from the 2020 cancer data. It is estimated that by the year 2040, there will be around 28,887,940 cases of cancer all over the world with Asia contributing the maximum (GloboCan n.d.). Figures 1.7a and 7b represent the estimated number of new cases by the year 2040 in the world and India respectively.

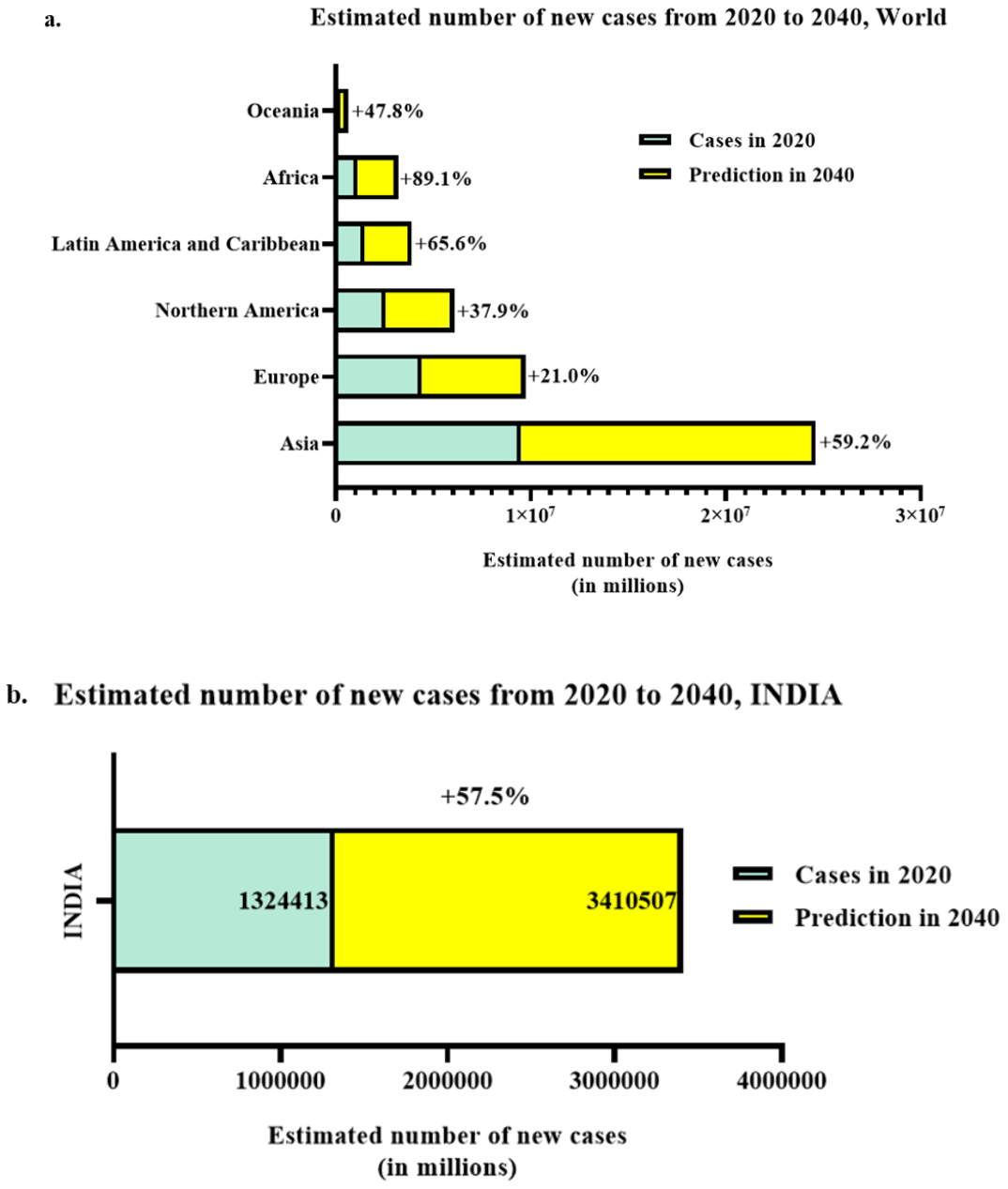



Figure 1.7 Cancer Statistics by GloboCan (a). Represents the estimated number of new cases from 2020-2040 in the world, (b). Represents the estimated number of new cases from 2020-2040 in India.

## 1.6. Molecular Foundations of Cancer

### 1.6.1. Genetic Regulation

Over the years a variety of mechanisms have been proposed to explain how cancer cell forms and proliferate. The majority of studies suggest that the development and progression of cancer are the consequence of the interaction between hundreds of genes which relies on the molecular interactions between the spatially organized genome and a broad class of proteins, including the transcription factors and chromatin remodelers (Repana et al. 2019) (Croce 2008) (Jin1 and , Yan Li1, Jesse R. Dixon1, Siddarth Selvaraj1, Zhen Ye1, Ah Young Lee1, ChiaAn Yen1, Anthony D. Schmitt1, Celso Espinoza1 2018). Cancer phenotypes like growth and proliferation are the outcome of both genetic and epigenetic alterations. However, the TP53 protein-encoding gene is the most frequently dysregulated in the majority of human tumors (Kandoth et al. 2013) and therefore it is regarded as the "gatekeeper of the genome" (Levine 1997). Depending on the quantity and types of DNA damage, a functional p53 protein transactivates a specific subset of its target genes to trigger cell cycle arrest and/or apoptosis. Scian and Coworkers have found that several mutants of p53 that arise in cancer are responsible for driving the development of more aggressive forms of the disease by transactivating genes that promote cell proliferation and tumor development (Scian et al. 2004). Murine double minute 2 (MDM2) is a protein that binds to p53 and blocks its function, relegating the nucleus-bound protein to the cell's cytoplasm. Additionally, MDM2 functions as a ubiquitin ligase. Through this mechanism, MDM2 aids in the destruction of functional p53 by directing it to the ubiquitin proteasomal system (UPS), thereby decreasing cellular p53 levels. Due to p53's cancer-preventing properties, malignant cells have developed numerous strategies to counteract its effects and avoid senescence and programmed cell death (Z. Xu et al. 2021). It was observed that in breast cancer protein kinases such as CHK1, CHK2 (Rad53), ATM (ataxia-telangiectasia mutated), and ATR (Rad53-related protein), that respond to DNA

damage sentinels like BRCA1, regulate p53 activity and stability (Bellazzo et al. 2018). The mutation in the p53 gene at codon 80 and codon 285 is linked to colorectal cancer (Katkoori et al. 2009).

Cancer signatures include the ability to divide in the absence of growth factor stimulation, the ability to divide in the presence of anti-growth signals, the ability to resist apoptosis, the ability to maintain telomere length over repeated cell division, angiogenesis stimulation, and the ability to invade surrounding tissues and colonize other parts of the body (Hanahan and Weinberg 2011). It is also hypothesized that all these characteristics are acquired and vary across the cancer spectrum. All these hallmarks may not appear all at once during cancer progression but may take different paths to the same destination. Many events influence gene regulation in cancer, beginning with insertions/deletions in the DNA sequence, inversions, translocations, single nucleotide variants, copy number variation, and gene fusions. The suppression of tumor suppressor genes and/or the activation of proto/oncogenes is a major contributor to cancer progression. This activation/suppression is commonly associated with epigenetic changes (Baylin and Jones 2016). The term "epigenetics" initially referred to how a fertilized zygote became a mature, complex organism. The definition of epigenetics was revised to focus on heritable features, with knowledge of gene expression mechanisms not related to nucleotide sequence alterations, but with DNA chemical modifications or structural and regulatory proteins bound to it. Discoveries about these pathways in early development may make it beneficial to revert to the native concept of "epigenetics." These epigenetic changes are divided into three categories: DNA and RNA methylations, histone modifications, and non-coding RNAs, which are thought to be the main regulatory mechanisms during cancer progression (Y. Lu et al. 2020).

### 1.6.2. Epigenetic Regulations

#### 1.6.2.1. DNA and RNA methylation

DNA methylation occurs in CpG islands (CGIs) in the 5′ promoter region of more than 50% of human genes, which is the most studied epigenetic process. It's important for X chromosome inactivation, embryonic development, genomic imprinting, epigenetic reprogramming, cell identity, and lineage specification. It silences genes by covalently binding methyl groups from SAM to the cytosine pyrimidine ring's 5 position 5-methylcytosine (m5C) can inhibit transcription factors from accessing DNA binding sites or recruit methyl-binding domain proteins to remodel chromatin, repressing gene expression (Baylin and Jones 2016). DNA methylation is catalyzed by a team effort including three different DNA methyltransferases (DNMTs): DNMT1, DNMT3a, and DNMT3b. Maintenance DNA methyltransferase 1 (DNMT1) preferentially methylates hemimethylated DNA during replication and is primarily important for maintaining the DNA methylation status. De novo methyltransferases, such as DNMT3a and DNMT3b, generate and maintain the precise DNA methylation status in the genome, yet they both have an equal preference for binding to unmethylated DNA (Y. Lu et al. 2020). Different kinds of methylation are linked to cancer progression like CpG-island hypermethylation of the BRCA1 gene is observed in ovarian cancer and breast cancer (Esteller et al. 2000). In 22 lung cancer patients the hypermethylation of the tumor suppressor gene *p16* was associated with lung cancer progression (Esteller et al. 1999). Methylation of the CpG island of GSTP1 (glutathione S-transferase) was found in 90% of patients with prostate cancer. Hypermethylation of CpG islands in the GSTP1 gene has been found in the plasma, urine, and ejaculate of males (Nakayama et al. 2004).

Methylation of the N-6 position of the adenosine residue, known as N6-methyladenosine (m6A), has been known since the 1970s and is quickly becoming a focus of study in the fields of epigenetics and cancer biology. The stop codon, 3'UTR, and internal long exons are enriched

for M6A modifications. It has an impact on the transcription, degradation, splicing, and translation of RNA. RNA modification proteins like methyltransferases and demethylases can affect cell proliferation, migration, and tumors, worsening the prognosis. Overexpression of METTL3 in gastric cancer (D. D. Yang et al. 2020), lung (Yuchan Li et al. 2022), pancreatic cancer (Yuan Li et al. 2022), and colon cancer patients (WEN et al. 2021) was connected with poor overall survival (Wang et al., 2020).

#### 1.6.2.2. Histone Modification

In chromatin, DNA is wrapped with histone octamer to create nucleosomes and "beads on a string" structure, which controls DNA sequence accessibility. Each histone octamer is made of a tetramer of histones 2A and 2B bordered by dimers of histones 3 and 4 (H4). These histone proteins have a globular C-terminal domain and a long N-terminal tail that is modified by methylation, acetylation, ubiquitylation, phosphorylation, SUMOylation, ADP ribosylation, citrullination, and biotinylation. Adding acetyl groups to several histone tail lysine residues causes acetylation. Adding an acetyl group neutralizes unmodified lysine's basic charge, weakening (Ilango et al. 2020). Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate lysine acetylation. HATs use acetyl CoA as a cofactor to transfer acetyl groups to lysine side chains. They counteract lysine's positive charge, which can decrease histone-DNA connections. HDAC enzymes reverse lysine acetylation, restoring its positive charge. This stabilizes local chromatin, consistent with HDACs being transcriptional repressors (Y. Lu et al. 2020). Altered global levels of histone acetylation have been long synonymous with cancer phenotype in a variety of cancers (Kurdistani 2007). Another class of important histone post-translation modifications in histone methylation. Methylation mainly occurs on the side chains of Arginine and/or Lysine residues. Depletion of hSETD1A in breast cancer cells downregulated numerous MMPs and reduced the metastatic phenotype, revealing a function for hSETD1A and H3K4 HMT in breast cancer progression (Salz et al. 2015).

Studies have shown that blocking histone H3K79 methylation preferentially reduces breast cancer's ability to grow, renew itself, and spread (L. Zhang et al. 2014).

#### 1.6.2.3. Non-coding RNAs

A large proportion of the human transcriptome comprises non-protein-coding RNAs that are encoded in intergenic, antisense, or overlapping areas with coding genes. It is estimated that over 98% of the human genome is made up of non-coding RNAs (ncRNAs), of which only a small fraction has been investigated thus far. As a result, there has been an explosion in the number of studies conducted over the past several decades that focus on the "dark matter" or non-coding transcriptome (Johnson et al. 2005) (Kapranov and St. Laurent 2012). At first, these ncRNAs were written off as "transcriptional noise;" however, recent developments in RNA sequencing technology and bioinformatics have shown that they play important roles in gene regulation (Hüttenhofer, Schattner, and Polacek 2005). Researchers are enthusiastic about the significance of ncRNAs' contributions to tumorigenesis and malignancy, despite divergent viewpoints on the effect of ncRNAs on cellular activity as a whole. Improved RNA sequencing methods have allowed researchers to examine the cancer transcriptome. Non-coding RNAs (ncRNAs) have been categorized based on their length, shape, and genomic position, and this technology allows us to acquire the sequences and frequencies of dysregulated ncRNAs in malignancies. Cancer-related ncRNAs can be divided into small ncRNAs (sncRNAs, < 200 nt) and long ncRNAs (lncRNAs, > 200 nt) based on size (M. Chen et al. 2021; Fabbri 2014).

## 1.7. miRNAs in Cancer

miRNAs were initially thought to be "junk transcripts," but those are critical mediators in biological robustness, buffering off small perturbations and ensuring organism homeostasis. In humans, miRNAs regulate nearly 60% of the protein-coding genes. Calin, Croce, and coworkers found the first evidence of a miRNA's (miR-15 and miR-16) role in cancer while trying to identify the gene responsible for the deletion of a specific region of the chromosome

in B cell chronic lymphocytic leukemias (Calin and Croce 2006). They suppress gene expression by complementarily binding to target mRNA's 3′ UTR (Lu et al. 2020). Since then, many miRNAs have been identified as tumor suppressors, oncogenes, or both concerning malignancies (Goodall and Wickramasinghe 2021). miRNAs range in length from 20 to 23 nucleotides, but only the first 7 or 8 nucleotides, known as the seed region are necessary for base pairing with target mRNAs. Those are generated from longer precursor transcripts by several, sequential cleavages. Therefore, a single miRNA can target several mRNAs, and vice versa, for example, ZEB1 mRNA has three binding affinities for miR-200a and five binding sites for miR-200b, the miRNAs can act synergistically to significantly inhibit the expression of the target, but a single miRNA binding to a target mRNA only results in a mild reduction in target expression (Jevšinek Skok et al. 2019). The human genome contains 38,589 unique miRNA molecules, 1917 of which have developed into functional forms (http://microrna.sanger.ac.uk/miRBase/). The microRNAs (miRNAs) are involved in crucial processes such as cell fate determination, cell proliferation, tumor growth, immune evasion, invasion, angiogenesis, and cell death. Also play important roles in many other cellular functions as well, including viral replication, neurotransmitter synthesis, circadian, immunological response, rhythm, insulin secretion, and many others (Goodall and Wickramasinghe 2021; B. Wang 2013; Yu Wang and Lee 2009). miRNAs are crucial during physiological settings for maintaining tissue homeostasis and regulating cellular signaling. These molecules work in tandem with other systems to counteract the effects of endocrine hormones as well as other stimuli (e.g., cytokines, chemokines, viral or stress conditions) observed in the cellular microenvironment, preventing the development of abnormal cellular proliferation, and regulating the differentiation of cells and mRNAs. miRNAs are constantly expressed in quiescent or proliferating cells and serve to regulate disorganized cellular replication and differentiation, thereby enabling the tissue to maintain homeostasis or respond

appropriately to stress or injury (Iorio and Croce 2012). However, cancer development can occur if the apoptotic and necrotic processes employed to destroy detected threats are insufficient or if aberrant cells can evade the formation of an immune response (Galvão-Lima et al. 2021). A possible biomarker is the most exciting use of miRNAs. Many studies have investigated the potential role of miRNAs in various areas of medicine. It could be quite useful as a biomarker in cancer (Lan et al. 2015). The word "biomarker" is used to describe a wide range of quantitative measures of bodily function and illness. In 2008, Lawrie et al. used miRNAs to analyze diffuse large B-cell lymphoma in patient serum, establishing their utility as biomarkers for cancer; since then, their potential application as biomarkers has been highlighted in the literature for a wide variety of disorders (Carmen Elena Condrat1,† et al. 2020). There are a few requirements that a reliable biomarker must meet. To begin, it must be accessible; that is, it must be found and measured with noninvasive techniques. Sensitivity is the most crucial factor, followed by specificity to the condition being studied. Finally, it needs to be able to be implemented in clinical settings following the study (Galvão-Lima et al. 2021) (Taylor 2018). Over the years many studies have been done to identify the potential biomarkers that are unique in different cancer types such as breast cancer, cervical cancer, prostate cancer, and lung cancer, and many studies have been done to identify the biomarkers that are the same for different cancers types, for example, miR-18a, miR-21, miR-143, miR-145, miR-218and miR-221 were found to be as important biomarkers in six different cancer types (Sharma and Gupta 2020). It is possible for miRNAs to be secreted into the extracellular space and then transferred to the circulating bodily fluid, such as the peripheral blood. It was revealed that 10 percent of all human miRNAs were detectable in blood plasma and were called circulating miRNAs. These miRNAs are extraordinarily stable and detected in plasma or serum because they are enclosed in extracellular vesicles or associated with specialized lipid proteins that prevent their digestion by RNase. Thus, these tiny molecules have the potential to function as

biomarkers for the diagnosis of cancer with liquid biopsies. These circulating miRNAs can be detected in saliva, cerebrospinal fluid, urine, breast milk, and sperm as well (Hao Wang et al. 2018). A list of some dysregulated miRNAs associated with cancer is shown in table 1.1.

Table 1.1 The table gives a list of some dysregulated miRNAs which are known to be associated with different types of cancers.

| **miRNA** | **Regulation** | **Cancer** | **Reference** |
|---|---|---|---|
| miR-34a, miR-34b miR-34c | Cell cycle arrest, apoptosis & senescence | Various types of cancers | (He1 et al. 2007) |
| miR-145 | Apoptosis | Various cancers like prostate cancer | (Suzuki et al. 2009) |
| miR-143/145 cluster | Transcriptional repression of miR-143/145 cluster | Various cancers like Pancreatic, Colorectal Adenocarcinoma | (OA Kent, K Fox-Talbot 2021) |
| miR-20a, miR-181b | Inverse correlation between MN1 and miRNAs | Acute myeloid leukemia(AML) patients | (Seipel et al. 2020) |
| miR-17~92 cluster | Controls the expression of E2F1, THBS1, CTGF, & PTEN | Various types of cancer, including B- Cell lymphoma & Breast cancer | (Dews et al. 2006)(Mogilyansky and Rigoutsos 2013)(O'Donnell et al. 2005)(Ventura et al. 2008)(Yulin Li1, Peter S. Choi1, Stephanie C. Casey1, David L. Dill2 2006) |

| | | | |
|---|---|---|---|
| miR-200c, miR-26, miR-29, miR-30, let-7 | Suppresses the expression of their genes | Nasopharyngeal carcinoma & lymphoma | (Chang et al. 2008; Xinwei Zhang#1, 2, Xiaohong Zhao#1, Warren Fiskus3, Jianhong Lin4, Tint Lwin1 et al. 2012) |
| miR-210, miR-155 | Repression of initiation of tumor growth | Various cancers like Head & neck tumor | (Cicchillitti et al. 2012) |
| miR-145 | Tumor progression | Breast Cancer, Colon cancer | (S. Pan et al. 2021) (Zhai and Ju 2011) |
| miR-188-5p | Proliferation, metastasis | HCC | (Fang et al. 2015) |
| miR-15b-5p | Induces ER stress and apoptosis | HCC | (W. Y. Pan et al. 2019) |
| miR-147 | Regulates ERBB3/AKT2/c-Myc and ERBB3/AKT2/Snail pathway | Bladder cancer | (Xiao Wang et al. 2016) |
| miR-21 | Proliferation, migration, EMT | Breast Cancer | (Hui Wang et al. 2019) |
| miR-34b, miR-451 | Regulates apoptosis, senescence, cell cycle arrest | Gastric cancer | (Bai and Wu 2019) (Hermeking 2010) |
| miR-141 | Inhibits cancer stem cell properties | Prostate cancer | (C. Liu et al. 2017) |
| hsa-miR-211, hsa-miR-670, hsa-miR-4770, hsa-miR-23c | Cancer progression | Endometrial Cancer | (X. Xu et al. 2019) |

| | | | |
|---|---|---|---|
| miR-217, miR-144, miR-129, miR-125b, miR-125a, miR-375<br><br>miR-328, miR-486, miR-194 | Role in cancer progression | Colon cancer | (Zhu et al. 2020) |
| hsa-miR-17-29 cluster (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-92-1 | Accelerates tumor development | Lung Cancer | (Lin, Yu, and Yang 2010) |
| miR-2, miR-92a, miR-155, miR-543, miR-27a, miR-200a, miR-20b, miR-371-373 cluster, miR-9, miR-183, miR-223, miR-200b, miR-124, miR-126, miR-148a, miR-26a, miR-199 family, miR-195, miR-27a, miR-375, miR-133b, miR-143, miR-125b | Tumor Growth | Esophageal Carcinoma | (Zarrilli et al. 2021) |

miRNAs can also function as epigenetic modulators by focusing on enzymes involved in epigenetic processes which include DNA methyltransferases (DNMTs), histone deacetylases (HDACs), and histone methyltransferases (EZH) (Sato et al. 2016) (Yingqin Li et al. 2019). Epigenetic mechanisms including DNA methylation, RNA modification, and histone modification are also involved in controlling miRNA expression. Demethylation of the HOXA10 promoter increased the overexpression of HOXA10 and miR-196b-5p, enhancing gastric cancer growth and invasion in vitro (L. Shao et al. 2018). In another experiment carried

out by Rennert and coworkers. it was found that by treating H1299 cells with BIX01294, an inhibitor of G9a methyltransferase, miR-106b-3p and miR-151a-3p were downregulated in lung cancer (Pang, Title, and Rennert 2014). MicroRNAs can alter genome-wide methylation by changing DNA methylation enzyme expression. miR-29b focuses on DNMTs and TETs to alter DNA methylation. miR-29b inhibition during early pig embryo development increased DNA methylation by overexpressing DNMT3A/B and TET1 and suppressor TET2/3 (Z. Zhang et al. 2018). In hepatocellular carcinoma patients, a large number of miRNAs were involved in H3-K9 methylation (W. Ding et al. 2017).

### 1.8. lncRNAs in Cancer

Long non-coding RNA transcripts are those that are longer than 200 nucleotides in length and do not have the potential to code for proteins. However, they may have shorter open reading frames (ORFs). Represent a sizable proportion (>80%) of all ncRNAs; their expression varies widelyacross tissues and cell types. First, lncRNA XIST and H19 were identified in 1990 (Bolha, Ravnik-Glavač, and Glavač 2017; Brannan et al. 2015; Brockdorff et al. 1992). The trimethylation of histone H3 at Lys27, an important epigenetic complex, was found to be regulated by XIST's direct binding to PCR2(J. Zhao et al. 2008). miRNAs typically negatively regulate gene expression by speeding the deadenylation and degradation of target mRNAs whereas lncRNAs can regulate gene expression in cis or trans by acting as scaffolds for regulatory protein complexes, by localizing to genomic DNA, or by altering genome architecture (shown in figure 1.8). The lncRNA can bind to the PRC2 complex, leading to the repression mark of gene expression known as lysine 27 (H3K27) methylation on histone H3 (Ana Luisa Pedroso Ayub, Debora D'Angelo Papaiz et al. 2016). Most lncRNAs with known functions regulate gene expression, usually at the transcriptional level. lncRNAs have been proven to exhibit oncogenic or tumor suppressor properties despite their recent discovery. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a long noncoding RNA

that has been linked to both gene silencing and gene activation, suggesting it may play a role as a molecular scaffold (Qiu et al. 2013) (Arun, Aggarwal, and Spector 2020). Recent breakthroughs in cancer transcriptome profiling and substantial evidence of the involvement of lncRNAs have linked some differentially expressed lncRNAs to diverse cancer types, all of which gain one or more dynamic structural alterations. Chromatin remodeling of lncRNA HULC is linked with the progression of Hepatocellular carcinoma (Panzitt et al. 2007). It was found that lncRNA UCA1 in gallbladder cancer cells interacted with Brahma related gene 1 (BRG1), a component of the chromatin SWI/SNF remodeling complex, and inhibited BRG1's ability to bind to the p21 promoter region (Xiujuan Wang et al. 2014).

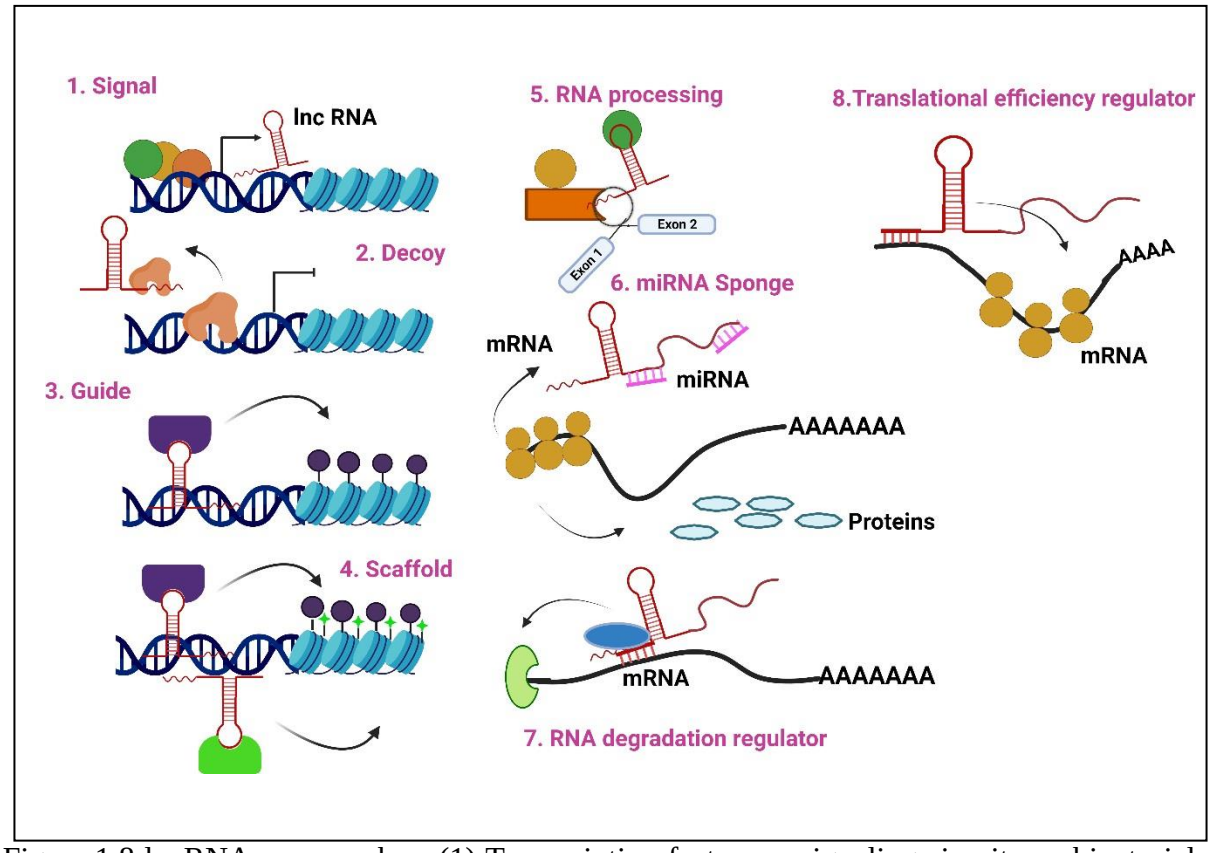



Figure 1.8 lncRNAs can work as (1) Transcription factors or signaling circuit combinatorial. (2) DNA-binding proteins like transcription factors. (3) Partner with chromatin-modifying enzymes to target genes. (4) Scaffold to bring proteins together. (5) Nuclear paraspeckle and help in processing mRNAs. (6) miRNA sponge. (7) Exosome-mediated RNA degradation. (8) Translation efficiency regulator.

HOTAIR is one of the first reported and characterized lncRNA involved in cancer progression (Bolha, Ravnik-Glavač, and Glavač 2017; R. A. Gupta et al. 2010). HOTAIR is shown to bind PRC2 and the LSD1-CoREST complex simultaneously to inhibit genes (Tsai et al. 2010).

Large intergenic non-coding RNAs (lincRNAs) are a subset of lncRNAs that promote the transcription of genes involved in metastasis and angiogenesis, two hallmarks of tumor growth (Meisler 2001). lncRNAs have also been studied to identify their role as potential biomarkers in cancers. Various cancer-specific lncRNA biomarkers such as SLC16A1-AS1 in lung cancer (H. Y. Liu et al. 2020), lncRNA MIAT in breast cancer (Ye et al. 2021), and others have been identified. Apart from this lncRNA such as SNHG16 have been identified as a common biomarker in twelve cancer types e.g., acute lymphoblastic leukemia, cervical cancer, colorectal cancer, diffuse large B-cell lymphoma, esophageal squamous cell carcinoma, gastric cancer, hepatocellular carcinoma, neuroblastoma, non-small cell lung cancer, oral squamous cell carcinoma, pancreatic cancer and retinoblastoma (Y. Xiao et al. 2020). lncRNAs can be found in nearly every part of a cell. Apart from their abundant localization in the nucleus and cytoplasm studies have identified the presence of lncRNAs in body fluids such as serum, blood, plasma, gastric juices, sperm, etc. known as circulating lncRNA which offers a new method for early, noninvasive cancer diagnosis (Qi, Zhou, and Du 2016).

### 1.9. lncRNA–miRNA–mRNA competing endogenous RNA network

Generally, ncRNAs are a part of elaborate networks of interconnections with other RNAs and proteins that can have far-reaching consequences for cell biology. The broad alteration of miRNA function and expression seen in human cancer has been used as a model system to investigate the non-coding genome's potential role in human disease. As seen earlier microRNAs a negative regulator of gene expression, prevent or slow the translation of their mRNA targets. Thus, microRNAs are typically considered to be dynamic regulatory elements, while their target mRNAs are considered to be repressed targets. Repression of target gene expression is typically the result of microRNA binding to microRNA response elements (MREs) on target RNA transcripts. The human genome contains roughly 20,000 protein-coding genes, many of which are heavily covered in MREs. As our ability to detect MREs on transcripts of coding genes improves; we can estimate how much regulation is mediated by microRNAs. More MREs mean better "communication" and more effective coregulation. miRNAs in RISCs can regulate target lncRNAs, reducing their structural and functional stability via imperfect base-pairing (L. L. Chen 2016) just as they can regulate target mRNAs. It has been found that miRNAs bind to lncRNA via miRNA response elements (MREs), which also compete with mRNA for the binding of miRNAs. These RNAs are known as competitive endogenous RNAs because they compete with miRNAs to prevent their target mRNAs from being regulated (ceRNAs) (Salmena et al. 2011) (shown in figure 1.9). The ability of an MRE in lncRNA to function as a ceRNA is contingent on its ability to bind miRNA in an incompletely complementary fashion. Small interfering RNAs (miRNAs) and long non-coding RNAs (lncRNAs) regulate each other by binding to complementary sites on each other. MicroRNA (miRNA)-long noncoding RNA (lncRNA) interactions are thought to play a crucial role in the regulation of their target genes by leading to the premature aging of the targeted lncRNAs. These connections create intricate webs of activity.

These interactions between miRNAs and lncRNAs can be direct or indirect, and they happen primarily in the cytosol and less frequently in the nucleus (Zur Hausen 2008).

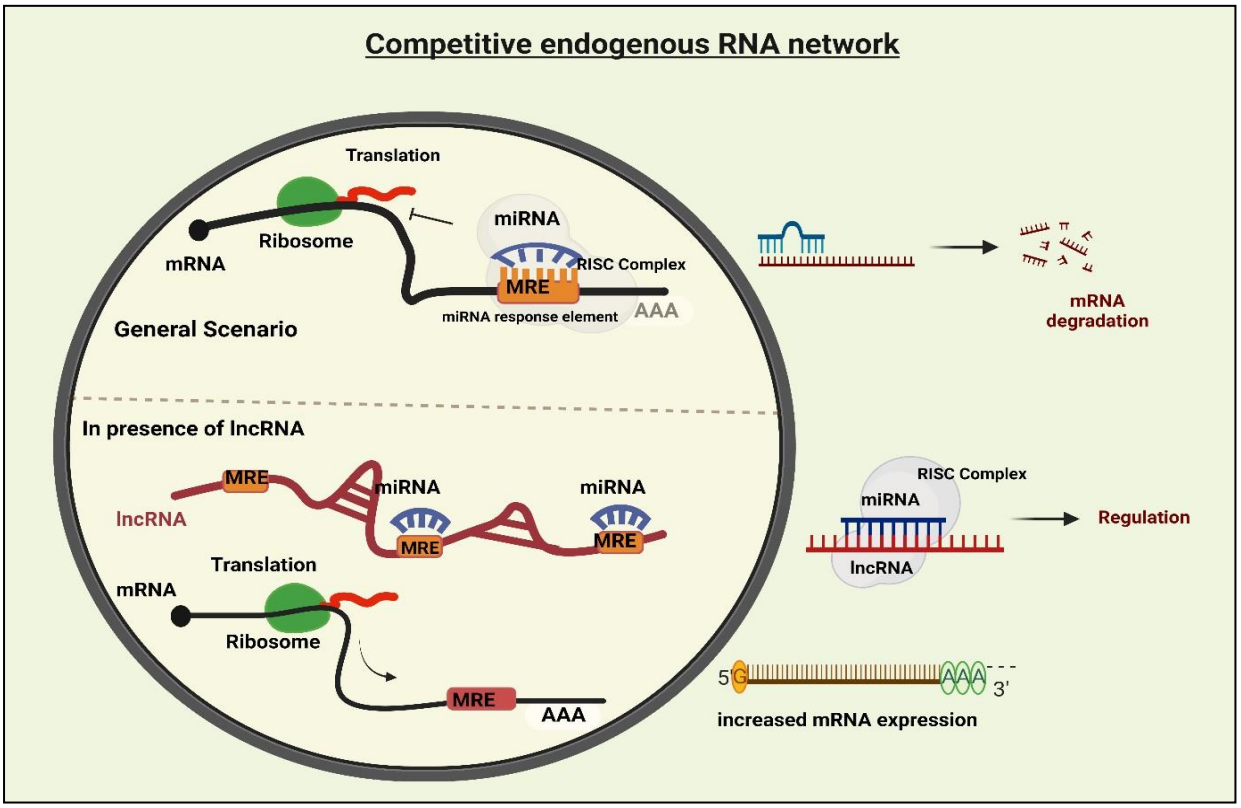



Figure 1.9 The schematic figure depicts the competing endogenous RNA regulatory mechanisms adopted by the lncRNAs in cancer progression. In a general scenario, miRNA binds at MRE (miRNA response element) sites present on mRNA and leads to mRNA regulation. In presence of lncRNA, these lncRNAs compete with mRNA for the binding of miRNAs leading to increased mRNA expression.

According to recent research (Ebert and Sharp 2010), lncRNA could also bind to miRNA and thereby "communicate" with other RNA targets. In many cases, miRNA regulates lncRNA and vice versa. The escalating number of research studies have centered on the functions of lncRNAs in epigenetic changes as well as other biological processes as the number of lncRNAs has grown. Notably, there is growing evidence that lncRNAs can act as natural miRNA decoys, implying they may serve as ceRNAs at the post-transcriptional level. LINCMD1, a muscle-specific lncRNA, is the first example of a lncRNA functioning as a ceRNA. It controls muscle differentiation by binding and sequestering microRNAs miR-133 and miR-135. The expression of the transcription factors mastermind-like 1 (MAML1) and myocyte enhancer factor 2C (MEF2C), which trigger muscle-specific gene expression, is typically suppressed by these

miRNAs. Since LINCMD1 acts as a miRNA decoy by preventing the release of these miRNAs, MAML1 and MEF2C are turned on (Dai et al. 2015). They can also act as miRNA sponges, capturing miRNAs for storage, or they may bind directly to the 3'-UTR of mRNAs to prevent miRNA-mediated mRNA decay. Because lncRNAs can regulate the expression of all other transcripts via the ceRNAs network, studying these networks may help us determine how lncRNAs and their ceRNA networks aided in the development of cancer. There is growing evidence that ceRNAs play a role in the development, spread, resistance to treatment, and relapse of cancer. Potential advances in diagnosis and treatment for cancer can be uncovered through the study of ceRNA networks. These ceRNA networks can be miRNA-mRNA, lncRNA-miRNA, and lncRNA-miRNA-mRNA. Rapid progress is being made in the creation of cutting-edge computational tools and experimental technologies that can provide putative predictions and validations of ceRNA interactions. Given that many of these regulate critical physiological processes, changes in the abundance or binding of specific miRNAs and lncRNAs will have a direct impact on how cells perform. To alleviate mRNA repression, lncRNAs can effectively remove and compete with miRNAs by binding to the same miRNA binding sites as their mRNA targets.

Approximately 10% of lncRNA genes have been found to host a miRNA in either an intron or an exon (Dunham et al. 2012). In combination with other RNA-binding proteins, studies have revealed that microRNAs can govern lncRNA stability and miRNA-mediated decay by binding to lncRNA. Guangle Zhang et al. studied miRNA-lncRNA interaction by establishing basic miRNA-lncRNA networks (BMLNs) for specific types of cancer and an individual miRNA-lncRNA network (ISMLN) for each disease sample (Zhang et al. 2018). Various studies have been done to understand the lncRNA-miRNA-mRNA network based on competitive endogenous RNA in many different cancer types such as oral cancer (Yin et al. 2020), Gastrointestinal cancer (Dastsooz et al. 2022), Non-small Cell Lung Cancer (M. Wang et al. 2020), Colorectal cancer (Ghasemi et al. 2020) and others to identify the potential biomarkers

and therapeutic targets. A list of some lncRNA-miRNA-mRNA network interactions in various cancer types is listed in table 1.2.

Table 1.2 The table gives a list of some dysregulated lncRNA-miRNA-mRNA axis that are known to be associated with different types of cancers.

| **lncRNA-miRNA-mRNA** | **Function/Role** | **Cancer** | **Reference** |
|---|---|---|---|
| GAS5-miR-196-5p-FOXO1A,<br>H19-miR-340-3p-YWHAZ,<br>H19-miR-152-DMNT1,<br>MALAT1-miR-145-VEGF | Proliferation(+)<br>Angiogenesis<br>Invasion(+)<br>Metastasis(+) | Breast Cancer | (Venkatesh et al. 2021) |
| ZFAS1-hsa-miR-150-5p-GINS1 | replication (+) | Hepatocellular Carcinoma | (S. Chen et al. 2022) |
| SNHG12↑-miR-195-5p-Notch2 | proliferation (+)<br>invasion (+)<br>migration(+) | Osteosarcoma | (S. Zhou et al. 2018) |
| H19↑-miR-200a-ZEB1, ZEB2<br>H19↑-miR-29b-3p- STAT3 | proliferation(+)<br>migration(+)<br>invasion(+) | Lung Cancer | (Y. Zhao et al. 2019)(Lihua Liu, Liu, and Lu 2019) |
| NBAT1↓-miR-21-<br>PTEN/PDCD4/TPM1/RECK | metastasis(-) | Osteosarcoma | (C. Yang et al. 2017) |
| HOTAIR↑-miR-331-3p-HER2 | proliferation(+)<br>metastasis(+) | Gastric cancer | (X. hua Liu et al. 2014) |
| SNHG4↑-miRNA-204-5p-RRM2 | growth(+)<br>metastasis(+) | Prostate Cancer | (CHENG1 et al. 2013) |

| | | | |
|---|---|---|---|
| FOXP4-AS1↑-miRNA-3184-5p-FOXP4 | | | |
| MAFG-AS1↑-miR-147b-NDUFA4 | Proliferation(+), Apoptosis(-) | Colorectal Cancer | (S. Cui et al. 2018) |
| HOTAIR -miR-203a-3p-β-catenin, GRG5 HOTAIR-miR-545-EGFR HOTAIR-miR-218-VOPP1 | proliferation(+) | Colorectal cancer | (Z. Xiao et al. 2018)(P. Li et al. 2017) |
| SNHG12-miR-320b-ZEB, KTN1-AS1-miR-23b-3p-HMGB2, MALAT1-miR-217- KRAS, AFAP1-AS1- miR-384-ACVR1, | proliferation(+), invasion(+), metastasis(+), tumor growth(+) survival(+) | Pancreatic Cancer | (Jichuan Xu et al. 2022) |

## 1.10. Gaps in existing research

Cancer research is a vast field that encompasses many different aspects of the disease, including its causes, diagnosis, treatment, and prevention. Despite significant advances in our understanding of cancer and the development of new treatments, many gaps in our knowledge need to be addressed to advance the field further. One major gap in cancer research is the lack of understanding of the underlying causes of cancer. While we know that certain genetic and environmental factors can increase the risk of developing cancer, we still don't fully understand the mechanisms by which these factors contribute to the development of the disease. This makes it difficult to develop targeted prevention strategies and identify new therapeutic targets for treatment. In this context, a major gap in cancer research is the lack of integration and analysis of large, diverse data sets. However, this data is often scattered across different

databases and sources, and there is an urgent need for more effective tools and techniques to integrate and analyze this data to gain a more comprehensive understanding of the disease. In this regard, the non-coding RNAs (ncRNAs) and their implications in cancer biology are relatively unexplored research fields despite their growing implications in this field of science. For example, there are still many lacunae in our understanding of the lncRNA-miRNA-mRNA axis in cancer, and further research is needed to understand its role in cancer development and progression fully. Some key areas in this domain where further research can enrich our knowledge include understanding the complex cross-talk existing between ncRNAs and how their expression pattern shapes during cancer pathogenesis, across diverse cancer types. Herein, it is known that lncRNAs and miRNAs can regulate gene expression by binding to target mRNAs and inhibiting their translation or promoting their degradation. However, the precise mechanism by which this occurs is not fully understood. Furthermore, identifying the specific lncRNAs and miRNAs that are dysregulated in different stages of cancer and understanding their roles in cancer development and progression is also poorly elucidated. Importantly, advancement in genome sequencing technologies has been a boon in obtaining data on the ncRNAs across cancer types, and by that, there has been progress in identifyinglncRNAs and miRNAs that are dysregulated in specific cancers. However, a holistic approach though possible with publicly available datasets is still missing. A comprehensiveunderstanding of the unique or common lncRNAs or miRNAs involved in cancer and the mechanisms by which they contribute to cancer have not yet been fully explored. Research channeled in this direction would help in determining the clinical utility of targetingthe lncRNA-miRNA-mRNA axis in cancer treatment. Importantly, based on growing evidence,the lncRNA-miRNA-mRNA axis is increasingly getting recognized as a promising therapeutictarget for cancer, therefore, more research is needed to identify putative targets/biomarkers, determine the most effective ways to target this axis for the treatment and to understand the

potential side effects of such therapies. Herein, it must be understood that cancer is a heterogeneous disease; therefore, delineating this complex set of interactions between lncRNAs-miRNAs and mRNAs in diverse cancer types would be useful for a future fundamental understanding of their modus operandi and appropriate therapy design. The current study, therefore, characterizes the expression pattern of the ncRNAs and mRNAs across diverse cancer types, obtaining data from publicly available databases and presenting the key molecules de-regulated.

## 1.11. Objectives of the Current Study

1. Understanding transcriptomic variations in different carcinomas and identification of networks of pathways responsible for cancer progression.

2. Elucidating the role of miRNAs and lncRNAs in the progression of carcinomas and identification of potential lncRNA/miRNA/mRNA axis across pan carcinomas.

# CHAPTER-II

## Research Materials and Methodology

This chapter describes the research approach used for our study. We described the research procedure along with the tools and databases used in our study. It begins with a detailed description of the database and the data selection criteria, followed by the methodology used for selecting and sorting the data types. The extraction and analysis of the complete RNA sequencing data of the various carcinoma patients comprising mRNAs, miRNAs, and lncRNAs data from the publicly accessible data portal are described.

## 2.1 Overview

The strategies for achieving the objectives of this study are illustrated in figure 2.1. It outlines the workflow we used to select, collect, and analyze the transcriptomic data for carcinoma patients from NCI's genomic data commons (GDC) which is a repository containing data shared through several cancer genome programs. mRNAs, miRNAs, and lncRNAs data were obtained from carcinoma patients. As it is shown in the workflow, the transcriptomic data of various carcinoma patients was downloaded from the GDC portal. The dysregulated mRNAs, miRNAs, and lncRNAs are identified through the Deseq2 suits of the program. Finally, the miRNA-mRNA and lncRNA-miRNA-mRNA axis were identified through network interaction studies and pathway analysis.

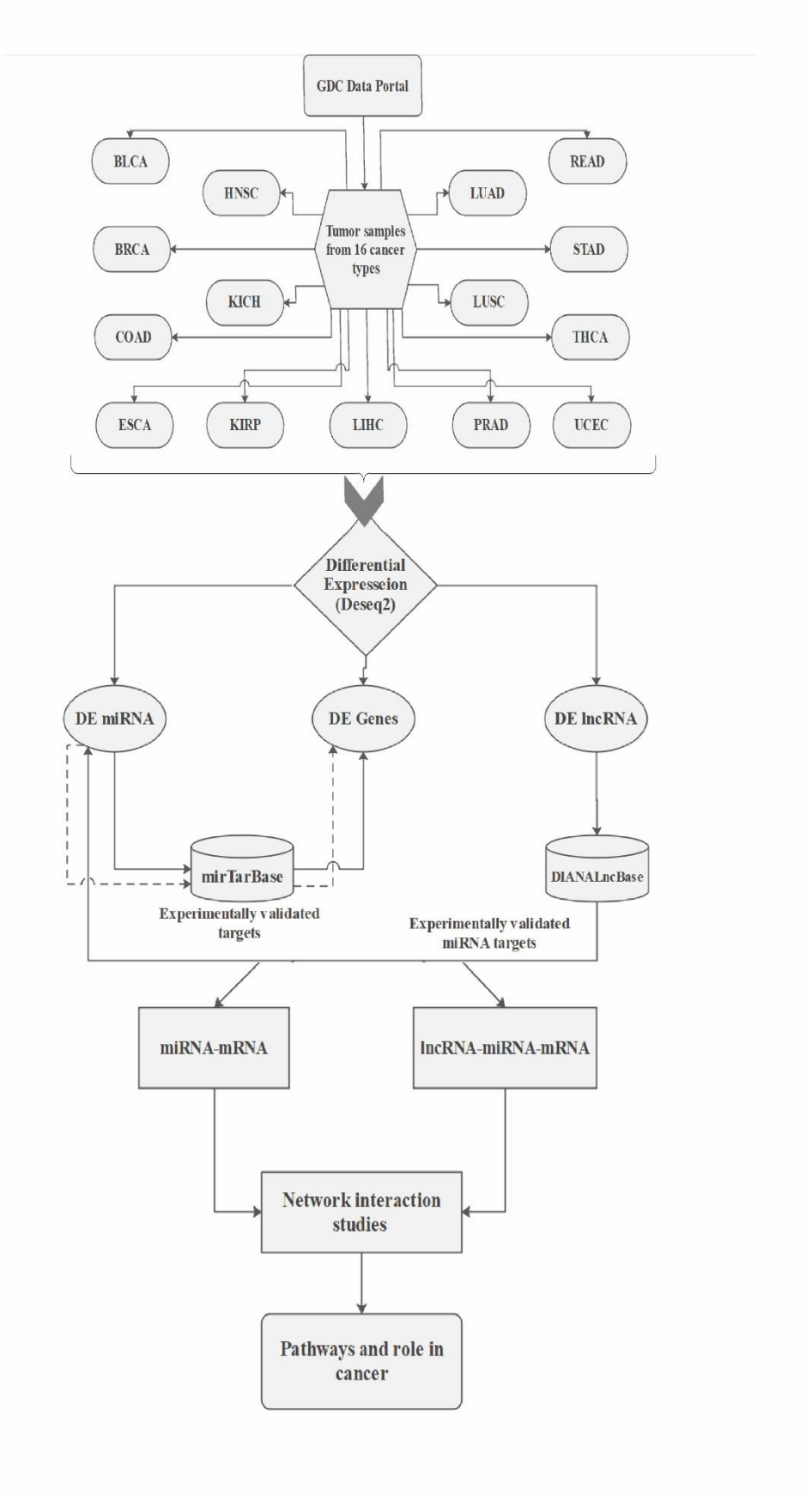



Figure 2.1 Workflow of the research methodology adopted in this study.

## 2.2. Datasets

### 2.2.1. GDC Portal

Genomic Data Commons (GDC), an NCI data-sharing platform, consists of information on patients suffering from different kinds of cancer worldwide. It is a knowledge base that provides researchers with standardized clinical, proteomic, epigenomic, and genomic data from multiple cancers, allowing them to better pinpoint abnormalities in cancer cells that may be pivotal in the development of cancer. The preparation of GDC databases began in June 2015 and is being regularly updated (https://portal.gdc.cancer.gov/repository). The latest version of GDC v36.0 was released on 12$^{th}$ December 2022. The data from multiple sources and in various formats is deposited in GDC by researchers working on patients suffering from different types of cancers. Currently, the GDC database contains 86,513 cancer patient data involving 67 primary sites from almost all parts of the human body. Within GDC these cancer data are organized into various categories under the tag "Programs" which consist of 22 different types of large and small repositories or databases that store cancer patient data. These 22 programs contain 74 projects. A project is a focused endeavor to explore a specific type(s) of cancer as part of a wider cancer research program. Out of the several programs provided within GDC, we have considered data under the TCGA program. The TCGA data is categorized based on the primary site i.e. site where cancer has occurred first.

### 2.2.2. TCGA program

The cancer genome atlas (TCGA) stands out as one of the most comprehensive and fruitful collaborative projects between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) which contains 20,000 original cancer and normal samples data from 33 common and 10 unusual cancers. The data relating to all 33 cancer types and the number of cases within each cancer type is tabulated in Table 2.1.

Table 2.1 List of all 33 TCGA-Cancer along with the number of cases in each available on the TCGA project.

| CANCER | CASES |
|---|---|
| TCGA-BRCA (Breast invasive carcinoma) | 1,097 |
| TCGA-GBM (Glioblastoma multiforme) | 166 |
| TCGA-OV (Ovarian serous cystadenocarcinoma) | 492 |
| TCGA-LUAD (Lung adenocarcinoma) | 519 |
| TCGA-UCEC (Uterine corpus endometrial carcinoma) | 542 |
| TCGA-KIRC (Kidney renal clear cell carcinoma) | 537 |
| TCGA-HNSC (Head and Neck squamous cell carcinoma) | 510 |
| TCGA-LGG (Brain Lower Grade Glioma) | 516 |
| TCGA-THCA (Thyroid carcinoma) | 507 |
| TCGA-LUSC (Lung squamous cell carcinoma) | 504 |
| TCGA-PRAD (Prostate adenocarcinoma) | 498 |
| TCGA-SKCM (Skin Cutaneous Melanoma) | 470 |
| TCGA-COAD (Colon adenocarcinoma) | 433 |
| TCGA-STAD (Stomach adenocarcinoma) | 439 |
| TCGA-BLCA (Bladder Urothelial carcinoma) | 412 |
| TCGA-LIHC (Liver hepatocellular carcinoma) | 377 |
| TCGA-CESC (Cervical squamous cell carcinoma and endocervical adenocarcinoma) | 307 |
| TCGA-KIRP (Kidney renal papillary cell carcinoma) | 291 |
| TCGA-SARC (Sarcoma) | 255 |
| TCGA-LAML (Myeloid Leukemias) | 200 |
| TCGA-PAAD (Pancreatic adenocarcinoma) | 178 |
| TCGA-ESCA (Esophageal carcinoma) | 184 |
| TCGA-PCPG (Pheochromocytoma and Paraganglioma) | 179 |
| TCGA-READ (Rectum adenocarcinoma) | 158 |
| TCGA-TGCT (Testicular Germ Cell Tumors) | 150 |
| TCGA-THYM (Thymoma) | 123 |
| TCGA-KICH (Kidney Chromophobe) | 113 |
| TCGA-ACC (Adrenocortical carcinoma) | 92 |
| TCGA-MESO (Mesothelioma) | 83 |
| TCGA-UVM (Uveal Melanoma) | 80 |
| TCGA-DLBC (Lymphoid Neoplasm Diffuse Large B-cell Lymphoma) | 48 |
| TCGA-UCS (Uterine Carcinosarcoma) | 57 |
| TCGA-CHOL (Cholangiocarcinoma) | 51 |

Out of 33 cancers, 22 cancer types belong to the carcinoma category. In this study, we consider 16 carcinomas as the control sample count was less than 10 for the remaining 6 carcinomas. Sixteen different carcinomas along with their primary site are represented in figure 2.2.

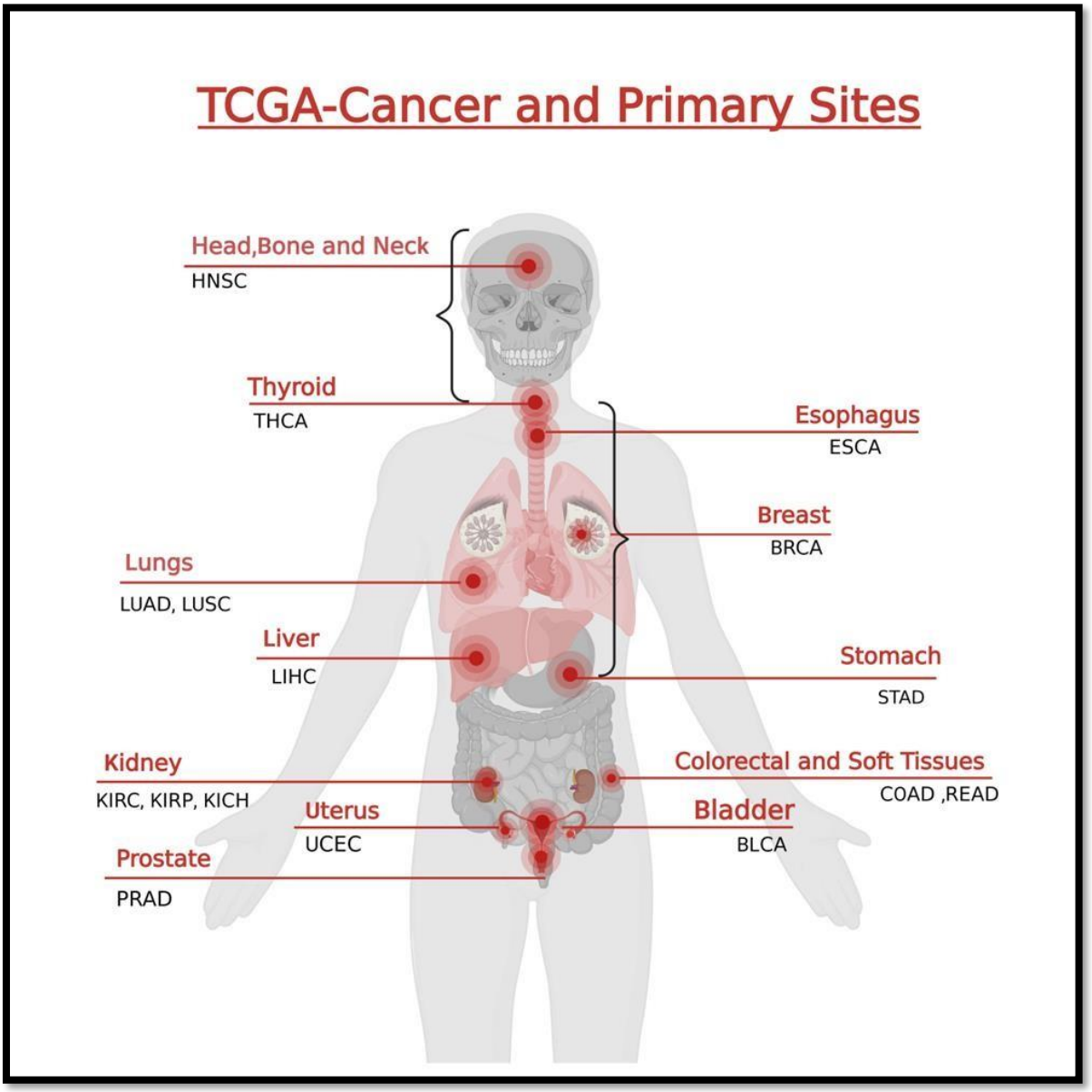



Figure 2.2 The figure depicts the sixteen different carcinomas along with their primary site which are considered for our study.

### 2.3. Data retrieval and data processing

The complete RNA expression data of sixteen different types of carcinomas were extracted through Bioconductor, a free, open-source software project. Bioconductor is used for the analysis and comprehension of genomic data generated by wet lab experiments in molecular biology which are primarily based on the statistical R programming, we have utilized NIH / NCI Genomic Data Commons Access, TCGA Workflow, TCGAbiolinks, and Limma for RNA sequence data retrieval and processing. The complete transcriptomic data comprising

of mRNAs, miRNAs, and lncRNAs data were downloaded and details of control and tumor samples are shown in Table 2.2.

Table 2.2 The data size of each sixteen carcinomas with tumor and control samples.

| Cancer Type | Total Sample | Control patient | Tumor |
|---|---|---|---|
| TCGA-BLCA (Bladder Urothelial Carcinoma) | 427 | 19 | 408 |
| TCGA-BRCA (Breast invasive carcinoma) | 1208 | 113 | 1095 |
| TCGA-COAD (Colon adenocarcinoma) | 326 | 41 | 285 |
| TCGA-ESCA (Esophageal carcinoma) | 195 | 11 | 184 |
| TCGA-HNSC (Head and Neck squamous cell carcinoma) | 564 | 44 | 220 |
| TCGA-KICH (Kidney Chromophobe) | 91 | 25 | 66 |
| TCGA-KIRC (Kidney renal clear cell carcinoma) | 606 | 73 | 533 |
| TCGA-KIRP (Kidney renal papillary cell carcinoma) | 322 | 32 | 290 |
| TCGA-LIHC (Liver hepatocellular carcinoma) | 421 | 50 | 371 |
| TCGA-LUAD (Lung adenocarcinoma) | 574 | 59 | 515 |
| TCGA-LUSC (Lung squamous cell carcinoma) | 553 | 51 | 502 |
| TCGA-PRAD (Prostate adenocarcinoma) | 549 | 52 | 497 |
| TCGA-READ (Rectum adenocarcinoma) | 104 | 10 | 94 |
| TCGA-STAD (Stomach adenocarcinoma) | 450 | 35 | 415 |
| TCGA-THCA (Thyroid carcinoma) | 564 | 59 | 505 |
| TCGA-UCEC (Uterine Corpus Endometrial Carcinoma) | 200 | 24 | 176 |

### 2.3.1. Data normalization

We analyze transcriptomic data through DESeq2 which provides differential expression analysis based on the negative binomial distribution. The DESeq2 package is designed for normalization, visualization, and differential analysis of high-dimensional count data. It makes use of empirical Bayes techniques to estimate priors for log fold change and dispersion and to calculate posterior estimates for these quantities (Rajkumar et al. 2015). Differential expression analysis was conducted for each of the sixteen cancer types using a fold change cut-off of ±1.5 and p-value of ≤0.05 (M. I. Love, Anders, and Huber 2014).

### 2.3.2. Analysis of Transcriptomic data

Gene ontology and functional analysis of dysregulated genes were analyzed using DAVID (D. W. Huang, Sherman, and Lempicki 2009) which is a web-accessible application that provides functional genomic annotations with graphical summaries. The functional annotated dysregulated genes are then classified into three gene ontology categories as GO_BP (Biological Process), GO_CC (Cellular Component), and GO_MF (Molecular Function). We identify “key genes” by using Venny which is an interactive tool used for comparing lists of elements and finding the one present in all (https://bioinfogp.cnb.csic.es/tools/venny/index.html). The dysregulated genes associated with all three functional categories are termed ‘key genes’.

### 2.3.3. Pathway analysis

The ClueGO module of Cytoscape (Shannon et al. 2003) was used to analyze large lists of genes to identify pathways. The ClueoGo module works by selecting gene ontology terms and pathways from numerous gene ontology (GO) data and then visualizing these components as networks that are organized according to their functions. Thereafter, clueGO will construct a dynamic network structure with gene lists that are of interest by using kappa score statistics to connect the terms in the network. ClueGO develops a network of GO and route terms that are

functionally ordered by the integration of GO terms as well as pathways from KEGG (Kanehisa and Goto 2000) and BioCarta (Nishimura 2001). The first thing that ClueGO does is generate a binary gene-term matrix with both the selected terms and the genes that are connected with them. The kappa score is then calculated based on this gene-term matrix, and it is this score that is used to define the nodes. The relevance of the term in terms of gene enrichment is reflected in the size of the nodes. For finding the enriched pathway we used a threshold of $p \leq 0.05$, which gave us the pathways that are more significant.

### 2.3.4. Functional annotation of miRNAs and lncRNAs

The potential targets of miRNAs and lncRNAs were obtained from miRTarBase (Chou et al. 2016) and Diana LncBase (Karagkouni et al. 2020) (Paraskevopoulou et al. 2018) respectively. miRTarBase is an experimentally validated miRNA–target interaction database (MTIs). miRTarBase 9.0 (September 2019) has 2,200,449 validated MTIs from 13,389 studies and CLIP-seq data. After manually examining relevant literature as well as data mining the text, miRTarBase has more than 3,500 MTIs. Validation of MTIs is done utilizing reporter assays, western blot, or microarray experiments with overexpression or knockdown of miRNAs. miRTarBase catalogs 3576 empirically verified MTIs in 17 species between 657 miRNAs and 2297 target genes. On the other hand, DIANA-LncBase (version 3) contains 500,000 entries, with 240,000 unique miRNA-lncRNA interactions across tissues and cell types. Interactions are experimentally verified by 15 low and high-throughput methods in 243 cell types/tissues, and 162 experimental conditions.

### 2.3.5. Statistical analysis

#### 2.3.5.1. Principal component analysis

Principal component analysis (PCA) is a form of unsupervised dimension reduction (Hartigan and Wong 1979) algorithm. The method takes a data set with multiple variables that may be correlated and transforms it into a set of values for a smaller number of linearly uncorrelated

principal components. Through the use of principal component analysis (PCA), we were able to reduce the dimensionality of the transcriptomic data, count the number of distinct clusters, and determine how these cancers are grouped according to gene expression, and miRNA expression.

#### 2.3.5.2. K-means clustering

K-means algorithm for unsupervised learning creates K clusters from a given dataset. The algorithm iteratively sorts data points into K categories using the features provided. The data is organized into groups defined by their shared characteristics (Moore 2001). The values of a set of characteristics are used to determine the cluster centers (or "centroids") for K groups. The centroid values are recalculated after each data point is assigned to the centroid that is geographically closest to it using the Euclidean distance (Hartigan and Wong 1979). To optimize the square error function, the algorithm repeats itself until a stop criterion is met, such as when no data point changes in clusters, the sum of distance is minimized, or a maximum number of iterations is reached (C. Ding 2004)

.

$$J(V) = \sum_{i=1}^{c} \sum_{j=1}^{c_i} (\|x_i - v_j\|)^2$$

J(V)=ΣΣ

Where,

‘||xi - vj||’ is the Euclidean distance between xi and vj

‘ci’ is the number of data points in the ith cluster

‘c’ is the number of cluster centers.

There are three most popular clustering methods available which can be used to find the most

optimal clusters. We applied all three to validate our results. To validate the optimal cluster, we use elbow (Shi et al. 2021)**,** Silhouette (scikit-learn 2017), and gap statistic method (Tibshirani, Walther, and Hastie 2001). The elbow algorithm aims todefine clusters in such a way that there are minimum variations between the clusters by keepingthem compact clusters and as small as possible. The bend (knee) in the plot is an indicator of the appropriate number of clusters whereas the Silhouette algorithm measures the quality and consistency of the clusters**.** The silhouette approach computes silhouette coefficients for each point to quantify similarity to its cluster and therefore gives a more precise cluster compared to others. It also gives the plot indicating the best clusters. The gap statistic is a comparison between the observed and expected total intracluster variation at various k values.

.

# CHAPTER-III

## Results and Discussion

### 3.1. Transcriptomic Profiling Carcinomas

The transcriptomic data of sixteen carcinomas are downloaded from the publicly accessible GDC portal to analyze the role of mRNA, miRNA, and lncRNAs in cancer progression. The details of the transcriptomic profile of mRNAs, miRNAs, and lncRNAs for each of these cancers are represented in the figure. 3.1A-C respectively.

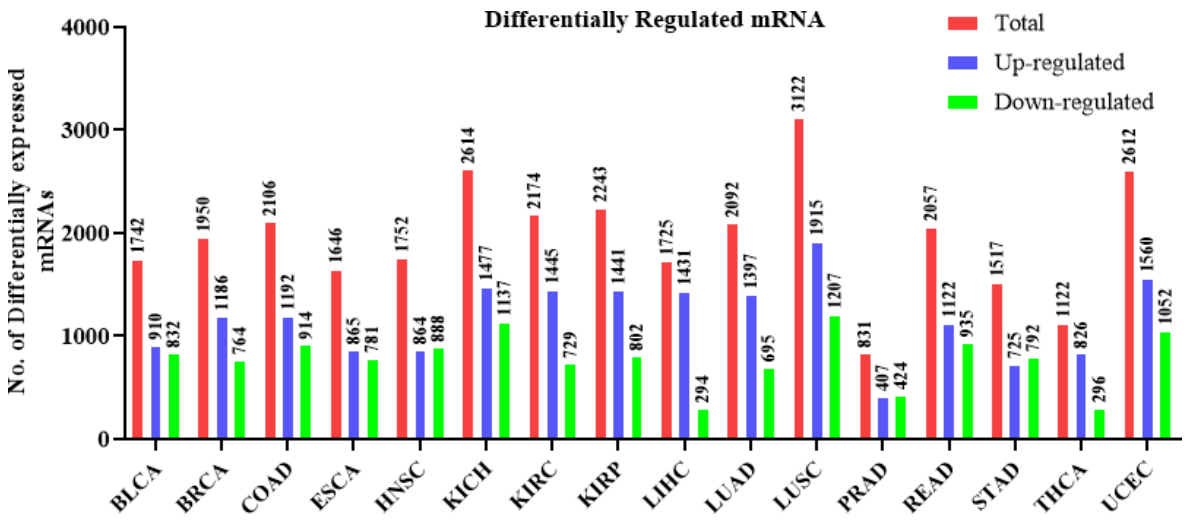



Figure 3.1A The bar graph represents the differentially expressed total, up-regulated, and down-regulated mRNAs in each cancer type.

It is observed that more genes are up-regulated (concerning down-regulated genes) in the majority of carcinomas except for HNSC, PRAD, and STAD where the number of down-regulated genes is higher than up-regulated genes (figure 3.1 A), indicating that positive regulation of genes leads to the progression of cancer. However, in most of the cancer types, quite a large variation in the number of up-regulated and down-regulated genes is observed. It is possible that due to the varied genomic profiles, different types of carcinoma show different prognoses. As it is observed in figure 3.1B, a larger number of miRNAs in READ, COAD, and UCEC is dysregulated. Interestingly, more miRNAs were down-regulated in the majority of carcinomas. It has been reported that due to transcriptional repression, genetic loss, defects in signaling pathways, and epigenetic silencing (Jun Lu et al. 2005; Jansson and Lund 2012) the miRNAs generally have a lower expression in tumors.

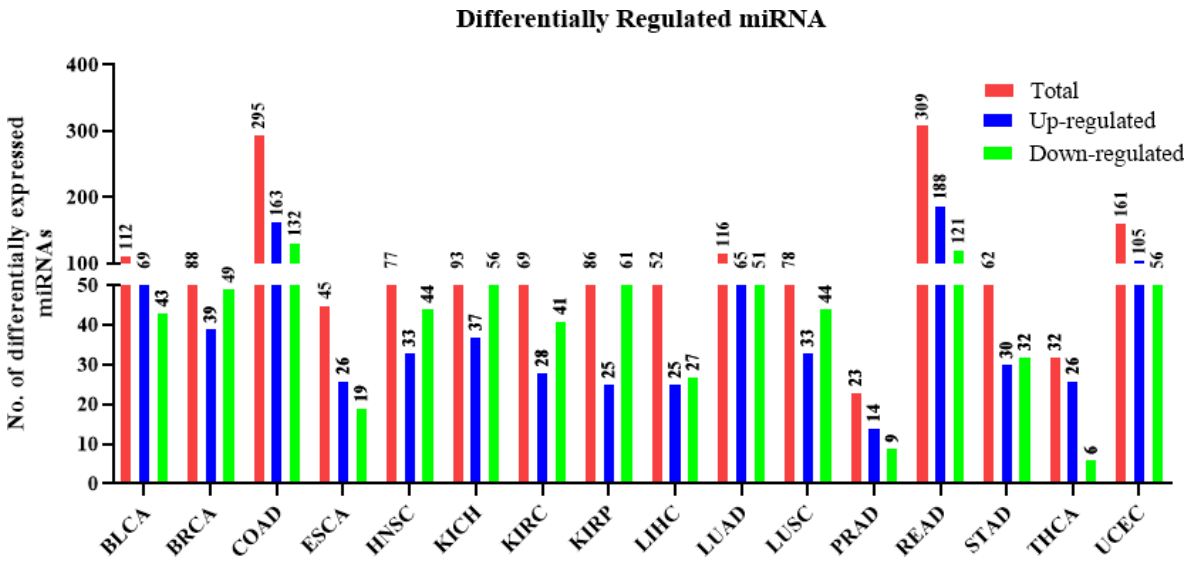



Figure 3.1B The bar graph represents the differentially expressed total, up-regulated, and down-regulated miRNAs in each cancer type.

We have also examined the lncRNA profiling of patient samples to understand its expression pattern in various carcinomas in Figure 3.1C.

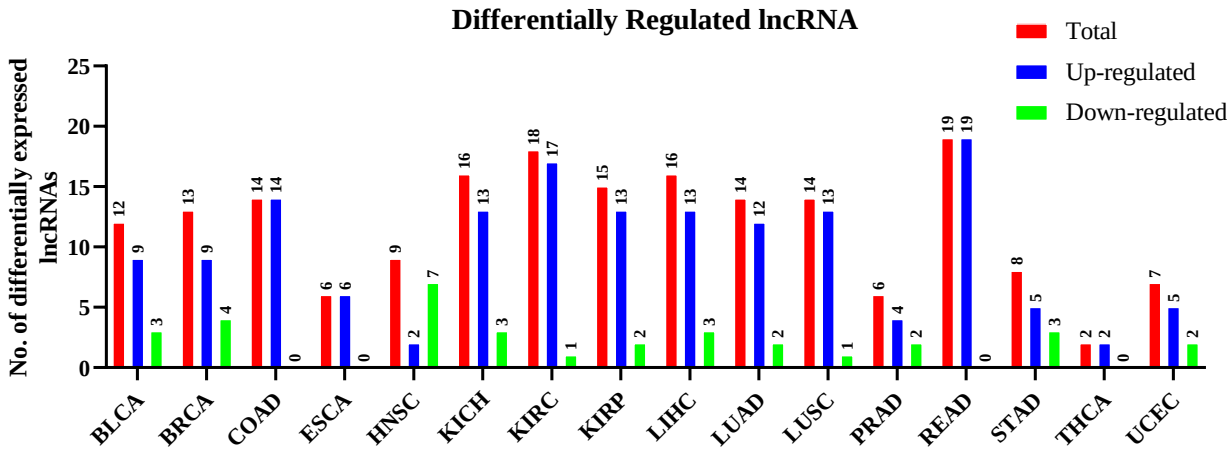



Figure 3.1C The bar graph represents the differentially expressed total, up-regulated, and down-regulated lncRNAs in each cancer type.

In comparison to mRNA and miRNA profiling, a very different expression pattern of lncRNAs was observed across the carcinomas. We observed that in all cancers there were fewer numbers of lncRNAs in total, and all of them had more up-regulated lncRNAs than down-regulated indicating that the upregulation of lncRNAs plays an important role in cancer progression.

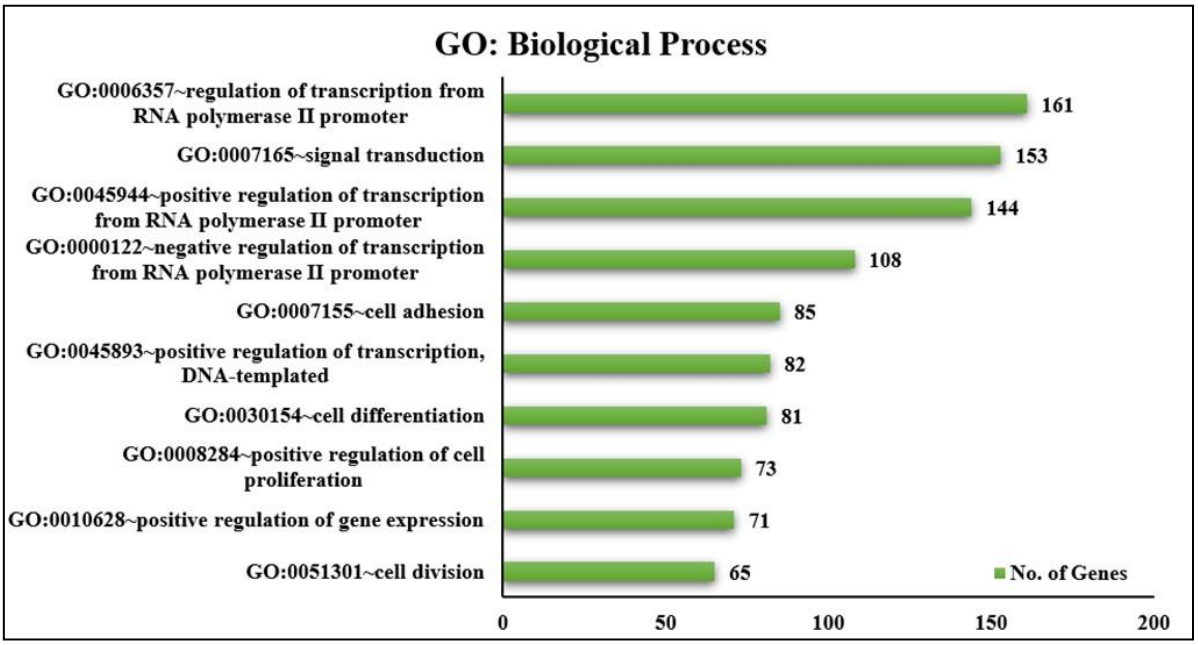
Some cancers like COAD, ESCA, KICH, KIRC, READ, and THCA had no down-regulated lncRNAs. This observation is possibly due to the fact that many lncRNAs remain uncharacterized so far. To date, out of ~16000 annotated lncRNAs, only around 400 are functionally characterized. In the following sections, we analyze the detailed role of miRNA and lncRNA in the gene expression of 16 carcinomas.

## 3.2. Bladder Urothelial carcinoma (BLCA)

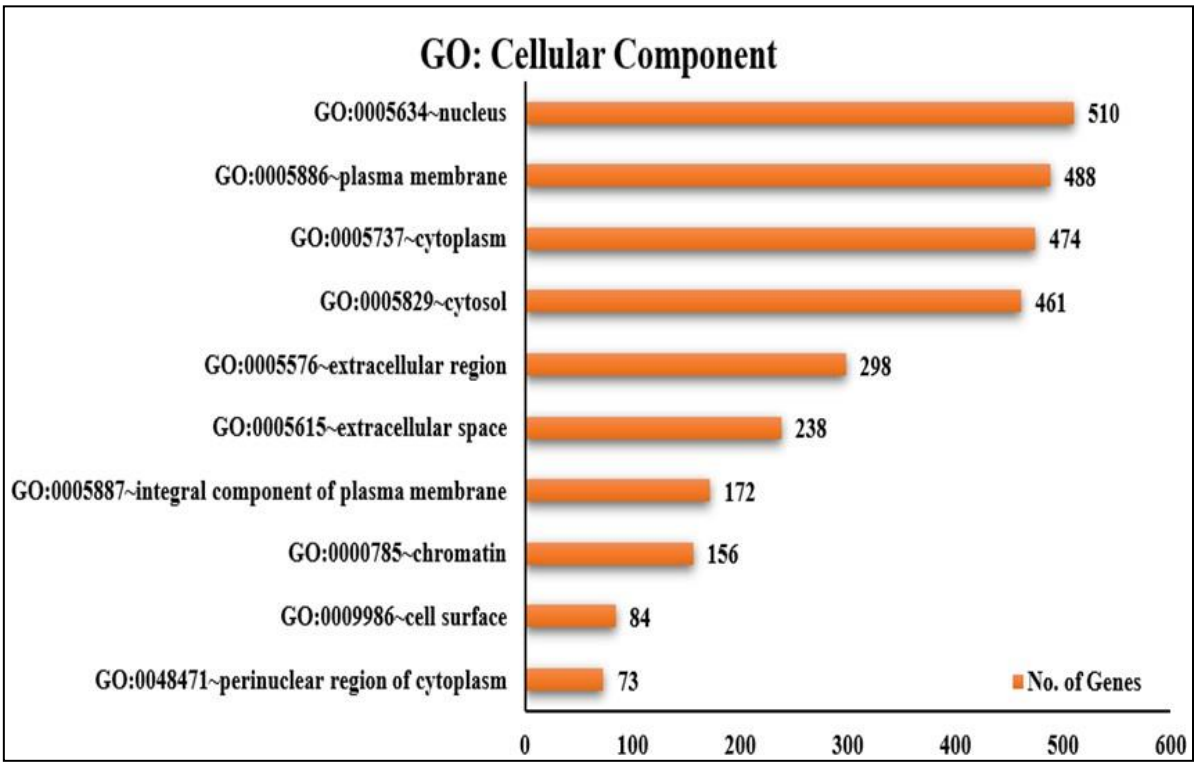
### 3.2.1. Differentially expressed mRNAs

Bladder cancer is one of the most common cancers affecting the urinary system which is also one of the most aggressive and potentially fatal carcinomas (Y. Ding et al. 2021). Typically, urothelial or transitional epithelium cells are the ones responsible for developing bladder cancer. Depending on how deeply it penetrates the muscle tissue, non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer are the two main categories of bladder cancer (MIBC). In total, transcriptomic data of 408 BLCA patients and 19 controls were analyzed. mRNA expression profile analysis revealed that a total of 910 genes are up-regulated whereas 832 genes were down-regulated. The functional annotated dysregulated genes are then classified into three gene ontology categories as GO_BP (Biological Process), GO_CC (Cellular Component), and GO_MF (Molecular Function) (GO), using DAVID. With the help of Venny, we identified the transcripts which are associated with all three functional categories, and those common transcripts (genes) are termed key genes. Here, using this procedure we have identified 1170 key genes (figure 3.2.1A). We have performed gene ontological analysis for each type of transcript (mRNA, miRNA,and lncRNA) of all sixteen cancer types and identified key genes in each case.

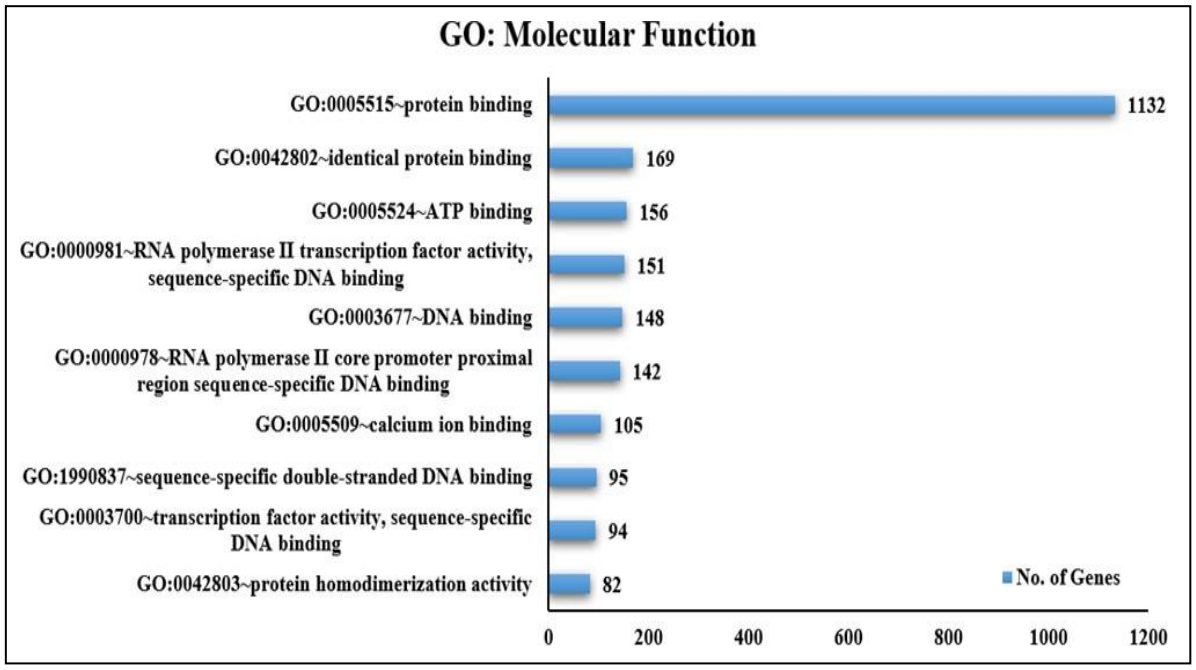
a.


GO: Biological Process
GO:0006357~regulation of transcription from RNA polymerase II promoter
161
GO:0007165~signal transduction
153
GO:0045944~positive regulation of transcription from RNA polymerase II promoter
144
GO:0000122~negative regulation of transcription from RNA polymerase II promoter
108
GO:0007155~cell adhesion
85
GO:0045893~positive regulation of transcription, DNA-templated
82
GO:0030154~cell differentiation
81
GO:0008284~positive regulation of cell proliferation
73
GO:0010628~positive regulation of gene expression
71
GO:0051301~cell division
65
No. of Genes
0
50
100
150
200

b.


GO: Cellular Component
GO:0005634~nucleus
510
GO:0005886~plasma membrane
488
GO:0005737~cytoplasm
474
GO:0005829~cytosol
461
GO:0005576~extracellular region
298
GO:0005615~extracellular space
238
GO:0005887~integral component of plasma membrane
172
GO:0000785~chromatin
156
GO:0009986~cell surface
84
GO:0048471~perinuclear region of cytoplasm
73
No. of Genes
0
100
200
300
400
500
600

c.




d.

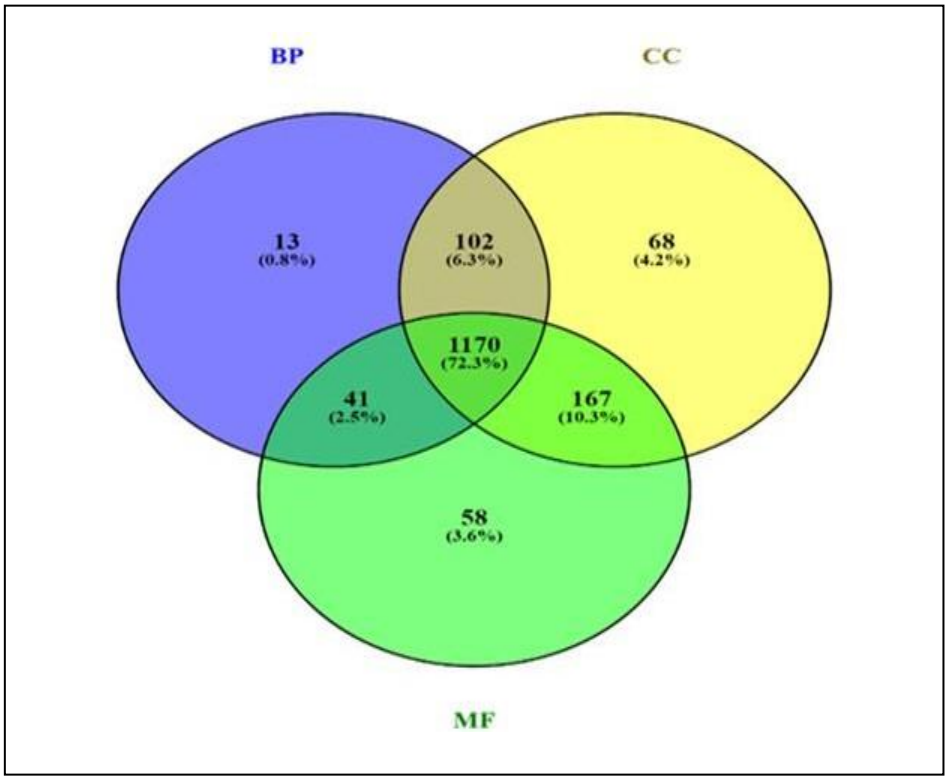



Figure Functional enrichment of the target genes regulated by mRNAs. (a) The top 10 enriched BP genes; (b) the top 10 enriched CC genes; (c) the top 10 enriched MF genes; (d) Venn diagram showing key genes obtained from GO.

To have a deeper insight, the pathways regulated by the key genes were analyzed and represented through ClueGO, a Cytoscape plugin. Pathways that were majorly regulated

included pathways in Cancer (70), MAPK signaling pathway (46), PI3K-Akt signaling pathway (44), calcium signaling pathway (44), and the pathways which were regulated by the least number of genes included cysteine and methionine metabolism (3), mitophagy (3), ABC transporters (4) shown in figure 3.2.1B. All pathways like PI3K-Akt, MAP kinase, and JAK-STAT Pathway have already been reported to play an important role in bladder cancer progression(Chestnut et al. 2020).

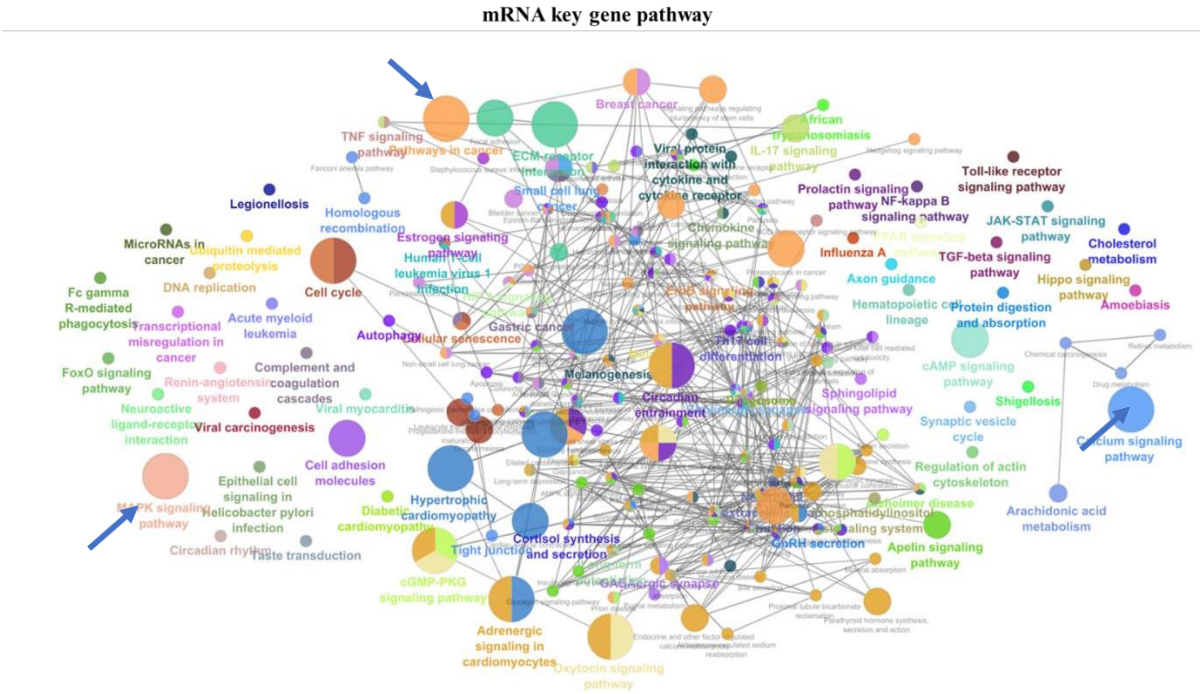



Figure 3.2.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in blue color.

### 3.2.2. Analysis and Construction of miRNA-mRNA network

Bladder cancer consisted of a total of 112 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (69) was higher than the down-regulated miRNAs (43) showing a similar pattern as of mRNAs. By using mirTarBase, we obtained the mRNA targets of these miRNAs and looked for the ones which are present in our data, and finally segregated the miRNA-mRNA correlated pairs consisting of opposite expression values. A total of 727 correlated pairs were obtained with 74 miRNAs (17 down-regulated and 57 up-regulated)

having 397 mRNA (243 down-regulated and 154 up-regulated) targets. Further, we classify these 397 mRNA targets into their GO terms which revealed the biological process, cellular components, and molecular function and led to the identification of 288 key genes shown in figure 3.2.2A.

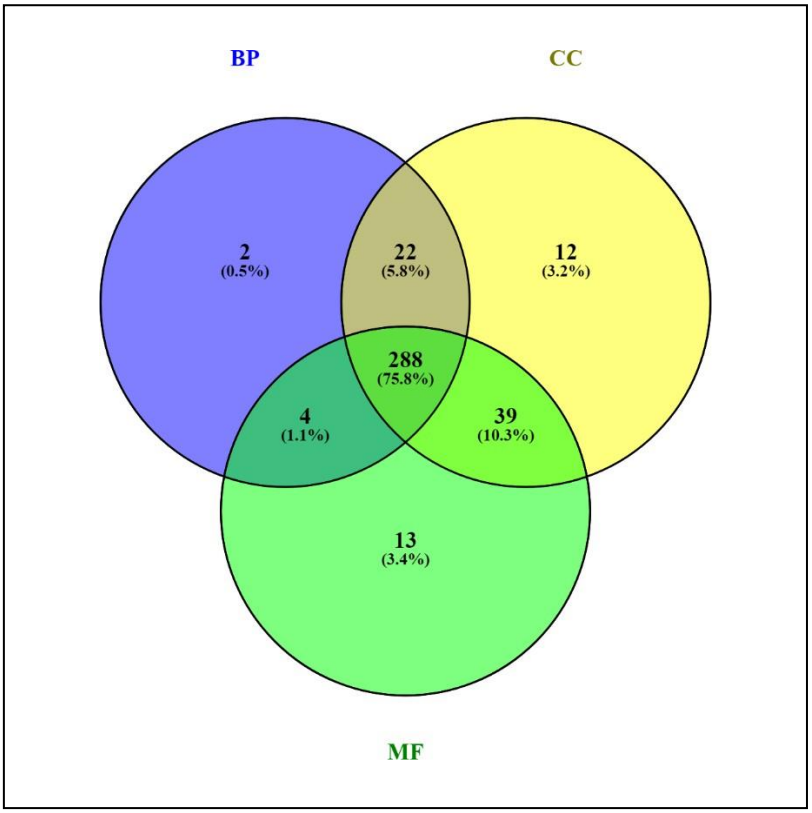



Figure 3.2.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

A total of 383 pathways were regulated by 288 key genes. Pathways that were majorly regulated included pathways in Cancer (70), MAPK signaling pathway (46), micro RNA in cancer (44), calcium signaling pathway (44), and the pathways that were regulated by the least number of genes included cysteine and methionine metabolism (3), Mitophagy (3), ABC transporters (4) shown in figure 3.2.2B. The majority of these pathways like PI3K-Akt, MAP kinase, and JAK-STAT have already been reported to play an important role in bladder cancer progression (Chestnut et al. 2021).

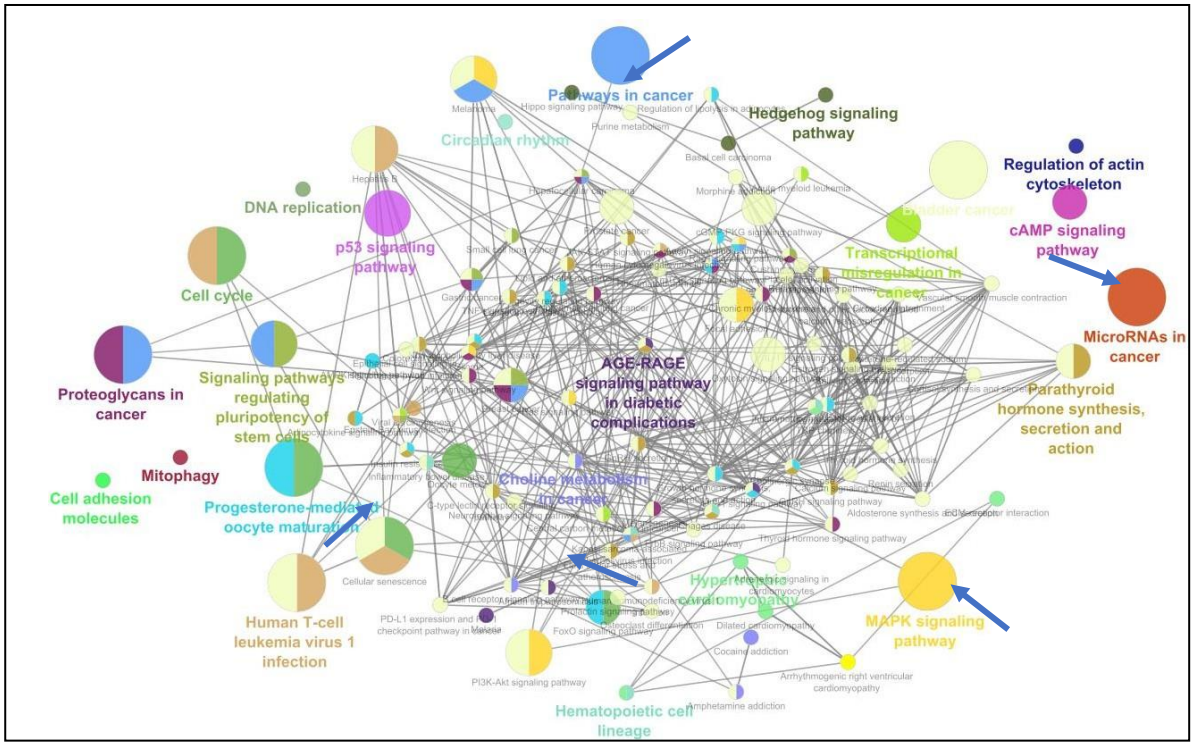



Figure 3.2.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated genes. Some of the important pathways are indicated by the arrow in blue color.

### 3.2.3. Analysis and Construction of lncRNA-miRNA-mRNA network

To understand the role of lncRNA-regulated mRNAs in Bladder cancer, we wanted to identify the differentially regulated expression of lncRNAs in bladder cancer. A total of 12 dysregulated (9 up-regulated and 3 down-regulated) lncRNAs were identified out of which only 5 (all up-regulated) lncRNAs were experimentally validated. The mRNA target of the lncRNAs was identified using DIANA LncBase V.3 and correlated those miRNA targets with the ones present in our dataset of dysregulated miRNAs, and finally identified their mRNA targets. A total of 427 axis of lncRNA-miRNA-mRNA were obtained which were correlated in their expression pattern. It consisted of 5 lncRNAs (up-regulated)-16 miRNAs (down-regulated)-192 mRNAs (up-regulated). Further, we classified these 192 mRNAs based on their functions related to biological processes, cellular components, and molecular function and identified 117 key genes as shown in figure 3.2.3A. These genes could be regulated by miRNA-mediated lncRNAs.

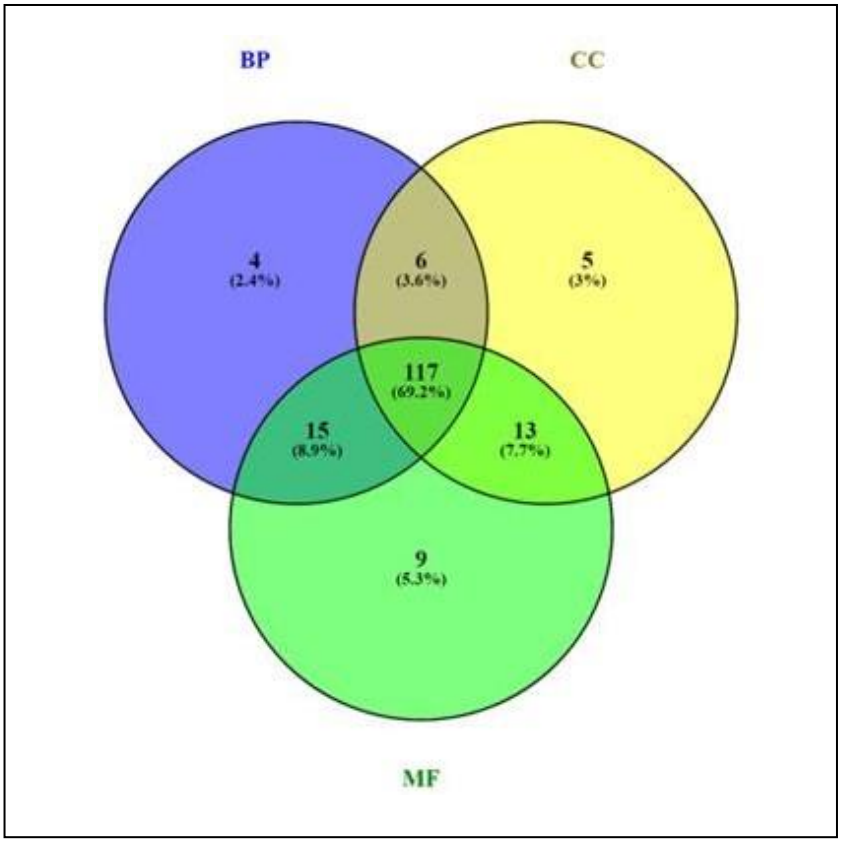



Figure 3.2.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

Out of the total of five up-regulated lncRNAs, lncRNA SNHG3, UCA1, and PVT1 were shown to be involved in bladder cancer proliferation and metastasis (M. Chen et al. 2021; Dai et al. 2020; Z. Ding et al. 2021; Lebrun et al. 2018; J. Luo et al. 2017). It has been found that upregulation of UCA1 might be involved in regulating cell cycle distribution via CREB through PI3-K dependent pathway. It may increase the chemoresistance of bladder cancer cells by regulating Wnt signaling. PVT1 is known to promote cell proliferation and suppress cell apoptosis (Taheri, Omrani, and Ghafouri-Fard 2018) whereas SNGH3 promotes bladder cancer proliferation and metastasis through miR-515-5p/GINS2 axis (Dai et al. 2020) but the role of lncRNA GHRLOS and C2orf27A have not been explored in bladder cancer. Finally, we identified the pathways regulated by key genes (shown in figure 3.2.3B. A). A total of 153 pathways were regulated by 117 genes. Pathways regulated by the maximum number of genes were cell cycle (18), pathways in Cancer (13), microRNAs in cancer (12), and whereas the

pathways regulated by the least number of genes were one carbon pool by folate (1), endocytosis (1), and AMPK signaling pathway (1). lncRNA SNHG3 is regarded as a regulator ofenergy metabolism and the role of lncRNA UCA1 in promoting glutamine metabolism has also been studied (H. J. Li et al. 2015).

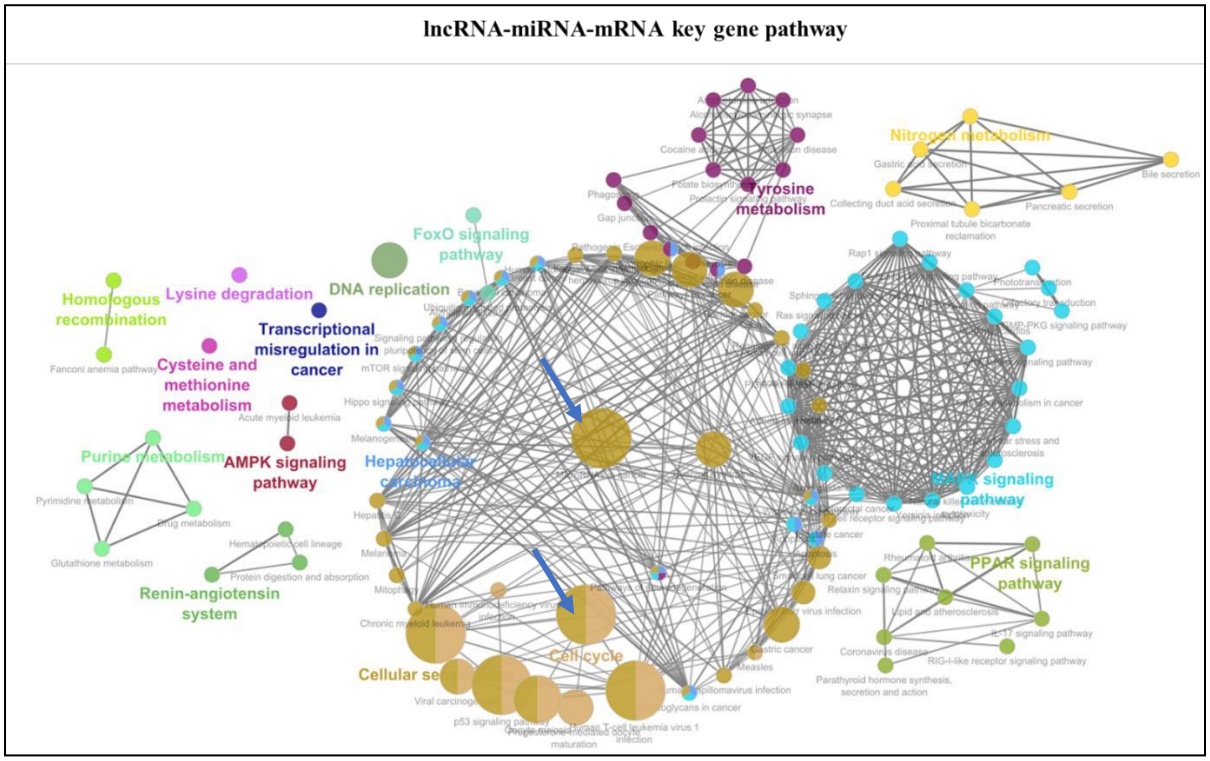



Figure 3.2.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in blue color.

In the case of the top KEGG pathways that are regulated by key genes consisted of pathways in Cancer, MAPK signaling, PI3K-Akt, cell signaling, and calcium signaling. Recent studies have shown that all these pathways are involved in bladder cancer progression (Chestnut et al. 2021).

## 3.3. Breast invasive carcinoma (BRCA)

### 3.3.1. Differentially expressed mRNAs

Breast cancer is one of the most common cancers observed in females. The ductal epithelium is the primary source for 85% of its cells, while the lobules of glandular tissue account for the

remaining 15%. In its earliest stages, cancer typically grows asymptomatically and is confined to a duct or lobule. However, as it develops, breast cancer can spread to the lymph nodes and other nearby breast tissue, and even to other organs. There were approximately 7.8 million people with breast cancer in the world by the end of 2020. For women, breast cancer is the leading cause of disability-adjusted life years (DALYs) lost worldwide. Breast cancer can affect women at any age after puberty, though it is more common in older age groups. Although extremely uncommon, breast cancer in men can also occur (NCI/NIH Male Breast Cancer n.d.). Transcriptomic data of BRCA consisted of a total of 515 patient samples and 59 control samples. The transcriptomic analysis showed out of a total of 1950 dysregulated genes, 1186 were up-regulated genes while 764 genes are down-regulated, and gene ontology analysis identifies 1236 key genes (shown in figure 3.3.1A).

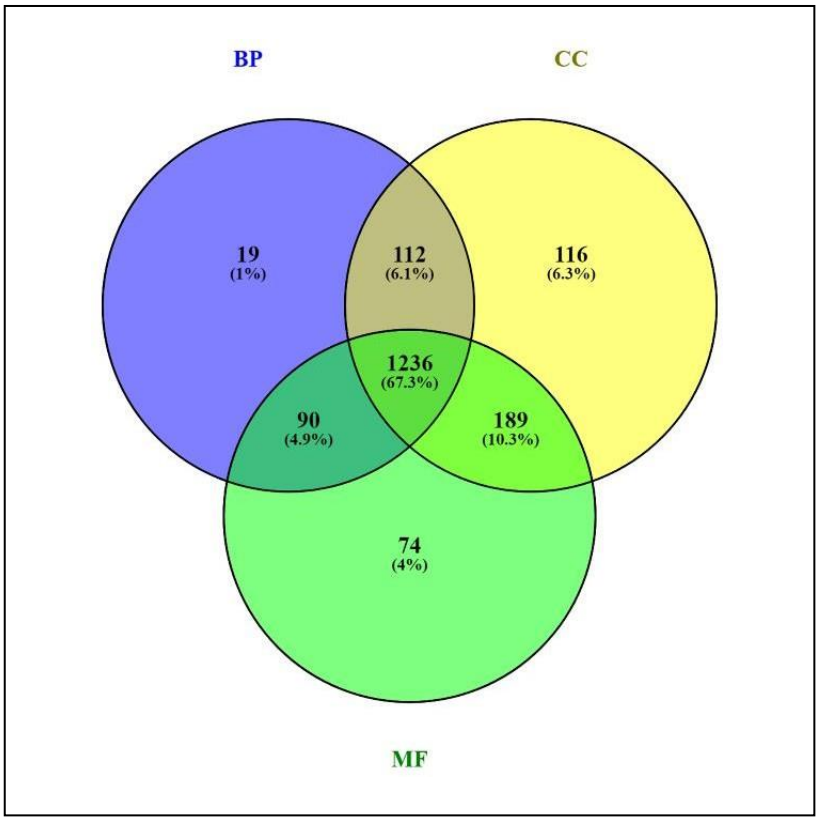



Figure 3.3.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

Pathway analysis revealed that maximum genes (61) are associated with pathways like pathways in Cancer, cytokine-cytokine receptor interaction (50), neuroactive ligand-receptor interaction (48), PI3K-Akt signaling pathway (46), and the minimum number of dysregulated genes (1) are associated with pathways like drug metabolism, RNA degradation, glutathione metabolism (figure 3.3.1B).

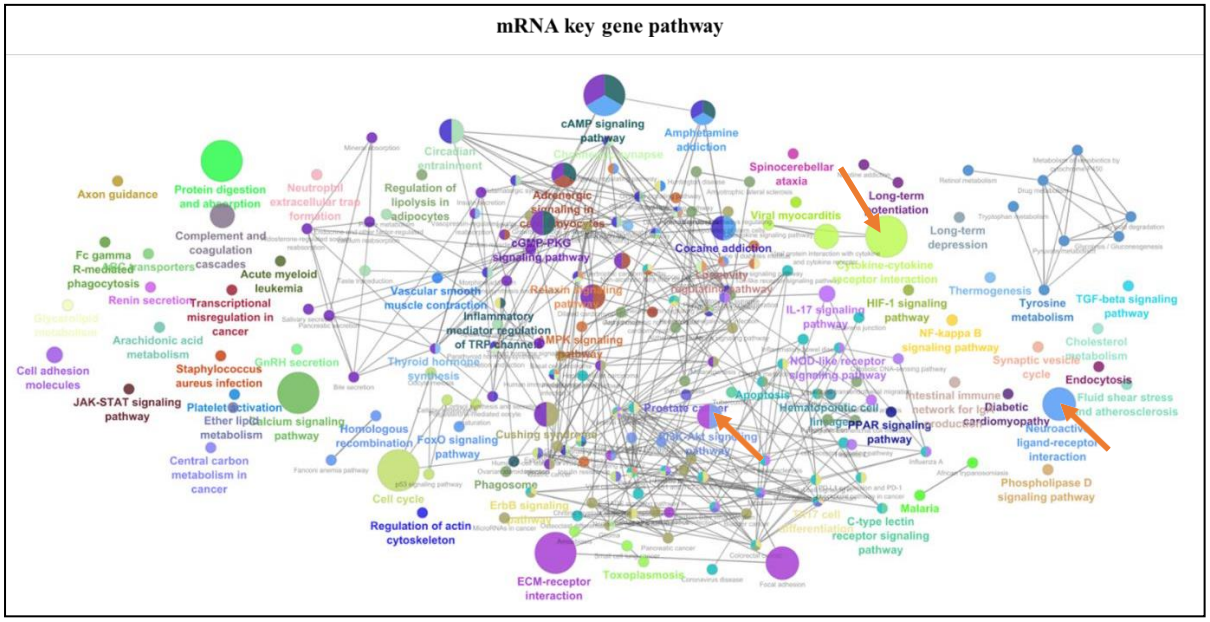



Figure 3.3.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in orange color.

### 3.3.2. Analysis and Construction of miRNA-mRNA network

Transcriptomic analysis of breast cancer patients contained a total of 88 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (39) was lower than the down-regulated miRNAs (49). Correlated studies of miRNA-mRNA interaction revealed a total of 894 correlated pairs with 82 miRNAs (48 down-regulated and 34 up-regulated) having 612 mRNA (165 down-regulated and 447 up-regulated) targets. Among the dysregulated genes GO analysis identifies 616 key genes (shown in figure 3.3.2A).

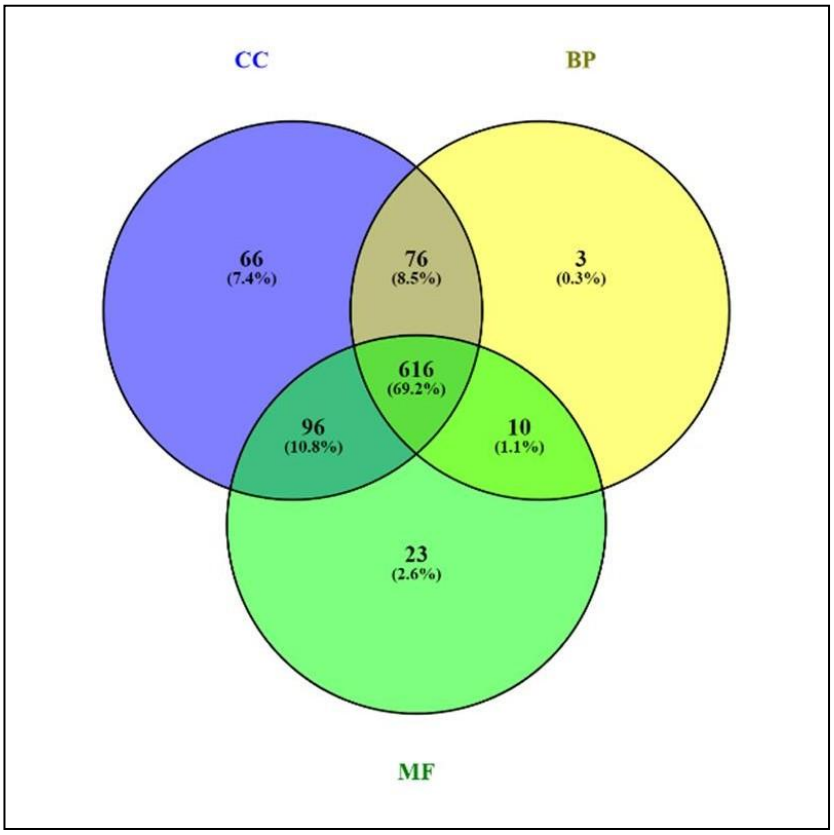



Figure 3.3.2A. Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

Pathways that were majorly regulated included pathways in Cancer (48), PI3K-Akt signaling pathway (26), MAPK signaling pathway (26), and cytokine-cytokine receptor interaction (24). drug metabolism, fatty acid degradation, and VEGF signaling pathway were regulated by the least number of genes (shown in figure 3.2.2B).

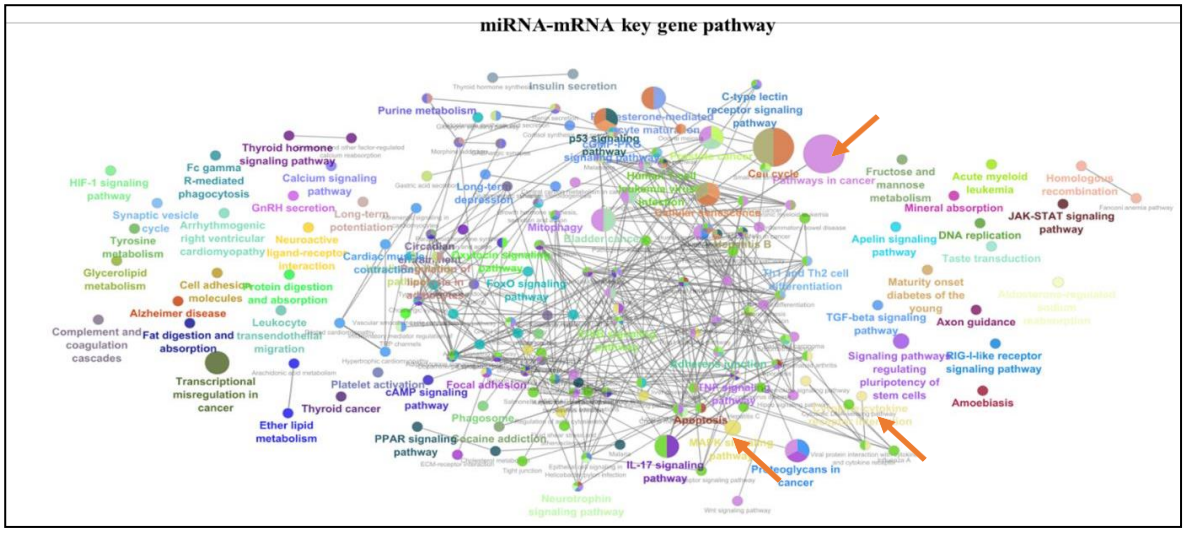



Figure 3.3.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in orange color.

### 3.3.3. Analysis and Construction of lncRNA-miRNA-mRNA network

A total of 13 differentially expressed (9 up-regulated and 4 down-regulated) lncRNAs were identified out of which only 6 (5 up-regulated and 1 down-regulated) lncRNAs were experimentally validated. The correlated study revealed 127 axis of correlated lncRNA-miRNA-mRNA. It consisted of 2 lncRNAs (1 up-regulated and 1 down-regulated), 11 miRNAs (5 up-regulated and 6 down-regulated), 127 mRNAs (86 up-regulated and 41 down-regulated) with 34 key genes (shown in figure 3.3.3A).

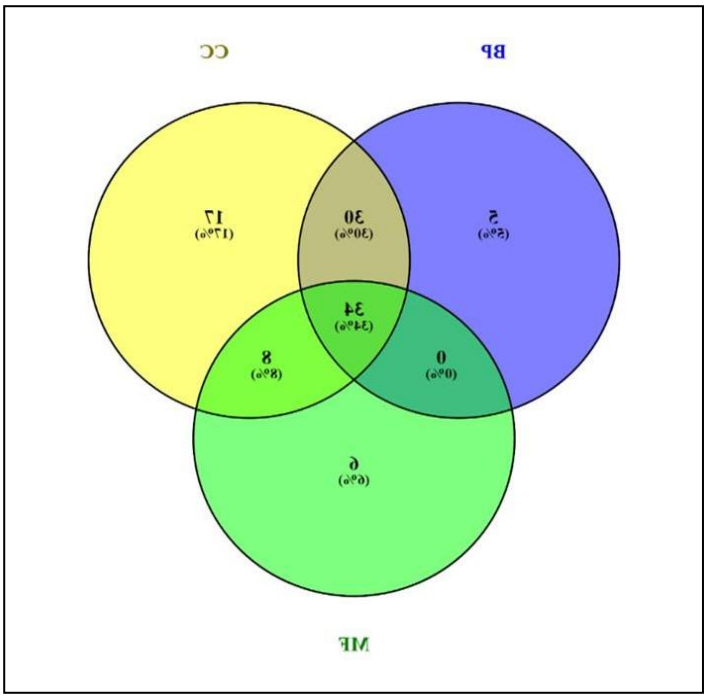


Figure 3.3.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The top dysregulated pathways consisted of pathways in Cancer (11), miRNA in cancer (9), cell cycle (8), cellular senescence (7), and dysregulated pathways with the least number of genes were lysine degradation (1), signaling pathways regulating pluripotency of stem cells (1). figure 3.3.3B shows a Cytoscape map representing the pathways regulated by thelncRNA-miRNA-mRNA correlated genes. All the dysregulated lncRNAs (SNHG3, UCA1, MIR155HG, EMX2OS, PVT1, and HOTAIR) have been well studied for their role in breast cancer and reported to be involved in breast cancer progression. Except for EMX2OS, all

lncRNAs are up-regulated. SNHG3 is identified as a potential oncogene and can act as a miRNA sponge to promote breast cancer through metabolic reprogramming. A positive correlation is found between the overexpression of SNHG3 with advanced tumor node metastasis (TNM) stage, histological grade, lymph node metastasis, estrogen receptor (ER), and human epidermal growth factor receptor 2 (Her-2) leading to migration, invasion, and cancer progression (Yan Li et al. 2020; B. Xu et al. 2020). In breast cancer cells, UCA1 can up-regulate PTP1B expression by sequestering miR-206 at the post-transcriptional level. miR-206 normally binds to the 3′UTR of PTP1B mRNA, thereby repressing PTP1B expression (Yi Li et al. 2019).

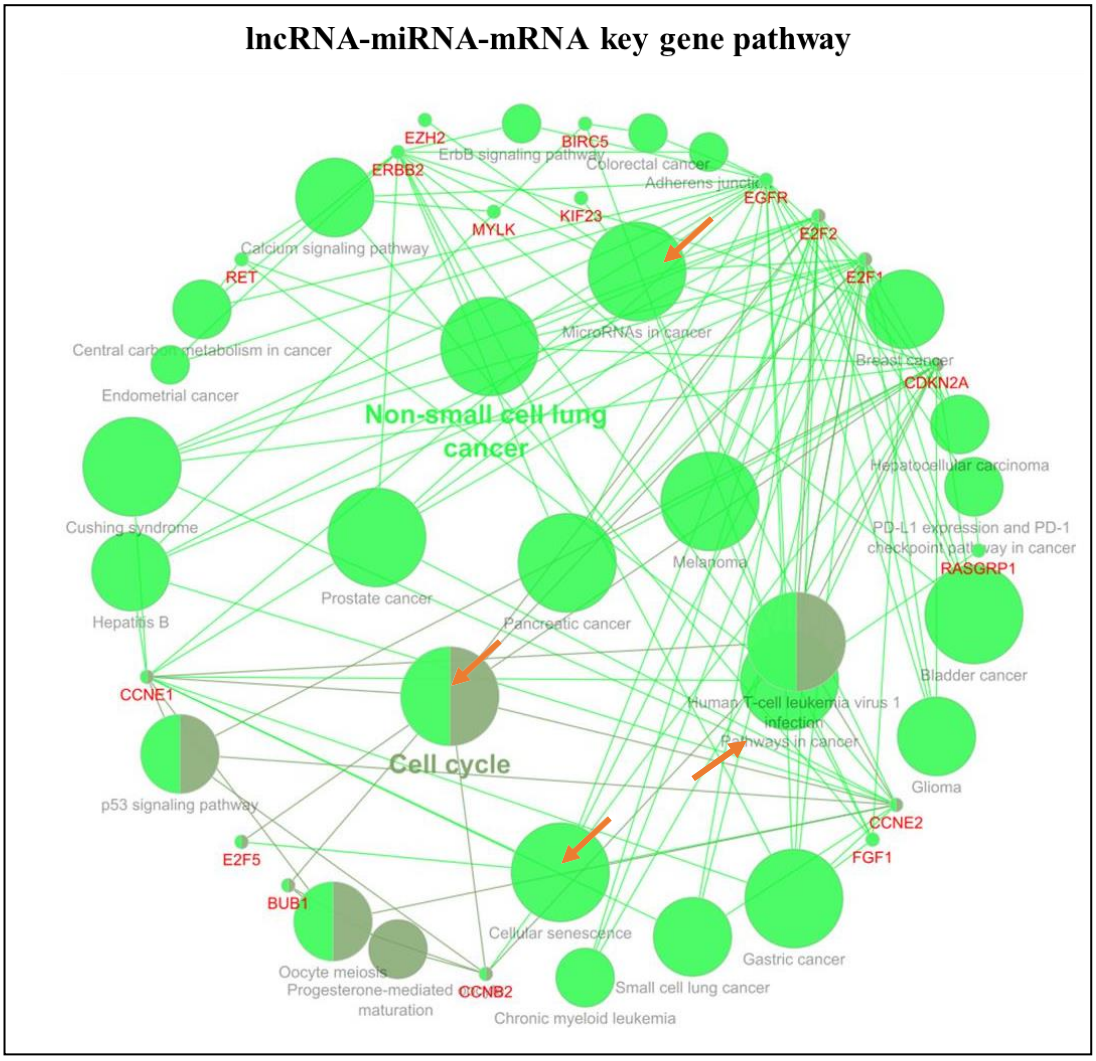



Figure 3.3.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in orange color.

## 3.4. Colon adenocarcinoma (COAD)

### 3.4.1. Differentially expressed mRNAs

Colon and rectal cancers are often lumped together because of the similarities between them and are collectively referred to as colorectal cancer or colon adenocarcinoma. One of the most prevalent cancers worldwide is colorectal cancer. Polyps form in the mucosal lining of the colon or at its terminus (called the rectum) and spread outward into the surrounding tissue, eventually forming blood vessels or lymph vessels from which cancer can spread to nearby lymph nodes or other locations (COAD n.d.; GDC TCGA-COAD n.d.). Transcriptomic data is obtained from 285 tumor patients and compared with 41 control samples. Out of a total of 2106 dysregulated genes, 1192 genes were up-regulated whereas 914 were down-regulated genes. 1233 dysregulated genes are found to be involved in BP, CC, and MF (key genes) as shown in figure 3.4.1A.

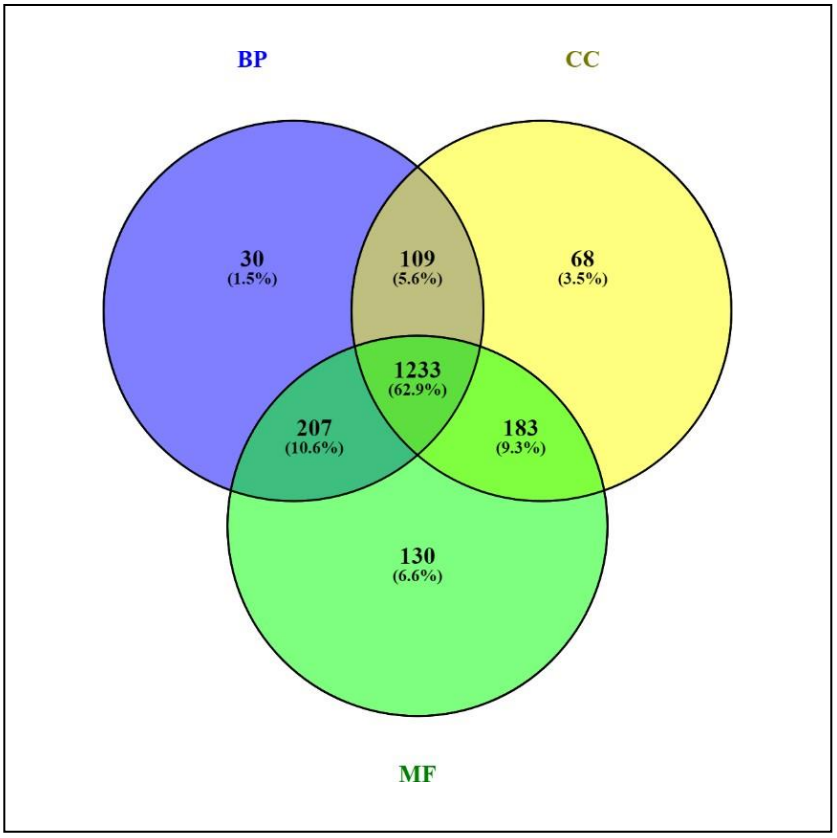



Figure 3.4.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The top regulated pathways that were regulated by maximum numbers of genes consisted of neuroactive ligand-receptor interaction (66), cytokine-cytokine receptor interaction (65), pathways in Cancer (58), and the least regulated pathways were hippo signaling pathway, glycosphingolipid biosynthesis, starch, and sucrose metabolism. Cytoscape map representing the pathways regulated by the mRNA genes in figure 3.4.1B.

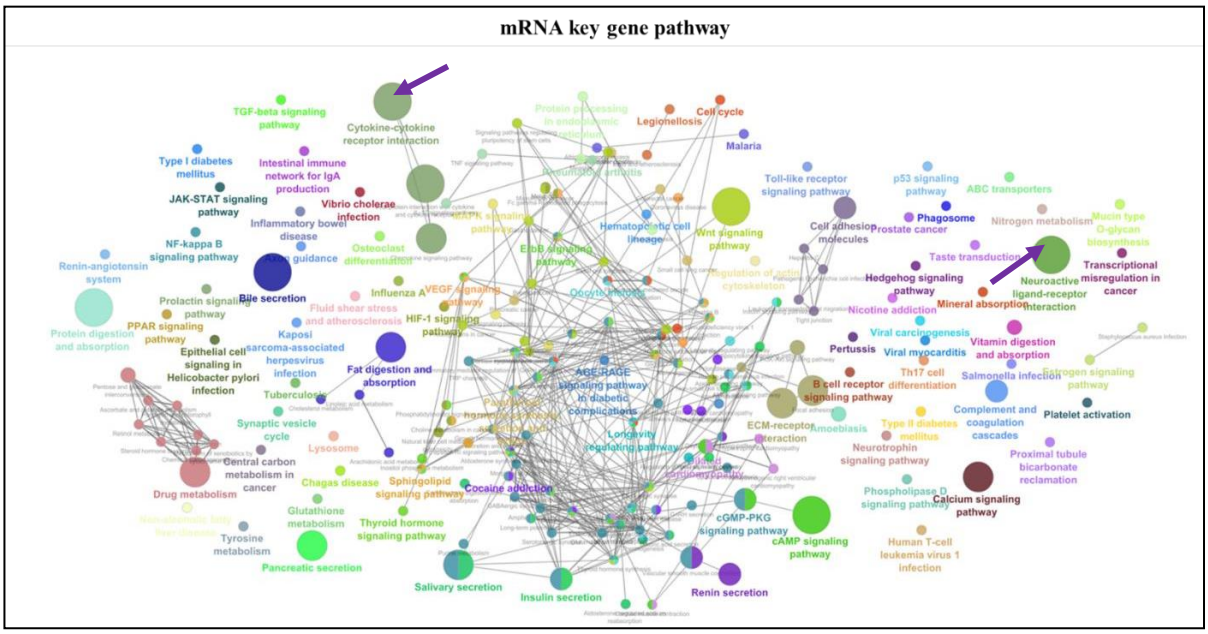



Figure 3.4.1B Cytoscape network showing the different pathways regulated by the mRNAkey genes. Some of the important pathways are indicated by the arrow in purple color.

### 3.4.1. Analysis and Construction of miRNA-mRNA network

Colon Adenocarcinoma consisted of a total of 295 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (163) was higher than the down-regulated miRNAs (132). Correlated studies of miRNA-mRNA interaction revealed a total of 2845 correlated pairs with 277 miRNAs (125 down-regulated and 152 up-regulated) having 1039 mRNA (515 down-regulated and 524 up-regulated) targets. Our analysis identified 603 miRNA-regulated key genes (shown in figure 3.4.2A).

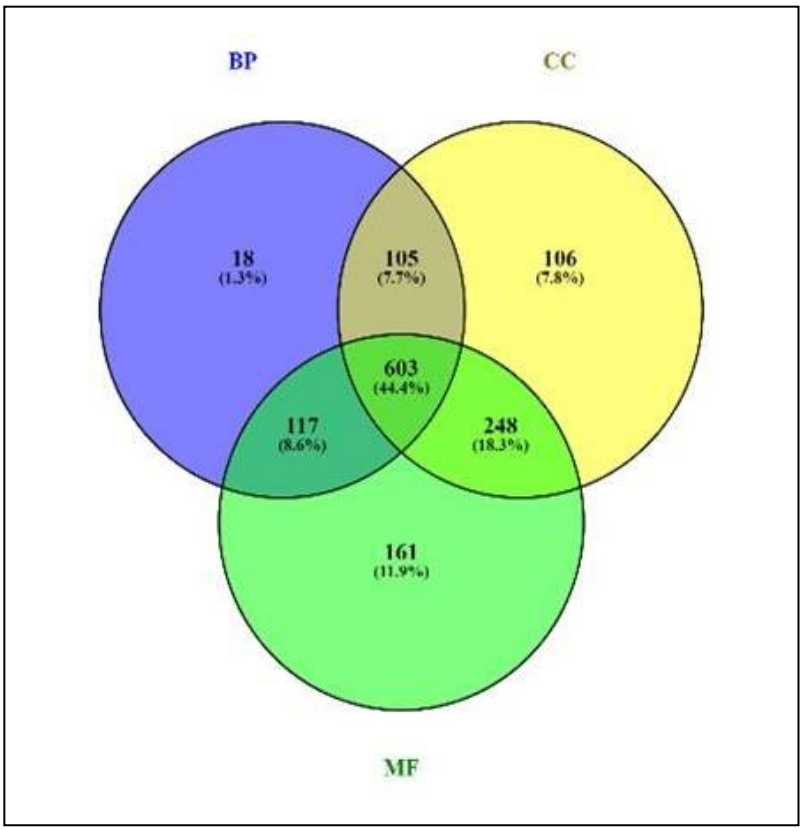



Figure 3.4.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

Through pathway analysis, we identified that cytokine-cytokine receptor interaction (47), pathways in Cancer (45), PI3K-Akt signaling pathway (35), and Wnt signaling pathway (31) are the major pathways that were being regulated by a large number of genes (shown in figure 3.4.2B). The pathways which were regulated by a single gene include steroid biosynthesis, synaptic vesicle cycle, glycerolipid metabolism, and taurine and hypotaurine metabolism.

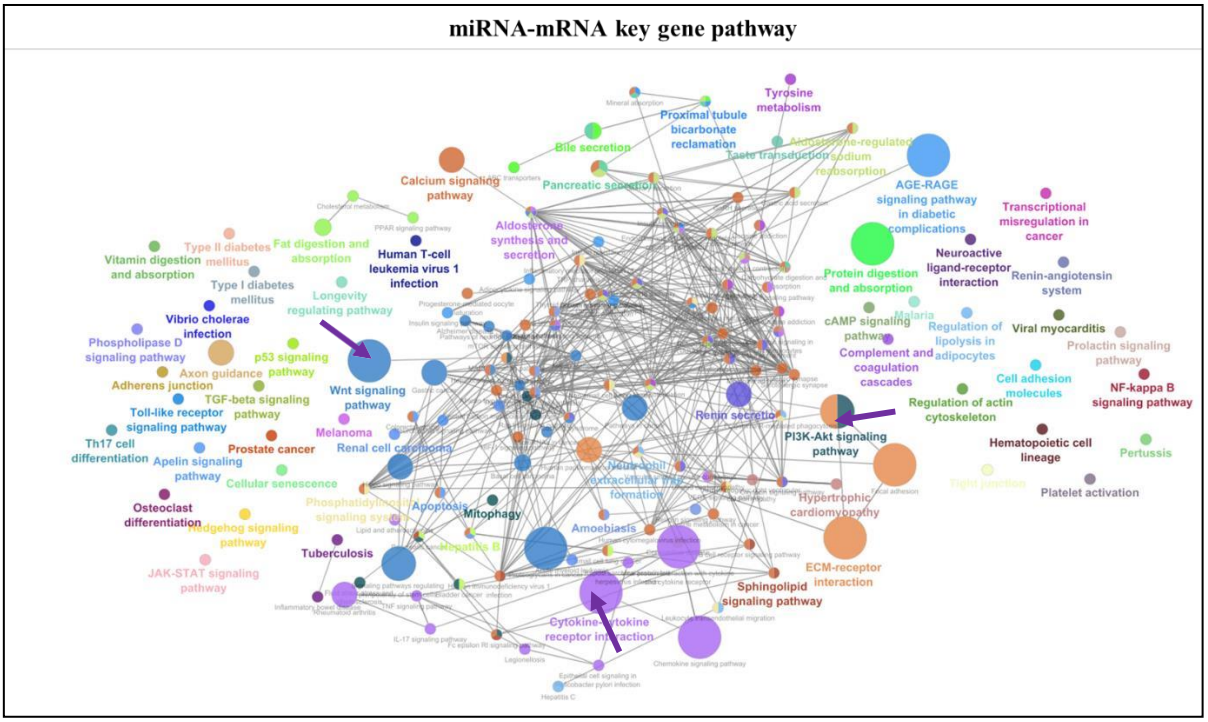



Figure 3.4.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in purple color.

### 3.4.2. Analysis and Construction of lncRNA-miRNA-mRNA network

In this carcinoma, transcriptomic data analysis revealed 17 up-regulated lncRNAs. Out of which 14 had experimentally validated targets. The correlated study revealed 3288 axis of correlated lncRNA-miRNA-mRNA which consisted of 15 up-regulated lncRNAs, 77 down-regulated miRNAs, and 435 up-regulated mRNAs. Among these 435 mRNAs, 186 are key genes (shown in figure 3.4.3A). The 15 lncRNAs are SNHG1, SNHG3, KCNQ1OT1, C9orf163, C2orf27A, MIR17HG, PVT1, TMEM105, H19, SNHG11, RMRP, UCA1, TSIX and HOTAIR.

lncRNAs SNHG1 (Avazpour et al. 2020), RMRP (Y. Chen et al. 2021), SNHG11(W. Xu et al. 2020), SNHG3(Weizhen et al. 2017), KCNQ1OT1(C. Chen et al. 2020), HOTAIR (Y. Huang, Wang, and Liu 2021), UCA1(Luan et al. 2020), C2orf27A (Yuan et al. 2021), and MIR17HG (Zhao et al. 2021) all these lncRNAs have been explored for their role in colorectal cancer progression. lncRNA TSIX is the antisense of XIST but its role has not been explored

in colorectal cancer. lncRNA TMEM105 is a novel DNA methylation signature that is associated with androgen receptor activity although its role has not been explored much. However, it is associated with the sonic hedgehog pathway in colorectal cancer (Ylitalo et al. 2021) whereas PVT1 is a well-studied lncRNA and is involved in the progression of colorectal cancer (Lai et al. 2021, Fang Liu et al. 2020). This lncRNA is considered a biomarker for multiple different cancer types. The role of C9orf163 has not been explored in colorectal cancer.

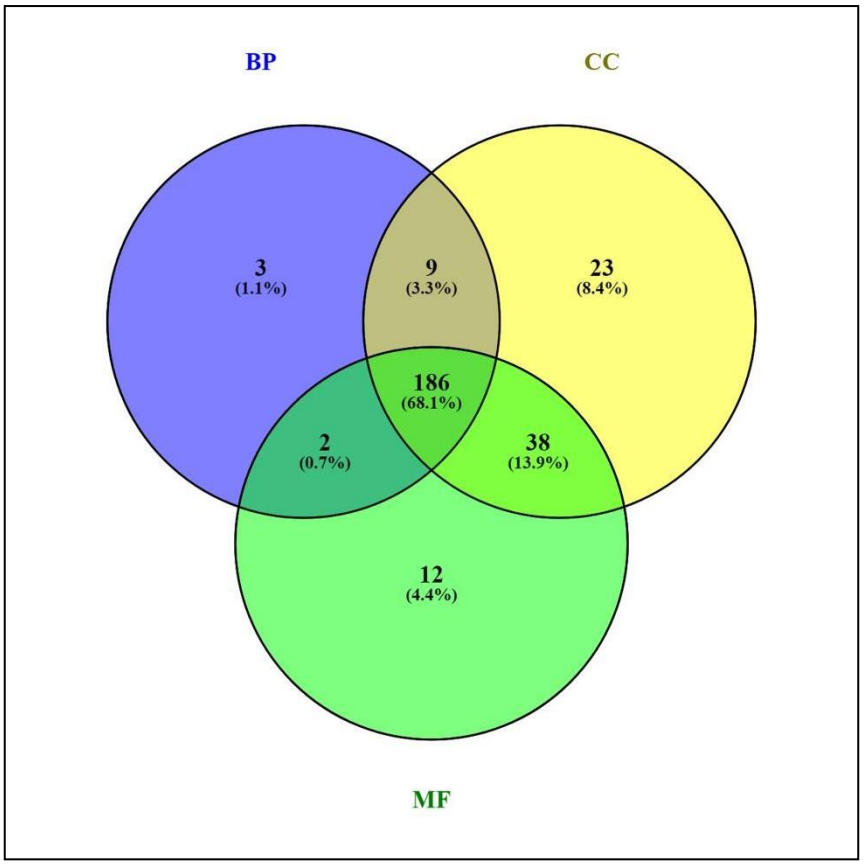



Figure 3.4.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

Through pathway analysis, we identified a total of 285 different pathways that were being regulated by these 186 key genes. The major pathways that were being regulated by the maximum number of genes included pathways in Cancer (17), human papillomavirus infection (13), microRNAs in cancer (12), and PI3K-Akt signaling pathway (11). The pathways which were

regulated by a single gene include mitophagy, AMPK signaling pathway, oxytocin signaling pathway, FoxO signaling pathway, and hedgehog signaling pathway (shown in figure 3.4.3B).

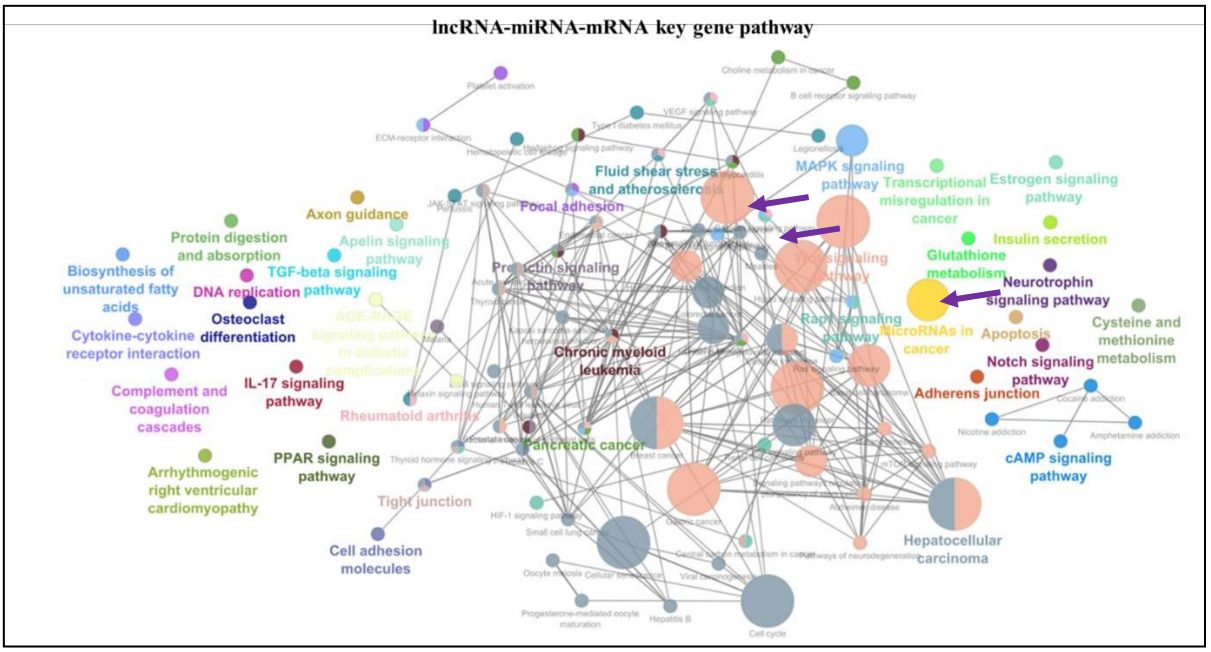



Figure 3.4.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in purple color.

## 3.5. Esophageal carcinoma (ESCA)

### 3.5.1. Differentially expressed mRNAs

Cancer of the esophagus and stomach, also known as esophageal carcinoma, typically develops in the esophagus, the long, hollow tube connecting the throat and the stomach. Food is transported from the mouth to the stomach where it is digested. Because the esophagus extends from the throat to the stomach, it provides a large space for digestion and allows the cancerous cell to amplify quickly, making it difficult to detect the symptoms of esophageal cancer. If the tumor is allowed to grow, it will eventually block the opening to theesophagus, making it difficult for the patient to swallow. ESCA consisted of transcriptomic data from a total of 184 patient samples and 11 control samples. The transcriptomic data analysis revealed a total of 1646 significantly dysregulated mRNAs with 865 up-regulated genes (865) and 781 down-regulated genes (781). GO analysis identified 1096 key genes (shown in figure 3.5.1A).

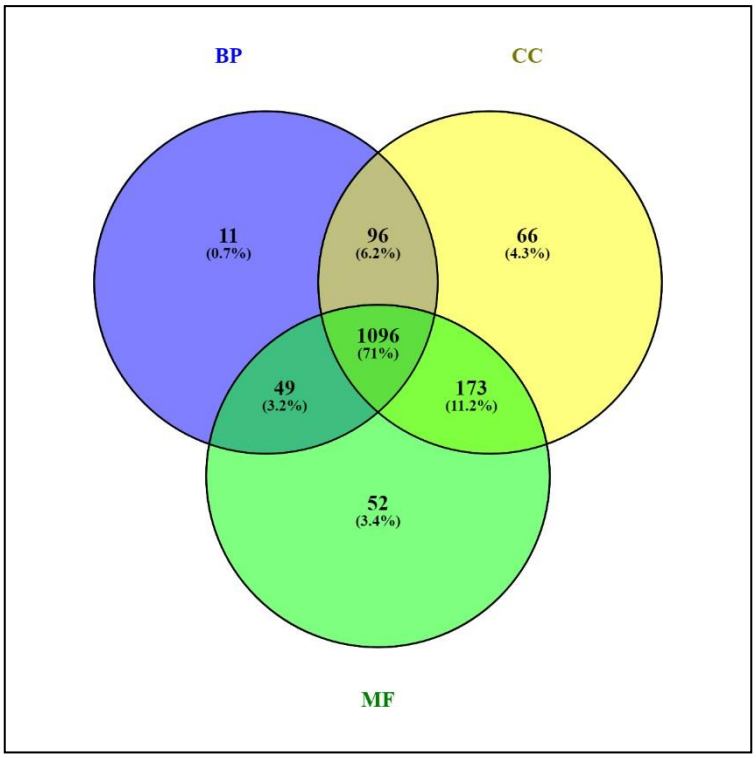



Figure 3.5.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

Through pathway analysis, we identified a total of 371 different pathways that were being regulated by 1096 key genes. pathways in Cancer (57), cytokine-cytokine receptor interaction (54), human papillomavirus infection (44), and PI3K-Akt signaling pathway (42) are the major pathways that were being regulated by these key genes (figure 3.5.1B). A few pathways were there which were regulated by single genes these include pathways like the renin-angiotensin system, protein processing in the endoplasmic reticulum, primary bile acid biosynthesis, and endometrial cancer. These pathways could play an important role in this specific cancer.

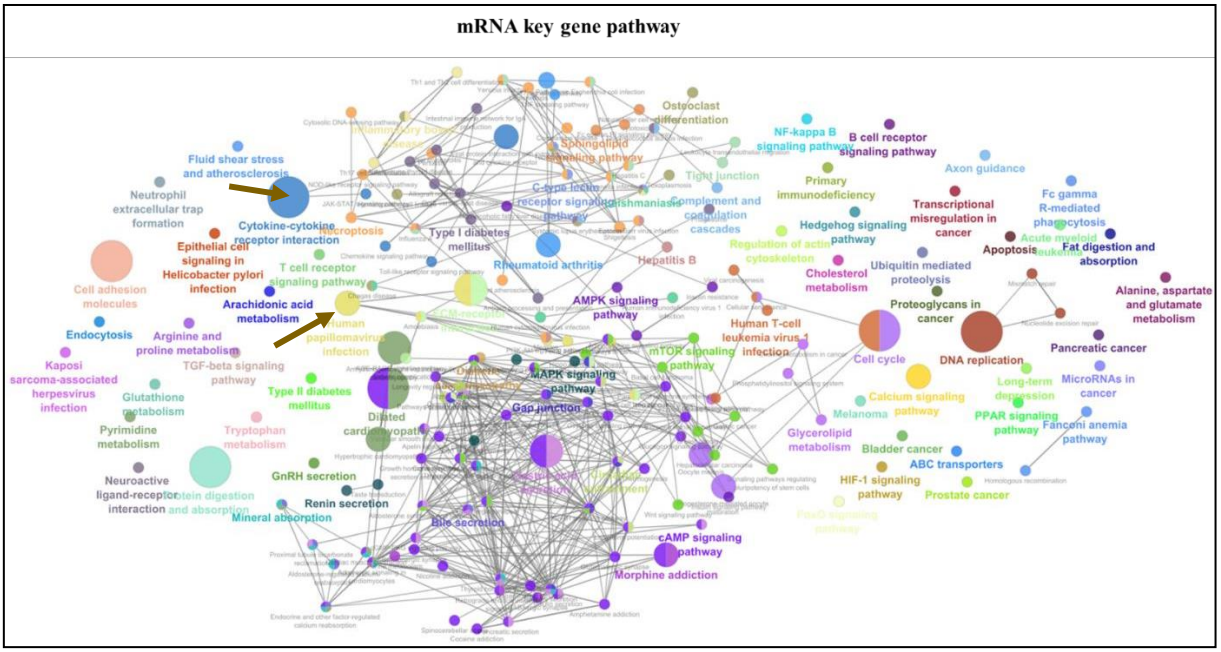



Figure 3.5.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in brown color.

### 3.5.2. Analysis and construction of miRNA-mRNA network

Transcriptomic data of esophagus adenocarcinoma consisted of 45 dysregulated miRNA transcripts. The number of up-regulated miRNAs (26) was higher than the down-regulated miRNAs (19). Correlated studies of miRNA-mRNA interaction identified 400 correlated pairs with 43 miRNAs (18 down-regulated and 25 up-regulated) with 301 mRNA (130 down-regulated and 171 up-regulated) targets with 181 key genes (figure 3.5.2A).

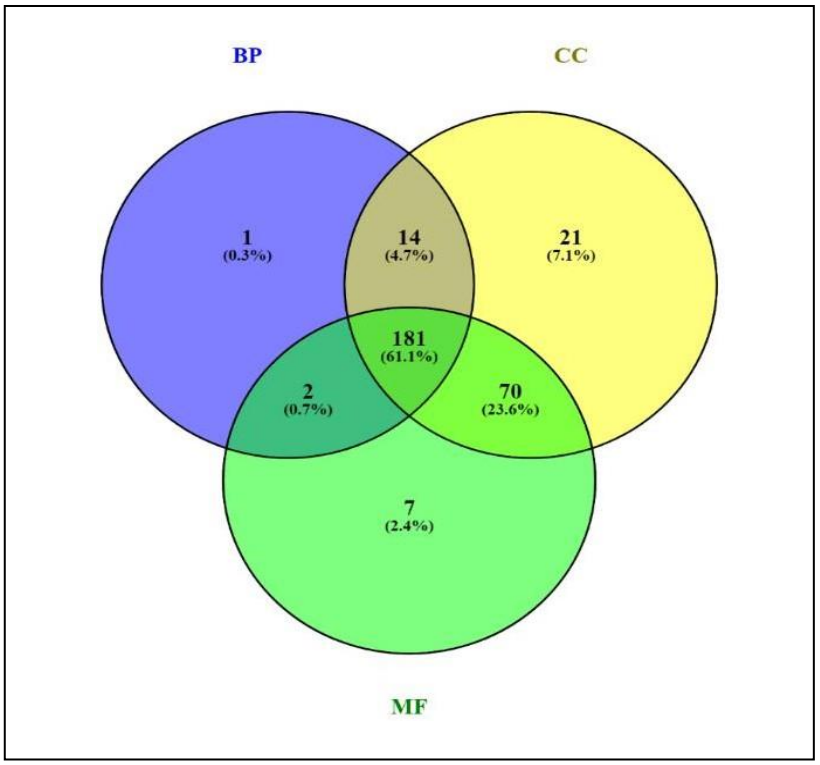



Figure 3.5.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

A total of 454 pathways were identified by Cytoscape analysis which was regulated by 349 key genes. Pathways in Cancer, cell cycle, human papillomavirus infection, and proteoglycans in cancer are the major pathways that were being regulated by the key genes. The pathways which were regulated by a single gene include the notch signaling pathway, cysteine, and methionine metabolism, pentose phosphate pathway, and glucagon signaling pathway (shown in figure 3.5.2B).

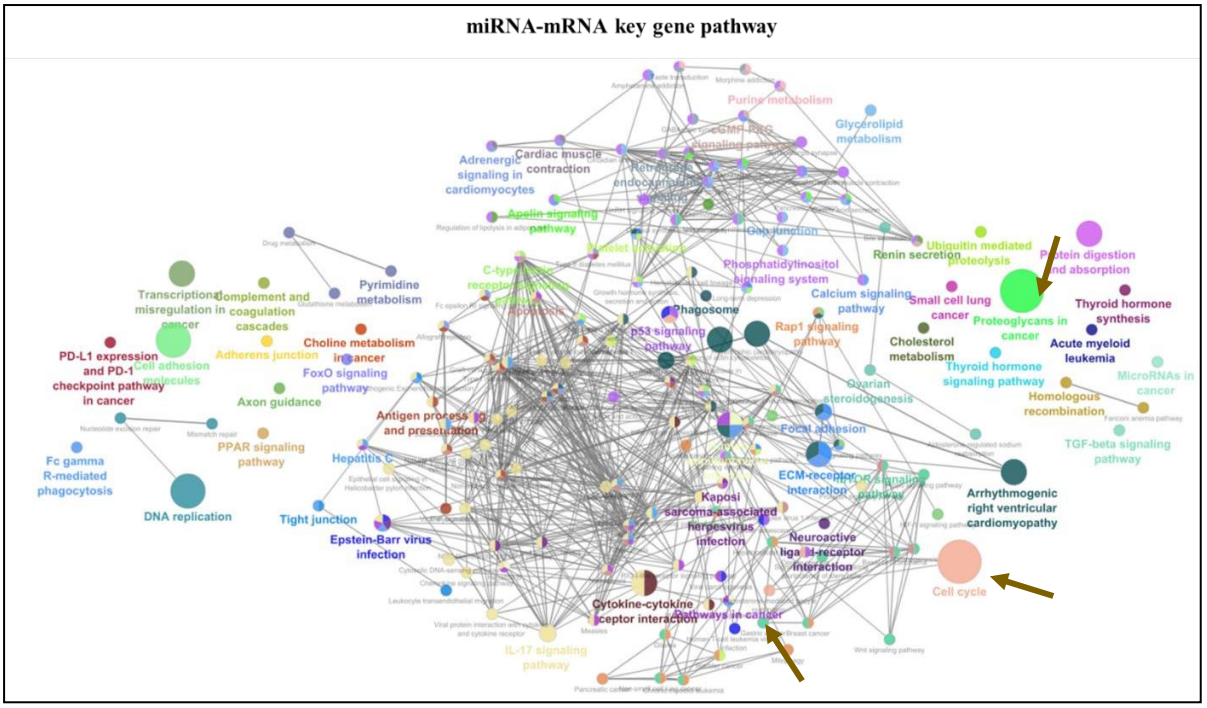



Figure 3.5.2B Cytoscape network showing the different pathways regulated by the miRNA-regulated mRNA key genes. Some of the important pathways are indicated by the arrow in brown color.

### 3.5.3. Analysis and construction of lncRNA-miRNA-mRNA network

Transcriptomic data analysis revealed that out of six up-regulated lncRNAs in ESCA cancer 4 lncRNAs (HCP5, HOTAIR, UCA1, and PVT1) had experimentally validated miRNA targets. We observed a total of 206 correlated lncRNA-miRNA-mRNA axis. It consisted of 4 lncRNAs (up-regulated), 9 miRNAs (down-regulated), and 129 mRNAs (up-regulated). Out of these 129 up-regulated genes, 104 are key genes (figure 3.5.3A). It has been shown that HCP5 promotes malignant cell behaviors in esophageal squamous cell carcinoma by altering the PI3K/AKT/mTOR signaling pathway (Jianyu Xu et al. 2021). HOTAIR is a prognostic biomarker for esophageal cancer and has been studied in other cancers as well. It is known to promote cancer cell progression (Lv et al. 2013). Numerous cancers including hepatocellular carcinoma, breast cancer, colorectal cancer, and gastric cancer have been shown to have substantial upregulation of UCA1, suggesting that UCA1 may serve as an important oncogene in human cancers. UCA1 regulates the mTOR, Wnt, or Akt signaling pathway which contributes to cancer progression when overexpressed. The mechanism of UCA1 in ESCA is unclear but it has been shown that its upregulation is associated with esophageal cancer progression (Jiao et al. 2016).

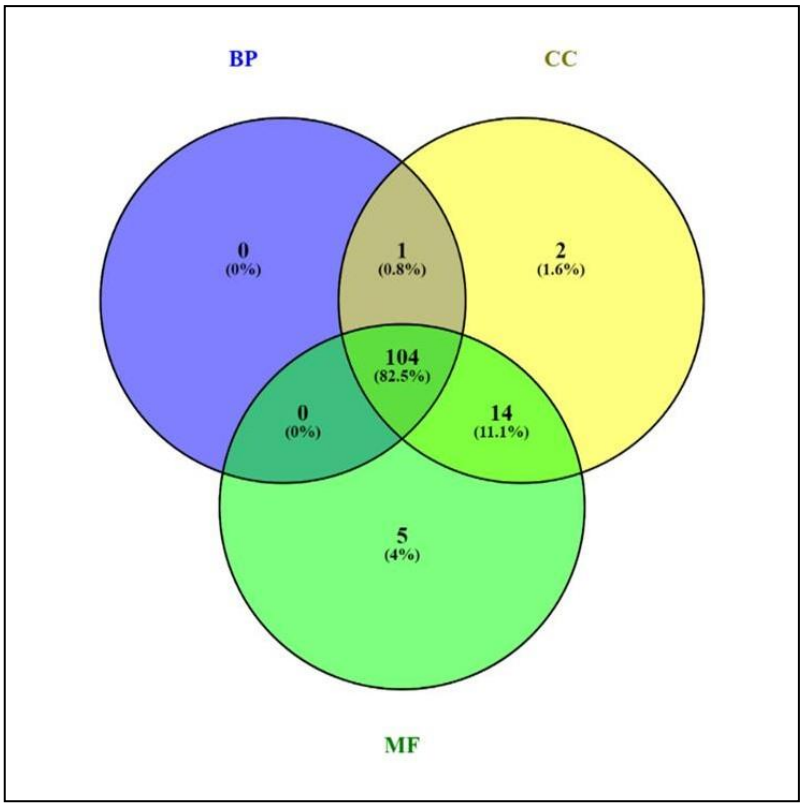



Figure 3.5.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

A total of 183 pathways were regulated by 104 key genes. cytokine-cytokine receptor signaling, cell cycle, pathways in Cancer, transcriptional misregulation in cancer, and human papillomavirus infection are the major pathways that were being regulated by dysregulated key genes (figure 3.5.3B). cysteine and methionine metabolism, GnRH signaling pathway, AMPK signaling pathway, and FoxO signaling pathway were regulated by single genes.

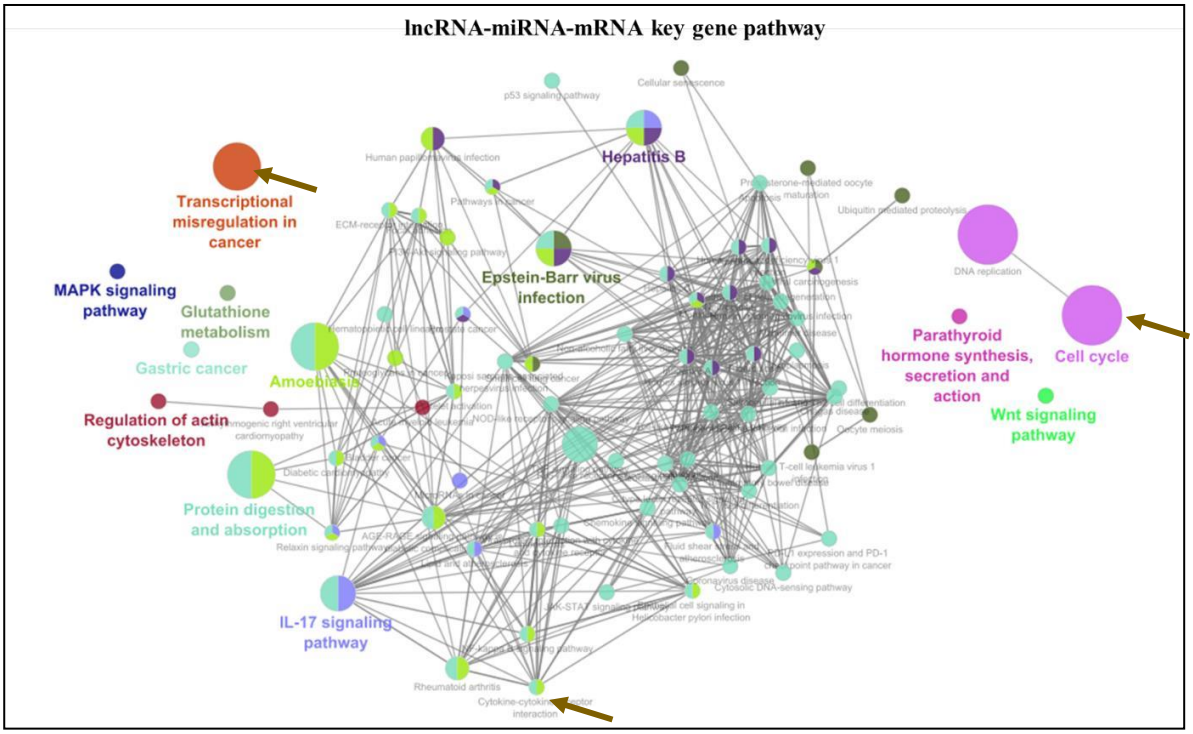



Figure 3.5.3B Cytoscape network showing the different pathways regulated by the lncRNA-regulated mRNA key genes. Some of the important pathways are indicated by the arrow in brown color.

The major pathways regulating esophageal cancer were cytokine-cytokine receptor signaling, Cell Cycle, and Pathways in Cancer. Although the role of human papillomavirus infection in ESCA is not very unclear understood, numerous meta-analyses have found that it is linked to the development of esophageal cancer (L. Guo et al. 2016).

## 3.6. Head and Neck squamous cell carcinoma (HNSC)

### 3.6.1. Differentially expressed mRNAs

When compared to other cancers, head and neck squamous cell carcinoma (HNSC) is distinct in its tumor progression because it remains locoregional for a long time and develops visceral metastases only at a late stage of the disease. Head and neck squamous cell carcinomas begin in the squamous cells that line the skin and mucous membranes of the head and neck (including the mouth, throat, and voice box). HNSC can also begin in the salivary glands, sinuses, muscles, or nerves of the head and neck, but these are much less common. Total transcriptomic data consisting of dysregulated mRNAs, miRNAs, and lncRNAs from 520 HNSC samples and

44 control samples. A total of 1752 dysregulated mRNAs contain 864 up-regulated and 888 down-regulated genes among which 990 are key genes (shown in figure 3.6.1A).

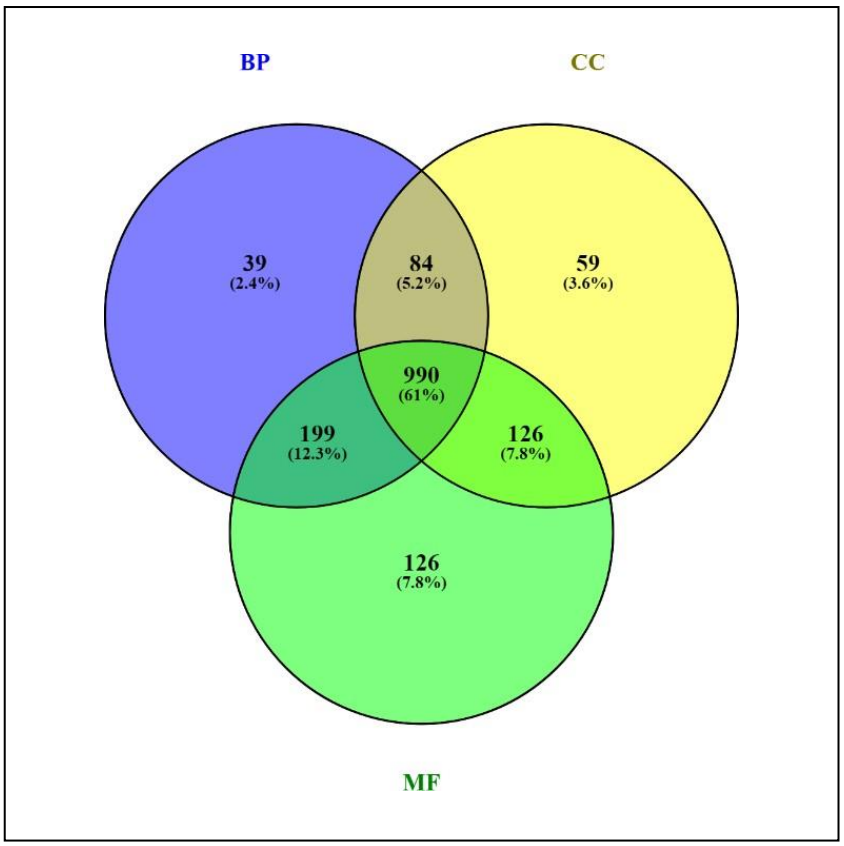



Figure 3.6.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

In HNSC, 339 pathways were regulated by 990 key genes. Here, key genes are majorly regulating pathways in Cancer, cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, and human papillomavirus infection (figure 3.6.1B). The pathways which were regulated by a single gene were the hedgehog signaling pathway, phototransduction, asthma, and peroxisome, which might play a role in this cancer.

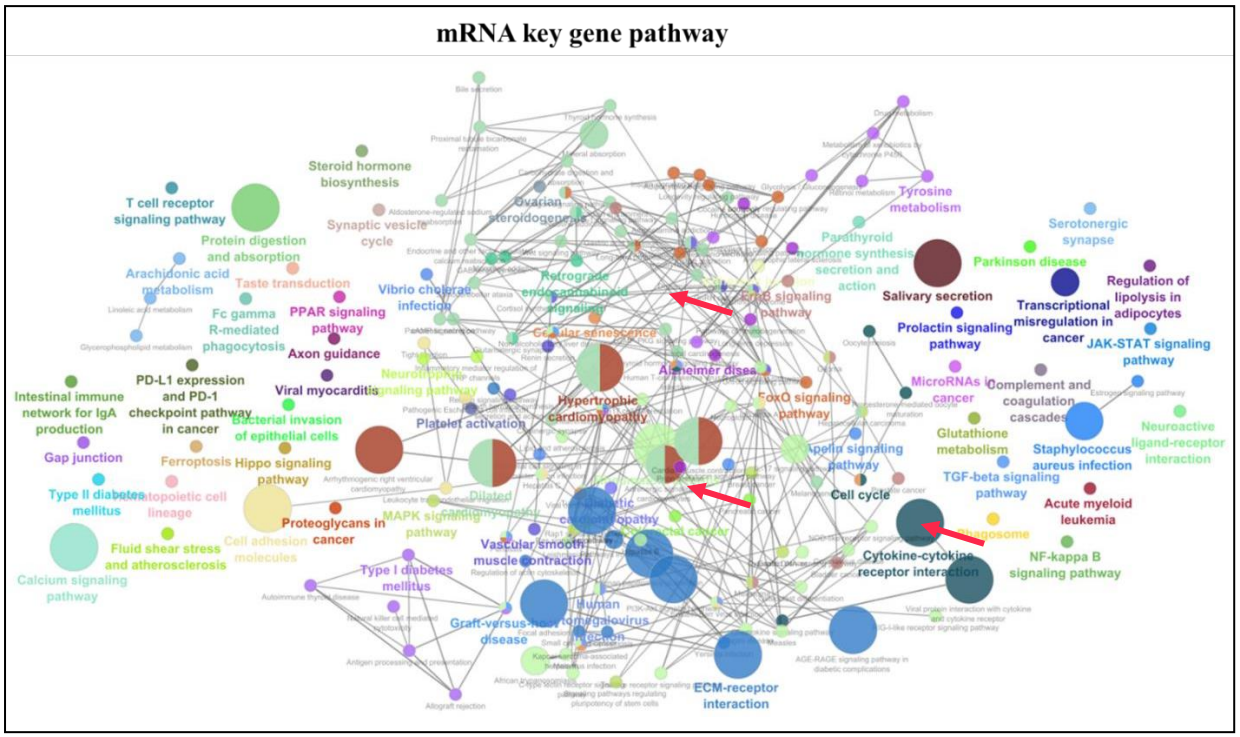



Figure 3.6.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

### 3.6.2. Analysis and construction of miRNA-mRNA

Transcriptomic data analysis showed 77 differentially expressed miRNA transcripts in HNSC. The number of up-regulated miRNAs (33) was lower than the down-regulated miRNAs (44). We have found 551 correlated miRNA-mRNA pairs in which 67 miRNAs (41 down-regulated and 26 up-regulated) positively correlated with 375 mRNAs (133 down-regulated and 242 up-regulated genes) targets. Out of these 375 mRNAs, 270 are key genes (figure 3.6.2A).

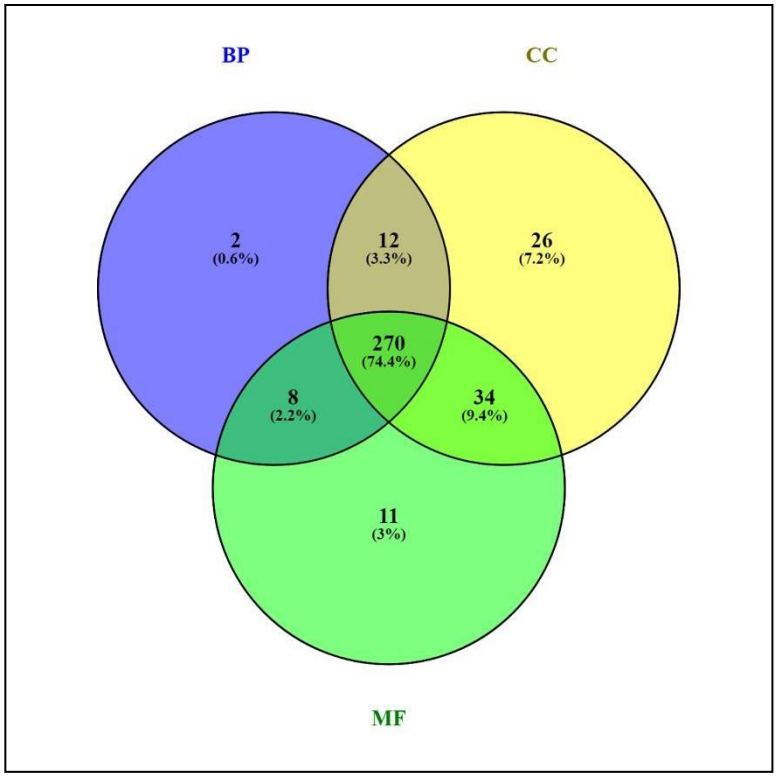



Figure 3.6.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

We have observed that a total of 204 pathways were regulated by 270 key genes. PI3K-Akt signaling pathway, human papillomavirus infection, and pathways in Cancer are major dysregulated pathways that are possibly regulated by miRNAs (shown in figure 3.6.2B). A few pathways being regulated by a single gene were insulin secretion, adherens junction, DNA replication, and hedgehog signaling pathway.

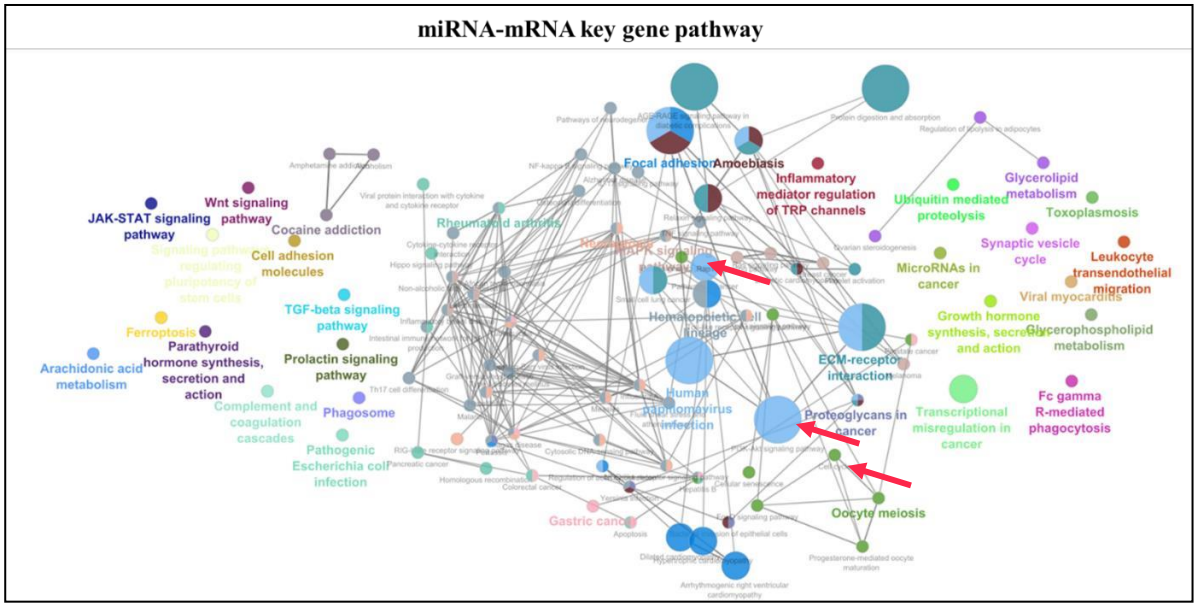



Figure 3.6.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

### 3.6.3. Analysis and construction of lncRNA-miRNA-mRNA network

We could find 9 differentially expressed lncRNAs from HNSC transcripts among which 6 (4 up-regulated and 2 down-regulated) had experimentally validated miRNA targets. The correlation analysis among lncRNA-miRNA-mRNA revealed 1681 axis of correlated lncRNA-miRNA-mRNA which consisted of 6 lncRNAs (2 down-regulated and 4 up-regulated), 36 miRNAs (19 down-regulated and 17 up-regulated) and 444 mRNAs (167 down-regulated and 277 up-regulated). A total of 130 key genes were identified (figure 3.6.3.A). lncRNA KCNQ1OT1 promoted cell proliferation, migration, and invasion of HNSC (Sun et al. 2020) other lncRNAs PART1 (Q. Yu et al. 2021), EMX2OS (Kozłowska et al. 2021), MIAT (F. Song, Yang, and Liu 2021) (Yu Wang et al. 2020), and MIR155HG (Cui et al. 2019) have not been explored for their functional role in HNSC but these lncRNAs have been studied in different squamous cell carcinomas like oral squamous cell carcinoma (OSCC) and esophagus squamous cell carcinomas(ESCC) and laryngeal squamous cell carcinoma (LSCC) which might have similar functionality with HNSC. Among head and neck carcinomas, laryngeal carcinoma is an extremely common tumor. One of the study results showed that

MIR155HG and miR155-5p were highly elevated in LSCC tissues and highly elevated in LSCC tissues and were linked to the TNM stage, pathological differentiation, and lymph node metastasis. Furthermore, MIR155HG overexpression enhanced carcinogenesis, while inhibition of MIR155HG and miR1555p had the opposite effect on LSCC cell proliferation, migration, and invasion in vitro (W. Cui et al. 2019). The role of lncRNA C20orf197 is not well explored in the case of HNSC.

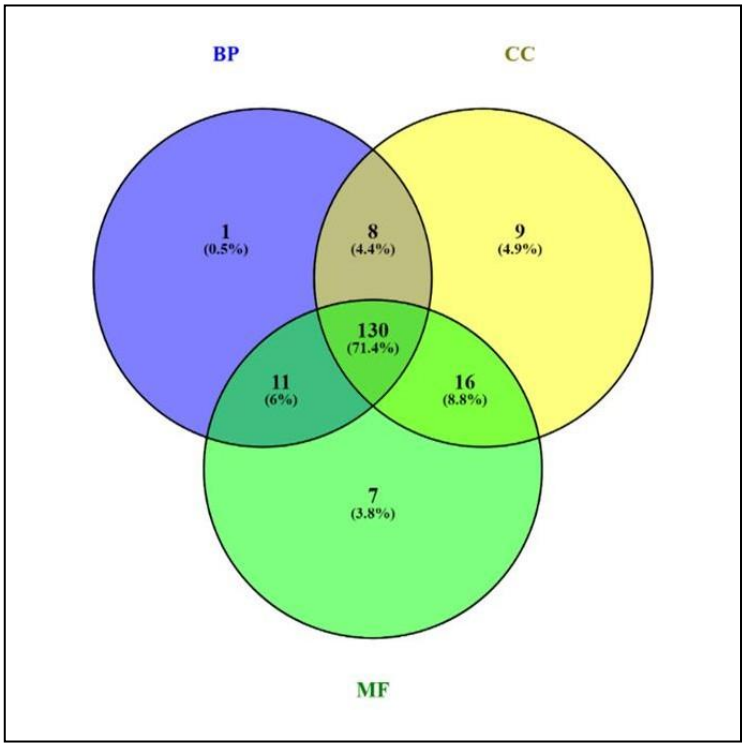



Figure 3.6.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 152 different pathways that were regulated by 130 key genes. Human papillomavirus infection, focal adhesion, PI3K-Akt signaling pathway, and ECM-receptor interaction (15) are major pathways that could be regulated through the lncRNA- miRNA-mRNA axis (figure 3.6.3B). The pathways that were regulated by single dysregulated genes are cysteine and methionine metabolism, ferroptosis, and glycerophospholipid metabolism.

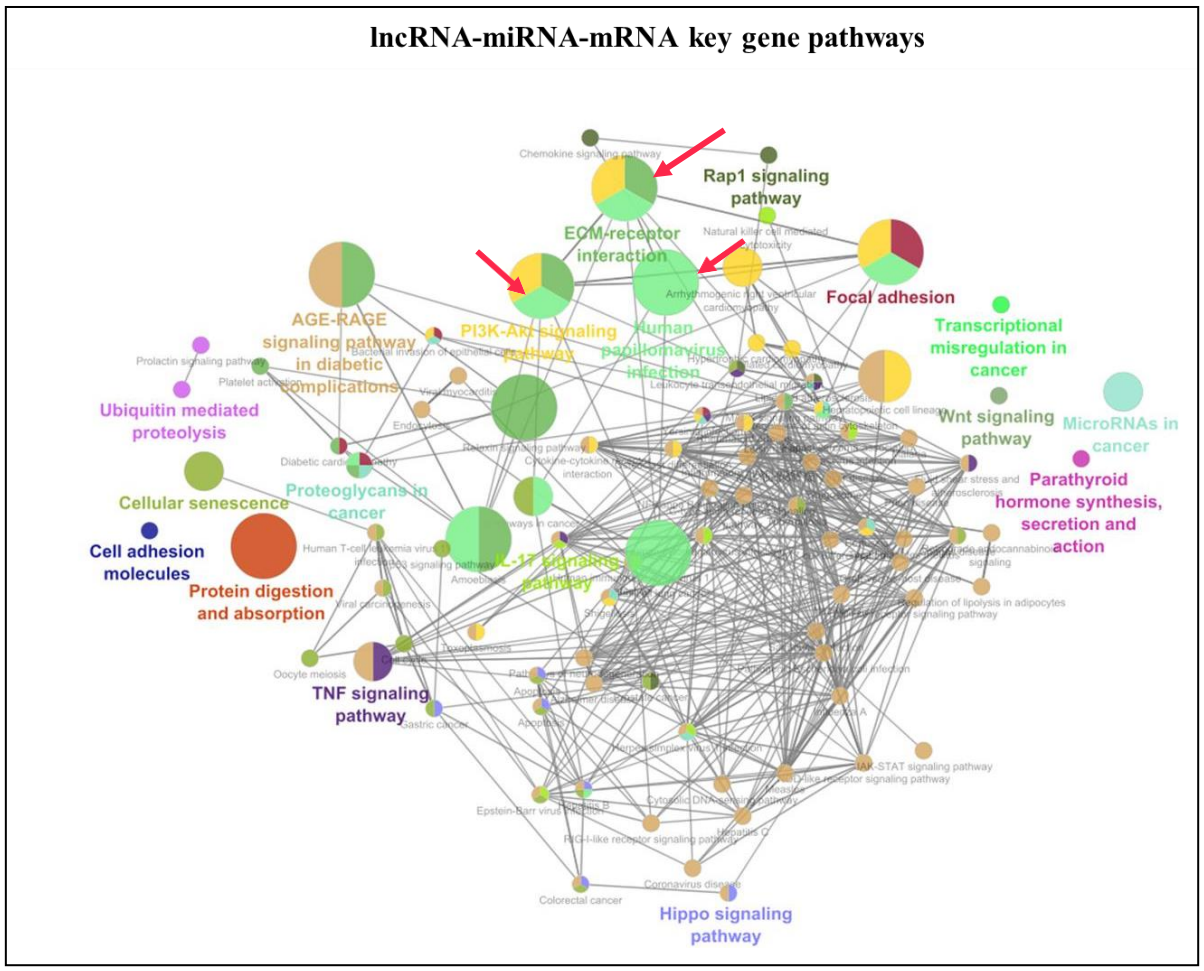



Figure 3.6.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

### 3.7. KIPAN- Pan-kidney cohort (KICH, KIRC, KIRP)

Renal cancer is a large heterogeneous group of cancers derived from renal tubular cells. Kidney cancer comprises dozens of distinct molecular and histopathological subtypes among which renal chromophobe carcinoma (KICH), renal clear cell carcinoma (KIRC), and renal papillary cell carcinoma (KIRP), are major subtypes. Out of these KIRC is the most common and comprises almost 75% of total kidney cancer. All these three together are known as pan-renal cell carcinoma (L. Chen et al. 2022).

### 3.7.1. Renal chromophobe carcinoma (KICH)

#### 3.7.1.1. Differentially expressed mRNAs KICH

This uncommon kind of kidney cancer, known as chromophobe renal cell carcinoma, develops in the cells that line the kidney's tiny tubules, which are responsible for filtering waste products from the blood and producing urine (Range, M, and Moser 2012). KICH is a type of genetic disorder known as Birt-Hogg-Dubé syndrome which runs in families (N. Gupta, Sunwoo, and Kotloff 2016). Because of its genetic makeup, researchers have fewer options for studying it. Despite its potential importance, little research has been done on this subtype of kidney cancer. Surgery is the sole current option for treatment. Total transcriptomic data for KICH consisted of a total of 66 tumor samples and 25 control samples. The transcriptomic data consisted of 2614 dysregulated genes with 1477 up-regulated and 1137 down-regulated genes. Out of these dysregulated genes, 1526 are key genes (figure 3.7.1.1A).

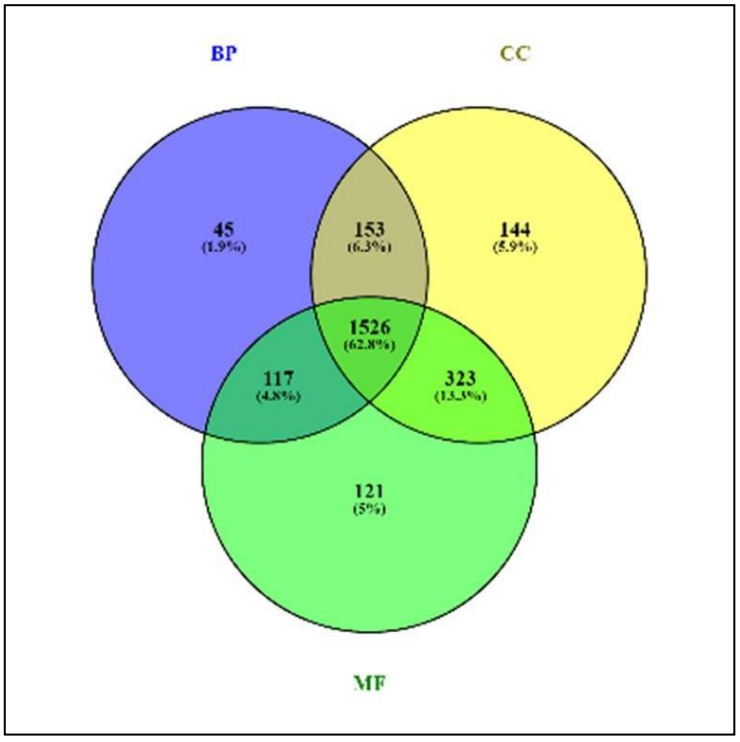



Figure 3.7.1.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed 304 different pathways were regulated by 1526 key genes. Among these pathways, pathways in Cancer, neuroactive ligand-receptor interaction, calcium signaling pathway, and PI3K-Akt signaling pathway are regulated by a larger number of key genes (figure 3.7.1.1B). Some of the pathways were regulated only by a single gene which includes the citrate cycle (TCA cycle), steroid biosynthesis, and N-Glycan biosynthesis.

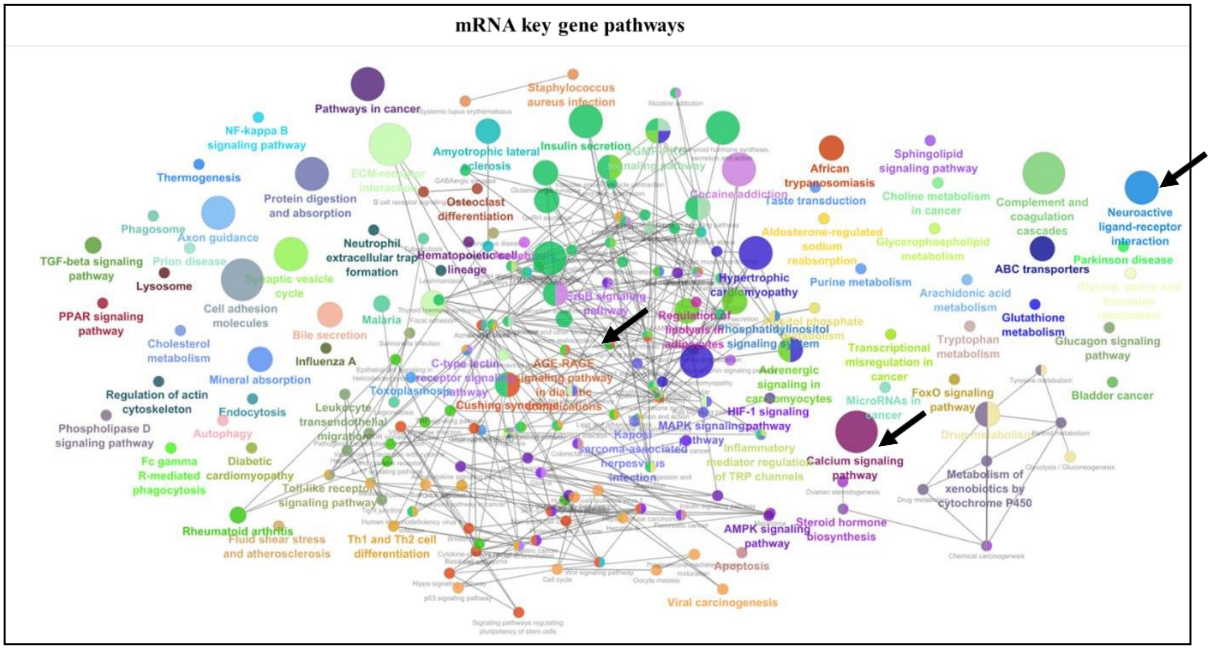



Figure 3.7.1.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

**3.7.1.2. Analysis and construction of miRNA-mRNA network** Differentially expressed transcriptomic data of KICH consisted of 93 miRNA transcripts.Among these dysregulated miRNAs, 90 miRNAs (54 down-regulated and 36 up-regulated)were found to be correlated with the expression of 822 genes (188 down-regulated and 634up-regulated) and 510 are key genes (shown in figure 3.7.1.2A).

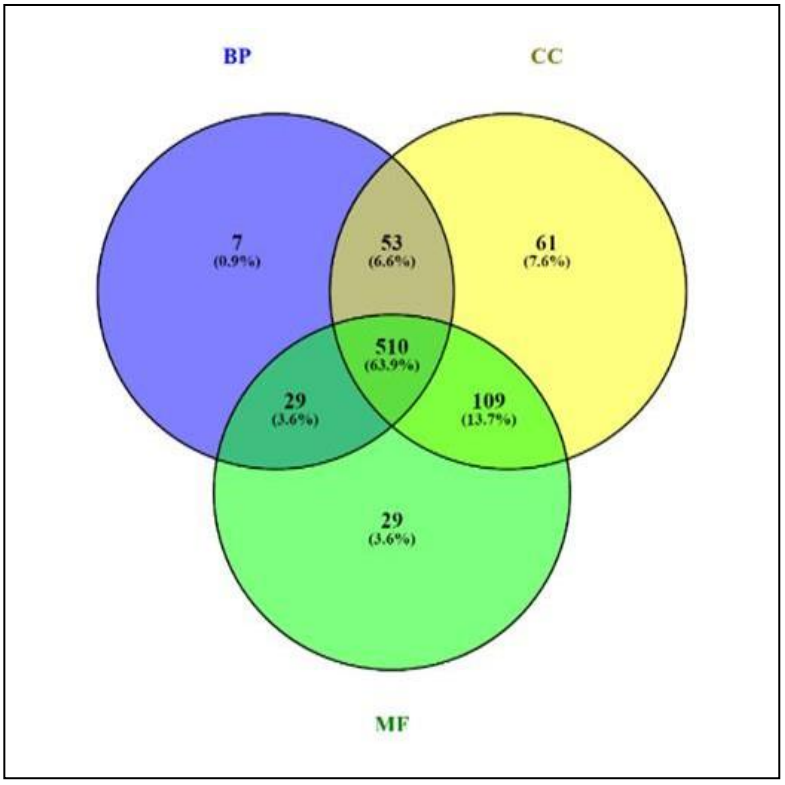



Figure 3.7.1.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed that 256 different pathways were regulated by 510 key genes and among the affected pathways, a larger number of key genes are involved in pathways in Cancer, MAPK signaling pathway, MicroRNAs in cancer, and PI3K-Akt signaling pathway (figure 3.7.1.2B) whereas single key gene affects pathway involving fructose and mannose metabolism, autoimmune thyroid disease, lysine degradation.

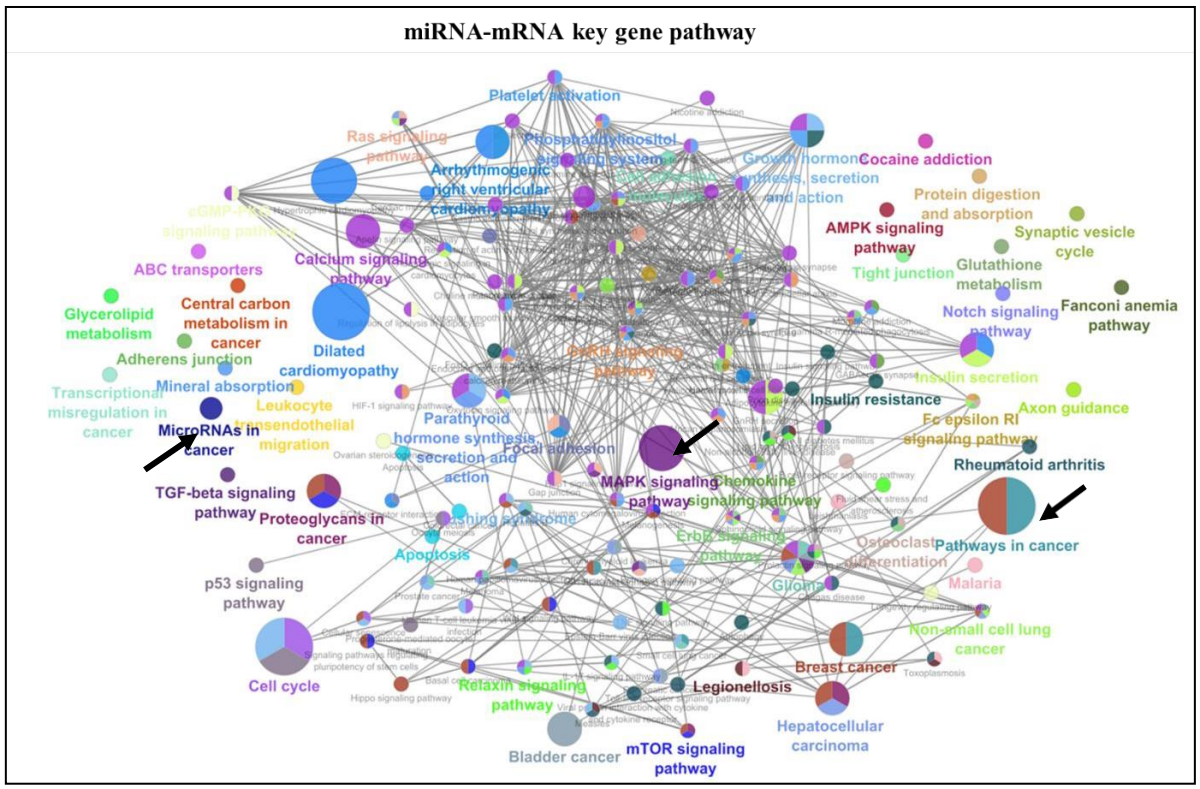



Figure 3.7.1.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.1.3. Analysis and construction of lncRNA-miRNA-mRNA network

Transcriptomic analysis of lncRNA identified 17 significantly differentially expressed lncRNAs out of which 8 (MEG3, SNHG3, PVT1, RMRP, HCP5, C1orf220, INE1, and PART1) lncRNA had experimentally validated miRNA targets. It has been reported that maternally expressed gene 3 (MEG3) encodes a 1.6 kb lncRNA that acts as an antitumor component in breast, liver, glioma, colorectal, cervical, gastric, lung, ovarian, and osteosarcoma cancer cells. MEG3 represses tumor growth by regulating p53, Rb, and miRNAs. MEG3 deregulation is linked to cancer development and progression, suggesting it may be a biomarker and therapeutic target (Al-Rugeebah, Alanazi, and Parine 2019). Upregulation of SNHG3 has been identified to increase the proliferation of renal cancer (C. Zhang et al. 2019), In one of the studies PVT1 upregulation has been linked to tumor stage progression in renal cancer (Bohosova et al. 2022), RMRP (Hao and Zhou 2022) (Yi Wang et al. 2019) and HCP5 (S. pu Hu et al. 2021), are also identified as a potential biomarker for

various human cancer types. C1orf220 is so far characterized as a gene of a putative uncharacterized protein. INE1 (Inactivation Escape 1) is linked to the X chromosome and has not been explored much. In our analysis, we obtained 1112 correlated lncRNA-miRNA-mRNA axis which consists of 8 up-regulated lncRNAs, 27 down-regulated miRNAs, and 347 up-regulated genes. Among these 347 genes, 191 are key genes (figure 3.7.1.3A).

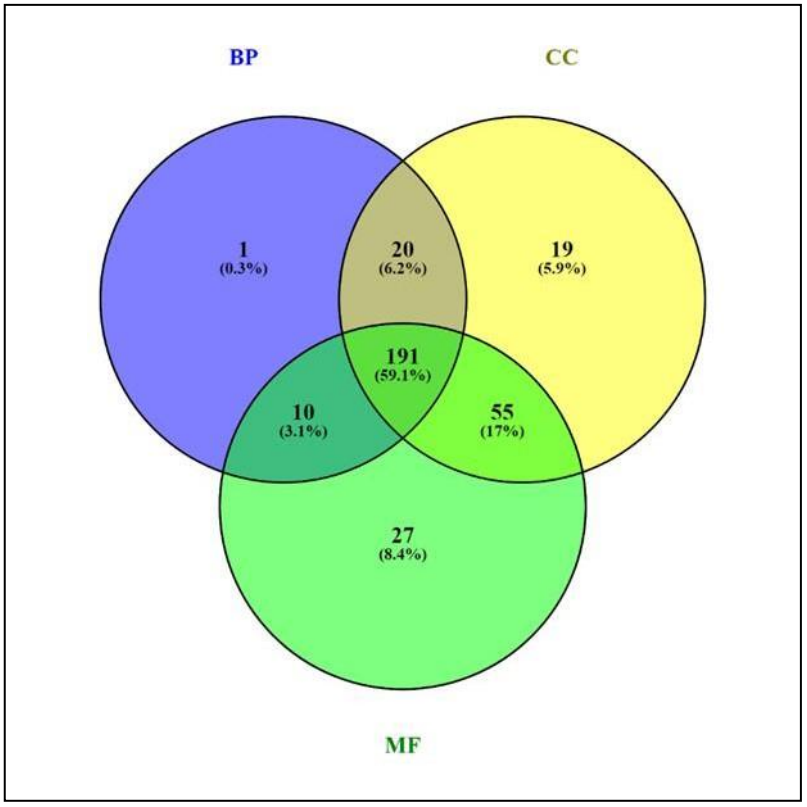



Figure 3.7.1.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed 196 dysregulated pathways that could be regulated by 191 key genes. Among these pathways in Cancer, cell cycle, miRNAs in cancer, and PI3K-Akt signaling pathway are regulated by a larger number of key genes while single gene regulated pathways involving mineral absorption, human immunodeficiency virus 1 infection, lysine degradation (figure 3.7.1.3B).

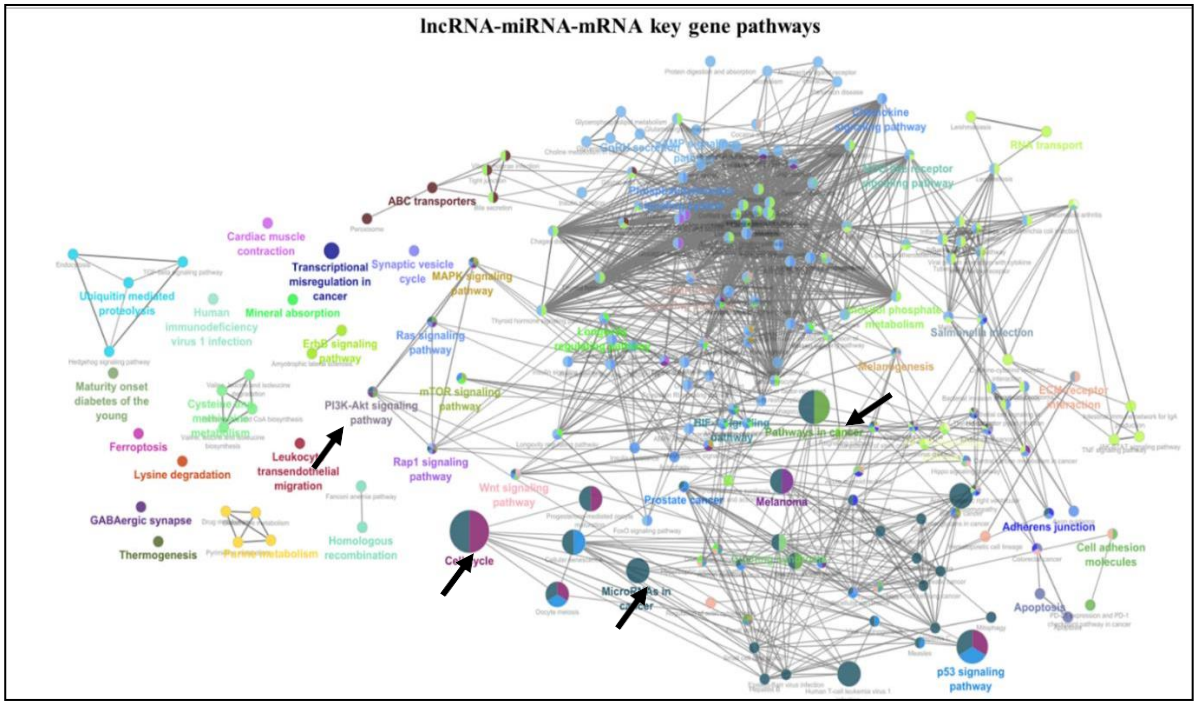



Figure 3.7.1.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.2. Kidney renal cell carcinoma (KIRC)

#### 3.7.2.1. Differentially expressed mRNAs

Kidney renal cell carcinoma is the most common type of kidney cancer. This cancer forms in the cells lining the small tubules in the kidney that filter waste from the blood and make urine. When detected early, most cases of kidney cancer can be treated effectively. However, survival rates are low when cancer has spread from the kidney to other parts of the body. Total transcriptomic data (mRNA, miRNA, and lncRNA) of KIRC was generated from 533 patient samples and 73 control samples. The transcriptomic data consisted of a total of 2174 dysregulated genes with 1445 up-regulated and 729 down-regulated genes which were categorized based on their GO and found 1278 key genes (shown in figure 3.7.2.1A).

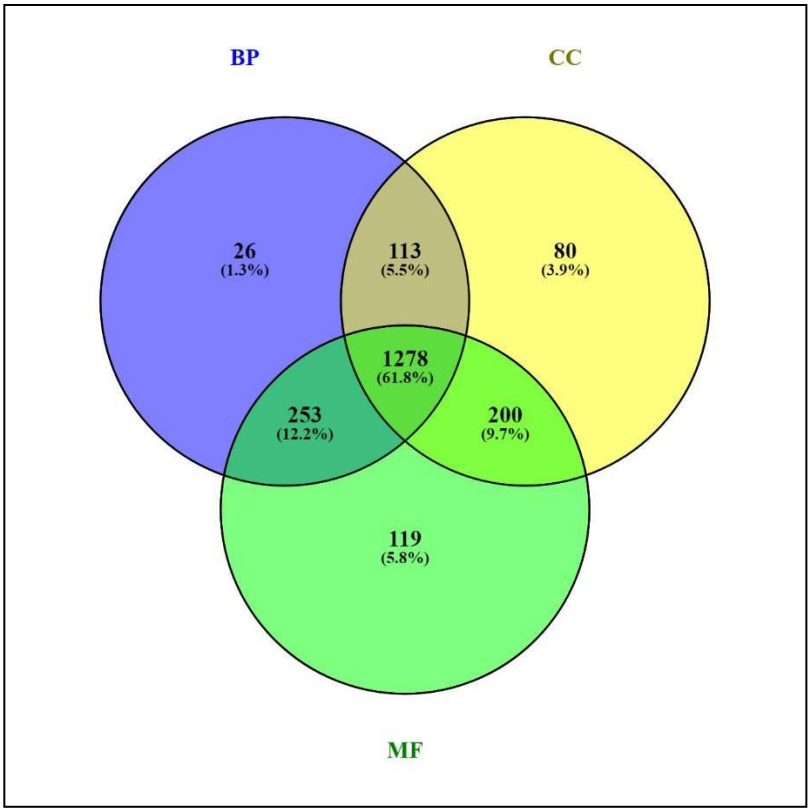



Figure 3.7.2.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 292 different pathways that were regulated by 1278 key genes. The pathways regulated by maximum key genes were cytokine-cytokine receptor interaction, pathways in cancer, neuroactive ligand-receptor interaction, and PI3K-Akt signaling pathway. Some of the pathways which were regulated by only a single gene included nicotinate and nicotinamide metabolism, renin-angiotensin system, and selenocompound metabolism (shown in figure 3.7.2.1B).

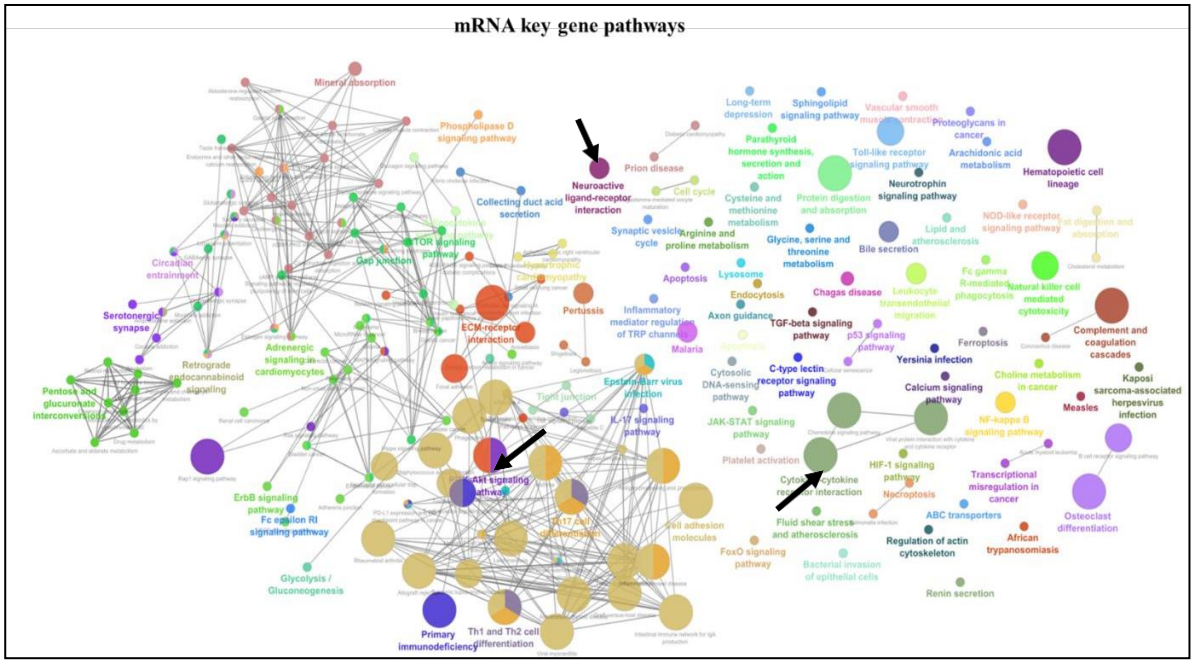



Figure 3.7.2.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.2.2. Analysis and construction of miRNA-mRNA network

KIRC consisted of a total of 69 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (28) was lower than the down-regulated miRNAs (41). We found a total of 909 correlated miRNA-mRNA with 65 miRNAs (41 down-regulated and 24 up-regulated) and 638 mRNAs (121 down-regulated and 517 up-regulated) targets. Among the 638 dysregulated mRNA targets, 404 were key genes (shown in figure 3.7.2.2A).

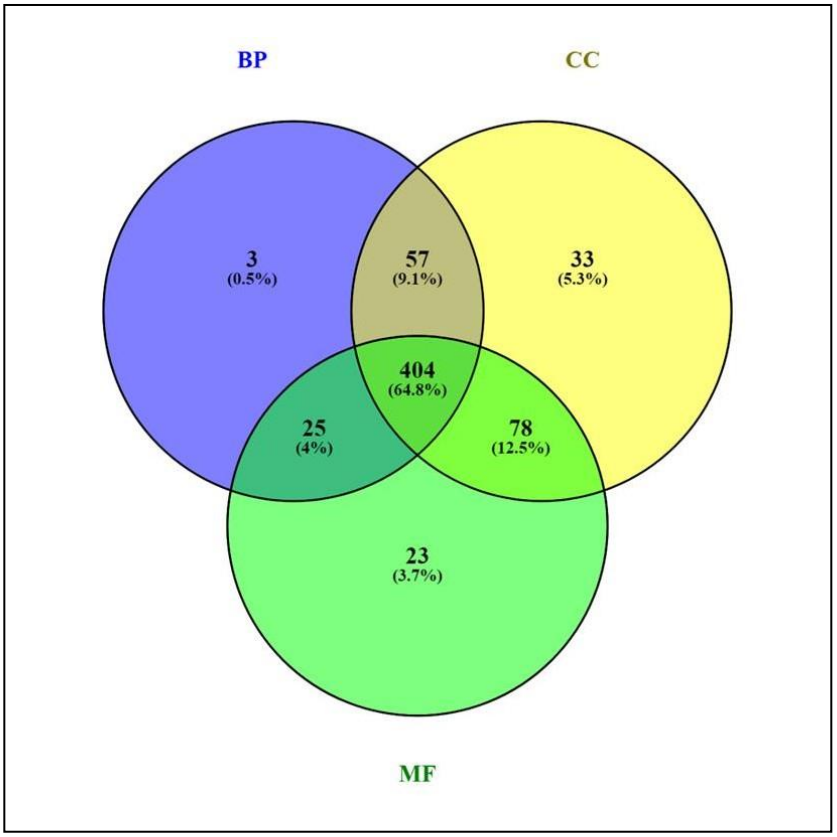



Figure 3.7.2.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 249 different pathways that were regulated by 404 key genes. The pathways regulated by maximum key genes included Pathways in Cancer, cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, and microRNAs in cancer. The pathways involving Fc gamma R-mediated phagocytosis, autophagy, and seleno compound metabolism were regulated by a single dysregulated gene (shown in figure 3.7.2.2B).

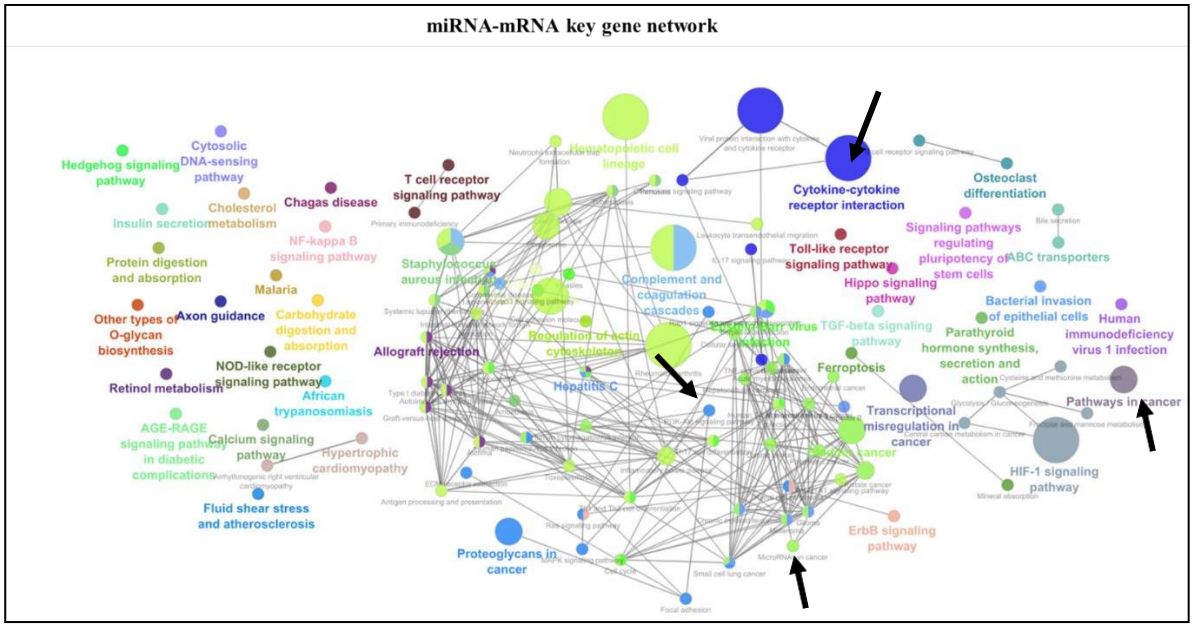



Figure 3.7.2.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.2.3. Analysis and construction of lncRNA-miRNA-mRNA network

We analyze the total transcriptome to understand the role of lncRNAs in regulated miRNA-mediated gene regulation. We obtained 18 differentially expressed lncRNAs. Out of this dysregulated lncRNA, 8 up-regulated lncRNAs had experimentally validated miRNA targets. Our analysis revealed an 1816 axis of correlated lncRNA-miRNA-mRNA. It consisted of 6 up-regulated lncRNAs (NEAT1, MALAT1, GAS5, MIR17HG, MIAT, and MIR155HG) which were regulating 34 miRNAs (down-regulated), and those miRNAs possibly regulated 299 key genes (out of a total of 510 dysregulated mRNA targets) (shown in figure 3.7.2.3A). Interestingly, it has been indicated that NEAT1 (lncRNA) can be considered a biomarker for renal cancer (Ning et al. 2017). Upregulation of MALAT1 is also linked with renal cancer progression (Hai min Zhang et al. 2015). The lncRNA growth arrest-specific transcript 5 (GAS5) is a novel lncRNA, which acts as a tumor suppressor. The downregulation of this lncRNA stops cell proliferation and induces apoptosis (Qiao et al. 2013). However, we observed that in renal cancer GASS is up-regulated. It has been shown that MIR17HG promotes aerobic glycolysis and liver metastasis. High MIR17HG expression predicted poor

prognosis in CRC patients with liver metastases (Zhao et al. 2021). MIAT (Qu et al. 2018), MIR155HG is an identified biomarker for multiple cancer types and its higher expression is associated with tumor progression (Peng et al. 2019).

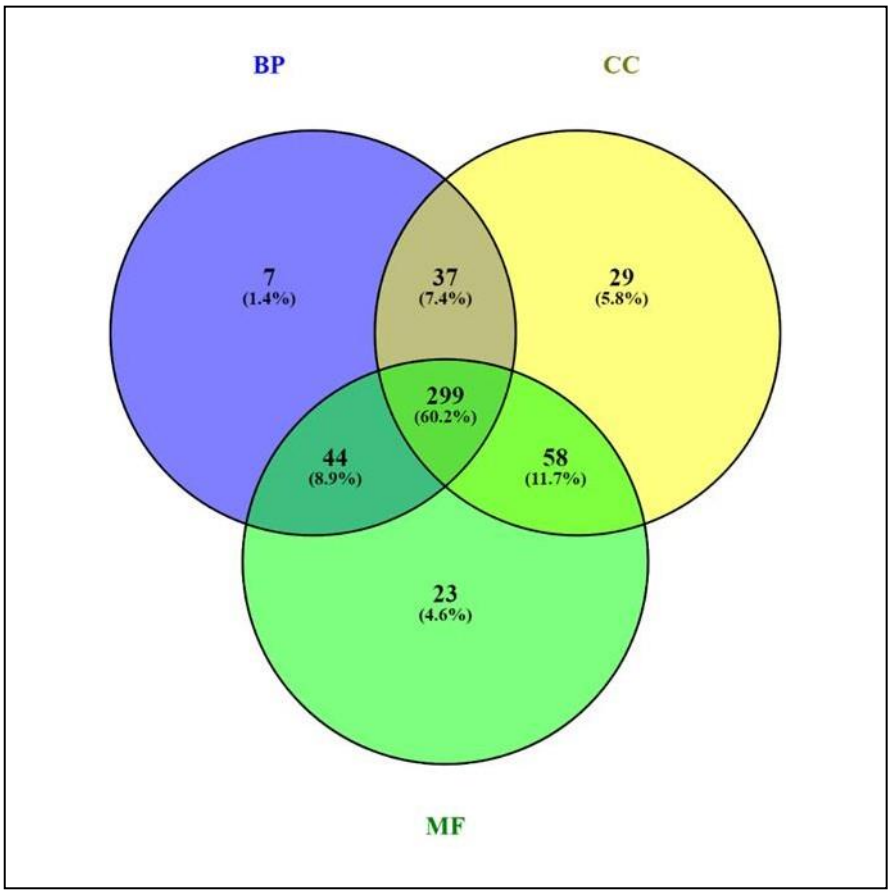



Figure 3.7.2.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 212 different pathways that were regulated by 299 key genes. Pathways like cytokine-cytokine receptor interaction, pathways in Cancer, hematopoietic cell lineage, and PI3K-Akt signaling pathway were found to be regulated by a large number of genes. Whereas pathways like a serotonergic synapse, Fc gamma R-mediated phagocytosis, and mTOR signaling pathway were regulated by a single gene (shown in figure 3.7.2.3B).

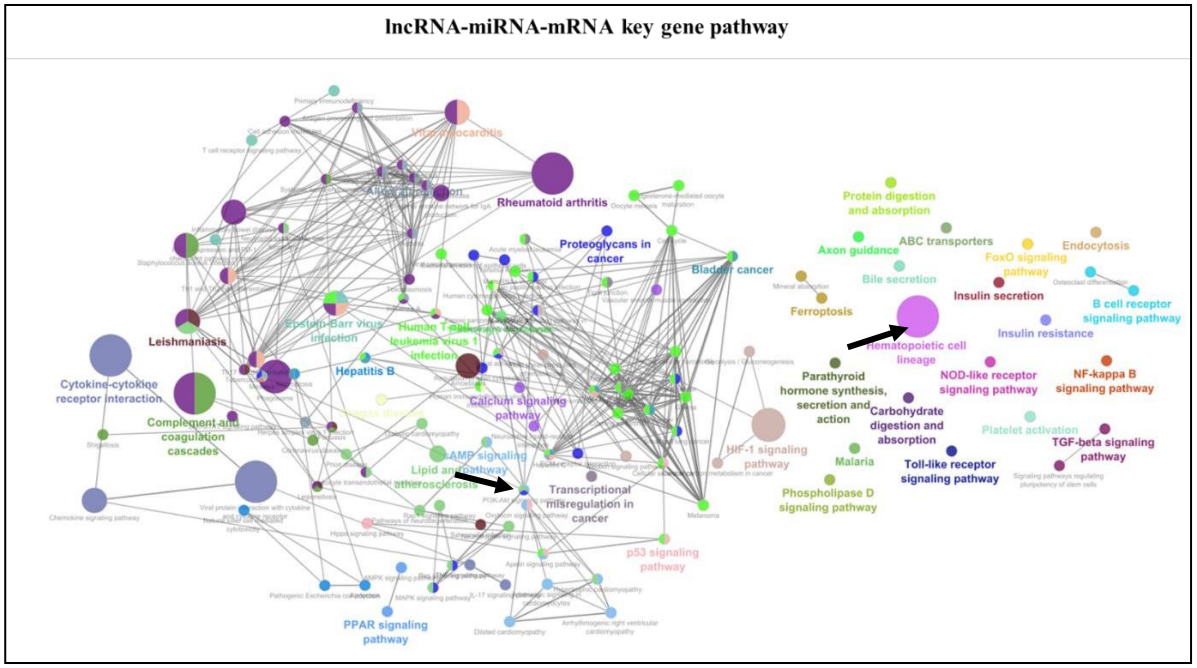



Figure 3.7.2.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.3. Papillary renal cell carcinoma (KIRP)

#### 3.7.3.1. Differentially expressed mRNAs

Total transcriptomic data (mRNA, miRNA, and lncRNA) for papillary renal cell carcinoma (KIRP) consists of 290 tumor samples and 32 control samples. The transcriptomic analysis identify 2243 dysregulated genes with 1441 being up-regulated and 802 being down-regulated. Through the gene ontology analysis, we have identified 1461 key genes (as shown in figure 3.7.3.1A).

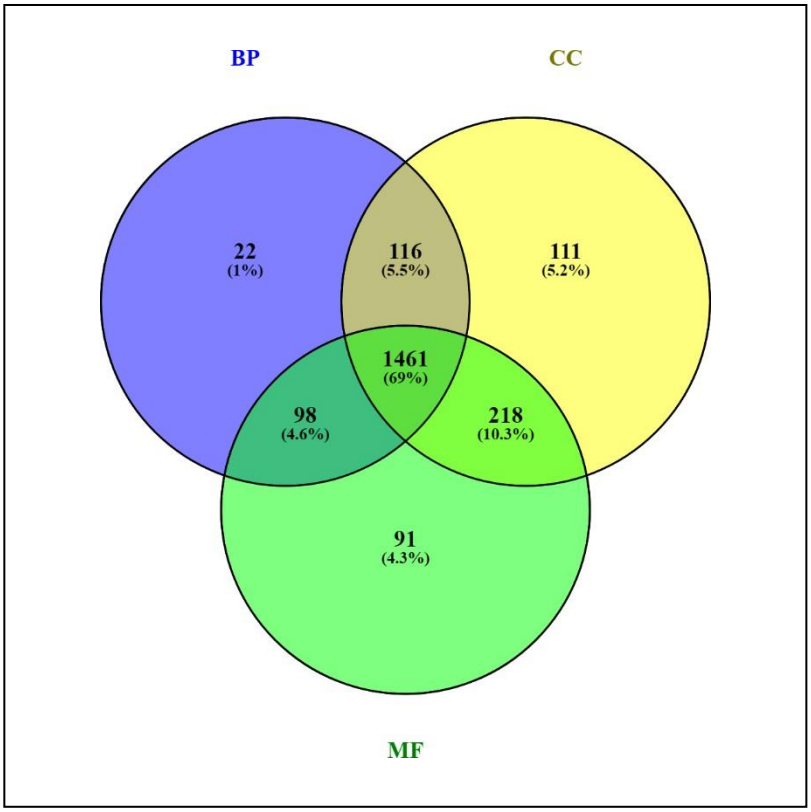



Figure 3.7.3.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 303 different pathways that were regulated by 1461 key genes. Few pathways like pathways in Cancer, cytokine-cytokine receptor interaction, calcium signaling pathway, and neuroactive ligand-receptor interaction are regulated by a larger number of key genes whereas the citrate cycle (TCA cycle), seleno compound metabolism, and glycosphingolipid biosynthesis pathways were regulated by a single gene (shown in figure 3.7.3.1B).

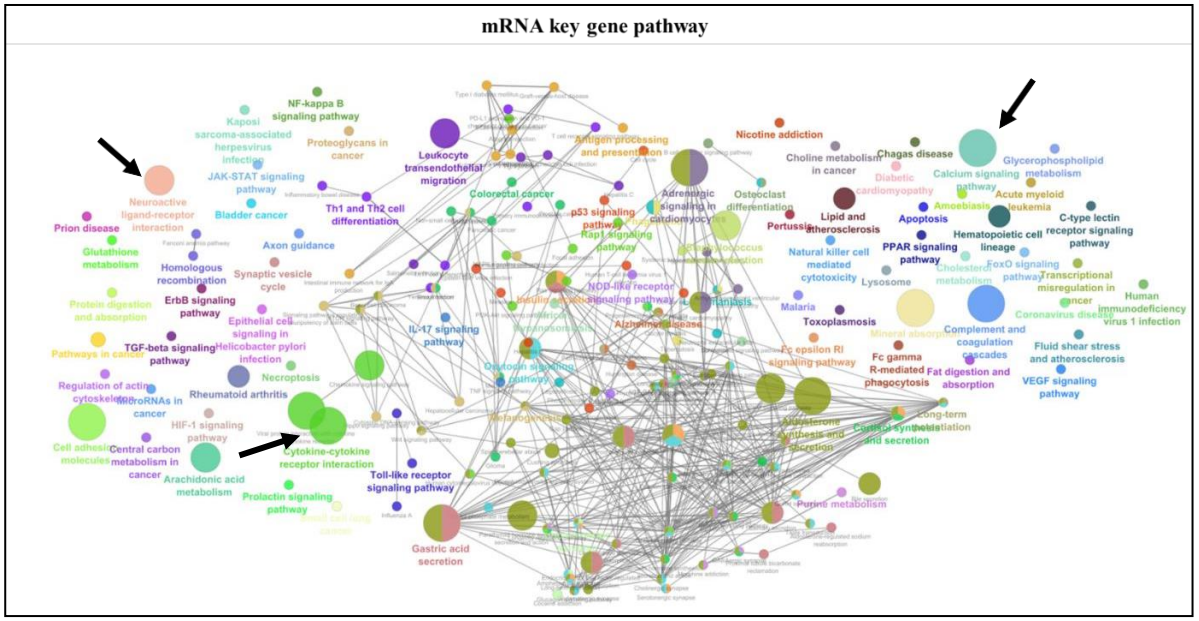



Figure 3.7.3.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.3.2. Analysis and construction of miRNA-mRNA network

KIRP consisted of a total of 86 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (25) was lower than the down-regulated miRNAs (61) a similar trend to the mRNA. Correlated studies of miRNA-mRNA interaction revealed A total of 930 correlated pairs with 84 miRNAs (60 down-regulated and 24 up-regulated) having 538 mRNA(136 down-regulated and 402 up-regulated) targets. A more number of miRNAs were regulated and found 404 key genes shown in figure 3.7.3.2A.

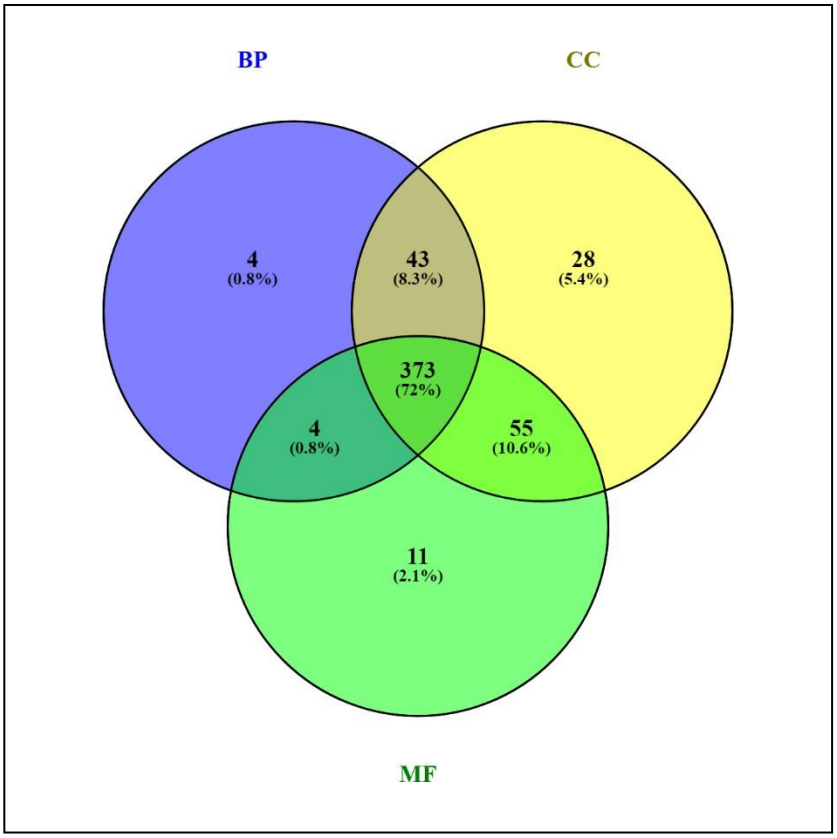



Figure 3.7.3.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 242 different pathways that were regulated by 373 key genes. The pathways regulated by maximum key genes were Pathways in Cancer, PI3K-Akt signaling pathway, microRNAs in cancer, and calcium signaling pathway. The pathways that were regulated by a single gene are cysteine and methionine metabolism mitophagy, and renin-angiotensin system (shown in figure 3.7.3.2B).

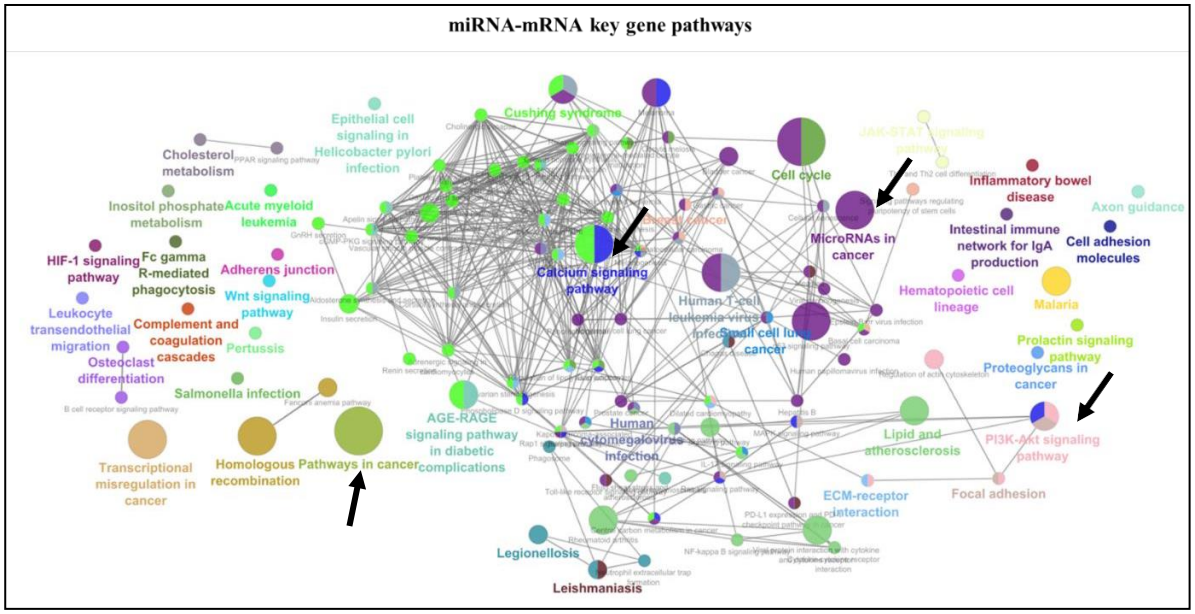



Figure 3.7.3.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.7.3.3. Analysis and construction of lncRNA-miRNA-mRNA network

In the transcriptomic analysis of lncRNA, we obtained a total of 15 differentially expressed lncRNAs that were present out of which only 11 had experimentally validated miRNA targets all of which are up-regulated. The correlated study revealed a 1290 axis of correlated lncRNA-miRNA-mRNA. It consisted of 11 lncRNAs (C2orf27A, HOTAIR, UCA1, SNHG12, TSIX, MIR155HG, SNHG3, PVT1, MIAT, and RMRP) all of them up-regulated regulating 25 miRNA and further by 323 mRNAs(up-regulated) and had 197 key genes shown in figure 3.7.3.3A. Chromosome 2 Open Reading Frame 27A (C2orf27A) is an uncharacterized protein. HOX transcript antisense RNA (HOTAIR) has been identified as a biomarker in cancers such as breast, colorectal, renal, gastric, and others (D. Li et al. 2020). lncRNA Urothelial Cancer Associated 1(UCA1) regulated SOX4 via miR129 to promote cell proliferation, and invasion, and inhibit apoptosis in RCC, making it a potential therapeutic target and prognostic marker (Q. Liu et al. 2018). Small Nucleolar RNA Host Gene 12 (SNHG12) has been identified to increase renal cancer progression by acting as ceRNA (H. Yu et al. 2021), TSIX function in cancer is not studied much. HLA Complex Group 27 (HCG27) identified lncRNA but the

the functional aspect is yet to be explored. Other lncRNAs such as MIR155HG, SNHG3, PVT1, MIAT, and RMRP role has been explored in renal cancer.

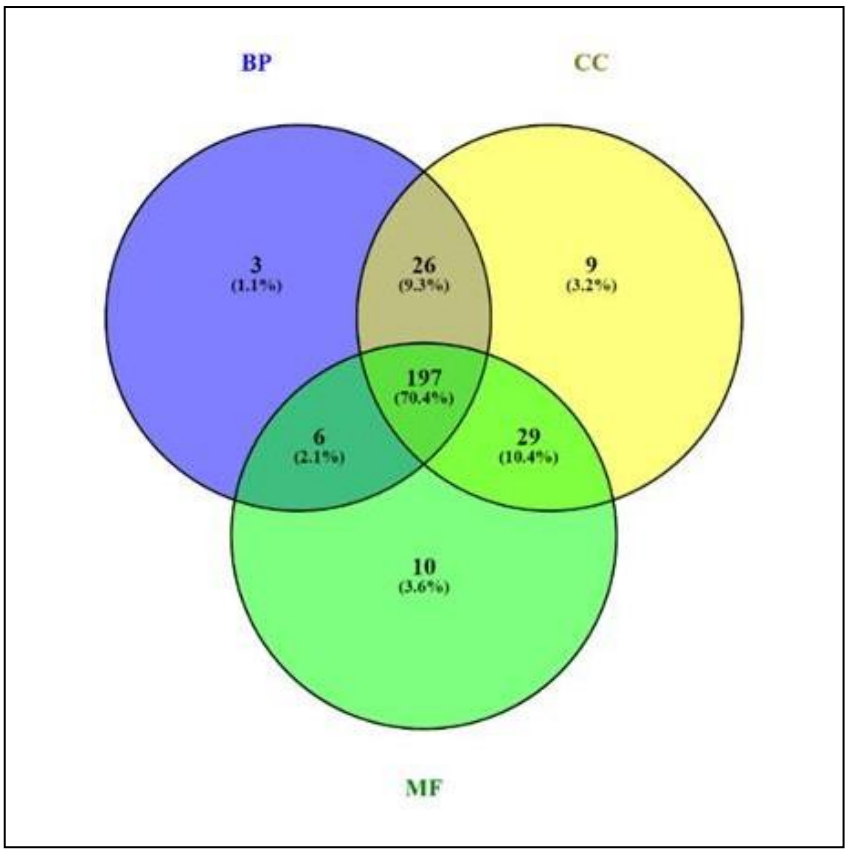



Figure 3.7.3.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 181 different pathways that were regulated by 197 key genes. The pathways regulated by maximum key genes included pathways in cancer, microRNAs in cancer, cell cycle, and transcriptional misregulation in cancer. Single gene-regulated pathways are base excision repair, GnRH secretion, and lysine degradation (figure 3.7.3.3B).

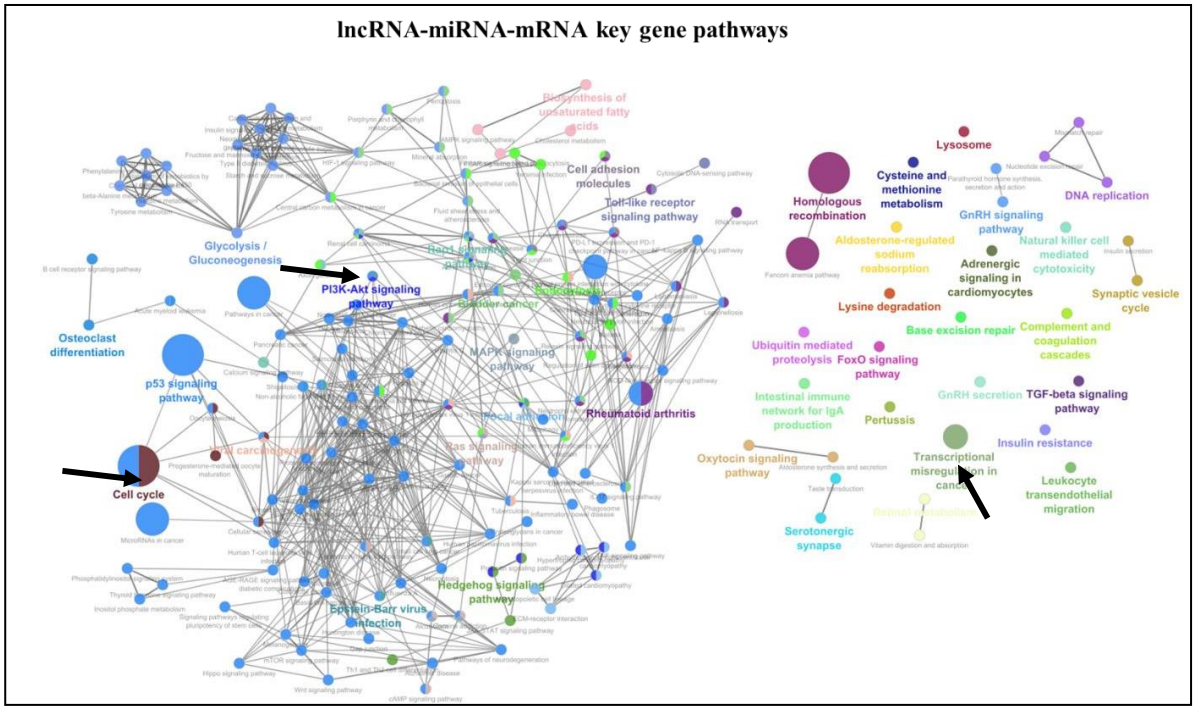



Figure 3.7.3.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

## 3.8. Liver hepatocellular carcinoma (LIHC)

### 3.8.1. Differential Expression of mRNA

There are several types of liver cancer. Hepatocellular carcinoma is the most common form of liver cancer. It has not been identified what causes this type of cancer, but it is found that it mostly occurs in people with chronic liver diseases, such as cirrhosis caused by hepatitis B or hepatitis C infection. Total transcriptomic data for LIHC were obtained from 371 tumor samples and 50 control samples. Transcriptomic analysis shows 1725 dysregulated mRNAs with 1431 up-regulated and 294 down-regulated genes with a total of 1091 key genes (figure 3.8.1A). The number of upregulated genes is significantly more than that of downregulated genes.

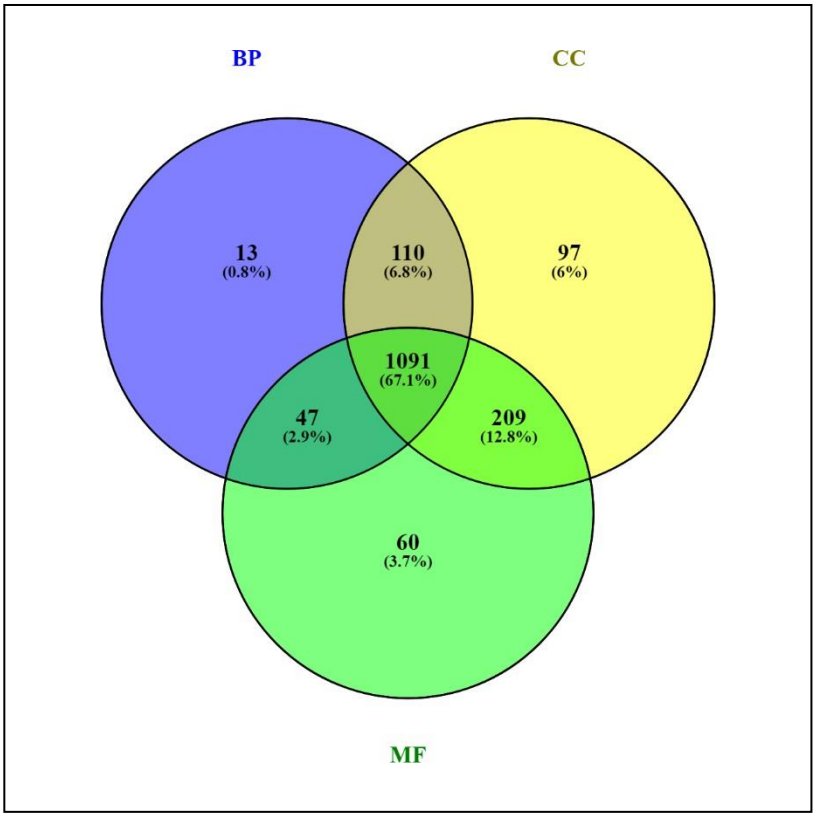



Figure 3.8.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 291 different pathways that were regulated by 1091 key genes. The pathways regulated by maximum key genes were pathways in cancer, neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, and human papillomavirus infection. The pathways that were regulated through a single gene are tryptophan metabolism and glycosaminoglycan biosynthesis (figure 3.8.1A).

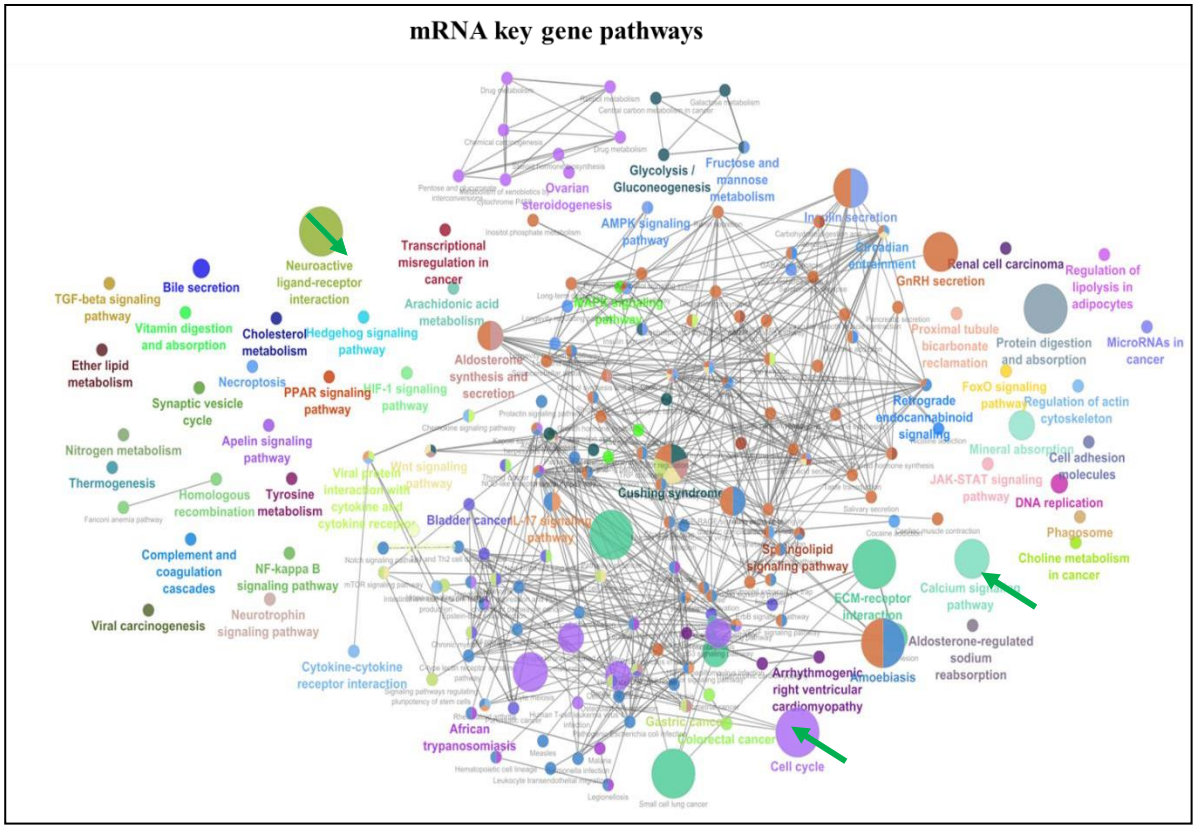



Figure 3.8.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in green color.

### 3.8.2. Analysis and construction of miRNA-mRNA network

LIHC consisted of 52 differentially expressed miRNA transcripts with 346 miRNA-mRNA correlated pairs. The correlated pairs contain 46 miRNAs (26 down-regulated and 20 up-regulated) and 240 mRNAs (50 down-regulated and 190 up-regulated) targets. Out of the correlated genes, 151 are key genes (shown in figure 3.8.2A).

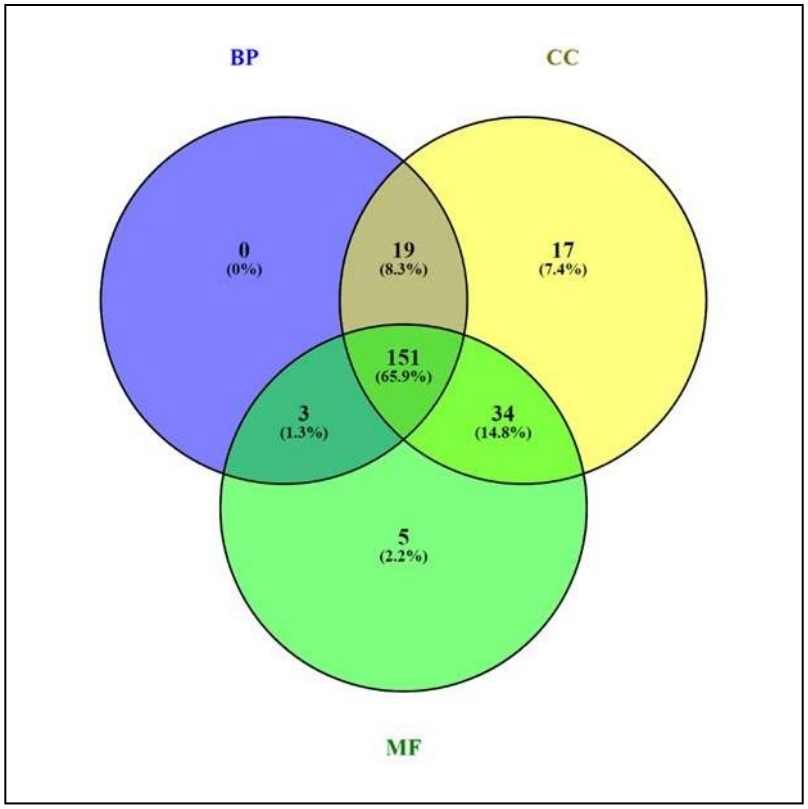



Figure 3.8.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 197 different pathways that were regulated by 151 key genes. The pathways which were regulated by maximum key genes included pathways in cancer, PI3K-Akt signaling pathway, and cellular senescence. Some of the pathways which were regulated by very few genes included pathways like the B cell receptor signaling pathway, mismatch repair DNA replication (shown in figure 3.8.2B).

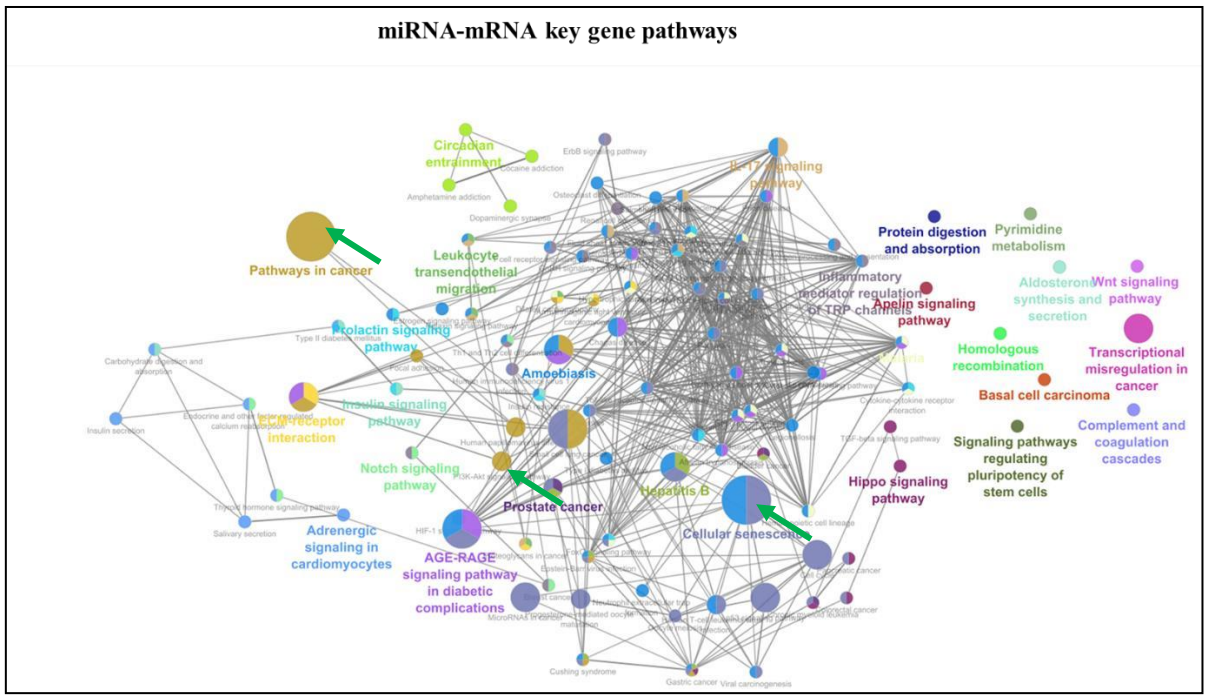



Figure 3.8.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in green color.

### 3.8.3. Analysis and construction of lncRNA-miRNA-mRNA network

To understand the role of lncRNA-regulated mRNA, we did the transcriptomic analysis and identified a total of 16 differentially expressed lncRNAs. Out of which 14 had experimentally validated miRNA (11 up-regulated and 3 down-regulated) targets. Our study revealed 152 axis of correlated lncRNA-miRNA-mRNA triplet. It consisted of 5 lncRNAs (1 down-regulated and 4 up-regulated), 11 miRNAs (8 down-regulated and 3 up-regulated), and 85 mRNAs (18 down-regulated and 67 up-regulated). Among a total of 85 lncRNAs-mediated dysregulated genes 55 are key genes (shown in figure 3.8.3A). Interestingly, it was identified that the downregulation of RMRP in HCC tissues is linked with poor prognosis. RMRP reduced HCC cell proliferation, migration, and invasion by inhibiting miRNA (C. Shao et al. 2020) which correlates with our study as well because RMRP is down-regulated here. Upregulation of SNGH1 is known to regulate the cancer progression in hepatocellular carcinoma via the regulatory network (Zheng and Yu 2021). C2orf27A has been reported as an important

biomarker for HCC cancer (Yuan et al. 2021). Chromosome 1 Putative Open Reading Frame 220 (C1orf220) is a putative uncharacterized protein.

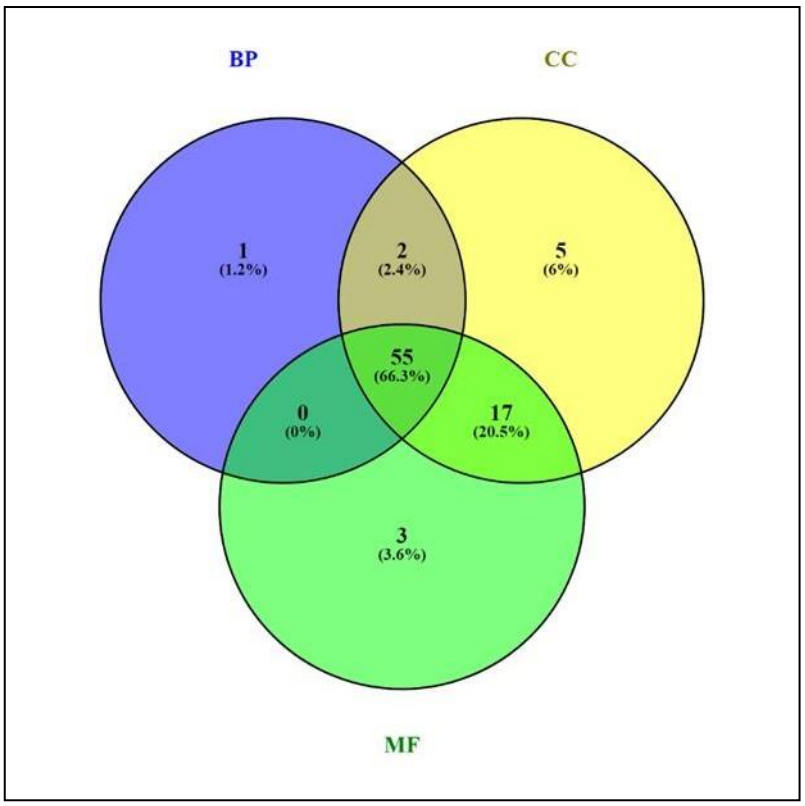



Figure 3.8.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 130 different pathways that were regulated by 55 key genes. Some of the pathways that were regulated by a large group of genes included pathways in cancer, cellular senescence, human papillomavirus infection, and microRNAs in cancer. The pathways that were regulated by a single gene are lysine degradation, JAK-STAT signaling pathway, and Rap1 signaling pathway, (shown in figure 3.8.3B).

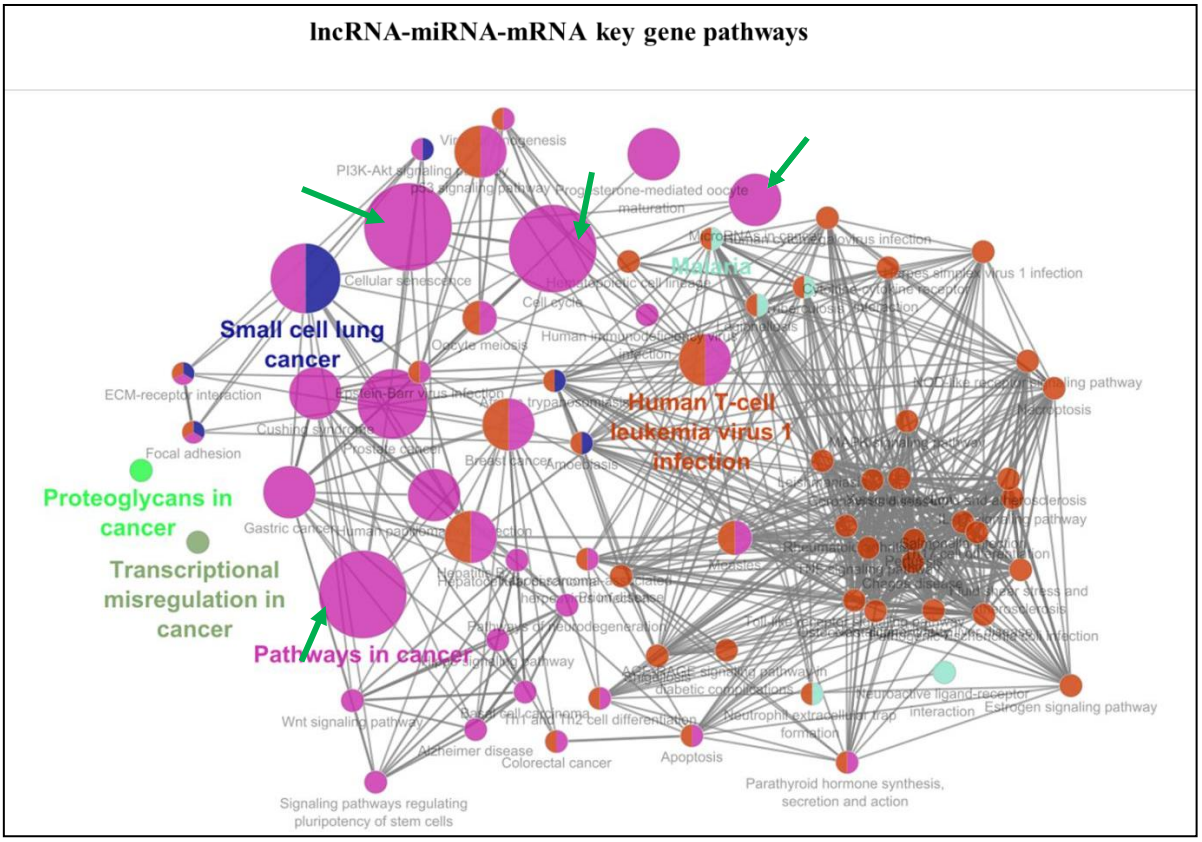



Figure 3.8.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in green color.

### 3.9. Lung adenocarcinoma (LUAD and lung squamous cell carcinoma (LUSC)

Lung cancer develops in the lung tissues, typically inside the cells that border the air passageways. Worldwide, lung carcinomas have the highest fatality rate of any cancer, male or female. Both small-cell and non-small-cell subtypes of lung cancer exist (NSCLC). These two cultivars have distinct growth patterns and require distinct care. There are two main types of lung cancer, although non-small cell is far more prevalent. Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the two kinds of non-small cell lung cancer (NSCLC). Although both are lung cancer it has been identified that both LUAD and LUSC are very different from each other in terms of prognosis, gene composition, and signaling pathways (Anusewicz, Orzechowska, and Bednarek 2020).

### 3.9.1. Lung Adenocarcinoma (LUAD)

#### 3.9.1.1. Differential Expression of mRNA

It is known that 40% of lung cancer cases belong to LUAD. Most patients with LUAD are not smokers, although this condition is also seen in those who regularly light up. The tumor tends to generate metastases at an earlier stage of the disease despite being placed more peripherally and growing more slowly than the other forms. Total transcriptomic data for LUAD consisted of a total of 515 tumor samples and 59 control samples. A total of 2092 dysregulated genes were obtained out of which 1397 were up-regulated and 695 were down-regulated genes which are significantly low with respect to the up-regulated ones. GO analysis shows that among the dysregulated genes, 1304 key genes (figure 3.9.1.1A).

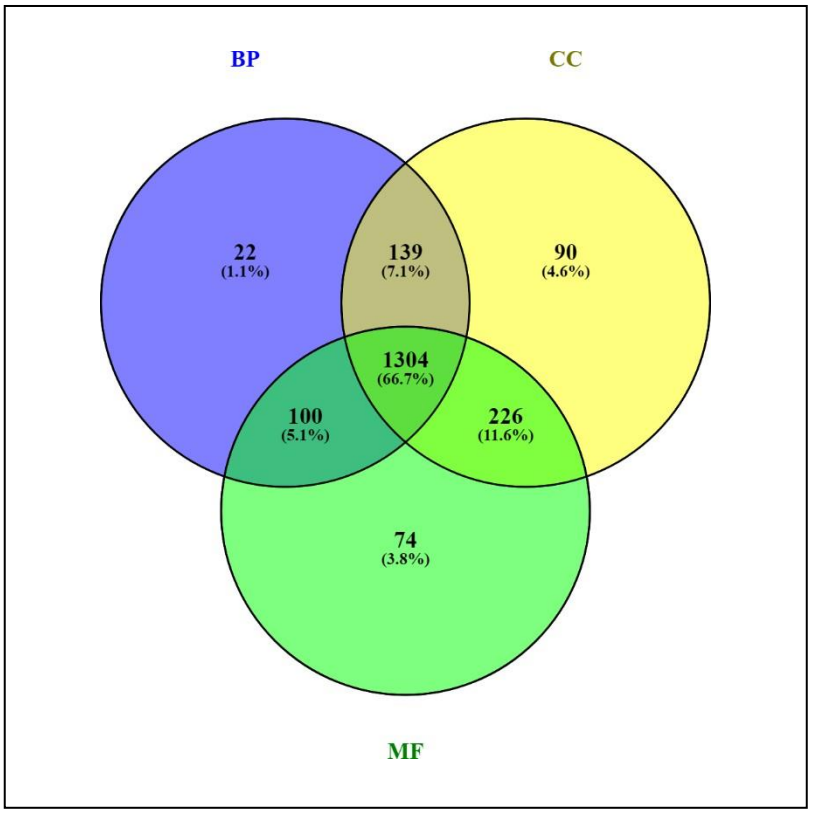



Figure 3.9.1.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 293 different pathways that were regulated by 1304 key genes. The pathways regulated by maximum key genes included pathways in cancer,

neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, and pathways of neurodegeneration. The pathways that were regulated by a single gene are primary bile acid biosynthesis, sphingolipid metabolism, and mRNA surveillance pathway (shown in figure 3.9.1.1B).

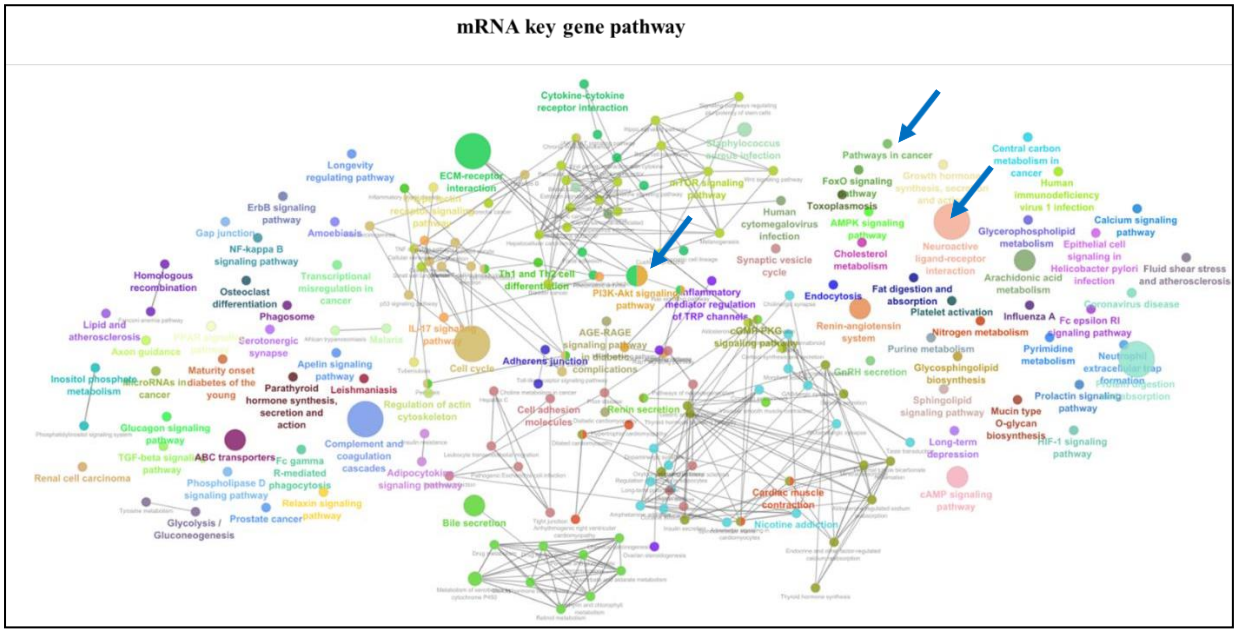



Figure 3.9.1.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in blue color.

### 3.9.1.2. Analysis and construction of miRNA-mRNA network

LUAD consisted of a total of 116 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (65) was higher than the down-regulated miRNAs (51). We observe a total of 1127 miRNA-mRNA correlated pairs with 110 miRNAs (50 down-regulated and 60 up-regulated) and 601 mRNA (176 down-regulated and 425 up-regulated) targets and found 423 key genes (shown in figure 3.9.1.2A).

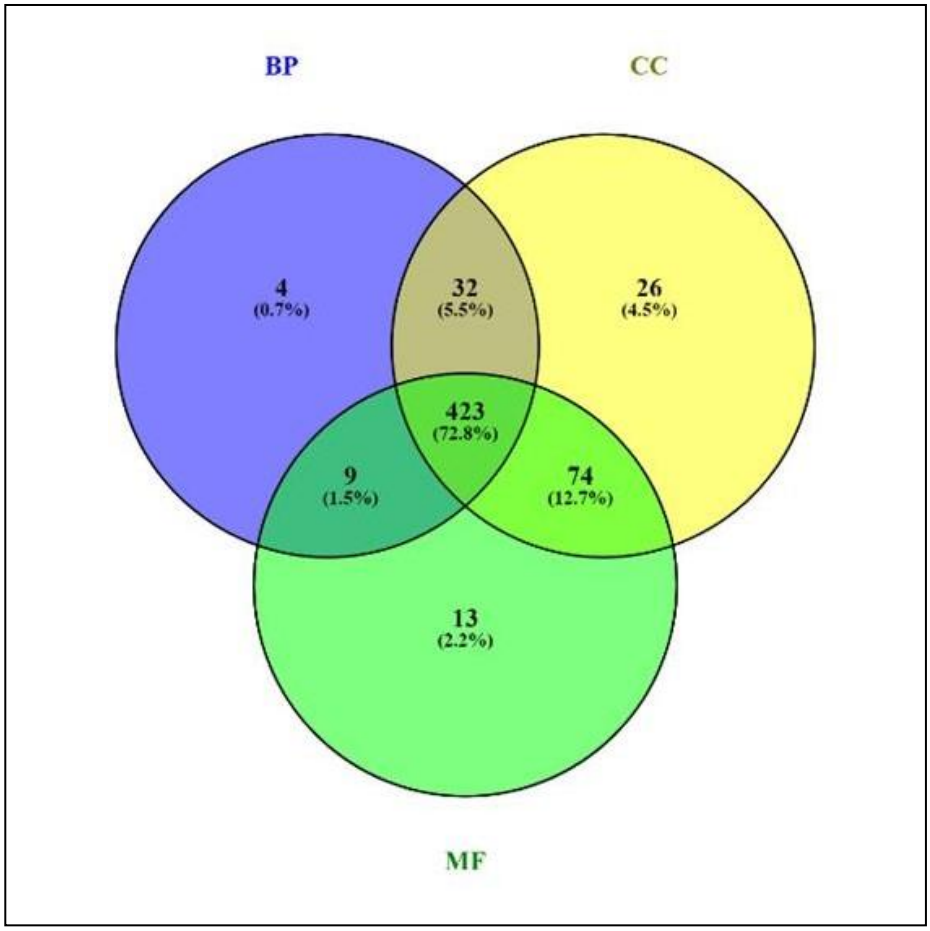



Figure 3.9.1.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 243 different pathways that were regulated by 423 key genes. pathways in Cancer, cell cycle, human T-cell leukemia virus 1 infection, PI3K-Akt signaling pathway had a large number of genes regulating them whereas some pathways were regulated by a single gene such as neurotrophin signaling pathway, proximal tubule bicarbonate reclamation, mineral absorption, and others shown in figure 3.9.1.2B.

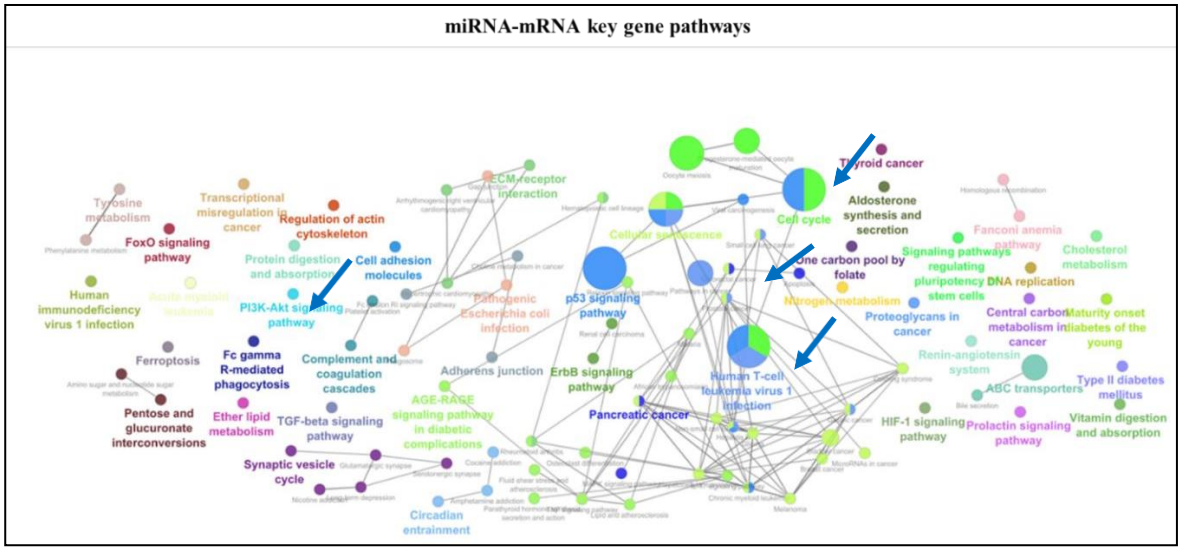



Figure 3.9.1.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in blue color.

### 3.9.1.3. Analysis and construction of lncRNA-miRNA-mRNA network

A total of 14 differentially expressed lncRNAs are present out of which only 11 had experimentally validated miRNA (10 up-regulated and 1 down-regulated) targets. The correlated study revealed 2978 axis of correlated lncRNA-miRNA-mRNA. It consisted of 9 lncRNAs(SNHG1, SNHG3, H19, MEG3, MIAT, PART1, UCA1, TMEM105, and PVT1) (all up-regulated) which are regulated by 34 miRNA (all down-regulated) and further these miRNAs were regulated by 384 mRNAs (all up-regulated) and had 253 key genes shown in figure 3.9.1.3A. LncRNA SNHG3 and SNHG1 both have been studied well for their potential role in Lung Cancer progression. It has been identified that when up-regulated they act as ceRNAs that targets tumor suppressor microRNAs (miRNAs) and mediate tumor progression (Liang Liu, Ni, and He 2018) (Z. Li et al. 2018). lncRNA H19 is responsible for the progression of lung adenocarcinoma via modulating miRNA-mRNA interaction and pathways (Lihua Liu, Liu, and Lu 2019). The exact molecular mechanism of MEG3 in lung adenocarcinoma is not well explored but it is identified as a biomarker for this disease (LV et al. 2021)(Z. Zhou, Zhang, and Xiong 2020a). lncRNA PART1, MIAT, and PVT1 are also known to promote lung adenocarcinoma (M. Li et al. 2017) (Z. Zhou, Zhang, and Xiong

2020b). Urothelial cancer associated 1 (UCA1) was first discovered in bladder cancer but its functional role has been found in multiple tumors and its expression is overly expressed in lung carcinomas (Tang et al. 2018). lncRNA TMEM105 function is not explored much.

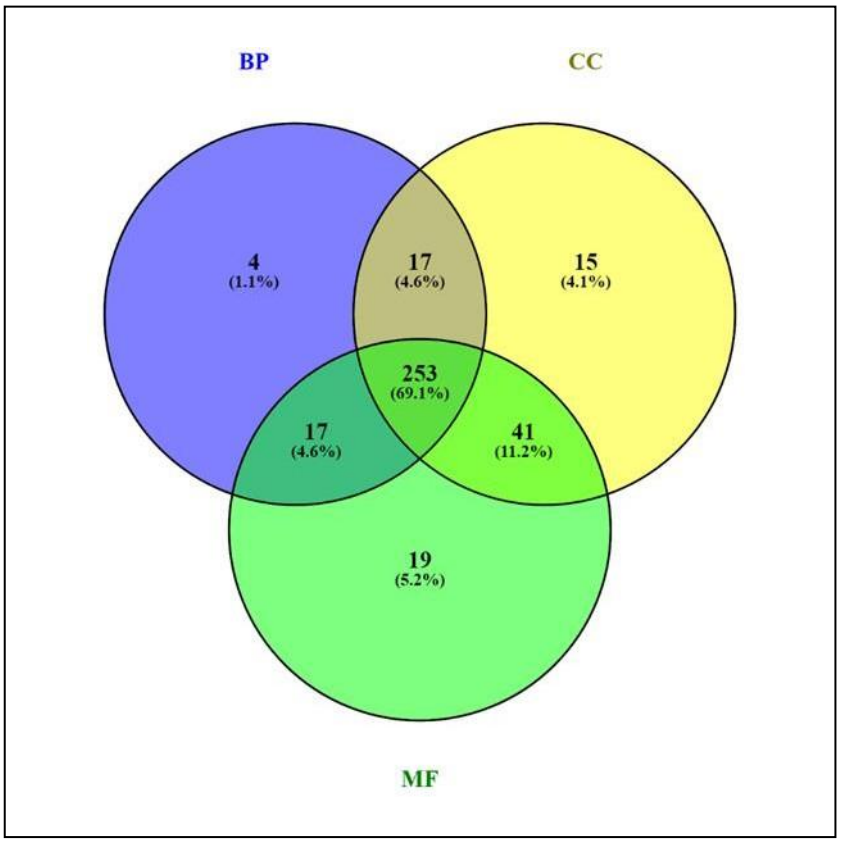



Figure 3.9.1.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 195 different pathways that were regulated by 253 key genes. Pathways like cell cycle, pathways in cancer, human T-cell leukemia virus 1 infection, and cellular senescence were regulated by a large number of genes whereaspathways like spinocerebellar ataxia, leishmaniasis, and lysine degradation were regulated bysingle gene as shown in figure 3.9.1.3B.

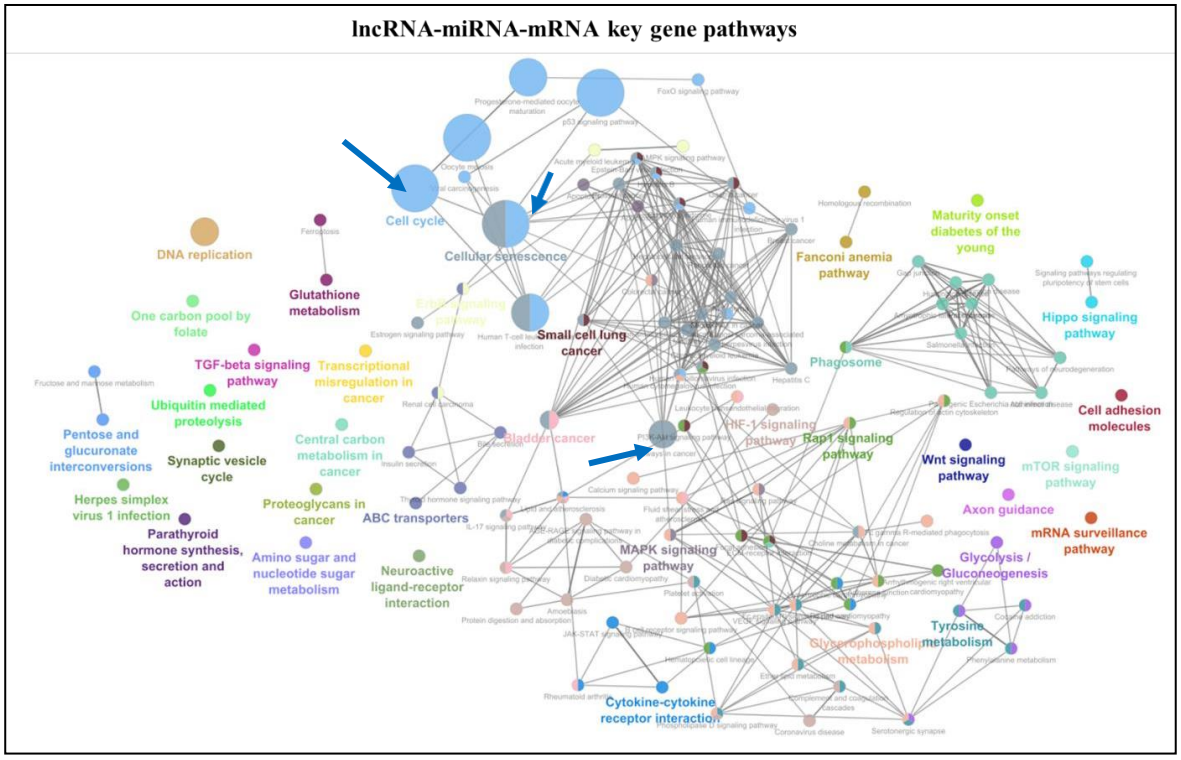



Figure 3.9.1.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in blue color.

## 3.9.2. Lung squamous cell carcinoma (LUSC)

### 3.9.2.1. Differential Expression of mRNA

LUSC is the second most common lung malignancy among tobacco smokers. Its pathogenesis is strongly associated with airway lesions that arise with smoking and is mostly located in the central parts of the lung. Total transcriptomic data (mRNA, miRNA, and lncRNA) for LUSC consisted of 502 tumor samples and 51 control samples. The transcriptomic data consisted of 3122 dysregulated mRNAs which had 1915 up-regulated and 1207 down-regulated mRNAs. It was found that 2042 are key genes (shown in figure 3.9.2.1A).

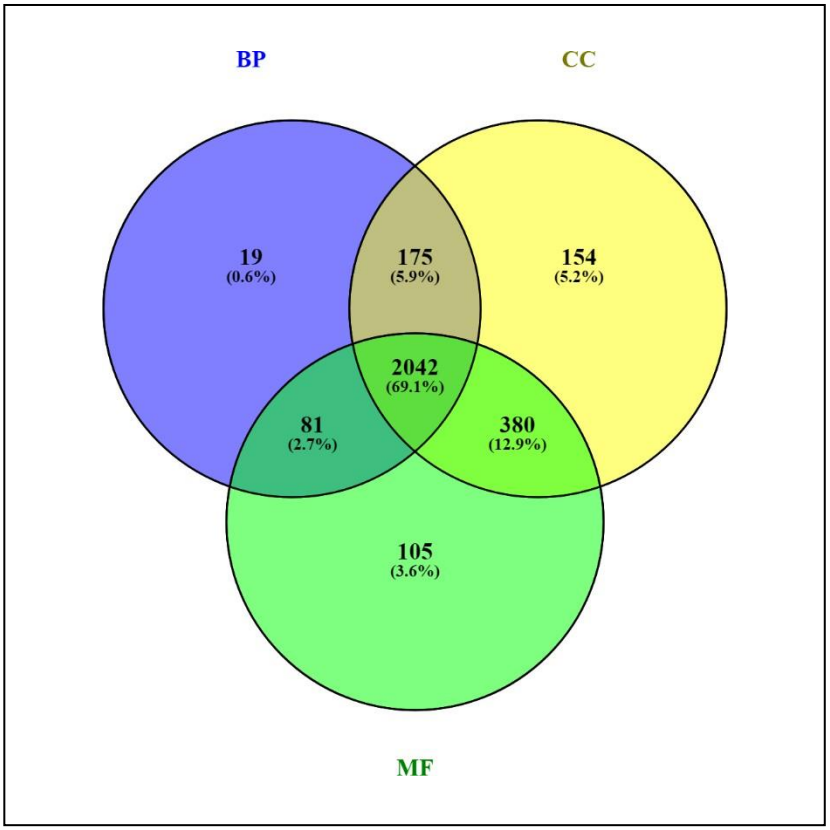



Figure 3.9.2.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 195 different pathways that were regulated by 253 key genes. pathways in Cancer, neuroactive ligand-receptor interaction, PI3K-Akt signaling pathway, human papillomavirus infection, and others were regulated by a large number of genes whereas pathways like N-Glycan biosynthesis, glycosaminoglycan biosynthesis, and RNA polymerase were regulated by a single gene (shown in figure 3.9.2.1B).

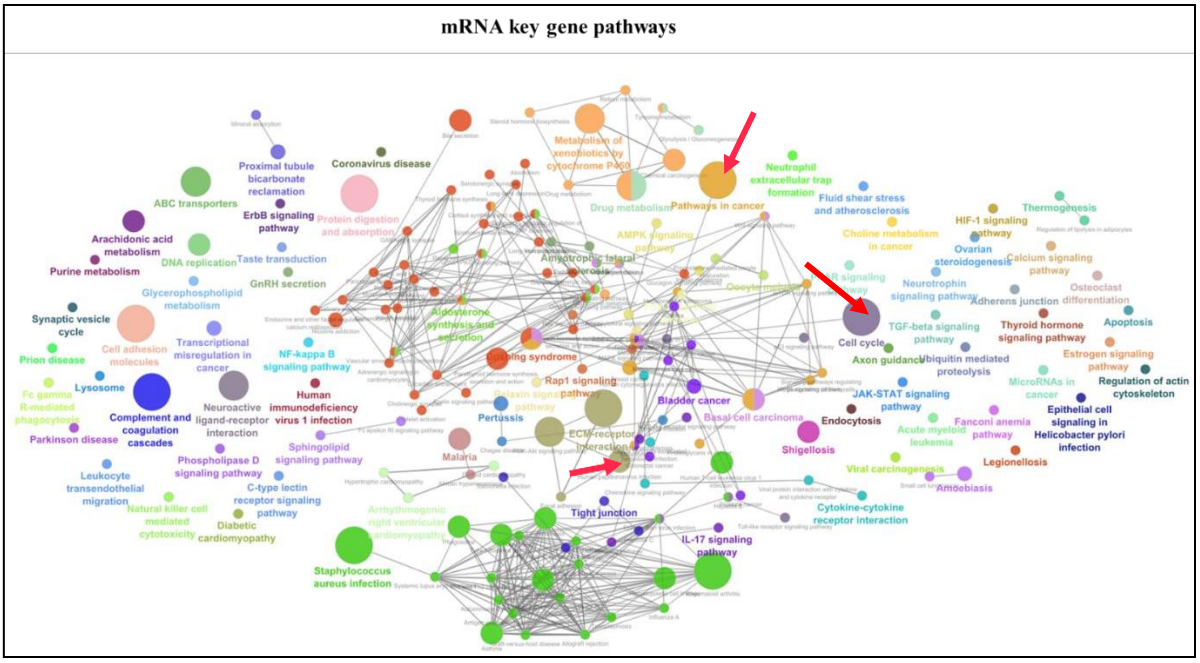



Figure 3.9.2.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

### 3.9.2.2. Analysis and construction of miRNA-mRNA network

LUSC consisted of a total of 78 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (33) was lower than the down-regulated miRNAs (44). We found a total of 1234 correlated miRNA-mRNA interactions with 73 miRNAs (44 downregulated and 29 up-regulated) and 775 mRNA (234 down-regulated and 541 up-regulated) targets. Among the genes, 423 are key genes (shown in figure 3.9.2.2A).

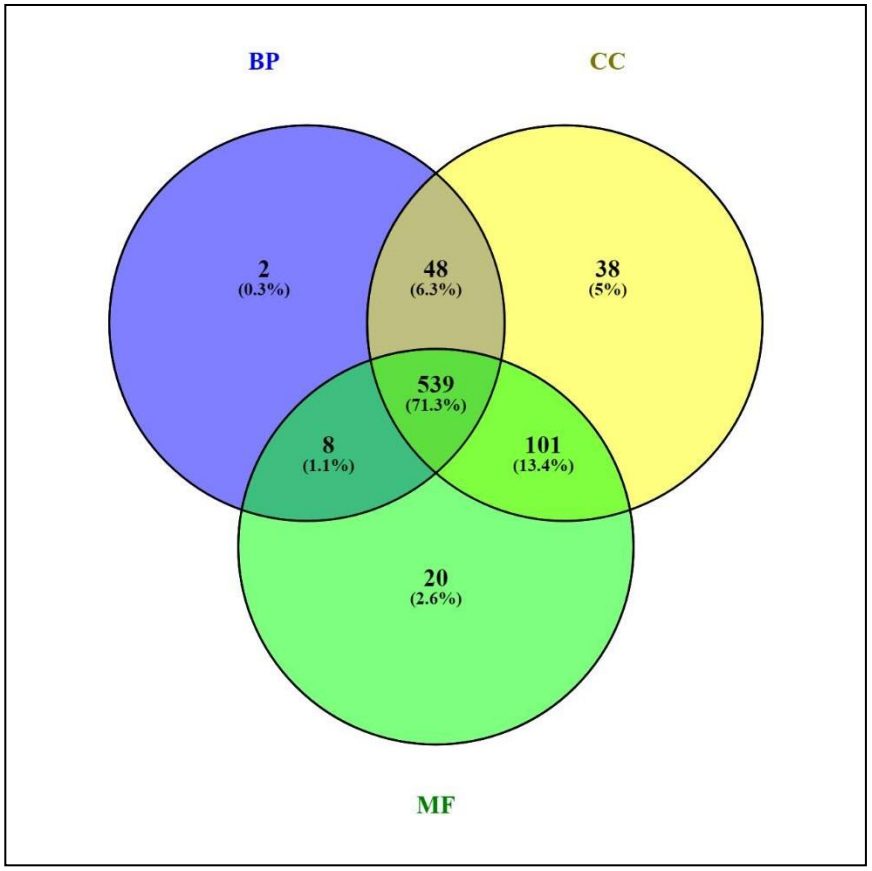



Figure 3.9.2.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 249 different pathways that were regulated by 539 key genes. Human papillomavirus infection, pathways in cancer, PI3K-Akt signaling pathway, and cell cycle were important pathways that are regulated by a large number of key genes. On the other hand, the RIG-I-like receptor signaling pathway, circadian rhythm, and synaptic vesicle cycle were regulated by a single gene as shown in figure 3.9.2.2B.

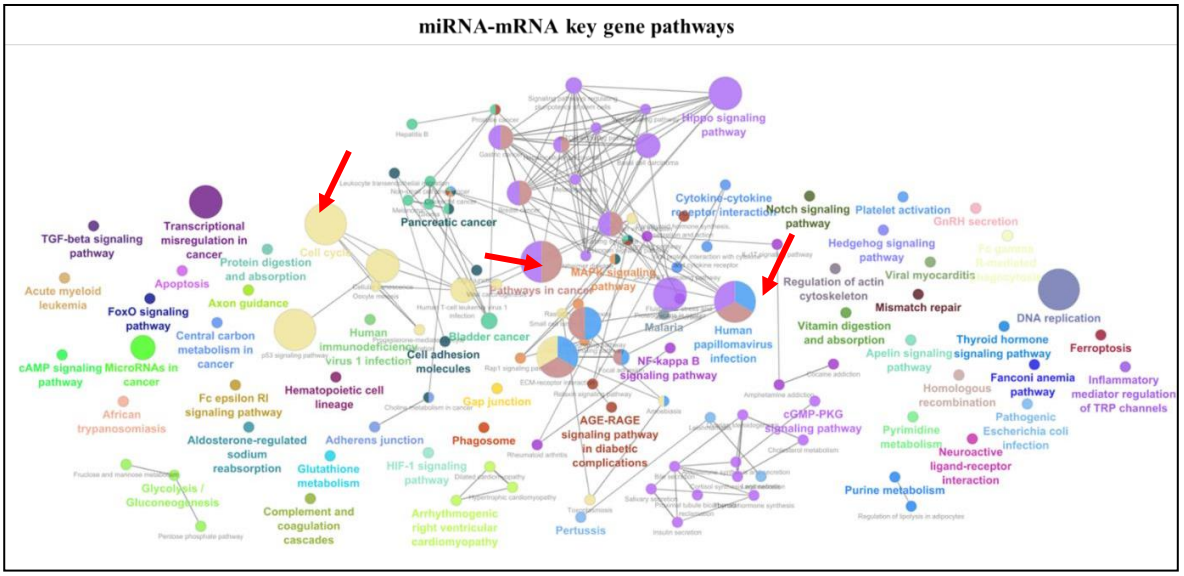



Figure 3.9.2.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

### 3.9.2.3. Analysis and construction of lncRNA-miRNA-mRNA

A total of 14 differentially expressed lncRNAs are present out of which 10 (9 upregulated and 1 downregulated) had experimental validated miRNA targets. We have identified 2748 axis of correlated lncRNA-miRNA-mRNA. It consisted of 10 lncRNAs (all upregulated), 25 miRNAs (all downregulated), and 305 key genes containing 460 mRNAs (upregulated). Interestingly, eight (SNHG3, MIAT, MEG3, H19, PART1, UCA1, SNHG1, and PVT1 ) out of ten experimentally validated lncRNAs in this carcinomas are also dysregulated in Lung adenocarcinomas, indicating similar lncRNA based gene regulation in both of these carcinomas. Reports indicated that DLEU1 has an oncogenic function in lung cancer via inhibiting apoptosis (S. Zhang et al. 2019).

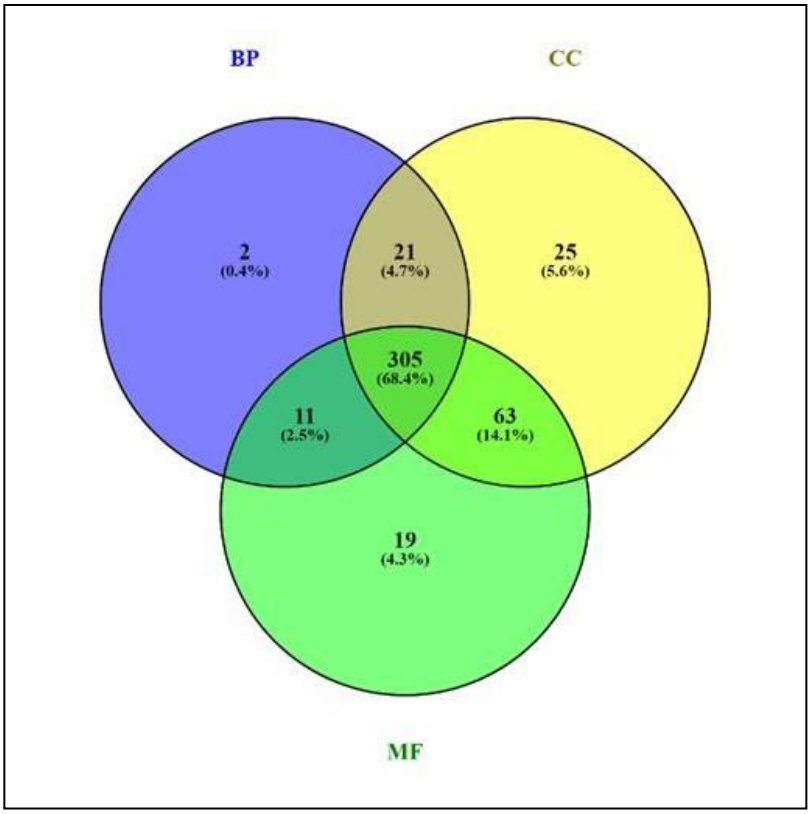



Figure 3.9.2.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 203 different pathways that were regulated by 305 key genes. The pathways regulated by maximum key genes include pathways in cancer, cell cycle, human papillomavirus infection, and PI3K-Akt signaling pathway. The pathways that were regulated by a single gene are ether lipid metabolism, herpes simplex virus 1 infection, and mRNA surveillance pathway (shown in figure 3.9.2.3B).

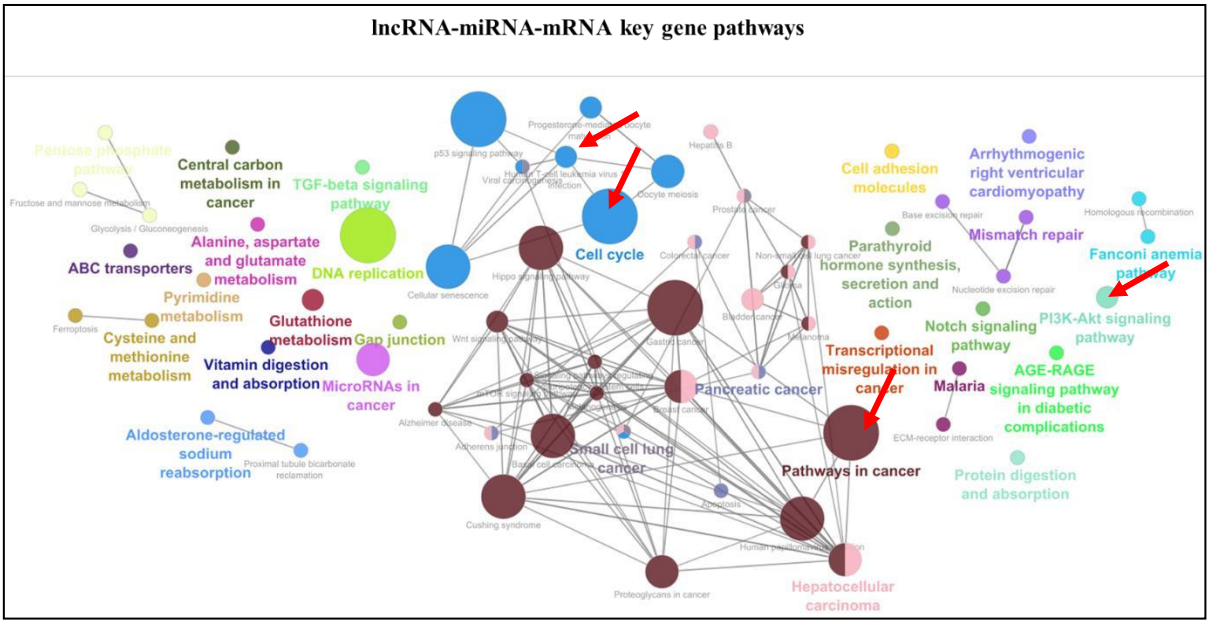



Figure 3.9.2.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in red color.

## 3.10. Pancreatic adenocarcinoma (PRAD)

### 3.10.1. Differential Expression of mRNA

Prostate adenocarcinoma is the most common form of cancer in men. It develops in the gland cells that make prostate fluid. Many prostate cancers grow slowly and are confined to the prostate gland, while some types of prostate cancer grow slowly and may need minimal or even no treatment, Other types are aggressive and can spread quickly. Total transcriptomic data for PRAD consisted of 497 tumor samples and 52 control samples. A total of 831 dysregulated mRNA were obtained out of which 407 were up-regulated and 427 down-regulated. GO study found 301 key genes as shown in figure 3.10.1A.

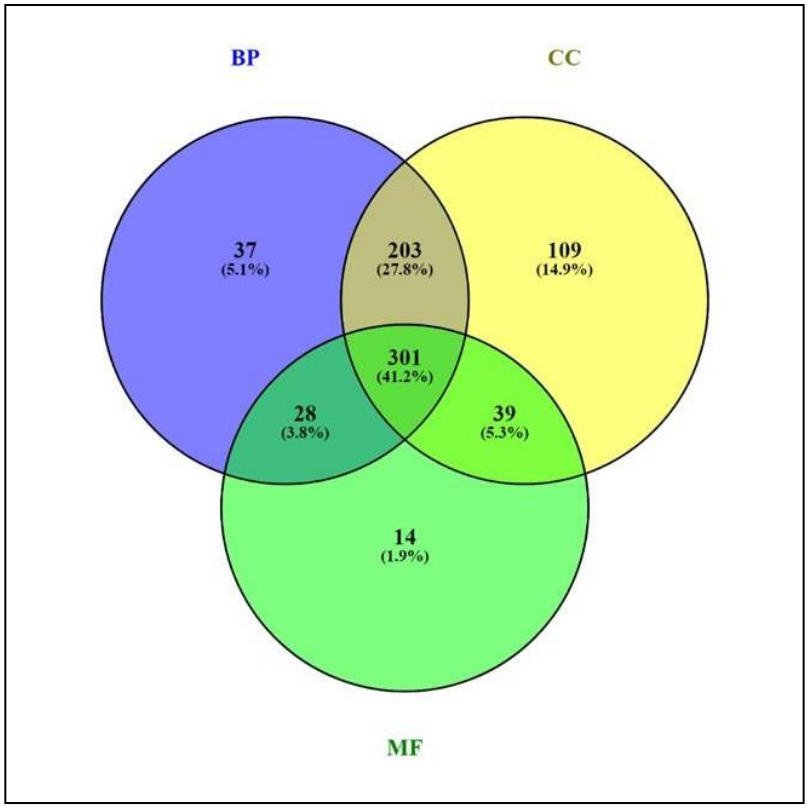



Figure 3.10.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 203 different pathways that were regulated by 305 key genes. The pathways regulated by the maximum number of key genes included pathways in Cancer, protein digestion and absorption, PI3K-Akt signaling pathway, pathways of neurodegeneration, and others. The pathways that were regulated by a single gene are the renin-angiotensin system, maturity-onset diabetes of the young, and the p53 signaling pathway (shown in figure 3.10.1B).

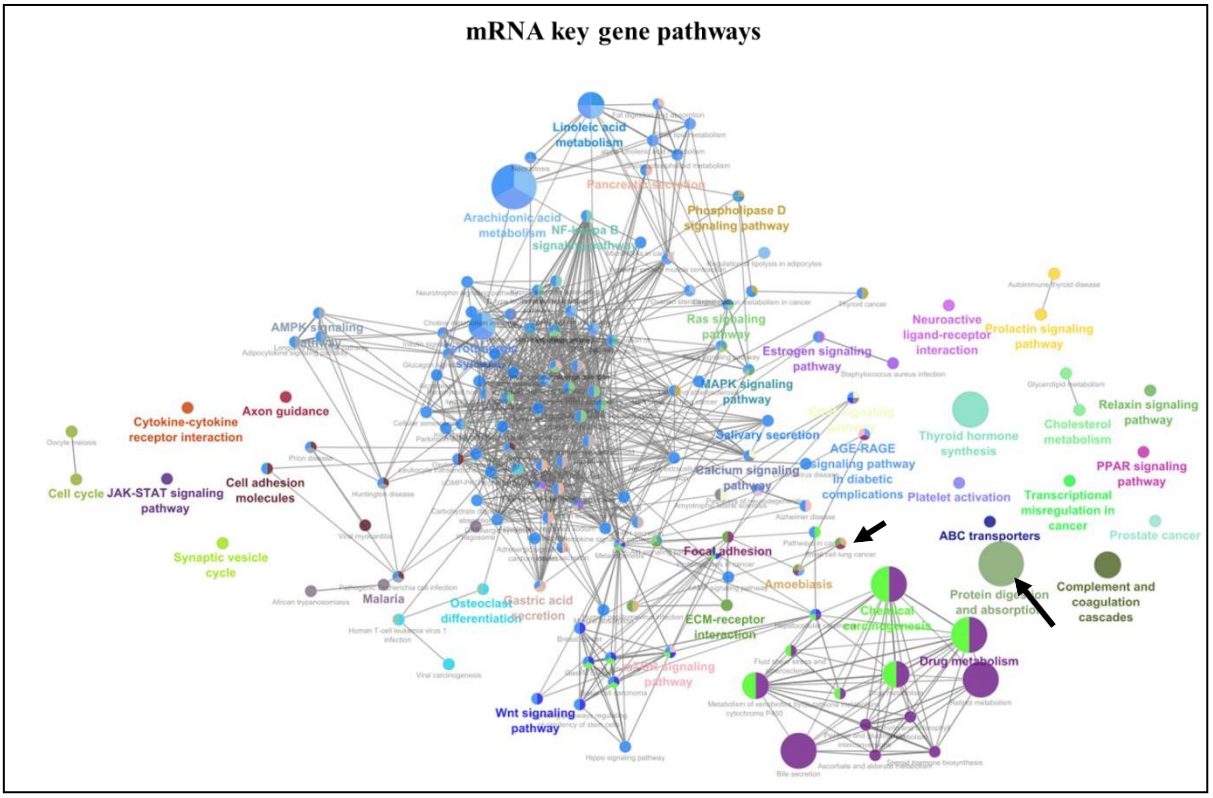



Figure 3.10.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.10.2. Analysis and construction of miRNA-mRNA network

In the case of PRAD, we observed a somewhat different pattern of miRNA-mRNA network from the above-mentioned carcinomas. Here, we observed 23 differentially regulated miRNAs, of which 14 were upregulated and 9 were downregulated. Among these 18 miRNAs (7 down-regulated and 10 up-regulated) participated in correlated interactions with 64 mRNAs (43 down-regulated and 21 up-regulated). Using the Cytoscape-clueGo module we obtained 113 pathways which were regulated by all 64 genes. Vascular smooth muscle contraction, focal adhesion, proteoglycans in cancer, and cGMP-PKG signaling pathway are the major pathways that could be regulated by miRNAs (shown in figure 3.10.2A).

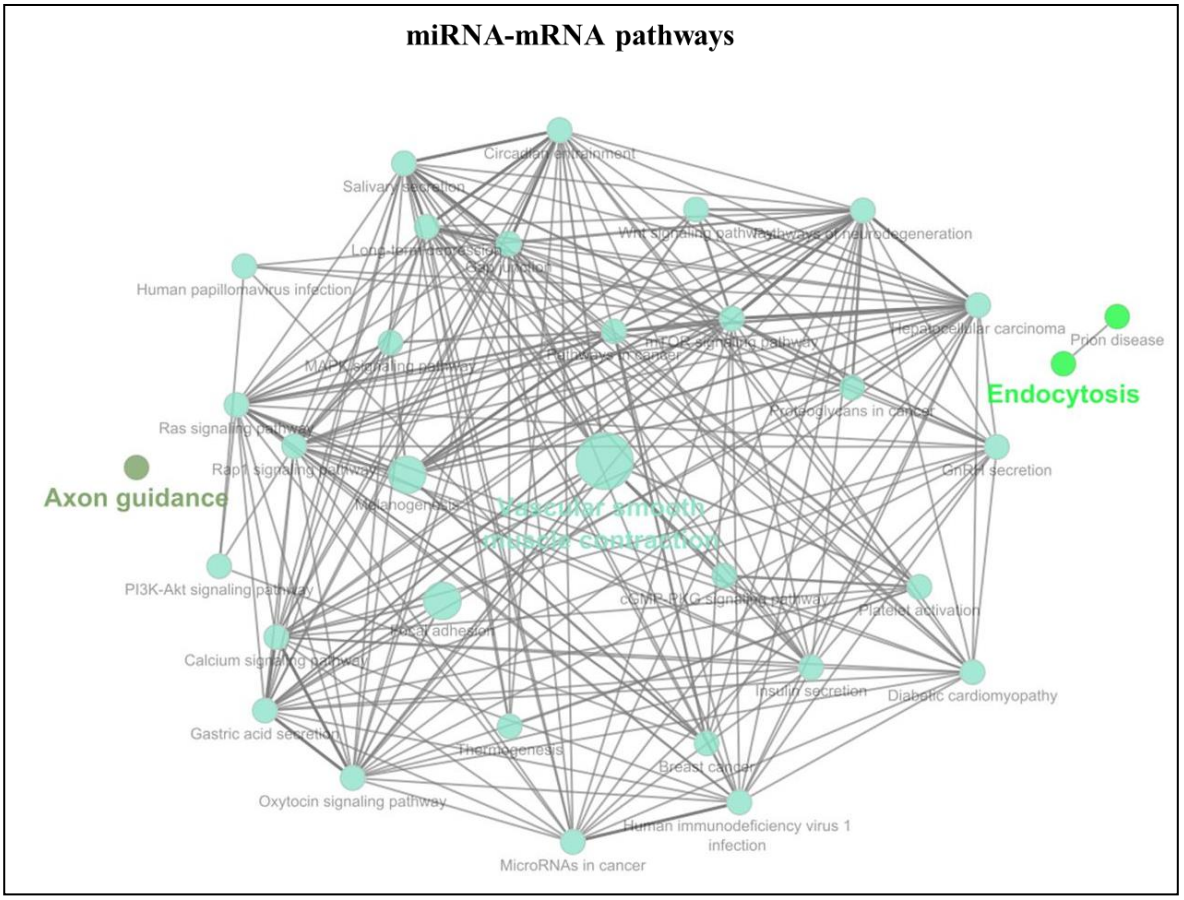



Figure 3.10.2A Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes.

### 3.10.3. Analysis and Construction of lncRNA-miRNA-mRNA network

The transcriptomic data analysis identified a total of 6 differently regulated lncRNAs present out of which 3 had experimentally validated miRNA (2 up-regulated and 1 down-regulated) targets. Our analysis revealed 22 axis of correlated lncRNA-miRNA-mRNA. It consisted of 2 lncRNAs (1 up-regulated and 1 down-regulated), 2 miRNAs (1 up-regulated and 1 down-regulated), and 22 mRNAs (12 down-regulated and 10 up-regulated). We observed that EMX2OS (down-regulated) and SNHG3(up-regulated) are the two lncRNAs that possibly participate in miRNA-mediated gene regulation. Interestingly, EMX2OS downregulation is associated with the regulation of cancer progression, migration, and invasion (Z. Wang et al. 2020). It is known that SNHG3 promotes prostate cancer progression by sponging microRNA-1827 via the Wnt/AKT/mTOR pathway (M. Hu et al. 2022). Through Cytoscape-ClueGO analysis we have found that 76 pathways were being regulated by these 22 dysregulated genes.

The pathways included the MAPK signaling pathway, focal adhesion, human immunodeficiency virus 1 infection, and proteoglycans in cancer (shown in figure 3.10.3A).

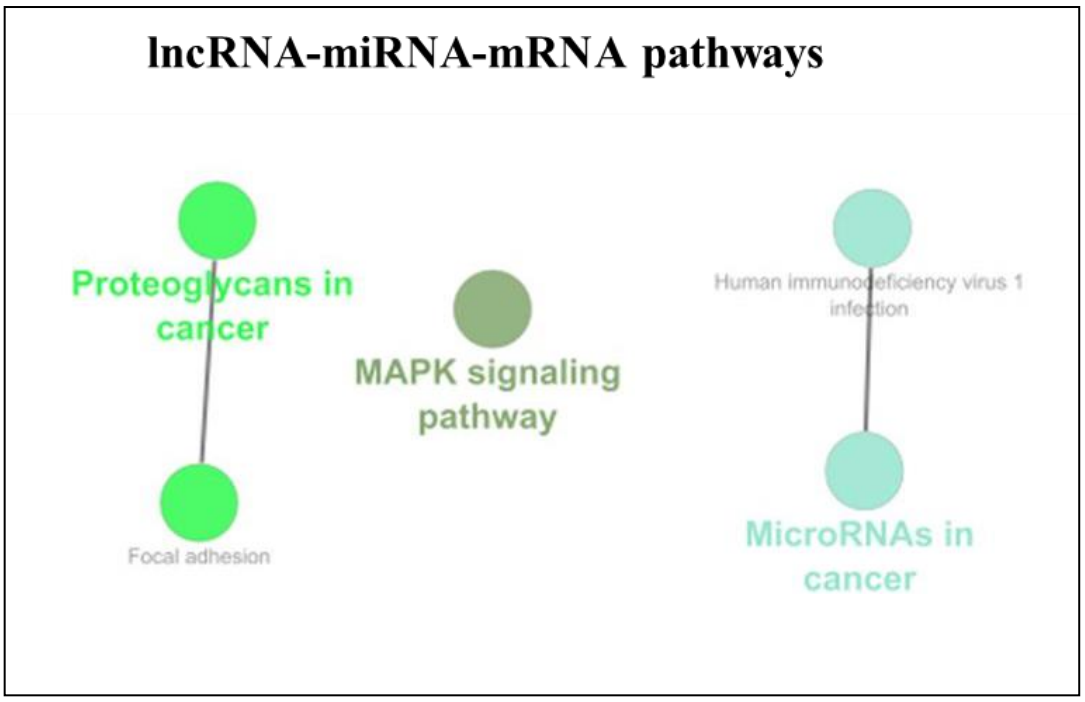



Figure 3.10.3A Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes.

## 3.11. Rectal adenocarcinoma (READ)

### 3.11.1. Differential Expression of mRNA

Rectal adenocarcinoma is a cancer that begins in the rectum. The rectum is the last several inches of the large intestine. It starts at the end of the final segment of your colon and ends when it reaches the short, narrow passage leading to the anus. It usually develops when cancer cells form in the rectum, which is part of the large intestine. Total transcriptomic data for READ consisted of 94 tumor samples and 10 control samples. It had a total of 2057 dysregulated mRNA with 1122 up-regulated and 935 down-regulated mRNAs. GO analysis has identified 1181 key genes (figure 3.11.1A).

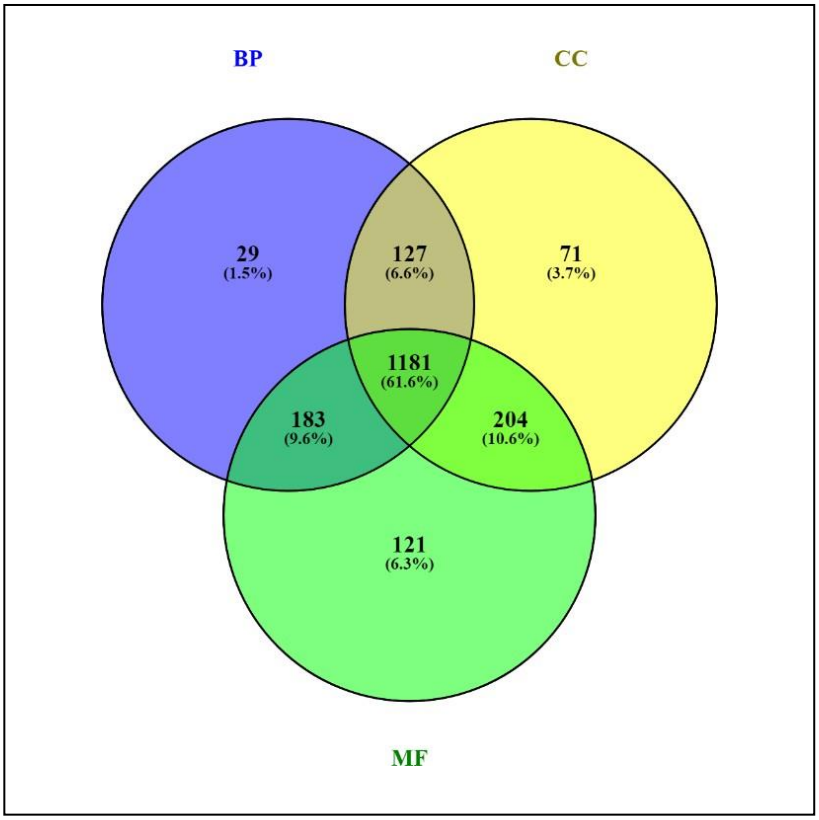



Figure 3.11.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 284 different pathways that were regulated by 1181 key genes. Cytokine-cytokine receptor interaction, pathways in Cancer, PI3K-Akt signaling pathway, and neuroactive ligand-receptor interaction were found to be regulated by a large number of genes whereas pathways like ubiquitin-mediated proteolysis, primary bile acid biosynthesis and SNARE interactions in vesicular transport were regulated by a single gene (shown in figure 3.11.1B).

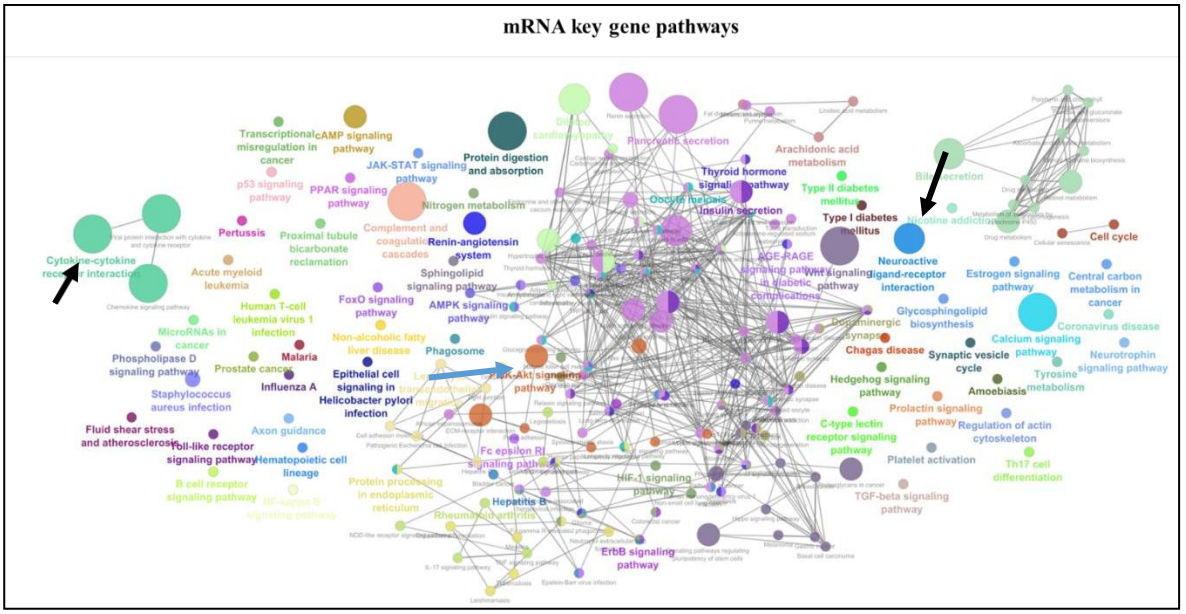



Figure 3.11.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.11.2. Analysis and construction of miRNA-mRNA network

READ consisted of a total of 309 differentially expressed miRNA transcripts. The number of up-regulated miRNAs (188) was higher than the down-regulated miRNAs (121). Out of these miRNAs, 293 (111 down-regulated and 182 up-regulated) miRNAs possibly participated in correlated type miRNA-mRNA interactions with 1044 mRNAs (565 down-regulated and 479 up-regulated) targets. Out of which 703 are key genes as shown in figure 3.11.2A.

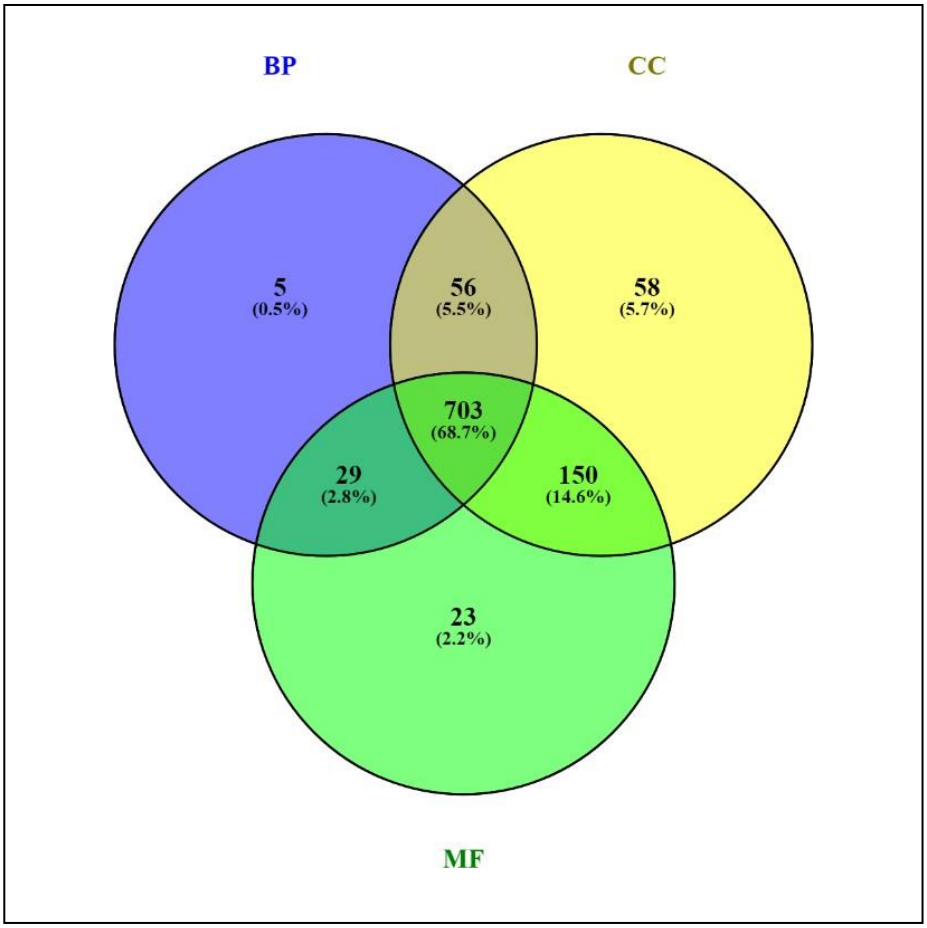



Figure 3.11.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 284 different pathways that were regulated by 703 key genes. Pathways in cancer, PI3K-Akt signaling pathway, and MAPK signaling pathway are the major pathways that are regulated by the larger number of key genes whereas glycosaminoglycan biosynthesis, biosynthesis of unsaturated fatty acids, and toll-like receptor signaling pathway are regulated by single key gene (shown in figure 3.11.2B).

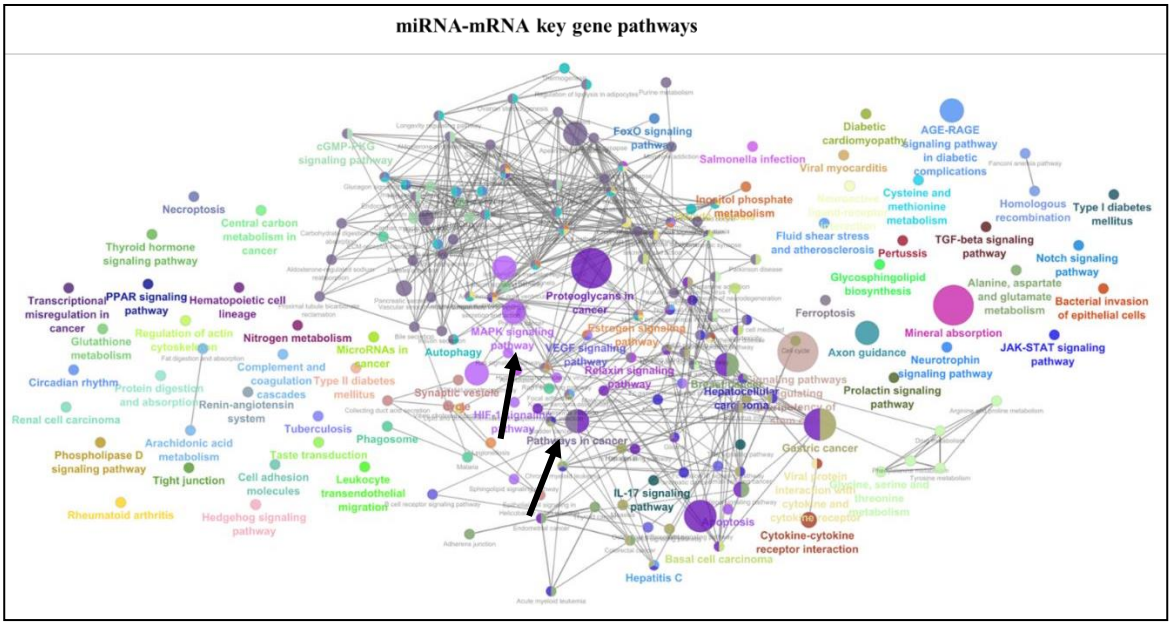



Figure 3.11.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.11.3. Analysis and construction of lncRNA-miRNA-mRNA network

A total of 19 differentially regulated lncRNAs are present out of which only 14 had experimentally validated miRNA (all up-regulated) targets. Our analysis revealed 3431 axis of correlated lncRNA-miRNA-mRNA. It consisted of 14 lncRNAs (upregulated), 63 miRNAs (downregulated), and 399 mRNAs (upregulated) with 244 key genes (shown in figure 3.11.3A). MIR17HG has already been reported to be involved in promoting colorectal cancer progression via miR-17-5p (Jie Xu et al. 2019). lncRNAs H19(Y. Zhang et al. 2020), SNHG3 (Weizhen et al. 2017), SNHG12(J. Z. Wang et al. 2017), KCNQ1OT1(C. Chen et al. 2020), SNHG1(Avazpour et al. 2020), C2orf27A (Gao et al. 2020), RMRP(Y. Chen et al. 2021), UCA1 (Luan et al. 2020), PVT1 (Wu et al. 2020), SNHG11(W. Xu et al. 2020) have potential role in renal cancer progression. C9orf163 (chromosome 9 putative open reading frame 163) is a putative uncharacterized protein. The function of transmembrane protein 105 (TMEM105) is not much explored.

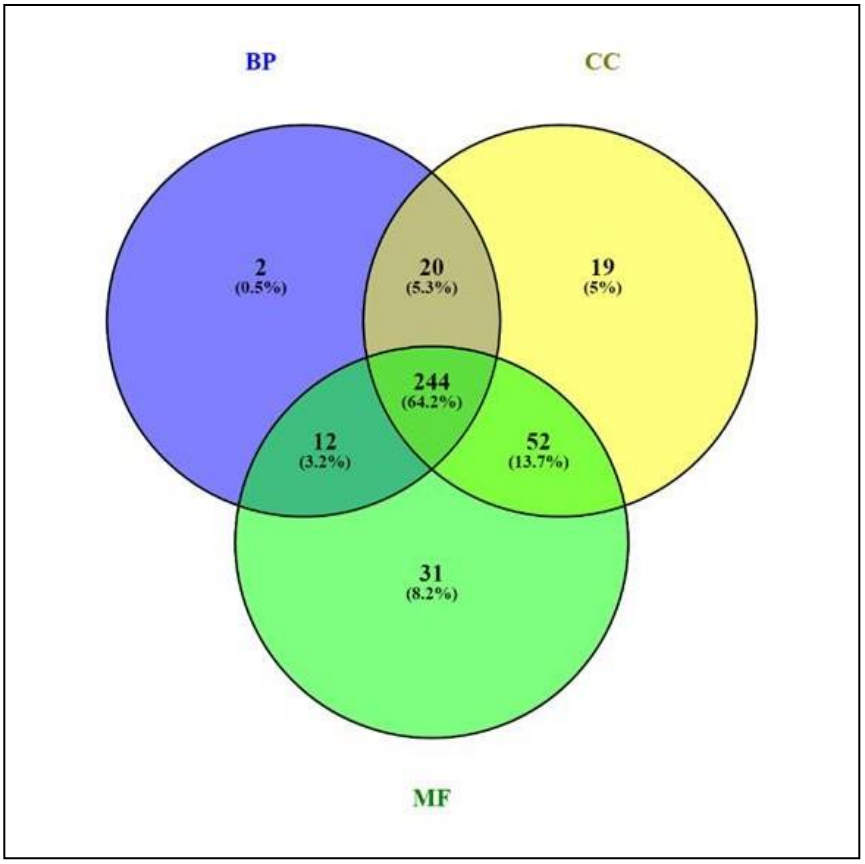



Figure 3.11.3A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 179 different pathways that were regulated by 244 key genes. Pathways in cancer, microRNAs in cancer, proteoglycans in cancer, and the Wnt signaling pathway are the key pathways that were regulated by a large number of genes. On the other hand fat digestion and absorption, NF-kappa B signaling pathway, and neuroactive ligand-receptor interaction pathway were regulated by a single gene (in figure 3.11.3B).

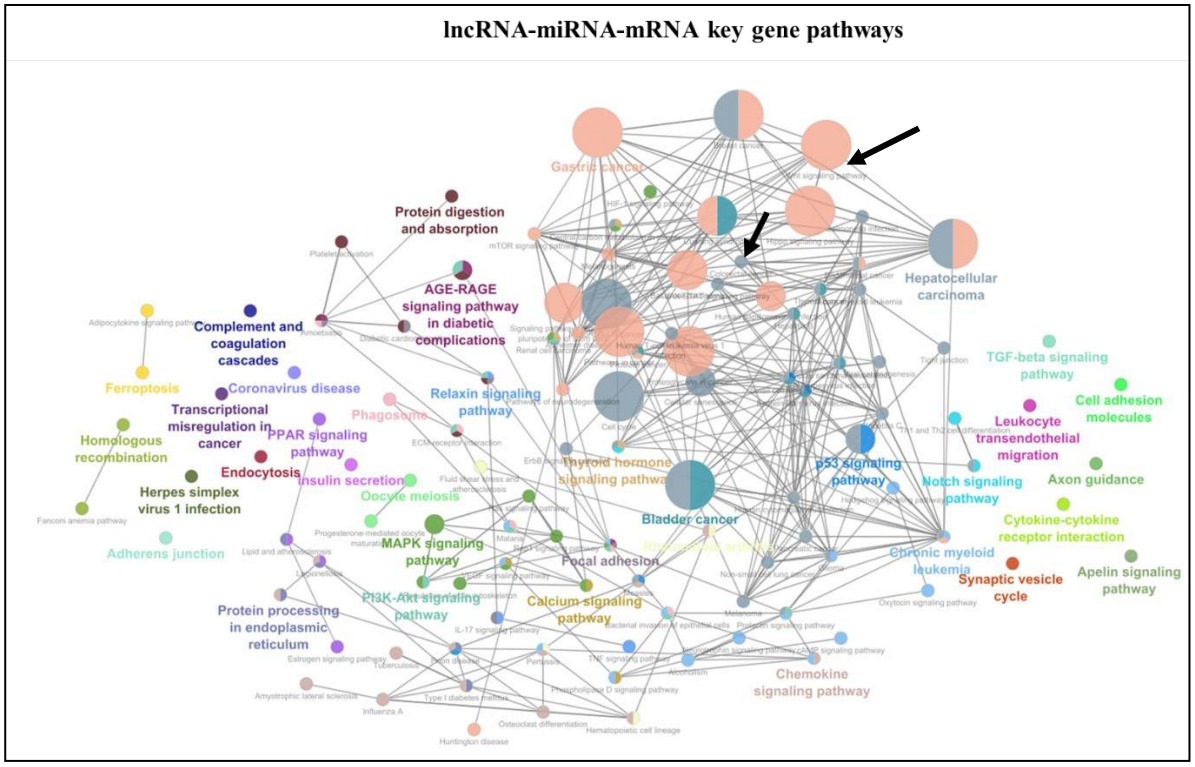



Figure 3.11.3B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

## 3.12. Stomach adenocarcinoma (STAD)

### 3.12.1. Differential Expression of mRNA

Stomach adenocarcinoma or gastric adenocarcinoma is a cancer of the stomach and its related parts. It begins when cells in the stomach start to grow out of control. Cancers starting in different sections of the stomach can cause different symptoms and tend to have different outcomes. Stomach cancer is different from other cancers that can occur in the abdomen, like cancer of the colon or rectum (large intestine), liver, pancreas, or small intestine. These cancers can have different symptoms, different outlooks, and different treatments. Stomach cancers tend to develop slowly over many years. Before true cancer develops, pre-cancerous changes often occur in the inner lining (mucosa) of the stomach. These early changes rarely result in symptoms, and hence remain undetected in the majority of cases. Total transcriptomic data for STAD are extracted from 415 tumor samples and 35 control samples. A total of 1517

dysregulated genes were present, of which 792 were down-regulated and 725 were up-regulated with 875 key genes (figure 3.12.1A).

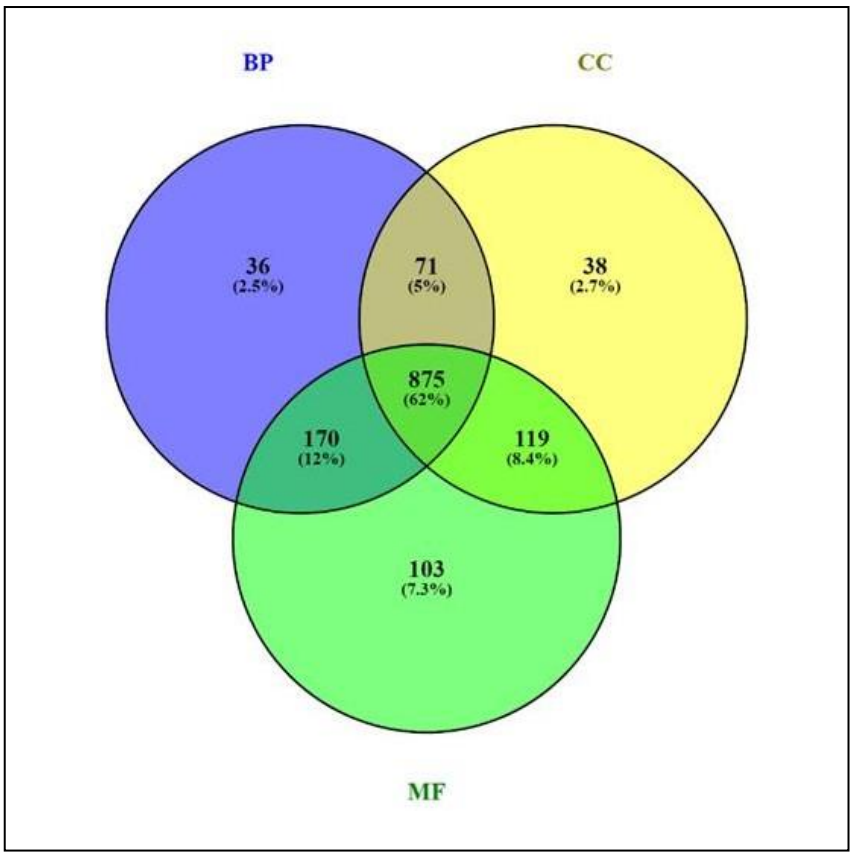



Figure 3.12.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 22 different pathways that were regulated by 875 key genes. Cytokine-cytokine receptor interaction, neuroactive ligand-receptor interaction, pathways in cancer, and PI3K-Akt signaling pathway are key pathways regulated by a larger number of key genes whereas hippo signaling pathway, Notch signaling pathway, and B cell receptor signaling pathway was regulated by a single key gene (figure 3.12.1B).

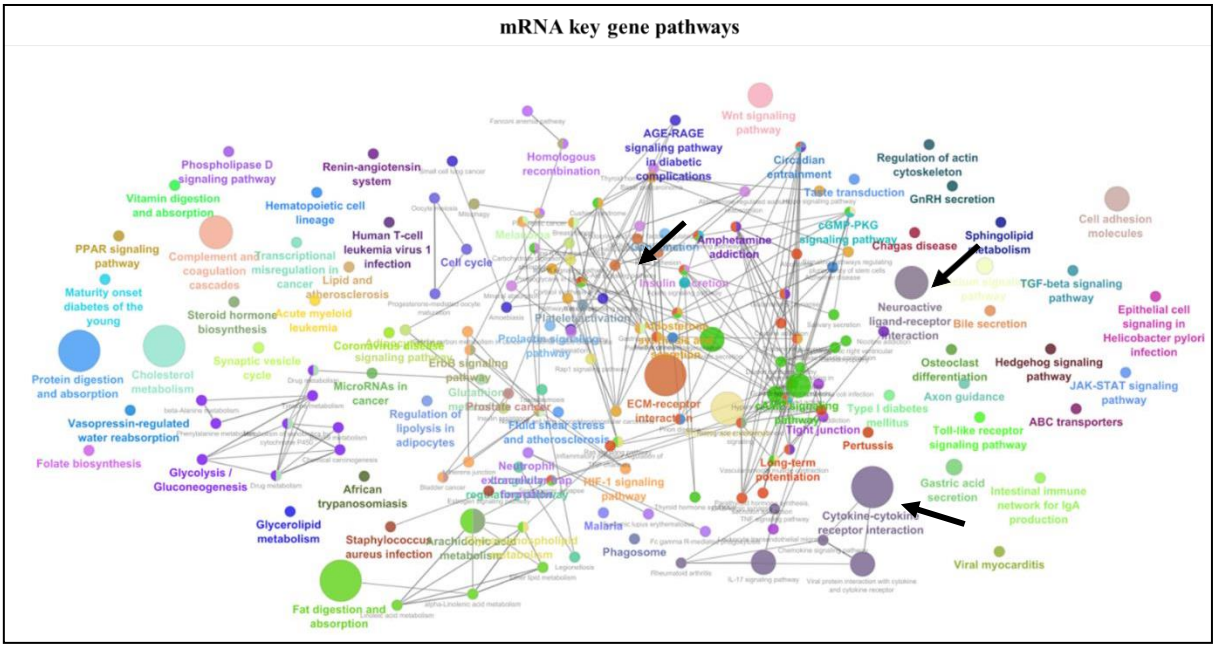



Figure 3.12.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.12.2. Analysis and construction of miRNA-mRNA network

STAD consisted of a total of 62 differentially expressed miRNA transcripts. A total of 490 correlated type miRNA-mRNA interactions was identified which were associated with 61 miRNAs (32 downregulated and 29 upregulated) and 313 mRNA (130 downregulated and 183 upregulated) targets with 224 key genes (figure 3.12.2A).

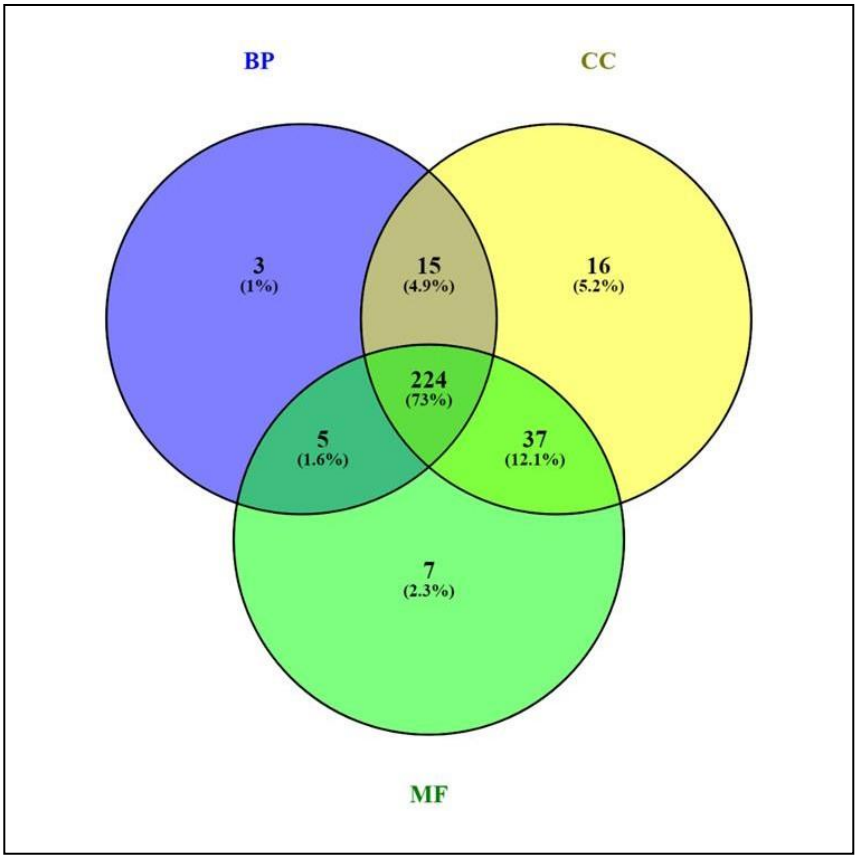



Figure 3.12.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 185 different pathways that were regulated by 224 key genes. PI3K-Akt signaling pathway, pathways in cancer, microRNAs in cancer, and MAPK signaling pathway were regulated by a large number of genes whereas pathways like vitamin digestion and absorption, vasopressin-regulated water reabsorption, ubiquitin-mediated proteolysis were regulated by few genes (figure 3.12.2B).

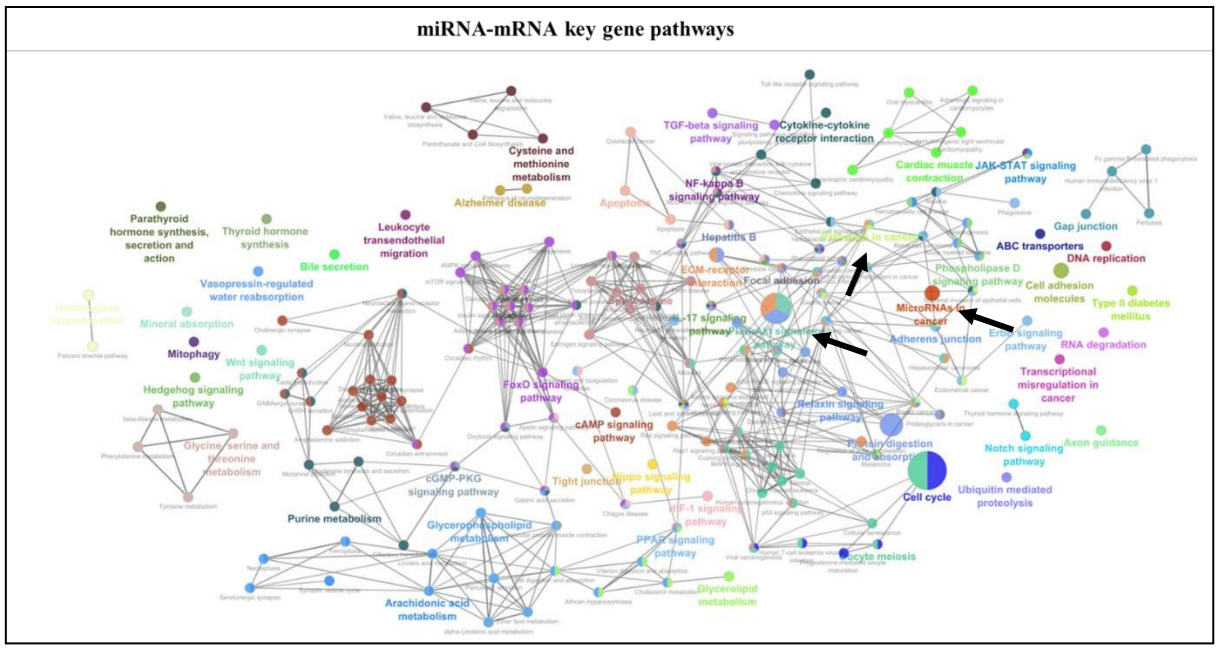



Figure 3.12.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.12.3. Analysis and construction of lncRNA-miRNA-mRNA network

To understand the role of lncRNA-regulated mRNAs in stomach cancer, we did the transcriptomic analysis and identified 8 differentially expressed lncRNAs. Out of these lncRNAs, 6 have experimentally validated miRNA (2 downregulated and 4 upregulated) targets. Our analysis revealed 375 axis of correlated lncRNA-miRNA-mRNA. It consisted of 5 lncRNAs (1 downregulated and 4 upregulated), 20 miRNAs (16 down-regulated and 4 upregulated), and 177 mRNAs (28 downregulated and 149 up-regulated) and Among these 177 dysregulated genes 126 are key genes (shown in figure 3.12.3A). It has been reported that PVT1 (Martínez-Barriocanal, Arango, and Dopeso 2020), H19 (F. Yang et al. 2012), UCA1 (A. Yang et al. 2021), and HOTAIR (Jie Zhang et al. 2020) are upregulated in various cancer while RMRP is downregulated (Y. Shao et al. 2016). All these lncRNAs are known as biomarkers and have been studied for their role in cancer progression.

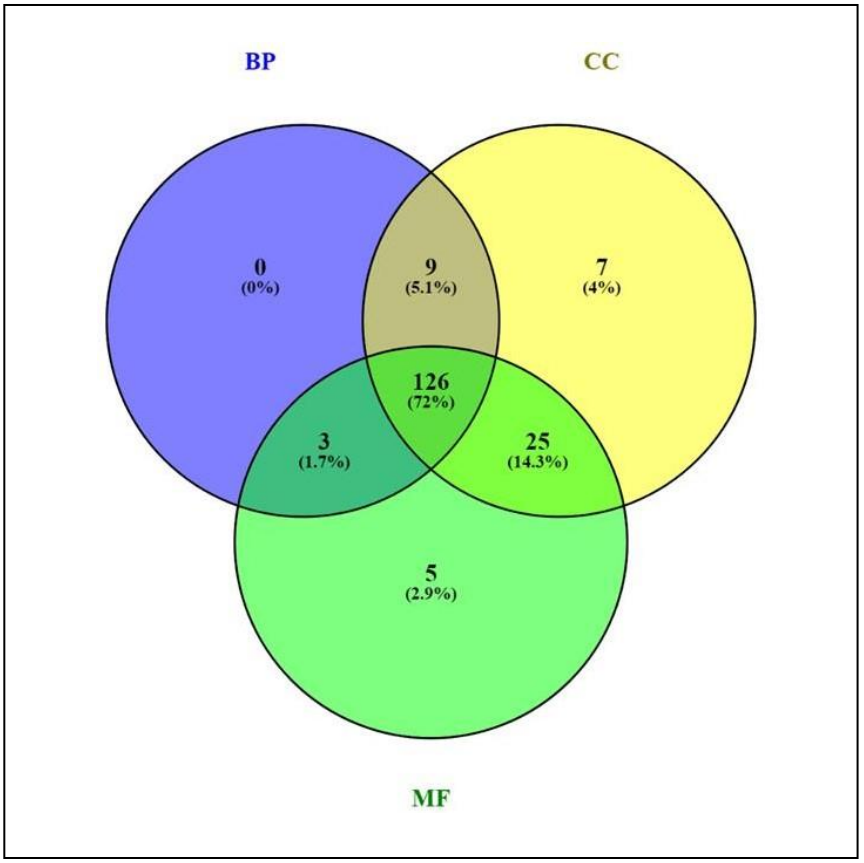



Figure 3.12.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 117 different pathways that were regulated by 126 key genes. PI3K-Akt signaling pathway, pathways in cancer, microRNAs in cancer, and cell cycle are the prominent dysregulated pathways that are regulated by a larger number of key genes. In contrary cysteine and methionine metabolism, thyroid hormone synthesis, and FoxO signaling pathways were regulated by a single gene (figure 3.12.3B).

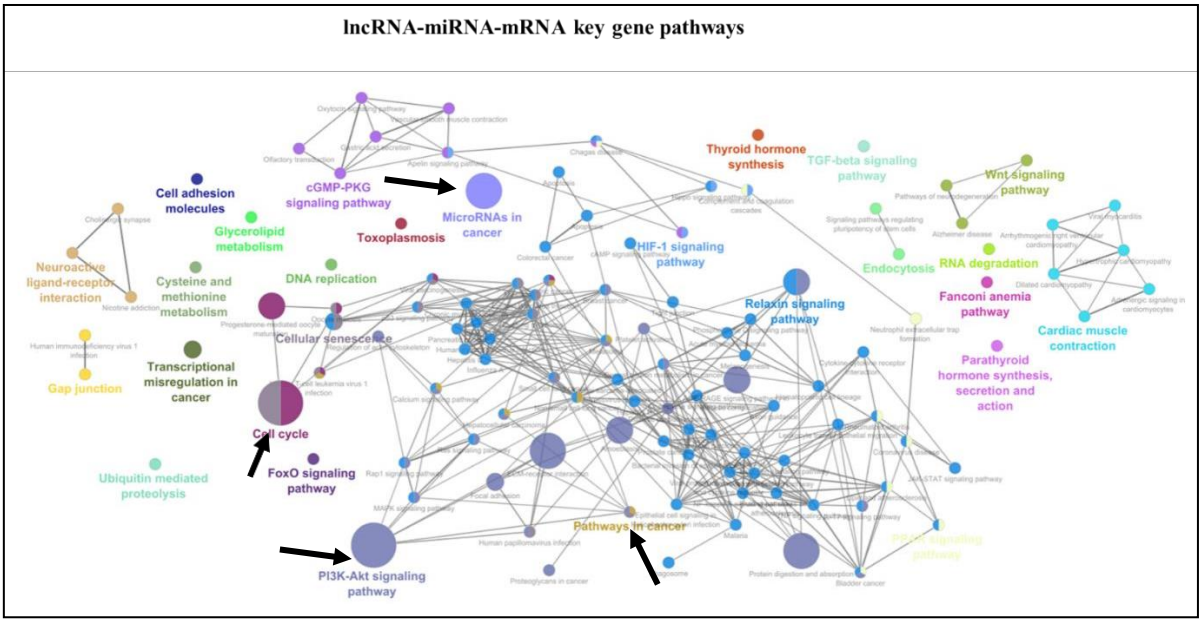



Figure 3.12.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

## 3.13. Thyroid carcinoma (THCA)

### 3.13.1. Differential Expression of mRNA

Thyroid cancer develops in the follicular cells of the thyroid. Thyroid cancer begins in the thyroid gland. This gland is in the front of the neck just below the larynx, which is called the voice box. The thyroid gland is part of the endocrine system, which regulates hormones in the body. The thyroid gland absorbs iodine from the bloodstream to produce thyroid hormones, which regulate a person's metabolism. Most thyroid cancers are highly curable. Total transcriptomic data of THCA were obtained from 505 tumors and 59 control samples. A total of 1122 dysregulated mRNA were present out of which 826 were up-regulated and 296 were down-regulated. Among the dysregulated genes 548 are key genes shown in figure 3.13.1A.

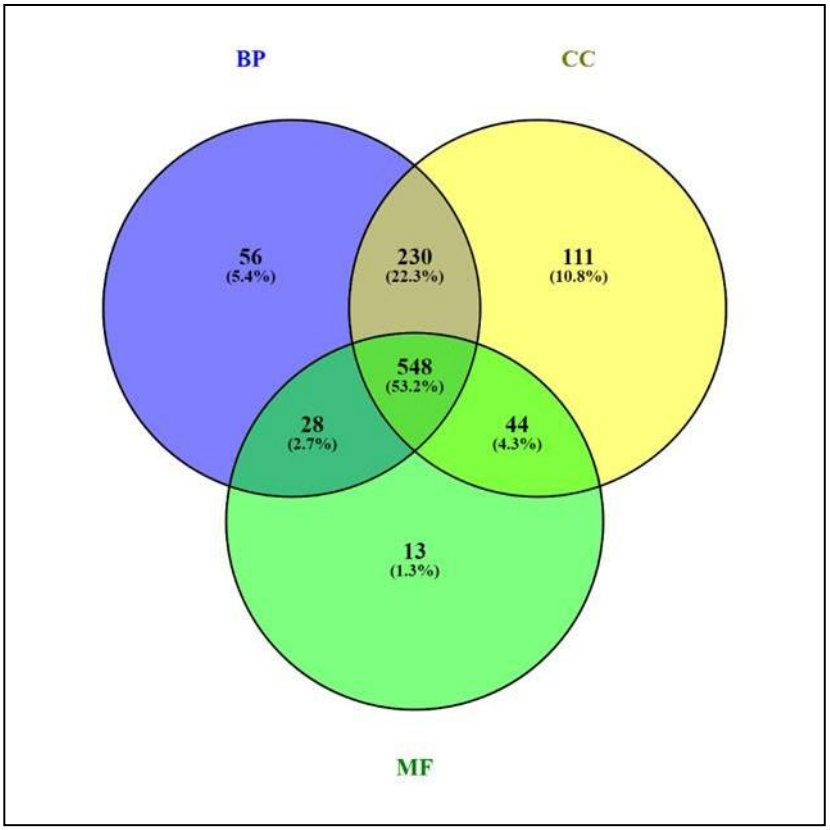



Figure 3.13.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 174 different pathways that were regulated by 548 key genes. pathways in Cancer, cytokine-cytokine receptor interaction, PI3K-Akt signaling pathway, and others were regulated by more genes. Some of the pathways that were found to be regulated by single genes were N-Glycan biosynthesis, glycosaminoglycan biosynthesis, sphingolipid metabolism, and others. As shown in figure 3.13.1B.

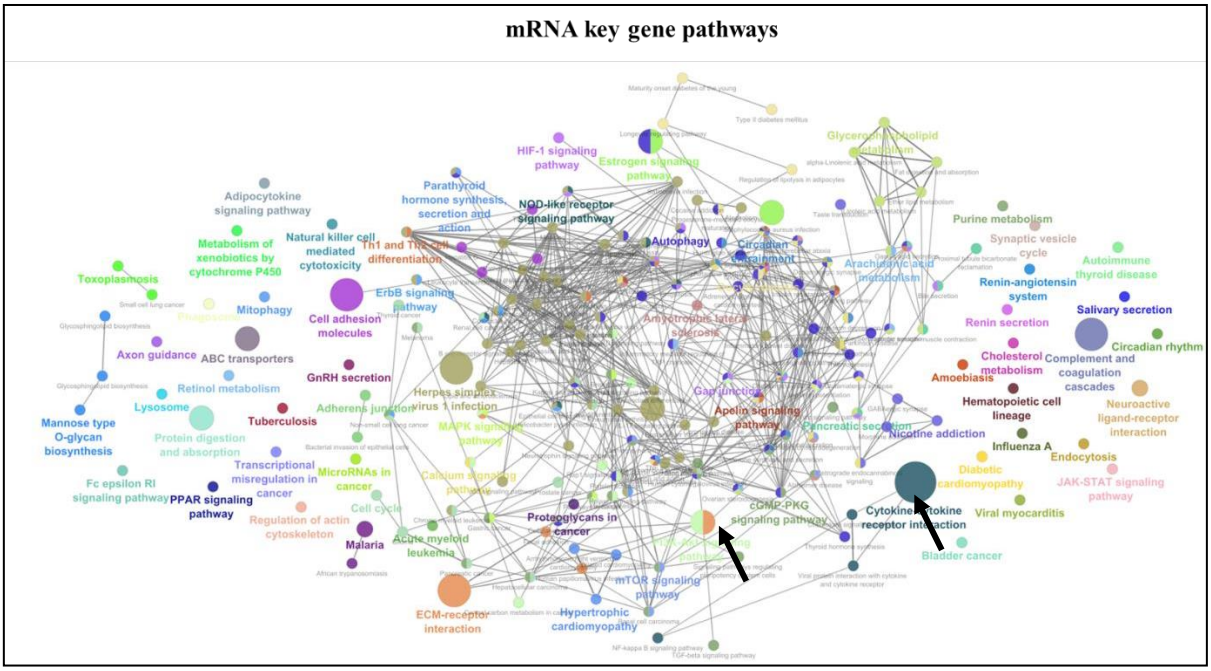



Figure 3.13.1B Cytoscape network showing the different pathways regulated by the mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.13.2. Analysis and construction of miRNA-mRNA network

THCA consisted of a total of 32 differentially expressed miRNA transcripts. The number of upregulated miRNAs (26) was significantly higher than the downregulated miRNAs (6), indicating that the up-regulation of genes could be associated with cancer progression. We have identified 69 correlated miRNA-mRNA interactions with 21 miRNAs (5 down-regulated and 16 up-regulated) and 50 mRNAs (38 down-regulated and 12 up-regulated). Since the number of dysregulated mRNAs war less, we consider all genes for pathway analysis. Here, we obtained a total of 156 possible dysregulated pathways which were regulated by these 50 genes. Pathways in Cancer, PI3K-Akt signaling pathway, cAMP signaling pathway, and estrogen signaling pathway were the key pathways that were regulated by a large set of genes. Single gene-regulated pathways were the TGF-beta signaling pathway, Wnt signaling pathway, and hippo signaling pathway (figure 3.13.2A). The transcriptomic data of THCA consisted of

2 differentially expressed lncRNAs (both up-regulated) but none of the two had any experimentally validated miRNA targets.

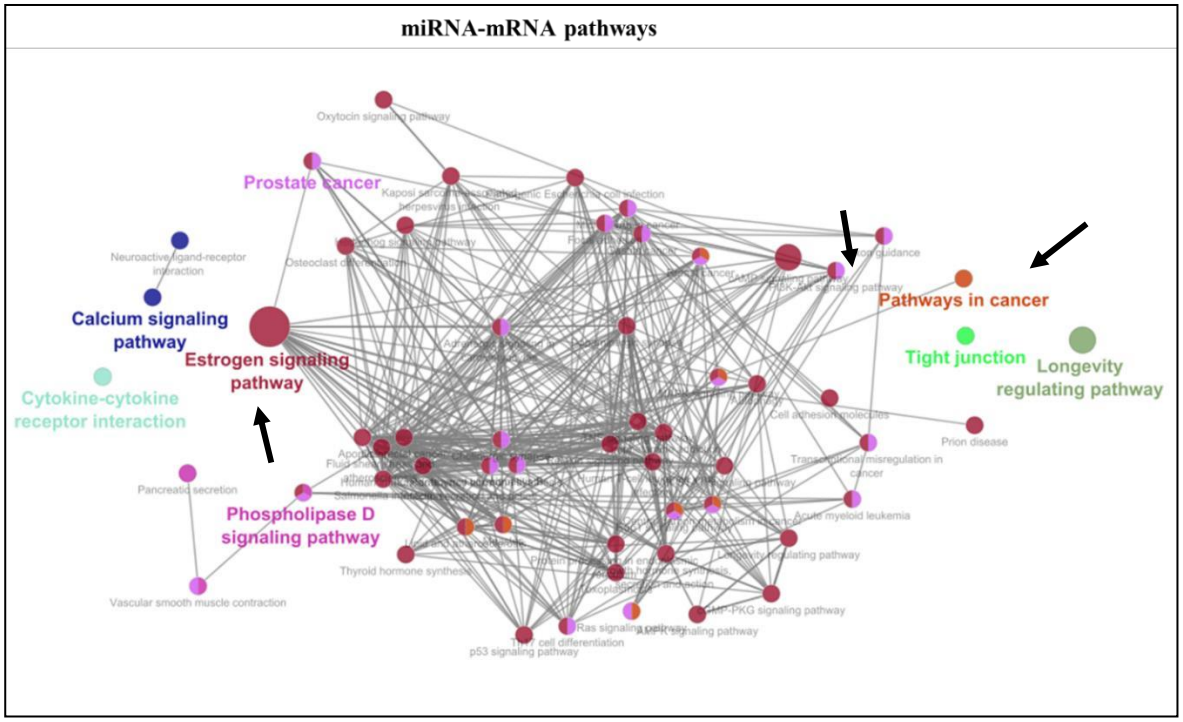



Figure 3.13.2A Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

## 3.14. Uterine corpus endometrial carcinoma (UCEC)

### 3.14.1. Differential Expression of mRNA

Uterine cancer develops in the cells that form the inner lining of the uterus, or the endometrium, and is one of the most common cancers of the female reproductive system. Total UCEC transcriptomic data were taken from 176 tumors and 24 control samples. The transcriptomic data consisted of a total of 2612 dysregulated mRNAs. The number of upregulated genes (1560) is significantly larger than the downregulated genes (1052). We have identified 1775 key genes (figure 3.14.1A).

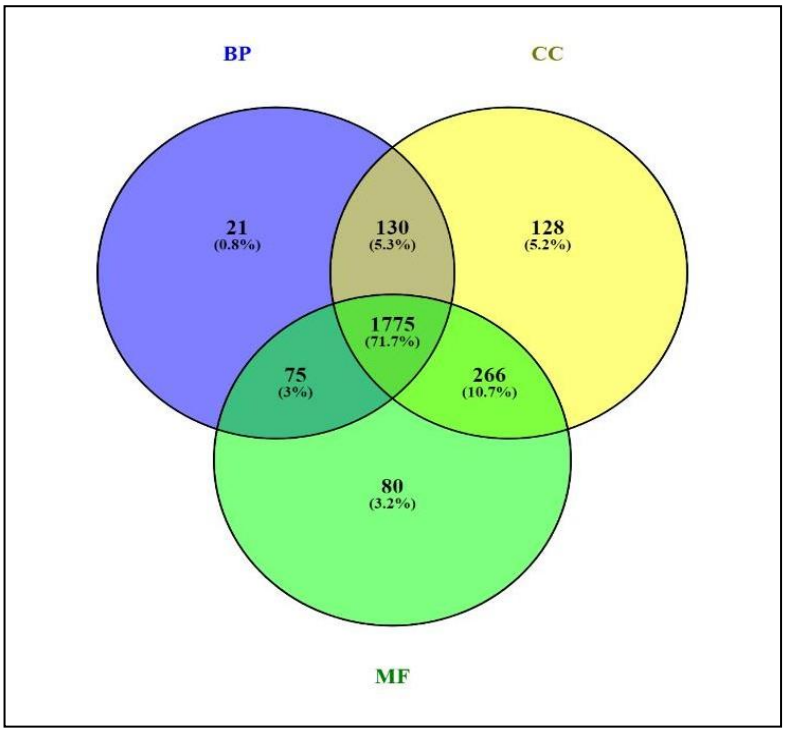



Figure 3.14.1A Venn diagram showing key genes obtained from gene ontological studies of mRNA.

The pathway analysis revealed a total of 301 different pathways that were regulated by 1775 key genes. MAPK signaling pathway, pathways in cancer, and PI3K-Akt signaling pathway were key pathways that were regulated by many key genes. Seleno compound metabolism, inositol phosphate metabolism, and thiamine metabolism pathways were regulated by a single gene (in figure 3.14.1B).

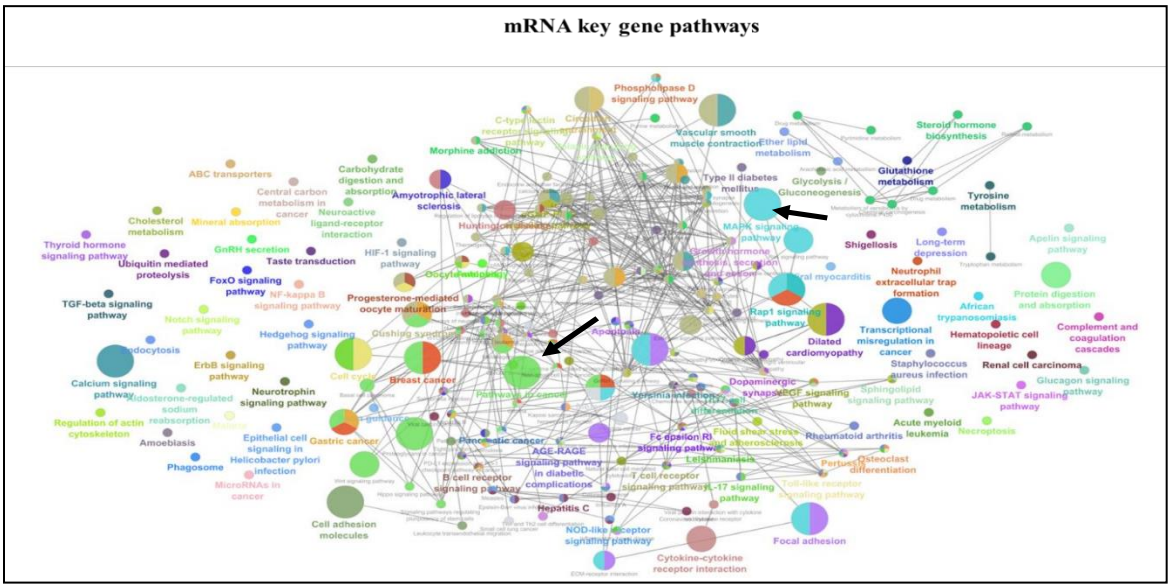



Figure 3.14.1B Cytoscape network showing the different pathways regulated by the mRNAkey genes. Some of the important pathways are indicated by the arrow in black color.

### 3.14.2. Analysis and construction of miRNA-mRNA network

UCEC consisted of a total of 161 differentially expressed miRNA transcripts. A total of 2041 correlated miRNA-mRNA interactions identified 155 miRNAs (56 downregulated and 99 upregulated) and 912 mRNAs (443 downregulated and 469 upregulated). Out of these possible miRNA-mediated 912 dysregulated genes, 642 are key genes (figure 3.14.2A).

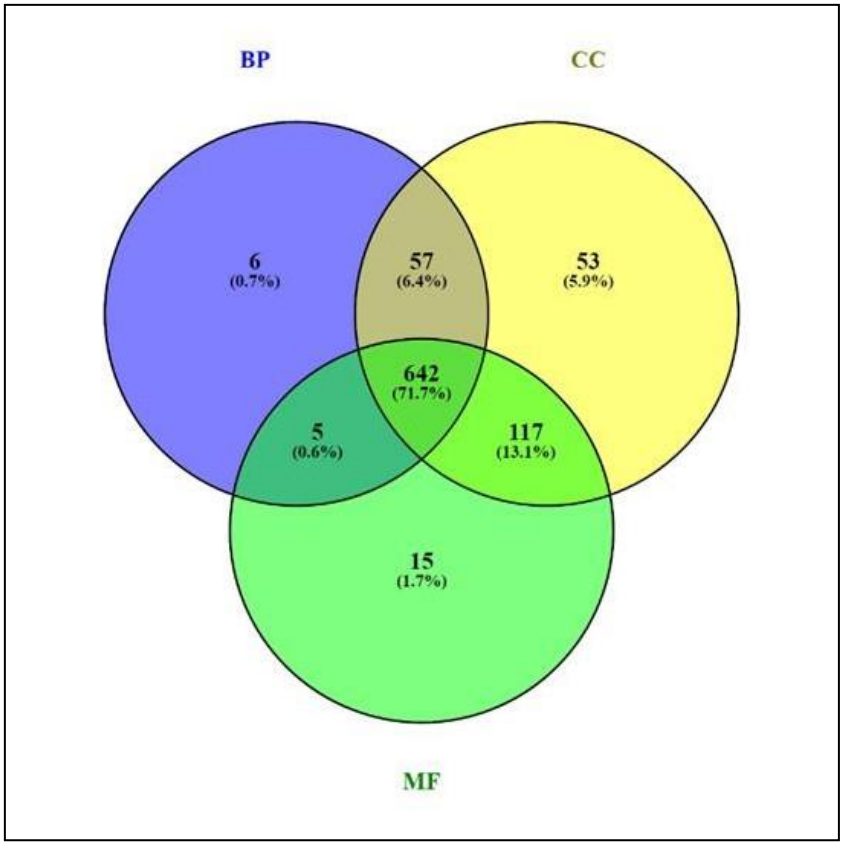



Figure 3.14.2A Venn diagram showing key genes obtained from gene ontological studies of miRNA regulated by mRNA.

The pathway analysis revealed a total of 280 different pathways that were regulated by 642 key genes. Pathways in cancer, microRNAs in cancer, PI3K-Akt signaling pathway, and proteoglycans in cancer are the key pathways that were regulated by a large number of key genes. However, nucleotide excision repair, collecting duct acid secretion, and steroid hormone biosynthesis pathways were regulated by a single gene (figure 3.14.2B).

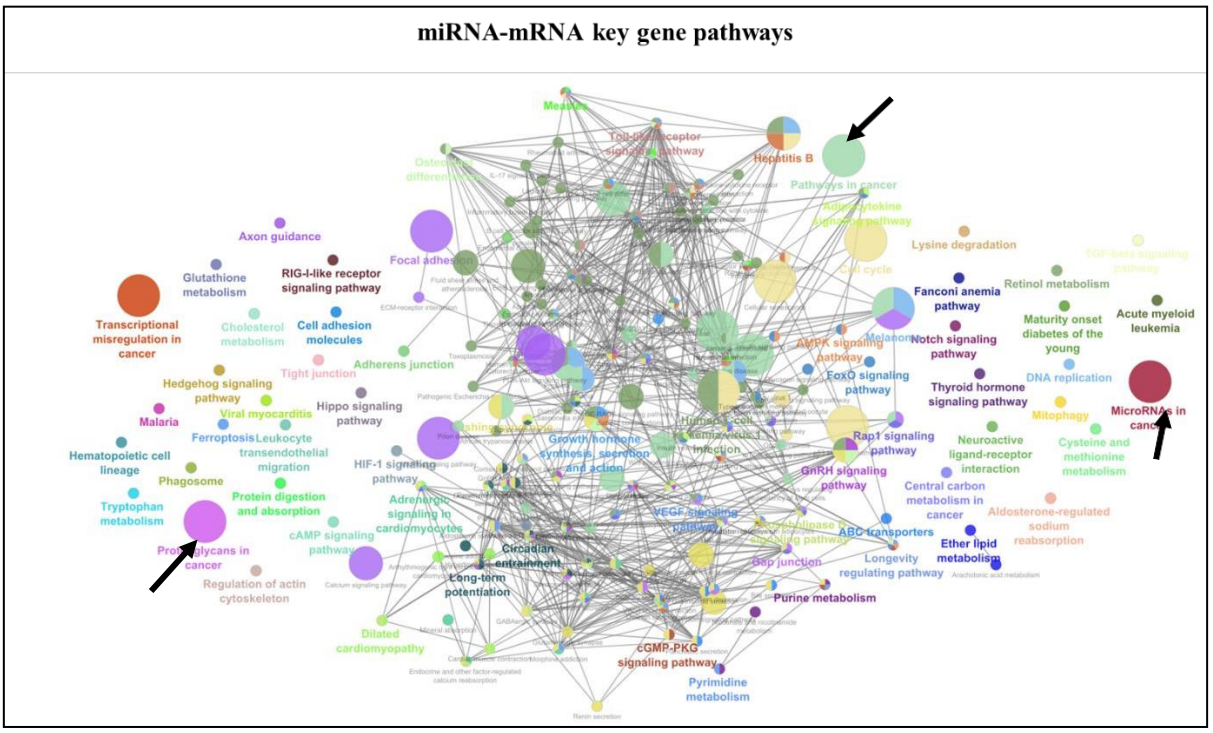



Figure 3.14.2B Cytoscape network showing the different pathways regulated by the miRNA-mediated mRNA key genes. Some of the important pathways are indicated by the arrow in black color.

### 3.14.3. Analysis and construction of lncRNA-miRNA-mRNA network

A total of 7 differentially expressed lncRNAs are present out of which only 3 (MEG3, PVT1, and MIR155HG) had experimentally validated miRNA (1 down-regulated and 2 up-regulated) targets. We could identify 695 correlated lncRNA-miRNA-mRNA axes. It consisted of 3 lncRNAs (1 down-regulated and 2 up-regulated), 34 miRNAs (13 down-regulated and 21 up-regulated), and 431 mRNAs (203 down-regulated and 228 up-regulated). Out of these 431 lncRNA-mediated dysregulated genes, 323 are key genes (shown in figure 3.14.3A). It has been reported that MEG3 is down-regulated and reduced levels of MEG3 inhibited EC proliferation through downregulation of the Notch signaling system (Q. Guo et al. 2016). The upregulation of PVT1 and MIR155HG is associated with cancer progression (F. Kong et al. 2018, Peng et al. 2019).

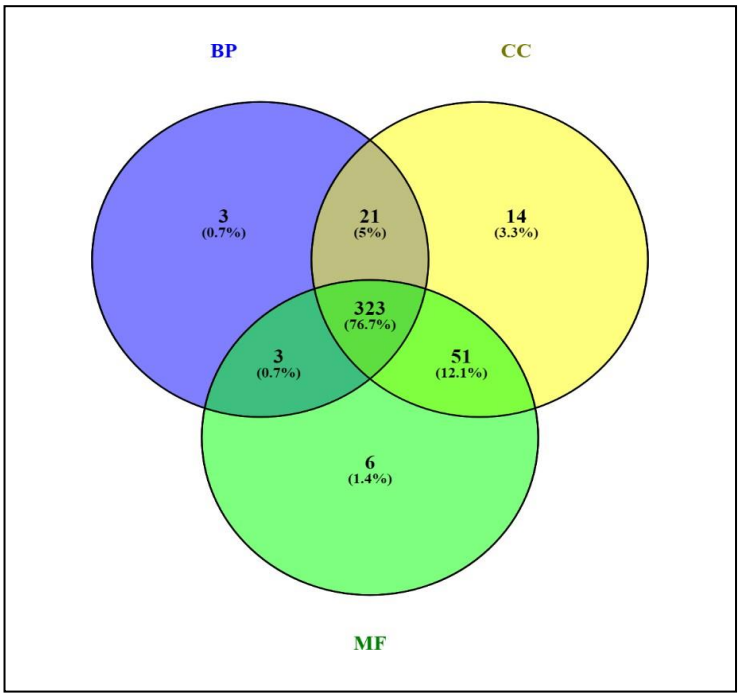



Figure 3.14.3A Venn diagram showing key genes obtained from gene ontological studies of lncRNA regulated by mRNA.

The pathway analysis revealed a total of 245 different pathways that were regulated by 323 key genes. Pathways in cancer, microRNAs in cancer, PI3K-Akt signaling pathway, and human T-cell leukemia virus 1 infection were regulated by a large number of genes whereas pathways like SNARE interactions in vesicular transport, cardiac muscle contraction, notch signaling pathway were regulated by single gene only (figure 3.14.3B).

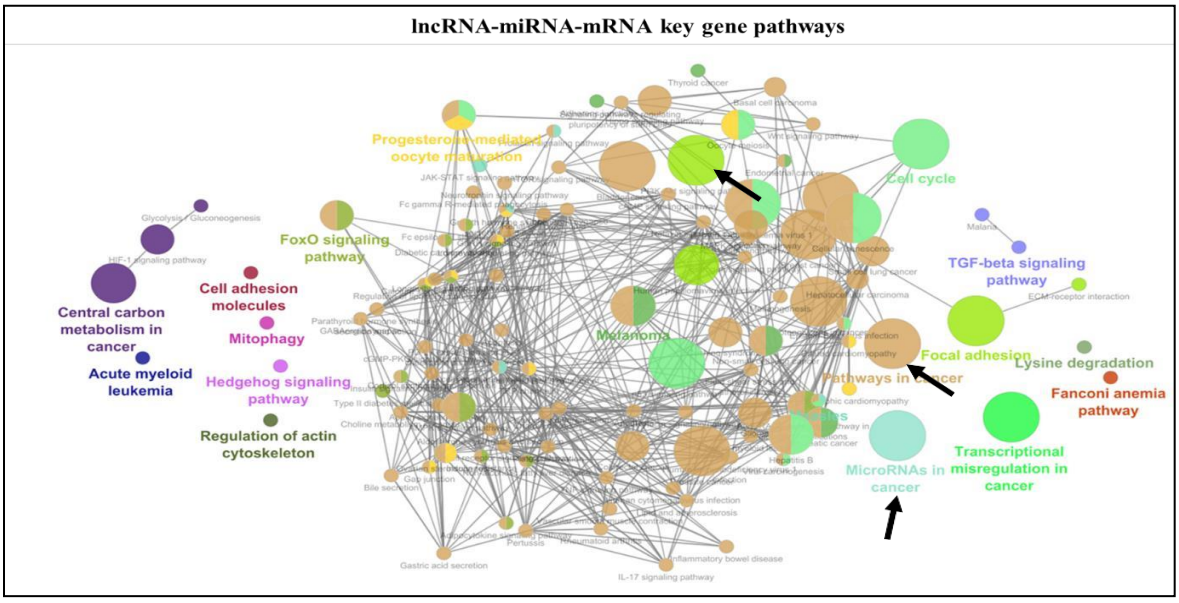



Figure 3.14.3B Cytoscape network showing the different pathways regulated by the lncRNA-mediated mRNA key genes. Some of the important pathways are indicated by thearrow in black color.

### 3.15. Pan carcinoma mRNA regulation

We have compared the significantly dysregulated genes in each carcinoma type and identified common and unique dysregulated mRNAs. Figure 3.15A shows the number of common and unique dysregulated genes across 16 carcinomas.

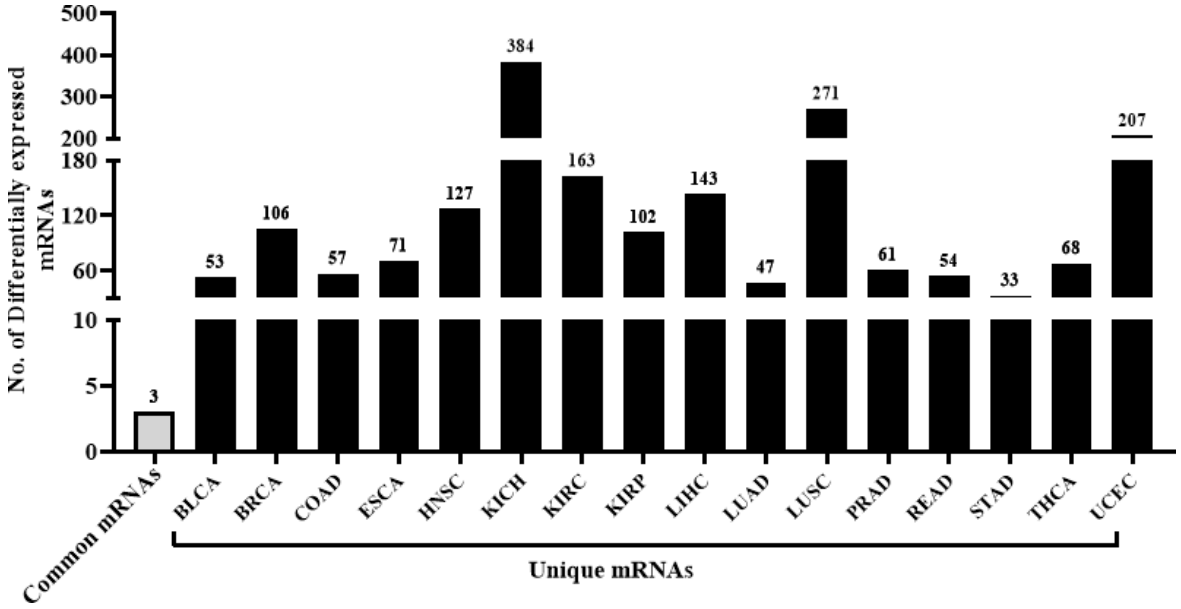



Figure 3.15A The bar graph summarizes the number of common and unique mRNAs in each cancer type. The grey represents the three mRNAs that are present in all sixteen cancer types.

Out of the pool of 7984 genes, a total of 1946 genes were found to be unique for different cancer types. It is observed that during the progression of KICH, LUSC, and UCEC significantly large number of unique genes are dysregulated which may indicate that the progression of these cancers could be different from other types of carcinomas. On the other hand, FAP, CTHRC1, and COL11A1 are the three commonly dysregulated genes across 16 different carcinomas. It is interesting to observe that all these genes were significantly up-regulated in all 16 cancers except for FAP in UCEC where it was found to have a down-

regulated expression. A heatmap representing the expression variation of the three genes in sixteen cancers is shown in figure 3.15B.

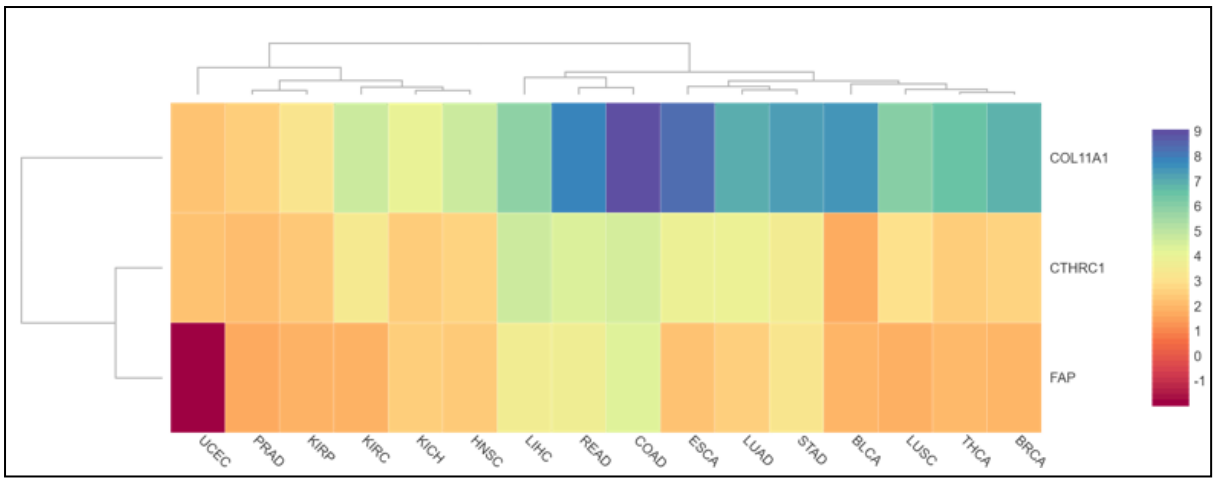



Figure 3.15B The heatmap represents the differential expression of the three commonly regulated genes that are present in all sixteen cancer types.

FAP (Fibroblast activation Protein Alpha), a post-prolyl peptidase, is expressed on the surface and has a role in extracellular matrix disintegration, inflammation, tissue remodeling, tumor formation, wound healing, and fibrosis. This protein regulates fibroblast proliferation throughout embryogenesis, postnatal tissue repair, and epithelium carcinogenesis. It is experimentally validated that FAP expression is low or undetectable in normal tissues but high in 90% of carcinomas (Fitzgerald and Weiner 2020). FAP overexpression causes breast, intestinal, lung, bladder, and ovarian cancers. Due to FAP's widespread expression, it is also considered a universal marker for cancer-associated fibroblasts (CAF) which is associated with cancer progression via survival, proliferation, angiogenesis, immunological suppression, and invasion (Orimo and Weinberg 2006; Östman and Augsten 2009; Sund and Kalluri 2009).

CTHRC1 (collagen triple helix repeat containing 1) is a glycosylated 28 kDa protein with a signal sequence. It has been demonstrated that CTHRC1 has a prominent role in the initiation and progression of various cancers including LUAD, PRAD, KIRC, BRCA, COAD, HNSC, STAD, LIHC, and UCEC (Mei et al. 2020). CTHRC1 overexpression is connected to shorter overall survival, higher CD8+ T cell infiltration, and enhanced tumor purity. It is also shown

that CTHRC1 may activate many signaling pathways, including TGF-, MAPK, Wnt, P13-Akt, and ERK. Thus, CTHRC1 is considered a good prognostic biomarker for predicting tumor recurrence and detecting metastasis (Mei et al. 2020; Pyagay et al. 2005; Sial et al. 2021).

COL11A1 encodes collagen XI's 1 chain, which is expressed by chondrocytes and osteoblasts but not quiescent fibroblasts. Mesenchyme-derived cancers, like scleroderma and keloids, and high-grade human gliomas/glioblastomas express COL11A1/(pro)collagen 11A1. COL11A1 is a particular biomarker of activated CAFs in numerous epithelial cancer types. It is not expressed in mesenchymal precursors or fibroblasts associated with non-cancerous diseases including inflammation and organ fibrosis. COLLA1 is already been considered as a biomarker in breast and lung cancer (Dongyu Jiaa, Zhenqiu Liub, Nan Dengb et al. 2017; Vázquez-Villa et al. 2015).

It is noticed that all three commonly expressed genes are related to cancer-associated fibroblasts (CAF), which promote cancer growth and metastasis. CAFs are implicated in fibrotic stromal programs across cancer types. Virchow and Duvall identified fibroblasts as collagen-producing connective tissue cells (Vijayashree, R. J. 2017). It is believed that normal fibroblasts lack metabolic or transcriptomic activity (T. Liu et al. 2019). When cancer cells develop in tissue, they exploit a chronic wound-healing response. Cancer fibrosis is a chronic host-healing reaction within tumors. It is found that cancer cells may attract activated fibroblasts. Cancer and immune cells draw tumor-associated fibroblasts (SAFs) to the tumor site. Tumor-associated fibroblasts (SAFs) are recruited to the tumor site in response to growth stimuli generated by cancer cells and immune cells that penetrate the tumor.

### 3.15.1. Important Regulatory Pathways

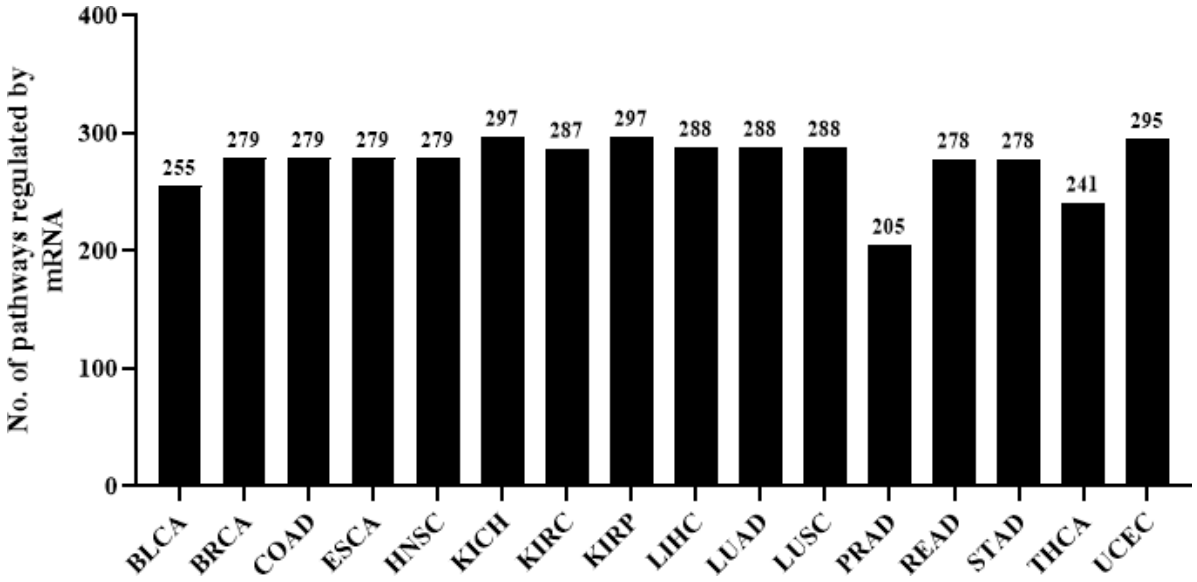



Figure 3.15.1 The bar graph represents the number of pathways that are regulated by each of the sixteen different cancer types.

All dysregulated genes across 16 carcinomas regulate a pool of 316 pathways. Out of this pathway, 169 pathways are present in all sixteen cancers. pathways in Cancer, MAPK signaling pathway (Braicu et al. 2019), PI3K-Akt signaling pathway (Rascio et al. 2021), calcium signaling pathway (Sadras, Monteith, and Roberts-Thomson 2021), human papillomavirus infection (Medda, Duca, and Chiocca 2021), and cytokine-cytokine receptor interaction pathways (Lee and Rhee 2017) to name a few which are common to all cancer type. These pathways are well characterized and known to be involved in cancer progression.We also found that basal transcription factors were specific to only the KICH cancer type which was regulated by TAF7L (TATA-box binding protein associated factor 7-like). This may play a role in spermatogenesis. One of the factors in regulating transcription initiation isTAF7, which is part of the TFIID complex. It regulates RNA polymerase II by interacting withand modifying the enzymatic activities of transcription factors. Its crucial role in cell proliferation is supported by the wide variety of roles it plays in the initiation of transcription.TAF7L is a TAF7 paralog with an unknown function and is known to be specifically expressedin the testis (Yazarloo et al. 2013).

### 3.16. Pan Carcinoma miRNA Regulation

miRNA dysregulation of each carcinoma type was compared to identify common and unique miRNAs that were present in sixteen different cancer types. The bar graph (figure 3.16A) depicts the number of common and unique miRNAs in various carcinomas.

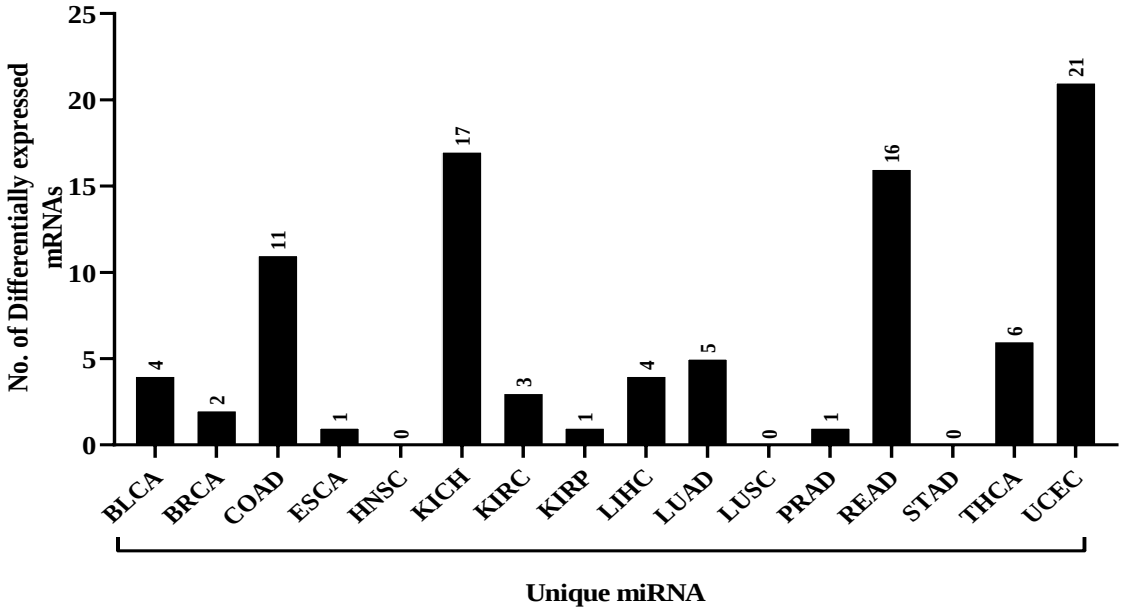



Figure 3.16A The bar graph summarizes the number of unique miRNAs in each of the sixteen cancer types. There was no common miRNA for all cancer. Some of the cancers did not have any unique miRNAs.

Out of the pool of 478 miRNAs, a total of 92 miRNAs were found to be of a unique type for different cancer types. It is apparent from figure 3.16a that there is no common dysregulated miRNA among all 16 carcinomas and on the other hand, HNSC, LUSC, and STAD did not have any unique miRNA, However, the larger number of unique miRNAs were dysregulated in UCEC, KICH, READ, and COADA carcinomas. It is found that hsa-miR-1-3p dysregulated in thirteen cancer types (BLCA, BRCA, COAD, ESCA, HNSC, KICH, KIRC, KIRP, LUAD, LUSC, READ, STAD, and UCEC). Interestingly, hsa-miR-1-3p is considered a biomarker in many different cancers including hepatocellular carcinoma where miR-1-3p suppresses the proliferation by targeting SOX9 (Hao Zhang et al. 2019). In the case of colorectal cancer, the proliferation and metastasis are suppressed by inhibiting YWHAZ-Mediated Epithelial–Mesenchymal Transition (Du et al. 2021). In bladder cancer, hsa-miR-1-3p functions as a

tumor suppressor leading to inhibition of proliferation, migration, and invasion (Junfeng Zhang et al. 2018). We observed that hsa-miR-133a-3p, hsa-miR-204-5p, hsa-miR-139-5p, and hsa-miR-195-5p were dysregulated in twelve carcinomas and heat map of these four miRNAs is shown in figure 3.16B.

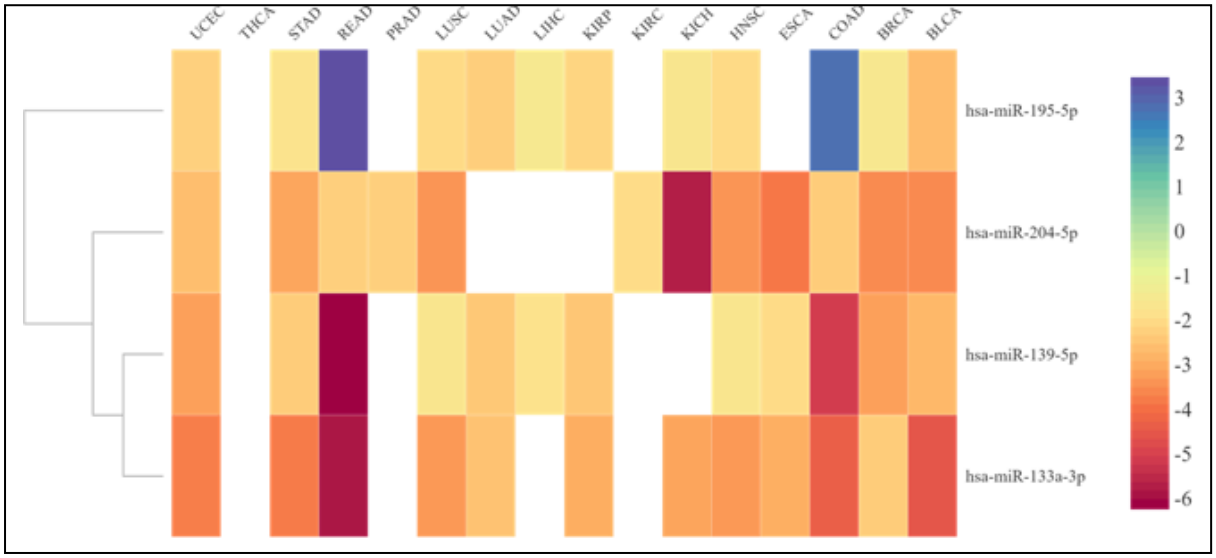



Figure 3.16B The heatmap represents the differential expression of three genes that are commonly regulated in twelve different cancer types.

As observed from the heatmap these four miRNAs are mostly found to be down-regulated in all the cancer types except for hsa-miR-195-5p which is up-regulated in COAD and READ. miR-133a-3p is up-regulated in several types of cancer, including breast (Sui et al. 2018), ovarian, and prostate cancer, colorectal cancer, gastric cancer, hepatocellular carcinoma, and pancreatic cancer. In these cancers, miR-133a-3p has been shown to promote cancer cell proliferation, migration, and invasion, and to inhibit apoptosis (programmed cell death). It has also been shown to play a role in cancer stem cell maintenance and drug resistance. In some cases, the expression of miR-133a-3p has been associated with poor prognosis in cancer patients. However, the exact mechanisms through which miR-133a-3p contributes to cancer are still being studied and are not fully understood (Hua et al. 2021) (B. Kong et al. 2021).

hsa-miR-204-5p is down-regulated in several types of cancer, including breast (B. S. Hong et al. 2019), ovarian (L. Hu et al. 2019), lung cancer (Liang et al. 2020), head and neck cancer

(Zhuang et al. 2020) and prostate cancer (T. Li, Pan, and Li 2016). In these cancers, miR-204-5p has been shown to inhibit cancer cell proliferation, migration, and invasion, and to promote apoptosis.

miR-139-5p is down-regulated in several types of cancer, including ovarian (Jiang et al. 2018), breast cancer (Krishnan et al. 2013), lung (DU et al. 2021), colorectal (Q. Li et al. 2016) and gastric cancer (Yuanbo Li et al. 2022) and is known to affect cancer progression.

hsa-miR-195-5p was associated with almost all cancer types such as lung (L. Li et al. 2020), breast, esophageal, pancreatic, gastric, colorectal, breast, ovarian, endometrial, bladder, prostate, etc (Q. Xu et al. 2022) (W. Yu et al. 2018).

### 3.16.1. Important Regulatory Pathways

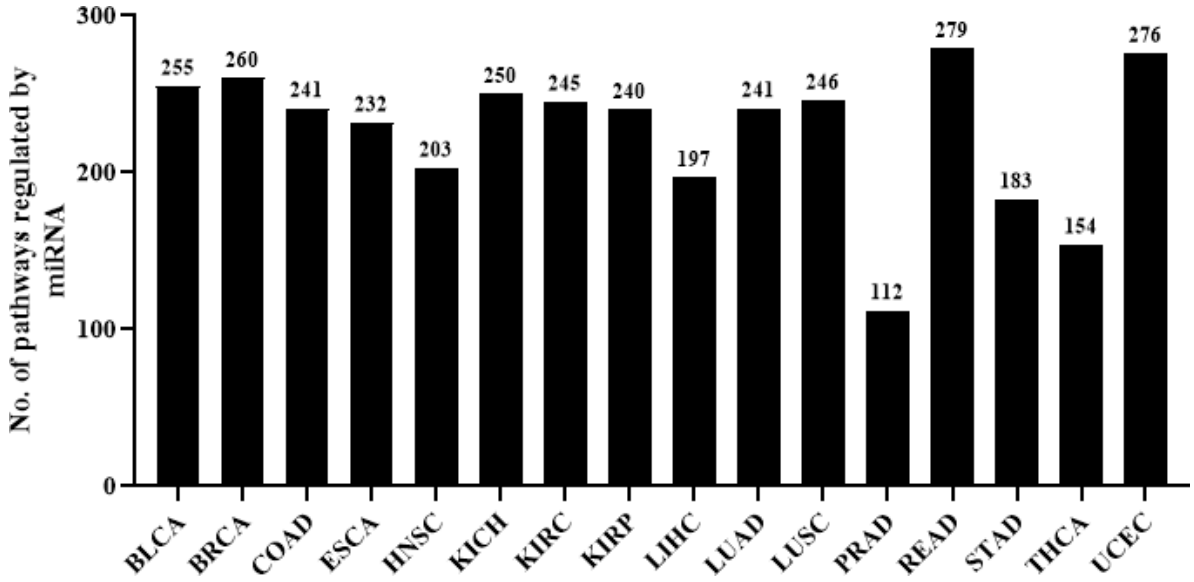



Figure 3.16.1 The bar graph represents the number of pathways that are being regulated by the miRNAs in each of the sixteen different cancer types.

In the case of pathways regulated by miRNA, we found a pool of 312 pathways out of which we found 54 pathways that were regulated by all sixteen cancer types. The primary bile acid biosynthesis pathway was unique in KIRC. This was regulated by the *HSD3B7* gene (Hydroxy-Delta-5-Steroid Dehydrogenase, 3 Beta- And Steroid Delta-Isomerase 7). It is involved in the initial stages of bile acid synthesis from cholesterol and is a member of the

short-chain dehydrogenase/reductase superfamily and is a known biomarker for the progression of renal cancers (Z. Hu and Wang 2022).

### 3.17. Pan carcinoma lncRNA regulation

We have analyzed all dysregulated lncRNAs in all the carcinomas and we obtained a total of 59 dysregulated lncRNAs out of which 20 were found to be unique in different cancer types. The number of total and unique dysregulated lncRNAs in each carcinoma is shown in figure 3.17A We did not find any commonly dysregulated lncRNA across carcinomas.

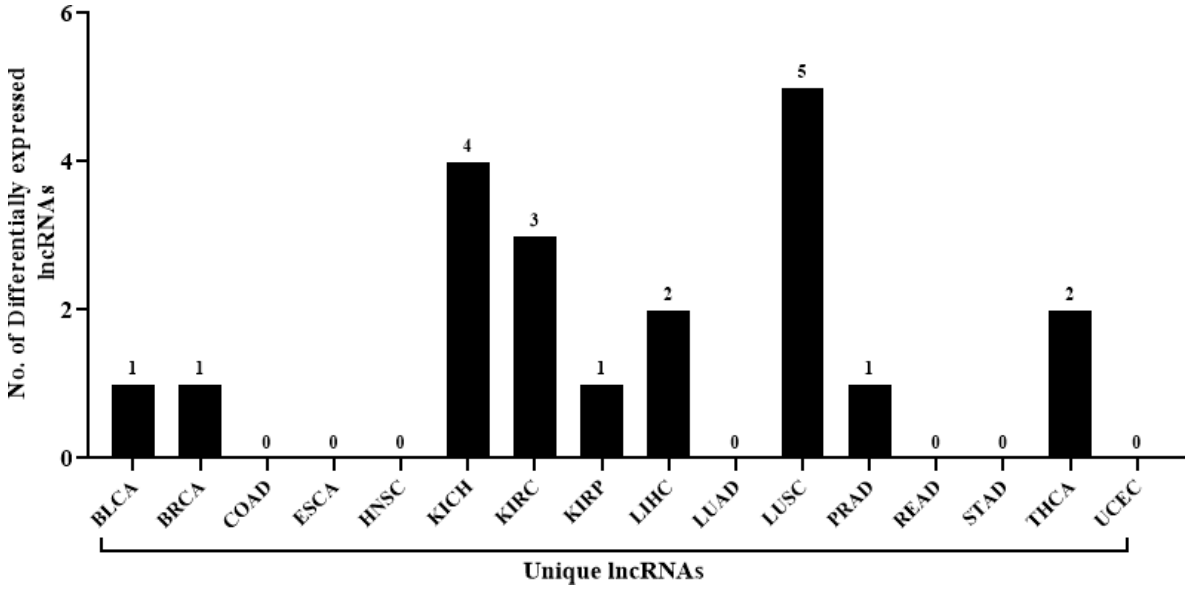



Figure 3.17A The bar graph summarizes the number of unique lncRNAs in each of the sixteen cancer types. There was no common lncRNA for all cancer. Some of the cancers didnot have any unique lncRNAs.

COAD, ESCA, HNSC, LUAD, READ, STAD, and UCEC did not have any unique lncRNAs while the rest nine had unique lncRNAs with LUSC having maximum dysregulated unique lncRNAs. A heatmap of all 59 dysregulated lncRNAs is shown in figure 3.17B.

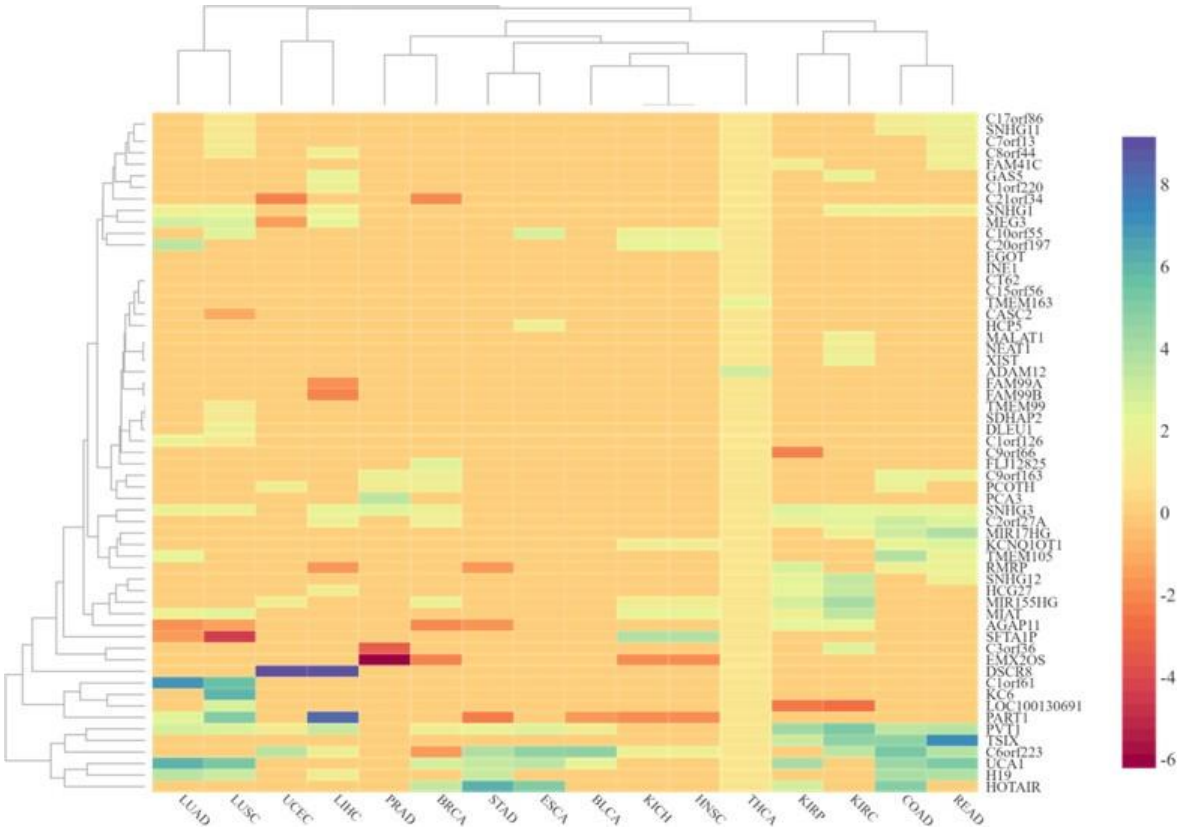



Figure 3.17B Heatmap representing the complete pool of the 59 dysregulated lncRNAs that are present across all sixteen cancer types.

From the expression profile, it is observed that lncRNAs are mostly up-regulated and very few of them are down-regulated. Although there is no common dysregulated lncRNA present across 16 carcinomas, PVT1 was up-regulated in thirteen carcinomas (BLCA, BRCA, COAD, ESCA, KICH, KIRC, KIRP, LIHC, LUAD, LUSC, READ, STAD, and UCEC).

We have categorized the dysregulated lncRNAs into a novel and experimentally validated category for each of the sixteen carcinomas (table 3.1). Interestingly, it is observed that novel lncRNA C6orf223 is present in 11 carcinomas. A deeper study is required to identify the interaction partner of this lncRNA and to understand its role in cancer progression.

Table 3.1. List of known and novel lncRNAs that are identified in each of the sixteen cancer types.

| Cancer | Experimentally validated lncRNA | Novel lncRNA |
|---|---|---|
| **BLCA** | PART1, PVT1, UCA1 | C6orf223 |
| **BRCA** | PVT1, UCA1, EMX2OS, SNHG3, AGAP11, PCOTH, C2orf27A, MIR155HG, HOTAIR | C6orf223, C21orf34, C9orf163, FLJ12825 |
| **COAD** | PVT1, UCA1, SNHG3, PCOTH, C2orf27A, HOTAIR, SNHG11, RMRP, SNHG1, KCNQ1OT1, MIR17HG, TMEM105, H19, TSIX | C6orf223, C9orf163, C17orf86 |
| **ESCA** | PVT1, UCA1, HOTAIR, HCP5 | C6orf223, C10orf55 |
| **HNSC** | PART1, EMX2OS, MIR155HG, KCNQ1OT1, MIAT, C20orf197, SFTA1P | C6orf223, C10orf55 |
| **KICH** | PART1, PVT1, SNHG3, PCOTH, RMRP, HCP5, EGOT, C15orf56, INE1, C1orf126, MEG3, CT62 | C6orf223, C10orf55, LOC100130691, C1orf220 |
| **KIRC** | PVT1, AGAP11, SNHG3, C2orf27A, MIR155HG, SNHG1, MIR17HG, TSIX, MIAT, NEAT1, MALAT1, GAS5, XIST, HCG27, SNHG12 | C6orf223, LOC100130691, C3orf36 |
| **KIRP** | PVT1, AGAP11, SNHG3, C2orf27A, MIR155HG, HOTAIR, RMRP, TSIX, MIAT, HCG27, SNHG12, C9orf66 | LOC100130691, FAM41C |
| **LIHC** | PART1, PVT1, SNHG3, C2orf27A, RMRP, SNHG1, H19, MEG3, GAS5, HCG27, FAM99B, FAM99A, DSCR8 | C6orf223, C1orf220, C8orf44 |
| **LUAD** | PART1, PVT1, UCA1, AGAP11, SNHG3, SNHG1, TMEM105, H19, MIAT, SFTA1P, C1orf126, MEG3, C1orf61 | C20orf197 |
| **LUSC** | PART1, PVT1, UCA1, AGAP11, SNHG3, SNHG11, SNHG1, C17orf86, H19, MIAT, SFTA1P, C1orf126, MEG3, C1orf61, CASC2, SDHAP2, C7orf13, TMEM99, DLEU1 | C10orf55, LOC100130691, C8orf44 |
| **PRAD** | EMX2OS, SNHG3, PCOTH, PCA3 | C9orf163, C3orf36 |
| **READ** | PVT1, UCA1, SNHG3, C2orf27A, SNHG11, RMRP, SNHG1, KCNQ1OT1, MIR17HG, TMEM105, H19, TSIX, SNHG12, C7orf13 | C6orf223, C9orf163, C17orf86, FAM41C, C8orf44 |
| **STAD** | PART1, PVT1, UCA1, AGAP11, HOTAIR, RMRP, H19 | C6orf223 |
| **THCA** | ADAM12, TMEM163 | |
| **UCEC** | PVT1, PCOTH, MIR155HG, MEG3, DSCR8 | C6orf223, C21orf34 |

From the table, we can see that lncRNA PVT1 is present in present in the majority of the cancer types. This is an identified oncogene and a biomarker. After PVT1, lncRNA PART1 is found to be common among cancer types. Among the novel ones lncRNAs C6orf223 and C9orf163 are found to be present in a majority of the cancer types. Although the functions of these lncRNAs have not been identified, research on these could help us with more biomarkers.

### 3.17.1. Important regulatory pathways

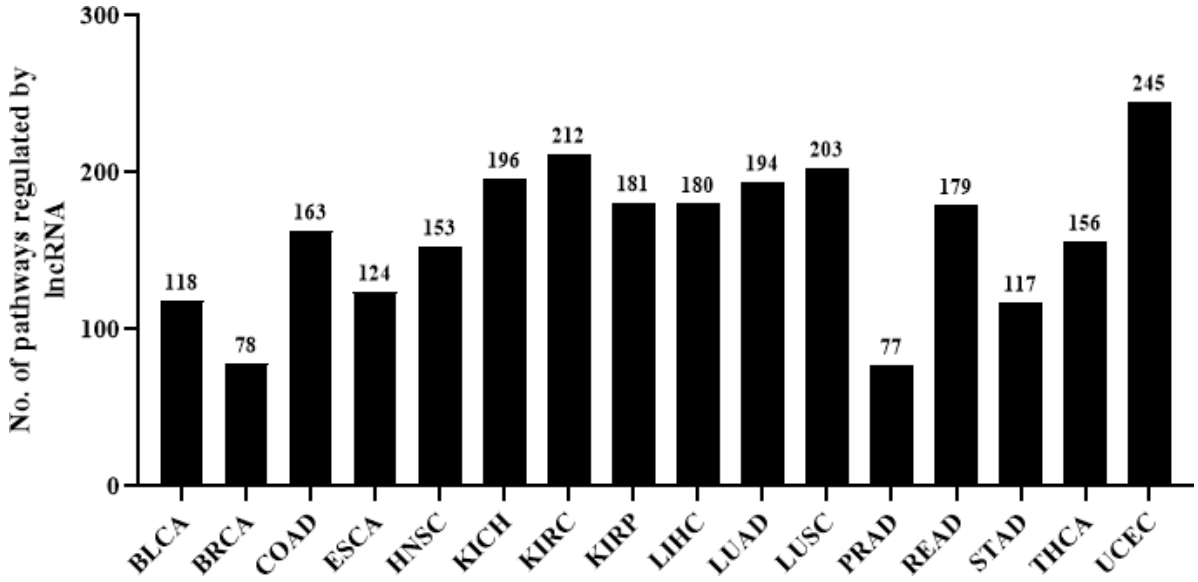



Figure 3.17.1 The bar graph represents the number of pathways that are being regulated by the lncRNAs in each of the sixteen different cancer types.

After identifying all dysregulated lncRNAs across 16 carcinomas, we try to understand the pathways that could be regulated by these lncRNAs. We obtained a pool of 293 pathways, out of which 15 pathways were found to be present in all sixteen cancer types and a total of 22 pathways were present unique in single carcinoma. A list of all pathways that were unique and common across sixteen carcinomas is represented in table 3.2 and table 3.3.

Table 3.2. List of 22 pathways that were unique and regulated by the single carcinoma type along with their gene and their possible regulatory role. The majority of the pathways are associated with the biosynthesis of various amino acids.

| Cancer | Pathway | Genes | Function/Role |
|---|---|---|---|
| BLCA | Phototransduction | CNGB1 (5.21) | Biomarker for bladder cancer(Hepburn et al. 2021) |
| KICH | Valine, leucine and isoleucine degradation | BCAT1 (3.45) | BCAT1 encodes a transaminase of branched-chain amino acids—valine, isoleucine, and leucine. BCAT1 expression represents a poor prognosis for cancer patients, and it serves as a potential marker for cancer immunotherapy (G. S. Li et al. 2022). |
| | Valine, leucine, and isoleucine biosynthesis | | |
| | Pantothenate and CoA biosynthesis | | |
| KIRC | Primary immunodeficiency | CD3D(3.26), CD8A(3.75), JAK3(2.39) | CD3D is strongly correlated with the immune score, immune checkpoint, and immune-infiltrated cells(Y. Yang et al. 2020). CD8A are preferred immune cells for targeting cancer (van der Leun, Thommen, and Schumacher 2020). JAK3 is known to promote the survival and proliferation of malignant cells(Vadivel et al. 2021) |
| | Primary bile acid biosynthesis | HSD3B7(1.76) | HSD3B7 is identified as a prognostic-related biomarker that predicts poor prognostic in renal carcinomas (Z. Hu and Wang 2022). It is responsible for bile acid synthesis. |
| | Arginine and proline metabolism | P4HA1(1.90) | P4HA1 promoted proliferation, migration, invasion, and EMT in renal cancer (Yang Li et al. 2022). |
| | Nicotinate and nicotinamide metabolism | CD38(2.55) | It has been identified as a potential immunological biomarker for predicting therapeutic response in patients with renal carcinomas (Carlisle et al. 2022) |
| LUAD | Mucin-type O-glycan biosynthesis | GALNT7(1.78) | It has been reported to promote breast cancer metastasis to the lung by hastening the initiation of metastasis and outgrowth into macrometastases within the lung parenchyma (Song et al. 2016) |
| | Other types of O-glycan biosynthesis | | |
| | Citrate cycle (TCA cycle) | PC(1.78) | Pyruvate carboxylase is essential for Lung cancer growth. Its knockdown induced multinucleation, inhibited cell proliferation and colony formation, and reduced tumor development (Sellers et al. 2015). |
| | Glycine, serine, and threonine metabolism | SHMT2 (1.50) | It's unclear how SHMT2 affects lung cancer, but its expression was higher in cancer cells as compared to normal tissues. LUAD tumor-infiltrating lymphocytes have elevated HMT2 expression indicating it is highly related to immune cell infiltration in LUAD, suggesting it may be a predictive biomarker (L. Luo et al. 2021). |
| | Glyoxylate and dicarboxylate metabolism | | |
| | Ascorbate and aldarate metabolism | UGDH(1.97) | UGDH promotes normal cellular development and migration by producing hyaluronic acid. UGDH is connected with epithelial cancer development, lymph node metastases, and poor patient survival (Saha et al. 2020). |
| LUSC | Basal transcription factors | TAF4B(1.74) | Although its role in regulating proliferation and apoptosis has been less thoroughly investigated, it is suspected that this gene plays a role in the development of ovarian, testicular, and head-and- |

| | | | |
|---|---|---|---|
| | | | neck cancers by regulating p53 activity (Ribeiro et al. 2014). |
| | Arginine biosynthesis | CPS1(3.70) | CPS1 is activated allosterically by N-acetyl glutamate to generate carbamoyl phosphate from bicarbonate, ammonia, and ATP. CPS1 transforms bicarbonate and ammonia into carbamoyl phosphate, depleting the cell's ammonia. Carbamoyl phosphate is a precursor for arginine and pyrimidine metabolism. CPS1 plays a function in the metabolism and cell proliferation of LKB1-inactivated lung adenocarcinomas, and its expression is linked to poor survival (Catherine Pham-Danis1, Sarah Gehrke1, Etienne Danis3, Andrii I. Rozhok4, Daniels3, Dexiang Gao3, Christina Collins1, José T. Di Paola1, Angelo D'Alessandro1, and DeGregor 2018). |
| | Proteasome | PSMD11(1.59) | PSMD11 was needed to build the proteasome complex in embryonic stem cells. It was found that PSMD11 was ubiquitinated at position K32 in LUSC tissues but not in normal lung tissues (X. Z. and M. Lu 2020). |
| READ | RNA polymerase | POLR3G(1.70) | POLR3G operates as a nuclear and cytosolic DNA sensor in innate immune responses and is crucial for stem cell maintenance. Its expression has been associated with lung squamous cell carcinoma, lung adenocarcinoma, stomach adenocarcinoma, esophageal carcinoma, kidney chromophobe, cholangiocarcinoma, colorectal adenocarcinoma, bladder urothelial carcinoma, and uterine corpus endometrial carcinoma (Van Bortle et al. 2022) |
| | Fatty acid degradation | ACSL6(5.80) | Its functional significance is unknown, though some bioinformatics study it was found that ACSL6 is up-regulated in cancer cells compared to healthy tissue (W. C. Chen et al. 2016) |
| UCEC | Vasopressin-regulated water reabsorption | ADCY3(-1.58), ADCY9(-1.59) | Through activation of the cAMP response element-binding protein (CREB) pathway, upregulation of adenylate cyclase 3 (ADCY3) enhances the tumorigenic capacity of cells (S. H. Hong et al. 2013). Adenylate cyclase downregulation has been associated with cancer progression (Orchel et al. 2012) |
| | Terpenoid backbone biosynthesis | HMGCR(1.69) | Plays an important role in cholesterol homeostasis and is involved in cell proliferation, differentiation, and survival(Schointuch et al. 2014) |
| | Thiamine metabolism | ALPL(2.09) | Alkaline Phosphatase plays a role in skeletal mineralization and adaptive thermogenesis. Its expression level has been detected in uterine cancer, endometrial cancer, and cervical cancer (Nozawa et al. 1981). |

Table 3.3. List of 15 pathways that were commonly regulated by all sixteen cancer types. These pathways are responsible for cancer dysregulation and are associated with cancer hallmarks.

| **Commonly Regulated Pathways regulated by lncRNA** |
| --- |
| cell cycle, pathways in Cancer, microRNAs in cancer, oocyte meiosis, progesterone-mediated oocyte maturation, hepatitis B, Pathways of neurodegeneration, human cytomegalovirus infection, coronavirus disease, proteoglycans in cancer, MAPK signaling pathway, Ras signaling pathway, parathyroid hormone synthesis, secretion, and action, Rap1 signaling pathway, focal adhesion |

From the above table, we can see that these pathways are commonly regulated in all sixteen cancer types. These can be used as potential biomarkers for cancer treatment.

## 3.18. Pan carcinoma Gene-noncoding RNA network

### 3.18.1. Pan-cancer miRNA-mRNA linked gene network

We have analyzed the correlated regulatory pair for miRNA-mRNA interaction in each cancer type. The correlated miRNA-mRNA pairs interaction for each cancer type is shown in figure 3.18.1A.

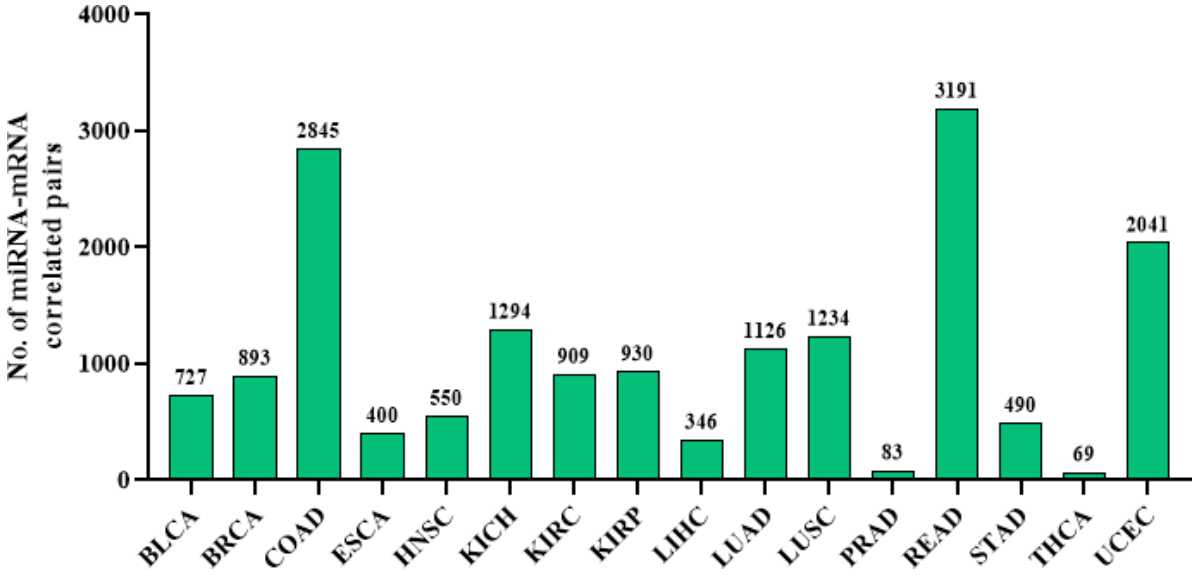



Figure 3.18.1A The bar graph represents the total number of unique miRNA-mRNA correlated pairs in each of the sixteen cancer types.

We obtained a total of 10399 correlated pairs which are possibly regulating the sixteen carcinomas. READ, COAD and UCEC have a significantly larger number of correlated mRNA-miRNA pairs. We observed that correlated hsa-miR-1-3p-CENPF, hsa-miR-1-3p-KIF2C, and hsa-miR-1-3p-KIF4A pairs present in eleven cancer types (BLCA, BRCA, ESCA, HNSC, KICH, KIRC, KIRP, LUAD, LUSC, STAD, UCEC). Here, we noticed that in each carcinoma hsa-miR-1-3p was down-regulated. Various reports indicated that hsa-miR-1-3p has a profound effect on the progression of various cancers including hepatocellular carcinoma, prostate, bladder, lung, and colorectal (ref). This miRNA targets different mRNA in different cancer and overexpression of this miRNA has been known to inhibit proliferation and induce apoptosis (Hao Zhang et al. 2019, Du et al. 2021, T. Li et al. 2019, J. Y. Wang et al. 2018). We observed that one of the targets of hsa-miR-1-3p is CENPF (chromosomal

segregation regulator) which is part of the centromere-kinetochore complex and the nuclear matrix during G2 of interphase. It accumulates during the cell cycle, peaks in the G2/M phase, and declines after mitosis (Varis, Salmela, and Kallio 2006) (Liao et al. 1995). It has been found to play an important role in various cancer progressions including prostate (P. Li et al. 2018), pancreatic (H. Chen et al. 2021), hepatocellular (H. Chen et al. 2022), Osteosarcoma (Zou et al. 2021) and breast cancer (J. Sun et al. 2019). The second potential target of hsa-miR-1-3p is KIF2C (Kinesin family member 2C) which is a microtubule-based motor protein that regulates mitosis and genome stability. KIF2C interacts with TIP150 and EB1 at microtubule plus ends, mediating microtubule dynamics. It also influenced bipolar spindle formation and chromosomal segregation, which aided cell division (Manning et al. 2007) (Gwon, Cho, and Kim 2012) (K. Jiang et al. 2009). Its role and functional importance have been identified in many cancer types including lung cancer (Bai et al. 2019), and colorectal cancer (Gnjatic et al. 2010). Kinesin Family Member 4A (KIF4A) are crucial intracellular transport proteins necessary for cellular function and morphology, including cell division (Zhu and Jiang 2005). Therefore, it can be postulated that these three miRNA-mRNA pairs could potentially act as biomarkers for these eleven carcinomas. Further, we also looked into the correlated pairs of miRNA-mRNAs which were unique for each cancer type. Figure 3.18.1B represents the unique correlated pairs for each cancer type.

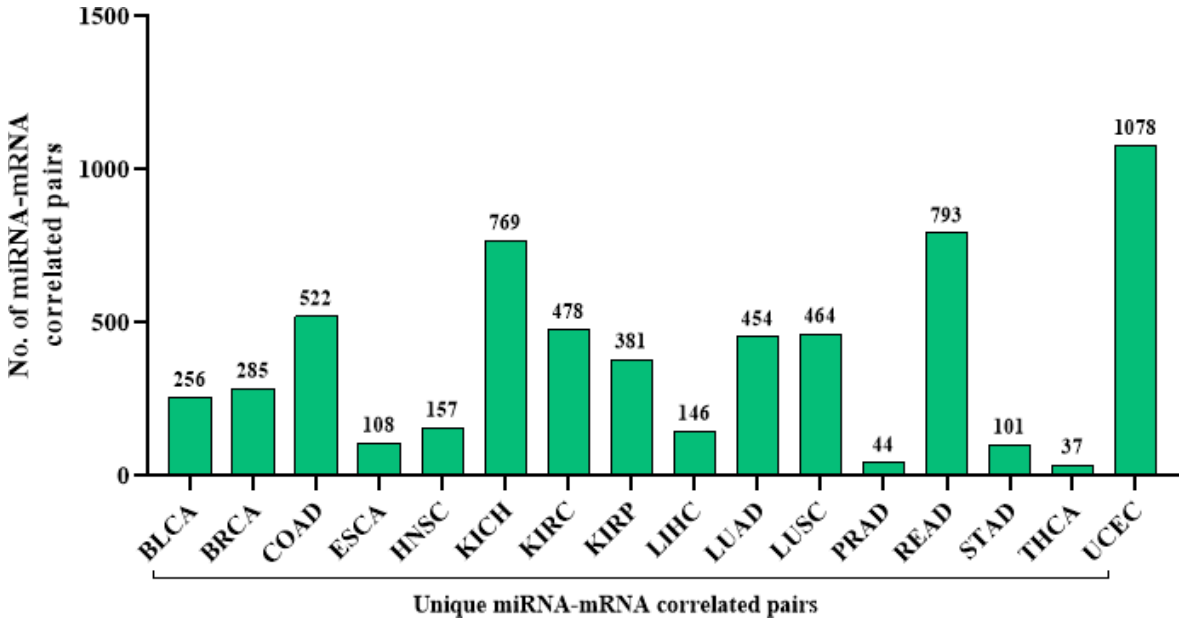



Figure 3.18.1B Represents the total number of unique miRNA-mRNA correlated pairs that were identified for each cancer type.

Our analysis shows that UCEC has the maximum number of unique correlated pairs of miRNA-mRNA whereas THCA has the minimum indicating a possible smaller number of miRNA-mediated gene regulations during cancer progression. These cancer-specific unique miRNA-mRNA axes can be used as potential targets for cancer-specific treatment.

### 3.1.1. Pan-cancer lncRNA-miRNA linked gene network

After comparing the dysregulated transcripts of 16 carcinomas, we have found out that READ has the maximum number of correlated lncRNA-miRNA-mRNA axis whereas THCA does not have any. The number of potential lncRNA-miRNA-mRNA axis in various carcinomas is shown in figure 3.18.2A.

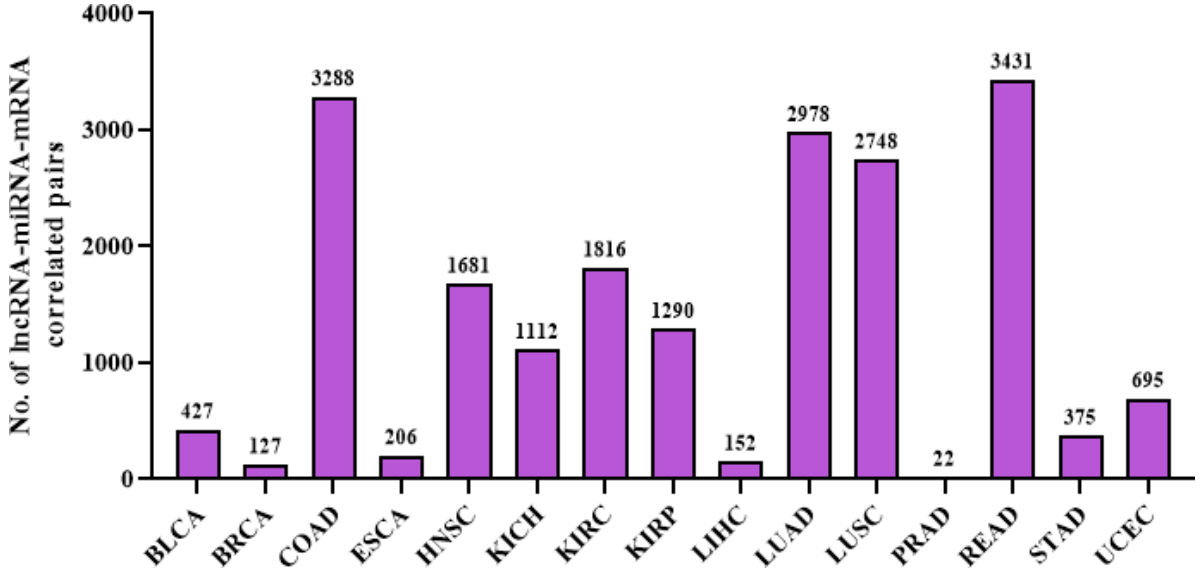



Figure 3.18.2A Represents the total number of lncRNA-miRNA-mRNA correlated axis that were observed for each cancer type. READ and COAD have the maximum number of axis.

We obtained a total of 14986 correlated lncRNA-miRNA-mRNA axis which were present in fifteen carcinomas. It has been observed that PVT1-hsa-miR-195-5p-BIRC5, PVT1-hsa-miR-195-5p-CEP55, PVT1-hsa-miR-195-5p-CLSPN, and PVT1-hsa-miR-195-5p-E2F7 axis were present in BLCA, BRCA, KICH, KIRP, LUAD, LUSC, STAD, and UCEC. In each of these eight carcinomas PVT1 (lncRNA) was up-regulated and the corresponding miRNA (hsa-miR-195-5p) and gene were down-regulated. PVT1 has been recently identified as a novel oncogene in multiple cancer types including oral squamous cell carcinoma (OSCC) non-small cell lung cancer (NSCLC), cholangiocarcinoma (CCA), osteosarcoma (OS), prostate cancer (PCa), gallbladder cancer (GBC), nasopharyngeal carcinoma (NPC), breast cancer (BC), gastric cancer (GC), renal cell carcinoma (RCC), esophageal cancer (EC), colorectal cancer (CRC), cervical cancer (CC), hepatocellular carcinoma (HCC), ovarian cancer (OC), pancreatic cancer (PC), lymphoma, multiple myeloma (MM), endometrial cancer, bladder cancer (BC), glioma thyroid cancer (TC), and leukemia (R. Li et al. 2022). PVT1 is closely associated with the onset, migration, development, and invasion, and is an emerging biomarker for the diagnosis and treatment of cancer (Bohosova, Kubickova, and Slaby 2021) (Martínez-Barriocanal, Arango, and Dopeso 2020) (Derderian et al. 2019). It has been reported that the

miR-15 family member miR-195-5p is down-regulated in a variety of cancers. These include breast cancer (Fangteng Liu, Dong, and Huang 2017), hepatocellular carcinoma, colorectal cancer, and endometrial carcinoma, cervical cancer (Shen, Cheng, and Wang 2017). It has been reported that PVT1 suppresses miRNA expression in tumorigenesis. PVT1 targets FGFR2 to suppress miR-195 expression, as seen in non-small cell lung cancer, pancreatic cancer, osteosarcoma, and endometrial carcinoma (Kong et al. 2018). Furthermore, PVT1 reduces miR-195 expression through direct sponging of miR-195 and by increasing histone H3K27me3 in the miR-195 promoter region.

The BIRC5 gene on chromosome 17 (17q25.3) is part of the inhibitor of apoptosis (IAP) gene family, which encodes anti-apoptotic proteins. This gene encodes the survivin protein, an IAP usually produced in fetal and adult proliferative cells. Most cancers overexpress survivin and BIRC5. A recent study indicates that BIRC5 could work as a biomarker in various cancers (Fäldt Beding et al. 2022). The 55 kDa centrosomal protein (CEP55) was found to regulate the final stage of cytokinesis, where two daughter cells separate. The recently discovered mitotic phosphoprotein CEP55 plays a crucial role in ensuring that the midbody structure continues to function normally. It has been identified that tumorigenesis and an increase in cell migration and invasion are both correlated with overexpression of CEP55, which causes cytokinesis abnormalities and increases the number of multinucleated cells. Its role has been studied in many different cancer types such as breast (Inoda et al. 2009), thyroid (Y. H. Li et al. 2020), and esophagus cancer (W. Jiang, Wang, and Jia 2017). CLSPN (Claspin) gene product initiates a cell cycle checkpoint and arrests the cell cycle in response to replicative stress or DNA damage. The protein is needed for normal S-phase DNA replication. Its overexpression in prostate cancer has been linked to tumor progression (Cai et al. 2021). The E2F transcription factors are crucial in regulating cell cycle progression. Several types of cancer, including those of the prostate (Y. Wang et al. 2020), liver (Ma et al. 2018), and cervical (Zong et al. 2019), have been linked to the oncogene E2F7. Also, we looked at the pairs which are unique and

present in each cancer type shown in Figure 3.18.2B.

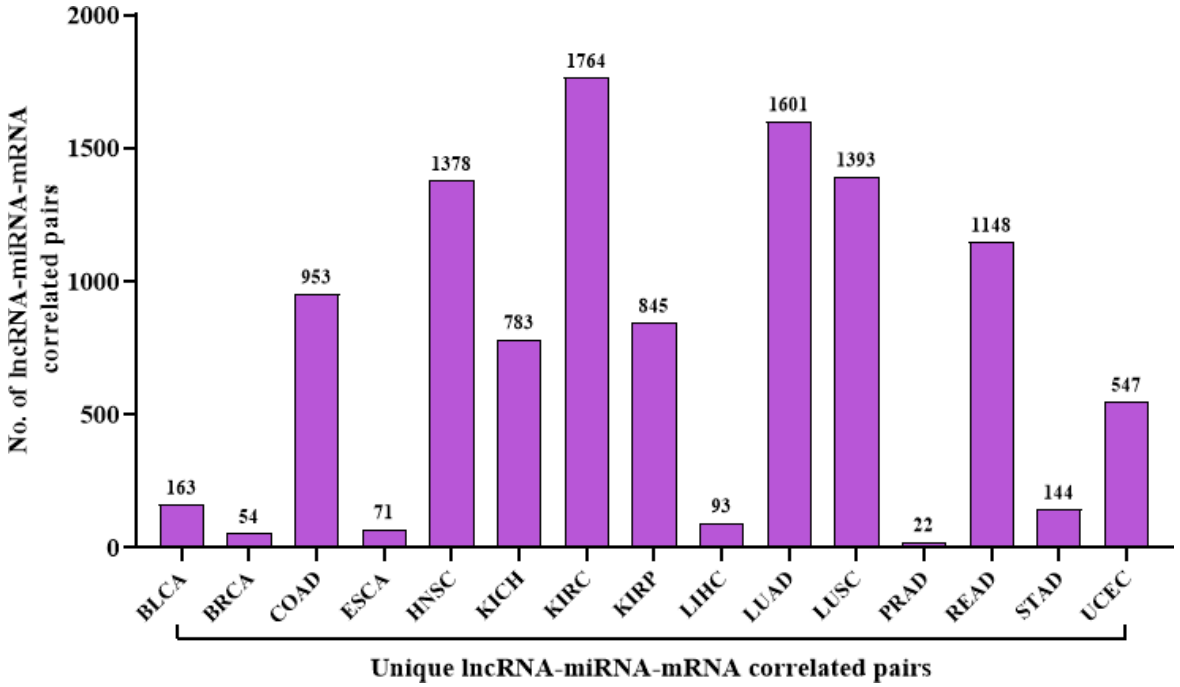



Figure 3.18.2B The bar graph represents the total number of unique lncRNA-miRNA- mRNA correlated axis for each cancer type.

From the above bar graph, we can see that KIRC (1764) has the maximum number of unique correlated pairs and PRAD (22) has the minimum.

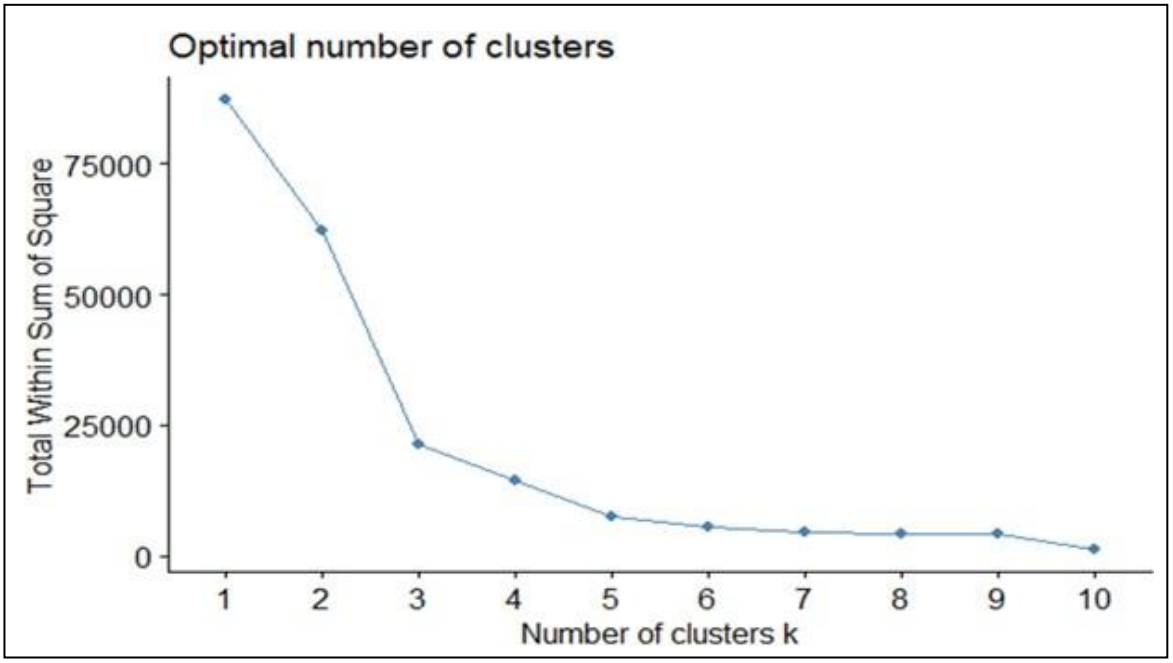
## 3.2. Transcripts-based clustering of carcinomas

Transcriptomic data analysis of 16 carcinomas revealed that these cancers are very different from each other albeit several carcinomas shows striking similarity. The significantly dysregulated transcripts are used to cluster carcinomas. We have separately clustered 16 carcinomas based on gene expression and miRNA expression. The dimensionality of gene expression data initially was reduced by principal component analysis. Principal component analysis, or PCA, is a technique for reducing the number of dimensions in large data sets by condensing a large collection of variables into a smaller set that retains the majority of the large set's information. The reduced data was then clustered through the k-mean clustering algorithm. The optimal number of clusters was determined and validated through three methods “Within-Cluster-Sum of Squared Errors” (WSS) or elbow method, gap-stat, and Silhouette method.

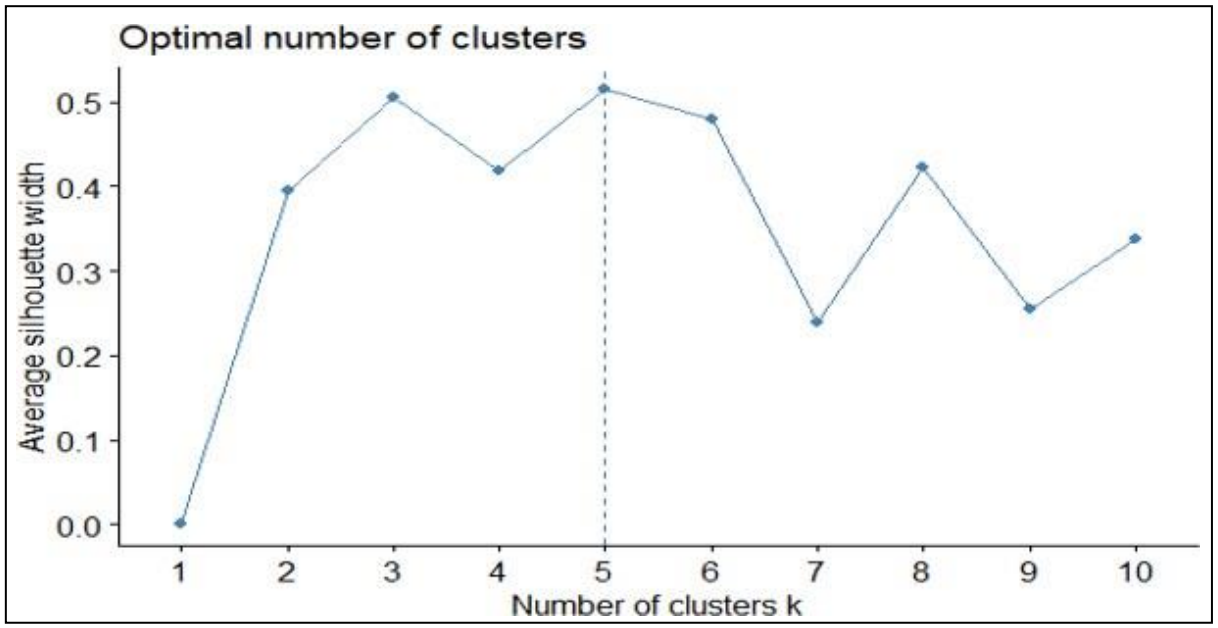
### 3.2.1. Clustering based on mRNA expression

Differentially regulated transcriptomic data across 16 carcinomas with 7984 dysregulated genes are subjected to principal component analysis (PCA) to reduce the dimensionality of the data. This data was then clustered using a k-mean clustering algorithm to identify gene expression based on closely associated carcinomas. As shown in figures 3.19.1A-B, both WSS and Silhouette techniques have suggested five optimum clusters. K means clustering with 5 optimum clusters of 16 carcinomas is shown in figure 3.19.1C.

A.



B.




C.

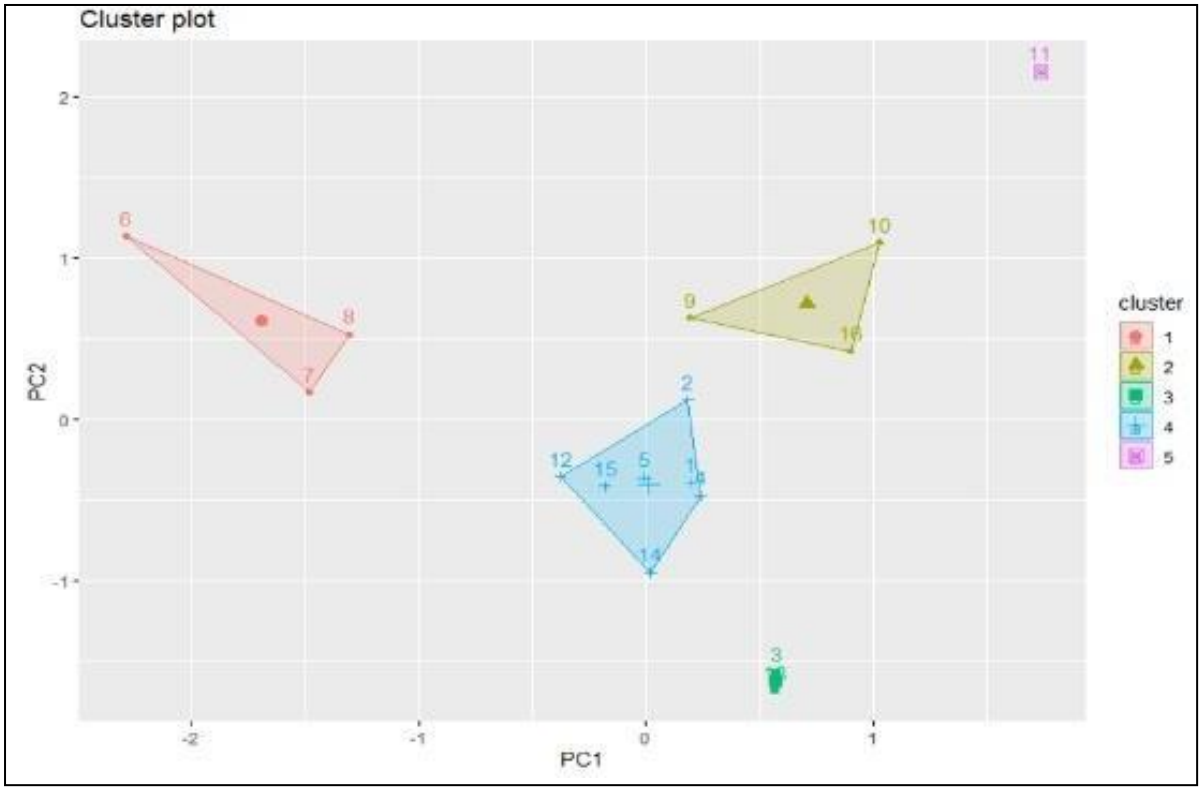



Figure 3.19.1 Principal component analysis (PCA) of 16 cancer types and identification of clusters based on mRNA expression. A) The clustering plot using the WSS method; B) Clustering plot using the silhouette method; C) Gives the clusters of cancer. We observed five clusters in which these sixteen carcinomas are grouped. Cluster-1 red color (KICH, KIRP, and KIRC), Cluster-2 dark green (LIHC, LUAD, and UCEC), Cluster-3 green color cancer (COAD and READ), Cluster-4 blue color cancer (BLCA, BRCA, ESCA, HNSC, PRAD, STAD, and THCA) and Cluster-5 pink color cancer (LUSC).

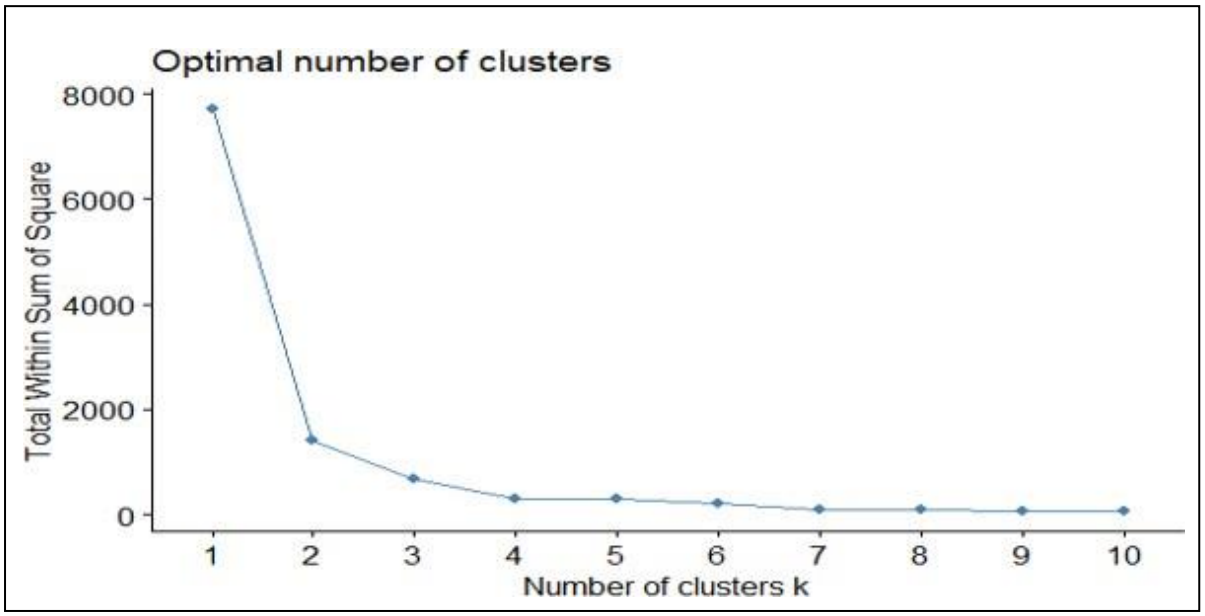
As depicted in figure 3.19.1 C, kidney-related carcinomas KICH, KIRP, and KIRC were clustered together indicating gene expression patterns of these cancers are similar. They had a total of 635 genes and 275 pathways in common. As expected, due to their similar gene expression patterns, two colorectal carcinomas (COAD and READ) are clustered together. These two cancers had 1626 genes and 273 pathways in common. It is observed that LIHC, LUAD, and UCEC carcinomas were clustered together with similar gene expression patterns although these three carcinomas originated from different tissue types and were located distantly from each other. Few studies indicated that the metastatic expression of endometrial cancer has been detected in lung and liver sites in patients (Miyazaki et al. 2018) (Ahmad, Raza, and Patel 2015) (Crespo et al. 1999). These three cancers LIHC, LUAD, and UCEC had 470 genes and 268 pathways in common. Interestingly, it is observed that gene expression patterns of lung-related carcinomas (LUAD and LUSC) are different since they are placed in different clusters. The largest cluster is formed with seven carcinomas that include BLCA, BRCA, ESCA, HNSC, PRAD, STAD, and THCA.

### 3.2.2. Clustering based on miRNA expression

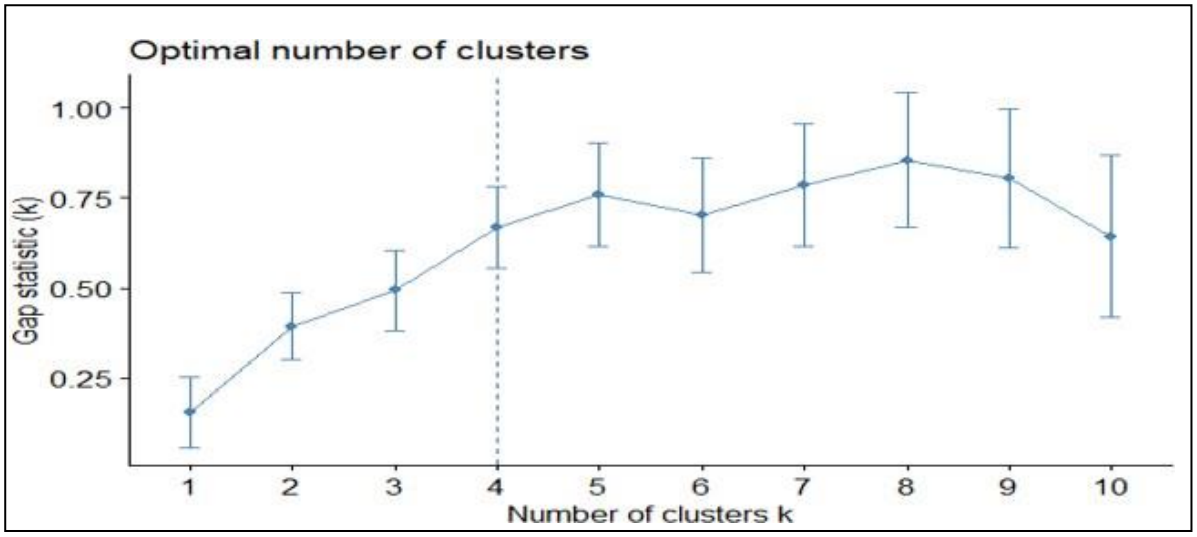
A total of 478 dysregulated miRNAs were considered for the clustering of 16 carcinomas. Both WSS and gap-stats algorithms predicted that dysregulated miRNAs data could lead us to four optimum clusters (figure 3.19.2 A-B). The miRNA expressions of kidney-related cancers (KICH, KIRP, and KIRC) are found to be similar as these carcinomas are clustered together. Here we found that these kidney-related cancers had 16 miRNAs and 173 pathways in common. Similarly, COAD and READ are grouped indicating similar miRNA regulation.For this cluster, we observed they had 265 miRNAs and 273 pathways in common. The thirdcluster was formed among BLCA, LUAD, and UCEC. These three cancers had 30 miRNAs

and 220 pathways in common. The miRNA expression pattern of the rest of the eight carcinomas was similar as they formed a fourth distinct cluster (figure 3.19.2C).

A.




B.



C.

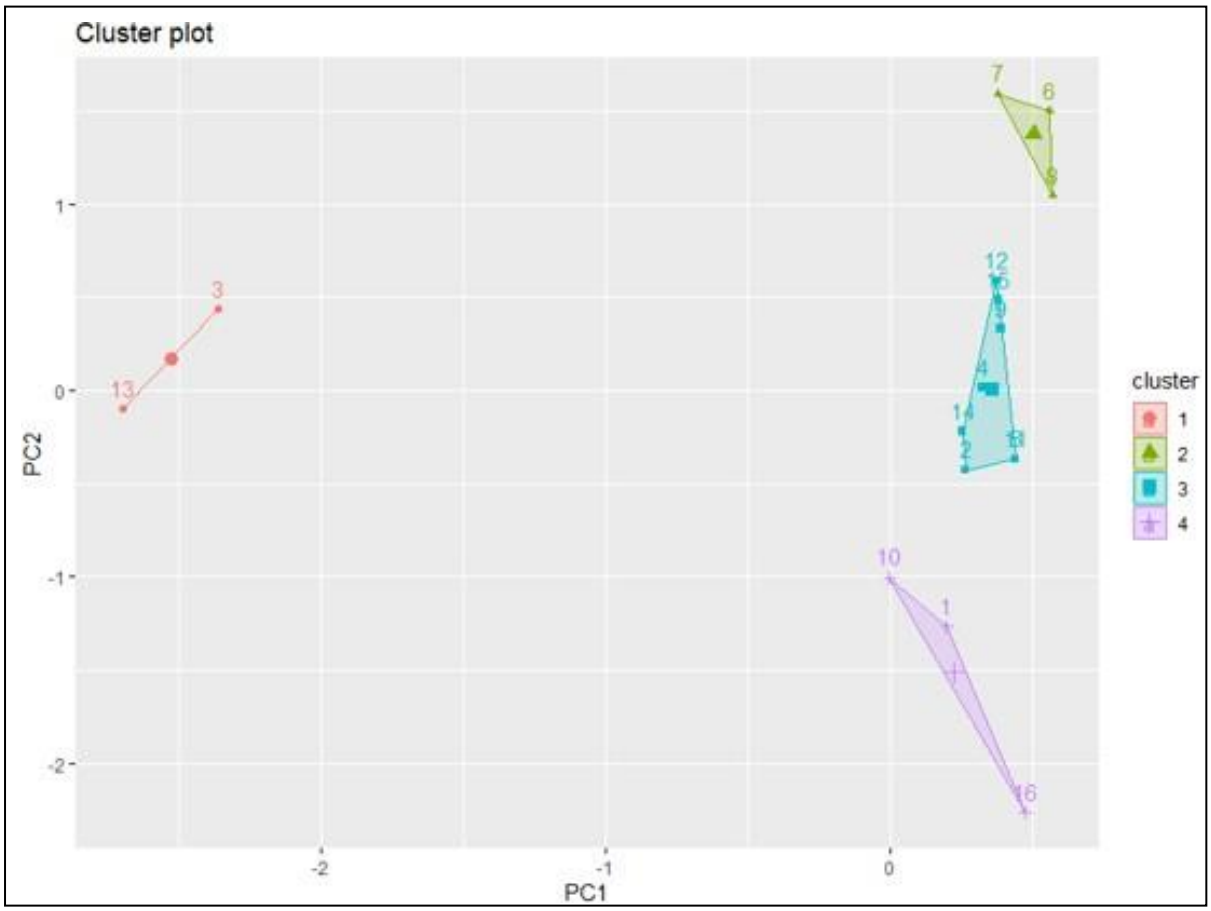



Figure 3.19.2 Principal component analysis (PCA) of 16 cancer types and identification of clusters based on miRNA expression. A) The clustering plot using the WSS method; B) Clustering plot using the Gap-stat method; C) Gives the clusters of cancer. We observed four clusters in which these sixteen carcinomas are grouped. Cluster-1 red color cancer (COAD and READ), Cluster-2 dark green color cancer (KICH, KIRP, and KIRC), Cluster-3 blue color cancer (LUSC, LIHC, BRCA, ESCA, HNSC, PRAD, STAD, and THCA) and Cluster-4 purple color cancer (BLCA, LUAD, and UCEC).

# CHAPTER- IV

## Conclusion and Future Perspective

## 4.1. Conclusion

It was previously believed that RNA served as the transporter of genetic information; however, recent developments in RNA research and the introduction of cutting-edge technologies have made it possible to acquire and satisfy a thirst for searching for novel regulatory RNAs in a variety of genomic data. Ever since the discovery of microRNAs, researchers have made significant efforts to profile the transcriptomes of numerous short regulatory RNAs under a wide variety of biological situations and in several different species. Although a single microRNA and lncRNA have the potential to regulate hundreds of thousands of distinct mRNAs, most transcripts are actively expressed and translated with the assistance of other mechanisms that work against the regulation exerted by the microRNA and lncRNA. This is necessary for a system to maintain homeostasis.

In this thesis, we aimed to understand the transcriptomic expression variation in pan-carcinomas. Our evaluation of transcript expression at various levels such as mRNA, miRNA, and lncRNA provided valuable insight into the important genes that are specifically involved in carcinoma progression. Our analysis showed that three mRNA encoding genes (FAP, CTHRC1, COL11A1) are universally expressed across all the sixteen cancer types and thus can have an important role in cancer progression. These genes give us the first indication of genes that might be altered in multiple cancer types. In our analysis, we found that the expression of these genes was higher, and the upregulation of these genes can be associated with cancer development or progression. We also tried to look for the important pathways regulated by de-regulated genes that could potentially be used as an important characteristic feature to define cancer. We identified a total of 169 pathways that were present in all sixteen carcinomas. These common pathways include the MAPK signaling pathway, PI3K-Akt signaling pathway, calcium signaling pathway, pathway of human papillomavirus infection, and cytokine-to-cytokine receptor interaction. We also observed that the pathway involving basal

transcription factors was specific to only TCGA-KICH which was regulated by the TAF7L gene; this gene's function is not explored much.

The analysis involving miRNAs indicated that miRNA hsa-miR-1-3p was present in thirteen out of 16 carcinomas. This miRNA has been found as an important biomarker in most cancer types. Although the exact function is yet to be explored, its downregulation is often linked with the proliferation of cancer and is known to inhibit apoptosis. Our analysis, therefore, emphasizes the importance of this miRNA in cancer as a whole. Further, in the case of lncRNAs, the number of experimentally validated lncRNAs was very few, but we found one lncRNA named PVT1, upregulated in thirteen cancer types. This lncRNA could therefore be an important biomarker associated with multiple cancer malignancies.

We also looked at important miRNA-mRNA and lncRNA-miRNA-mRNA regulatory axis across the Pan-Carcinomas that could probably serve as an important biomarker or target. Our analysis showed a correlated expression of hsa-miR-1-3p-CENPF, hsa-miR-1-3p-KIF2C, and hsa-miR-1-3p-KIF4A in eleven cancer types (BLCA, BRCA, ESCA, HNSC, KICH, KIRC, KIRP, LUAD, LUSC, STAD, UCEC). Again, the miRNA- hsa-miR-1-3p was found to be commonly present in all three pairs indicating that this miRNA is important in pan-carcinoma studies. Although many miRNA-mRNA correlated pairs in different cancer types have been identified and studied, the importance of these three is yet to be explored, which could potentially come across as an important biomarker for these eleven cancer types. While analyzing the important lncRNA-miRNA-mRNA axis, we have found four important axes PVT1-hsa-miR-195-5p-BIRC5, PVT1-hsa-miR-195-5p-CEP55, PVT1-hsa-miR-195-5p-CLSPN and PVT1-hsa-miR-195-5p-E2F7 which were present in eight cancer types (BLCA, BRCA, KICH, KIRP, LUAD, LUSC, STAD, and UCEC). Importantly, PVT1 was upregulated and common in all four pairs, miRNA hsa-miR-195-5p was downregulated, and it was also common across all eight cancer types. The sum ary of our observation is schematically shown

in figure 4.1. We thus observe that specific lncRNAs and miRNAs can have important implications across multiple cancer types, and our study provides a platform for future exploration of their specific function and inclusion as biomarkers or targets. The majority of the ncRNA-mRNA axis identified by us have not been explored for their potential role in cancer progression. These could prove to be important for future pan-cancer analysis.

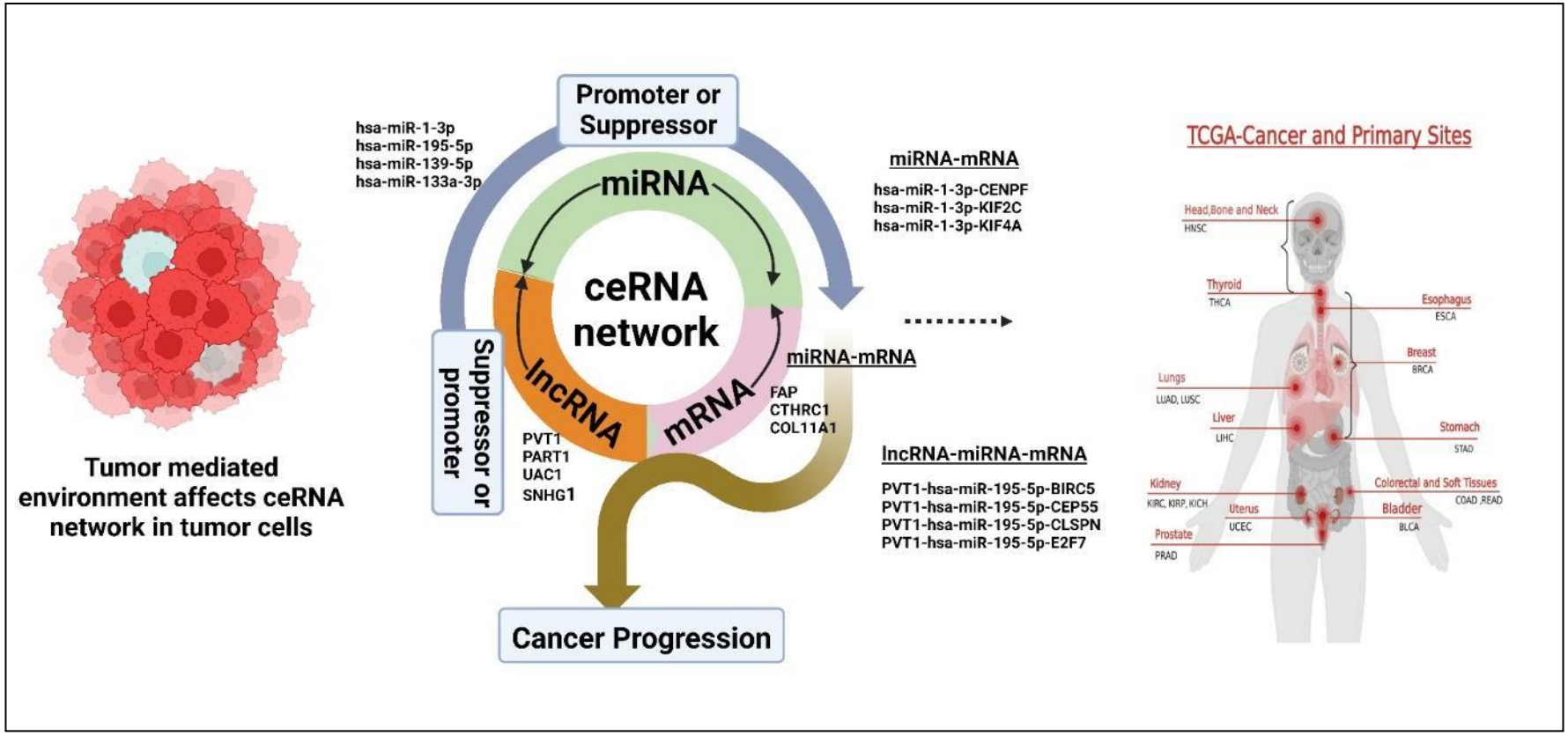



Figure 4.1 Summary of the functional role of mRNAs, miRNAs, and lncRNAs in 16 carcinoma progression. The important regulatory axis of miRNA-mRNA and lncRNA-miRNA-mRNAs obtained in our study could play an important role in the majority of the carcinoma progression.

## 4.2. Future scope of the research

Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) are both important regulatory molecules that play a key role in various biological processes, including gene regulation and cell proliferation. Dysregulation of lncRNA and miRNA expression has been implicated in the development and progression of various types of cancer. There is growing interest in the potential use of lncRNAs and miRNAs as diagnostic biomarkers for cancer. For example, the expression levels of certain lncRNAs and miRNAs have been found to be differentially expressed in cancer tissue compared to normal tissue, and these differences may be detectable in biofluids such as blood, urine, and/or saliva. These non-invasive sampling methods make lncRNA and miRNA-based biomarkers a potentially attractive option for cancer diagnosis. In addition to their potential use as diagnostic biomarkers, lncRNAs and miRNAs are also being explored as therapeutic agents for cancer treatment. For example, lncRNAs and miRNAs that are overexpressed in cancer cells may be targeted with small molecules or RNA interference techniques to inhibit their expression and potentially inhibit cancer growth. In our study, a large number of novel lncRNAs was identified; however, their functions are not identified so far. *In silico* and wet lab, studies could identify their functions as well as their potential role in carcinoma progression. There is still much research that needs to be done to fully understand the role of lncRNAs and miRNAs in cancer and to develop effective lncRNA and miRNA-based diagnostic and therapeutic approaches. However, the potential of lncRNAs and miRNAs in cancer diagnosis and treatment is an active area of research, and it is likely that we will see significant progress in this field in the coming years. There is growing evidence suggesting that the lncRNA-miRNA axis plays a major role in cancer development and progression and that targeting this axis may have therapeutic potential for cancer treatment. For example, lncRNAs have been shown to modulate miRNA expression and function, and targeting lncRNAs may affect miRNA-mediated gene regulation in cancer cells. Similarly, miRNAs have been shown to target lncRNAs and

regulate their expression, and targeting miRNAs may in turn affect lncRNA-mediated gene regulation in cancer cells. The exact role of the lncRNA-miRNA axis in cancer thus opens up a new field ofresearch that is not yet fully understood. Future, *in vitro* and *in vivo* studies could provide valuable insights into the mechanisms by which lncRNAs and miRNAs regulate gene expression in cancer cells and how this regulation may be disrupted in cancer. The transcriptomic data of cancer patients can also be explored in various directions. One such could be cancer stage-specific studies which can offer focused insight into cancer progression, enhance detection, and tailor treatments which can help to give personalized therapy studies that reshape cancer understanding and care, leading to better outcomes. Overall, the future scope of our study will include the development of effective diagnostic and therapeutic approaches targeting specific molecules or the ncRNA-mRNA axis.

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# APPENDIX

# Appendix I

## List of Publications

1. Heena Saini, Mahima Choudhary, **Harshita Sharma**, Shibasish Chowdhury Sudeshna Mukherjee, Rajdeep Chowdhury. "Chloroquine induces transitory attenuation of proliferation of human lung cancer cells through regulation of mutant P53 and YAP". Molecular Biology Reports (2022). DOI: 10.1007/s11033-022-08072-y (Impact Factor 2.742).

2. Abhilasha Srivastava, **Harshita Sharma**, Simran Khanna, Tejasvini Sadhu Balasundaram, Shibasish Chowdhury, Rajdeep Chowdhury and Sudeshna Mukherjee. "Interleukin-6 Induced Proliferation Is Attenuated by Transforming Growth Factor-β-Induced Signaling in Human Hepatocellular Carcinoma Cells". Frontiers in Oncology (2022). DOI: 10.3389/fonc.2021.811941. (Impact Factor 5.738).

3. **Harshita Sharma**, Divya Niveditha, Rajdeep Chowdhury, Sudeshna Mukherjee, Shibasish Chowdhury. "A genome-wide expression profile of noncoding RNAs in human osteosarcoma cells as they acquire resistance to cisplatin". Discover Oncology (2021). DOI: 10.1007/s12672-021-00441-6. (Impact Factor 4.67).

4. Yash T. Katakia, Niyati P. Thakkar1, Sumukh Thakar, Ashima Sakhuja, Raghav Goyal, **Harshita Sharma**, Rakshita Dave, Ayushi Mandloi, Sayan Basu, Ishan Nigam, Bhanu V. R. Kuncharam, Shibasish Chowdhury, Syamantak Majumder. "Dynamic alterations of H3K4me3 and H3K27me3 at ADAM17 and Jagged-1 gene promoters cause an inflammatory switch of endothelial cells". Journal of Cellular Physiology (2022). DOI: 10.1002/jcp.30579. (Impact Factor 6.513).

5. Heena Saini, **Harshita Sharma**, Sudeshna Mukherjee, Shibasish Chowdhury, Rajdeep Chowdhury. "Verteporfin disrupts multiple steps of autophagy and regulates p53 to sensitize osteosarcoma cells". Cancer Cell International. DOI: 10.1186/s12935-020-01720-y. (Impact Factor 6.436).

6. Divya Niveditha, **Harshita Sharma**, Anirudha Sahu, Syamantak Majumder, Rajdeep Chowdhury, Shibasish Chowdhury. "Drug Tolerant Cells: An Emerging Target With Unique Transcriptomic Features". Cancer Informatics DOI:10.1177/1176935119881633 (Impact Factor 2.056).

7. Divya Niveditha, **Harshita Sharma**, Syamantak Majumder, Sudeshna Mukherjee, Rajdeep Chowdhury, Shibasish Chowdhury. "Transcriptomic analysis associated with reversal of cisplatin sensitivity in drug-resistant osteosarcoma cells after a drug holiday". BMC Cancer. DOI:10.1186/s12885-019-6300-2 (Impact Factor 4.4).

# Appendix II

# Biography

## Prof. Shibasish Chowdhury

Prof. Shibasish Chowdhury obtained a master's degree in physical chemistry from Calcutta University. Then, he shifted to biophysics and obtained a Ph.D. degree from the Molecular Biophysics Unit (MBU) at the Indian Institute of Science, Bangalore on "Computer modelling studies on G-rich unusual DNA structure". Subsequently, entered into the protein folding field and worked as a postdoctoral research fellow in the Department of Chemistry and Biochemistry, University of Delaware, the USA for three years. Then, He joined the Department of Biological Science, BITS Pilani as a lecturer in 2004, after that promoted to Assistant Professor (2006-2012) and then Associate Professor at the same department (2013- 2020) and then Professor at the same department (2020-Till date). His broad research area is computational bioinformatics which includes analyses of biomolecular structures and structure-function relationships using model building and computational techniques, Modelling of RNA structures, Cancer Genomics, and Transcriptomic

## Prof. Sudeshna Mukherjee

Prof. Sudeshna Mukherjee Chowdhury is currently working as an Associate Professor at, the Department of Biological Sciences, BITS Pilani, Pilani campus, Rajasthan. She did herPh. D. at Chittaranjan National Cancer Institute (CNCI, Kolkata) under JadavpurUniversity. Her graduate research was primarily focused on elucidating the molecularmechanisms of carcinogenesis with special emphasis on cell cycle dysregulation andchromosomal aberrations on embryonic fibroblasts. After her graduation, she movedon to the USA where she did her post-doctoral research at the Department of

Biochemistry, Tufts School of Medicine, Boston, USA. The research was focused on Polyoma Middle T Antigen mediated signaling in cancer. She joined BITS-Pilani in July 2017 with the DST-SERB Young Scientist Grant. Post-completion of that project, shegot a regular position as an Assistant Professor. Her expertise is in the field of cancer biology specifically epithelial to mesenchymal transition. She is interested in studying the role of cytokines in the tumor microenvironment and their cross-talk in EMT and cancer progression. She has received projects from various government funding authorities like - (i) SERB, Department of Science and Technology (SERB-DST) (ii) BITS, Research Initiation Grant (iii) SERB, Department of Science and Technology (SERB-DST) (iv) BITS, Additional Competitive Research Grant (v) CSIR, Council of Scientific and Industrial Research as well. Findings from her works have been published in reputed international scientific journals. At present, she is guiding 5 Ph.D. students and has guided more than 10 fM.Tech. Bio-sciences students for the fulfillment of their dissertation.

**Harshita Sharma**

Ms. Harshita Sharma has a dual integrated degree Bachelor of Technology (B. Tech) and Master of Technology (M. Tech) in Biotechnology and Bioinformatics Centre for Converging Technologies, University of Rajasthan, Jaipur Rajasthan. Currently, she is pursuing her doctoral thesis under the guidance of Prof. Shibasish Chowdhury, associate professor at Birla Institute of Technology and Science, Pilani, Pilani campus. During the period of Ph.D. research, she was awarded the BITS Pilani Research Fellowship. Her research interest includes Bioinformatics data analysis, Network analysis, Transcriptomic data analysis, and NGS data analysis.