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Supervisor

Prof. Dr. Filiz ÖZBAŞ GERÇEKER

by

Bodour ALASSIL

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miRNA EXPRESSION ANALYSIS IN BREAST CANCER

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ABSTRACT

miRNA EXPRESSION ANALYSIS IN BREAST CANCER

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Ph.D. in Biology
Supervisor: Prof. Dr. Filiz ÖZBAŞ GERÇEKER
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The role of microRNAs (miRNAs) in breast cancer has gained significant attention. This study explores specific miRNAs (miR-551a, miR-1238, miR-4534, miR-1294) in breast cancer, evaluating their expression levels in 82 samples from 41 patients (41 tumor and 41 normal tissues) using real-time PCR and identifying their target genes and pathways through bioinformatics, using tools like miRDB and TargetScan, and databases like topGO and EnrichR. Results showed that miR-1294 and miR-551a were lower in tumor tissues than in normal ($p<0.05$), while miR-4534 was overexpressed in tumors ($p<0.05$). No significant difference was observed in miR-1238 levels, excluding it from further analysis. MiR-1294, acting as a tumor suppressor, potentially targets BRCC3, MYCL, YOD1 genes in Hippo and p53 signaling pathways. MiR-551a, also a tumor suppressor, targets ERbB4, MEF2C, CRB2, TMPRSS2 in pathways like sphingolipid metabolism, Notch, MAPK, and ErbB signaling. Conversely, miR-4534, with oncogenic function, targets SOX6, ADCY1, ESRRG, ZNF142 in ion transport, and glutamatergic and cholinergic synapse signaling. In conclusion, miR-551a and miR-1294 are potential tumor suppressors suitable for therapeutic applications, while miR-4534, an oncogene in breast cancer, merits further study as a diagnostic biomarker.

Keywords: Breast cancer, miR-1294, miR-551a, miR-4534, Bioinformatics

ÖZET

MEME KANSERİNDE miRNA EKSPRESYON ANALİZİ

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Meme kanserinde mikroRNA'ların (miRNA) rolüne dikkat çeken bu çalışma, özellikle miR-551a, miR-1238, miR-4534, ve miR-1294'ün rollerini araştırmıştır. 41 hastadan alınan 82 örnek (41 tümör ve 41 normal doku) üzerinde gerçek zamanlı PCR ile miRNA ifade seviyeleri değerlendirilmiş ve miRDB, TargetScan araçlar ile topGO ve EnrichR veritabanları kullanılarak hedef genler ve yolları belirlenmiştir. Elde edilen sonuçlar, miR-1294 ve miR-551a'nın tümör dokularında normal dokulara kıyasla anlamlı derecede düşük olduğunu ($p<0.05$), miR-4534'ün ise tümör dokularda normal dokulara göre aşırı ifade edildiğini göstermiştir ($p<0.05$). MiR-1238 seviyelerinde tümör ve normal dokular arasında anlamlı bir fark bulunmadığı için sonraki analizlerden çıkarılmıştır. MiR-1294 tümör baskılayıcı olarak Hippo ve p53 sinyal yollarında yer alan BRCC3, MYCL, YOD1 genlerini hedeflemekte, miR-551a da tümör baskılayıcı olarak sfingolipid metabolizması, Notch, MAPK, ve ErbB sinyal yolarında etkili olan ERbB4, MEF2C, CRB2, TMPRSS2 gibi genleri hedeflemektedir. Buna karşılık, miR-4534'ün onkojenik işlev gösterdiği ve iyon taşınımı, glutamaterjik ve kolinergik sinaps sinyal yollarında yer alan SOX6, ADCY1, ESRRG, ZNF142 gibi genleri hedeflediği düşünülmektedir. Sonuç olarak, miR-551a ve miR-1294'ün tümör baskılayıcı fonksiyonlarıyla terapötik uygulamalar için uygun olduğu, miR-4534'ün ise meme kanseri ilerlemesinde onkojenik bir rol oynayarak tanı biyomarkerı olarak daha fazla incelenmeye değer olduğu ortaya çıkmıştır

Anahtar Kelimeler: Meme Kanseri, miR-1294, miR-551a, miR-4534, Biyoinformatik

''Dedicated to my family''

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LIST OFABBREVIATIONS

ADAMDEC1	ADAM-like Decysin-1
ADCY1	Adenylate Cyclase 1
	Advanced Glycation End-products - Receptor for
AGE-RAGE	Advanced Glycation End-products
Ago	Argonaute Protein
AJCC	American Joint Committee on Cancer
AKT1	Protein Kinase B
ALL	Acute Lymphoblastic Leukemia
ASOs	Antisense Oligonucleotides
ATG2B	Autophagy Related 2B
BC	Breast Cancer
BCL2L13	BCL2-like 13
BioGRID	Biological General Repository for Interaction Datasets
BP	Biological Process
BPH	Benign Prostatic Hyperplasia
BRCA1/2	Breast Cancer Gene 1/2
BRCC3	BRCA1/BRCA2-Containing Complex Subunit 3
CA-125	Cancer Antigen 125
CA15-3	Cancer Antigen 15-3
CAV1	Caveolin-1
CC	Cellular Component
CCK8	Cell Counting Kit-8
CCND2	Cyclin D2
ccRCC	Clear Cell Renal Cell Carcinoma
CDK2	Cyclin-Dependent Kinase 2
CDK4	Cyclin-Dependent Kinase 4
CDK6	Cyclin-Dependent Kinase 6
CEA	Carcinoembryonic Antigen

ceRNA	Competing Endogenous RNA
cfDNA	Cell-free DNA
circRNAs	Circular RNAs
CKB	Creatine Kinase, Brain-type
CLDN14	Claudin-14
CNB	Core Needle Biopsy
COAD	Colon Adenocarcinoma
COPII	Coat Protein Complex II
CpG	Cytosine-Phosphate-Guanine
CRB2	Crumbs Cell Polarity Complex Component 2
CRC	Colorectal Cancer
Ct	Cycle Threshold
ΔΔCt	Delta Delta Cycle Threshold Method
CTNNB1	β-Catenin
DAAM2	DAAM Family RhoGEF
DCIS	Ductal Carcinoma In Situ
DEGs	Differentially Expressed Genes
DGCR8	DiGeorge Syndrome Critical Region 8
DLG4	Discs Large Homolog 4
DNA	Complementary DNA
dNTPs	Deoxynucleotide Triphosphates
DVL1	Disheveled Segment Polarity Protein 1
DVL2	Disheveled Segment Polarity Protein 2
EGFR	Epidermal Growth Factor Receptor
EOC	Epithelial Ovarian Cancer
ER	Estrogen Receptor
ErbB	Erb-B2 Receptor Tyrosine Kinase
ERbB4	Human Epidermal Growth Factor Receptor 4
ESCC	Esophageal Squamous Cell Carcinoma
ESRRG	Estrogen-Related Receptor Gamma
FAK	Focal Adhesion Kinase
FGFR1	Fibroblast Growth Factor Receptor 1
FNA	Fine Needle Aspiration

FOXK1	Forkhead Box K1
FOXO1	Forkhead Box Protein O1
FOXO3a	Forkhead Box O3a
GABA	Gamma-Aminobutyric Acid
GC	Gastric Cancer
GEO	Gene Expression Omnibus
GLIPR2	Glioma Pathogenesis-Related Protein 2
GLOBOCAN	Global Cancer Repository
GNAI2	Guanine Nucleotide-Binding Protein, Alpha Inhibiting Activity Polypeptide 2
GNAI3	Guanine Nucleotide-Binding Protein, Alpha Inhibiting Activity Polypeptide 3
GNAS	Guanine Nucleotide-Binding Protein, Alpha Stimulating
GNB1	Guanine Nucleotide-Binding Protein, Beta Polypeptide 1
GO	Gene Ontology
GRB2	Growth Factor Receptor-Bound Protein 2
HBEGF	Heparin-Binding EGF-Like Growth Factor
HCC	Hepatocellular Carcinoma
HER2	Human Epidermal Growth Factor Receptor 2
HER4	Human Epidermal Growth Factor Receptor 4 (Alternative Name for ERbB4)
HNSCC	Head and Neck Squamous Cell Carcinoma
HOXA6	Homeobox A6
HOXA9	Homeobox A9
IBC	Inflammatory Breast Cancer
IDC	Invasive Ductal Carcinoma
IGF1R	Insulin-like Growth Factor 1 Receptor
IL-15	Interleukin-15
ILC	Invasive Lobular Carcinoma
IRS2	Insulin Receptor Substrate 2

JAK-STAT	Janus Kinase/Signal Transducer and Activator of Transcription
KEGG	Kyoto Encyclopedia of Genes and Genomes
Ki-67	Marker of Proliferation Ki-67
LCIS	Lobular Carcinoma in Situ
LHX2	LIM-homeobox gene 2
lncRNAs	Long non-coding RNAs
LSCC	Laryngeal Squamous Cell Carcinoma
MAPK	Mitogen-Activated Protein Kinase
MEF2C	Myocyte Enhancer Factor 2C
MF	Molecular Function
miRDB	MicroRNA Database
miRNAs	MicroRNAs
MMP-9	Matrix Metallopeptidase 9
MRI	Magnetic Resonance Imaging
mTOR	Mammalian Target of Rapamycin
MYC	MYC Proto-Oncogene, bHLH Transcription Factor
MYCL	MYC-Like Proto-Oncogene
ncRNAs	Non-coding RNAs
NRG1	Neuregulin 1
NRG2	Neuregulin 2
NRG3	Neuregulin 3
OC	Ovarian Cancer
OS	Osteosarcoma
OSCC	Oral Squamous Cell Carcinoma
PCR	Polymerase Chain Reaction
PDAC	Pancreatic Ductal Adenocarcinoma
PDCD4	Programmed Cell Death 4
PET	Positron Emission Tomography
PI3K/AKT/mTOR	Phosphoinositide 3-Kinase/Protein Kinase B/mammalian Target of Rapamycin
PI3K-Akt	Phosphatidylinositol 3-Kinase-Akt
PIM1	Proviral Integration of Moloney virus 1

PKM2	Pyruvate Kinase M2
PLK1	Polo-like Kinase 1
PPI	Protein-Protein Interaction
PPIN	Protein-Protein Interaction Network
PR	Progesterone Receptor
pre-miRNA	Precursor miRNA
pri-miRNA	Primary miRNA
PRL-3	Phosphatase of Regenerating Liver-3
PTEN	Phosphatase and Tensin Homolog
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
RAD51	AD51 Recombinase
RAS	Rat Sarcoma
RISC	RNA-Induced Silencing Complex
RNU6NB	Small Nuclear RNA U6 Non-coding B
ROX	Reference Rhodamine X
SKP2	S-phase Kinase-Associated Protein 2
SOX15	SRY-box Transcription Factor 15
SOX4	SRY-Box Transcription Factor 4
SOX6	SRY-Box Transcription Factor 6
STAT3	Signal Transducer and Activator of Transcription 3
STRING	Search Tool for the Retrieval of Interacting Genes/Proteins
TCGA	The Cancer Genome Atlas
TFs	Transcription Factors

CHAPTER 1
INTRODUCTION

Breast cancer (BC) is a common invasive neoplasm in women and has continuously attracted international attention because of its high mortality rate among women worldwide (Harbeck et al., 2019). For example, in 2020, the World Health Organization (WHO) reported that breast cancer accounted for 24.5% of all cancers, with 2,3 million cases and 685.000 deaths among women worldwide. Researchers expected that with the population growth in 2040, breast cancer cases might reach 3 million with a mortality of 1 million (Arnold et al., 2022).

Despite the advances in early diagnosis and treatment of breast cancer, a third of patients still experience cancer progression and metastasis even during the treatment. In the shed of the fact that breast cancer is heterogeneous at the molecular level, breast cancer tumors pose formidable challenges to various therapeutic innovations. While existing chemotherapeutic regimens have improved survival rates, there is a propensity for some breast tumors to develop resistance post-initial response in addition to the accompanying side effects. Consequently, it is imperative to elucidate molecular targets within breast cancer that may be pivotal for the genetic modulation of the disease and sensitize tumors to therapeutic agents (Leone & Leone, 2015).

MicroRNAs (miRNAs) are a class of non-coding RNA (ncRNAs), single-stranded that include 19 to 24 nucleotides approximately, and changes in their expression levels have been implicated in the pathogenesis of many diseases, most notably “cancer.” Accumulating evidence has unveiled the capacity of miRNAs to induce anomalies in gene expression, culminating in the initiation, progression, and metastasis of cancer as well as resistance to treatment. These miRNAs are known to modulate key signaling pathways by upregulation or downregulation, engendering alterations in the expression profiles of critical genes such as oncogenes and tumor suppressor genes and fostering tumorigenesis (Reddy, 2015).

It is commonly acknowledged that myriad cellular signaling pathways and molecular networks regulate cell cycle progression, genomic stability, and gene expression

profiles. That means signaling pathways affect cellular homeostasis, survival, and apoptosis, leading to cancer formation, progression, and metastasis (Jang & Lee, 2021). Research endeavors have shed light on the role of miRNAs in these pathways, with several investigations unraveling correlations between miRNA dysregulation and breast cancer phenotypes regarding cancer initiation, progression, and metastasis (Reddy, 2015) MiR-1294, miR-551a, miR-1238, and miR-4534 have been reported to be dysregulated in different cancer types based on previously published studies (Li et al., 2012; Nip et al., 2016; Wu et al., 2020; Yin et al., 2019).

This study aims to explore the roles of miR-1294, miR-551a, miR-1238, and miR-4534 in the development of breast cancer. Through bioinformatics tools, this research seeks to provide a comprehensive overview of these microRNA target genes and better understand their specific cellular functions, molecular targets, and mechanistic dynamics within the broader genomic landscape of breast cancer.

1.1 Breast Cancer

Breast cancer is a heterogeneous disease on a molecular level that occurs when abnormal cells in breast tissue grow uncontrollably, forming tumors. Types of breast cancer can be distinguished based on their histological and clinical characteristics. Typically, abnormal cell growths begin in the ducts or lobules of the glands, mainly in the milk ducts, before spreading to adjacent breast tissues. At this stage, the cancer is called “In-situ,” which is the benign form of cancer. After cancer disseminates to the other tissues forming the invasive form of cancer is known as “Carcinoma.” The carcinoma form might spread to lymph nodes or other organs through “metastasis,” posing health risks and life-threatening consequences (Harbeck et al., 2019).

1.1.1 Breast Anatomy

The breasts, bilateral structures present in both males and females, are positioned anteriorly on the chest wall, above the pectoralis major and serratus anterior muscles. Breasts exhibit differential development in females after puberty, largely driven by hormonal influences. Each breast is composed of the body, which constitutes the main bulk, and the axillary tail extends towards the axilla, delimited by the lateral border of the sternum, the mid-axillary line, between the second to sixth ribs.

The nipple is located at the center of each breast and has a cylindrical structure surrounded by a pigmented area known as the “areola,” which is equipped with

sebaceous glands secreting an oily substance for lubrication during breastfeeding. The areola, which surrounds the nipple, is equipped with sebaceous glands secreting an oily substance for lubrication during breastfeeding. It also serves as the conduit for the lactiferous ducts, responsible for milk transportation from the mammary glands to the exterior. These mammary glands are at the core of breast anatomy, specialized sweat glands pivotal in milk production during lactation.

Encircled by myoepithelial cells, these alveoli facilitate the expulsion of milk into the lactiferous ducts. These ducts then converge and expand, culminating in lactiferous sinuses, where milk is stored before excretion through the nipple to infants. The breast is also composed of adipose tissue, connective tissue, blood vessels, nerves, and lymphatic vessels. Adipose tissue variability determines breast size, which varies among women, while connective tissue forms suspensory ligaments, offering structural support. Vascular supply and venous drainage are orchestrated by branches originating from the internal thoracic, lateral thoracic, and intercostal arteries and veins.

Nervous innervation is derived from the anterior and lateral cutaneous branches of the fourth to sixth intercostal nerves. Coming to the lymphatic drainage system, approximately 75% is directed towards the axillary lymph nodes, with the remaining 25% channeling towards the parasternal and other regional nodes. This system in breast anatomy is responsible for regulating fluid and immune function. Moreover, the cancer cells spread through the lymph system in breast cancer metastasis, as lymphatic vessels can help cancer cells reach regional lymph nodes and spread to other parts of the body (Bazira et al., 2022; Darlington, 2015). Figure 1.1

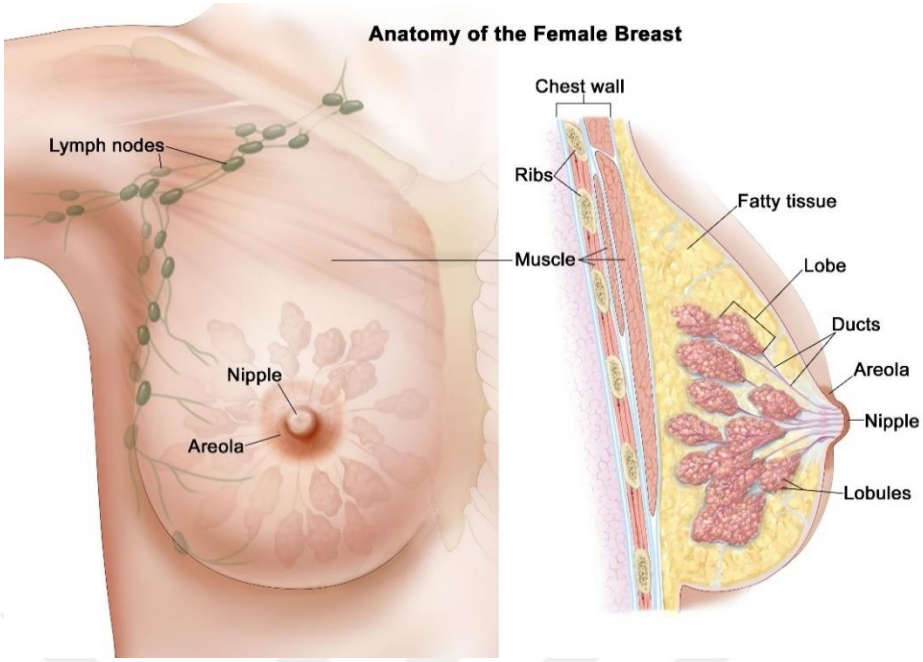

Figure 1.1. Breast anatomy (National Cancer Institute, n.d.)

1.1.2 Breast Physiology

The breast's main function is to develop the glands that support reproduction by producing milk and regulating hormones. The hormones, like estrogen progesterone and growth hormone, control breast tissue growth and duct branching. When it comes to nourishing newborns, the mammary glands are involved in the process of lactation by an endocrine hormone "prolactin." Prolactin, secreted by the pituitary gland, regulates milk production after childbirth by stimulating the cells within the glands to produce and secrete milk (Bazira et al., 2022).

The initiation of breastfeeding involves the baby suckling at the nipple, which triggers a reflex called the "letdown reflex." This reflex stimulates oxytocin release when nipple stimulation causes contraction of cells around alveoli, allowing milk to flow easily through ducts. Coming to estrogen and progesterone hormones produced by ovaries influence breasts' size, vascularity, and sensitivity. Moreover, these hormones are involved in controlling cycles and can impact cyclic changes that occur in breast tissue (Bazira et al., 2022).

1.1.3 Breast Cancer Etiology

The cause of breast cancer can result from a combination of genetics and non-genetic factors. The inherited mutations of genes account for about 5 to 10% of breast cancer

cases, most importantly, mutations in the breast cancer gene 1 (BRCA1) and breast cancer gene 2 (BRCA2) in addition to other less common genes like phosphatase, tensin homolog (PTEN), and tumor protein P53 (TP53).

Having a family history of breast cancer among relatives doubles the risk while carrying mutations in the BRCA1 or BRCA2 gene increases the risk to reach 70%. Race and ethnicity might be another possible risk of breast cancer; for example, Caucasian and African American women under 45 face higher incidence and mortality rates.

Being a woman increases breast cancer 100 times as the disease is more prevalent in women compared to men. Furthermore, advancing age is another factor with an increased incidence rate after reaching 50 years old. Hormonal factors such as the onset of menstruation, menopause, never giving birth (nulliparity), and hormone replacement therapy are recognized as crucial for initiating and increasing the risk of breast cancer.

Factors such as lifestyle choices and personal behaviors like contraception, excessive alcohol consumption, obesity, and smoking raise the breast cancer risk, even without a family history of breast cancer. Furthermore, reproductive factors like not having children, giving birth at an age, starting menstruation early, and experiencing menopause later can also boost the chance for women to have breast cancer (Harbeck et al., 2019).

1.1.4 Breast Cancer Epidemiology

Breast cancer remains the major cause of cancer-related fatalities in women. In 2020, breast cancer constituted 24,5% of all cancer cases, with 2.26 million diagnosed women and 685,000 deaths (WHO, 2023.).

As stated by the Global Cancer Repository "GLOBOCAN" report about the prevalence of breast cancer in populations over five years "2016-2020," stating that breast cancer incidence was approximately 30.3% among cancer types and was responsible for 15.5% of deaths of females worldwide. Looking into ethnicity, Asian females were reported to have the highest breast cancer incidence rate, 42.2%, followed by African females with a rate of 25.8% and a 7.9% incidence rate in Latin America and the Caribbean women (Łukasiewicz et al., 2021).

Concerning mortality rates, African patients diagnosed with breast cancer experienced 12.5%, while patients from Latin America and the Caribbean had an estimated mortality rate of 8.5% (Sung et al., 2021). Survival rates in regions with established healthcare systems were higher over five years for localized and regional breast cancer cases than in less developed countries, highlighting the importance of ensuring access to high-quality healthcare services for everyone.

Projections suggest that by 2030, the breast cancer incidence rate will increase worldwide, with approximately 2.7 million and about 0.87 million associated deaths annually (Łukasiewicz et al., 2021), while in 2040, the cases are anticipated to reach 3 million with a mortality of 1 million (Arnold et al., 2022).

Breast cancer is also a common health problem in Türkiye, like the case on the global level. According to the official statistics released in 2018 by the Turkish Ministry of Health, 24,518 women were diagnosed with breast cancer. The incidence rate of breast cancer was 26% of the other cancer types and is expected to reach 25,351 in 2021 (Turkish Ministry of Health, 2018). According to the Globocan report, in 2020, there were 24,175 breast cancer cases, which accounted for 10.3% of all cancer cases (Figure 1.2) (The Global Cancer Observatory, 2021).

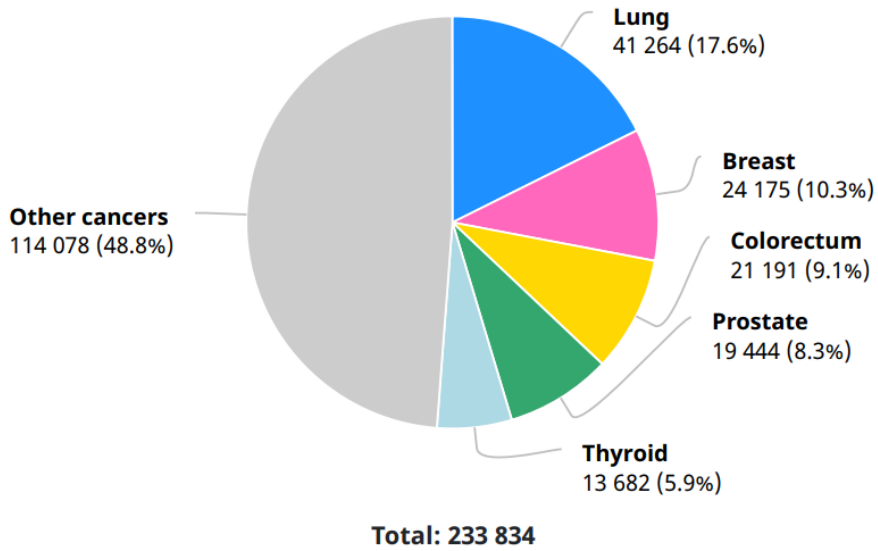

Figure 1.2. Cancer Types Incidence Rate in Türkiye in 2020 based on Globocan (The Global Cancer Observatory, 2021)

1.1.5 Staging in Breast Cancer

Breast cancer staging represents the cancer development that employs the (TNM) system, a vital framework for precisely characterizing the extent and spread of the cancer defined by the American Joint Committee on Cancer (AJCC). The TNM acronym stands for Tumor (T), Node (N), and Metastasis (M), collectively encompassing critical indicators for diagnosis and treatment planning. The updated TNM system, active in 2018, utilizes pathological and clinical staging methodology. The pathological staging expresses the process of examining tissue after surgical removal. Clinical one relies on biomarkers in staging the cancer (American Joint Committee on Cancer, 2018).

Tumor (T) is the element that evaluates the primary tumor's size and extent. It ranges from "T0," which means no discernible tumor, to "T4", an advanced tumor potentially involving the chest wall or skin. While "Tis" indicates a non-invasive carcinoma within ducts or lobules, and "T1" signifies a small invasive tumor without lymph node involvement. Transitioning to Node (N) that refers to Node involvement pertains to the presence and extent of cancer within nearby lymph nodes. It spans from "N0, "meaning no regional lymph node involvement, to "N3," which indicates extensive lymph node involvement. For instance, N0 denotes the absence of nodal metastasis, while N1 to N3 indicates increasing degrees of lymph node involvement. Metastasis (M) indicates whether cancer cells have spread to distant organs. "M0" signifies no distant metastases, while "M1" denotes the presence of distant metastases. For instance, M0 indicates no distant spread, while M1 signifies the presence of metastatic disease. The biomarkers used to identify the clinical staging are the status of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). Meaning if the value of these biomarkers is positive or negative. Also, clinical staging considers the grade of cancer (G) represented by Ki-67, which refers to how the cancer cells look like the normal ones (Giuliano et al., 2018).

The numeric staging (0, 1, 2, etc.) denotes the progression of the cancer as follows:

a) **Stage 0 (Tis, N0, M0):** Denotes non-invasive carcinoma confined to ducts or lobules, with no evidence of lymph node involvement or distant metastasis of cancer. This stage carries a highly favorable prognosis.

b) **Stage I (T1, N0, M0):** Small, invasive tumors without lymph node involvement or distant spread are identified. Early detection and intervention are pivotal, leading to favorable outcomes (Giuliano et al., 2018).
c) **Stage II (T2 or T3, N0, M0):** Encompasses larger tumors or those with local extension; however, still confined to the breast without involving lymph nodes or distant sites. Treatment necessitates a comprehensive approach, including surgery, radiation, chemotherapy, and hormone therapy (Giuliano et al., 2018).
d) **Stage III (T0-3, N1-3, M0):** Reflects advanced disease with extensive lymph node involvement, yet the primary tumor may vary in size. Multimodal therapies are essential to target the primary tumor and affected lymph nodes (Giuliano et al., 2018)
e) **Stage IV (T4, any N, M1):** Indicates metastatic disease, with cancer cells disseminated to distant organs. Treatment focuses on palliation and improving quality of life, involving systemic therapies and targeted treatments (Giuliano et al., 2018).

1.1.6 Breast Cancer Types

The categorization of a type of breast cancer depends on the particular cells within the breast that experience a malignant transformation. Breast cancer is broadly classified into two categories based on its severity: invasive/carcinoma (advanced stage), representing the severe form, and non-invasive (early stage), which is treatable, also known as "in situ," refers to the early stage of cancer. Each breast cancer type has its unique biological behavior and response to treatment modalities (Weigelt et al., 2010).

1.1.6.1. Ductal carcinoma in situ (DCIS)

DCIS is an early-stage cancer that occurs when abnormal cells are discovered within the breast milk duct lining but have not spread beyond the ducts into the surrounding breast tissue. It is a non-invasive form of breast cancer that makes up approximately 20% of all breast cancer cases (Weigelt et al., 2010).

1.1.6.2. Invasive ductal carcinoma (IDC)

IDC represents the most common form of breast cancer, making up about 70–80% of diagnoses. It takes place in the milk ducts and involves the spread of abnormal cells away from their initial location. This type of cancer can also metastasize to other parts of the body, which is why it is called infiltrative ductal carcinoma(Weigelt et al., 2010).

1.1.6.3. Lobular carcinoma in situ (LCIS)

LCIS is considered a non-invasive form of breast cancer, occurs when there are abnormal cells in the lobules. Approximately 1% of all breast cancer cases are of this type. While it seldom evolves into invasive cancer, the presence of LCIS in one breast increases the likelihood of developing breast cancer in either one of the breasts (Weigelt et al., 2010).

1.1.6.4. Invasive lobular carcinoma (ILC)

ILC originates in the milk-producing lobules of the breast. It can spread to surrounding tissues and, in some cases, by way of blood and lymph systems, reaching other parts of the body. This type makes up about 10–15% of breast cancer diagnoses, which is why it is considered the second most common type of invasive breast (Weigelt et al., 2010).

1.1.6.5 Triple-negative breast cancer (TNBC)

TNBC is a subtype that is identified by the inexistence of estrogen receptors (ER-), progesterone receptors (PR-), or HER2, which causes traditional hormone therapies to be ineffective. TNBC makes up 10–15% of breast cancer cases and usually does not respond to hormonal therapy owing to the lack of hormone receptors (Weigelt et al., 2010).

1.1.6.6 Inflammatory breast cancer (IBC)

IBC is an aggressive type of breast cancer whose cases account for less than 5% of all cases. It is characterized by fast-growing cancer cells infiltrating the skin and lymph vessels of the breast. Differing from other types, IBC lacks a distinct, palpable tumor. Instead, symptoms become visible when cancer cells obstruct lymph vessels (Weigelt et al., 2010).

1.1.7 Breast Cancer Diagnosis

Diagnosing breast cancer requires a manifold approach to make sure that effective treatment and improved patient outcomes are attained. Starting with the physical examination, health staff check breasts, lymph nodes, lumps, skin changes, nipple discharge, and areas of hardness or tenderness. This hands-on examination is the first attempt of inquiry into detecting any palpable abnormalities that provide instant information about the physical traits of the breast tissue (Jafari et al., 2018).

Mammography is an essential tool for early cancer detection. It is done by low-dose X-ray imaging of breast tissue, focusing on identifying microcalcifications and early-stage tumors. Despite this method's effectiveness, it may not provide sufficient sensitivity for women with dense breast tissue; supplementary techniques are required in these cases (Sardanelli et al., 2010). Moving to 3D mammography, known as "Tomosynthesis," is an advanced mammography technique. Tomosynthesis provides a three-dimensional view of breast tissue. This technology is promising in increasing detection rates, this being especially true in women with dense breast tissue, where traditional mammography may prove ineffective (Jafari et al., 2018). Shifting to the beast magnetic resonance imaging (MRI) method that utilizes strong magnetic fields and radio waves to obtain detailed images. It especially proves valuable in assessing high-risk patients and cases of dense breast tissue. The sensitivity of MRI compared to mammography is greater, especially in detecting invasive cancers (Jafari et al., 2018; Sardanelli et al., 2010).

Histopathology tests like fine needle aspiration (FNA) and core needle biopsy (CNB) are essential techniques in diagnosing breast lesions. Cellular material is extracted from FNA for a preliminary microscopic assessment of cell structure. CNB, on the other hand, presents a more detailed view of the lesion's structure and cellular composition, which is rather helpful for complex lesions. CNB is the preferred method for definitive diagnosis and detailed characterization of breast lesions, which provides important information for treatment planning (Hoda, 2007). Furthermore, liquid biopsy is an innovative and minimally invasive diagnostic approach in breast cancer assessment. It is done by analyzing the circulating tumor markers or cell-free DNA (cfDNA) particles found in bodily fluids, such as blood or plasma. These markers may include mutated genes, chromosomal rearrangements, or abnormal protein expressions, which provide critical genetic and molecular information about the tumor. Liquid biopsy enables real-time monitoring of the tumor's genetic profile, offering insights into its evolution and the potential emergence of drug resistance (García-Saenz et al., 2017).

Assessing cancer metastasis is essential for staging and the treatment plan. Bone scans and computerized tomography (CT) scan techniques are adopted for this purpose. Other techniques, such as ultrasound and positron emission tomography (PET), may be employed to further evaluate breast lesions and assess metastasis. Detecting any

potential metastatic spots guarantees an extensive understanding of the disease's reach and guides treatment strategies accordingly (Jafari et al., 2018). Genetic testing for inherited mutations, especially in genes like BRCA1 and BRCA2, offers crucial insights for risk assessment, treatment planning, and family counseling. Diagnosing individuals with a hereditary predisposition to breast cancer is important for customized treatment strategies and long-term management (Jafari et al., 2018). Blood tests in breast cancer diagnosis involve the analysis of specific biomarkers and substances indicative of tumor presence, type, and aggressiveness. These tests provide a non-invasive approach to gathering valuable information about the disease's status, aiding in early detection, treatment planning, and therapeutic efficacy monitoring (Duffy, 2006; Jafari et al., 2018).

1.1.8 Breast Cancer Biomarkers

Tumor markers are substances produced by the body in response to cancer cells and play a crucial role in assessing breast cancer's progression and response to treatment. These markers are measurable in bodily fluids like blood, offering valuable insights into therapeutic interventions' effectiveness (Barzaman et al., 2020). Starting with Cancer antigen 15-3 (CA15-3), a protein produced by breast cancer cells, can be measured in the bloodstream. In an individual, the levels of CA15 3 are typically below or equal to 25 U/mL. However, elevated levels of CA15-3 in cases of metastatic breast cancer indicate an advanced stage of the disease. CA15-3 is utilized to assess the effectiveness of treatments. If the levels decrease, it suggests a response to treatment, whereas higher levels could signal a recurrence (Song et al., 2020).

Moving to carcinoembryonic antigen (CEA), a glycoprotein found to be elevated in various cancers, including breast cancer. CEA typically presents in low levels in the blood of healthy adults (2.5 ng/mL) and shows elevated levels in patients with advanced breast cancers, usually stage 3 or stage 4. Conversely, patients in the early stages of breast carcinoma exhibit lower levels of CEA expression. This correlation between CEA levels and breast cancer stage provides valuable insights into disease severity. Post-effective breast cancer treatment usually gradually decreases CEA levels (Nam et al., 2019).

In addition, cancer antigen 125 (CA-125), a crucial blood test, aids in predicting the severity of breast cancer. High CA-125 levels indicate metastatic breast cancer and the

likelihood of cancer recurrence. Furthermore, the CA-125 test plays a vital role in post-treatment assessments, determining the effectiveness of the treatment protocol. Notably, CA-125 levels show a positive correlation between breast and ovarian cancer, making it a significant diagnostic, predictive, and prognostic biomarker in breast cancer oncology. The normal CA-125 range is less than 35 U/mL (Li et al., 2020).

Humanized epidermal growth factor receptor 2 (C-erbB-2), commonly known as HER 2, is an antigen protein in breast cancer. While under-expressed in normal cells, it is overexpressed in cancerous cells, making it a crucial tumor marker for cancer identification (Prat et al., 2020). is a pivotal protein present on the surface of breast cells. When overexpressed, indicating an excess of the HER2 gene, it leads to aggressive tumor growth and an elevated risk of recurrence. Approximately 20–25% of breast cancers are categorized as HER2-positive, signifying an abundance of HER2 receptors. This often results in more aggressive tumor behavior, necessitating specialized treatment approaches. Normal HER2 levels are typically determined to be 0 to 2+ (negative), but values of 3+ or higher are considered HER2-positive. Testing HER2 status involves analyzing tissue samples from biopsies or surgical procedures. The results of HER2 testing are instrumental in guiding treatment decisions. Targeted therapies like trastuzumab (Herceptin) have significantly improved outcomes for individuals with HER2-positive breast cancers (Kabakçı et al., 2021; Wolff et al., 2018).

Another biomarker known as a marker of proliferation (Ki-67), a nuclear antigen, is a pivotal proliferation biomarker. The MKI67 gene encodes Ki-67, which is essential for cell growth. Elevated levels of Ki-67 expression indicate extensive tumor progression, while low levels signify early-stage cancer or the absence of cancer. WHO categorizes Ki-67 into grades 1 (G1), 2 (G2), and 3 (G3). G1 denotes slow-growing tumors that are less likely to spread to other areas of the breast. G2 represents a state where tumors are more likely to grow and invade other areas. Additionally, moderately differentiated tumors can be analyzed through G2. In G3, abnormal cells grow rapidly, indicating poorly differentiated cancer cells. The Ki-67 index test quantifies the level of actively dividing cells in the breast that express the Ki-67 protein. A Ki-67 index of less than 2% corresponds to G1, where 2% of cells exhibit division. G2 indicates that 3 to 20% of cells divide in every 100 tumor cells, while a Ki-67 index of ≥ 20% corresponds to G3, denoting rapid cell growth. This biomarker

is instrumental in determining the aggressiveness and progression of breast cancer. Normal Ki-67 levels in normal breast tissue are less than 3% (Menon et al., 2019).

The status of the hormonal receptors, such as estrogen receptors (ER), is a pivotal biomarker in assessing breast cancer. This biomarker identifies the presence of estrogen receptors on the surface of cancer cells, signifying their responsiveness to hormonal therapies. In breast cancer, ER-positive tumors exhibit a higher affinity for estrogen, promoting their growth and proliferation. Conversely, ER-negative tumors lack these receptors, rendering them less susceptible to hormone-based treatments. Determining ER status aids clinicians in tailoring therapeutic strategies, particularly in administering endocrine therapies like tamoxifen and aromatase inhibitors. According to guidelines established by the American Society of Clinical Oncology (ASCO), tumors with greater than or equal to 1% of cells demonstrating positive ER staining are considered ER-positive, while those with less than 1% positivity are classified as ER-negative (Nicolini et al., 2018).

Similarly, progesterone receptor (PR) is a vital biomarker in breast cancer, determined through IHC analysis. Tumors are PR-positive if at least 1% of cell nuclei exhibit positive staining. PR-positive tumors often respond well to endocrine therapies, while PR-negative tumors may require different approaches. PR is found in over 50% of ER-positive breast tumors but rarely in ER-negative ones. It is considered a key prognostic factor, positively correlating with overall survival, treatment failure, or progression. Lower PR levels in ER-positive tumors signal more aggressive disease, poorer prognosis, and higher recurrence risk (Allison et al., 2020).

1.1.8 Breast Cancer treatment

Breast cancer treatment strategies vary based on factors such as the type, stage, and size of the cancer. The available options encompass surgery, chemotherapy, radiotherapy, and hormone therapy. In the realm of surgery, a range of approaches are employed, each tailored to the invasiveness of breast cancer. Lumpectomy, for instance, selectively removes the tumor and surrounding healthy cells, making it suitable for smaller tumors. In the case of larger tumors, chemotherapy is administered prior to lumpectomy to facilitate tumor shrinkage before removal (Mutebi et al., 2020). Moving on to mastectomy this procedure involves the comprehensive removal of the affected breast, encompassing lobules, fatty tissue, ducts, and skin. Additionally,

sentinel node biopsy focuses on removing a limited number of lymph nodes to assess metastasis. If the nodes prove clear, further removal is not deemed necessary. Axillary lymph node dissection takes the process a step further by removing several lymph nodes. Should cancer be detected in sentinel nodes, additional nodes from the armpit may be excised. Moreover, individuals with a family history of breast cancer might consider a prophylactic bilateral mastectomy to mitigate future risks (Lovelace et al., 2019).

Meanwhile, radiation therapy deploys high-energy X-ray beams to eradicate cancer cells. This treatment can be administered either externally or internally post-surgery, directly targeting any remaining cancerous cells. Generally, external radiation follows a lumpectomy. It may be provided either before (neoadjuvant) or after (adjuvant) surgery. It is imperative to note that radiation therapy may lead to side effects such as fatigue, skin changes, and, in rare cases, long-term effects on the heart or lungs and potential secondary cancers in other organs (He et al., 2018).

The chemotherapy treatment method is designed to target fast-growing cells with drugs administered via injection or orally. Neoadjuvant therapy, administered before surgery, effectively reduces tumor size, aiding in complete removal. Adjuvant chemotherapy, administered post-surgery, targets potentially undetectable cancer cells, significantly reducing recurrence risk, especially for high-risk or metastatic cases. However, it is crucial to know that chemotherapy may entail side effects, including nausea, fatigue, hair loss, nail color changes, nerve damage, mouth sores, and hair loss (Abotaleb et al., 2018).

Finally, hormone therapy, “endocrine therapy,” intervenes by disrupting hormone receptors in cancer cells. This action either prevents hormones from binding or inhibits hormone production. Currently, three types of drugs are given to hormone-sensitive breast cancer patients: selective estrogen receptor modulators, selective estrogen receptor down regulators, and aromatase inhibitors. The crosstalk between estrogen receptors and growth factors causes endocrine resistance. Due to de novo resistance, some patients with breast cancer who are estrogen receptor-positive cannot receive endocrine therapy. Whereas acquired resistance was reported for patients who were initially positively responding to endocrine therapy (Giuliano et al., 2019). Hormone therapy resistance is a significant hurdle in treating hormone-sensitive breast carcinomas. While ER and PR-targeted therapy is effective, the interplay between

estrogen and growth factor receptors contributes to endocrine resistance. Combined hormonal therapy targeting both receptors shows promise. Triple-negative breast cancer that lacks estrogen, progesterone, and HER2 receptors has a challenging prognosis as it is resistant to hormonal therapy. Current therapies rely on targeting these receptors, highlighting the need for additional molecular markers. Endocrine therapy faces resistance due to de novo or acquired factors, limiting effectiveness (Al-Mahmood et al., 2018). Therefore, identifying novel target molecules and combining therapies could illuminate resistance mechanisms, offering potential solutions in breast cancer treatment.

1.1.9 Signaling Pathways in Breast Cancer

In the vast intricacies of cellular communication, signaling pathways serve as the conduits for information transfer and processing, guiding every aspect of cellular function. At its core, a signaling pathway is a sequential cascade of molecular events initiated by external cues that facilitate communication between and within cells (Alberts et al., 2002).

Starting with ligands, these molecular harbingers often originate from outside the cellular environment. Their primary role is to bind specifically to cellular receptors, instigating the entire signaling process. Receptors, typically proteinaceous in nature and anchored on the cell surface, are tailored to recognize these specific ligands. These receptors undergo conformational changes upon ligand binding, creating a domino effect of intracellular events (Lemmon & Schlessinger, 2010).

Genes are central to the progression of these pathways, the foundational blueprints of life. Genes encode for proteins, many of which are integral components of these signaling cascades. Some genes produce the receptors that detect the initial signals, while others might encode for intracellular molecules that relay these signals further. Moreover, the final cellular responses, such as transcriptional changes, are governed by the activation or repression of specific target genes, underscoring the gene-centric nature of cellular signaling. This cascade does not just flow unidirectionally; it comprises multiple branches, feedback loops, and checkpoints. Effector proteins, often the terminal components of these pathways, transduce the processed signal into tangible cellular responses. These could manifest as changes in gene expression,

alterations in metabolic pathways, or even behavioral adjustments of the cell in response to its environment (Alberts et al., 2002).

The span of cellular signaling is not confined to the individual cell. Cells communicate over varying distances: from autocrine signaling, where cells respond to signals, they produce themselves, to paracrine signaling, affecting nearby cells, and even endocrine signaling, where molecules like hormones traverse through the bloodstream to influence distant cells. Juxtacrine signaling adds another layer to this complexity, necessitating direct cell-to-cell contact for information transfer (Lemmon & Schlessinger, 2010). The ramifications of signaling pathways are monumental. They regulate cell growth, differentiation, and even programmed cell death. They allow cells to sense and adapt to changes, whether it is a fluctuating nutrient concentration or the presence of a foreign toxin. In multicellular organisms, they ensure that the cacophony of billions of cells translates into a harmonious symphony, enabling tissues and organs to function seamlessly (Alberts et al., 2002).

Nevertheless, as in any sophisticated system, aberrations can be catastrophic. Dysregulated signaling can culminate in unchecked cellular proliferation, a precursor to cancer. Such misdirection might confuse the immune system, leading to self-targeting and autoimmune disorders. Given their profound influence, it is no surprise that signaling pathways have become the bullseye for therapeutic interventions. Drugs meticulously designed to modulate specific components of these pathways can potentially reverse, halt, or even prevent diseases (Lemmon & Schlessinger, 2010). In the cancerous milieu, malignant cells employ these signaling tactics for survival, recruiting vascular support, or eluding immune surveillance. This interaction, reflecting an orchestra's engagement with its audience, can dictate disease progression or regression (Hanahan & Weinberg, 2011). Prominent signaling pathways in breast cancer are shown in (Figure 1.3) and include the following: Phosphoinositide 3-kinase/Protein Kinase B/ mammalian target of rapamycin (PI3K/AKT/mTOR) pathway. A vital signaling mechanism that connects oncogenes with various receptor types and governs numerous crucial cellular activities. This pathway is considered the most activated signaling pathway in human cancer (Huang et al., 2014).

The Rat Sarcoma (RAS) pathway is also a crucial cellular signaling cascade that regulates various cellular processes, such as growth, differentiation, and survival. Ras genes are among human cancers' most mutated oncogenes. Mutations in Ras genes

can lead to the production of constitutively active Ras proteins. A constitutively active Ras protein leads to continuous signaling down its pathways, even without external growth signals, resulting in unchecked cell proliferation, resistance to apoptosis (programmed cell death), and other cancer-associated behaviors (Stephen et al., 2014). The Janus kinase/signal transducer and activator of transcription (JAK-STAT) pathway consists of various intracellular molecules crucial for signal pathways activated by growth factors and cytokines. It serves as a bridge between extracellular signals and intracellular transcriptional responses, playing a significant role in immune cell functions and inflammatory processes (O'Shea et al., 2015). The transforming growth factor beta receptor 1 (TGFBR1) pathway highlights the adaptability of signaling, especially in conditions such as hepatocellular carcinoma, showcasing its role in modulating cellular growth and differentiation in different cancer progression (Moore-Smith & Pasche, 2011).

The Wnt and hippo pathways are arbitrators in tissue regeneration and organ size control, foundational for maintaining tissue equilibrium (Moya & Halder, 2018). The tumor protein p53 (p53) pathway, often termed the "guardian of the genome," is indispensable for preserving genomic stability and mediates DNA repair, cell cycle arrest, and apoptosis. P53 dysregulation is common in cancers (Kastenhuber & Lowe, 2017). The focal adhesion kinase (FAK) signaling is prominent in cell migration, adhesion, and survival; it plays roles in processes like angiogenesis and wound recovery (Sulzmaier et al., 2014). The sphingolipid metabolism pathway underscores cell growth, differentiation, and stress responses, adding another dimension to the signaling matrix (Hannun & Obeid, 2017). The notch and Erb-B2 Receptor Tyrosine Kinase (ErbB) pathways are pivotal in cell fate determination and growth and play central roles in development and disease states (Yarden & Pines, 2012). The phosphatase and tensin homolog (PTEN) pathway act as a counterweight to PI3K signaling; this pathway illustrates the delicate balance of cellular processes and is crucial as a tumor suppressor (Song et al., 2012).

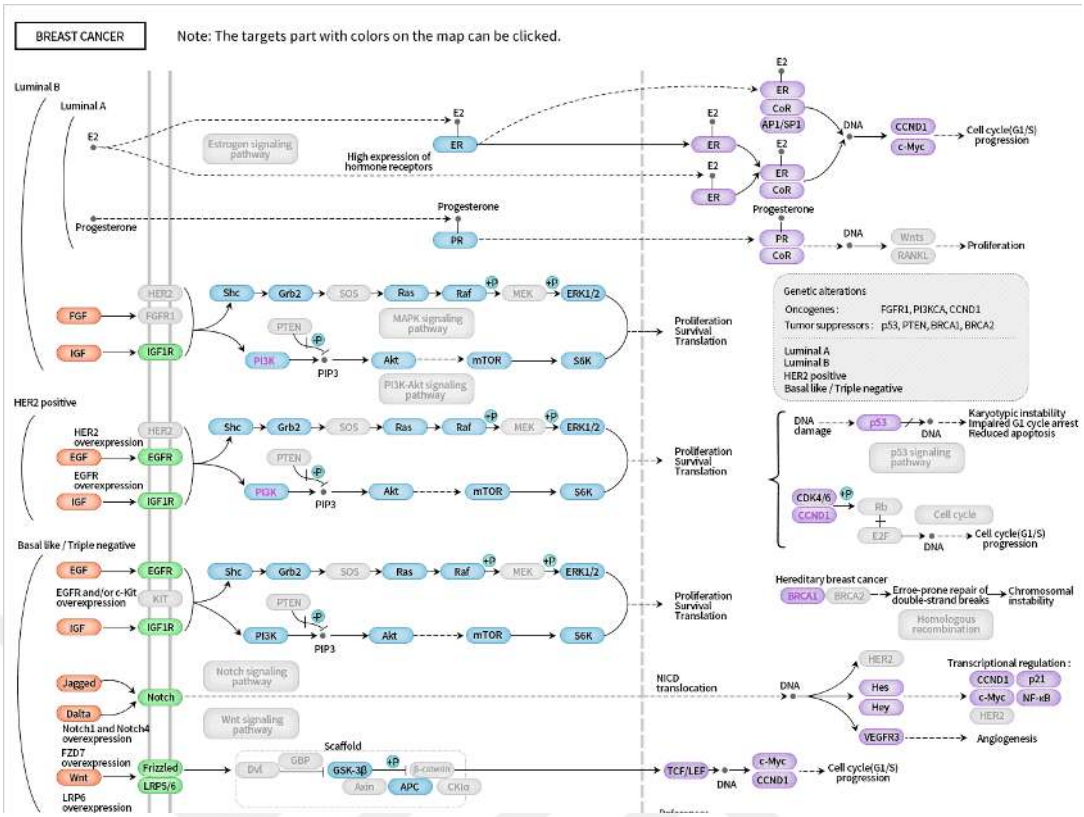

Figure 1.3. Breast Signaling Pathways (KEGG PATHWAY: Breast Cancer - Homo Sapiens (Human), n.d.)

1.2. MicroRNA

MicroRNAs (miRNAs) are small RNA molecules generated internally within the cell, serving as regulators of gene expression after the transcription process. MiRNAs are single stranded, approximately 18 to 25 nucleotides long. Unlike other RNA types, miRNAs do not undergo translation to create proteins. Instead, they are pivotal in controlling gene expression across a wide spectrum of physiological, developmental, and pathological processes. MiRNAs are found in plants, green algae, animals, and certain viruses and are actively engaged in RNA silencing and the post-transcriptional control of gene expression (Ambros, 2004). This regulatory function is achieved through the destabilization and degradation of target messenger RNA (mRNA) molecules, along with the inhibition of mRNA translation (Fabian et al., 2010). The dysregulation of miRNAs expression may lead to abnormal expression patterns of coding genes that result in changes in the encoded protein formation and, subsequently, the malfunctioning of the whole cellular machinery (Gebert & MacRae, 2018).

1.2.1 Discovery of microRNAs

In 1993, two research teams investigated the developmental cycle stages of the nematode Caenorhabditis elegans (*C. elegans)*. During their studies, they discovered the first miRNA, "lin-4." The study showed that lin-4 does not code for any protein. Instead, it produces two distinct small RNAs, with the longer one hypothesized to be a precursor to the shorter one (Lee et al., 1993). These RNAs came from lin-4 and showed antisense complementarity in several parts of the untranslated segment of the lin-14 mRNA (Lee et al., 1993; Wightman et al., 1993). The discovery of let-7, the second miRNA, also took place in *C. elegans*. However, homologues of let-7 were subsequently identified in various other species (Pasquinelli et al., 2000). Soon after, numerous miRNA genes were discovered in various species, creating a registry that serves as a comprehensive database of documented miRNAs and a definitive reference for miRNA nomenclature (Griffiths-Jones, 2004). This registry later evolved into miRBase, which is now the principal reference database for discovered miRNAs. It includes sequence information, annotations, and links to databases containing predicted and validated target genes for miRNAs (Kozomara & Griffiths-Jones, 2014). The elucidation of the RNA interference (RNAi) pathway awarded a Nobel Prize in 2006 unveiled a pivotal mechanism underpinning miRNA-mediated gene silencing. Within this context, miRNAs guide the RNA-induced silencing complex (RISC) to target mRNA molecules, inducing their degradation or translation (Zamore, 2006).

1.2.2 MicroRNA Genomics

The discovery and identification of small RNAs have improved over the years. MiRNAs have been identified in over 271 diverse organisms, including a broad spectrum of animals, plants (Jones-Rhoades et al., 2006), and even viruses (Grundhoff & Sullivan, 2011). As of the latest update miRBase version (version 23) in August 2022, the platform recorded 38,589 mature miRNAs in 271 species, including 2,742 specifics to humans. This rapid increase in the catalog is primarily attributed to the advancement and broader accessibility of sequencing techniques (miRBase, 2022) This suggests that approximately 3–4% of human genes are responsible for miRNA encoding.

Evolutionarily speaking, miRNAs demonstrate a high level of conservation (Bartel, 2004). As evidence, about 55% of miRNAs found in *C. elegans* share similarities with

those in humans (Ibáñez-Ventoso et al., 2008). The appearance of complex, multi-celled life seems to happen around the same time as the development of miRNA processes. This suggests that miRNAs play a key role in the evolution of advanced life forms (Lee et al., 2007).

In terms of genomic placement, miRNAs exhibit diversity. Nearly half of the miRNAs neighbor other miRNAs, forming polycistronic miRNA groups that are transcribed together. Some have distinct genomic regions with promoter areas (Kim et al., 2009). These miRNAs or their clusters can be embedded in exons or introns of protein-coding and non-coding genes (T. Du & Zamore, 2005). MiRNAs are expressed in all tissue types, and their profiles differ across tissues (Krol et al., 2010); also, many miRNAs change in expression during an organism's growth and often mark key development stages (Bartel, 2004). Moreover, it is widely accepted that a single miRNA type can regulate multiple target genes. In contrast, numerous miRNAs can collaboratively control the expression of a single target mRNA (Lewis et al., 2003).

1.2.3 MicroRNA Biogenesis and Mechanism of Action

MiRNA biogenesis is a complex, tightly regulated cellular process essential for post-transcriptional gene expression regulation. These small, non-coding RNA molecules play crucial roles in various biological processes, including development, differentiation, and disease progression (Bartel, 2009).

The biogenesis of miRNAs initiates in the nucleus, as miRNAs are transcribed from genomic DNA by RNA polymerase II to form a long primary miRNA (pri-miRNA) molecule. The pri-miRNA molecule contains a stem-loop (hairpin) structure, with a 33-bp double-helix stem and a terminal loop, and flanking single-strand sequences, which are several hundreds or thousands of nucleotides long (Lee et al., 2003). These pri-miRNAs are recognized and processed by a nuclear microprocessor complex consisting of the RNAse III enzyme, known as "Drosha" and its cofactor "DiGeorge syndrome critical region 8 (DGCR8)" (Denli et al., 2004). The microprocessor complex cuts the pri-miRNA near the base of the stem-loop structure, creating a precursor miRNA (pre-miRNA) that looks like a hairpin and is about 70 nucleotides long (Lee et al., 2004).

The pre-miRNA is then actively transported from the nucleus to the cytoplasm by the nuclear export factor Exportin-5 (XPO5), a member of the nuclear transport receptor

family (Lund et al., 2004). In the cytoplasm, the pre-miRNA is further processed by another RNase III enzyme called “Dicer,” along with its cofactor, “TAR RNA-binding protein (TRBP),” which is not required for effective dicing of the pre-miRNA but acts to physically bridge the Dicer to argonaute protein (Ago) (Chendrimada et al., 2005). Dicer cleaves the pre-miRNA near the terminal loop, producing a double-stranded RNA duplex consisting of the mature miRNA and its complementary passenger strand known as the “miRNA*” (star strand) (Matranga et al., 2005).

The argonaute protein wraps around the duplex, creating what's known as “RNA-induced silencing complex” or RISC. This complex includes proteins like Dicer, TRBP, and Ago. Here, Ago works with Dicer and TRBP to untangle the duplex's strands. It keeps one strand, called the guide strand (or miRNA), and lets go of the other, called the passenger strand (or miRNA*), which is then broken down (Liu et al., 2004).

The RISC, guided by mature miRNA, targets mRNA molecules through partial base-pairing interactions, leading to translational repression or mRNA degradation, ultimately regulating gene expression (Bartel, 2004). RISC is instrumental in RNA interference, with Ago at its core (Filipowicz et al., 2008). Figure 1.4 provides an overview of miRNAs modulating mRNA expression. Typically, miRNA target sites are in the 3’ UTR of mRNA but can also be in the coding region or 5’ UTR repressing mRNA translation or promoting its degradation (Bernstein et al., 2001). MiRNAs might have alternative roles, like enhancing target mRNA translation (Vasudevan et al., 2007), activating gene transcription (Place et al., 2008), or preventing mRNA translation inhibition (Eiring et al., 2010).

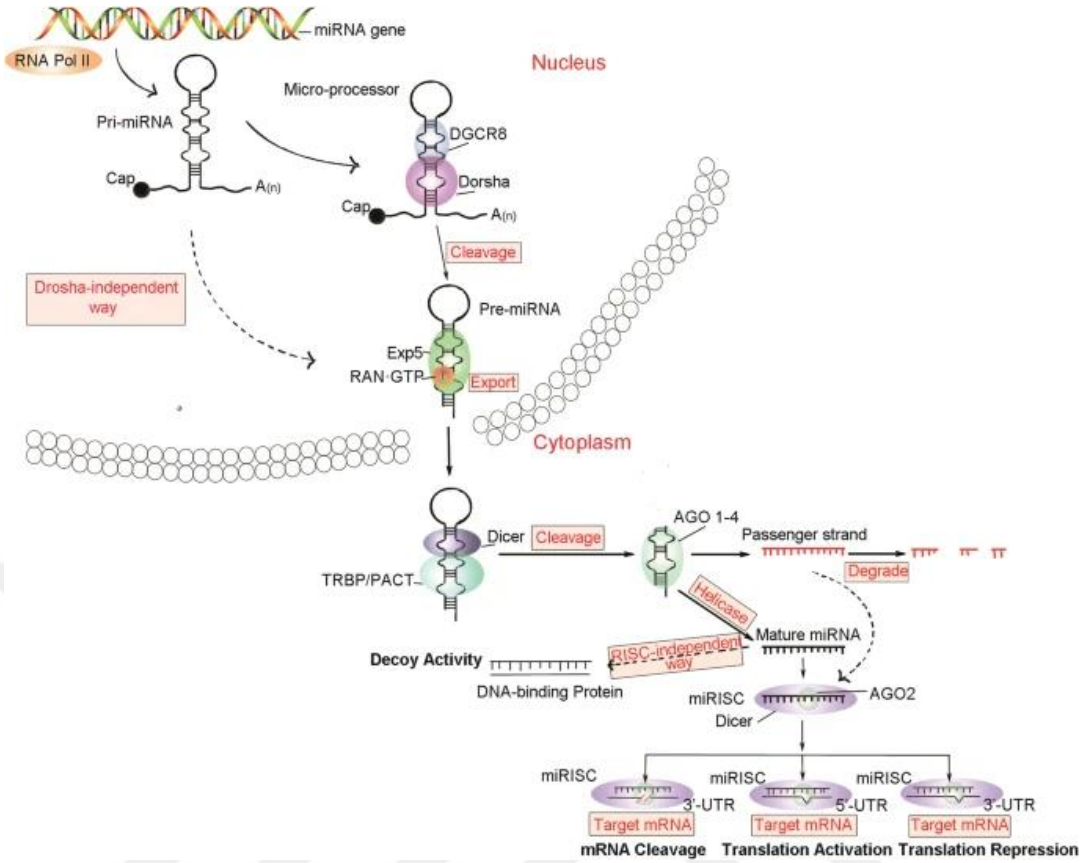

Figure 1.4. MicroRNA Biogenesis pathway (Si et al., 2019)

1.2.4 Mechanisms Regulating miRNA Transcription

MiRNAs control gene expression levels by targeting various genes affecting developmental transitions and disease progression. The complexity surrounding their regulation has been an area of focused research, given their complicated roles with numerous cellular processes (Breving & Esquela-Kerscher, 2010).

Gene transcription is often regulated like that of typical protein-coding genes, but this regulation varies across different tissues and developmental stages (Davis & Hata, 2009). Studies have shown that the promoter regions of miRNAs are like those of typical genes as they have parts like the TATA box and cytosine-phosphate-guanine (CpG) regions and are controlled by Key transcription factors (TFs) (Corcoran et al., 2009; Ozsolak et al., 2008)**.** On another front, miRNAs, like typical genes, have their expression influenced by epigenetics. For instance, acetylation of the DNA coding for miRNAs can activate their transcription, while methylation can turn off gene activity. (Sato et al., 2011)

Another aspect to consider in regulating miRNA transcription is the “transcriptional start site” (TSS). The TSS is the starting point for transcription and is surrounded by core promoters that initiate the process. MiRNAs, similar to protein-coding genes, have varied TSSs and associated core promoters, which affect the miRNAs’ transcription patterns. (Marson et al., 2008).

Furthermore, long non-coding RNAs (lncRNAs) play a critical role in regulating miRNA transcription. lncRNAs are a type of non-coding RNA that can affect both the concentration and activity of miRNAs. Some lncRNAs contain miRNAs within their structures, whereas others influence miRNA levels by interacting directly with the transcriptional apparatus. A notable mechanism involves the competing endogenous RNA (ceRNA) effect, wherein lncRNAs act as “sponges” to capture and regulate the availability of miRNAs (Quinn & Chang, 2015)

1.3 MicroRNAs and Breast Cancer

MiRNAs play a crucial role in post-transcriptional regulation and silencing of mRNA molecules by binding to complementary sequences in target mRNAs’ 3'-untranslated region (3'-UTR). Consequently, imbalances in miRNAs expression can result in altered gene expression, either through overexpression or underexpression (Loh et al., 2019). MiRNAs can be classified as oncogenes “oncomiRs” if they silence tumor-suppressor genes1.2.5, or as tumor-suppressor miRNAs “tumor suppressive miRNAs” when they counteract oncogenes. However, this distinction is not always clear-cut, as some miRNAs can influence both oncogenes and tumor-suppressor genes, with their ultimate effect depending on various factors (Barbato et al., 2017)

MiRNAs, whether tumor-suppressing or oncogenic, govern various cellular pathways critical to breast cancer development, such as the apoptotic response, cell proliferation, and metastasis. Oncogenic miRNAs, when overexpressed, silence vital tumor suppressor genes, leading to the formation and progression of breast cancer. Conversely, a decrease in the expression of tumor suppressor miRNAs can elevate oncogene levels, further promoting breast cancer. Thus, tumor suppressors and oncogenic microRNAs significantly influence the progression and development of breast cancer (Loh et al., 2019).

Moreover, miRNAs affect the cellular signaling pathways by modulating the expression of essential genes, thereby guiding cellular responses from growth to apoptosis. Any disturbance in these pathways by miRNAs can influence cell

proliferation, differentiation, and survival, emphasizing their significant impact on cell fate (Jang & Lee, 2021).

Oncogenic miRNAs, such as miR-21, miR-155, miR-182, miR-10b, miR-27a, miR-9, and miR-19a, propel cancer growth by inhibiting breast cancer tumor suppressor genes. For instance, the overexpression of miR-21 and miR-155 in breast cancer suppresses genes like tropomyosin 1 (TPM1), timp metallopeptidase inhibitor 3 (TIMP3), programmed cell death 4 (PDCD4), and PTEN, as well as forkhead box O3 (FOXO3a), signal transducer and activator of transcription 3 (STAT3), and cascade-3. These suppressed genes otherwise act as brakes on cell growth, so their inhibition leads to increased cell proliferation, angiogenesis, and diminished apoptosis, fundamental factors in tumor development. On the other hand, tumor suppressor miRNAs, including let-7a, let-7b, let-7c, miR-145, miR-200, miR-205, and miR-335, act to inhibit oncogenes, thus countering breast cancer growth (Wang & Luo, 2015).

miRNAs also serve as intricate molecular switches in cellular processes. By interacting with specific mRNA targets, they can either inhibit mRNA translation or promote its degradation. Such regulation is complex; a single miRNA can target several genes, while multiple miRNAs might interact with a singular gene. This balance between oncogenic miRNAs and tumor suppressor miRNAs is instrumental in determining cancer onset, progression, and potential metastasis (Wang & Luo, 2015).

1.3.1 MicoRNAs in Cancer Metastasis

MiRNAs are recognized as key players in regulating cancer metastasis, which is considered the leading reason for death in individuals with solid tumors. Research indicates that certain miRNAs can inhibit the spread of various cancers, known as “metastamiR”, while other miRNAs can promote cancer metastasis by controlling various genes (Raval et al., 2022; Tavazoie et al., 2008)

For instance, miR-335, which has been observed to target SRY-Box transcription factor 4 (SOX4), reduces metastasis in breast cancer, and its loss was observed to be correlated with poorer outcomes (Tavazoie et al., 2008). In contrast, circulating levels of miR-200c/141, a promising marker for breast tumor metastasis, have been seen to be elevated during cancer progression (Loh et al., 2019). Moreover, the downregulation of miR-200 promotes metastatic breast cancer by elevating the

expression of the zinc finger E-box-binding homeobox 2 (ZEB2) gene (Singh & Mo, 2013).

1.3.2 MicroRNAs as Biomarkers in Breast Cancer

MiRNAs have recently gained prominence as potential biomarkers in cancer diagnosis due to their crucial role in gene expression regulation and the notable dysregulation witnessed in malignant cells (Yang & Liu, 2020). Their stability in blood, body fluids, and other tissues makes them ideal candidates for early detection and monitoring, even in samples stored for extended periods (Meng et al., 2013).

A specific set of miRNAs, namely miR-642b-3p, miR-1202-5p, miR-1207-5p, miR-1225-5p, miR-4270-5p, and miR-4281-3p, showed increased presence in the blood plasma of patients diagnosed with stage I breast cancer (Hamam et al., 2016). On the other hand, the concentration of serum miR-1825-3p was distinctly downregulated in initial-stage gliomas, and its downregulation was associated with the tumor's aggressive nature and less-than-optimal patient outcomes (Wang et al., 2018).

MiRNAs are utilized to differentiate various cancer subtypes, as miRNAs are upregulated in one kind of breast cancer, whereas they are downregulated in other types of breast cancer. The elevated level of miR-342 expression was observed in ER and HER2+ breast carcinoma (Lindholm et al., 2019). Whereas underexpression of miR-342 was identified in TNBC. Hence, miR-342 can be used as a biomarker to identify different types of breast cancers (Romero-Cordoba et al., 2018).

Emerging techniques, like the two-dimensional analysis method, optimize the sensitivity and accuracy of miRNA detection, enhancing the potential of early breast cancer diagnosis. Such innovations and the understanding that genes demonstrate tissue-specific behaviors emphasize their crucial role across various cancer types (Yan et al., 2023).

1.3.3 MicroRNA as Therapeutic Agents and Mediated Therapy Resistance

MiRNAs have shown immense promise as therapeutic targets and tools for cancer management. Their significance spans a range of functions in the cancer development process, including controlling cancer stem cells and promoting or inhibiting cancer, making their therapeutic potential quite significant (Szczepanek et al., 2022).

For example, while miR-155 can foster tumor cell proliferation, it may also render them more vulnerable to certain treatments. An interesting observation was made in breast cancer patients who underwent ionizing radiation therapy. Ionizing radiation functions by initiating double-stranded DNA breaks in tumor cells, which can be mended through DNA homologous recombination. However, increased certain enzymes can lead to resistance to this treatment. A breakthrough study by Gasparini et al. showed that miR-155 hinders the activity of AD51 Recombinase (RAD51), a pivotal protein for DNA homologous recombination. This inhibition enhances the sensitivity of triple-negative breast cancer to ionizing radiation. Intriguingly, despite the oncogenic properties of miR-155 in breast cancer, patients with elevated levels of this miRNA showed a higher overall survival rate (Tavazoie et al., 2008)

In therapeutic strategies, one approach involves using miRNA analogs or synthesized miRNAs to elevate tumor-suppressing miRNA levels or decrease oncomiRs levels in cancer cells. Preliminary studies indicate that nanoparticles show promise as effective delivery agents for these miRNA analogs, ensuring their stability and targeted delivery to cancer cells (Menon et al., 2022).

Another innovative technique involves using antisense oligonucleotides (ASOs) to target and inhibit specific oncomiRs. These synthetic nucleic acids bind to complementary miRNA sequences, resulting in the inactivation of the oncomiRs (Xiong et al., 2021) However, miRNA-based treatments have challenges, including efficient delivery, stability, and safety. Ongoing research focuses on improving these aspects, while closely monitoring potential side effects and risks (Szczepanek et al., 2022).

MiR-1294 is known for its tumor-suppressing capabilities in various cancers and is being investigated for its therapeutic potential (Mao et al., 2023). While MiR-551a presents a dual role, acting as a tumor suppressor in gastric cancer while promoting cancer progression in head and neck squamous cell carcinoma, suggesting its nuanced potential in cancer therapies (Karanam et al., 2015; Li et al., 2012). Regarding miR-4534 that is often upregulated in cancers and is an "oncomiR" that could be targeted in therapy due to its role in tumor growth and metastasis (Nip et al., 2016b; Inoue et al., 2021). Lastly, miR-1238's downregulation in glioblastoma and non-small cell lung cancer impacts cell proliferation and invasion, highlighting its therapeutic target potential (Shi et al., 2015; Luo et al., 2022).

MiRNAs are instrumental in modulating drug resistance in cancer therapy. Their roles encompass regulating drug efflux, altering drug targets, affecting DNA repair, cell cycle regulation, and apoptosis evasion (Si et al., 2019). For example, miR-27a notably regulates drug efflux by enhancing ATP-binding cassette transporters, decreasing intracellular drug concentrations (Dong et al., 2019). Meanwhile, miR-125 b's overexpression diminishes HER2 protein levels in breast cancer cells, impacting the effectiveness of trastuzumab (Crosby et al., 2009). DNA repair mechanisms are amplified with elevated miR-210, increasing resistance to therapies like chemotherapy and radiation (Kim et al., 2009). Furthermore, miR-221 and miR-222 regulate the cell cycle, making cells less prone to treatments targeting rapid cell division (Kim et al., 2023). Finally, miRNAs such as miR-221 and miR-222 are known to influence radiation sensitivity via the PTEN pathway (Li et al., 2014), while others impact pathways like PI3K/Akt related to chemotherapy resistance (Si et al., 2019).

1.3.4 The Role of Bioinformatics in microRNA Analysis in Breast Cancer

Bioinformatics refers to computational biology, where biological problems are solved using computational techniques. Bioinformatics has started to play a role in understanding cancer's molecular intricacies. It aids in identifying potential therapeutic targets, prognostic indicators, and, most importantly, in understanding the subtle dynamics of miRNAs within the landscape of cancer (Chen et al., 2020; Mondanizadeh et al., 2020).

Critical databases, such as miRBase and the gene expression omnibus (GEO), serve as foundational repositories for miRNA sequences and gene expression datasets, respectively (Barrett et al., 2013). These platforms, alongside resources like the Cancer Genome Atlas (TCGA) and the Kyoto Encyclopedia of Genes and Genomes (KEGG), facilitate a comprehensive analysis of tumor-related data, empowering researchers to perform intricate differential expression and pathway enrichment analyses (Kanehisa & Goto, 2000; Tomczak et al., 2015). The Gene Ontology (GO) database is an integral part of these analyses, which provides a structured and controlled vocabulary to describe gene and gene product attributes across species (Ashburner et al., 2000).

Bioinformatics is especially useful for studying miRNA-mRNA and protein interactions. Understanding these interactions is key to grasping how diseases like cancer develop. Tools like STRING and BioGRID help researchers learn more about

these interactions and find ways to intervene in cancer's progression (Szklarczyk et al., 2019).

1.4 Objective

The study's main objective was to clarify the roles of miR-1294, miR-551a, miR-1238, and miR-4534 in breast cancer development. By integrating bioinformatics analyses, the research aimed to deeply understand how these miRNAs influence the complex nature of breast cancer. Since these miRNAs have connections to various cancers, the study thoroughly investigated their specific functions, primary molecular targets, and interactions within the breast cancer genome.

Bioinformatics tools were employed to pinpoint and validate the primary molecules influenced by these altered miRNAs in breast cancer. These miRNA alterations were then evaluated concerning cancer signals, assessing any links to treatment resistance.

Additionally, the study explored the potential of these miRNAs as indicators of the disease's future progression. By comparing their levels with patient outcomes, the research sought to determine whether these miRNAs could predict disease outcomes and treatment responses or offer new therapeutic avenues in breast cancer.

CHAPTER 2
LITERATURE REVIEW

2.1. MicroRNA-1294 (miR-1294)

In research studies, miR-1294 has become notable for its inherent capacity to suppress tumors across various cancer forms. Additionally, miR-1294 is under investigation as a potential diagnostic indicator and therapeutic tool.

In the first study on the function of miR-1294 by Liu et al. in 2015, it was discerned that miR-1294 expression was considerably downregulated in human esophageal squamous cell carcinoma (ESCC) tissue when compared to corresponding normal tissue. This was evidenced using quantitative qRT-PCR on samples from 79 ESCC patients. The study revealed that lower miR-1294 expression was correlated with poorer prognosis. In vitro analysis showed that downregulation of miR-1294 upregulated the oncogene c-MYC, suggesting c-MYC as a target gene for miR-1294. Taken together, these findings underscore the potential prognostic implications of miR-1294 downregulation in ESCC, which may be attributed to its diminished inhibitory action on c-MYC protein function (Liu et al., 2015).

In 2018, Zhang et al. explored the role of miR-1294 in platinum-resistant ovarian cancer (OC). Using qRT-PCR, they measured the expression of miR-1294 in 30 OC tissues and cell lines. Their research involved cell transfection methods to modulate miR-1294 levels and various assays to study cell behaviors. The results indicated that miR-1294 dysregulation influenced OC cisplatin resistance through the regulation of the insulin-like growth factor 1 receptor (IGF1R) gene. Notably, a reduction in IGF1R led to reduced cell proliferation, migration, and invasion. Furthermore, elevating miR-1294 levels combated cisplatin resistance. These findings underscore miR-1294's potential both as a diagnostic tool and a therapeutic strategy for OC, particularly for those unresponsive to cisplatin (Zhang et al., 2018).

In gastric cancer (GC), Shi et al. (2018) identified a significant downregulation of miR-1294 in GC tissues compared to their adjacent normal counterparts. Using qRT-PCR on samples from 82 GC patients, the study reinforced this observation. The low miR-1294 levels correlated strongly with specific clinicopathological parameters, including

larger tumor size, lymph node metastasis, and distant metastasis. Further emphasizing its clinical importance, the study noted that patients with reduced miR-1294 levels faced a poorer cancer prognosis, highlighting its potential as a prognostic marker in GC (Shi et al., 2018)

In a study by Guo et al. (2018), miR-1294 expression was analyzed in 69 epithelial ovarian cancer (EOC) tissues and cell lines using qRT-PCR. Results showed reduced miR-1294 expression associated with lymph node metastasis, and decreased survival rate in EOC patients. Using cell counting kit-8 (CCK8) and flow cytometry, the study found that miR-1294 inhibits EOC cell growth and progression. Additionally, miR-1294 was identified as an independent prognostic indicator for EOC, emphasizing its potential as a therapeutic target (Guo et al., 2018).

Zhang et al. (2018) investigated miR-1294's expression in osteosarcoma (OS) cell lines and found it to be significantly downregulated, correlating with poorer survival rates in OS patients. In vitro tests demonstrated that miR-1294 upregulation inhibited OS cell proliferation and invasion. Through the luciferase reporter assay and Western blot analysis, the research proved homeobox A9 (HOXA9) as a direct target gene for miR-1294 (Zhang et al., 2018).

MiR-1294 is notably downregulated in glioma tissues and cell lines. Overexpression of miR-1294 reduces glioma cell growth and invasiveness while enhancing responsiveness to temozolomide. The research identified the Xenopus kinesin-like protein 2 (TPX2) as a target gene for miR-1294, a link further verified using a xenograft model, underscoring miR-1294's therapeutic potential for glioma (Chen et al., 2018).

Wang et al. (2018) studied miR-1294's role in oral squamous cell carcinoma (OSCC) using samples from 24 patients and cell lines. The study revealed that miR-1294 was significantly downregulated in cancer tissues and cell lines. Reduced levels of miR-1294 in OSCC were associated with larger tumors, increased lymphatic involvement, and venous infiltration. Importantly, miR-1294 directly targets the c-Myc oncogene, with an evident inverse relationship between c-Myc mRNA and miR-1294 levels in OSCC samples. Elevating miR-1294 levels inhibited OSCC cell growth and movement, underscoring its potential to curb tumor growth by targeting c-Myc (Wang et al., 2018).

In 2019, following evidence proving miR-1294's role as a tumor suppressor, researchers started to explore the regulation mechanisms of miR-1294, particularly through circRNAs. CircRNAs are a unique type of RNA molecule that forms a covalently closed continuous loop, differentiating them from the more familiar linear RNAs which have 5' or 3' ends. Some circRNAs act as miRNA sponges, possessing binding sites for miRNAs and sequestering these miRNAs to prevent them from repressing their target mRNAs. For instance, Xu et al. (2019) found that circ_0030235 was upregulated in pancreatic ductal adenocarcinoma (PDAC) cell lines and cancerous tissues. This circRNA proved to sponge miR-1294, which was found to be downregulated in pancreatic ductal adenocarcinoma tissues. The reduced expression of miR-1294 was associated with a higher tumor stage and positive lymph node invasion (Xu et al., 2019).

In a study cell renal cell carcinoma (ccRCC) cell lines by Pan et al. (2019) found that miR-1294 was downregulated and its reduced expression was associated with poor patient survival. Elevating the expression of miR-1294 reduced the proliferation, colony formation, and invasion of ccRCC cells. Furthermore, they established that homeobox A6 (HOXA6) is a target of miR-1294, with HOXA6 being negatively regulated by miR-1294 (Pan et al., 2019).

In a 2020 study by Wang et al., the role of miR-1294 in GC, particularly its involvement in epithelial–mesenchymal transition (EMT), was explored using samples from 60 patients and cell lines. They observed miR-1294 to be downregulated in GC, with a higher expression linked to longer overall survival. Through methods like Western blotting and dual luciferase assays, it was demonstrated that miR-1294 directly targets the forkhead box K1 (FOXK1) gene, altering its expression. FOXK1, recognized for its involvement in cell proliferation and tumorigenesis, is frequently upregulated in various cancers (Wang et al., 2020).

Another proof of miR-1294 downregulation in gastric cancer comes from a 2020 study by Wu et al. They examined 30 GC patients and their cancer cells, finding that the downregulation of miR-1294 led to the upregulation of the protein kinase B (AKT1) gene and dysregulation of the PI3K/AKT/mTOR signaling pathway (Wu et al., 2020).

In their study analyzing 15 tumor tissues, 20 normal tissues, and several cell lines, Yang et al. (2020) identified a pronounced downregulation of miR-1294 in laryngeal

squamous cell carcinoma (LSCC). Significantly, miR-1294 directly targets the epidermal growth factor receptor (EGFR) gene; thus, its decreased presence leads to enhanced EGFR expression. Overexpression or mutation of EGFR is known to significantly propel tumor progression by stimulating cell proliferation, migration, adhesion, and angiogenesis while concurrently inhibiting apoptosis. This relationship emphasizes the crucial influence of diminished miR-1294 levels on LSCC progression and behavior, especially through its modulation of EGFR (Yang et al., 2020).

In a comprehensive study conducted on cell lines, Yuan et al. (2020) identified a marked downregulation of miR-1294 in osteosarcoma cells. Notably, when the levels of miR-1294 were experimentally elevated, significant reductions in cellular processes such as proliferation, migration, and invasion were observed. The main finding from their investigation pinpointed that miR-1294 targets and modulates the pyruvate kinase M2 (PKM2) signaling cascade - a key enzyme in glycolysis known to be frequently overexpressed in various cancers, promoting cell growth and motility. In vivo experiments reinforced the pivotal role of the miR-1294/PKM2 pathway in osteosarcoma progression suggesting miR-1294's potential as a therapeutic target (Yuan et al., 2020).

Another study on 30 esophageal cancer patients, conducted in 2020 by Zong et al., found that miR-1294 was downregulated in esophageal cancer and identified polo-like kinase 1 (PLK1) as its direct target (Zong et al., 2020)

In a study carried out by Chen et al. (2020) involving 30 osteosarcoma patients and various cell lines, it was elucidated that miR-1294 can be sponged by circRNAs (circ_0005198). Circ_0000885, which is elevated in OS, can reduce the expression level of FGFR1 by targeting miR-1294. This interaction subsequently activates the RAS signaling pathway, further advancing the progression of OS (Chen et al., 2020).

Hua et al. (2020) focused on the role of miR-1294 in temozolomide-resistant glioma cells from 30 patients. They identified that miR-1294 was downregulated in these resistant cells. By restoring miR-1294 levels, the sensitivity of glioma cells to temozolomide significantly increased, indicating the pivotal role of miR-1294 in mediating drug resistance in glioma (Hua et al., 2020)

In a study on 94 hepatocellular cancer tissues, Chen et al. (2021) found that the circRNA (circ-PRKCI) influences the behavior of HCC cells by sponging miR-1294

in hepatocellular carcinoma (HCC) cells. Moreover, reduced levels of miR-1294 were linked to the enhancement of tumor development and spread in HCC. Increased expression of miR-1294 showed a decrease in cell growth and an increase in cell death in HCC. Moreover, miR-1294 was identified as a regulator of target forkhead box K1 (FOXK1) expression levels, a gene that is upregulated in liver cancer cells and governs glycolysis, ultimately affecting the viability of these cells (Chen et al., 2021)

The first study on miR-1294 in breast cancer was conducted by Chen et al. in 2022 on 30 patients. They found that miR-1294 was significantly downregulated in breast cancer tissues, and its reduced expression was associated with a poorer prognosis in patients. In vitro analysis demonstrated that miR-1294 inhibited breast cancer cell proliferation, migration, invasion, and tumor formation in animal models. On a molecular level, miR-1294 exerted these tumor-suppressive effects by modulating the extracellular signal-regulated kinase (ERK) signaling pathway. Based on these findings, miR-1294 emerges as a potential tumor suppressor in breast cancer and may offer a new avenue for therapeutic intervention (Chen et al., 2022).

In a study of 44 patients with esophageal squamous cell carcinoma by Liang et al. in 2022, miR-1294 expression levels were decreased in tumor tissues. The target genes of miR-1294 were linked to both cell cycle arrest and the phosphoinositide 3-kinase-Akt signaling pathway. Reduced levels of miR-1294 in ESCC were correlated with increased tumor size, lymphatic penetration, spread to lymph nodes, and vein infiltration. The findings highlight miR-1294's crucial role in limiting the malignancy of ESCC cells, pointing to its potential therapeutic significance (Liang et al., 2022).

Li et al. (2022) found that in non-small cell lung cancer (NSCLC), a low expression of miR-1294 was linked with advanced TNM staging and distant metastasis. Patients with a lower miR-1294 expression had a notably reduced overall survival rate compared to those with a higher expression. Molecular investigations indicated that the circRNA (circPLK1) directly suppresses miR-1294 activity, thereby influencing the progression of NSCLC via the miR-1294/high mobility group protein A1 (HMGA1) axis. When circPLK1 was overexpressed, NSCLC cells exhibited increased malignancy characteristics, such as elevated proliferation, migration, and invasion, while apoptosis decreased. The findings emphasize the potential of the circPLK1/miR-1294/HMGA1 pathway as a target for therapeutic strategies in NSCLC treatment (Li et al., 2022).

Qin and Wang (2023) observed downregulation of miR-1294 and an upregulation of the long non-coding RNA (ASAP1-IT1) in 30 paired hepatocellular carcinoma and adjacent non-tumor tissues. ASAP1-IT1 functioned as a molecular absorbent for miR-1294, subsequently raising the levels of the transforming growth factor beta receptor 1 (TGFBR1), which miR-1294 targets. Decreasing ASAP1-IT1 levels led to significant reductions in HCC cell behaviors, including proliferation, migration, and invasion. Moreover, the pro-tumoral effects of ASAP1-IT1 were reversed when the miR-1294/TGFBR1 pathway was inhibited (Qin & Wang, 2023).

In 2023, Moa et al. discussed the relationship between miR-1294 and cancer therapy. According to the CADDIE https://exbio.wzw.tum.de/caddie/drug-lookup, several medications might influence 6 genes downstream of miR-1294. These include Palifermin, Heparin, Regorafenib, Ponatinib, and Lenvatinib, which target FGFR1. Fostamatinib acts on polo-like kinase 1 (PLK1) and proviral integration of Moloney virus 1 (PIM1). Cetuximab, Lidocaine, Gefitinib, Erlotinib, and Lapatinib are associated with EEGFR. Insulin and Mecasermin interact with IGF1R. Finally, Arsenic Trioxide (ATO) and Resveratrol (RESV) influence AKT serine/threonine kinase 1 (AKT1) (Mao et al., 2023).

Cen et al. (2023) explored the role of miR-1294 in acute lymphoblastic leukemia (ALL) by examining samples from 15 children diagnosed with ALL as well as cell lines. Their findings revealed a significant upregulation of miR-1294 in the bone marrow cells of ALL patients when compared to healthy individuals. Additionally, there was a marked reduction in the expression of the SRY-box transcription factor 15 (SOX15) gene in these ALL-bone marrow cells. Importantly, miR-1294 was found to directly target and inhibit SOX15, leading to the activation of the Wnt/β-Catenin signaling pathway. This activation subsequently enhances ALL cell proliferation, decreases apoptosis, and impacts the disease's progression (Cen et al., 2023).

2.2. MicroRNA-551a (miR-551a)

In the scientific literature, miR-551a is documented to possess diverse functionalities across different cancer types, having been reported to exhibit upregulation in some and downregulation in others.

The first study on miR-551a, conducted by Li et al. in 2012, involved an analysis of four normal gastric tissues alongside 30 malignant tissues, and GC cell lines for in-depth investigation. By employing Western blotting and q RT-PCR, the researchers

reported a significant downregulation of miR-551a in the gastric carcinoma samples compared to normal tissue. MiR-551a was found to target and upregulate the phosphatase of regenerating liver-3 (PRL-3) gene, known for its association with metastasis in gastric cancer. Through the transfection of GC cell lines with miR-551a mimics, Li et al. observed a significant suppression in cell migration and invasion, indicating the miRNA's potential role as a tumor suppressor (Li et al., 2012).

In their 2015 study of head and neck squamous cell carcinoma, Karanam et al. focused on a cohort of 118 patients, 41 of whom experienced distant metastasis. Notably, the study revealed significant upregulation of miR-551a in patients who developed distant metastasis. In vitro analysis demonstrated that overexpression of miR-551a promoted cell proliferation, migration, and invasion, indicating its role in enhancing cancer aggressiveness. The discovery that miR-551a targets the glioma pathogenesis-related protein 2 (GLIPR2) gene was particularly significant, as this interaction is believed to contribute to tumor growth. The ability of miR-551a to modulate GLIPR2 expression highlights its importance in tumor progression and its potential as a marker for poor prognosis. This paves the way for its use as both a biomarker and a therapeutic target in head and neck squamous cell carcinoma (Karanam et al., 2015).

In the investigation led by Loo et al. in 2015, extensive analyses were conducted on colorectal cancer cell lines and animal models to decode the mechanisms of liver metastasis, a critical factor in patient mortality. Through a rigorous screening of 661 miRNAs, it was established that miR-551a found to be downregulated in colorectal cancer, plays a crucial role in deterring the spread of cancer to the liver. The research uncovered that miR-551a exerts its suppressive effects by targeting creatine kinase, brain-type (CKB), a pivotal enzyme that facilitates the survival of metastasizing cancer cells by regulating their energy metabolism. Clinically, this finding was substantiated by the lower expression levels of miR-551a in liver metastases when compared to the primary colon tumors. Therapeutic interventions designed to augment miR-551a levels or to inhibit CKB function led to a significant decrease in metastatic proliferation in their mouse model experiments. Thus, miR-551a's downregulation is intricately linked with colon cancer progression showing its tumor suppressor role in colorectal cancer (Loo et al., 2015).

In 2017, Du and Sha evaluated the role of miR-551a in the progression of ovarian cancer by analyzing 15 matched pairs of tumor tissues and adjacent normal tissues

from 15 patients, along with cell lines. The researchers found that miR-551a was notably downregulated in ovarian cancer specimens and cell lines. Further, they observed that increased expression of miR-551a led to reduced cell proliferation and enhanced apoptosis rates. Significantly, miR-551a was identified to target the insulin receptor substrate 2 (IRS2) gene, an oncogene that plays a vital role in ovarian cancer progression through the activation of the IRS2/PI3K/Akt signaling pathway. The research also delved into the effects of demethoxycurcumin, a derivative of curcumin known for its instability yet potent anti-proliferative effects on various cancer cell types in vitro. They elucidated that demethoxycurcumin was able to inhibit cell proliferation and induce apoptosis in ovarian cancer cells, while concurrently deactivating the IRS2/PI3K/Akt axis. Notably, treatment with demethoxycurcumin led to an upregulation of miR-551a, culminating in the suppression of IRS2 activity and thereby thwarting the progression of ovarian cancer cells (Du & Sha, 2017).

MiR-551a was first studied in the context of breast cancer in a 2019 study by Anuj et al. The investigation encompassed 24 tumor tissues and cell lines, revealing that miR-551a expression was significantly downregulated in breast cancer tissues and cell lines. Utilizing a suite of methodologies, including the overexpression of miR-551a in cell lines and xenograft mouse models, along with promoter luciferase assays, the researchers uncovered that miR-551a binds directly to the 3′ UTR of focal adhesion kinase (FAK) mRNA. This interaction leads to a reduction in FAK expression, which is otherwise typically upregulated in breast tumors. The results highlighted a considerable decrease in miR-551a levels within tumor samples, corresponding with increased FAK expression. Remarkably, the overexpression of miR-551a curtailed the invasive and migratory capabilities of cancer cells by diminishing the activity of matrix metallopeptidase 9 (MMP-9), cementing its role as a tumor suppressor. Moreover, they identified an intricate regulation mechanism orchestrated by c-Fos—properly named Proto-oncogene c-Fos—which binds to the miR-551a promoter, thereby enhancing its expression. These insights underscore the potential of miR-551a as a therapeutic target, opening avenues for new treatments aimed at FAK in breast cancer management (Anuj et al., 2019).

The research by Kang et al. offers valuable insights into the role of miR-551a in cancer cell resistance to chemotherapy. The study demonstrates that the miR-551a was overexpressed in Hep3B liver cancer cells leading to a notable increase in resistance

to the chemotherapeutic agent 5-Fluorouracil (5-FU). This resistance is correlated with heightened cell survival, sphere formation, and an indication of stem-like qualities in the cancer cells, suggesting that miR-551a could be influencing the fundamental properties that contribute to tumor aggressiveness and recurrence. Crucially, miR-551a exerts its effects by suppressing the transcription factor myocyte enhancer factor 2C (MEF2C). MEF2C is pivotal in the differentiation and maturation of neural stem/progenitor cells, among other cellular functions. The study posits that targeting the miR-551a/MEF2C interaction could enhance the effectiveness of 5-FU and provide a novel approach to combat drug resistance in cancer therapy (Kang et al., 2019).

In 2020, Wu et al. conducted research to explore the prognostic significance of miRNAs in colon cancer, specifically in relation to 5-Fluorouracil (5-FU) chemotherapy. They utilized miRNA expression data from the Broad Institute's Genomic Data Analysis Center (GDAC) Firehose, focusing on miRNA-gene interactions following 5-FU treatment and employing colon adenocarcinoma (COAD) data to analyze these relationships in depth. Within this dataset, miR-551a was identified as having a significant association with drug response and prognosis. These findings suggest that miR-551a regulation might serve as an important prognostic indicator for colon cancer patients undergoing 5-FU chemotherapy, underscoring the potential of miRNAs, possibly functioning as tumor suppressor miRNAs, in colorectal cancer therapeutic strategies (Wu et al., 2020).

Chen et al. conducted a study on hepatocellular carcinoma in 2021, to analyze the patterns of miRNA expression in a cohort of 344 HCC patients using data from The Cancer Genome Atlas (TCGA). They employed volcano plot analyses to identify differentially expressed miRNAs between HCC and normal. They found that miR-551a was upregulated in HCC and identified it as a key biomarker, with its increased expression indicating a poor prognosis. Furthermore, bioinformatics analyses underscored the oncogenic role of miR-551a and suggested its potential as a therapeutic target in HCC (Chen et al., 2021).

Another study, conducted by Karanam et al. in 2023, analyzed miRNA profiles in 94 tumor specimens from patients with head and neck squamous cell carcinoma (HNSCC) who were treated with postoperative radiotherapy. Their comparative analysis highlighted that miR-551a was significantly overexpressed in patients with

distant metastases and poor survival outcomes. Utilizing HNSCC cell lines, the study elucidated the oncogenic role of miR-551a by demonstrating its effects on cell proliferation, migration, and invasion assays. Intriguingly, miR-551a was found to target the mRNA of glioma pathogenesis related 2 (GLIPR2), a gene known to negatively regulate autophagy. By modulating this pathway, miR-551a not only enhanced tumor growth and invasiveness but also increased radio-resistance in HNSCC cells. These findings suggest that targeting miR-551a to regulate GLIPR2 expression might be a viable therapeutic strategy to inhibit tumor-promoting autophagy in HNSCC (Karanam et al., 2023).

2.3. MicroRNA-4534 (miR-4534)

Previous scientific research has consistently indicated that miR-4534 is frequently found to be present at elevated levels across a diverse array of cancerous tissues when compared to normal tissues. Given its consistent upregulation in such a wide variety of malignancies, miR-4534 is increasingly being characterized as an "oncomiR."

The foundational study exploring miR-4534's role in prostate cancer was published by Nip et al. in 2016. They examined 84 matched pairs of clinical samples from patients, along with additional benign and malignant specimens. Employing qRT-PCR, methylation-specific PCR, and a suite of functional assays, the team uncovered that miR-4534 is consistently overexpressed in prostate cancer tissues and cell lines compared to normal counterparts. They revealed a correlation between miR-4534 overexpression and the methylation status of cancer cells, suggesting its potential as a biomarker for distinguishing between malignant and benign tissues. The study further demonstrated that miR-4534 downregulates PTEN—a known tumor suppressor gene in prostate cancer—altering pathways critical for cell proliferation, survival, migration, cancer metastasis, and the formation of new blood vessels through the PI3K/Akt pathway. Inhibiting miR-4534 function in cancer cells resulted in tumor-suppressive effects such as cell cycle arrest, apoptosis, and reduced migration and invasion highlighting miR-4534's role as an “OncomiR” and its therapeutic targeting potential (Nip et al., 2016).

Another study on miR-4534 in prostate cancer was conducted by Jiang et al., in 2019 and involved 78 prostate cancer patients. The researchers employed qRT-PCR to confirm the oncogenic role of miR-4534, demonstrating its upregulation in cancerous tissues. The findings showed that miR-4534 facilitates the proliferation, migration,

invasion, and angiogenesis of prostate cancer cells, primarily through the suppression of its target gene, Vasohibin 1 (VASH1). The tumor-suppressive properties of VASH1 were further evidenced by its observed downregulation in prostate cancer tissues and cell lines. The study provided compelling evidence that the long noncoding RNA "RBMS3-AS3" may impede the progression of prostate cancer by sequestering miR-4534, thus reducing its oncogenic effects (Jiang et al., 2019).

In 2021, Inoue et al. examined the impact of TP53 deficiency in colorectal cancer cells on fibroblast autophagy, with an emphasis on the miR-4534. They compared human colorectal cancer cell lines, namely HCT116 with an intact TP53 gene and HT29 with a mutated version of TP53. Their observations revealed that autophagy in fibroblasts was significantly inhibited when they were co-cultured with HCT116 cells lacking TP53. They discovered that this effect was mediated by an increase in miR-4534 within exosomes from the TP53-deficient HCT116 cells. MiR-4534 targets and suppresses the expression of the autophagy-related gene, autophagy related 2B (ATG2B), which is essential for the early stages of autophagosome formation, crucial for the induction of autophagy, and for maintaining cellular homeostasis. This insight underscores a new pathway by which TP53 mutations in cancer cells may influence the tumor microenvironment, fostering an environment that supports cancer progression through the suppression of autophagy in fibroblasts, mediated by miR-4534's action on ATG2B (Inoue et al., 2021).

Öztürk et al.'s 2022 study on 83 individuals, including 37 with prostate cancer, 31 with benign prostatic hyperplasia (BPH), and 15 healthy controls, found elevated levels of miR-4534 in the plasma of the cancer and BPH groups using qRT-PCR. This overexpression, linked to lymph node metastasis, indicates miR-4534's potential as a biomarker for these conditions. However, it's unclear if it can differentiate between prostate cancer and BPH. The study also suggests a possible oncogenic role for miR-4534 in disease progression (Öztürk et al., 2022).

The study by Chai et al. in 2023 unveiled significant insights into the molecular dynamics of triple-negative breast cancer (TNBC) by examining the roles of miR-4534 and ADAM-like Decysin-1 (ADAMDEC1). Through the analysis of RNA sequencing data sourced from the GEO database, the researchers identified a notable upregulation of miR-4534 in TNBC tissues. This elevation was associated with decreased survival rates among patients, pointing to miR-4534's potential impact on the progression of

the disease. Further exploration using the Linked Omics database revealed a contrasting expression pattern for ADAMDEC1; its upregulation appeared to be a favorable marker in chemosensitive TNBC, corresponding with enhanced patient survival. The interplay between miR-4534 and ADAMDEC1 was clarified by correlation analysis, which positioned miR-4534 as a potential upstream inhibitory regulator of ADAMDEC1. This relationship underscores the influence of miR-4534 on chemotherapeutic responsiveness and, subsequently, on patient survival outcomes (Chai et al., 2023).

2.4. MicroRNA-1238 (miR-1238)

Like the previously mentioned miRNAs, miR-1238 is also found to be aberrant in various cancers and plays a role in disrupting the expression of essential genes.

The first study on miR-1238 was conducted by Balaguer et al. in 2011, focusing on colorectal cancer (CRC). The study utilized 54 CRC tissues and 20 normal colonic tissues. Employing microarray analysis and validation by qRT-PCR, the researchers found that miR-1238 was overexpressed in colorectal cancer (Balaguer et al., 2011).

Within the realm of non-small cell lung cancer research, Shi et al. (2015) targeted the largely unexplored role of miR-1238. Their research included 50 patient tissue samples and revealed that miR-1238 expression was diminished in 62% of NSCLC cases. This reduction was associated with an upregulation of LHX2 expression, indicating an inverse relationship between the two. LHX2, recognized for its involvement in a variety of biological processes such as hematopoiesis, neural development, and cancer cell proliferation, was identified as a pivotal element in NSCLC pathology. The study's significant discovery was supported by qRT-PCR and Western blot analyses, which demonstrated that overexpressing miR-1238 in NSCLC cell lines decreased the mRNA and protein levels of LHX2, leading to suppressed cell proliferation. The inverse correlation between miR-1238 and LHX2 not only elucidates the molecular mechanisms of NSCLC but also suggests miR-1238 as a potential biomarker and therapeutic target, given its regulatory effect on LHX2 expression and the consequent impact on tumor cell growth (Shi et al., 2015)

Delving into the genetic underpinnings of primary glioblastoma (pGBM), Yan et al.'s 2015 research revealed pivotal differences in miRNA expression profiles across two subgroups with distinct responses to temozolomide (TMZ). Utilizing microarray analysis on 82 tissue samples and further qRT-PCR validation on 78 additional

specimens, they distinguished the TMZ-resistant (TCR) subgroup with overexpression of miR-1238 and unfavorable prognosis from the TMZ-sensitive (TCS) subgroup that responded better to treatment. Notably, miR-1238 was part of a seven-miRNA classifier that effectively differentiated between these subgroups, indicating a potential correlation between miR-1238 overexpression and increased chemoresistance and adverse outcomes in pGBM cases (Yan et al., 2015). In 2019, Yin et al. conducted the same study and proved the same results by measuring miR-1238 expression in GBM cell lines, as well as in a collection of primary and recurrent GBM tissue samples, alongside sera from 13 patients. They observed a significant increase in miR-1238 levels in both TMZ-resistant exosomes and cells and in the sera of GBM patients. The study added to the molecular reason by reporting that miR-1238 could counteract TMZ resistance by targeting and suppressing the caveolin-1 (CAV1) gene that acts as a negative regulator of EGFR activation. This suggests that miR-1238 may be involved in the pathogenesis of glioma and could potentially serve as a therapeutic target (Yin et al., 2019).

In a study focusing on cholangiocarcinoma (CCA), Xu et al. examined samples from 58 patients and CCA lines and found that the miR-1238 was downregulated, suggesting a tumor-suppressive role. Their methodology encompassed a variety of experimental approaches including qRT-PCR, cell transfection, and luciferase reporter assays, among others, to investigate the cellular and molecular dynamics. Results highlighted the upregulation of circular RNA circ_0005230 in both CCA tissues and cell lines, establishing a connection with the progression and prognosis of the disease. Specifically, they discovered that circ_0005230 acts as a molecular sponge for miR-1238, thus influencing the expression of the gene BCL2-like 13 (BCL2L13), which has implications for cell proliferation and apoptosis resistance. The findings propose that targeting circ_0005230 could offer a new therapeutic strategy for CCA, potentially impacting the disease's clinical management (Xu et al., 2019).

In the study conducted by Shan et al. (2020) on prostate cancer, they examined 90 cancer tissues and adjacent non-tumor prostate tissues, as well as cell lines. Using a combination of bioinformatics and functional assays, and confirming with qRT-PCR, the researchers discovered that miR-1238 was downregulated in both cancer tissues and cell lines. They also identified that circ_0005100 (circFMN2) was overexpressed in prostate cancer, playing an oncogenic role. Shan et al. found that circFMN2

effectively functions as a sponge absorbing miR-1238, which facilitates an increase in LIM-homeobox gene 2 (LHX2) expression—a gene notably elevated in PCa tissues. This interaction impedes the typical inhibitory effect of miR-1238 on LHX2 mRNA, leading to its increased presence in PCa cells. The study further demonstrated that the introduction of miR-1238 mimics could counteract the pro-proliferative effects of excess circFMN2, suggesting miR-1238's potential tumor-suppressive effects in PCa. These findings support the circFMN2/miR-1238/LHX2 regulatory pathway as a promising target for PCa therapy (Shan et al., 2020).

MiR-1238 was found to be downregulated in non-small cell lung cancer, as reported by Luo et al. in their 2021 study. The study, which included analysis from 42 pairs of primary NSCLC and adjacent normal tissues, utilized qRT-PCR to assess miR-1238 expression levels. The research also reported that miR-1238 targets and regulates the expression of the claudin-14 gene (CLDN14), which appears to promote proliferation and metastasis in cancer cells. Through luciferase reporter and RNA immunoprecipitation assays, the team revealed that circKIF4A directly binds to and acts as a sponge for miR-1238. Therefore, circKIF4A may regulate NSCLC progression by sequestering miR-1238, preventing it from targeting other genes involved in cancer development. This research not only advances our understanding of the circKIF4A/miR-1238/CLDN14 axis but also underscores the tumor-suppressive capacity of miR-1238 (Luo et al., 2022).

In 2023, Mao et al through their study on miR-1238's involvement in colorectal cancer (CRC). The study included 20 paired CRC tissues and demonstrated that miR-1238 was typically found at lower levels in CRC tissues compared to normal tissues, indicating a potential tumor suppressive function. The researchers highlighted that circSKA3, a circular RNA upregulated in CRC, has a binding affinity for miR-1238, which in turn interacts with YTH N6-methyladenosine RNA binding protein 2 (YTHDF2). The study’s results showed that when miR-1238's expression was artificially increased, there was a marked reduction in CRC cell proliferation, migration, and invasion—processes crucial for cancer metastasis. However, this suppressive effect of miR-1238 on cancer cells could be counteracted by the overexpression of YTHDF2. The intricate mechanism uncovered by Mao et al. suggests that by sponging miR-1238, circSKA3 indirectly elevates YTHDF2 expression, thus promoting CRC progression (Mao et al., 2023).

CHAPTER 3
MATERIALS AND METHODS

3.1 Material

3.1.1. Tissue Samples

The study encompassed an analysis of 82 tumor and histologically adjacent normal tissue samples (distance from the tumor greater than or equal to 5 cm), which were procured from 41 female breast cancer patients who visited Gaziantep University Şahinbey Research and Practice Hospital, Department of General Surgery between 2019 and 2021. These patients were not undergoing chemotherapy or radiotherapy at the time of sampling. A written, signed, and informed consent was obtained from all participants before tissue sampling. The tissue samples were preserved in tubes containing RNA Later solution (Thermo Fisher Scientific, USA) and stored at -80 °C until further analysis.

This study was approved by the Ethics Committee of Gaziantep University (date: 09/01/2019, decision number: 2019/20).

3.2 Methods

3.2.1. MicroRNA Extraction and Quantification

MicroRNA from fresh frozen tissue samples was extracted using the Hybrid-R™ miRNA isolation kit (GeneAll, South Korea), as shown in Table 3.1, according to the manufacturer's instructions. The RNA was quantified using a MaestroNano Spectrophotometer (Maestrogen, Taiwan), and the RNA concentrations were adjusted to a standard concentration for uniformity across samples. The samples were then stored at -80°C until further use.

Table 3.1. Hybrid-R™ miRNA kit content

Parameters	**Producer**
RiboEx™	
Column type B	

Column type W	
2 mL Collection tubes	
1.5 mL Microcentrifuge tubes	Catalog No: 325-150
SW1 Buffer	
RBW Buffer	GeneAll, South Korea
RNW Buffer	
Nuclease-free water	

3.2.2 Reverse Transcription and Cyclic DNA (cDNA) Synthesis

Total RNA was reverse transcribed into complementary DNA (cDNA) using the ABT™ Single miRNA qPCR Assay product (ABT, Türkiye) as per the manufacturer's instructions, with specific details provided in Table 3.2.

Table 3.2. ABT™ Single miRNA qPCR Assay product.

Component	Producer
miR-cDNA Synthesis Kit	Catalog No: Q07-01-20
miR-qPCR MasterMix	ABT, Türkiye

The miR-cDNA Synthesis Kit included in the assay contains all necessary components for the reverse transcription reactions, including reverse transcriptase, RNase inhibitor, miRNA-specific Stem-Loop primer, and dNTPs, with volumes specified in Table 3.3.

Table 3.3. The volume of reverse transcription used per sample.

Component	Volume
miR-cDNA Synthesis Kit	9 µL
miRNA Example	1 µL

Total Reaction Volume	**10 µL**

The prepared samples were transferred to the PCR device and cDNA synthesis was performed according to the protocol given in Table 3.4. The synthesized cDNA samples obtained after the application were kept at +4 °C to be used in the Real-Time PCR process. While stock cDNA samples were stored at -20 °C.

Table 3.4. cDNA synthesis protocol

Step	Temperature (°C)	Duration (min)	Cycle
Step 1	25	10	one
Step 2	37	20	one
Step 3	85	5	one
Step 4	4	∞	∞

3.2.3 Real-Time Polymerase Chain Reaction (RT-qPCR)

The 7500 Fast Real-Time PCR system (Applied Biosystems, USA) was used for qPCR with an ABT™ Single miRNA qPCR Assay kit (ABT, Türkiye). The miR-qPCR Master Mix included in the product contains all the components required for Real-Time PCR reactions, including antibody-mediated hot-start Taq DNA Polymerase, forward and reverse primers, dNTPs, MgCl2, SYBR-Green, the reference Rhodamine X (ROX), enhancer, and stabilizer. The specific volumes of components utilized in the Real-Time PCR reaction can be found in Table 3.5.

Table 3.5. Real-Time PCR reaction mix

Component	Volume
miR-qPCR Master Mix	9 µL
cDNA Sample	1 µL
Total Reaction Volume	**10 µL**

The prepared samples were subjected to Real-Time qPCR following the protocol outlined in Table 3.6, with each sample analyzed in duplicate.

Table 3.6. Real-Time qPCR gene expression protocol

Step	Temperature (C°)	Duration (min)	Cycle
Initial Denaturation	95	10 min	one
Denaturation	95	10 sec	40
Annealing	60	25 sec	
Melting Curve	60-95	2 sec/digit	-

The comparative Ct method was utilized for data analysis in the Real-Time qPCR software. The relative expression levels of miR-551a, miR-1238, miR-1294, and miR-4534 were normalized against the endogenous control gene "RNU6NB" as using the ΔΔCt method (Livak & Schmittgen, 2001):

$\Delta\Delta Ct_{\text{target gene}} = (Ct_{\text{target gene, tumor tissue}} - Ct_{\text{endogenous control gene, tumor tissue}}) - (Ct_{\text{target gene, normal tissue}} - Ct_{\text{endogenous control gene, normal tissue}})$.

Ct = Cycle Threshold value, Target gene = miR-551a, miR-1238, miR-1294 or miR-4534, Endogenous control gene = RNU6B

Relative gene expression (fold change) was then calculated according to the following method (Livak & Schmittgen, 2001);

Relative Gene Expression (fold change) = $2^{-\Delta\Delta Ct}$

3.2.4 Statistical Analysis

The paired t-test was used to compare the expression levels of miRNAs between tumor tissues and their normal counterparts. Moreover, the evaluation of the association between clinicopathological factors and miRNA expression levels was performed using Pearson's Chi-Square and Fisher's tests. Statistical analysis was conducted using SPSS software (version 22.0, IBM Corp). A p-value of less than 0.05 was considered significant.

3.2.5 microRNA-mRNA Target Identification

To elucidate the interactions between miRNAs and their mRNA targets, a two-pronged predictive approach was employed, leveraging the capabilities of the miRDB and miRWalk databases. The analysis included miRNAs that exhibited significant differential expression between tumor and normal tissues only.

miRDB served as the primary platform for predicting potential targets. This online database utilizes high-throughput sequencing data to annotate functional miRNA-target interactions. The results, encompassing target rank, score, gene symbol, and descriptions, were filtered using a threshold score of >80, aligning with the database authors' recommendations (Chen & Wang, 2020).

miRWalk toll reinforced the target identification process by providing a robust, integrative analysis that combines machine learning predictions with experimentally verified miRNA-mRNA interactions. It references multiple databases, including TargetScan, miRDB, and MiRTarBase, to ensure a comprehensive target list. Selection criteria for miRWalk included a stringent score >0.95 and cross-verification across at least two of the mentioned databases (Sticht et al., 2018).

Subsequently, the potential targets identified from both miRDB and miRWalk were merged, ensuring the removal of duplicates, and preserving the highest-scoring interactions for further analysis. This combined list of potential miRNA-mRNA targets sets the foundation for more in-depth subsequent analyses, aimed at uncovering the regulatory roles of miRNAs in tumor biology.

3.2.6 Functional Enrichment Analysis of microRNAs

In advanced transcriptomic investigations, a vital component is the execution of functional enrichment analysis. This process entails a thorough examination of genes to assess their prevalence in relation to cellular operations, biological mechanisms, and molecular pathways. This essential step is instrumental in ascertaining the potential biological functions that may be affected as a consequence of variations in gene expression levels. Functional enrichment analysis is to compare the enrichment of any type of biological relevant labels such as gene ontology terms in a list of genes to determine biological functions that are enriched which provides a better understanding of the underlying biological process, molecular functions, and wherein the cell do theses encoded proteins perform their functions. The entire biological systems of the

organisms are crucially dependent on the accurate function of the proteins encoded by their corresponding genes(Webber, 2011). Gene ontology (GO) Resource is the world's largest source of information on the functions of genes which are divided into three distinct categories: molecular function (<F) cellular component (CC) and biological process (BP). It is an initiative that provides a unified representation of a comprehensive computational model of biological systems along with gene knowledgebase and resources that range from molecular to organism level and across a multi-species spectrum (Carbon et al., 2021). Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis offers insights into the complex network of molecular interactions and biochemical routes impacted by gene expression alterations (Kanehisa & Goto, 2000)**.** To perform functional enrichment analysis on the respective target list of each miRNA, "enrichR" R package was used and the target list was provided as input for the package to connect to the enrichR database for the analysis (Kuleshov et al., 2016). EnrichR is a gene enrichment analysis web-based tool and R-based package that allows various types of enrichment and visualization of the collective functional analysis on the provided gene list. It features various libraries including GO, KEGG, and PANTHER (Thomas et al., 2022).

3.2.7 Protein-Protein Network Analysis

Protein-protein interactions (PPIs) are fundamental to virtually every process within a cell, influencing cellular physiology in both health and disease (Rao et al., 2014). To elucidate the complex networks governing cellular functions, it is imperative to analyze PPIs systematically. Protein-protein interaction networks (PPINs) provide a mathematical and visual representation of the physical contacts between proteins, which are critical for understanding the mechanisms underlying cellular processes (Cho et al., 2004). The STRING database (version 11.0) was utilized for the analysis of PPIs. STRING offers a comprehensive aggregation of known and predicted protein interactions, including both direct (physical) and indirect (functional) associations. These interactions are sourced from a variety of methods: genomic context prediction, high-throughput laboratory experiments, conserved co-expression, automated text-mining, and existing knowledge in curated databases (Szklarczyk et al., 2019). The default parameters for network analysis in STRING, as recommended by the database guidelines (Szklarczyk et al., 2019), were applied to ensure the broadest discovery potential while maintaining high confidence levels.

To identify PPIs pertinent to our study, we entered a list of differentially expressed genes (DEGs) into the STRING database, adhering to established protocols to ensure data integrity. The resulting networks enabled us to pinpoint key hub proteins for subsequent investigation and validation.

CHAPTER 4
RESULT

4.1 Clinicopathological Characteristics of Breast Cancer Patients

Drawing on hospital data for histological and biochemical analyses, the study revealed a split in age demographics with 21 patients aged 50 years or above (51%) and 20 patients below 50 years of age (49%). A predominant segment of the cohort was diagnosed at initial cancer stages; 28 patients were in stages I and II (68%) and 13 were in stages III and IV (32%). This detailed breakdown of the stages of cancer at diagnosis is further illustrated in Table 4.1.

Only a marginal fraction of patients, 2 individuals (5%), engaged in smoking, and none of the patients reported alcohol consumption. Tumor localization was nearly equally divided, with 20 patients exhibiting right-sided tumors (49%) and 21 with left-sided tumors (51%). Distant metastasis was absent in the majority of cases, with 38 patients (93%) having no metastasis (M0), contrasting with 3 patients (7%) who presented with metastatic disease (M1). Regarding tumor size, a larger proportion, 34 patients, had tumors measuring less than 50 mm (83%), while 7 patients had tumors measuring 50 mm or larger (17%).

A small percentage, 4 patients, confirmed a family history of cancer (10%), whereas the remaining 37 patients had no such history (90%). Hormone receptor analysis showed that estrogen receptors were positive in 30 patients (73%) and negative in 11 patients (27%). Progesterone receptors followed a similar pattern, with 29 patients testing positive (71%) and 12 negative (29%).

Delving into the specifics of histological categorization, invasive ductal carcinoma was identified as the most frequent type among the patients, affecting 33 individuals (80%). Other types included invasive lobular carcinoma in 4 patients (10%), invasive breast carcinoma in 3 patients (7%), and invasive medullary carcinoma in 1 patient (2%). Lymph node involvement was assessed as N1 (indicating the presence in one or more lymph nodes or underarms) in 33 patients (80%), N2 (involvement of internal

mammary lymph nodes) in 5 patients (12%), and N3 (indicative of widespread metastasis) in 3 patients (7%).

Table 4.1. Characteristic features of the breast cancer patients analyzed in this study.

Clinicopathological factor		Number	Percentage
Phase	I and II	28	68%
	III and IV	13	32%
Age	≥50 years	21	51%
	<50 years	20	49%
Gender	Man	0	0%
	Woman	41	100%
Smoking	Yes	02	5%
	No	39	95%
Alcohol use	Yes	0	0%
	No	41	100%
Tumor Localization	Right	20	51%
	Left	21	49%
Distant Metastasis	M0 (No Metastasis)	38	93%
	M1 (Metastasis)	03	7%
Family History	Yes	04	10%
	No	37	90%
Tumor Size (mm)	Less than 50mm	34	83%
	50mm and above	7	17%
Lymph Node Metastasis	N1	33	80%
	N2	5	12%
	N3	3	7%
ER (Estrogen receptor)	Positive	30	73%
	Negative	11	27%
PR (Progesterone receptor)	Positive	29	71%
	Negative	12	29%
CEA	Normal	34	83%
	High	7	17%
CA-125	Normal	38	93%
	High	03	7%
CA 15-3	Normal	40	98%
	High	1	2%
C-erbB-2	+1	4	10%
	+2	6	15%
	+3	11	27%
	Negative	20	49%
Ki-67	G1 (lower than 2%)	1	3%
	G2 (3-20%)	19	46%
	G3 (more than 20%)	21	51%
Histological Type	Invasive breast carcinoma	3	7%
	Invasive ductal carcinoma	33	80%
	Invasive lobular carcinoma	4	10%
	Invasive medullary carcinoma	1	2%

In the realm of tumor markers, carcinoembryonic antigen (CEA) levels were found to be within the normal range (0 to 2.5 µg/L) for 34 patients (83%), while elevated levels were seen in 7 patients (17%). For Cancer Antigen 125 (CA-125, with a normal reference of 0 to 35), 38 patients had normal levels (93%), with an elevated count in 3 patients (7%). The results for Cancer Antigen 15-3 (CA15-3, with a normal reference level of 30 U/mL) were normal in 40 patients (98%), and high in 1 patient (2%). Through evaluating the expression of receptor tyrosine-protein kinase (C-erbB-2), it was observed that 4 patients rated at +1 (10%), 6 at +2 (15%), 11 at +3 (27%), and 20 patients were negative (49%).

Ki-67, a prognostic marker in breast cancer, showed varied levels with 1 patient classified as G1 (less than 2%, indicating low proliferative activity), 19 as G2 (3 to 20%), and 21 as G3 (more than 20%), suggesting a higher proliferation index. These gradings underscore the diverse nature of tumor growth rates within the studied population.

4.2 Expression Analysis of miR-551a, miR-1294, miR-4534, and miR-1238

In our study involving 41 breast cancer patients, we utilized the fold change "$2^{-\Delta\Delta Ct}$" method to analyze the expression changes of various miR-1294, miR-551a, miR-4534, and miR-1238 in tumor tissues compared to the adjacent normal tissues. This approach provided us with valuable insights into the differential expression of miRNAs in cancerous conditions. We conducted qRT-PCR analysis on each miRNA for all samples from both tumor and normal tissues, using RNU6 as an internal control to ensure the robustness of our findings.

In our findings, the average ΔCt value for miR-1294 in tumor tissues was (0.4±0.24), which was lower than in normal tissues (0.1±0.58). The fold change calculated was (0.82±0.13), indicating a significant downregulation of miR-1294 in tumor tissues ($p=0.004$), as detailed in Figure 4.1.

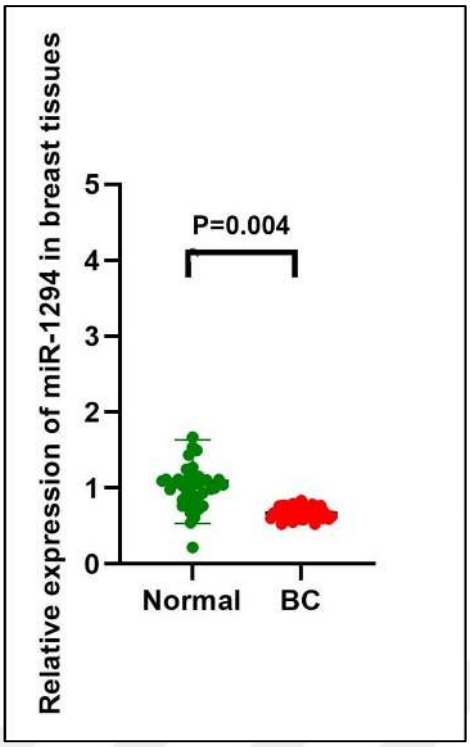

Figure 4.1. Relative expression of miR-1294 in breast cancer tissues compared to normal ones using qRT-PCR.

For miR-551a, we observed an average ΔCt value of (-1.36±0.38) in tumor tissues, compared to (-1.7±0.9) in normal tissues. This resulted in a fold change of (0.84±0.38), signifying a statistically significant decrease in miR-551a expression in tumor tissues compared with the normal tissues (p=0.038), as presented in Figure 4.2.

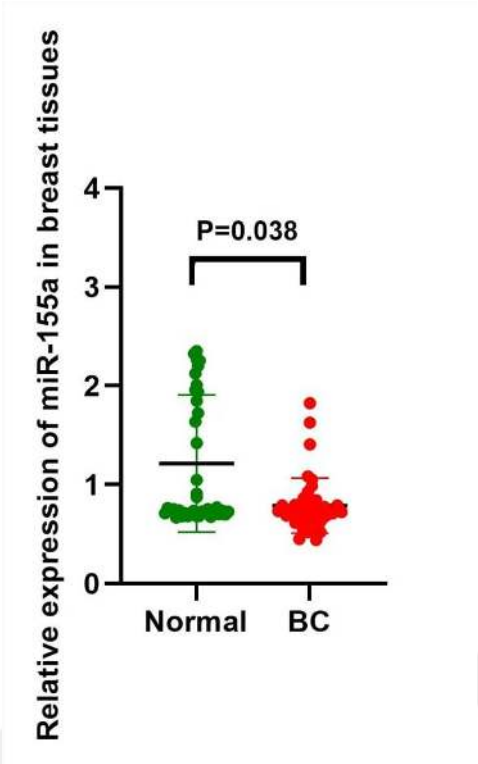

Figure 4.2. Relative expression of miR-551a in breast cancer tissues compared to normal ones using qRT-PCR.

Regarding miR-4534, our data showed a contrasting trend with an average ΔCt value of (-5.39±0.43) in tumor tissues, versus (-4.86±1.02) in normal tissues, resulting in a fold change of (1.5±0.43). This indicates a significant increase in miR-4534 expression in tumor tissues (p=0.003), as documented in Figure 4.3.

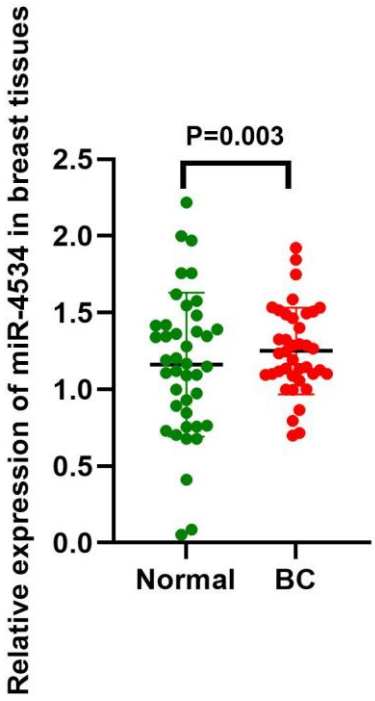

Figure 4.3. Relative expression of miR-4534 in breast cancer tissues compared to normal ones using qRT-PCR.

Finally, for miR-1238, we recorded an average ΔCt value of (2.29±0.75) in tumor tissues and (2.52±0.71) in normal tissues, with a fold change of (1.35±0.78). However, this did not translate into a statistically significant difference in miR-1238 expression between tumor and normal tissues (p=0.076), as represented in Figure 4.4.

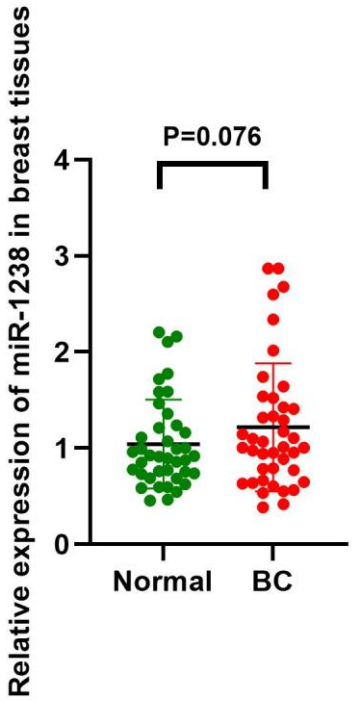

Figure 4.4. Relative expression of miR-1238 in breast cancer tissues compared to normal ones using qRT-PCR.

4.3 Correlation of miR-1294, miR-551a, miR-4534, and miR-1238 Expression Levels with Clinical and Pathological Data

In this study, we explored the relationship between the expression levels of miR-1294, miR-551a, miR-4534, and miR-1238 and a range of clinicopathological factors. This exploration was underpinned using Pearson Chi-Square and Fisher's Exact tests, which allowed us to rigorously assess any potential associations.

We began by categorizing breast cancer into Phases I and II versus Phases III and IV, recognizing that the stage of cancer is fundamental to understanding its progression and informing treatment strategies. Age was another key variable, with the division into under 50 and 50 years and above groups to account for the varying hormonal influences at different life stages. Smoking status was also factored into our analysis, given its broad health implications and potential impact on cancer progression. Additionally, tumor localization, whether right or left, was considered for its potential influence on surgical approaches and prognosis.

Distant metastasis, a crucial marker of cancer severity, was classified as M0 for none and M1 for present, reflecting its significance in the overall cancer journey. The

histological type of the tumor was categorized to understand the cellular characteristics that could influence treatment and outcomes. Family history was noted for its potential genetic implications, while tumor size was recorded as less than 50mm or 50mm and above, given its importance in staging and gauging cancer aggressiveness.

We also included the status of Estrogen and Progesterone receptors and the expression of biomarkers like CEA, CA-125, CA 15-3, and C-erbB-2. Each of these markers offers insights into the biological behavior of the cancer. The Ki-67 index was also analyzed to understand the proliferation rate of the cancer cells.

However, the results of our statistical analysis revealed that none of the studied miRNAs' expressions showed a significant association with these clinicopathological factors. This was indicated by p-values above 0.05 for all the studied miRNAs, as detailed in Tables 4.2 for miR-1294, Table 4.3 for miR-551a, Table 4.4 for miR-4534, and Table 4.5 for miR-1238. This finding suggests that the expression of these miRNAs was not significantly different across any of the clinicopathological groups we studied.

Table 4.2. Relationship between miR-1294 expression levels and clinicopathological features of patients.

Clinicopathological Factor	Patients (41)	Low (39)	High (2)	P Value
Phase				**1.000**
I and II	28	26	2	
III and IV	03	13	0	
Smoking				**1.000**
Yes	02	2	0	
No	39	37	2	
Tumor Localization				**1.000**
Right	21	20	1	
Left	20	19	1	
Distant Metastasis				**1.000**
M0 (No)	38	36	2	
M1 (Yes)	03	3	0	
Histological Type				**1.000**
I	05	5	0	
II and III	36	34	2	
Family History				1.000
Yes	04	4	0	
No	37	35	2	
Tumor Size (mm)				**0.65**
Less than 50mm	34	32	2	
50mm and above	07	7	0	

EP (Estrogen receptor)				**1.000**
Positive	30	28	2	
Negative	11	11	0	
PR (Progesterone receptor)				**1.000**
Positive	29	27	2	
Negative	12	12	0	
Lymph Node Metastasis				**1.000**
N1	33	31	2	
N2/3	08	8	0	
CEA				**0.32**
Normal	34	33	1	
High	07	6	1	
CA-125				**1.000**
Normal	38	36	2	
High	03	3	0	
CA 15-3				**1.000**
Normal	40	38	2	
High	01	1	0	
C-erbB-2				**1.000**
Positive	20	19	1	
Negative	21	20	1	
Ki-67				**0.23**
20 and below	20	18	2	
Over 20	21	21	0	

Table 4.3. Relationship between miR-551a expression levels and clinicopathological features of patients.

Clinicopathological Factor	Patients (41)	Low (33)	High (8)	P Value
Phase				**1.000**
I and II	28	23	5	
III and IV	13	10	3	
Smoking				**0.34**
Yes	02	0	2	
No	39	33	6	
Tumor Localization				**0.45**
Right	20	15	5	
Left	21	18	3	
Distant Metastasis				**1.000**
M0 (No)	38	30	8	
M1 (Yes)	03	3	0	

Histological Type				**1.000**
I	05	4	1	
II and III	36	29	7	
Family History				**0.165**
Yes	04	2	2	
No	37	31	6	
Tumor Size (mm)				**0.66**
Less than 50mm	34	27	7	
50mm and above	07	6	1	
EP (Estrogen receptor)				**0.412**
Positive	30	23	7	
Negative	08	1	7	
PR (Progesterone receptor)				**0.34**
Positive	29	22	7	
Negative	12	11	1	
Lymph Node Metastasis				**1.000**
N1	33	26	7	
N2/3	08	7	1	
CEA				**1.000**
Normal	34	27	7	
High	07	6	1	
CA-125				**1.000**
Normal	38	30	8	
High	01	1	0	
CA 15-3				**1.000**
Normal	40	32	8	
High	01	1	0	
C-erbB-2				**0.13**
Positive	20	14	6	
Negative	21	19	2	
Ki-67				**0.13**
20 and below	20	14	6	
Over 20	21	19	2	

Table 4.4. Relationship between miR-4534 expression levels and clinicopathological features of patients.

Clinicopathological Factor	Patients (41)	Low (2)	High (39)	P Value
Phase				**1.000**
I and II	28	2	26	
III and IV	13	0	13	
Smoking				**1.000**

Yes	02	0	02	
No	39	2	37	
Tumor Localization				**0.49**
Right	21	2	19	
Left	20	0	20	
Distant Metastasis				**1.000**
M0 (No)	38	2	36	
M1 (Yes)	03	0	03	
Histological Type				**1.000**
I	05	0	05	
II and III	36	2	34	
Family History				**1.000**
Yes	04	0	04	
No	37	2	35	
Tumor Size (mm)				**0.65**
Less then 50mm	34	2	32	
50 mm and above	07	0	07	
EP (Estrogen receptor)				**0.47**
Positive	30	1	29	
Negative	11	1	10	
PR (Progesterone receptor)				**0.55**
Positive	29	1	28	
Negative	12	1	11	
Lymph Node Metastasis				**1.000**
N1	33	2	31	
N2/3	08	0	08	
CEA				**0.32**
Normal	34	1	33	
High	07	1	06	
CA-125				**1.000**
Normal	38	2	36	
High	03	0	03	
CA 15-3				**1.000**
Normal	40	2	38	
High	01	0	01	
C-erbB-2				**1.000**
Positive	20	1	19	
Negative	21	1	20	
Ki-67				**0.49**
20 and below	20	0	20	
Over 20	21	2	19	

Table 4.5. Relationship between miR-1238 expression levels and clinicopathological features of patients.

Clinicopathological Factor	Patients (41)	Low (17)	High (24)	P Value
Phase				**1.000**
I and II	28	11	17	
III and IV	13	6	7	
Smoking				**0.5**
Yes	02	0	2	
No	39	17	22	
Tumor Localization				**0.41**
Right	21	10	11	
Left	20	7	13	
Distant Metastasis				**0.56**
M0 (No)	38	15	23	
M1 (Yes)	03	2	1	
Histological Type				**0.38**
I	05	1	4	
II and III	36	16	20	
Family History				**0.63**
Yes	04	1	3	
No	37	16	21	
Tumor Size (mm)				**0.45**
Less than 50 mm	34	12	22	
50 mm and above	07	5	2	
EP (Estrogen receptor)				**0.74**
Positive	30	13	17	
Negative	11	4	7	
PR (Progesterone receptor)				**1.000**
Positive	29	12	17	
Negative	12	5	7	
Lymph Node Metastasis				**0.43**
N1	33	15	18	
N2/3	08	2	6	
CEA				**1.000**
Normal	34	14	20	
High	07	3	4	
CA-125				**0.064**
Normal	38	14	24	
High	03	3	0	
CA 15-3				**1.000**
Normal	40	17	23	
High	01	0	1	
C-erbB-2				**0.53**
Positive	20	7	13	

Negative	21	10	11	
Ki-67				**0.74**
20 and below	20	6	14	
Over 20	21	11	10	

4.4 microRNA-mRNA Target Identification

This investigation led to the discovery of specific target genes related to the altered expression levels of significantly downregulated miRNAs, namely of miR-1294 and miR-551a, and the elevated levels of miR-4534. The gene selection methodology was rooted in the utilization of a benchmark score of over 80 in the miRDB database, corroborated by their simultaneous presence in both TargetScan and miRDB, as verified by mi Walk. This methodology facilitated the formation of unique gene clusters for in-depth analysis by amalgamating data from these databases.

Concerning miR-1294, the initial screening in miRDB identified a potential list of 163 genes. This list was refined through cross-validation with Mir Walk, which pinpointed 44 overlapping genes, ultimately leading to an expanded list of 168 genes. Among these, genes like BRCA1/BRCA2-containing complex subunit 3 (BRCC3), MYC proto-oncogene, bHLH transcription factor (MYC), MYCL proto-oncogene, bHLH transcription factor (MYCL), cyclin D2 (CCND2), and deubiquitinase YOD1 (YOD1) were prominent. Regarding miR-551a, an initial exploration in miRDB yielded 5 gene targets, and further examination using miRWalk highlighted 56 genes that were consistent with those in both TargetScan and miRDB, resulting in a focused group of 18 target genes. This group prominently featured genes such as crumbs cell crumbs 2 (CRB2), myocyte-specific enhancer factor 2C (MEF2C), and Human Epidermal Growth Factor Receptor 4 (ERbB4). In the case of miR-4534, the exploration in miRDB uncovered 168 genes, and a subsequent cross-referencing with miRWalk refined this to 124 overlapping genes, culminating in a comprehensive list of 193 target genes, including notable ones like SRY-box transcription factor 6 (SOX6) and adenylate cyclase 1 (ADCY1).

4.5 Gene Ontology Analysis

Functional enrichment analysis was performed individually for the target genes of each dysregulated miRNA.

4.5.1 Gene Ontology Analysis for miR-1294

In this section of the analysis, we present the gene ontology analysis results for miR-1294, focusing on biological processes (BP), molecular functions (MF), and cellular components (CC) with a p-value less than 0.05.

Starting with biological processes, miR-1294 shows significant enrichment in the regulation of lipid biosynthetic processes, Wnt signaling pathways, including both canonical and non-canonical aspects, and regulation of cholesterol storage. Gene silencing by RNA, a critical mechanism for gene expression control, also appears as a target, with post-transcriptional gene silencing by RNA and mRNA cleavage in gene silencing showing notable involvement. Additionally, processes involved in cell cycle regulation, such as the positive regulation of G1/S transition, are highlighted, along with aspects of renal system development as shown in Figure 4.5**.**

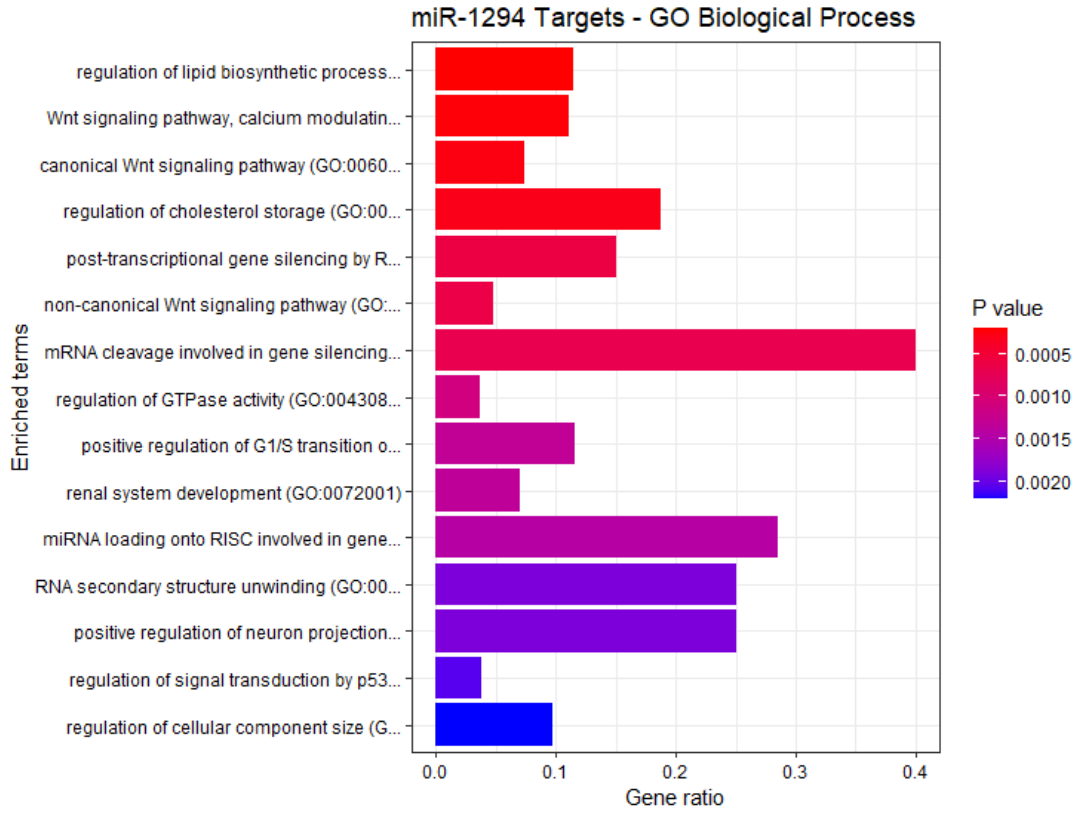

Figure 4.5. Biological process GO classification analysis of miR-1294 target genes.

Moving to molecular functions, miR-1294 target genes are significantly enriched in metallopeptidase activity and Wnt-activated receptor activity. Binding activities, including GTPase, ATP, and adenylyl ribonucleotide binding, are also prominently affected. The data shows the miRNA's influence on protein interactions, as indicated

by the enrichment in protein heterodimerization activity. Furthermore, the miRNA's role extends to RNA processing and regulation, with functions such as double-stranded RNA binding and RNA polymerase II transcription regulator activity being significantly involved as stated in Figure 4.6.

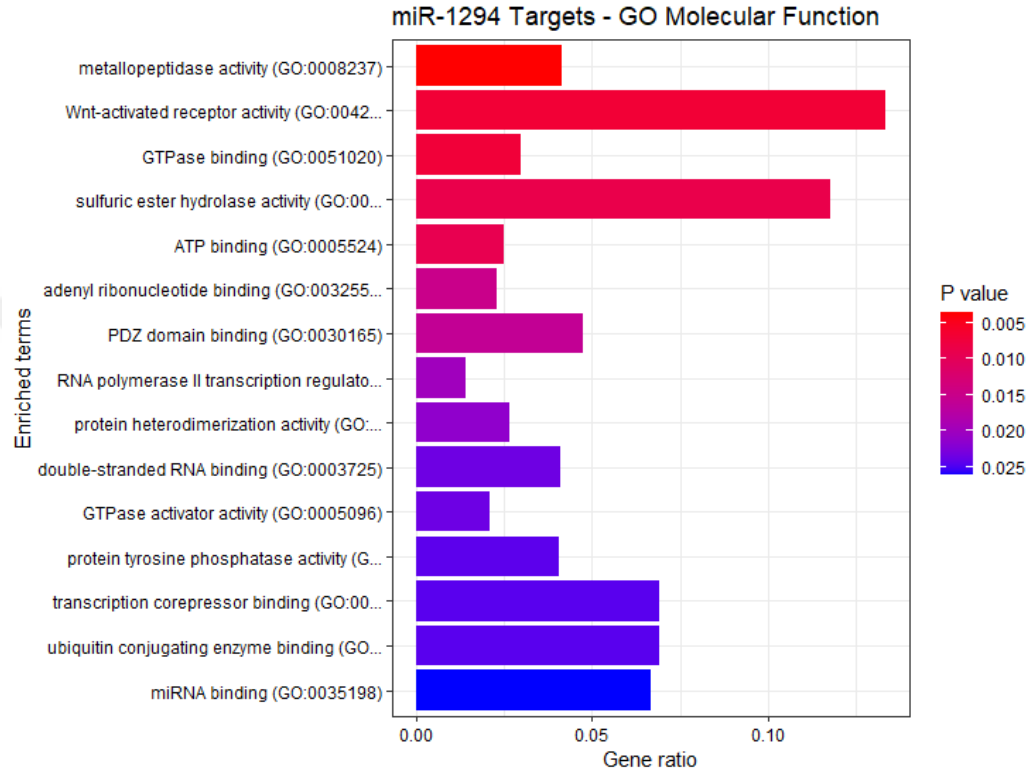

Figure 4.6. Molecular Function MF classification analysis of miR-1294 target genes.

In terms of cellular components, miR-1294 targets show a significant association with the RISC complex and RISC-loading complex, which are integral to RNA-induced silencing. The nucleus and various cellular projections like actin-based cell projections and filopodia are also significantly enriched. Structures associated with protein transport and sorting, such as Coat Protein Complex II (COPII) vesicle coat, and those involved in cellular responses to stress, like cytoplasmic stress granules and autophagosomes, are affected by miR-1294 as mentioned in Figure 4.7.

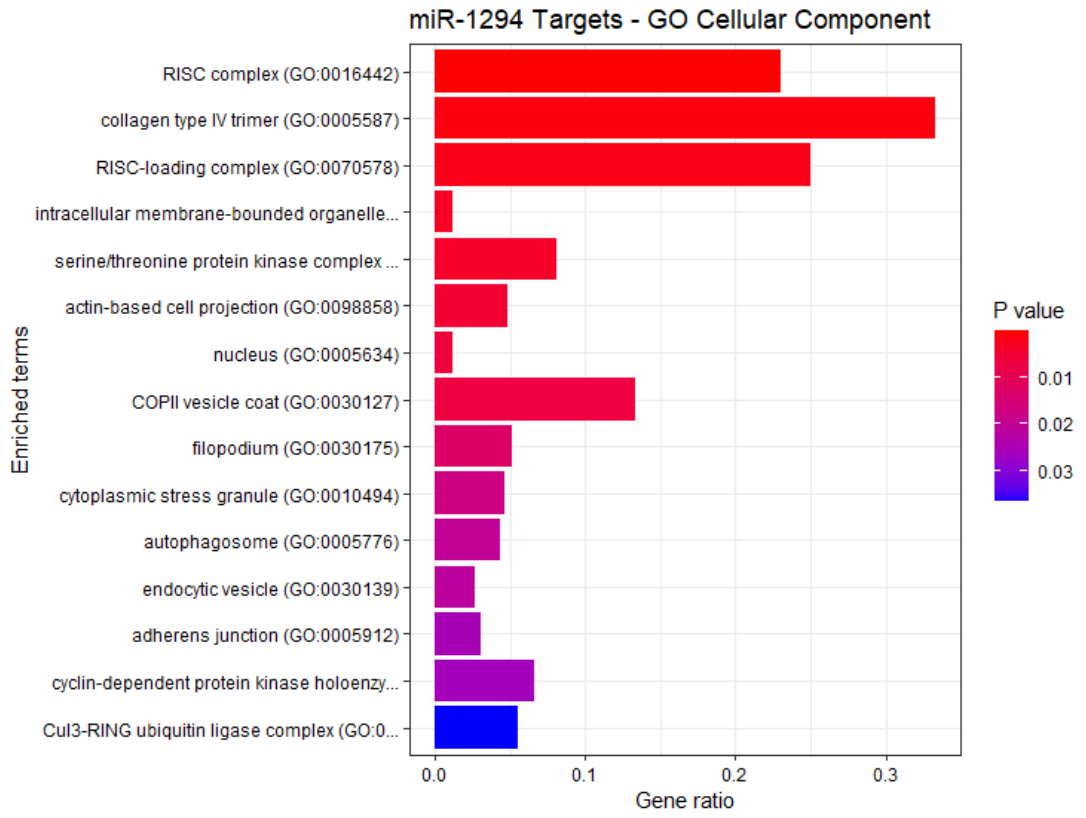

Figure 4.7. Cellular Component CC classification analysis of miR-1294 target genes.

4.5.2 Gene Ontology Analysis for miR-551a

In the present analysis, miR-551a has been linked with distinct cellular processes, molecular functions, and cellular components with p-values falling below the threshold of 0.05, indicating significant enrichment.

For biological processes, miR-551a appears to play a crucial role in the regulation of cardiac muscle cell development and differentiation, with a clear emphasis on the positive regulation of these processes. This involvement extends to the broader scope of circulatory system development. The miRNA also shows a significant influence on chondrocyte differentiation, underlining its potential role in skeletal development. Moreover, the data points to miR-551a's participation in the intricacies of cell adhesion and neuron differentiation, essential for tissue organization and nervous system development as per Figure 4.8.

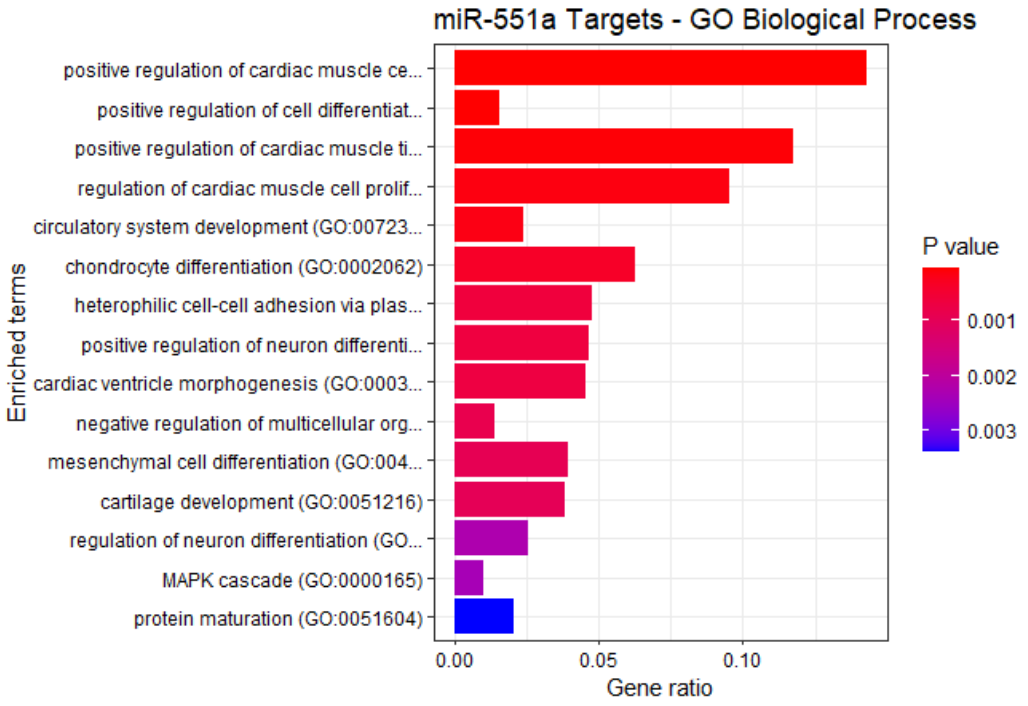

Figure 4.8. Biological process GO classification analysis of miR-551a target genes.

In the molecular function category, miR-551a targets are notably enriched in activities related to enzyme inhibition, such as aspartic-type endopeptidase inhibitor activity, indicating a regulatory control over proteolytic processes. The binding activities to specific DNA regions and various enzymatic activities, including those related to the metabolism of hyaluronan, are also markedly involved. Additionally, the miRNA's targets are implicated in crucial binding interactions essential for transcription and post-transcriptional regulation, including RNA polymerase binding and mRNA 3'-UTR AU-rich region binding as illustrated in Figure 4.9.

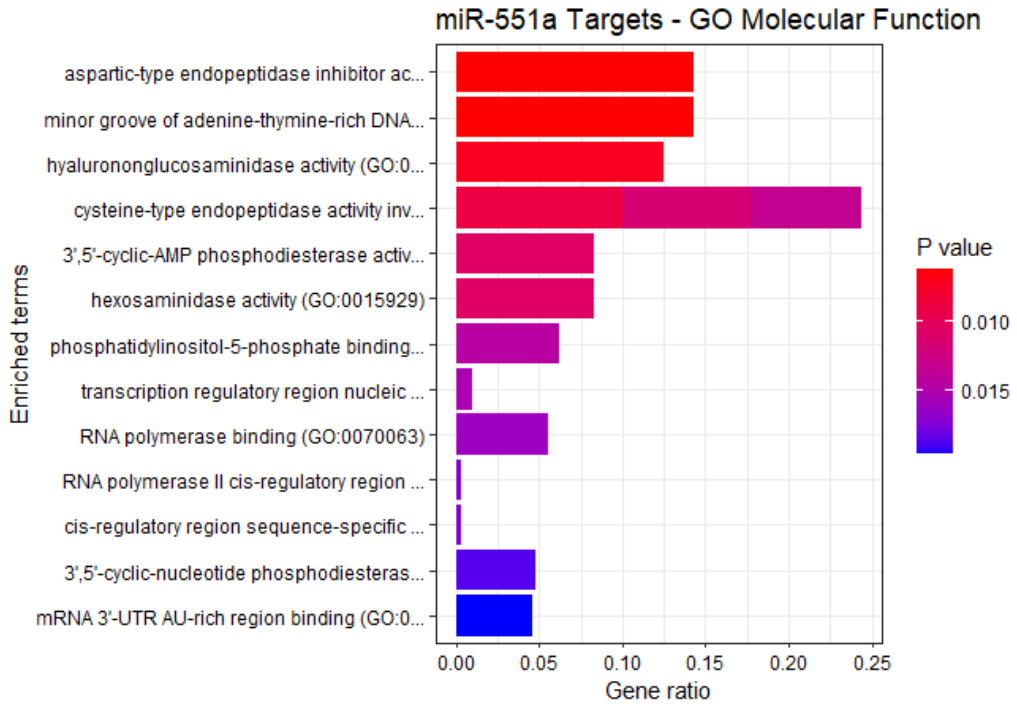

Figure 4.9. Molecular Function MF classification analysis of miR-551a target genes.

Considering cellular components, miR-551a target enrichment is significant in structures like the organelle outer membrane and the nuclear outer membrane, pointing to a role in the regulation of intracellular transport and compartmentalization. The involvement extends to components critical for cellular architecture and communication, such as the axon and myofibril, as well as to the nucleus itself, which is central to cellular function as per Figure 4.10.

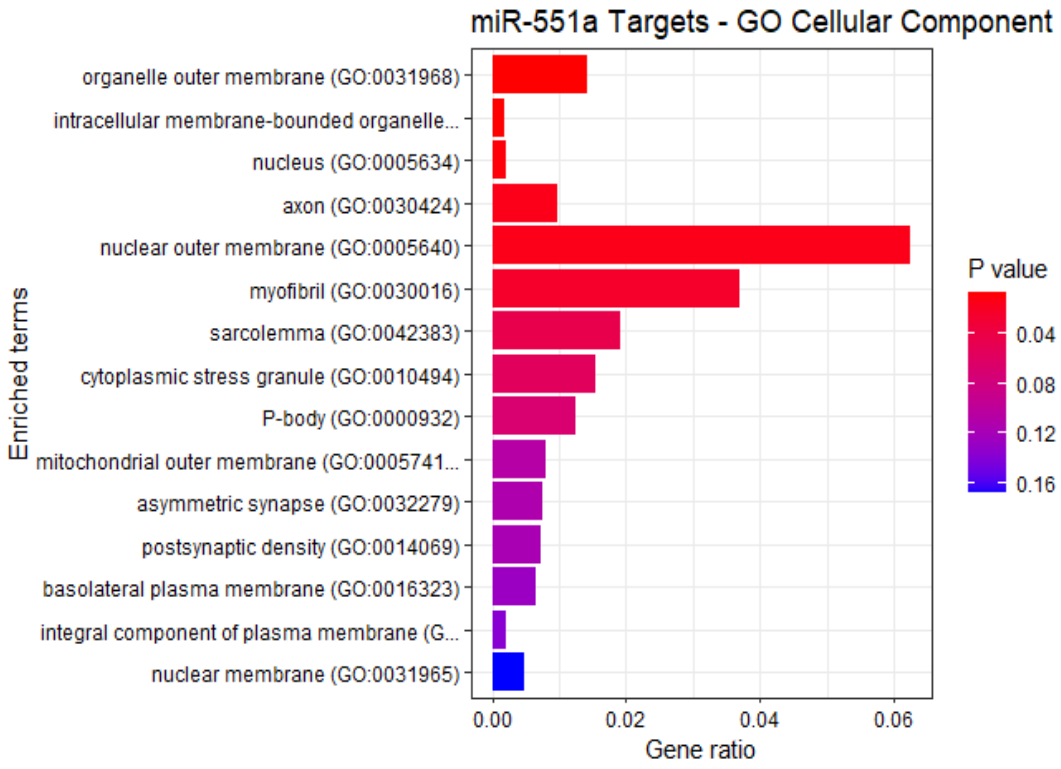

Figure 4.10. Cellular Component CC classification analysis of miR-551a target genes.

4.5.3 Gene Ontology Analysis for miR-4534

In biological processes, miR-4534 target genes show significant enrichment in the regulation of cation channel activity and neuromuscular synaptic transmission, suggesting a crucial role in the control of ion flux across membranes and neuronal communication, respectively. These processes are essential for the proper functioning of both the nervous and muscular systems. Also noteworthy is the miR-4534 association with the regulation of sodium ion transmembrane transport, a fundamental aspect of maintaining cellular ion balance and generating action potentials as per Figure 4.11.

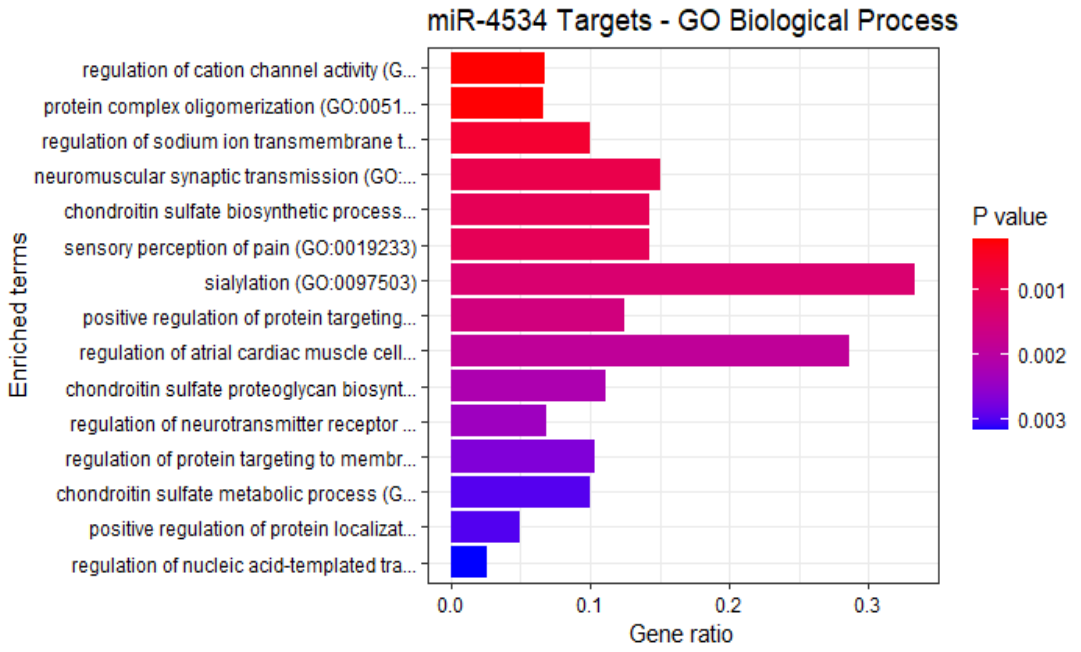

Figure 4.11. Biological process GO classification analysis of miR-4534 target genes.

Turning to molecular functions, miR-4534 displays a strong influence on voltage-gated sodium channel activity, indicating a potential regulatory role in neuronal excitability and muscle contractions. The significant enrichment in sialyltransferase activity points to a role in the modification of glycoproteins, affecting a variety of cellular interactions and signaling pathways. These molecular functions are integral to the proper execution of complex cellular responses, particularly in the nervous system as shown in Figure 4.12.

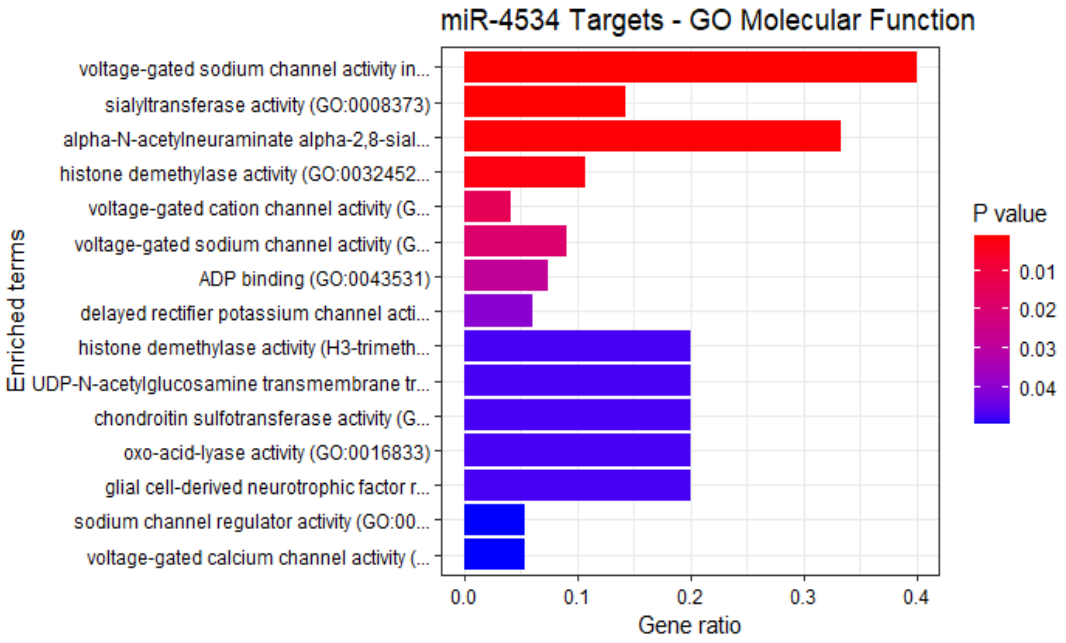

Figure 4.12. Molecular Function MF classification analysis of miR-4534 target genes.

Regarding cellular components, the analysis reveals a pronounced enrichment of miR-4534 targets within the Golgi membrane, which is central to protein processing and trafficking. There is also a significant association with the postsynaptic density, a specialized area of the cell essential for signal transduction in neurons. The involvement of miR-4534 in these components suggests its importance in the regulation of synaptic activity and neuronal plasticity as illustrated in Figure 4.13.

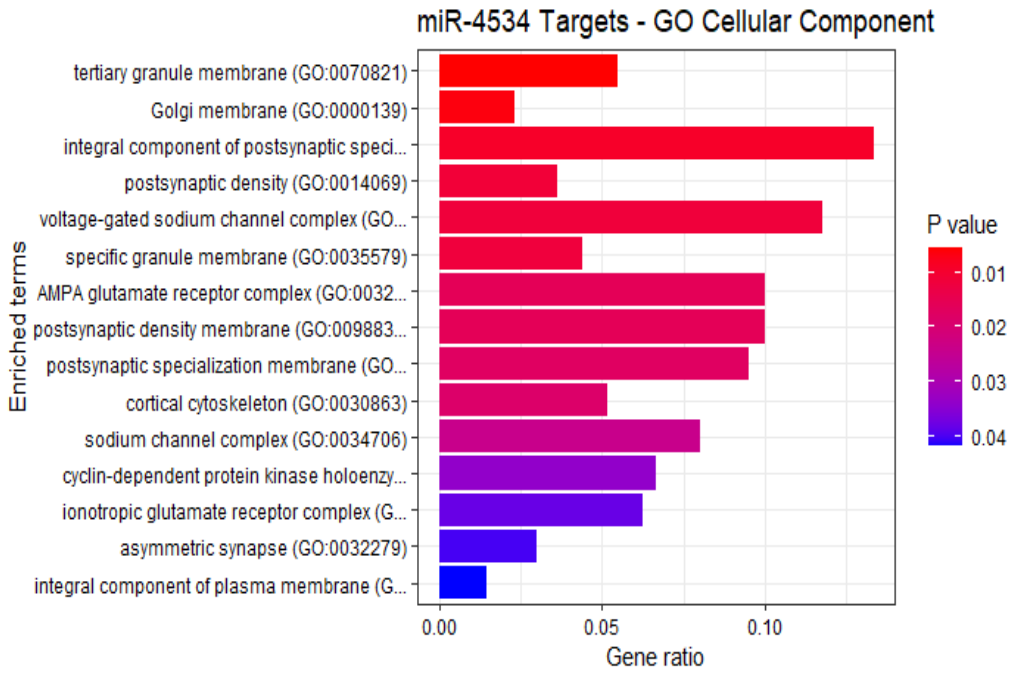

Figure 4.13. Cellular Component CC classification analysis of miR-4534 target genes.

4.6 KEGG Pathway Analysis

The KEGG pathway analysis elucidated a distinct profile of biological pathways enriched for targets of miR-1294, miR-551a, and miR-4534, underscoring its potential regulatory breadth across various physiological and pathological processes. In the KEGG analysis depicted in the accompanying photos, the bar graph illustrates a subset of enriched pathways characterized by significant gene ratio involvement. These pathways have P values below the threshold of 0.05, indicating statistical significance.

4.6.1 KEGG Pathway Analysis for miR-1294

The KEGG pathway analysis has revealed a compelling landscape of biological pathways that are significantly associated with the targets of miR-1294 shown in Figure 4.14. Foremost, the Hippo signaling pathway stands out with the highest gene ratio, illuminating miR-1294's possible pivotal role in the regulation of cell proliferation, apoptosis, and organ size control. This pathway's significance is further underscored by its strong statistical association, denoted by the red color coding for a P value less than 0.001.

Adjacent to this, the generic pathways in the cancer category reflect a broad involvement of miR-1294 across oncogenic processes, suggesting a role in tumorigenesis and cancer progression. This is complemented by significant

enrichments in the Wingless (Wnt) signaling pathway, a crucial mediator of cellular communication and differentiation, and hepatocellular carcinoma, indicating a potential specificity of miR-1294 in liver cancer pathophysiology. The graph further delineates miR-1294's association with discrete cancer types, including breast cancer and gastric cancer, highlighting its potential as a biomarker or therapeutic target in these malignancies. Notably, the gene ratios in these pathways are substantial, and the P values are well below the 0.05 threshold, reinforcing the robustness of these associations. Additionally, the signaling pathways regulating pluripotency of stem cells show significant enrichment, presenting miR-1294 as a factor in stem cell maintenance and differentiation, which could have far-reaching implications for developmental biology and regenerative medicine.

The advanced glycation end-products - receptor for advanced glycation end-products (AGE-RAGE) signaling pathway in diabetic complications and the mammalian target of rapamycin (mTOR) signaling pathway are also statistically significant, the former suggesting involvement of miR-1294 in the molecular mechanisms underlying diabetic complications, and the latter implicating it in key processes of cell growth and metabolism. Moreover, the analysis identifies significant associations with basal cell carcinoma and acute myeloid leukemia, providing a potential link between miR-1294 and these specific cancer types, while adherents junction signifies its role in cell-cell adhesion and signal transduction.

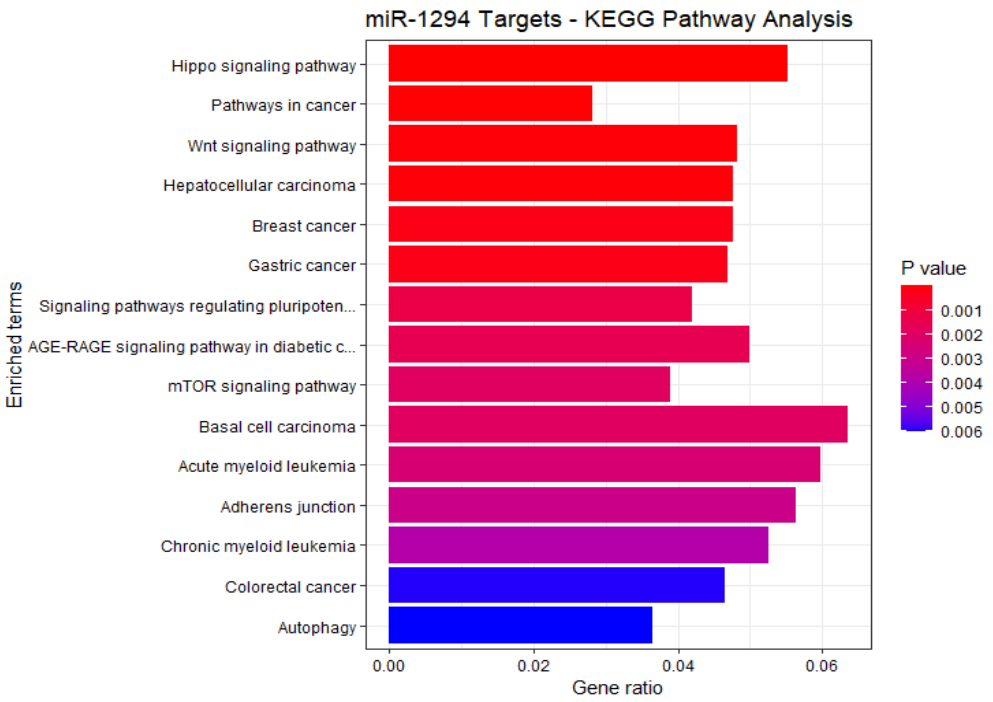

Figure 4.14. KEGG pathways analysis of miR-1294 target genes. Red color pathways are significant.

4.6.2 KEGG Pathway Analysis for miR-551a

Moving to the KEGG pathway analysis for miR-551a targets has delineated a series of pathways that are significantly associated with the interaction of miR-551a, as depicted in Figure 4.15. The bar graph elucidates the gene ratio and P value of each pathway, with a lower P value indicating a stronger association with miR-551a targets.

The analysis revealed a notable enrichment of miR-551a targets in the parathyroid hormone synthesis, secretion, and action pathway, suggesting a potential regulatory role of miR-551a in calcium homeostasis and bone metabolism. This pathway displayed the highest gene ratio, with a corresponding P value in the most significant range, less than 0.025. Another pathway with a significant association with miR-551a targets was the mitogen-activated protein kinase (MAPK) signaling pathway, well-known for its role in various cellular processes, including proliferation, differentiation, and apoptosis. The sphingolipid metabolism pathway also showed a substantial gene ratio, pointing towards miR-551a's involvement in the complex lipid signaling mechanisms and possibly in cell death and survival.

miR-551a's influence extends to the Notch signaling pathway, which is crucial for cell-cell communication and embryonic development. The mitophagy pathway, responsible for the selective degradation of mitochondria by autophagy, also exhibited

a noteworthy association, suggesting a role for miR-551a in cellular energy homeostasis and quality control. The Erb-B2 Receptor Tyrosine Kinase (ErbB) signaling pathway, implicated in the development and progression of various cancers, displayed a significant enrichment of miR-551a targets. Likewise, the morphine addiction pathway indicated an association with miR-551a, potentially affecting neural pathways involved in addiction and pain perception.

The tumor necrosis factor (TNF) signaling pathway, a mediator of inflammation and immune response, along with the purine metabolism pathway, central to cellular energy transfer, also showed significant associations with miR-551a targets. Interestingly, the analysis highlighted the breast cancer pathway, where miR-551a targets were significantly enriched, suggesting a noteworthy link between miR-551a and the molecular etiology of breast cancer. This could have implications for understanding the disease's pathogenesis and for the development of targeted therapies.

Other pathways such as the apelin signaling pathway, implicated in cardiovascular homeostasis, and fluid shear stress and atherosclerosis pathway, relevant to vascular biology, were also represented. The enrichment of miR-551a targets in the breast cancer pathway is particularly significant, providing a potential molecular link that warrants further investigation into the role of miR-551a in breast cancer biology.

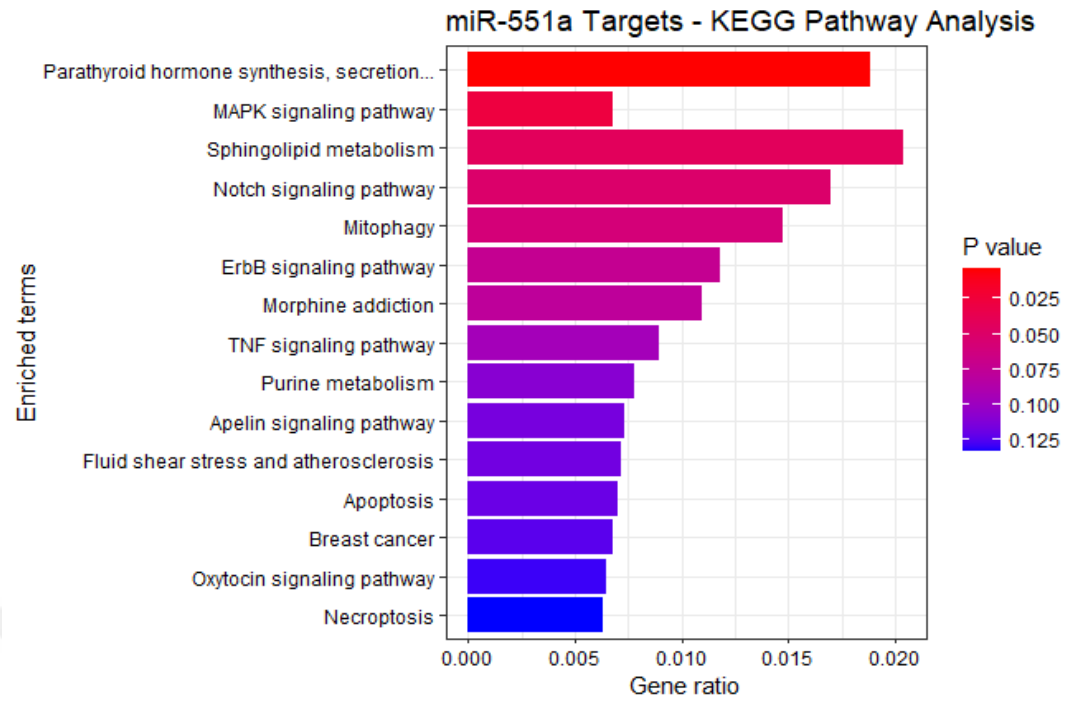

Figure 4.15. KEGG pathways analysis of miR-551a target genes. Red color pathways are significant.

4.6.3 KEGG Pathway Analysis for miR-4534

Finally, the results of the KEGG pathway analysis for miR-4534, as illustrated in Figure 4.16, provide a comprehensive profile of miR-4534 target enrichment across various biological pathways. The accompanying bar graph displays the gene ratio and P value for each pathway, with varying color intensities indicating different levels of statistical significance.

Prominently, the glutamatergic synapse pathway, which is central to the most prevalent neurotransmitter system in the brain, shows a high gene ratio with a P value less than 0.02, suggesting a significant regulatory potential of miR-4534 in neural signaling and synaptic plasticity. The Kaposi sarcoma-associated herpesvirus infection pathway and the human cytomegalovirus infection pathway, both critical in viral pathogenesis and host-pathogen interactions, are markedly enriched for miR-4534 targets. These findings highlight a possible role for miR-4534 in the modulation of host responses to these viral infections.

Additionally, the cholinergic synapse pathway, which mediates key physiological processes including muscle activation and cognition, and the phosphatidylinositol 3-

kinase-Akt (PI3K-Akt) signaling pathway, a fundamental route for cell cycle progression and survival, show substantial enrichment of miR-4534 targets. This suggests an involvement in processes ranging from cellular communication to cancer progression. The enrichment within the gamma-aminobutyric acid (GABAergic) synapse pathway points to a potential influence of miR-4534 on inhibitory neurotransmission, with implications for neurological disorders. Similarly, the presence of miR-4534 in the morphine addiction pathway could indicate a role in pain pathways and addictive behaviors. Further analysis reveals significant enrichment in the Wnt signaling pathway, which is essential for cell proliferation and differentiation, and the ascorbate and aldarate metabolism pathway, suggesting a role in antioxidant processes and cellular metabolism.

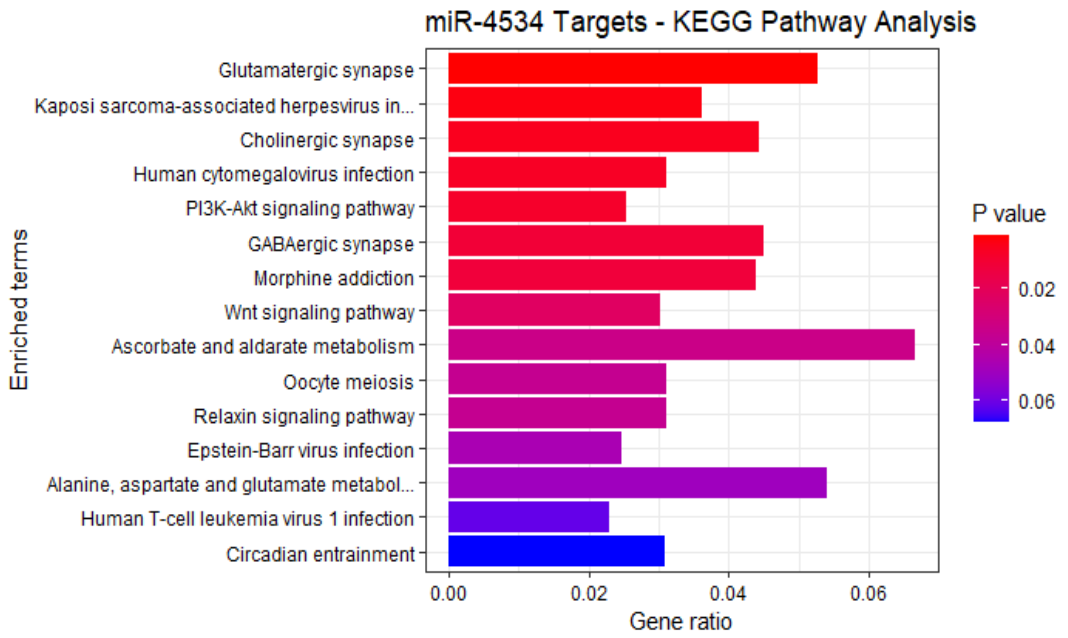

Figure 4.16. KEGG pathways analysis of miR-4534 target genes. Red color pathways are significant.

4.7 Protein-Protein Interaction (PPI) Analysis

4.7.1 Protein-Protein Interaction (PPI) Analysis for miR-1294

The protein-protein interaction (PPI) analysis, conducted using STRING to explore the functional partners of proteins targeted by the downregulated miR-1294, revealed an intricate network as shown in Figure 4.17.

The PPI network features a detailed map, annotating the complex interactions between miRNA targets and other proteins. This network differentiates between various types of interactions. Interactions that are known from curated databases are displayed with light blue lines, while those that have been experimentally determined are shown with purple lines. Additionally, the network illustrates predicted interactions based on gene neighborhood, gene fusions, and gene co-occurrence, represented by green, orange, and red lines, respectively. In the heart of the network are central nodes, depicted as colored spheres, which are direct targets of miR-1294. These central nodes are encircled by two layers of interactors: the first shell is represented by colored nodes, and the second shell by white nodes, each layer indicating subsequent tiers of protein interactions. The diverse colors of these nodes signify the presence or absence of known 3D structures for the respective proteins. The network effectively showcases the intricate relationships and predictive modeling aspects, providing a comprehensive view of the interaction dynamics within the PPI network. Central to the network are several nodes representing proteins known to play critical roles in cellular processes. These include MYC, BRCA1/BRCA2-containing complex, subunit 3 (BRCC3), cyclin D2 (CCND2), MYC-like (MYCL), and YOD1 deubiquitinase (YOD1).

Of particular interest is the gene CCND2, a major target of miR-1294, which is linked to a variety of genes including CDK6 (Cyclin-dependent kinase 6), SKP2 (S-phase kinase-associated protein 2), CDK2 (Cyclin-dependent kinase 2), and CDK4 (Cyclin-dependent kinase 4). Also, the STRING analysis showed interactions among the identified proteins and the p53 signaling pathway. This association was highlighted by the presence of specific proteins within the network that are documented in the STRING database for their involvement in the p53 pathway.

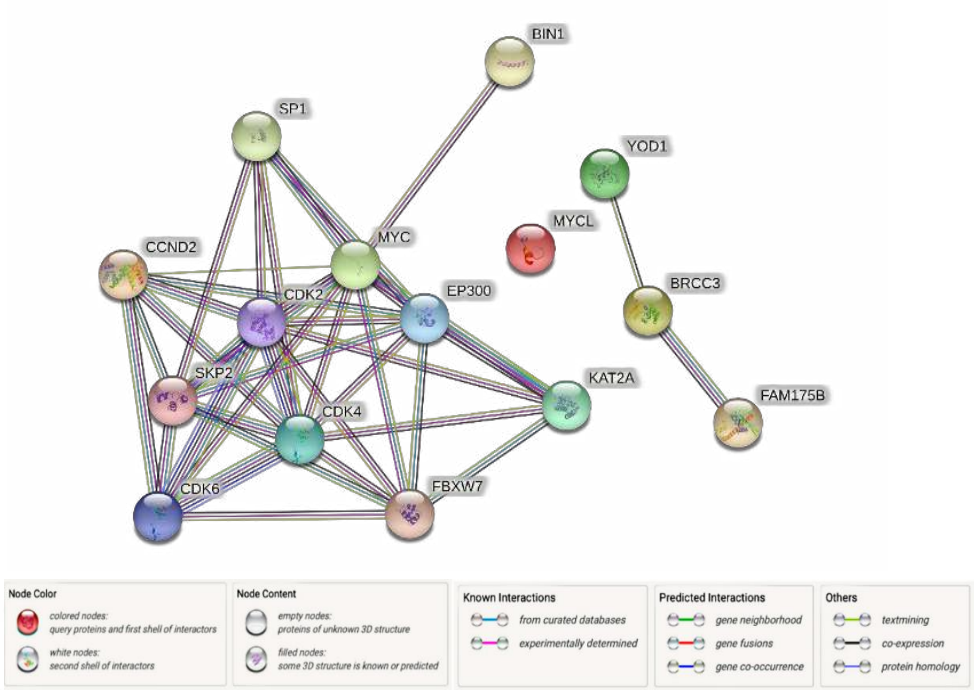

Figure 4.17. Protein-Protein Interaction analysis of miR-1294. CCND2 has many contacts with various other proteins.

4.7.2 Protein-Protein Interaction (PPI) Analysis for miR-551a

The PPI network analysis pertaining to miR-551a targets has yielded a complex interaction map as illustrated in Figure 4.18. Central to the network is the human epidermal growth factor receptor 4 (HER4) or ERbB4. The network displays multiple interactions between ERbB4 and members of the neuregulin family, including neuregulin 1 (NRG1), neuregulin 2 (NRG2), neuregulin 3 (NRG3), and SCH1. Additionally, ERbB4 interacts with discs large homolog 4 (DLG4), Growth Factor Receptor-Bound Protein 2 (GRB2), and heparin-binding EGF-like growth factor (HBEGF). Other top target genes of miR-551a are myocyte enhancer factor 2C (MEF2C), crumbs cell polarity complex component 2 (CRB2), and transmembrane protease, serine 2 (TMPRSS2). STRING analysis showed that these target genes are implicated in the negative regulation of the Interleukin-15 (IL-15)-mediated signaling pathway, ERbB signaling pathway, and Epidermal Growth Factor Receptor (EGFR) signaling pathway.

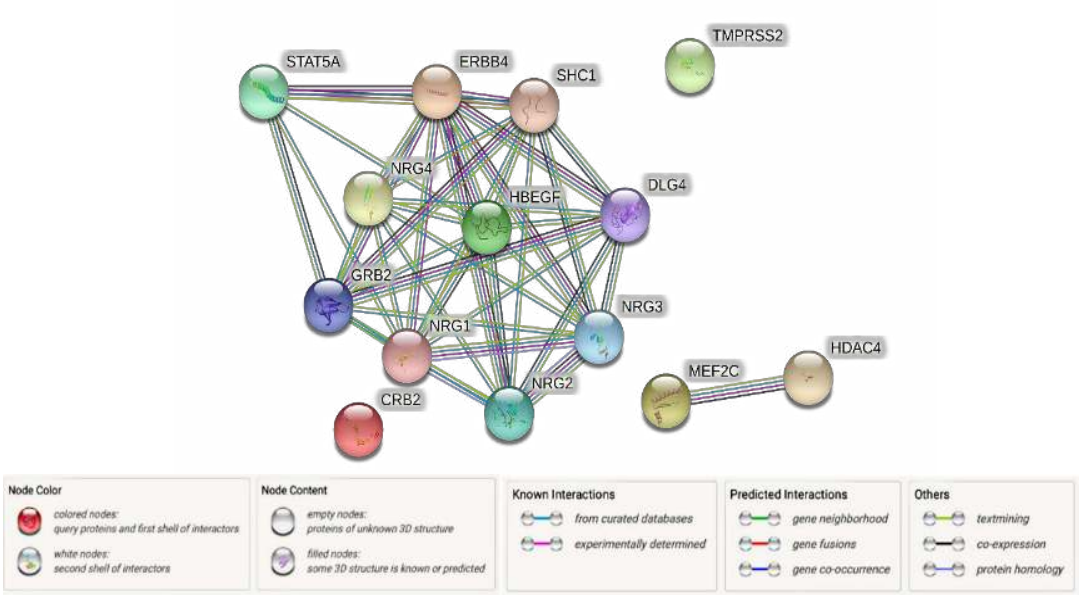

Figure 4.18. Protein-Protein Interaction analysis of miR-551a. ERbB4 has many contacts with various other proteins, especially the NRG family.

4.7.3 Protein-Protein Interaction (PPI) Analysis for miR-4534

The PPI network for miR-4534, illustrated in the Figure 4.19, presents a detailed map of protein interactions, providing insights into the potential biological roles of miR-4534. This network identifies several key proteins and complexes that could be integral to understanding the functional impact of miR-4534 on cellular processes.

This analysis shows that at the heart of the network is β-catenin (CTNNB1), which is essential in the Wnt signaling pathway. Its interaction with disheveled segment polarity proteins (DVL1 and DVL2) and DAAM family RhoGEFs (DAAM2) suggests a potential mechanism by which miR-4534 might influence Wnt signaling and, consequently, various developmental and pathological processes. The network also showed adenylate cyclases 1 (ADCY1) and its family (ADY2, ADCY9) connects with a central node is a cluster of proteins consisting of guanine nucleotide-binding proteins (G proteins) like GNAS, GNAI2, GNAI3, and GNB1. This cluster forms a dense network of interactions indicative of miR-4534's potential involvement in G protein-coupled receptor signaling pathways, which mediate a variety of physiological responses. Furthermore, the network includes the SRY-box transcription factor 6 (SOX6) and the forkhead box protein O1 (FOXO1). The presence of zinc finger protein 142 (ZNF142) suggests additional regulatory layers that might be influenced by miR-4534 in gene expression dynamics.

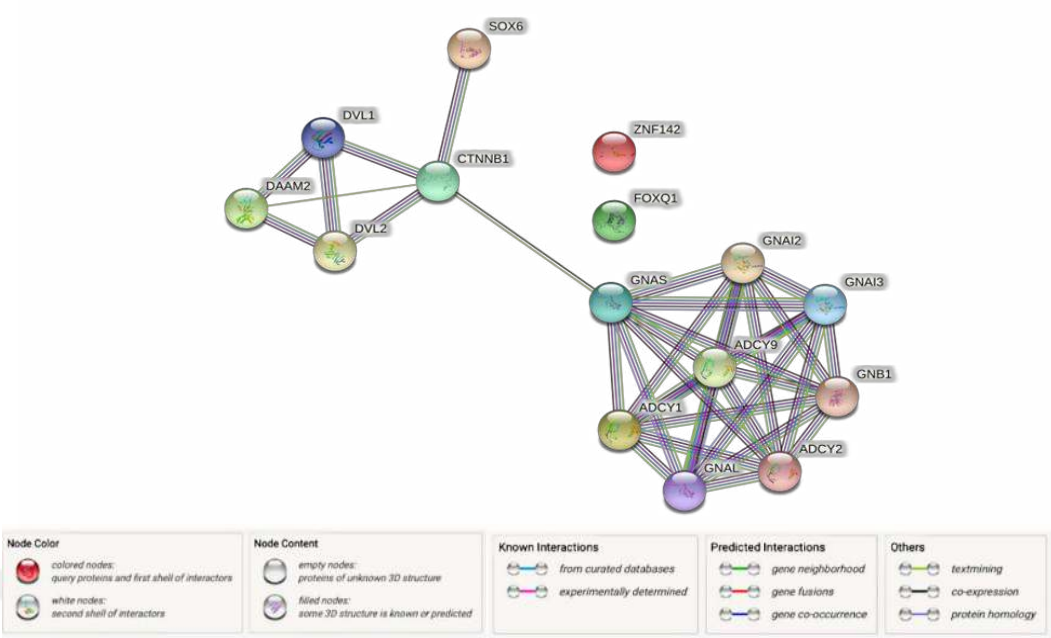

Figure 4.19. Protein-protein Interaction analysis of miR-4534. ADCY1 has many connections with various other ADCY protein family and G-proteins family.

CHAPTER 5
DISCUSSION

Our study represents a pioneering effort in breast cancer research, with a special focus on the microRNAs miR-1294, miR-551a, miR-4534, and miR-1238, particularly within the Turkish population. Despite the global scarcity of research on the impact of these specific miRNAs in breast cancer tissues, our research has utilized a novel, asymmetrical pipeline of experimental technologies, applicable both in vitro and in vivo, to delve into their roles in breast cancer progression and metastasis. Employing advanced bioinformatics tools, we systematically analyzed the dysregulation of miR-1294, miR-551a, and miR-4534 in breast cancer tumors. This comprehensive analysis was aimed at identifying their target genes and understanding the resultant protein-protein interactions. Such understanding is crucial for elucidating the mechanisms through which these miRNAs influence biological pathways, particularly those contributing to the proliferation and metastasis of cancer cells. A central aspect of this study was to explore the potential of these dysregulated miRNAs as therapeutic agents or diagnostic biomarkers in breast cancer. The feasibility of using these miRNAs in early disease detection and their impact on the metastatic behavior of breast cancer cells were critically examined. This exploration included assessing whether these miRNAs could be effectively utilized as therapeutic factors in treatment strategies, thereby offering new avenues in breast cancer management and treatment.

5.1. MiR-1294 Downregulation in Breast Cancer

This investigation notably demonstrates a significant reduction in miR-1294 expression within breast cancer tissues compared to adjacent normal tissues, evidenced by a statistically significant P-value of 0.004.

Our findings echo the pattern of pronounced downregulation of miR-1294 in breast cancer, a trend also noted in other malignancies. The decreased expression of miR-1294 has been documented in several cancers, such as esophageal squamous cell carcinoma, ovarian cancer, gastric cancer, osteosarcoma, glioma, oral squamous cell carcinoma, non-small cell lung cancer, hepatocellular carcinoma, and specifically, in breast cancer (Chen et al., 2022; Chen et al., 2020; Guo et al., 2018; Hua et al., 2020;

Li et al., 2022; Liu et al., 2015; Qin & Wang, 2023; Shi et al., 2018; Wang et al., 2020; Wang et al., 2018; Wu et al., 2020; Yuan et al., 2020; Zhang et al., 2018; Zhang et al., 2018).

In contrast, a study investigating acute lymphoblastic leukemia in a pediatric cohort of 15 children noted an anomalous increase in miR-1294 levels, targeting the SOX15 gene (Cen et al., 2023). This finding deviates from our observations and suggests the need for caution in generalizing results due to the small sample size of the leukemia study.

Overall, the downregulation of miR-1294 in our study supports its classification as a tumor suppressor, aligning with previous research in various cancers. However, it's important to note a key divergence in our results compared to prior studies. Predominantly, miR-1294 downregulation has been associated with poorer prognosis and adverse clinical outcomes. For instance, in esophageal squamous cell carcinoma, a decrease in miR-1294 correlates with a poorer prognosis (Liu et al., 2015). Similarly, in ovarian cancer, its dysregulation affects cisplatin resistance and cellular activities like proliferation, migration, and invasion (Zhang et al., 2018). Gastric cancer studies have shown that lower levels of miR-1294 are linked with larger tumor sizes and more advanced stages (Shi et al., 2018), and in osteosarcoma, its downregulation is linked to poorer survival rates, while upregulation inhibits cancer cell growth and invasion (Zhang et al., 2018). Oral squamous cell carcinoma also shows reduced miR-1294 levels associated with more aggressive tumor characteristics (Wang et al., 2018). In glioma, overexpression of miR-1294 could reduce cell growth and enhance responsiveness to treatment (Chen et al., 2018). This pattern is somewhat mirrored in non-small cell lung cancer and hepatocellular carcinoma, where low expression of miR-1294 is linked with advanced stages of the disease and enhanced tumor development, respectively (Chen et al., 2022; Li et al., 2022). These studies collectively indicate a strong link between miR-1294 expression and cancer prognosis. Our findings, however, did not demonstrate a significant correlation between clinicopathological factors and the downregulation of miR-1294. This discrepancy may largely be attributed to the small sample size and specific characteristics of our study cohort. For instance, our study included only three cases of metastasis, and the majority of the tumors were less than 50 mm in size. Such a sample composition limits the generalizability of our results, particularly in establishing relationships between

tumor characteristics and miR-1294 expression. It's crucial to acknowledge that our findings do not negate the possibility of a correlation; rather, they highlight the necessity of conducting studies with larger and more varied sample sizes. Our study's constraint emphasizes the importance of more extensive research to fully understand the implications of miR-1294 downregulation and its interactions with various genes and signaling pathways in cancer.

Examining the role of miR-1294 as a tumor suppressor reveals its interaction with various genes and key signaling pathways across different cancers. In esophageal cancer, its downregulation leads to the overexpression of the oncogene c-MYC (Liu et al., 2015) Similarly, in ovarian cancer, miR-1294 influences IGF1R expression (Zhang et al., 2018), while in osteosarcoma, it modulates HOXA9 and PKM2 (Yuan et al., 2020; Zhang et al., 2018). The effects of miR-1294 downregulation extend to TPX2 in glioma (Chen et al., 2018) and to c-Myc in oral squamous cell carcinoma (Wang et al., 2019a). Its influence in non-small cell lung cancer impacts HMGA1 (C. Li et al., 2022), and in hepatocellular carcinoma, it focuses on FOXK1 (Chen et al., 2021). Additionally, in acute lymphoblastic leukemia, miR-1294 modulates SOX15 (Cen et al., 2023).

Beyond these gene interactions, miR-1294 significantly modulates key pathways in various cancers. It affects cell cycle arrest and the PI3K/AKT signaling pathway in esophageal squamous cell carcinoma (Liang et al., 2022) and impacts the epithelial–mesenchymal transition in gastric cancer (Wang et al., 2019b). In hepatocellular carcinoma, it modulates the PI3K/AKT/mTOR pathway (Wu et al., 2020b). The influence of miR-1294 in acute lymphoblastic leukemia is observed in the Wnt/β-Catenin signaling pathway (Cen et al., 2023). Moreover, miR-1294 is crucial in affecting pathways like RAS and JAK/STAT across these cancer types. The dysregulation of these essential pathways, influenced by miR-1294, is pivotal in modulating cancer initiation and progression (Mao et al., 2023).

In this research, we conducted a comprehensive bioinformatics analysis to elucidate the mechanistic role of miR-1294 in breast cancer, a domain where such information was previously lacking. Our extensive analysis, which included identification of target genes, KEGG pathway mapping, Gene Ontology (GO) assessment, and protein-protein interaction (PPI) analysis, revealed that miR-1294 regulates several key genes in breast cancer, including BRCC3, CCND2, MYC, MYCL, and YOD1.

Particularly, BRCC3, a component of the DNA damage response mechanism within the BRCA1-BRCA2 complex, exhibits upregulation in tumor tissues and has been linked with cancer metastasis (Zhang & Zhou, 2018). Elevated levels of CCND2, MYC, and MYCL, commonly observed in breast cancer and other malignancies, are known to play roles in cancer progression (Flem-Karlsen et al., 2018; Li et al., 2019; Xu et al., 2010). YOD1, although not previously reported in breast cancer, is overexpressed in various other cancers (Zhang et al., 2022). This aligns with earlier research identifying MYC as a miR-1294 target (Wang et al., 2018).

Our study indicates that the downregulation of miR-1294 in breast cancer might lead to the increased expression of these genes. The GO and functional analysis connected miR-1294's potential target genes with processes integral to cancer development such as lipid synthesis, cholesterol storage, Wnt signaling, GTPase activity, metallopeptidase activity, and cyclin binding (Fields & Stawikowski, 2016; Litan & Langhans, 2015; Sevinsky et al., 2018; Sukocheva & Wadham, 2014; Tauro & Lynch, 2018). KEGG pathway analysis further emphasized the involvement of these genes in critical pathways like the Hippo and mTOR signaling pathways (Mao et al., 2023). The Hippo pathway's role in breast cancer, vital for colonization, molecular biology, and treatment response, was particularly highlighted (Kyriazoglou et al., 2021). Disruption in Hippo signaling has been implicated in the progression of breast cancer through interaction with the P53 oncogenic pathway (Duffy et al., 2018).

Additionally, the PPI analysis elucidated that genes in the network are enriched in the p53 signaling pathway, a key player in various cancers including breast cancer. Mutations in this pathway are known to drive cancer onset, growth, and metastasis (Duffy et al., 2018). CCDN2, targeted by miR-1294 and a part of the Hippo pathway, interacts with several genes such as CDK6, SKP2, CDK2, and CDK4, involving the cullins family essential for the formation of ubiquitin ligases (E3). These E3 ligases, crucial in tumor suppression, have been associated with breast cancer cell invasion and migration through the p53 pathway (Ohta & Fukuda, 2004; Wang et al., 2021).

However, it is important to acknowledge the limitations of our analysis, primarily the reliance on existing databases and the need for experimental validation of these findings. Future studies should focus on experimental validation of these targets in clinical samples.

In conclusion, this research not only enhances our understanding of miR-1294's role in breast cancer but also emphasizes the importance of detailed bioinformatics analyses in uncovering complex molecular interactions in cancer biology. Our findings offer potential new targets for therapeutic intervention and could guide the development of more effective treatment strategies for breast cancer. In the broader context of cancer research, this study contributes to the intricate mosaic of understanding breast cancer's molecular biology, underscoring the P53 pathway's involvement in miR-129 regulation and revealing the previously unrecognized targeting of the Hippo signaling pathway and its associated gene CCND2 by miR-1294.

5.2. MiR-551a downregulation in breast cancer

In our analysis of miR-551a's expression in breast cancer tissues, we observed a notable decrease in its expression in breast cancer samples compared to the adjacent normal tissues. This downregulation, statistically significant with a p-value of 0.038, aligns with findings from various cancer research studies, yet also presents important contrasts, emphasizing miR-551a's multifaceted role.

Echoing our findings, previous research in ovarian cancer reported a reduction in miR-551a in breast cancer, linking it to its role in inhibiting cell growth, movement, and invasive capabilities. This is evidenced by targeting the IRS2 gene, which is involved in the PI3K/AKT signaling pathway (Du & Sha, 2017). Similar patterns of downregulation were observed in gastric and colorectal cancers, where miR-551a's levels were reduced in tumor tissues associating with tumor-suppressive effects, targeting PRL-3 and CKB genes, respectively (Li et al., 2012b; Loo et al., 2015). In the context of breast cancer, miR-551a's decrease was further attributed to the activation of the FAK pathway (Anuj et al., 2019), paralleling findings about reduced miR-551a levels in breast cancer (Kang et al., 2019).

In stark contrast, studies on metastatic head and neck squamous cell carcinoma (HNSCC) presented divergent outcomes. MiR-551a was found to be upregulated in patients with distant metastasis, acting as an oncogene and promoting processes such as cell proliferation, migration, invasion, and radio-resistance, with GLIPR2 identified as a target gene (Karanam et al., 2015). Additionally, hepatocellular cancer research demonstrated miR-551a's upregulation, where it functioned as a carcinogen and correlated with poor overall survival (Chen et al., 2021).

These diverse findings underscore the complexity of miR-551a's function in cancer biology. While our study and others suggest miR-551a as a tumor suppressor in certain types, the contrasting evidence in HNSCC and hepatocellular cancers indicates a more nuanced, context-specific role. MiR-551a's function appears to vary significantly across different cancer types, influenced by the specific molecular and cellular environments. This duality, acting as both a tumor suppressor and an oncogene, highlights the need for a deeper understanding of miR-551a's role, considering the unique characteristics of each cancer type.

In this bioinformatics investigation, we extensively analyzed the molecular dynamics associated with miR-551a in breast cancer, focusing on identifying its potential target genes. Our findings highlight ERbB4, MEF2C, CRB2, and TMPRSS2 as key targets. ERbB4, a gene encoding an epidermal growth factor receptor family member, is linked to proliferative breast tumors and various other cancers (Hu et al., 2021; Kawahara & Simizu, 2022; Schubert et al., 2023) The study also shed light on CRB2, a component of the crumbs cell polarity complex, which is upregulated in glioblastoma and could act as a carcinogenic factor (Wang et al., 2022). Additionally, TMPRSS2, a transmembrane serine protease, has been found overexpressed in breast cancer, correlating with poor prognosis in ER-positive patients (Kang et al., 2019). The gene MEF2C was also identified as a significant target of miR-551a in breast cancer (Kang et al., 2019).

Gene Ontology analysis revealed the involvement of miR-551a's target genes in critical biological processes, particularly in cell differentiation and circulatory system development. This suggests that miR-551a downregulation might lead to the upregulation of key genes involved in cardiac muscle development, possibly contributing to cardiac dysfunction in breast cancer patients (da Costa et al., 2021; Demissei et al., 2020). The molecular function analysis linked these genes to activities such as endopeptidase inhibition and hyaluronan glucosaminidase activity, both associated with breast cancer progression (Sarkar et al., 2023).

KEGG pathway analysis indicated that miR-551a downregulation affects pathways like Sphingolipid metabolism, Mapk signaling, Notch signaling, and ErbB signaling. Disturbances in the Sphingolipid metabolism pathway is known to contribute to tumor growth in various cancers, including breast cancer (Companioni et al., 2021; Pani & Dasgupta, 2023). The aberrant activation of the Notch signaling pathway, essential for

cell differentiation, is observed in breast cancer, influencing tumor initiation, progression, and stem cell activity (Pandey et al., 2023). Both Mapk and ErbB signaling pathways are also excessively active in breast cancer cases (Naik, 2019; Wang, 2017)

PPI analysis identified a noteworthy interaction between ERbB4 and the NGR gene family, highlighting its overexpression in breast cancer and potential as a target for diagnosis and therapy (Vulf et al., 2023). This study emphasizes ERbB4 as a primary target of miR-551a, especially within the Mapk and ErbB signaling pathways, and underscores the role of miR-551a as a tumor suppressor. The downregulation of miR-551a could lead to the upregulation of ERbB4. Overall, this research underscores the downregulation of miR-551a in breast cancer, affecting key signaling pathways and highlighting its potential targeting of CRB2 and TMPRSS2, with a particular emphasis on ErbB4.

5.3. MiR-4534 Upregulation in Breast Cancer

The notable increase of miR-4534 in tumor tissues compared to adjacent normal ones in our breast cancer study, which is indicated by a p-value of 0.003, reinforces its classification as an "oncomiR." This consistent elevation of miR-4534 in various malignancies is further supported by its upregulation across a broad range of cancers.

In prostate cancer, the overexpression of miR-4534 in cancer, as demonstrated in previous studies, parallels our results. This overexpression, associated with changes in the methylation status of cancer cells and the downregulation of the PTEN gene, highlights miR-4534's influence on key cellular pathways such as PI3K/Akt (Jiang et al., 2019; Nip et al., 2016).

Further supporting our results, a study involving prostate cancer and benign prostatic hyperplasia patients indicated elevated miR-4534 levels in plasma, suggesting its biomarker potential (Öztürk et al., 2022). This is consistent with our findings, highlighting miR-4534's role in cancer biology and its potential as a diagnostic marker.

Similarly, in colorectal cancer, research has shown that miR-4534 is upregulated in TP53-deficient cells targeting and suppressing the autophagy-related gene ATG2B (Inoue et al., 2021). This finding echoes our observation of miR-4534's upregulation and offers insights into its potential impact on the tumor microenvironment, particularly in the context of autophagy modulation.

In triple-negative breast cancer, a significant upregulation of miR-4534 was also noted (Chai et al., 2023), aligning with our breast cancer study. This upregulation, associated with decreased survival rates, reinforces the role of miR-4534 in the progression of aggressive cancer types and is in line with our observations.

Collectively, these findings across various cancer studies resonate with our research, underscoring the pivotal role of miR-4534 as an oncogenic factor. The consistency of miR-4534 upregulation across different cancer types not only confirms its critical role in oncogenesis but also suggests its potential as a therapeutic target and a biomarker for cancer diagnosis and prognosis. These studies not only support our findings but also provide a deeper understanding of miR-4534's role in promoting oncogenic processes.

In our comprehensive analysis of breast cancer at the molecular level, we identified a set of top target genes for miR-4534, including SOX6, ADCY1, DAAM2, ESRRG, FOXQ1, and ZNF142. These genes, integral to various cellular functions and cancer development, present a nuanced understanding of miR-4534's role in oncogenesis.

SOX6, pivotal in organogenesis and cell differentiation, is recognized for its tumor-suppressive properties in breast cancer. This gene is notably downregulated in breast cancer contexts, implicating a crucial role in tumorigenesis (Wei et al., 2022). ADCY1, known to be promoter-methylated and downregulated in breast cancer, is associated with improved clinical outcomes when its mRNA levels are elevated, suggesting its potential as a prognostic marker (Li et al., 2017). DAAM2, whose downregulation has been documented in pancreatic adenocarcinoma, may play a similar role in breast cancer (Zhu et al., 2017). ESRRG serves as a tumor suppressor and is implicated in metabolic regulation, specifically in inhibiting glycolysis and promoting oxidative phosphorylation; its downregulation in breast cancer further signifies its functional importance (Eichner et al., 2010). FOXQ1's expression level inversely correlates with overall survival in breast cancer, making it a key factor in patient prognosis (Elian et al., 2021). Finally, ZNF142, marked by a significant mutation burden, is observed in a considerable fraction of breast cancer cases, highlighting its potential role in cancer pathophysiology (Aravind et al., 2018; Yan et al., 2021).

Our findings suggest that the upregulation of miR-4534 in breast cancer may lead to the suppression of these top target genes. Gene Ontology analysis reveals that miR-

4534's target genes are predominantly involved in regulating cation channel activity, protein complex oligomerization, and sodium ion transmembrane transport. This is particularly relevant, as alterations in ion channel function, especially cation channels, are increasingly recognized for their roles in cancer development, including breast cancer (Jiang et al., 2021). KEGG pathway analysis further delineates miR-4534's impact on several critical signaling pathways, including PI3K-Akt, glutamatergic synapses, cholinergic synapses, and relaxin signaling, with the PI3K-Akt pathway already identified as a target for miR-4534 (Zhao et al., 2020). Disruptions in glutamatergic and cholinergic synapse signaling pathways are linked to cellular growth, tumor proliferation, and endocrine resistance in breast cancer, emphasizing the significance of ion channels in these processes (García-Gaytán et al., 2022; Jiang et al., 2021; Willard & Koochekpour, 2013).

Protein-protein interaction analysis reveals that ADCY1, as a target gene of miR-4534, interacts with the adenylate cyclase and G-protein families, crucial in regulating various cellular processes associated with tumorigenesis, angiogenesis, and metastasis. The involvement of ADCY1 in glutamatergic and cholinergic synapse signaling pathways further underscores its role in these critical biological processes (Fan et al., 2019; Zou et al., 2019).

CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

In summary, our study proposes that the oncogenic potential of upregulated miR-4534 in breast cancer manifests through the disruption of key signaling pathways, most notably the glutamatergic and cholinergic synapses signaling pathways. This disruption, along with the modulation of top target genes like ADCY1, aligns with existing literature, reinforcing the need for further investigations, particularly in vivo studies, to confirm these targets and pathways influenced by miR-4534 and to explore potential therapeutic interventions in breast cancer.

In conclusion, the findings of this study contribute significantly to our understanding of the roles played by microRNAs, specifically miR-1294, miR-551a, and miR-4534, in the pathophysiology of breast cancer. The observed downregulation of miR-1294 and miR-551a in breast cancer specimens underscores their potential function as tumor suppressive agents. This study posits that miR-1294 likely targets and regulates genes such as BRCC3, MYCL, and YOD1, which play pivotal roles in the regulation of the Hippo and p53 signaling pathways. Similarly, the reduced expression of miR-551a in breast cancer tissue implicates its involvement in the modulation of genes including ERbB4, MEF2C, CRB2, and TMPRSS2, which are integral to various critical cellular pathways, namely cardiac muscle regulation, Sphingolipid metabolism, Notch signaling, Mapk signaling, and ErbB signaling.

Conversely, this study identifies an oncogenic attribute in miR-4534, as evidenced by its heightened expression in breast cancer tissues. This upregulation suggests its involvement in the regulation of genes associated with ion transport, and glutamatergic and cholinergic synapse signaling pathways, thereby highlighting its potential as a novel therapeutic target in breast cancer treatment. The study further proposes that miR-4534 may target genes such as SOX6, ADCY1, ESRRG, and ZNF142, suggesting these as key areas for future research. Collectively, these findings offer a nuanced understanding of the molecular mechanisms underlying breast cancer and pave the way for the development of targeted therapeutic strategies.

6.2. Recommendations

This research provides a solid foundation for further experimental exploration of the target genes proposed, beyond the scope of the bioinformatics analysis utilized here. While the computational analysis has been invaluable, empirical validation is imperative for confirming the involvement of miR-1294, miR-551a, and miR-4534 in gene regulation. Advancing these studies into in vivo environments, especially using mouse models, would enable a detailed examination of the impact of these miRNAs and their targets on tumor development, metastasis, survival rates, and their interactions within the tumor microenvironment. For future research, it is recommended to increase the sample size and diversify the sample characteristics, including variations in metastasis, tumor dimensions, and cancer-related biomarkers. Such an approach would facilitate a more thorough investigation into the correlation between these miRNAs and clinicopathological attributes, enhancing our understanding of their biological roles and their specific effects on breast cancer progression.

BÖLÜM 7
GENİŞLETİLMİŞ TÜRKÇE ÖZETİ

7.1 Giriş

7.1.1 Meme Kanseri Epidemiyolojisi

Meme kanseri, kadınlarda kansere bağlı ölümlerin başlıca sebeplerinden biri olmaya devam etmektedir. 2020 yılında, meme kanseri tüm kanser vakalarının %24,5'ini oluşturmuş, 2,26 milyon kadın teşhis edilmiş ve 685.000 ölümle sonuçlanmıştır (WHO, 2023).

Global Kanser Deposu "GLOBOCAN" raporu, beş yıl boyunca "2016-2020" meme kanseri yaygınlığı hakkında şu ifadeleri içermektedir: Meme kanseri vakaları, kanser türleri arasında yaklaşık %30,3 oranında görülmüş ve dünya genelinde kadın ölümlerinin %15,5'inden sorumlu olmuştur. Etnik köken açısından bakıldığında, Asyalı kadınlarda meme kanseri vaka oranı en yüksek olup %42,2 olarak rapor edilmiş, onu %25,8 ile Afrikalı kadınlar ve Latin Amerika ile Karayipler'deki kadınlarda %7,9 oranında vakalar takip etmiştir (Łukasiewicz et al., 2021).

Ölüm oranlarına ilişkin olarak, meme kanseri teşhisi konulan Afrikalı hastalar %12,5 oranında, Latin Amerika ve Karayipler'den hastalar ise tahmini %8,5 oranında ölüm oranı yaşamıştır (Sung et al., 2021). Gelişmiş sağlık sistemlerine sahip bölgelerde, yerel ve bölgesel meme kanseri vakalarında beş yıllık hayatta kalma oranları, daha az gelişmiş ülkelerden daha yüksektir. Bu durum, herkes için yüksek kaliteli sağlık hizmetlerine erişimin sağlanmasının önemini vurgulamaktadır.

Tahminlere göre, 2030 yılına kadar dünya genelinde meme kanseri vaka oranı artacaktır, ve buna göre yıllık yaklaşık 2,7 milyon vaka ve yaklaşık 0,87 milyon ilişkili ölüm beklenmektedir (Łukasiewicz et al., 2021), 2040 yılında ise vakaların 3 milyona ulaşması ve 1 milyon ölümle sonuçlanması öngörülmektedir (Arnold et al., 2022).

Meme kanseri, Türkiye'de de dünya genelinde olduğu gibi yaygın bir sağlık sorunudur. Türkiye Sağlık Bakanlığı'nın 2018 yılında yayınladığı resmi istatistiklere göre, 24.518 kadına meme kanseri teşhisi konulmuştur. Meme kanseri vaka oranı, diğer kanser türlerinin %26'sını oluşturmuştur ve 2021 yılında 25,351'e ulaşması beklenmektedir

(Türkiye Sağlık Bakanlığı, 2018). Globocan raporuna göre, 2020 yılında, 24.175 meme kanseri vakası, tüm kanser vakalarının %10,3'ünü oluşturmuştur (Şekil 1.2) (The Global Cancer Observatory, 2021).

7.1.2 Meme Kanserinde Sınıflandırma

Meme kanseri iki ana kategoride incelenir: invaziv ve noninvazivdır.

A. İnvaziv meme kanserleri: Oluşmaya başladığı bölgede kalmayarak yayılım gösteren meme kanserleridir.

- **Duktal invaziv meme kanseri**: Aynı zamanda "Memenin İnvaziv Duktal Karsinomu" olarak bilinir ve en yaygın meme kanseri çeşididir. Yüz meme kanseri vakasından 70 -80'inde görülür. Süt kanallarının duvarındaki hücrelerden başlayarak çevre meme dokusuna yayılan kanser türüdür. İnvazif duktal meme kanseri, hücrelerin kanallardan çıkıp lenf düğümlerine veya vücudun diğer bölgelerine yayılma potansiyeline sahiptir.
- **Lobüler invaziv meme kanseri**: Aynı zamanda "Memenin İnvaziv Lobüler Karsinomu" olarak adlandırılır ve her 10 meme kanserinden birinde görülür. Genellikle 45-55 yaş arasında tanı konulan kanserlerde rastlanır. Meme loblarının duvarındaki hücrelerden başlayarak çevre meme dokusuna yayılan kanser türüdür.

B. Noninvaziv meme kanseleri: Başladıkları yerde kalan ve yayılmayan kanserlerdir. Meme kanserinde başka bir sınıflandırma daha vardır. Nadiren rastlanan kanserler özel tip, sık rastlananlar ise özel olmayan tip olarak tanımlanır (Weigelt et al., 2010).

7.1.3 Meme Kanserinde Evrelendirme

Kanserde tanı konulduktan sonra evrelendirme en iyi tedavinin seçilebilmesi açısından önemlidir. Kanserin evrelendirilmesi amacı ile TNM sistemi geliştirilmiştir.

- **T** tümör boyutu, meme içinde ve çevre organlara yayılımı,
- **N** lenf benzlerinin durumunu,
- **M** kanserin metastaz durumunu ifade eder.

T. N ve M'den sonra gelen bazı rakam ve harfler tümör, lenf nodu ve metastaz hakkında detaylar için kullanılır (American Joint Committee on Cancer, 2018).

7.1.4 MikroRNA

MikroRNA (miRNA)'lar yüksek seviyede korunan DNA bölgelerinden kodlanan, transkripsiyon sonrası gen ekspresyonunu kontrol eden, küçük kodlanmayan RNA molekülleridir. İlk miRNA (Lin-4), 1993 yılında Lee vd. ile Wightman vd. tarafından ayrı laboratuvarlarda eş zamanlı olarak keşfedilmiştir. Ortalama 20 ile 23 nükleotidden oluşan bu miRNA'ların insan genomundaki sayısının binlerce olduğu tahmin edilmektedir. miRNA'ların hücre bölünmesi, hücre tipinin farklılaşması, hücrenin gelişimi ve hücrenin ölümü gibi olaylarda düzenleyici olarak işlev gördüğü bilinmektedir. Bu yüzden miRNA'nın ekspresyonundaki değişimlerin hastalık gelişiminde etken olduğu düşünülmektedir. miRNA'lar hücre içerisindeki sinyal yolaklarına etki etmektedirler. Genel anlamda hücre içerisindeki sinyal yolakları hücresel fonksiyonlar üzerine anahtar görevi üstlenirken, çevresel etmenler ve gen ekspresyonları ile kontrol edilmektedirler. Sinyal yolakları hücrelerin normal bir şekilde gelişimi ve tamiri için oldukça önemlidir. Bundan dolayı bu yolaklarda meydana gelebilecek herhangi bir düzensizlik ya da aktivite bozukluğu başta kanser gibi birçok hastalığa neden olabilmektedir (Gebert & MacRae, 2018).

7.1.5 MikroRNA Biyogenezi

miRNA'lar, kısa kodlanamayan RNA'lar sınıfından olup, gen ekspresyonunun düzenlenmesinde rol oynarlar. İlk başta DNA sekansından pri-miRNA'lar (primer miRNA) olarak transkript edilir daha sonra da pre-miRNA'ya, son olarak da fonksiyonel yapıdaki miRNA'ya dönüşürler. miRNA biyogenezi, çekirdekte RNA polimeraz II enzimi vasıtasıyla transkripsiyon sonucunda pri-miRNA eldesi ile başlamaktadır. Oluşan bu pri-miRNA hairpin yapısında yani saç tokası şeklindedir. Hairpin yapısı RNaz III enzimi ve onun ko-faktörü olan DGCR8 pasha vasıtası ile oluşan mikroprosesör ile etkileşim göstererek 60-70 nükleotidlik parçalar haline dönüşerek pre-miRNA oluştururlar. Nükleusta pre-miRNA oluşması işleminden sonra pre-miRNA XPO5 molekülü aracılığı ile sitoplazmaya taşınır ve bir RNaz III enzimi tarafından ortalama 20-25 nükleotidlik çift sarmal halinde olgun miRNA'ya çevrilir.

Helikaz enzimi tarafından açılan miRNA, daha sonra olgun miRNA'ya bağlanacak olan iplik RISC ile RNA'ya yüklenerek uyarılmış susturma komleksini oluşturmaktadır. mRNA'ya bağlanan bölge çekirdek olarak isimlendirilen bölge ile hedefe bağlanırken miRNA ve mRNA'ya bağlanan proteinler hedef seçiminde önemli rol oynamaktadır.

Mikro RNA'lar, genellikle hedef mRNA'ların 3'UTR (çevrilmeyen bölge) gibi translasyona uğramayan bölgelerine bağlanarak mRNA yıkımını tetikler ve sonuç olarak translasyonu baskılarlar. Bununla birlikte, miRNA'ların 5'UTR, kodlama dizileri ve gen promotörleri gibi diğer bölgelerle de etkileşimde bulunduğu belgelenmiştir. Özel koşullar altında, miRNA'lar sadece translasyon baskılama ve mRNA yıkımını indüklemekle kalmaz, aynı zamanda translasyonu aktive ederek gen ekspresyonunu düzenlerler. Hedef genlerle miRNA'ların dinamik etkileşimi, bu moleküllerin konsantrasyonları ve subhücresel konumları gibi faktörlere bağlıdır ve bu faktörler, miRNA-mRNA etkileşimlerinin affinitesini ve hedef mRNA miktarını etkiler. Ek olarak, miRNA'lar hücre dışı sıvılarla salgılanabilir ve eksozomlar gibi veziküller aracılığıyla veya Argonaute gibi proteinlere bağlanarak hedef hücrelere taşınabilirler. Hücre dışına salınan miRNA'lar, hücreler arası iletişimde rol alan kimyasal haberci moleküller olarak işlev görürler. Bu süreçler, miRNA'ların biyolojik işlevlerinin ve hücre içi iletişim mekanizmalarının anlaşılmasında kritik öneme sahiptir, Şekil 1.4 (Bartel, 2004).

7.1.6 MikroRNA ve Kanser

Bugüne kadar, farklı meme kanseri türlerinde miRNA profilleme çalışmaları yapılmış olup ifade düzeyi önemli derecede farklılık gösteren miRNA' lar bildirilmiştir. Mikro RNA'lar, meme kanseri başta olmak üzere birçok insan kanseri ile ilişkilendirilen anormal ifadeler sergilemektedir. Bunlar, hücre çoğalması, farklılaşma ve apoptoz gibi süreçlerde kritik roller üstlenmektedir. Örneğin, bazı miRNA'ların meme kanseri hücrelerinin invazif ve metastatik özelliklerini düzenlediği tespit edilmiştir. Tümör baskılayıcı özellikler gösteren miRNA'lar, meme kanserinin invazyonunu ve metastazını inhibe edebilmektedir. Ayrıca, çeşitli miRNA'ların hücre döngüsünü modüle ederek hücre çoğalmasını etkilediği veya apoptoz süreçlerini düzenlediği gözlemlenmiştir (Barbato et al., 2017).

Meme kanserindeki miRNA ekspresyon düzeylerinin incelendiği çalışmalarda değişken sonuçlar elde edilmiş olup, bu heterojenite klinikopatolojik faktörlerle ilişkili olabilir. Araştırmalar, miRNA'ların onkogenezde rol oynayabileceğini göstermektedir. Örneğin, miR-21 ve miR-155'in artan ifadesi ve miR-200 serisi, miR-10b, miR-125b ve miR-145'in azalan ifadesi, miRNA'ların tümör baskılayıcı veya onkogenik roller üstlenebileceğine işaret etmektedir. Proonkogenik veya prometastatik miRNA'ların artan ifadesi veya antionkogenik veya antimetastatik miRNA'ların azalan ifadesi,

tümör metastazını teşvik edebilir. miR-7, miR-128a, miR-210 ve miR-51-3p gibi miRNA'lar meme kanserinin ilerlemesi üzerinde etkili olabilmektedir. miR-373 ve miR-520c'nin artan ifadesi, CD44 ifadesini inhibe ederek metastaz sürecini desteklerken, miR-21'in tropomiyosin 1 gibi antionkogenik işlev gösteren hedeflere odaklanması, bu alanın karmaşıklığını ve önemini vurgulamaktadır (Loh et al., 2019).

7.1.7 Meme Kanserinde Biyobelirteç Olarak MikroRNA'lar

MikroRNA'lar, kanser teşhisinde önemli biyobelirteçler olarak kendini göstermiştir. Gen ifadesinin düzenlenmesindeki kritik rolleri ve kötü huylu hücrelerde gözlenen belirgin düzensizlikler, miRNA'ları erken teşhis ve izlem için ideal adaylar haline getirmektedir (Yang & Liu, 2020; Meng et al., 2013). Özellikle, miR-642b-3p, miR-1202-5p ve diğerleri gibi belirli miRNA'lar, erken evre meme kanseri hastalarının kan plazmasında tespit edilmiştir (Hamam et al., 2016), miR-1825-3p gibi diğerleri ise gliomalarda farklı modeller göstermektedir (Wang et al., 2018). MiRNA ifadesi aracılığıyla kanser alt tiplerinin ayrımı, özellikle miR-342 ile ilgili tanısal içgörüler sunmaktadır (Lindholm et al., 2019; Romero-Cordoba et al., 2018). Gelişmiş tespit teknikleri, miRNA'ların tanı potansiyelini artırmaya devam etmektedir (Yan et al., 2023).

7.1.8 Terapötik Ajanlar ve Tedavi Direnci Aracılığıyla MikroRNA

MiRNA'lar, kanser kök hücrelerini kontrol etmekten, kanser ilerlemesini etkilemeye kadar kanser yönetiminde hayati bir rol oynamaktadır (Szczepanek et al., 2022). MiR-155'in, üçlü negatif meme kanserinin iyonlaştırıcı radyasyona duyarlılığını etkilemesi örneğinde olduğu gibi, terapötik hedefler olarak da hizmet etmektedirler (Tavazoie et al., 2008). MiRNA analogları ve antisan anlamlı oligonükleotitler (ASO'lar) gibi yaklaşımlar, terapötik uygulamalar için araştırılmaktadır (Menon et al., 2022; Xiong et al., 2021). MiR-1294, miR-551a, miR-4534 ve miR-1238, çeşitli kanserlerdeki miRNA'ların çeşitli rollerini göstererek, yeni terapötik yollar sunmaktadır (Mao et al., 2023; Karanam et al., 2015; Li et al., 2012; Nip et al., 2016b; Inoue et al., 2021; Shi et al., 2015; Luo et al., 2022).

Tedavi direnci bağlamında, miRNA'lar, ilaç atılımını düzenleyerek, ilaç hedeflerini değiştirerek ve DNA onarımı ile hücre döngüsü düzenlenmesi gibi çeşitli mekanizmalarla ilaç direncini modüle eder (Si et al., 2019; Dong et al., 2019; Crosby et al., 2009; Kim et al., 2009; Li et al., 2014). Bu karmaşık etkileşim, miRNA'ların

hem terapötik ajanlar, hem de tedavi direnci faktörleri olarak potansiyelini vurgulamakta ve meme kanseri tedavi ve yönetimindeki gelişen manzara içindeki önemlerini ortaya koymaktadır.

7.1.7 Araştırmanın Amacı

Bu çalışma, miR-1294, miR-551a, miR-1238 ve miR-4534'ün meme kanseri bağlamında rollerini, bu miRNAların çeşitli kanser türlerindeki bilinen katılımlarının ötesine geçerek incelemeyi amaçlamaktadır. Bu araştırma, söz konusu miRNAların özel hücresel işlevlerini, moleküler hedeflerini ve meme kanseri genel genomik manzarası içerisindeki mekanistik etkileşimlerini daha iyi anlamayı hedeflemektedir. Dahası, bu miRNAlar ile ana kanserle ilişkili sinyal yolları arasındaki potansiyel ilişkileri ortaya çıkarmayı ve terapötik direnç üzerindeki etkilerini değerlendirmeyi hedeflemektedir. Bu araştırma, önemli bir bilgi eksikliğini gidermeyi ve meme kanseri patogenezinin daha kapsamlı bir incelemesine katkıda bulunmayı amaçlamakta olup, potansiyel olarak yeni terapötik yaklaşımların ve prognostik biyobelirteçlerin tanımlanmasına öncülük edebilir.

7.2 Kaynak Özetleri

7.2.1. MikroRNA-1294 (miR-1294)

Liu ve arkadaşları 2015 yılındaki miR-1294 işlevi üzerine yaptıkları ilk çalışmada, miR-1294 ifadesinin insan yemek borusu yassı hücreli karsinomu dokusunda, karşılık gelen normal dokuya göre önemli ölçüde düşük olduğu tespit edildi. Bu durum, 79 kanser hastasından alınan örneklerde RT-PCR kullanılarak kanıtlandı. Çalışma, düşük miR-1294 ifadesinin kötü prognoz ile ilişkili olduğunu ortaya koydu. İn vitro analizlerde, miR-1294'ün düşürülmesinin onkojen c-MYC'i yükselttiği, bu durumun c-MYC'i miR-1294 için bir hedef gen olarak önerdiği gösterildi. Toplamda, bu bulgular, kanserde de miR-1294 düşürülmesinin prognozik etkilerinin potansiyelini vurgulamakta ve bunun c-MYC protein fonksiyonu üzerindeki inhibe edici etkisinin azalmasına bağlanabileceğini öne sürmektedir (Liu et al., 2015).

2018'de Zhang ve arkadaşları, platin dirençli over kanseri içinde miR-1294'ün rolünü incelediler. qRT-PCR kullanarak, 30 kanser dokusu ve hücre hattında miR-1294 ifadesini ölçtüler. Araştırmaları, miR-1294 seviyelerini modüle etmek için hücre transfer etme yöntemlerini ve hücre davranışlarını incelemek için çeşitli testleri içeriyordu. Sonuçlar, miR-1294 düzensizliğinin kanserde sisplatin direncini, IGF1R geninin düzenlenmesi yoluyla etkilediğini gösterdi. Özellikle, IGF1R'de bir azalmanın

hücre proliferasyonunu, migrasyonunu ve invazyonunu azalttığı gözlemlendi. Dahası, miR-1294 seviyelerini yükseltmek, sisplatin direnciyle mücadele etti. Bu bulgular, özellikle sisplatine yanıt vermeyen durumlar için miR-1294'ün kansere ilişkin hem bir tanı aracı hem de terapötik bir strateji olarak potansiyelini vurgulamaktadır (Zhang et al., 2018).

Zhang ve arkadaşları (2018), osteosarkom (OS) hücre hatlarında miR-1294'ün ifadesini incelemiş ve bu ifadenin önemli ölçüde düşük olduğunu, OS hastalarında daha düşük hayatta kalma oranlarıyla ilişkili olduğunu bulmuştur. İn vitro testler, miR-1294'ün yükseltilmesinin OS hücrelerinin çoğalmasını ve invazyonunu engellediğini göstermiştir. Luciferaz raportör deneyi ve Western blot analizi aracılığıyla araştırma, miR-1294 için doğrudan hedef gen olan homeobox A9 (HOXA9)'ı kanıtlamıştır (Zhang et al., 2018).

miR-1294, glioma dokularında ve hücre hatlarında belirgin şekilde düşük ifade edilmektedir. miR-1294'ün aşırı ifade edilmesi, glioma hücrelerinin büyümesini ve invazivliğini azaltırken, temozolomideki yanıt verme yeteneğini artırmıştır. Araştırma, miR-1294 için hedef gen olan Xenopus kinesin benzeri protein 2 (TPX2)'yi tanımlamıştır ve bu bağlantı, miR-1294'ün glioma için terapötik potansiyelini vurgulayan bir ksenogreft modeli kullanarak doğrulanmıştır (Chen et al., 2018).

Wang ve arkadaşları (2018), 24 hastadan ve hücre hatlarından alınan örnekleri kullanarak oral skuamöz hücreli karsinom (OSCC)'da miR-1294'ün rolünü incelemiştir. Çalışma, miR-1294'ün kanser dokularında ve hücre hatlarında önemli ölçüde düşük ifade edildiğini ortaya çıkardı. OSCC'de miR-1294 seviyelerinin düşük olması, daha büyük tümörler, artmış lenfatik katılım ve venöz infiltrasyon ile ilişkilendirildi. Önemli olarak, miR-1294, c-Myc onkogenini doğrudan hedef alıyor ve OSCC örneklerinde c-Myc mRNA ve miR-1294 seviyeleri arasında açık bir ters ilişki bulunmaktadır. miR-1294 seviyelerini yükseltmek, OSCC hücrelerinin büyümesini ve hareketini engellemiş, c-Myc'i hedef alarak tümör büyümesini engelleme potansiyelini vurgulanmaktadır (Wang et al., 2018).

2019'da, miR-1294'ün tümör baskılayıcı bir rol oynadığını kanıtlayan bulguların ardından, araştırmacılar özellikle circRNA'lar aracılığıyla miR-1294'ün düzenleme mekanizmalarını araştırmaya başladı. CircRNA'lar, 5' veya 3' uçlarına sahip daha

tanıdık doğrusal RNA'lardan farklı olarak, kovalent olarak kapalı sürekli bir döngü oluşturan benzersiz bir RNA molekülü türüdür. Bazı circRNA'lar, miRNA süngerleri olarak hareket eder ve miRNA'ların hedef mRNA'ları baskılamalarını engellemek için bu miRNA'lar için bağlanma yerlerine sahiptir. Örneğin, Xu ve arkadaşları (2019), circ_0030235'in pankreatik duktal adenokarsinom (PDAC) hücre hatlarında ve kanserli dokularda yükseldiğini bulmaktadır. Bu circRNA, pankreatik duktal adenokarsinom dokularında düşük bulunan miR-1294'ü süngerlediği kanıtlanmıştır. miR-1294'ün azalmış ifadesi, daha yüksek tümör evresi ve pozitif lenf düğümü invazyonu ile ilişkilendirilmektedir (Xu et al., 2019).

2019 yılında Pan ve arkadaşları tarafından yapılan bir çalışmada, böbrek hücreli karsinom (ccRCC) hücre hatları üzerinde miR-1294'ün düşük ifade edilmesinin kötü hasta sağkalımı ile ilişkili olduğu bulunmuştur. Araştırmada, miR-1294 ifadesinin artırılmasının ccRCC hücrelerinin çoğalmasını, koloni oluşumunu ve invazyonunu azalttığı gözlemlenmiştir. Ayrıca, homeobox A6 (HOXA6)'nın miR-1294'ün bir hedefi olduğu ve HOXA6'ın miR-1294 tarafından negatif olarak düzenlendiği belirlenmiştir (Pan et al., 2019).

Cen ve arkadaşları (2023 tarafından yapılan bir çalışmada, Akut Lenfoblastik Lösemi (ALL) tanısı almış 15 çocuk ve hücre hatları üzerinde miR-1294'ün rolü incelenmiştir. Bulgular, ALL hastalarının kemik iliği hücrelerinde sağlıklı bireylere göre miR-1294 seviyelerinde önemli bir yükseliş olduğunu göstermiştir. Bu hücrelerde ayrıca SOX15 gen ifadesinde belirgin bir azalma saptanmıştır. Araştırmacılar, miR-1294'ün doğrudan SOX15'i hedef alarak inhibe ettiğini ve bunun sonucunda Wnt/β-Katenin sinyal yolunun aktivasyonuna yol açtığını bulmuşlardır. Bu aktivasyonun, ALL hücrelerinin çoğalmasını artırdığı, apoptozisi azalttığı ve hastalığın ilerlemesini etkilediği saptanmıştır (Cen et al., 2023).

7.2.2. MikroRNA-551a (miR-551a)

Bilimsel literatürde, miR-551a'nın farklı kanser türlerinde çeşitli işlevleri olduğu belgelenmiştir ve bazılarında yukarı yönlü düzenlenme, diğerlerinde ise aşağı yönlü düzenlenme gösterdiği bildirilmiştir.

miR-551a üzerine yapılan ilk çalışma, 2012 yılında Li ve arkadaşları tarafından gerçekleştirildi ve bu çalışma, dört normal mide dokusu ile 30 tümör doku ve GC hücre

hatlarının derinlemesine incelenmesini içermektedir. Western blot ve qRT-PCR kullanan araştırmacılar, mide karsinomu örneklerinde normal dokuya kıyasla miR-551a'nın önemli ölçüde düşük düzeyde ifade edildiğini bildirmiştir. miR-551a'nın, mide kanserinde metastazla ilişkili bilinen karaciğer yenilenmesi fosfatazı-3 (PRL-3) genini hedef alarak yukarı yönlü düzenlediği bulunmuştur. GC hücre hatlarının miR-551a benzerleriyle transfekte edilmesiyle, hücre migrasyonu ve invazyonunda önemli bir azalma gözlemlendi, bu da miRNA'nın bir tümör baskılayıcısı olarak potansiyel rolünü göstermektedir (Li et al., 2012).

Karanam ve arkadaşlarının 2015 yılındaki çalışmasında, baş ve boyun skuamöz hücreli karsinomu olan 118 hastanın bir kohortu incelenmiş ve bunlardan 41'inin uzak metastaz yaşadığı belirlenmiştir. Çalışma, uzak metastaz geliştiren hastalarda miR-551a'nın önemli ölçüde yukarı yönlü düzenlendiğini ortaya koymuştur. İn vitro analizler, miR-551a'nın aşırı ifadesinin hücre proliferasyonu, migrasyon ve invazyonunu teşvik ederek kanser agresifliğini artırdığını göstermiştir. Bu bağlamda, miR-551a'nın GLIPR2 genini hedef aldığı ve bu etkileşimin tümör büyümesine katkıda bulunduğuna inanıldığı keşfi özellikle önemlidir. miR-551a'nın GLIPR2 ifadesini düzenleme yeteneği, tümör ilerlemesindeki önemini vurgulamakta ve baş ve boyun skuamöz hücreli karsinomunda potansiyel bir biyobelirteç ve terapötik hedef olabileceğini işaret etmektedir (Karanam et al., 2015).

Loo ve arkadaşları tarafından 2015'te yürütülen araştırmada, hasta ölümlerinde kritik bir faktör olan karaciğer metastazının mekanizmalarını çözmek için kolorektal kanser hücre hatları ve hayvan modelleri üzerinde geniş analizler yapılmıştır. 661 miRNA'nın titiz bir taramasından sonra, kolorektal kanserde aşağı yönlü düzenlenen ve kanserin karaciğere yayılmasını engellemede önemli bir rol oynayan miR-551a'nın belirlendiği tespit edilmiştir. Araştırma, miR-551a'nın, metastaz yapan kanser hücrelerinin enerji metabolizmasını düzenleyerek hayatta kalmasını sağlayan kritik bir enzim olan kreatin kinaz, beyin tipi (CKB) hedef alarak baskılayıcı etkilerini ortaya koymuştur. Klinik olarak, bu bulgu, karaciğer metastazlarında miR-551a'nın ifade düzeylerinin primer kolon tümörlerine kıyasla daha düşük olduğu ile desteklenmiştir. miR-551a seviyelerini artırmaya veya CKB işlevini inhibe etmeye yönelik terapötik müdahaleler, fare modeli deneylerinde metastatik proliferasyonda önemli bir azalma sağlamıştır. Böylece, miR-551a'nın düşük düzeyde ifade edilmesi, kolorektal kanserde tümör

baskılayıcı rol oynadığını ve bu durumun kolon kanseri ilerlemesiyle yakından ilişkili olduğunu göstermektedir (Loo et al., 2015).

2017 yılında, Du ve Sha, 15 hastadan alınan 15 eşleşen tümör dokusu ve bitişik normal dokuların yanı sıra hücre hatları üzerinde yapılan analizlerle miR-551a'nın yumurtalık kanserindeki ilerlemesindeki rolünü değerlendirmiştir. Araştırmacılar, miR-551a'nın yumurtalık kanseri örnekleri ve hücre hatlarında belirgin bir şekilde düşük düzeyde ifade edildiğini bulmuştur. Ayrıca, miR-551a'nın artan ifadesinin hücre proliferasyonunu azalttığı ve apoptoz oranlarını arttırdığı gözlemlenmiştir. Önemli olarak, miR-551a'nın IRS2/PI3K/Akt sinyal yolunun aktivasyonu yoluyla yumurtalık kanseri ilerlemesinde önemli bir rol oynayan bir onkojen olan insülin reseptör substratı 2 (IRS2) genini hedeflediği belirlenmiştir. Araştırma ayrıca, in vitro çeşitli kanser hücre tiplerinde güçlü anti-proliferatif etkilere sahip olmasına rağmen kararsızlığı ile bilinen demetoksikurkuminin etkilerini de incelemiştir. Demetoksikurkuminin yumurtalık kanseri hücrelerinde hücre proliferasyonunu inhibe ettiği ve apoptozu indüklediği, aynı zamanda IRS2/PI3K/Akt eksenini devre dışı bıraktığı açıklanmıştır. Özellikle, demetoksikurkumin tedavisi ile miR-551a'nın yukarı yönlü düzenlenmesi, IRS2 aktivitesinin baskılanması ve böylece yumurtalık kanseri hücrelerinin ilerlemesinin engellenmesi ile sonuçlanmıştır (Du & Sha, 2017).

Karanam ve arkadaşları tarafından 2023 yılında gerçekleştirilen başka bir çalışma, postoperatif radyoterapi gören baş ve boyun skuamöz hücreli karsinom (HNSCC) hastalarından alınan 94 tümör örneğindeki miRNA profillerini analiz etmiştir. Karşılaştırmalı analizleri, miR-551a'nın uzak metastazları olan ve kötü sağkalım sonuçlarına sahip hastalarda önemli ölçüde fazla ifade edildiğini vurgulamıştır. HNSCC hücre hatlarını kullanarak yapılan bu çalışma, miR-551a'nın hücre çoğalması, migrasyon ve invazyon deneylerindeki etkilerini göstererek onkogenik rolünü açıklığa kavuşturmuştur. İlginç bir şekilde, miR-551a'nın gliom patogenezinde ilişkili 2 (GLIPR2) adlı mRNA'yı hedef aldığı bulunmuştur. Bu gen, otofajiyi negatif olarak düzenlemesiyle bilinir. Bu yolu modüle ederek miR-551a, sadece tümör büyümesini ve invazivliğini artırmakla kalmamış, aynı zamanda HNSCC hücrelerinde radyo-dirençliliği de artırmıştır. Bu bulgular, miR-551a'yı hedef alarak GLIPR2 ifadesini düzenlemenin, HNSCC'de tümör teşvik edici otofajiyi inhibe etmek için uygulanabilir bir tedavi stratejisi olabileceğini önermektedir (Karanam et al., 2023).

7.2.3. MikroRNA-4534 (miR-4534)

Önceki araştırmalar miR-4534'ün, normal dokulara kıyasla çeşitli kanserli dokularda sıklıkla yüksek seviyelerde bulunduğunu göstermiştir. Böyle geniş çeşitlilikteki malignitelerde sürekli olarak artan düzeyleri göz önünde bulundurularak, miR-4534 giderek bir "onkomiR" olarak nitelendirilmeye başlanmıştır.

miR-4534'ün prostat kanserindeki rolünü inceleyen temel çalışma, 2016 yılında Nip ve arkadaşları tarafından yayımlanmıştır. Araştırmacılar, hastalardan alınan 84 eşleşmiş klinik örnek yanında, normal ve tümör örnekleri incelemişlerdir. qRT-PCR, metilasyon-spesifik PCR ve bir dizi fonksiyonel deney kullanarak, miR-4534'ün prostat kanseri dokularında ve hücre hatlarında normal karşılıklarına göre sürekli olarak aşırı ifade edildiğini keşfetmişlerdir. miR-4534'ün aşırı ifadesi ile kanser hücrelerinin metilasyon durumu arasında bir bağlantı ortaya çıkararak, normal ve tümör dokular arasında ayrım yapmak için bir biyobelirteç olarak potansiyelini öne sürmüşlerdir. Araştırma ayrıca, miR-4534'ün PTEN'i—prostat kanserinde bilinen bir tümör baskılayıcı gen—azaltarak hücre çoğalması, migrasyonu, kanser metastazı ve PI3K/Akt yoluyla yeni kan damarlarının oluşumu için kritik yolları değiştirdiğini göstermiştir. Kanser hücrelerinde miR-4534 fonksiyonunu engellemek, hücre döngüsü durması, apoptoz ve migrasyonun ve invazyonun azalması gibi tümör baskılayıcı etkilerle sonuçlanmış ve miR-4534'ün "OncomiR" olarak rolünü ve terapötik hedefleme potansiyelini vurgulamıştır (Nip et al., 2016).

Öztürk ve arkadaşları 'nın 2022'deki çalışması, 37'si prostat kanseri, 31'i iyi huylu prostat hiperplazisi (BPH) ve 15'i sağlıklı kontrol olmak üzere 83 birey üzerinde yapılmıştır. Çalışma, qRT-PCR kullanarak kanser ve BPH gruplarında miR-4534'ün plazma seviyelerinin yükseldiğini bulmuştur. Lenf nodu metastazı ile bağlantılı olan bu aşırı ifade, miR-4534'ün bu durumlar için bir biyobelirteç olarak potansiyelini işaret etmektedir. Ancak, prostat kanseri ile BPH arasında ayrım yapabilme konusunda belirsizlik bulunmaktadır. Çalışma ayrıca, miR-4534'ün hastalık ilerlemesindeki olası onkojenik rolünü öne sürmektedir (Öztürk et al., 2022).

Chai ve arkadaşları 'nın 2023'teki çalışması, üçlü negatif meme kanseri (TNBC) moleküler dinamiklerine dair önemli içgörüler sunarak, miR-4534 ve ADAM-benzeri Decysin-1 (ADAMDEC1) rollerini incelemiştir. GEO veritabanından alınan RNA dizileme verilerinin analiziyle, araştırmacılar TNBC dokularında miR-4534'ün

belirgin bir şekilde artan düzeylerini tespit etmişlerdir. Bu artış, hastalar arasında azalmış hayatta kalma oranlarıyla ilişkilendirilmiş, böylece miR-4534'ün hastalığın ilerlemesine potansiyel etkisine işaret etmiştir. Linked Omics veritabanı kullanılarak yapılan daha ileri araştırmalar, ADAMDEC1 için zıt bir ifade modelini ortaya çıkarmıştır; kemoterapiye duyarlı TNBC'de yükselmesi, hastaların artan hayatta kalma oranlarıyla uyumlu görünen olumlu bir belirteç olarak görülmüştür. miR-4534 ve ADAMDEC1 arasındaki etkileşim, korelasyon analiziyle açıklığa kavuşturulmuş ve miR-4534, ADAMDEC1'in olası bir yukarı akış inhibe edici regülatörü olarak konumlandırılmıştır. Bu ilişki, miR-4534'ün kemoterapötik yanıt verilebilirliği ve ardından hastaların hayatta kalma sonuçları üzerindeki etkisini vurgulamaktadır (Chai et al., 2023).

7.2.4. MikroRNA-1238 (miR-1238)

Daha önce bahsedilen miRNAlar gibi, miR-1238 de çeşitli kanserlerde anormal bulunmuş ve temel genlerin ifadesini bozma rolü oynamıştır.

miR-1238 üzerine ilk çalışma Balaguer ve arkadaşları tarafından 2011 yılında, kolorektal kanser (CRC) üzerine odaklanarak yapılmıştır. Çalışmada, 54 CRC dokusu ve 20 normal kolon dokusunu kullanılmıştır. Mikroarray analizi ve qRT-PCR ile doğrulama yaparak, araştırmacılar miR-1238'in kolorektal kanserde aşırı ifade edildiğini bulmuştur (Balaguer et al., 2011).

Küçük hücreli olmayan akciğer kanseri araştırmalarında, Shi ve arkadaşları (2015) büyük ölçüde keşfedilmemiş miR-1238'in rolüne odaklanmıştır. Araştırmaları 50 hasta dokusu örneğini içerir ve miR-1238 ifadesinin %62 NSCLC vakasında azaldığını ortaya koyar. Bu azalmayı, LHX2 ifadesinin yükselmesiyle ilişkilendirirler ve bu iki arasında ters bir ilişkiyi ortaya koyarlar. Buna göre, hematopoez, nöral gelişim ve kanser hücresi çoğalması gibi çeşitli biyolojik süreçlerde rol alan LHX2, NSCLC patolojisinde önemli bir unsur olarak tanımlanır. Çalışmanın bulgusu, qRT-PCR ve Western blot analizleriyle desteklenmiş ve NSCLC hücre hatlarında miR-1238'in aşırı ifade edilmesinin LHX2'nin mRNA ve protein seviyelerini azalttığı, böylece hücre çoğalmasını baskıladığı gösterilmiştir. miR-1238 ve LHX2 arasındaki ters korelasyon, miR-1238' i NSCLC'nin moleküler mekanizmalarını aydınlatmanın yanı sıra, aynı zamanda, LHX2 ifadesi üzerindeki düzenleyici etkisi ve tümör hücre büyümesi

üzerindeki sonuçları göz önünde bulundurularak, potansiyel bir biyobelirteç ve terapötik hedef olarak önermektedir (Shi et al., 2015).

Primer glioblastoma'nın (pGBM) genetik temellerine dair detaylı bir inceleme yapan Yan ve arkadaşlarının 2015 tarihli araştırması, temozolomid (TMZ)'e farklı tepkiler veren iki alt grup arasında miRNA ifade profillerinde belirgin farklılıklar olduğunu ortaya koymuştur. 82 doku örneğinde mikroarray analizi ve 78 ek örnek üzerinde qRT-PCR doğrulaması yapan araştırmacılar, miR-1238'in aşırı ifade edildiğini ortaya koymuştur. Yine araştırmacılar, olumsuz prognoza sahip TMZ-dirençli (TCR) alt grubu, tedaviye daha iyi yanıt veren TMZ-duyarlı (TCS) alt gruptan ayırmıştır. Özellikle, miR-1238'in, bu alt gruplar arasında etkili bir şekilde ayrım yapabilen yedi miRNA sınıflandırıcısının bir parçası olması dolayısıyla, miR-1238'in aşırı ifadesi ile artan kemoterapi direnci ve pGBM vakalarındaki olumsuz sonuçlar arasında potansiyel bir ilişki olduğunu göstermiştir (Yan et al., 2015).

2019 yılında Yin ve arkadaşları aynı çalışmayı yürütmüş ve GBM hücre hatlarında ve birincil ve tekrarlayan GBM doku örnekleri yanı sıra 13 hastadan alınan serumlarda miR-1238 ifadesini ölçerek aynı sonuçları doğrulamıştır. TMZ dirençli ekzosomlar ve hücrelerde ve GBM hastalarının serumlarında miR-1238 seviyelerinde önemli bir artış gözlemlenirken, çalışma, aynı zamanda miR-1238'in EGFR aktivasyonunun negatif düzenleyicisi olan caveolin-1 (CAV1) genini hedefleyerek ve baskılayarak TMZ direncine karşı koyabileceğini bildirerek moleküler nedeni genişletmiştir. Bu, miR-1238'in glioma patogenezinde yer aldığını ve potansiyel olarak bir terapötik hedef olarak hizmet edebileceğini göstermektedir (Yin et al., 2019).

Kolanjiokarsinom (CCA) üzerine odaklanan bir çalışmada, Xu ve arkadaşları 58 hastadan ve CCA hatlarından alınan örnekleri incelemiş ve miR-1238'in düşük düzeyde ifade edildiğini, bu durumun tümör baskılayıcı bir rolü olduğunu öne sürmüştür. Araştırmacılar qRT-PCR, hücre transfeksiyonu ve lusiferaz raporlayıcı deneyleri dahil olmak üzere çeşitli deneysel yaklaşımları kullanarak hücresel ve moleküler dinamikleri incelemişlerdir. Sonuçlar, hem CCA dokularında hem de hücre hatlarında dairesel RNA circ_0005230'un yükselmesini vurgular ve hastalığın ilerlemesi ve prognozuyla bir bağlantı kurar. Araştırmacılar, özellikle, circ_0005230'un miR-1238 için bir moleküler sünger işlevi gördüğünü ve böylece hücre çoğalması ve apoptoz direnci ile ilgili BCL2 benzeri 13 (BCL2L13) geninin

ifadesini etkilediğini keşfettiler. Bulgular, circ_0005230'u hedeflemenin CCA için yeni bir terapötik strateji sunabileceğini ve hastalığın klinik yönetimini etkileyebileceğini önermektedir (Xu et al., 2019).

Shan ve arkadaşları (2020) tarafından prostat kanseri (PCa) üzerine yürütülen çalışmada, 90 kanser dokusu ve bitişik olmayan tümör prostat dokuları ile hücre hatlarını incelemiştir. Biyoinformatik ve fonksiyonel testlerin bir kombinasyonunu kullanarak ve qRT-PCR ile doğrulayarak, araştırmacılar hem kanser dokularında hem de hücre hatlarında miR-1238'in düşük düzeyde ifade edildiğini keşfetmişlerdir. Ayrıca, circ_0005100 (circFMN2) adlı genin prostat kanserinde aşırı ifade edildiğini ve onkojenik bir rol oynadığını belirlemişlerdir. Shan ve arkadaşları , circFMN2'nin miR-1238'i emen bir sünger gibi işlev gördüğünü ve böylece LIM-homeobox gen 2 (LHX2) ifadesinde bir artışa olanak tanıdığını bulmuşlardır; bu gen, PCa dokularında belirgin bir şekilde yükselmiştir. Bu etkileşim, miR-1238'in LHX2 mRNA üzerindeki tipik inhibe edici etkisini engelleyerek, PCa hücrelerindeki varlığını artırmıştır. Çalışma, miR-1238 taklitlerinin uygulanmasının, fazla circFMN2'nin pro-proliferatif etkilerini dengeleyebileceğini ve miR-1238'in PCa'daki potansiyel tümör baskılayıcı etkilerini öne sürerek, circFMN2/miR-1238/LHX2 düzenleyici yolunun PCa tedavisi için umut verici bir hedef olduğunu desteklemektedir (Shan et al., 2020).

miR-1238'in, 2021 yılında Luo ve arkadaşları tarafından yürütülen çalışmada küçük hücreli olmayan akciğer kanserinde düşük düzeyde ifade edildiği bulunmuştur. 42 çift primer NSCLC ve bitişik normal dokuların analizini içeren çalışma, miR-1238 ifade düzeylerini değerlendirmek için qRT-PCR kullanmıştır. Araştırma ayrıca, miR-1238'in kanser hücrelerinde çoğalma ve metastazı teşvik eden bir gen olan kladin-14 genini (CLDN14) hedeflediğini ve düzenlediğini bildirmiştir. Luciferase raporlayıcı ve RNA immünopresipitasyon testleri yoluyla ekip, circKIF4A'nın miR-1238'e doğrudan bağlandığını ve onu bir sünger gibi işlev gördüğünü ortaya koymuştur. Bu nedenle, circKIF4A, miR-1238'i hapsederek ve kanser gelişiminde yer alan diğer genleri hedeflemesini engelleyerek NSCLC ilerlemesini düzenleyebileceği saptanmıştır. Bu araştırma, sadece circKIF4A/miR-1238/CLDN14 ekseninin anlaşılmasını ilerletmekle kalmaz, aynı zamanda miR-1238'in tümör baskılayıcı kapasitesini de vurgulamıştır (Luo et al., 2022).

2023 yılında, Mao ve arkadaşları miR-1238'in kolorektal kanser (CRC) üzerindeki etkisiyle ilgili çalışmalarını yürütmüşlerdir. 20 çift CRC dokusu içeren çalışma, miR-1238'in CRC dokularında normal dokulara kıyasla genellikle daha düşük seviyelerde bulunduğunu, bu durumun potansiyel bir tümör baskılayıcı işlevi olduğunu göstermiştir. Araştırmacılar, CRC'de yükseltilmiş bir dairesel RNA olan circSKA3'ün miR-1238 için bağlanma afinitesine sahip olduğunu ve bu durumun YTH N6-metiladenozin RNA bağlama proteini 2 (YTHDF2) ile etkileşime girdiğini vurgulamışlardır. Çalışmanın sonuçları, miR-1238'in ifadesinin yapay olarak artırıldığında, kanser metastazı için kritik olan CRC hücre çoğalması, migrasyonu ve invazyonunda belirgin bir azalma olduğunu göstermiştir. Ancak, miR-1238'in kanser hücreleri üzerindeki bu baskılayıcı etkisi, YTHDF2'nin aşırı ifade edilmesiyle dengelenebilir. Mao ve arkadaşları tarafından ortaya çıkarılan karmaşık mekanizma, miR-1238'i süngerleyerek circSKA3'ün dolaylı olarak YTHDF2 ifadesini yükselttiğini ve böylece CRC ilerlemesini teşvik ettiğini öne sürmektedir (Mao et al., 2023).

7.3 Materyal ve Yöntem

7.3.1 Doku Örnekleri

Bu çalışma, Gaziantep Üniversitesi Şahinbey Araştırma ve Uygulama Hastanesi, Genel Cerrahi Anabilim Dalı'nda 2019-2021 yılları arasında 41 kadın meme kanseri hastasından temin edilen 82 tümör ve histolojik olarak yanındaki normal doku örneklerinin (tümörden 5 cm veya daha fazla mesafede) analizini kapsamaktadır.

Bu hastalar örnek alınma zamanında kemoterapi veya radyoterapi tedavisine başlamamış durumdadırlar. Doku örneklemesi öncesinde tüm katılımcılardan yazılı, imzalı ve bilgilendirilmiş onay alınmıştır. Doku örnekleri, RNA Later çözeltisi içeren tüplerde korunmuş ve daha sonraki analizlere kadar -80 °C'de saklanmıştır. Bu çalışma Gaziantep Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından onaylanmıştır (Karar no: 2019/20).

Bu çalışmada, Tablo 4.1'de belirtildiği gibi, 21 hasta (%51) 50 yaş veya üzerindeyken, 20 hasta (%49) 50 yaşın altındadır. Hastaların çoğu kanserin erken evrelerindedir. 28 hasta (%68) evre I ve II'de sınıflandırılırken, 13 hasta (%32) evre III ve IV'tedir. Yalnızca iki hasta (%5) sigara içtiğini bildirmiş ve hiçbiri alkol tüketicisi değildir. Tümör lokalizasyonu eşit şekilde dağılmıştır: 21 hasta (%51) sağ tarafta ve 20 hasta (%49) sol tarafta tümörlere sahiptir.

Hastaların çoğunda (38, %93) uzak metastazı (M0) yokken, yalnızca 3 hastada (%7) metastatik kanser (M1) vardır. Tümör boyutu açısından, çoğu hasta (34, %83) 50 mm'den küçük tümörlere sahipken, yedi hasta (%17) 50 mm ve üzeri tümörlere sahiptir. Bu hastalar arasında, 4'ü (%10) ailede kanser vakası bildirirken, 37'si (%90) bildirmemiştir.

Hormon reseptör durumu için, 30 hasta (%73) pozitif östrojen reseptörü (ER), 11 hasta (%27) negatif göstermiştir. Progesteron reseptörleri (PR) için, 29 hasta (%71) pozitifken, 12 hasta (%29) negatiftir. Meme kanserinin histolojik tipleri açısından, çoğunlukla 33 hasta (%80) invaziv duktal karsinoma, dört hasta (%10) invaziv lobüler karsinoma, üç hasta (%7) invaziv meme karsinoması ve 1 hasta (%2) invaziv medüller karsinoma sahiptir. Lenf düğümü metastazı için, 33 hasta (%80) N12 evresinde, 5 hasta (%12) N2 ve 3 hasta (%7) N3 evresindedir.

Kanser belirteçleri açısından, 34 hasta (%83) normal karsinoembriyonik antijen (CEA) seviyelerine sahipken, 7 hasta (%17) anormal seviyelerdedir. Kanser Antijeni 125 (CA-125) seviyeleri 38 hastada (%93) normal ve 3 hastada (%7) yüksektir. Kanser antijeni 15-3 (CA15-3) seviyeleri 40 hastada (%98) normalken, yalnızca 1 hasta (%2) yüksek seviyeler göstermiştir.

Reseptör tirozin-protein kinazı (C-erbB-2) için, 4 hasta (%10) +1, 6 hasta (%15) +2, 11 hasta (%27) +3 ve 20 hasta (%49) negatif olarak puanlanmıştır. Ki-67 indeksi açısından, 1 hasta (%3) G1 derecesinde, 19 hasta (%46) G2 ve 21 hasta (%51) G3'tedir.

7.3.2 MikroRNA İzolasyonu

Taze donmuş doku örneklerinden toplam RNA, üretici talimatlarına göre Tablo 3.1'de belirtildiği gibi Hybrid-R™ miRNA izolasyon kiti (GeneAll, Güney Kore) kullanılarak ekstrakte edilmiştir. RNA, bir MaestroNano Spektrofotometre (Maestrogen) kullanılarak kuantifiye edilirken ve RNA konsantrasyonları, örnekler arasında birliktelik sağlamak için standart bir konsantrasyona ayarlanmıştır. Daha sonra örnekler, ileri kullanımlar için -80°C'de saklanmıştır.

7.3.4 cDNA Sentezi ve Real-Time PCR

Bu çalışmada A.B.T.™ Single miRNA qPCR Assay ürünü kullanılarak hem cDNA çevrimi hem de Real-Time PCR çalışması gerçekleştirilmiştir. Ürün içeriği Tablo 3.2'de verilmiştir.

Bu aşamada kullanılan Single miRNA qPCR Assay ürününün içerisinde hazır halde bulunan miR-cDNA Synthesis Kit, reverse transkripsiyon reaksiyonlarını gerçekleştirmek için gerekli olan tüm bileşenleri (reverse transkriptaz, RNaz inhibitörü, miRNA'ya özgü Stem-Loop primer, dNTP) içermektedir (Tablo 3.2).

Hazırlanan örnekler PCR cihazına aktarılarak Tablo 3.3 ve 3.4'te verilen protokole göre cDNA sentez işlemi gerçekleştirilmiştir.

Uygulama sonrası elde edilen cDNA örneklerinin bir kısmı Real-Time PCR işleminde kullanılmak üzere +4 °C'de bekletilmiştir. Stok cDNA örnekleri -20 °C'ye alınarak saklanmıştır.

7.3.5 Real-Time PCR Çalışması

Real-Time PCR uygulamaları Single miRNA qPCR Assay kiti kullanılarak yapılmıştır. Ürününün içerisinde bulunan miR-qPCR Master Mix, Real-Time PCR reaksiyonlarını gerçekleştirmek için gerekli olan tüm bileşenleri (antikor aracılı hot-start Taq DNA Polimeraz, forward ve reverse primer, dNTP, $MgCl_2$, SYBR-Green, ROX, enhancer ve stabilizatör) içermektedir. Kontrol gen olarak RNU6NB kullanılmıştır. Real-Time PCR reaksiyonu için kullanılan bileşen hacimleri Tablo 3.5'da verilmiştir.

Hazırlanan örnekler Real-Time PCR cihazına aktarılarak Tablo 3.6'de verilen protokole göre gen ekspresyonu işlemi gerçekleştirilmiştir. Bu aşamada örnekler 2 tekerrürlü olarak analiz edilmiştir.

7.3.6 Veri Analizi

Real-Time PCR yazılımındaki verileri analiz etmek için karşılaştırmalı Ct yöntemi kullanılmıştır.

Endojen kontrol genine (RNU6B) normalize edilen hedef genlerin ekspresyon seviyelerinin hesaplanması, ΔΔCt yöntemi uygulanarak yapılmıştır.

Tümörlü ve normal dokulardaki miR-551a, miR-1238, miR-1294 ve miR-4534 ekspresyon düzeylerini karşılaştırmak ve farkı ortaya koymak için Paired t testi uygulanmıştır. Kategorik değişken olarak gruplandırılan klinik ve patolojik veriler ile miR-551a, miR-1238, miR-1294 ve miR-4534 ekspresyon düzeyleri arasındaki ilişki Pearson Ki-Kare ve Fishers' Exact testleri kullanılarak belirlenmiştir. Analizler SPSS

(Versiyon 22.0; IBM Corp.) programı kullanılarak yapılmıştır. 0,05'den küçük p değerleri anlamlı olarak kabul edilmiştir.

7.3.7 mikroRNA-mRNA Hedef Tanımlaması

Biyoinformatik analizi sadece, daha önce uygulanan ifade analizinde, tümör ve normal dokular arasında önemli farklılıklar gösteren miRNAlar üzerinde gerçekleştirilmiştir. MikroRNA'ların hedefleri, miRWalk (Stichtet al., 2018) ve miRDB (Chen & Wang, 2020) olmak üzere iki çevrimiçi veritabanı kullanılarak belirlenmiştir. miRDB tarafından önerildiği gibi, 80'den büyük bir hedef puan eşiği kullanılmıştır. 0.95'ten yüksek puan alan ve en az iki veritabanında listelenen hedefler seçilmiştir. miRDB ve miRWalk'tan elde edilen hedeflerin birleştirilmiş listesi oluşturulmuş ve daha sonraki analizler için kullanılmıştır.

7.3.8 mikroRNA'ların Fonksiyonel Analizi

Transkriptomik çalışmaların temel bir adımı olan fonksiyonel zenginleştirme analizi, çeşitli biyolojik işlevler ve süreçlerdeki genlerin zenginleşmesini değerlendirmek için yapılmıştır (Webber, 2011). Bu analiz için EnrichR R paketi, oluşturulan hedef listesini girdi olarak kullanır. Zenginleştirme için parametreler, Gene Ontolojisi (GO), Kyoto Gen ve Genom Ansiklopedisi (KEGG) ve PANTHER (Thomas et al., 2022) gibi kütüphaneleri içerecek şekilde ayarlanmıştır.

7.3.9 Protein-Protein Ağ Analizi

Protein-protein etkileşim ağları (PPIN'ler), bilinen ve tahmin edilen protein-protein etkileşimleri hakkında bilgi sağlayan STRING veritabanı kullanılarak analiz edilmiştir (Nadaradjane et al., 2018).

7.4 Bulgular

7.4.1 miR-551a, miR-1294, miR-4534 ve miR-1238'ın İfadesi ve Klinikopatolojik Faktörlerle İlişkisi

miR-551a'nın ortalama ΔCt değeri, tümör dokularında -1.36±0.38, normal dokularda ise -1.7±0.9 olarak not edilmiştir ve buna karşılık gelen katlanma değişimi 0.84±0.38'dir. Dikkat çekici bir şekilde, miR-551a ifadesi, tümör dokularında normal dokulara kıyasla anlamlı bir şekilde düşük seviyededir (p=0.038) (Şekil 4.1). Buna karşın, miR-1294'ün ortalama ΔCt değeri tümör dokularında 0.4±0.24, normal dokularda ise 0.1±0.58 olarak gözlemlenmiş ve buna karşılık gelen katlanma değişimi 0.82±0.13 olarak saptanmıştır. miR-1294 ifade seviyesindeki istatistiksel olarak

anlamlı düşüş, tümör dokularında normal dokulara göre belirgindir (p=0.004) (Şekil 4.2). Bu, tümör mikroçevresinin potansiyel olarak miR-1294 ve miR-551a'yı baskılayabileceğini ve tümör örneklerinde normal örneklerle kıyaslandığında ifadelerinin azalmasına neden olabileceğini göstermektedir. miR-4534 ile ilgili olarak, ortalama ΔCt değeri tümör dokularında -5.39±0.43 ve normal dokularda -4.86±1.02 olarak kaydedilmiş, katlanma değişimi ise 1.5±0.43'tür. miR-4534 ifade seviyesindeki istatistiksel olarak anlamlı artış, tümör dokularında normal dokulara göre belgelenmiştir (p=0.003) (Şekil 4.3), bu da tümör örneklerinde bir yükselişe işaret etmektedir. miR-1238 için, ortalama ΔCt değeri tümör dokularında 2.29±0.75, normal dokularda 2.52±0.71 olarak kaydedilmiş ve hesaplanan katlanma değişimi 1.35±0.78'dir. miR-1238 ifade seviyesi arasında tümör ve normal dokular arasında istatistiksel olarak anlamlı bir fark bulunmamıştır (p=0.076) (Şekil 4.4).

miR-1294, miR-4534 ve miR-1238 hakkındaki bilgiler öncü niteliktedir, çünkü bu miRNAlar daha önce meme kanseri bağlamında incelenmemiştir ve bu durum, anılan mikroRNA'ların bu hastalık için yeni biyobelirteçler olarak kullanılabileceğini ortaya koymaktadır. miR-551a, miR-1294, miR-1238 ve miR-4534'ün ifade düzeyleri ile meme kanseri hastalarındaki çeşitli klinikopatolojik faktörler arasındaki potansiyel ilişkiyi incelemek için bir iki-kare testi yapılmıştır. Analizde dikkate alınan faktörler arasında yaş, sigara kullanımı, alkol kullanımı, aile öyküsü, evre, histolojik tip, tümör boyutu, tümör lokalizasyonu, uzak metastaz, lenf nodu metastazı ve ER, PR, CEA, CA-125, CA 15-3, C-erbB-2 ve Ki-67 gibi diğer meme kanseri biyobelirteçleri bulunmaktadır.

Analiz sonuçları, klinikopatolojik faktörler ile miR-551a, miR-1294, miR-1238 ve miR-4534'ün ifade düzeyleri arasında istatistiksel olarak anlamlı bir ilişki olmadığını göstermiştir ($p > 0.05$). Bu bulgular, incelenen klinikopatolojik faktörlerin, çalışılan meme kanseri hastası örneğinde bu miRNAların ifade düzeylerini önemli ölçüde etkilemeyebileceğini düşündürmektedir (Tablo 4.2, 4.3, 4.4, 4.5).

7.4.2 MikroRNA-mRNA Hedef Tanımlama

Bu çalışma, ekspresyonu azaltılmış miRNAlar olan miR-1294 ve miR-551a ile birlikte yükseltilmiş miRNA olan miR-4534 ile ilişkili hedef genleri tanımlamıştır. Seçim kriterleri, miRDB veritabanında 80'in üzerinde bir hedef puanı kullanmayı ve genlerin hem TargetScan hem de miRDB veritabanlarında bulunmasını, miRWalk veritabanı

üzerinden doğrulanmasını içermektedir. Bunun ardından, ilgili veritabanlarından elde edilen gen listelerini entegre ederek benzersiz hedef gen setleri sentezlenmiştir. miR-1294 için, miRDB'den 163 hedef genin başlangıç havuzu çıkarılmıştır.

miRWalk ile karşılaştırıldığında, 44 genin ortak olduğu bulunmuştur. Bu birleştirme, BRCA1/BRCA2 içeren kompleks alt birimi 3 (BRCC3), MYC, MYCL, siklin D2 (CCND2) ve deubiquitinase YOD1 (YOD1) dahil olmak üzere toplam 168 hedef gene ulaşılmasını sağlamıştır. miR-551a için, miRDB 5 hedef gen katkıda bulunmuş, miRWalk ise TargetScan ve miRDB veritabanlarıyla ortak olan 56 geni ortaya çıkarmıştır. Bu, Crumbs Hücre crumbs 2 (CRB2), miyosit özgül arttırıcı faktör 2C (MEF2C), Erb-b2 reseptör tirozin kinaz 4 (ERbB4), transmembran protein 2 (TREM2) ve transmembran proteaz, serin 2 (TMPRSS2) gibi dikkate değer örnekler içeren 18 hedef genin kesin bir koleksiyonunu oluşturmuştur.

miR-4534 ile ilgili olarak, miRDB veritabanında 168 hedef gen tanımlanmıştır. miRWalk aracılığıyla yapılan çapraz doğrulama, 193 hedef gene ulaşan bir koleksiyonu oluşturan, ortak 124 geni göstermiştir: SRY-kutu transkripsiyon faktörü 6 (SOX6), adenilat siklaz 1 (ADCY1), morfogenez 2'nin dağılmış aktivatörü (DAAM2), östrojenle ilgili reseptör gamma (ESRRG) ve çatal kafa kutu Q1 (FOXQ1), çinko parmak protein 142 (ZNF142).

7.4.3 Gen Ontolojisi ve KEGG Analizi

Meme kanserinin altında yatan mekanizmaları anlama çabasında olan bu araştırma, miR-1294, miR-551a ve miR-4534'ün etkilerini, detaylı GO ve KEGG yol analizleriyle ortaya koymuştur. Sunulan sonuçlar, 0.05'ten düşük p-değerine sahip bulguları dikkate almaktadır.

miR-1294 üzerine yapılan çalışma, RNA-endüklü sessizleştirme kompleksi (RISC) ve kolajen tip IV trime gibi farklı hücresel bileşenleriyle ilişkili genler dizisini ortaya koymuştur (Şekil 4.5, 4.6, 4.7). Moleküler fonksiyon analizi, protein tirozin fosfataz aktivitesi ve transkripsiyon korepresör bağlanmasının miR-1294 tarafından düzenlenen genlerde önemli ölçüde zenginleştiğini göstermiştir. Ayrıca, KEGG yol analizi, miR-1294'ün azalmasının, Hippo sinyal yolu ve kanser yolları dahil olmak üzere çoklu biyolojik yollarda sapmalara yol açtığını ortaya koymuştur (Şekil 4.14).

miR-551a'nın rollerini inceleyen çalışmalar, kardiyak kas hücresi çoğalmasının pozitif düzenlenmesi ve doku büyümesi gibi biyolojik süreçlerde zenginleşme olduğunu ortaya çıkarırken; miR-551a'nın hedef genleri, aspartik-tip endopeptidaz inhibitör aktivitesi, kardiyak kas düzenlemesi, hücre farklılaşması, dolaşım sistemi gelişimi, adenin-timin zengin DNA'nın minör oluğuna bağlanma ve hiyalüronan glukozaminidaz aktivitesinde önemli ölçüde zenginleşme göstermiştir (Şekil 4.8, 4.9, 4.10). KEGG yol analizi, miR-551a'nın azalmasının, mitojen-aktive protein kinaz (Mapk) sinyal yolu, Notch sinyal yolu, eritroblastik lösemi viral onkojen homologu (ErbB) sinyal yolu ve Sfingolipid metabolizma yolunda yer alan genlerin yükseltilmesiyle ilişkili olduğunu göstererek, bu yollarda önemli bir etkiye işaret etmektedir (Şekil 4.15).

miR-4534'ün GO sonuçları, nöromusküler sinaptik iletim ve kondroitin sülfatın biyosentetik süreci gibi çeşitli biyolojik süreçlerde zenginleştiğini ortaya koyar. Gerilim kapılı sodyum kanal aktivitesi, protein kompleksi oligomerizasyonu ve sialiltransferaz aktivitesi, miR-4534 tarafından düzenlenen genler arasında önemli ölçüde yaygındır (Şekil 4.11, 4.12, 4.13). KEGG yol analizi, miR-4534'ün, Glutamaterjik sinapslar, Wnt, relaksin ve PI3K-Akt sinyal yolları dahil olmak üzere çok sayıda kritik biyolojik yola potansiyel etkileri olduğunu göstermiştir (Şekil 4.16).

7.4.4 Protein-Protein Etkileşim (PPI) Analizi

miRNAların meme kanseri ilerlemesindeki rolünü araştırmak, araştırmanın ayrılmaz bir yönü olmaya devam etmektedir. Bu çalışma, miRNAlar ve hedef genleri arasındaki etkileşimi PPI analizi yoluyla inceleyerek, dikkat çekici bulgular ortaya koymaktadır.

Düşük düzeyde ifade edilen miR-1294'ü araştırmak, monosit diferansiyasyonunun negatif regülasyonu, histon deübikitinasyonu, lys63-spesifik deübikitinaz aktivitesi ve siklin bağlanması gibi kritik süreçlerde yer alan BRCC3, CCND2, MYC, MYCL ve YOD1 gibi üst hedef genleri ortaya çıkarmıştır. miR-1294'ün üst hedef proteinleri arasında sıkça karşılaşılan CCND2, Siklin-bağımlı kinaz 6 (CDK6), S-faz kinaz ilişkili protein 2 (SKP2), Siklin-bağımlı kinaz 2 (CDK2) ve Siklin-bağımlı kinaz 4 (CDK4) gibi çeşitli genlerle ilişkilendirilmiştir, Şekil 4.17. Ayrıca, analiz bu genlerin p53 sinyal yoluyla önemli bir ilişkisi olduğunu göstermiştir.

Düşük düzeyde ifade edilen miR-551a'nın üst hedef genleri, ERbB4, MEF2C, CRB2 ve TMPRSS2 olarak belirlenmiş ve bu genlerin Interlökin-15 (IL-15) aracılı sinyal

yolu, ERbB sinyal yolu, epidermal büyüme faktörü reseptörü (EGRF) bağlanması gibi en zararlı işlevlerle ilişkili ağlara sahip olduğu tespit edilmiştir. miR-551a'nın üst hedef proteinleri arasında yer alan ERbB4, büyük diskler homolog 4 (DLG4), büyüme faktörü reseptörüne bağlı protein 2 (GRB2), heparin bağlayıcı EGF-benzeri büyüme faktörü (HBEGF), nöroregülinler (NRG) gen ailesi ve SCH1 gibi çeşitli genlerle ilişkilendirilmiştir, Şekil 4.18. ERbB4'ün etkileşimlerinin çoğu, NRG protein ailesinin İmmünoglobulin (Ig)-benzeri bölgesiyle gerçekleşmektedir.

Artmış miR-4534'ün üst hedef genleri olan SOX6, ADCY1, DAAM2, ESRRG, FOXQ1 ve ZNF142, kanonik Wnt sinyal yolu, cAMP biyosentetik süreci, GTPaz aktivitesi, G-protein beta/gama-alt birimi kompleksi bağlanması ve lipoliz regülasyonu gibi çoğunlukla işlevlerle ilişkili ağlara sahip olduğu belirlenmiştir. ZNF142 ve FOXQ1'ün ağ içinde birbirleriyle veya diğer proteinlerle etkileşimi olmadığı bulunmuştur (Şekil 4.19).

SOX6'nın katenin beta 1 (CTNNB1) ile etkileşimleri olduğu ve CTNNB1'in diğer proteinlerle, örneğin dishevelled segment polarite protein 1 (DVL1), dishevelled ile ilişkili morfogenez aktivatörü 2 (DAAM2) ve dishevelled segment polarite protein 2 (DVL2) ile etkileşimleri olduğu tespit edilmiştir. Öte yandan, adenilat siklaz 1 (ADCY1), adenilat siklaz protein ailesiyle ve G protein ailesiyle, G protein alt birimi alfa I2 (GNAI2) ve diğerleriyle etkileşimleri olduğu bulunmuştur.

7.5 Tartışma

Meme kanseri dokularında miR-1294, miR-551a ve miR-4534'ün etkisi üzerine sınırlı sayıda çalışma bulunmaktadır. Bu çalışmada, in vitro ve in vivo deneysel teknolojilerden oluşan asimetrik bir boru hattı birleştirerek, bu miRNAların meme kanseri ilerlemesi ve metastazında oynadığı rol incelenmiştir. Ayrıca, meme kanseri tümörlerindeki düzensizleşmiş miRNAları incelemek için biyoinformatik analizler; hedef genlerini, protein-protein etkileşimlerini, bu etkileşimlerin biyolojik yolları nasıl etkilediğini ve kanser hücrelerinin çoğalması ve metastazına nasıl katkıda bulunduğunu belirlemeye yönelik kullanılmıştır.

Çalışmamız, meme tümör dokularında miR-1294'ün normal dokulara göre önemli ölçüde düşük olduğunu açıkça ortaya koymaktadır. Bu gözlem, yemek borusu skuamöz hücreli karsinomu, mide kanseri, epitelyal yumurtalık kanseri, pankreatik

duktal adenokarsinom ve küçük hücreli olmayan akciğer kanseri gibi 15 farklı kötü huylu hastalıkta miR-1294'ün tutarlı bir şekilde düşük olduğunu gösteren önceki raporlarla uyumludur. miR-1294 için bildirilen yollar PI3K/AKT/mTOR, RAS ve JAK/STAT yollarıdır. Bu bağlamda tanımlanan miR-1294 hedef genleri, PI3K/AKT/mTOR, RAS ve JAK/STAT yolları gibi hayati sinyal kaskadlarını düzenlemede önemlidir. Ayrıca, düşük miR-1294 ifadesi, sisplatin ve temozolomide artan direnç ile ilişkilidir (Mao et al., 2023).

Bu çalışmada elde ettiğimiz bulgulara benzer şekilde, literatürde miR-1294'ün meme kanserinde düşük olduğunu ve hücresel çoğalmayı, migrasyonu ve invazyonu hücre dışı sinyal düzenlenmiş kinaz (ERK) sinyal yolunu modüle ederek inhibe ettiğini gösteren bir çalışma daha bulunmaktadır (Chen et al., 2022). Bu gözlemlerle uyumlu olarak, yapılan araştırmalar, hepatosellüler karsinomda sağlıklı dokulara kıyasla miR-1294'ün düşük olduğunu vurgulamıştır. miR-1294'ün düşük ifade düzeyi, onkogenik hedeflerine olan inhibe edici etkileri azaltarak hepatosellüler kanserin ilerlemesine olanak tanır. Bu bağlamda, miR-1294 hepatosellüler karsinomda tümör baskılayıcı bir rol üstlenmektedir (Qin & Wang, 2023). Dikkate değer olarak, akut lenfoblastik lösemi olan 15 çocuk üzerinde yapılan bir çalışmada, beklenmedik bir miR-1294 yükselmesi ve SOX15'i hedef alması gözlemlenmiştir (Cen et al., 2023). Bu çalışmada kullanılan küçük örneklem kullanımı nedeniyle, sonuçların güvenilirliği şüphelidir.

Bu çalışma, çeşitli kanser türlerinde miR-1294'ün tümör baskılayıcı özelliklerine dair kanıtlar sunmaktadır. Meme kanserinde miR-1294'ün mekanistik rolünü anlamak için, bioinformatik analizler gerçekleştirilirken, BRCC3, CCND2, MYC, MYCL ve YOD1'i miR-1294 için hedef genler olarak belirlenmiştir. DNA hasar tepkisinde rol alan BRCA1-BRCA2 kompleksinin bir alt birimi olan BRCC3, tümör dokularında artmıştır ve kanser metastazıyla ilişkilendirilmiştir (Zhang & Zhou, 2018). CCND2, MYC ve MYCL'in meme kanseri ve diğer kanser türlerinde aşırı ifade edildiği ve kanser ilerlemesine katkıda bulunduğu bildirilmiştir (Xu et al., 2010; Flem-Karlsen et al., 2018; Li et al., 2019).

YOD1, meme kanserinde rapor edilmemiş olmasına rağmen, diğer kanser türlerinde aşırı ifade edilmiştir (Zhang et al., 2022). MYC'nin daha önce miR-1294 için bir hedef olarak bildirildiği belirtilmiştir (Wang et al., 2018). Araştırma bulguları, meme kanserinde miR-1294'ün azaldığını ve bunun sonucunda BRCC3, CCND2, MYC,

MYCL ve YOD1 genlerinin artış gösterdiğini ortaya koymaktadır. miR-1294'ün önerilen hedef genlerinin GO ve fonksiyonel analizi, lipid sentezi, kolesterol depolama, Wnt sinyalizasyonu, GTPaz aktivitesi, metallopeptidaz aktivitesi ve siklin bağlanmasını düzenlemede rol aldığını göstermektedir.

Bu süreçler çeşitli çalışmalarda kanser gelişimiyle ilişkilendirilmiştir (Sukocheva & Wadham, 2014; Litan & Langhans, 2015; Fields & Stawikowski, 2016; Sevinsky et al., 2018; Tauro & Lynch, 2018; Wang et al., 2020). Ayrıca, yürütülen KEGG yolak analizi, bu genlerin Hippo sinyalizasyon yolu, mTOR sinyalizasyon yolu, kanser yolları ve Wnt sinyalizasyon yolu gibi yollardaki rollerini pekiştirmektedir. miR-1294'ün Wnt sinyalizasyon yolu ve mTOR sinyalizasyon yolundaki rolü daha önce bildirilmiştir (Li et al., 2023; Mao et al., 2023). Hippo yolunun meme kanseri üzerindeki etkisi, kolonizasyon, moleküler biyoloji ve tedavi yanıtı açısından önemli olduğu vurgulanmaktadır (Kyriazoglou et al., 2021).

Hippo sinyalizasyonunun düzensizliği, P53 onkojenik yolakla etkileşerek meme kanseri ilerlemesine ve tümörijenez potansiyelini artırarak katkıda bulunmaktadır (Raj & Bam, 2019). Yapılan PPI analizi, ağdaki genlerin, meme kanseri dahil pek çok kanserde hayati öneme sahip olan p53 sinyalizasyon yolunda zenginleştiğini ortaya koymuştur. Bu yolaktaki sapmalar, meme kanserinin başlangıcını, büyümesini ve yayılmasını tetikleyebilir (Duffy et al., 2018).

miR-1294 tarafından hedeflenen ve Hippo sinyalizasyon yolunda yer alan protein CCDN2, CDK6, SKP2, CDK2 ve CDK4 gibi çeşitli genlerle etkileşime girer. CCDN2'nin etkileşimlerinin çoğu, ubiquitin ligazları (E3) için iskeleler olarak görev yapan cullins ailesini içerir. Bu E3 ligazları, tümör baskılayıcılar için hayati öneme sahip olup, tümörijenezde önemli bir rol oynamaktadır. Ayrıca, E3'ün p53 yolakları üzerinden meme kanseri hücre invazyonu ve migrasyonuyla ilişkili olduğu bildirilmiştir (Ohta & Fukuda, 2004; Wang et al., 2021).

Bu çalışma, miR-1294'ün azalmasının, meme kanserinde tümör baskılayıcı olarak işlev gören ve önemli biyolojik yolları ve işlevleri etkileyen miR-1294'ün BRCC3, MYCL, CCND2 ve YOD1 gibi potansiyel hedeflerini vurgulamaktadır. Ayrıca, araştırma P53 yolaklarında miR-1294 düzenlemesinin rolünü ve daha önce

tanınmamış olan Hippo sinyalizasyon yolu ve ilişkili gen CCND2'nin miR-1294 tarafından hedeflenmesini ortaya koymaktadır.

Bu çalışma, farklı açılardan belli sınırlılıklar taşır. Özellikle klinikopatolojik faktörlerin analizinin sınırlı örnek sayısı göz önüne alındığında, klinikopatolojik faktörler ile miR-1294 ifadesi ve diğer incelenen miRNAlar arasında güvenli bir ilişki kurulamamaktadır. Gelecekte, özellikle in vivo deneyler kullanılarak yürütülecek olan daha fazla araştırmanın, miR-1294 ile ilişkili hedef genlerin ve yolların belirlenmesi açısından hayati olduğu vurgulanmalıdır. Bu durum, bu çalışmanın odak noktası olan diğer miRNAlar için de geçerlidir.

Bu çalışmada, miR-551a'nın ekspresyon analizi, meme kanseri dokularında, bitişik normal dokulara göre anlamlı bir düşürücü düzenleme gösterdiği saptanmıştır. Bu bulgu, yumurtalık kanserinde yapılan önceki bir çalışmayla uyumludur; bu çalışmada miR-551a'nın düşürüldüğü ve hücre proliferasyonunu, migrasyonu ve invazyonunu inhibe ederek bir tümör baskılayıcı olarak hareket ettiği gösterilmiştir. Belirlenen hedef gen IRS2 olup, PI3K/AKT sinyal yolunda rol oynamaktadır (Du & Sha, 2017). Benzer şekilde, meme kanserinde miR-551a'nın düşük düzeyde ifade edildiği ve Fokal adezyon kinazı (FAK) yolunu hedef alarak bu yolun yukarı yönlü düzenlenmesine katkıda bulunduğu bulunmuştur. FAK yolu, meme karsinomunda ortaya çıkan terapötik hedeflerden biri olarak değerlendirilmektedir (Anuj et al., 2019).

Bu çalışmayla uyumlu olarak, meme kanseri üzerine yapılan araştırmalar, meme kanserinde miR-551a ifade düzeyinin düşük olduğunu kanıtlamıştır (Kang et al., 2019). Bu bulguların aksine, metastatik baş ve boyun skuamöz hücreli karsinomu (HNSCC) olan hastalarda miR-551a üzerine yapılan bir çalışma farklı sonuçlar sunmuştur. Araştırma, miR-551a'nın bu kanserde yukarı regüle edildiğini ve hücre proliferasyonu, migrasyonu, invazyonu ve radyo-direnç gibi süreçleri teşvik eden bir onkojen olarak belirtmiştir. Araştırmacılar, HNSCC bağlamında miR-551a'nın hedef geni olarak GLI patogenez ile ilişkili 2'yi (GLIPR2) Tespit edilmiştir (Karanam et al., 2023).

Bir karşıt çalışma, miR-551a'nın hepatosellüler kanserde yükseltilmiş bir ifade düzeyine sahip olduğunu, bir kanserojen olarak hareket ettiğini ve kötü genel sağkalım oranları ile ilişkili olduğunu göstermiştir (Chen et al., 2021). miR-551a'nın

fonksiyonunun karmaşık yapısı, onkolojik ve tümör baskılayıcı özellikler de dahil olmak üzere, farklı kanser türlerindeki çeşitli davranışlarına atfedilebilir. Bu davranışlar, her malignitenin içsel olarak sahip olduğu doku özgülü bağlam tarafından belirlenir. miR-551a'nın meme kanseriyle ilişkili moleküler mekanizmaları kapsamlı bir şekilde anlamak için biyoenformatik analizler yapılmıştır.

Bu araştırma, miR-551a tarafından potansiyel olarak hedef alınan en üst genler olarak ERbB4, MEF2C, CRB2 ve TMPRSS2'yi belirlemiştir. Epidermal büyüme faktörü (EGFR) reseptör ailesi üyesini kodlayan ERbB4, proliferatif meme tümörleri ve diğer kanser türleriyle ilişkilendirilmiştir (Hu et al., 2021; Kawahara & Simizu, 2022; Schubert et al., 2023). Embriyonik gelişimi düzenleyen ve kırıntı hücre kutup karmaşasının bir bileşeni olan CRB2'nin, glioblastomada ifadesinin yukarı yönlü düzenlendiği tespit edilmiştir. Bu bulgu, CRB2'nin bir kanserojen olarak işlev görebileceğine işaret etmektedir (Wang et al., 2022). Transmembran bir serin proteaz olan TMPRSS2, meme kanseri hücrelerinde aşırı derecede ifade edilmekte ve bu durum potansiyel olarak karsinogenez sürecini teşvik edebilmektedir. Bunun yanı sıra, ER-pozitif hastalarda TMPRSS2 ifadesinin kötü prognostik bir gösterge ile ilişkili olduğu belirlenmiştir (Ozyurt et al., 2023).

MEF2C, meme kanserinde miR-551a için bir hedef gen olarak da tanınmıştır (Kang et al., 2019). Önerilen miR-551a hedef genlerinin Gen Ontolojisi (GO) analizi, bu genlerin kritik biyolojik süreçlerde, özellikle hücre farklılaşması ve dolaşım sistemi gelişiminde rol aldıklarını göstermiştir. Bu bulgular, miR-551a'nın düşük ifade düzeyinin, kalp kası gelişimi ve farklılaşmasında yer alan önemli genlerin ifadesinin artmasına yol açabileceğini ve bu durumun meme kanserinde kardiyak disfonksiyona potansiyel katkıda bulunabileceğini düşündürmektedir (Demissei et al., 2020; da Costa et al., 2021). miR-551a'nın hedef genlerinin moleküler fonksiyon analizi, endopeptidaz inhibitör aktivitesi ve hiyaluronan glikozaminidaz aktivitesi gibi işlevleri ortaya çıkarmıştır. Bu işlevler, meme kanseri ilerlemesiyle ilişkilendirilmiştir (Sarkar et al., 2023).

KEGG yolak analizi, miR-551a'nın düşük düzeyde ifade edilmesinin Sfingolipid metabolizma yolunu, Mapk sinyal yolağını, Notch sinyal yolağını ve ErbB sinyal yolağını düzensiz hale getirdiğini göstermiştir. Sfingolipid metabolizma yolunun düzensizliği, meme kanseri de dahil olmak üzere çeşitli kanser türlerinde tümör

büyümesine katkıda bulunmakta olduğu bilinmektedir (Companioni et al., 2021; Pani ve Dasgupta, 2023).

Hücre farklılaşması için kritik olan Notch sinyalizasyon yolakları, meme kanserinde anormal şekilde aktive edilmiştir. Bu yolakların etkileri, tümör başlangıcına, ilerlemesine ve meme kanseri kök hücre aktivitesine kadar uzanmaktadır (Pandey et al., 2023). Mapk ve ErbB sinyalizasyon yolakları, meme kanserinde aşırı aktive edilmiş olarak bilinmektedir (Wang, 2017; Naik, 2019).

PPI analizi, ERbB4'ün NGR gen ailesiyle bağlantı kurduğunun tespit edildiğini göstermiştir. ERbB4 ile NGR ailesi arasındaki bağlantı daha önce de rapor edilmiş ve bu bağlantının meme kanserinde aşırı ifade edildiği ve tanı ile tedavi hedefleri olarak belirlendiği gösterilmiştir (Vulf et al., 2023). Bu çalışmadan elde edilen biyoinformatik analiz, ErbB4'ü Mapk ve ErbB sinyalizasyon yolakları içinde miR-551a'nın birincil hedef geni olarak belirlemiştir. Ayrıca, ErbB4'ün NGR ailesi ile etkileşimi, miR-551a'nın meme kanserinde bir tümör baskılayıcısı olarak önemli rolünü daha da vurgulamaktadır ve bu durumun ErbB4'ün artışına yol açabileceğini düşündürmektedir. Sonuç olarak, bu çalışma, miR-551a'nın meme kanserinde düşürüldüğünü ve böylece meme kanserinde birçok kritik sinyalizasyon yolaklarının düzensizleştiğini belirtmektedir. Ayrıca, miR-551a'nın CRB2 ve TMPRSS2'yi hedef alabileceğini ve özellikle ErbB4'ün önemli olduğunu öne sürmektedir.

Bu çalışma, meme kanseri dokularında normal dokulara kıyasla miR-4534'ün önemli ölçüde yükseltilmiş bir ifade düzeyine sahip olduğunu bildirmiştir. Literatüre dayanarak yapılan araştırmalarda, kanserle ilişkili tüm çalışmalarda miR-4534'ün ifade düzeyinin sürekli olarak arttığı tespit edilmiştir. Bu araştırmadaki gözlem, miR-4534 hakkındaki önceki bulgularla çeşitli kanser türleri arasında tutarlıdır. Örneğin, prostat kanseri üzerine geniş çapta yapılan araştırmalar bu sonucu sürekli olarak desteklemektedir. Bir çalışma, miR-4534'ün prostat kanseri dokularında arttığını ve VASH1'i baskılayarak prostat kanseri anjiyojenezi teşvik ettiğini göstermiştir (Jiang et al., 2020).

Bu görüşü daha da güçlendiren başka bir çalışmada, prostat kanserinde miR-4534'ün aşırı ifadesine dikkat çekilmiştir. Onkolojik etkilerinin bir bölümü, PTEN yolunun düşük düzeyde ifade edilmesine bağlanmıştır. Bu baskılama, kanser hücrelerinde

hücre çoğalmasını ve hayatta kalma oranını artırabilir, bu da mevcut araştırmada gözlemlenen miR-4534'ün onkolojik etkilerini yansıtmaktadır (Nip et al., 2016). Ek olarak, miR-4534'ün artışı, sağlıklı kontrollere kıyasla benign prostat hiperplazisinde de açıkça görülmektedir. Çalışma ayrıca, artan miR-4534 ifadesinin lenf nodu metastazı ile ilişkisini rapor etmiş ve kanser ilerlemesindeki kritik rolünü vurgulamıştır (Öztürk et al., 2022). Mevcut araştırma bulgularını destekler bir biçimde, kolorektal tümör oluşumu bağlamında, p53 eksikliği gösteren dokularda miR-4534'ün ifadesinin arttığı tespit edilmiştir. Bu çalışma, miR-4534'ün tümör stromasında ATG2B'yi baskılayarak otofajiye olan düzenleyici etkisini ön plana çıkarmaktadır (Inoue et al., 2021).

Benzer bir bağlamda, RNA dizileme metodolojisi kullanılarak gerçekleştirilen diğer bir çalışma, üçlü negatif meme kanserinde miR-4534'ün düzeylerinin arttığını ve bu durumun mevcut bulgularla uyumlu olduğunu tespit etmiştir. Ayrıca, söz konusu araştırma miR-4534'ü, ADAMDEC1 geninin yukarı yönlü düzenlenmesinde etkili bir faktör olarak belirlemiştir. Bu bağlamda, miR-4534'ün bağışıklık göstergeleri ile negatif korelasyon göstermesi, olumsuz prognoz için potansiyel bir açıklama olarak değerlendirilmekte ve bu bulgular mevcut literatürle uyumlu bir görünüm sergilemektedir (Chai et al., 2023). Çeşitli kanser türlerinde miR-4534'ün düzeylerinin artışı, bu molekülün kanser gelişimi ve prognozunda kritik bir biyomarker olarak potansiyelini vurgulamaktadır.

MiR-4534'ün meme kanseri biyolojisindeki rolünün daha detaylı bir şekilde keşfedilmesi amacıyla yapılan biyoinformatik analizler, SOX6, ADCY1, DAAM2, ESRRG, FOXQ1 ve ZNF142 genlerinin miR-4534 için potansiyel hedefler olduğunu ortaya koymaktadır. SOX6, organ gelişimi ve hücre farklılaşması, dahil olmak üzere kök hücre oluşumu için kilit bir rol olarak tanınmaktadır. Önceki çalışmalar, özellikle meme kanserinde SOX6'nın tümör baskılayıcı işlevlerine işaret etmiş ve meme kanserinde aşağı yönlü düzenlendiği bildirilmiştir (Wei etal., 2022). Ayrıca, ADCY1, meme kanserinde promotör metilasyonu ve düşük ifade düzeyleri gösteren bir gen olarak bilinmektedir. ADCY1'in artmış mRNA seviyeleri ile meme kanseri hastalarında gözlemlenen olumlu sonuçlar arasında önemli bir ilişki bulunmaktadır. (Li et al., 2017).

Ayrıca, DAAM2'nin pankreatik adenokarsinomda düşük düzeyde ifade edildiği rapor edilmiştir (Zhang et al., 2022). ESRRG, bir tümör baskılayıcı olarak tanımlanmış ve meme kanserinde aşağı yönlü düzenlendiği gösterilmiştir. Mekanizması, glikolizi inhibe ederek ve oksidatif fosforilasyonu güçlendirerek işler (Eichner et al., 2010).

Ayrıca, FOXQ1'in düşük ifadesinin, meme kanserinde daha kötü genel sağkalımla ilişkili olduğu bildirilmiştir, bu da onu prognozda önemli bir faktör haline getirmektedir (Elian etl., 2021). Son olarak, ZNF142, meme kanseri örneklerinin dörtte birinden fazlasında bildirilen bir mutasyon yükü ile öne çıkmaktadır (Aravind et al., 2018; Yan et al., 2021). Sonuçlar, miR-4534'ün meme kanserinde yükseldiğini ve SOX6, ADCY1, DAAM2, ESRRG, FOXQ1 ve ZNF142'yi düşürdüğünü öne sürmektedir.

GO analizi aracılığıyla yapılan araştırma, miR-4534 tarafından önerilen hedef genlerin kation kanal aktivitesini düzenleme, protein kompleksi oligomerizasyonunu ve sodyum iyonunun transmembran taşımasını düzenleme konularında sorumlu olduğunu belirtmektedir. İyon kanallarında meydana gelen değişiklikler, özellikle kation kanal aktivitesindeki değişiklikler, meme kanseri dahil olmak üzere malignitelerle ilişkilendirilmiştir (Jiang et al., 2021). KEGG yol analizi, miR-4534'ün PI3K-Akt, glutamaterjik sinapslar, kolinergik sinapslar ve relaksin sinyal yolları üzerindeki etkisini vurgulamaktadır. PI3K-Akt sinyal yolu daha önce miR-4534 için bir hedef yol olarak tanımlanmıştır (Zhao et al., 2020).

Önemli bir nokta olarak, glutamaterjik sinaps ve kolinergik sinaps sinyal yollarının düzensizliğinin, hücre büyümesi, tümör proliferasyonu ve meme kanserinde endokrin direncini etkilediği bilinmektedir. Ayrıca, glutamaterjik ve kolinergik yolların işleyişinde iyon kanallarının oynadığı kritik rolün altının çizilmesi de önemlidir (Willard & Koochekpour, 2013; Jiang et al., 2021; García et al., 2022).

PPI analizi, miR-4534'ün hedef geni olan ADCY1'in, adenilat siklaz ve G-protein aileleriyle etkileşime geçtiğini göstermektedir. Bu aileler, tümörogenez, anjiyogenez ve metastaz ile bağlantılı bir dizi hücresel süreci yönetmektedir. Önemli olarak, bu araştırma, ADCY1'in glutamaterjik ve kolinergik sinaps sinyal yollarında bir hedef gen olduğunu ifade etmektedir (Fan et al., 2019; Zou et al., 2019).

Sonuç olarak, çalışma, artan ifade olan miR-4534'ün onkojenik olabileceğini ve farklı sinyal yollarının karmaşık ağını bozarak meme kanserinin ilerlemesine sebep olabileceğini belirlemiştir. Özellikle glutamaterjik sinaps ve kolinergik sinaps sinyal yolları en önemli olanlardır. Ayrıca, çeşitli genler üzerindeki etkisi, özellikle ADCY1 hedef geni üzerindeki etkisi daha önce bildirilmiştir. Sonuçlarımız, meme kanserinin yönetiminde miR-4534 seviyelerini düzenlemenin potansiyel terapötik etkilerini vurgulamakla birlikte, özellikle in vivo araştırmalar yaparak miR-4534'ün hedef genlerini ve yollarını doğrulamanın gerekliliğine de dikkat çekmektedir.

7.6 Sonuç ve Öneriler

7.6.1 Sonuç

Sonuç olarak, bu çalışma miR-1294, miR-551a ve miR-4534 gibi miRNAların meme kanserindeki rolleri hakkında değerli bilgiler sunmaktadır. miR-1294 ve miR-551a'nın meme kanseri dokularında gözlemlenen düşük düzeyde ifade edilmesi, bunların tümör baskılayıcı olarak potansiyellerini vurgulamaktadır. Özellikle, çalışma miR-1294'ün, Hippo ve p53 sinyal yollarında önemli rol oynayan BRCC3, MYCL ve YOD1 gibi genleri hedef aldığını öne sürmektedir. Ayrıca, meme kanserinde düşük seviyede ifade edilen miR-551a'nın ERbB4, MEF2C, CRB2 ve TMPRSS2 gibi genleri hedefleyebileceği bulunmuştur.

Bu genler, kardiyak kas düzenlemesi, Sfingolipid metabolizması, Notch sinyallemesi, Mapk sinyallemesi ve ErbB sinyallemesi gibi çeşitli yollarında önemli roller oynamaktadır. Çalışma, meme kanserinde artmış ifadesi nedeniyle miR-4534'ün onkojenik bir rolü olduğunu belirtmektedir. İyon taşınması, glutamaterjik ve kolinergik sinaps sinyal yollarında yer alan genleri hedef alarak, terapötik bir hedef olarak potansiyelini vurgulamaktadır. Çalışma, miR-4534'ün potansiyel hedefleri olarak SOX6, ADCY1, ESRRG ve ZNF142 gibi genleri önermektedir.

7.6.2 Öneriler

Bu çalışmadan elde edilen içgörüler, araştırmacılara önerilen hedef genleri laboratuvar ortamında daha detaylı incelemek için sağlam bir başlangıç noktası sunmaktadır. Bu çalışmada gerçekleştirilen biyoenformatik analizler paha biçilmez olsa da, miR-1294, miR-551a ve miR-4534 tarafından potansiyel olarak düzenlendiği belirlenen hedef genlerin deneysel doğrulaması hayati önem taşımaktadır. Bu araştırmaları canlı modellerde, özellikle fare modellerinde genişleterek, bu miRNAların ve hedef

genlerinin tümör büyümesi, metastaz, genel sağ kalım ve tümör mikroçevresindeki rollerini inceleme imkanı sağlanacaktır.

Gelecekteki çalışmalar, örnek sayısını arttırmayı ve metastaz, tümör büyüklüğü ve kanser biyokimyasal işaretçileri gibi farklı patolojik özelliklere sahip örnekleri dahil etmeyi düşünmelidir. Bu, incelenen miRNAlar ile klinikopatolojik faktörler arasındaki ilişkinin kapsamlı bir değerlendirmesini sağlayacak ve böylece bu miRNAların biyolojik işlevlerini ve meme kanseri üzerindeki etkilerini daha iyi anlamamıza yardımcı olacaktır.

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