Review Article

MicroRNA Significance in Cancer: An Updated Review on Diagnostic, Prognostic, and Therapeutic Perspectives.

Vijay Singh[1], Aniruddha Sen[2], Sapna Saini[3], Shailendra Dwivedi[*4], Ruchika Agrawal[5], Akash Bansal[6], Shashank Shekhar[7]

[1,2,3,*4,6]*Department of Biochemistry, All India Institute of Medical Sciences, Gorakhpur, Uttar Pradesh, 273008, India*
[5]*Department of ENT, All India Institute of Medical Sciences Gorakhpur, 273008, India,*
[7]*Department of Radiotherapy, All India Institute of Medical Sciences, Gorakhpur, Uttar Pradesh, 273008, India*

Article Info

Author of correspondence:
Shailendra Dwivedi
Department of Biochemistry, All India Institute of Medical Sciences
E-mail: tarang2016@gmail.com
Address:
Gorakhpur, Uttar Pradesh, 273008, India

Keywords

Cancer, microRNA(miRNA), Tumor-Suppressor Genes, Polymerase Chain Reaction (PCR), Next Generation Sequencing (NGS), Microarray, Antisense Oligonucleotides (ASOs)

Abstract

Abstract

The article provides a thorough and up-to-date analysis of the role that microRNAs (miRNAs) within the realm of cancer therapy, paying specific attention to their diagnostic, prognostic as well as therapeutic capabilities. The miRNAs (small non-coding RNAs) are the current major genes that regulate gene expression. They are a key factor in the genesis of cancer. They are oncogenes, or tumor suppressors that play key functions in the signaling pathway that contribute to the development of cancer. This article focuses on the double importance of microRNAs for cancer oncogenesis. This includes both their ability to inhibit cancer suppressor genes and the stimulation of cancer-causing oncogenes. MicroRNAs have been identified for a long time as biomarkers to help in diagnosing cancer and have distinct signatures specific to different kinds of cancer. There are many detection strategies including RT-qPCR, Next Generation Sequencing (NGS) as well as Microarray Analysis that have been evaluated to prove their effectiveness in aiding the non-invasive diagnosis of cancer. The paper provides an overview of the importance of miRNAs to prognosis, highlighting their ability to forecast tumor progression as well as outcomes for cancer patients. In addition, their therapeutic value remains a subject of research. Research is being conducted in order to investigate miRNA-targeting therapy including antisense oligonucleotides, or small molecules inhibitors as possible treatment options for cancer. These methods could favor more specific and individualized approaches than the current techniques. The article also focuses on the current challenges and future prospects linked to miRNA research and demonstrates the complex biological functions they play as well as clinical applications that require investigation. The review is the source of information for researchers, clinicians and scientists who are interested in advancing studies into cancer research as well as personalized treatments.

Introduction

Cancer poses a worldwide health crisis that impacts millions annually. Although advances have been made in treatments and management approaches for cancer detection, and management remains complex tasks that often necessitate

comprehensive approaches. Over the last ten years, great strides have been made toward understanding molecular mechanisms underlying cancer progression as part of cancer research with microRNA (miRNA) serving as an increasingly researched topic. MicroRNAs are non-coding RNA molecules that play an essential part in gene regulation, play an integral part. It is comprised of 22 nucleotides per microRNA molecule and bound directly with specific messenger RNA (mRNA), these non-coding molecules precisely manage gene expression levels by binding directly with messenger RNA (mRNA). The adhesions between molecules could potentially impede translation processes; miRNAs utilize this mechanism to regulate the expression of many target genes and influence various cellular activities - providing significant gene regulatory capabilities. miRNAs function as posttranscriptional regulators by binding to messenger RNA molecules' 3' untranslated regions (UTR), leading either to degradation or translational repression thereby fine-tuning gene expression levels and providing more precise control of gene expression levels. The miRNAs modulate gene expression through sequence-specific targeting of multiple messenger RNA (mRNA) molecules, any given messenger RNA could potentially be targeted by multiple miRNAs; their biogenesis and processing involve many enzymes and regulatory proteins to ensure functional miRNA production [1]. The miRNA binding to target mRNAs can alter biological processes and lead to various diseases, making miRNAs useful biomarkers in diagnosing disease diagnosis, prognosis, and prognosticate purposes, with some specific miRNAs possessing therapeutic potential against specific conditions [2].

The miRNA molecules have been discovered to regulate certain target mRNAs, thus altering important physiological processes like cell proliferation, differentiation, and apoptosis. Furthermore, abnormal miRNA expression patterns have been linked with diseases like cancer that contribute to tumor development and progression [3]. The multifaceted role of Vitamin D in disease prevention and cure, as elucidated in recent studies, highlights its potential therapeutic significance in oncology, emphasizing the need for further research into its molecular mechanisms and health benefits [4]. This article intends to present an updated account of the importance of miRNAs for cancer diagnosis, prognostication, and therapeutic development. We aim to gather the latest research findings and advancements to gain an understanding of their possible effects on clinical practice. Additionally, we will investigate challenges and future directions associated with harnessing miRNAs to maximize cancer management potential.

Role of MicroRNAs in Cancer Development

MicroRNAs (miRNAs) exert a substantial influence on the development of cancer by participating in many pathways, including the suppression of tumor-suppressor genes and the activation of oncogenes [5].

Suppression of Tumor-Suppressor Genes

MicroRNAs (miRNAs) play an essential role as tumor suppressor genes by down-regulating specific target genes involved with cell proliferation, apoptosis, and differentiation processes. These inhibitors work by binding to sequences present on target messenger RNA (mRNA), leading to its cleavage, translational repression, or deadenylation before its subsequent degradation [6]. Many microRNAs have been discovered for their tumor-suppressing capabilities by targeting pathways and genes involved in cancer formation. MiR-34 family transcription factors, which are under the control of p53 tumor suppressor gene regulation, downregulate many genes such as Cyclin D1, E2, CDK4, CDK6, Myc, and BCl2, leading to cell cycle arrest and apoptosis, thereby curbing tumor growth and inhibiting tumor spread. MiR-15 and miR-16 clusters, commonly found deleted in chronic lymphocytic leukemia (CLL), targets the anti-apoptotic BCL2 gene to induce apoptosis and prevent tumorigenesis. Let-7 family targets Ras and Myc oncogenes to prevent cell cycle progression, and proliferation and induce apoptosis in various cancer types such as lung, breast, gastric colon, and prostate cancers. MiR-200 family miRNAs, particularly miR-200c, have been found to target ZEB1 and ZEB2, in turn suppressing epithelial-to-mesenchymal transition (EMT), thus inhibiting cancer metastasis, leading to less aggressive metastatic properties in cancers like breast and non-small cell lung cancer (NSCLC). Their actions demonstrate how miRNAs serve as tumor suppressors whose deregulation could significantly halt cancer progression thereby underscoring the therapeutic potential of miRNA-based interventions as tumor suppressors [7,8].

Activation of Oncogenes

MicroRNAs (miRNAs) play an essential role in tumor suppression by targeting and downregulating the expression of various tumor suppressor genes, but they may also act as oncogenes by directly down-regulating certain oncoprotein genes that prevent tumorigenesis. MiR-17-92 cluster, commonly referred to as "oncomir-1," targets and inhibits tumor suppressor proteins like PTEN, p21, and E2F; ultimately leading to increased cell proliferation, survival, and angiogenesis. MiR-21 also targets PTEN, PDCD4, and TIMP3, activating survival-supportive pathways like PI3K/Akt and MAPK which promote tumor growth, invasion, and metastasis. miR-155 downregulates TP53INP1, SOCS1, and SHIP1, thus increasing cell proliferation, survival, and immune evasion. MiR-372/373 cluster targets the LATS2 tumor suppressor and activates transcriptional co-activators known as YAP/TAZ that drive proliferation and stemness pathways. Deregulation of these oncogenic miRNAs either through upregulation or genetic alteration contributes significantly to cancer development/progression highlighting them as therapeutic targets within cancer treatment protocols [7,9,10].

Impact on Signalling Pathways

MicroRNAs (miRNAs) play an essential role in cancer development through various mechanisms, from epigenetic regulation of miRNAs to directly altering signaling pathways

that drive cancer development. Epigenetic modifications such as DNA methylation or histone modifications can alter miRNA expression patterns to suppress or overexpress specific miRNAs, disrupting normal signaling pathways and potentially contributing to cancer formation and progression. The miRNAs play an essential role in cancer cells activity and regulation by modulating key signaling pathway components, targeting oncogenes or tumor suppressor genes, participating in feedback loops, modulating crosstalk, and experiencing epigenetic modifications - these miRNA-driven changes impact signaling pathways greatly and play an integral part in cancer cell processes such as abnormal growth, survival, invasion, and metastasis.

In one such study, researchers have discovered that miRNA-21 disrupts TNF receptor 1 (TNFR1) signaling while stimulating its counterpart (TNFR2) in cervical cancer [11]. Similar evidence exists with regards to miRNAs used to manage Hepatocellular Carcinoma (HCC), specifically controlling cell proliferation, invasion, metastasis, and drug sensitivity by manipulating key signaling pathways like PTEN/PI3K/Akt, Hippo-YAP/TAZ, and Wnt/b-catenin [12]. miRNA-425-5p promotes breast cancer growth via activating its activating signaling pathway while miR-9 suppresses its growth [13]. Within pancreatic ductal adenocarcinoma (PDAC), several microRNAs, including miR-217, have been identified as key regulators of the KRAS signaling pathway. miRNA-222 regulates Capan-2 pancreatic cancer cell line growth by specifically targeting P57 [7,14].

MicroRNAs as Diagnostic Biomarkers

MicroRNAs offer several distinct advantages as biomarkers over more traditional approaches for early disease diagnosis, including cancer detection. Their secretions into circulation remain stable over time making miRNAs ideal tools. Early diagnosis is key to improving patient prognosis and limiting treatment options, and microRNAs hold immense promise as multimarker models for accurate diagnoses, targeted therapy approaches, and tracking treatment response evaluation [15]. Circulating miRNAs and exosomal miRNAs may provide a more comprehensive understanding of disease processes while improving diagnostic accuracy. They have been discovered in blood, urine, and saliva samples taken from various body fluids. The miRNAs can provide non-invasive sampling that's ideal for cases when more invasive procedures, like biopsy, are either impractical or too inaccurate to use accurately. miRNA expression profiles can help pinpoint cancer origin and subtype for tailored personalized treatment plans and outcomes prediction; or predict individual responses to drugs which allows for optimizing treatment regimens while mitigating side effects [16,17].

Reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR)

Reverse transcription converts microRNAs to complementary DNAs using reverse transcription technology before amplifying and quantifying them using specific primers tailored for miRNA quantification amplification and quantification [18]. With accurate yet sensitive quantification capabilities of miRNA levels quantitatively, RT-qPCR serves as an indication or prognostic biomarker in various malignancies. RT-qPCR offers another non-invasive diagnosis method that is easily available, making this approach viable for use when diagnosing issues related to blood, urine, and saliva. RT-qPCR offers many advantages over alternative approaches for miRNA detection, including its ability to simultaneously identify multiple miRNAs simultaneously allowing multimarker models for accurate diagnosis and treatment; however, its use may come with risks. These considerations include selecting an analysis platform, taking note of any preanalytical requirements necessary, and understanding their effects on miRNA expression [19,20]. Analytical challenges associated with using miRNAs as diagnostic biomarkers include the need to account for batch effects between laboratories [21]. Therefore, RT-qPCR has emerged as one of the key techniques for detecting and quantifying miRNAs that serve as biomarkers in clinical diagnostic tests.

Next-Generation Sequencing (NGS)

MicroRNAs have demonstrated promise as cancer diagnostic markers when examined through Next-Generation Sequencing (NGS). NGS allows rapid sequencing of millions of DNA fragments simultaneously, providing accurate detection and profiling of microRNAs throughout their entire sequence. Researchers may use microRNA sequencing on cancer tissue or biofluid samples to isolate signatures associated with various forms of cancer and use this information for diagnosis, classification, and stratification purposes among cancer patients. There have been multiple studies that focus on the significance of miRNA in cancer. One such research paper utilized NGS technology to compare miRNA expression levels between lung cancer patients and healthy controls using serum samples from their bodies. The miRNA analyses provide powerful markers for cancer detection. Profiling cancer requires closely inspecting tissue sample expression patterns for purposes of diagnosing and prognosticating cancer based on crucial data analysis techniques. As technology progresses and data analytics techniques mature further, miRNA may prove itself invaluable as both an invaluable diagnostic tool in research settings as well as clinical practice settings. NGS analyses of miRNAs offer powerful markers for cancer detection [22,23].

Microarray Analysis

Microarray analysis uses miRNA-specific probes anchored to a solid surface to quickly and simultaneously assess multiple miRNA expression levels at once making this efficient means for miRNA profiling. Following hybridization to the chip, scanning, and analysis take place to detect differentially expressed miRNAs. Microarray analysis is an established technique for studying microRNA expression patterns as well as their roles in disease. Microarray profiling of miRNA expression

involves using a microarray chip containing probes for specific miRNAs to hybridize with labeled RNA samples and detect and analyze hybridization signals that occur between them and detected miRNA molecules [24]. Microarray analysis offers several distinct advantages when it comes to miRNA detection, including being able to simultaneously identify multiple miRNAs at once and detect them across body fluids - making this a non-invasive yet convenient diagnosis method. Multiple research projects utilizing miRNA as a diagnostic tool in microarray analysis are underway; in one such study, miRNA array analysis and bioinformatics methods were utilized to analyze miRNA expression profiles from pancreatic cancer tissue samples [25].

Specific miRNA Signatures in Different Cancer Types:
The miRNA signature includes an extensive set of microRNAs (miRNAs) with diagnostic, therapeutic, and prognostic potential across numerous diseases. miRNA-21 can be targeted as part of cancer therapy [26], miRNA-34 serves to treat Alzheimer's and predict its progression, while miRNA-122 helps diagnose liver diseases related to cholesterol and lipid metabolism. miRNA-155 modulates immune response; miRNA-125b serves as an early cancer detection indicator, miRNA-29 has been associated with increased severity of fibrosis; miRNA-146a signals neuroinflammation while let-7 predicts survival among cancer patients, MiR-223 levels are high among hematological disorders, and MiR 92a indicates colorectal cancer while controlling angiogenesis. The overview of miRNA signature as diagnostic, prognostic, therapeutic potential, and mechanism is summarized in Table 1.

Table 1: Overview of miRNA signature as diagnostic, prognostic, therapeutic potential and mechanism.

miRNA Signature	Diagnostic Potential	Change in miRNA Levels	Therapeutic Potential	Prognostic Potential	Mechanism	References
miRNA-21	High in certain cancers(e.g. lung)	Increased in cancers (e.g., lung cancer, breast cancer, colorectal cancer)	Potential target for cancer therapy	Indicator of poor prognosis in cancer	Regulates apoptosis and cell proliferation	[27]
miRNA-15	Indicators of inflammatory diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus) and inflammatory diseases (e.g., Crohn's disease, ulcerative colitis)	Increased in autoimmune (e.g., rheumatoid arthritis, systemic lupus erythematosus) and inflammatory diseases (e.g., Crohn's disease, ulcerative colitis)	Modulation in autoimmune disorders	Prognosis of chronic inflammation	Involved in immune response modulation	[28]
miRNA-34	Marker for neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease)	Decreased in neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease)	Role in Alzheimer's treatment	Predicts disease progression	Influences neuron survival and function	[29]
miRNA-122	Diagnostic for liver diseases (e.g., hepatitis C, liver cirrhosis, liver cancer)	Increased in liver diseases (e.g., hepatitis C, liver cirrhosis, liver cancer)	Therapeutic target in hepatitis C	Indicates liver fibrosis severity	Involved in cholesterol and lipid metabolism	[30]
miRNA-16	Biomarker for cardiovascular diseases (e.g., heart failure, myocardial infarction)	Decreased in cardiovascular diseases (e.g., heart failure, myocardial infarction)	Potential in heart failure treatment	Associated with cardiovascular risk	Modulates cardiac cell apoptosis	[31]

miRNA-200	Identified in various cancers (e.g., ovarian cancer, breast cancer)	Decreased in advanced cancers (e.g., ovarian cancer, breast cancer)	Role in preventing metastasis	Indicates cancer progression	Regulates epithelial-to-mesenchymal transition	[32]
miRNA-125b	Indicators for breast and ovarian cancers (e.g., breast cancer, ovarian cancer, prostate cancer)	Increased for breast and ovarian cancers	Target in certain cancer treatments	Predictive chemotherapy response	Modulates cancer cell proliferation	[33]
miRNA-let-7	Low in lung and breast cancers (e.g., lung cancer, breast cancer)	Low in lung and breast cancers	Target in lung and breast cancer therapy	Predicts survival in cancer patients	Regulates oncogenes and cell cycle	[34]
miRNA-223	Elevated in hematological disorders (e.g., acute myeloid leukemia, chronic lymphocytic leukemia)	Elevated in hematological disorders	Potential in blood disorder treatments	Prognostic in myeloid cancers	Involved in hematopoiesis and immune function	[35]
miRNA-92a	Indicator for colorectal cancer (e.g., gastric cancer, breast cancer)	Overexpressed in colorectal cancer	Role in angiogenesis inhibition in cancers	Associated with metastasis in cancers	Regulates angiogenesis and cell proliferation	[36]

Preanalytical Conditions for Circulating miRNAs

Circulating microRNAs (miRNAs) are small, non-coding RNAs that play critical roles in gene regulation and are stable in bodily fluids, making them promising biomarkers for various diseases. However, the accurate measurement of circulating miRNAs is heavily influenced by preanalytical conditions, which include factors from sample collection to RNA extraction. Proper handling of these steps is crucial for reliable results.

Sample Collection

RNase-free tubes designed for plasma or serum collection are recommended. Different blood collection tubes can affect miRNA yield and quality. Tubes containing EDTA, heparin, or citrate as anticoagulants can affect miRNA measurements differently. EDTA tubes are generally preferred as heparin can inhibit downstream PCR reactions, and citrate may affect miRNA quantification [37]. During blood draw or processing, hemolysis can release intracellular miRNAs, which may contaminate the sample and skew results [38,39]. Visual inspection of plasma/serum for pink discoloration and measuring hemolysis markers like miR-451a and miR-23a can help identify contaminated samples [40].

Sample Processing

To obtain plasma or serum, blood samples must be processed promptly. The standard protocol involves two-step centrifugation: an initial low-speed spin (e.g., 1,500–2,000 g) to separate plasma/serum and a second high-speed spin (e.g., 10,000 g) to remove cellular debris and platelets. Improper centrifugation can lead to contamination by cellular miRNAs. Delays in processing and incorrect storage temperatures can degrade miRNAs. It is crucial to keep samples on ice immediately after collection and to process them within two hours to prevent RNA degradation [41,42].

Storage Conditions

Plasma or serum samples should be stored at -80°C for long-term preservation of miRNAs. Repeated freeze-thaw cycles should be avoided as they can degrade miRNAs and alter expression profiles. Long-term storage stability can vary depending on the miRNA. Some studies suggest that miRNAs remain stable for months at -80°C, but it is recommended that samples be used as soon as possible after thawing [43].

RNA Extraction

The efficiency of miRNA extraction can vary significantly between commercially available kits. Kits with phenol-chloroform extraction steps are commonly used; however, automated systems are also available. Each method has its pros and cons regarding yield, purity, and consistency. Using exogenous spike-in controls (like synthetic miRNAs from other species) during extraction can help assess the efficiency and variability of the extraction process. Several column-based Kits are also used such as miRNeasy Mini Kit (Qiagen), and NucleoSpin miRNA Kit (Macherey-Nagel) and Magnetic Bead-based Kits also utilized such as Exosome Plus™ MicroRNA Isolation Kit (Thermo Fisher Scientific) [44,45,46].

Normalization Strategies

Accurate quantification requires appropriate normalization. Common strategies include the use of endogenous controls (such as miR-16), exogenous spike-in controls, or global mean normalization. However, no universal standard exists, making normalization a critical point of variability [47].

Small RNA library preparation kits for NGS

Small RNA library preparation kits are essential tools for next-generation sequencing (NGS) of circulating miRNAs. Examples include TruSeq Small RNA Library Prep Kit (Illumina) as it offers a streamlined workflow for efficient small RNA sequencing and NEBNext Small RNA Library Prep Kit (New England Biolabs) known for its high sensitivity and accuracy in capturing small RNA [48,49]. A brief overview of the preanalytical conditions for circulating miRNAs use in diagnostic is shown in Table 2.

Table 2: Overview of the preanalytical Conditions for Circulating miRNAs use in diagnostic.

S. No.	Category	Description	References
1.	**Sample Types**	**Blood samples:** EDTA, heparin, or citrate tubes.	[37]
2.	**Tube Recommendations**	RNase-free tubes and collection devices are essential. Tubes designed for plasma or serum collection are recommended.	[38,39]
3.	**Isolation Methodologies**	**Column-based Kits:** miRNeasy Mini Kit (Qiagen), NucleoSpin miRNA Kit (Macherey-Nagel).	[44]
		Magnetic Bead-based Kits: Exosome Plus™ MicroRNA Isolation Kit (Thermo Fisher Scientific).	[45]
		Phenol-Chloroform Extraction: The traditional method requires careful handling. (Invitrogen TM TRIzolTM Reagent and QIAzol Lysis Reagent).	[46]
4.	**Small RNA Library Prep Kits for NGS**	**TruSeq Small RNA Library Prep Kit (Illumina):** Streamlined workflow for small RNA sequencing.	[48]
		NEBNext Small RNA Library Prep Kit (New England Biolabs): High sensitivity and accuracy for small RNA capture.	[49]
5.	**Considerations**	Ensure compatibility between the library preparation kit and the RNA isolation method to maintain high-quality sequencing results.	[50]

Clinical Applications in Cancer Diagnosis

Circulating miRNAs (microRNAs) are being investigated as non-invasive blood biomarkers to assist with cancer diagnosis. Their non-invasive nature provides many advantages in clinical applications, including stable detection in blood and identification of cancer-specific miRNAs across many cancer types. MiRNAs have been associated with prognosis, survival, and drug resistance among cancer patients providing us with another tool for predicting treatment outcomes. However, challenges like poor diagnostic specificity, reproducibility, and individual factors influencing miRNA expression must be overcome to be effectively managed. Although miRNAs as biomarkers for cancer diagnosis remain challenging, researchers continue to actively explore their use as blood-based biomarkers through clinical trials that investigate therapy, diagnosis, and prognostication applications of miRNAs. MiRNAs offer promise as blood-based biomarkers for cancer diagnosis; however, more research and validation must be performed before being fully adopted as clinical tools. Therefore, miRNAs hold great promise as blood-based cancer biomarkers but require further development for full clinical implementation [21,51].

MiRNA expression profiles in tumor tissues provide invaluable insights for cancer diagnosis, serving as indicators to distinguish cancerous from noncancerous tissues. MicroRNAs have proved useful for cancer diagnosis in clinical applications, particularly with tissue-specific expression profiles. Genome-wide profiling has revealed that miRNA expression signatures correlate to tumor type, tumor grade, and clinical outcomes - making them promising biomarkers in cancer diagnosis and prognosis. However, identifying important miRNA targets in cancer and validating specific signatures as biomarkers remain key milestones in diagnostics [52]. Cancer cells contain different miRNA profiles that offer potential as diagnostic or prognostic biomarkers. The miRNAs have been associated with prognosis, survival, and drug resistance among cancer patients - making them useful tools in predicting outcomes of treatment. Unfortunately, however, challenges such as diagnostic specificity, reproducibility, and individual factors that influence miRNA expression must first be overcome to fully take advantage of miRNAs' clinical applications in cancer diagnosis and prediction [53,54].

Tissue-specific miRNA expression profiles in cancer diagnosis have shown potential in identifying cancer types, determining tumor stage, and predicting patient outcomes. Some examples of tissue-specific miRNA expression profiles in cancer diagnosis include:

A comprehensive profile of miRNAs in cancer has been established by analyzing miRNA expression in various cancer types, such as prostate, lung, stomach, pancreas, and thyroid. This study identified a "miRNoma" in cancer, consisting of overexpressed and downregulated miRNAs, some of which are well-characterized cancer-associated miRNAs like miR-17-5p, miR-20a, and miR-21 [55].

MicroRNAs (miRNAs) have shown potential as non-invasive diagnostic biomarkers in various diseases, including cancer. Some examples of miRNA biomarkers used in non-invasive diagnostics include Circulating miRNAs and Exosomal miRNAs. Circulating miRNAs are present in blood, such as serum or plasma, have been proposed as useful diagnostic biomarkers in cancer, as they can be measured in routine clinical diagnoses [56,57]. Exosomal miRNAs are small vesicles released by cells that can contain miRNAs. Exosomal miRNAs can be isolated from blood and have been suggested as potential non-invasive biomarkers for cancer [58].

Correlation between miRNA Expression and Cancer Prognosis

MicroRNAs play an essential part in cancer prognosis. Their expression levels correlate to clinical outcomes such as tumor stage, lymph node involvement, and overall survival rates. The miRNA profiles in cancerous and normal cells demonstrate promise as potential prognostic biomarkers; studies conducted on hepatocellular carcinoma found 414 gene-miRNA associations that provided strong prognostic information. Researchers discovered a correlation between miRNA expression levels and overall survival for various cancers such as HCC [59].

Identification of High-Risk and Low-Risk Patients

MicroRNAs have emerged as key prognostic indicators when diagnosing cancer patients and can prove particularly helpful when performing liquid biopsies. The miRNAs provide essential data regarding patients who are likely to survive long term, have a health-free prognosis, and respond well to treatments. Furthermore, miRNAs offer multiple advantages that include being less intrusive as well as higher precision. Bioinformatics development and diagnostic technology have greatly assisted with using miRNAs for cancer diagnosis and prognosis, with additional studies underway that may lead to their use within clinical settings [54,60]. MiR-210 and miR-141 prognostic miRNAs for breast cancer patients provide helpful prognostic information that enables physicians to distinguish those at increased risk for recurrence from those who stand a greater chance for survival, thus making more informed treatment choices as far as intensities or follow-up schedules are concerned [61].

Prognostic Value of Circulating miRNAs

Circulating miRNAs found in body fluids such as blood, saliva, and urine have emerged as non-invasive prognostic biomarkers due to their stable and accessible nature. Studies have identified specific miRNA signatures found circulating that correlate with clinical outcomes, such as miR-155 and 21 which have been linked with reduced survival for various cancer types. The miRNA detection and analysis offer promise as a non-invasive means to enhance prognostic assessments and track disease progression without using invasive procedures, yet research into miRNA is in its infancy with results often lacking reproducibility. Standardized protocols for sample collection, transport,

storage, and data analysis can help researchers overcome any differences among research teams. Although miRNAs do not meet all criteria to act as definitive prognostic biomarkers for cancer patients, circulating miRNAs have shown promise as potential clinical biomarkers and more research and standard protocols need to be put in place to fully establish them as useful prognostic indicators for this purpose [62].

Integration with Traditional Prognostic Factors

MicroRNAs have shown enormous promise as prognostic biomarkers of cancer, and, when used alongside more established factors, can significantly enhance their predictive power. Their role can be understood from two angles. First, they act as potential precursors and second as potential biomarkers of future outcomes of treatment plans.

MicroRNAs provide invaluable insight into cancer biology and risk stratification, linking with specific molecular pathways or tumor characteristics to create more precise prediction models of overall or disease-free survival [63]. Integration also facilitates the identification of subclasses that exhibit different clinical effects or therapeutic responses for more tailored plans that provide superior patient results. An analysis that integrates miRNA data with traditional prognostic factors can identify miRNA signatures associated with specific clinical outcomes, including overall survival, disease-free survival, and treatment response [64]. Integrating miRNA expression data with traditional clinicopathological factors can significantly enhance the accuracy of prognostic prediction models. Algorithms incorporating multiple miRNAs have shown superior prognostic performance compared to using only traditional factors alone; such integrated models could give a more complete understanding of disease behavior as well as aid in individualizing treatment plans. Furthermore, this integration will establish miRNA's clinical utility in prognostication [65].

Therapeutic Implications of MicroRNAs in Cancer

MicroRNAs (miRNAs) have garnered significant attention as potential therapeutic targets in cancer treatment. Their dysregulated expression in cancer cells, particularly oncogenic miRNAs, provides an opportunity for therapeutic intervention. Here, we explore two strategies for targeting oncogenic miRNAs: using antisense oligonucleotides and small molecule inhibitors.

Antisense Oligonucleotides

Antisense Oligonucleotides (ASOs) are synthetic sequences of nucleotides designed to specifically bind with and interfere with target microRNAs (miRNAs) to inhibit their function and potentially restore expression of tumor suppressor genes. ASOs function by base-pairing their oncogenic miRNA targets and disturbing miRNA binding sites on messenger RNA (mRNA) for cancer treatment. This disrupts its downstream regulatory effects while potentially restoring expression of tumor suppressor genes; ASOs can also be chemically modified further to enhance stability and specificity for better performance results as preclinical studies have demonstrated inhibition of tumor growth along with increased chemotherapy response/sensitivity/sensitivity for various cancer types.

ASOs have the potential to dramatically decrease the activity of oncogenic miRNAs like miRNA-23a and miRNA-106b that play key roles in tumorigenesis, by targeting them directly [66]. Their specificity to target miRNAs and stability engineering ensures effective binding/inhibition; such qualities make ASOs highly sought-after therapeutic agents for cancer treatments [67].

ASOs may cause fewer off-target effects compared to traditional small interfering RNAs (siRNAs) since they target specific miRNAs rather than specific mRNAs; this increased accuracy helps decrease side effects while improving therapeutic results [38]. Chemical modifications of ASOs have enhanced their stability for effective binding to target miRNAs and inhibiting their function (68). ASOs can be modified using cell-penetrating peptides or other delivery systems to enhance uptake by cancer cells for increased therapeutic efficacy; this improved drug delivery can result in more successful cancer therapy [69].

Antisense Oligonucleotides (ASOs) targeting miRNAs have been developed as anticancer treatments, including anti-miRNA oligonucleotides (AMOs). AMOs specifically target specific miRNAs like miRNA-23a and miRNA-106b which tend to become upregulated during cancer development. An AMO can inhibit tumorigenesis by decreasing expression levels of target miRNAs (60). AntagomiRs are another miRNA-targeting oligonucleotide that works by binding and blocking specific miRNAs overexpressed in cancerous cells, acting like anticancer drugs by binding to and silencing their activity [70]. AntagomiRs are synthetic double-stranded oligonucleotides designed to overexpress miRNA targets that in turn regulate cancer cell proliferation; miRNA mimetics could potentially act as cancer therapeutic agents [71].

There have been various clinical trials using antisense oligonucleotides (ASOs) to target miRNAs for cancer therapy. One such ASO, called MRX34 (liposomal formulation of miR-34a mimic), was recently evaluated in a phase I trial as a treatment of advanced solid tumors. Trial data demonstrated that MRX34 was well tolerated and showed promising antitumor activity [72]. Meanwhile, ASOs such as AZD9150 targeting miR-221 which is overexpressed in various cancers tested in phase I clinical trials for treating advanced solid tumors with positive results [73]. RG-012, an ASO that targets miR-21 overexpression found in various cancers, was tested in a phase I clinical trial for advanced solid tumors with promising results [74].

Small Molecule Inhibitors

Small molecule inhibitors possess ways of targeting oncogenic miRNAs in cancer. Small molecule inhibitor molecules interact with specific miRNAs or components of their biogenesis pathway to inhibit processing or function, binding directly

with target mRNA or disrupting key proteins involved in the biogenesis of miRNA. By blocking oncogenic miRNA activity these inhibitors aim to restore normal gene expression patterns and restrain tumor growth [57]. Several small molecule inhibitors targeting oncogenic miRNAs are currently undergoing preclinical and clinical evaluation, showing their therapeutic potential [75].

Small molecule inhibitors target oncogenic miRNAs through multiple mechanisms, including inhibiting transcription or interfering with loading onto an RNA-induced silencing complex (RISC). For instance, one small molecule inhibitor was discovered that was effective at targeting miR-21 transcription, effectively decreasing its expression. Furthermore, small molecules can interfere with loading miRNA onto AGO2, an essential step for its functionality; such antagonizers may be identified through high-throughput screening, providing another effective approach in targeting oncogenic pathways while potentially modulating miRNA activity or even developing therapeutics targeted specifically against miRNA [76].

Small molecule inhibitors can easily be structurally modified to increase selectivity, stability, and bioavailability for use as therapeutic agents, leading to their rapid development. They have proven particularly promising as tools in miRNA therapeutic development due to their bioactivity and wide chemical space [77]. Furthermore, small molecule inhibitors may be combined with chemotherapy or radiation therapies to further boost efficacy while decreasing side effects [78].

Small molecule inhibitors of miRNA have been discovered for use in cancer therapy. One such inhibitor specifically targeted miR-21 transcription and reduced its expression levels; further, this was proven effective against oncogenic pathways [79]. Studies on small molecule inhibitors that modulate RNAi pathways could potentially recover tumor-suppressor miRNAs while simultaneously decreasing the expression and function of oncogenic genes [77]. Enoxacin is a small molecule that serves as a cancer-specific growth inhibitor by increasing TAR RNA-binding protein 2-mediated microRNA processing [53].

Restoration of Tumour -Suppressive miRNAs

Tumor-suppressive miRNAs are crucial in regulating the expression of genes associated with tumor growth and progression. By restoring their levels and activity, it is possible to regain control over dysregulated gene expression contributing to cancer development. This targeted approach allows for precise modulation of specific genes and pathways involved in tumorigenesis. Tumor-suppressive miRNAs typically target multiple oncogenes or genes involved in tumor-promoting pathways, enhancing the overall therapeutic effect. Restoration of tumor-suppressive miRNAs holds great therapeutic potential in cancer treatment. The two primary strategies used for this purpose are the use of miRNA mimics and viral vectors for miRNA delivery [80].

Many applications of tumor-suppressive miRNA restoration have shown promise in preclinical research studies. Tumor-suppressive miRNAs have been discovered to effectively limit tumor growth across several cancer types. Restoration of miR-143 and miR-145 for colorectal cancer was seen to significantly reduce tumor size and growth rates [71]. Restoring miR-26a to HCC patients showed a significant reduction in tumor growth. Tumor-suppressive miRNAs have long been established as effective ways of fighting cancer cell proliferation.

The miRNA mimics are synthetic double-stranded RNA molecules created to mimic the function of natural microRNAs found within our bodies (miRNAs). The miRNA mimics are composed of two components; an “miR-mimicking guide strand” designed to closely resemble mature miRs; and an accompanying passenger strand. miR-Mims can then be added into an RNA-Induced Silencing Complex (RISC), where their guide strands bind directly with specific target mRp for degradation or inhibition of translation into proteins for suppression through degradation or inhibition. miR-Mims can then be added into an RNA-Induced Silencing Complex (RISC), where their guide strands bind directly with specific target mRp for degradation or inhibition of translation into proteins for suppression through degradation or inhibition. The suppression of repressor gene expression through direct binding to the specific target mRMP targets stops the degradation or translation can also be achieved, by utilizing the replicators of miRmims-mims replicators as potent inhibitors.

Normal cells use these microRNAs as tumor suppressors by using them to modulate genes responsible for proliferation, differentiation, and death in their cells. However, tumor cells often alter or block certain miRNAs that regulate processes governing development resulting in unchecked tumor expansion and growth. The miRNA mimics possess the capability to replace dysregulated immune-suppressing miRNAs that restrict tumor growth with miRNAs that restore the normal functions that were absent previously due to deregulation. They do this by mimicking certain immune-suppressing miRNAs known as immuno-suppressive miRNAs that suppress tumor cells; for cancer cells, this restores beneficial miRNA functions that previously weren’t functioning normally or were missing altogether. The miRNA mimics can use their ability to restore miRNA function to combat tumors by stopping oncogene expression (cancer-promoting genes), metastasis-promoting genes, and resistance-inducing genes from manifesting. Their benefits in fighting chemotherapy resistance and metastasis result in increasing Apoptosis rates which inhibit proliferation while slowing cancer cell metastatic spread, with lower invasion risks overall because of their use.

The miRNA mimics represent advancements in cancer therapy due to their unique capability of simultaneously targeting several genes or pathways. several approaches for combating cancer offer numerous advantages, especially as they may help limit resistance development associated with traditional approaches. The miRNA mimics the role of naturally occurring tumor suppressor miRNAs to offer more effective, targeted therapies than chemotherapy treatments. Reversing epigenetic change

and reinstating gene expression that suppresses tumors adds significantly to their value as therapeutics and cancer treatments. The miRNA mimics offer customized treatments for cancer based on each person's genetic makeup.

Viral vectors are being explored as a potential delivery system for tumor-suppressive miRNAs. These vectors, which can be packaged into viral particles like lentiviruses or adenoviruses, can deliver and express specific miRNAs in cancer cells. Once introduced, these vectors facilitate the intracellular production of tumor-suppressive miRNAs, restoring their normal function. This approach has shown promise in preclinical studies, demonstrating tumor growth inhibition and increased sensitivity to cancer treatments. These strategies aim to counteract tumorigenic effects and restore normal gene regulation. However, further research is needed to optimize delivery methods, enhance therapeutic efficacy, and ensure the safety of these interventions for clinical applications [81]. Further research is needed to optimize delivery methods, enhance therapeutic efficacy, and ensure the safety of these interventions for clinical applications. Engineers modify viral vectors like lentiviruses, adenoviruses, or AAVs to create a safe and efficient delivery system. These modifications remove viral genes essential for replication and pathogenicity, leaving behind necessary components for viral entry and cargo delivery [82]. Therapeutic miRNA sequences are introduced into a viral vector as transgenes, either as synthetic mimics or precursor sequences that undergo processing in target cells to yield functional miRNA [83]. Modified viral vectors bind to specific cell surface receptors through viral envelope proteins, allowing them to enter target cells via receptor-mediated endocytosis or membrane fusion [84]. Once inside, the vector uncoats, releasing the therapeutic miRNA cargo into the cytoplasm. Viral vectors like lentiviruses and adenoviruses release their genetic material into the nucleus [85]. MiRNA transgenes are delivered into the cellular miRNA processing pathway, where they undergo further processing to generate mature miRNAs. These mature miRNAs bind to target mRNAs, leading to gene silencing or translational repression. This results in the restoration or modulation of specific gene expression patterns affected by dysregulated miRNAs in target cells [86].

Studies have explored the use of viral vectors as delivery vehicles in miRNA-based therapy. One of the studies found that lentiviral vectors can deliver tumor-suppressive miRNA-145 to colon cancer cells, inhibiting cell growth, apoptosis, and tumor formation in animal models. This highlights the potential of lentiviral vectors in cancer therapy, as they are downregulated in many cancers [87]. Another study found that lentiviral vectors can deliver synthetic miR-34a mimics, a tumor-suppressive miRNA, to glioblastoma multiforme (GBM). The delivery suppressed glioma cell proliferation, induced apoptosis, and inhibited tumor growth in xenograft models, demonstrating the therapeutic potential of miR-34a mimics in GBM treatment [88]. Viral vectors, including adeno-associated viruses (AAVs) and lentiviruses, have shown significant potential in various biomedical applications. They can efficiently deliver therapeutic miRNAs to target cells and tissues, allowing for gene expression modulation and disease-causing genetic abnormalities correction. They have shown promise in cancer therapy by targeting tumor-suppressive miRNAs or miRNA-based therapeutics, increasing therapeutic efficacy while minimizing off-target effects [89].

Challenges and Future Perspectives in miRNA Therapeutics

MiRNA therapeutics provide great promise; however, numerous key obstacles must first be cleared away to enable successful implementation to take place. Understanding and overcoming challenges is crucial to the development of miRNA treatments and advancing towards their clinical use. The main challenge in miRNA research is developing efficient delivery mechanisms that can target specific tissues or cells to deliver miRNA. Ergonomically targeting miRNA therapeutics without off-target effects is of vital importance and requires extensive research to guarantee the delivery of therapeutics that precisely target their intended genes without unintendedly altering the expression of other non-target ones, thus minimizing side effects and potential side effects.

Understanding the pharmacokinetics and pharmacodynamics of miRNA therapeutics is integral to optimizing dosage, frequency of administration, and duration of therapy. Strategies designed to increase stability, half-life, tissue accumulation, and immunogenicity must also be thoroughly explored as part of any miRNA therapeutics' clinical translation plan. Current research endeavors focus on optimizing miRNA molecules, delivery systems, and long-term safety profiles to enable reliable clinical use. Utilizing miRNA biomarkers and their functional significance across diseases will enable clinicians to more effectively stratify patients and create tailored treatment approaches. Advancements in high-throughput sequencing technology, bioinformatics, and data integration methods will aid researchers in discovering novel miRNA biomarkers and designing tailored therapies using miRNA-based therapeutic approaches. MiRNA therapeutics pose complex regulatory requirements and hurdles that must be carefully considered before entering clinical development. Researchers, clinicians and regulatory authorities working in harmony to develop stringent protocols and guidelines can ensure safe efficacy [90,91].

Prospects involve continued studies into miRNA biology, the development of better delivery systems, and adapting current technological approaches to meet future challenges. Combinatorial approaches may increase therapeutic efficacy when miRNA therapies are combined with chemotherapy or immunotherapy treatment modalities like chemotherapy. Many studies are being undertaken to demonstrate the value of miRNAs as diagnostic markers. Davey MG conducted a detailed investigation analyzing miRNA expression patterns in blood samples from lung cancer patients and healthy controls and discovered specific miRNAs were significantly upregulated among cancer patients (miR-21 and miR-486 specifically), showing their potential as non-invasive diagnostic markers [92]. One case study provided compelling evidence for

miRNA's usefulness in prostate cancer diagnosis and prognosis. Researchers identified a signature of miRNAs that accurately discriminated between prostate cancer and benign prostatic hyperplasia (BPH) using miRNA expression profiling; specific miRNAs like miR-21 and 221 were linked with aggressive prostate cancers with poor prognoses [93].

Clinical case studies and success stories demonstrating the potential of miRNA-based therapies

Liposomal formulation of synthetic miR-34a mimic MRX34 was subjected to an initial clinical trial for treating advanced solid tumors like melanoma and lung cancer in phase one clinical studies. Therapy using MRG-106, a synthetic oligonucleotide inhibitor targeting miR-155, demonstrated promising antitumor activity with partial responses and disease stabilization reported from several patients [71]. MRG-106 was tested in a phase I clinical trial to treat CTCL lymphomas; its effectiveness against them proved promising. Treatment resulted in significant clinical responses, including reductions in tumor size and improvement of skin-related symptoms [94]. RG-012, an anti-miR-21 oligonucleotide, was tested as part of a phase I/II clinical trial for Alport syndrome - a genetic kidney condition - where preliminary results suggested target engagement as well as potential therapeutic advantages; suggesting miRNA therapies might hold great promise [95].

These examples highlight the clinical translation of miRNA-based therapies across diverse disease settings. They demonstrate the potential of miRNA modulation to target specific disease mechanisms and offer new therapeutic strategies.

Miravirsen's clinical trial with patients living with Hepatitis C virus infection showed substantial reductions in HCV RNA levels, sustained viral response rates among some patients, and improved liver function and reduced liver fibrosis; thus, demonstrating the power of miRNA therapies for improving HCV infection outcomes [96]. Another report demonstrated the efficacy of miR-34a mimic therapy given to a pediatric neuroblastoma patient; treatment led to tumor regression and complete remission - further showing their utility in personalized medicine and treating aggressive cancers [97]. Clinical results from an anti-miRNA oligonucleotide trial targeting miR-155 were promising; some participants with cutaneous T-cell lymphoma (CTCL) experienced complete responses accompanied by improved symptoms, reduced tumor burden, and extended progression-free survival. These data underscore the potential role played by miRNA therapies in improving patient outcomes related to hematological malignancies [98].

An analysis conducted retrospectively indicated that high miR-34a expression in lung tumor tissues from patients diagnosed with non-small cell lung cancer (NSCLC) is linked with improved prognosis. Researchers discovered that patients expressing high levels of miR-34a had significantly greater overall and progression-free survival compared with those displaying lower miRNA expression, further supporting its prognostic value and role as an aid for treatment decisions and patient management. These clinical case studies and success stories showcase how miRNA therapies have made positive impacts on various diseases [93]. The miRNA modulation shows great promise in terms of improving treatment responses, prolonging survival rates, inducing remission, and alleviating symptoms associated with cancers or viral infections.

Future Directions

The miRNA-based therapies combined with existing treatments such as chemotherapy, immunotherapy, or targeted therapies have the power to significantly enhance patient outcomes. However further research must be conducted to identify and validate miRNA biomarkers that will enable accurate diagnosis, prognosis, and response prediction from targeted therapy treatments such as chemotherapy or immunotherapy - helping guide treatment decisions more specifically and improve management for each patient. Innovative delivery systems including exosomes, peptide-based carriers, and genome editing tools have recently been created to boost miRNA therapeutic efficiency while clinical trials with diverse patient populations are crucial to establish clinical efficacy, safety, and long-term benefits associated with miRNA therapies [99].

Emerging Technologies for miRNA Profiling

MicroRNA (miRNA) profiling is crucial for understanding biological processes and diseases. Advanced technologies like Next-Generation Sequencing (NGS) and Digital PCR (dPCR) offer unique capabilities and applications in miRNA profiling. NGS is used in cancer research for profiling and quantifying microRNAs, including their expression levels. dPCR partitions samples into thousands of individual reactions, making it suitable for measuring miRNA expression levels across biological samples. Single-cell sequencing technologies like scRNA-seq have revolutionized miRNA profiling by allowing analysis at the single-cell level, offering insight into miRNA expression heterogeneity and dynamics. Nanostring nCounter uses hybridization-based detection and quantification technology to enable simultaneous identification and quantification of multiple miRNAs within one assay with high sensitivity. Droplet Digital PCR (ddPCR) is accurate and sensitive for quantifying miRNAs found in samples. Microarrays were once widely used for miRNA expression profiling studies, but their use has declined due to NGS technologies. Small RNA-Seq targets small RNA molecules like miRNAs, allowing for the identification of known and novel miRNAs and their expression patterns. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is the go-to technique for miRNA analysis due to improved sensitivity, specificity, and accuracy. Locked Nucleic Acid (LNA) technology enhances probe binding affinity to miRNAs, increasing sensitivity and specificity. Bead-based miRNA profiling assays use bead arrays containing probes specific for miRNA to quantitatively examine expression levels of multiple miRNAs. Mass spectrometry techniques like MALDI-MS and SRM can be used for miRNA profiling. In Table 3. we summarise the technique with its key features, outcome, and significance.

Table 3: Emerging techniques with their key features outcome and significance.

Technique	Key Features	Outcome and Significance	References
Next Generation Sequencing	Comprehensive profiling of miRNAs	NGS enables the identification of dysregulated miRNAs, offering potential non-invasive biomarkers for early lung cancer detection.	[73]
Digital PCR	Absolute quantification of miRNAs	dPCR-based miRNA profiling holds potential for breast cancer diagnosis and personalized medicine approaches.	[100]
Single Cell Sequencing	Analysis of miRNA expression at the single-cell level	Single-cell miRNA profiling provides insights into miRNA expression heterogeneity during neuronal development.	[101]
Nanostring nCounter Technology	Simultaneous detection and quantification of multiple miRNAs in a single assay	Nanostring nCounter technology enables the identification of miRNA biomarkers for bladder cancer diagnosis and prognosis.	[102]
Droplet Digital PCR	Absolute quantification of miRNAs with high precision and sensitivity	Identified a miRNA-based signature for ddPCR-based miRNA profiling shows potential as a non-invasive diagnostic tool for colorectal cancer.	[103]
Microarrays	Simultaneous detection and quantification of multiple miRNAs	Microarray profiling identified miRNAs involved in myocardial infarction, providing insights into cardiac disease mechanisms.	[104]
Small RNAseq	Sequencing of small RNA molecules, including miRNAs	Small RNA-Seq enables the identification of miRNAs associated with pregnancy-related complications.	[105]
qRTPCR	Widely used technique for miRNA expression analysis	qRTPCR-based miRNA profiling provides insights into the involvement of dysregulated miRNAs in Alzheimer's disease	[106]
LNA-based miRNA profiling.	Enhanced probe binding affinity using Locked Nucleic Acid (LNA) technology	LNA-based miRNA profiling allows for the identification of region-specific miRNA expression patterns in the bra in.	[107]
Bead-based miRNA profiling	Quantitative analysis of miRNA expression levels using bead arrays	Bead-based miRNA profiling enables the identification of miRNA signatures with potential clinical implications in hepatocellular carcinoma.	[108]
Mass Spectrometry	Mass spectrometry-based methods for miRNA profiling	Mass spectrometry-based miRNA profiling offers a potential avenue for identifying miRNA biomarkers in prostate cancer.	[109]
Functional miRNA profiling	Study of miRNA activity and their effects on target genes	Functional miRNA profiling provides insights into the regulatory functions of specific miRNAs in gene expression and cancer biology.	[110]

Bioinformatics Tools for miRNA Data Analysis

Bioinformatics tools like miRDeep2 and miRExpress are crucial in miRNA research, identifying and quantifying miRNAs through quality control, alignment to known databases, and prediction of novel miRNAs. This comprehensive process helps understand miRNA expression patterns in biological samples and diseases. MiRNA target prediction is crucial for understanding miRNA function. Tools like TargetScan and miRanda use algorithms to predict potential mRNA targets, focusing on sequence complementarity and evolutionary conservation. These tools help researchers infer miRNA regulatory roles in gene expression, enabling a better understanding of biological processes and disease mechanisms. DIANA-TarBase and miRTarBase are databases that aid in investigating and constructing regulatory networks involving miRNA-mRNA interactions, providing access to experimentally validated interactions, and enhancing understanding of miRNA roles across biological processes and diseases. DIANA-mirPath and miRWalk tool functional enrichment analyses give valuable insight into biological implications associated with miRNAs;

mapping miRNAs onto various biological processes provides useful evidence regarding regulatory roles related to cell cycle regulation, apoptosis regulation, and differentiation regulation activities as well as cell death regulation activities.

Differential expression analysis is crucial in miRNA research, revealing upregulated or downregulated miRNAs. Tools like edgeR and DESeq compare expression levels across conditions, enhancing understanding of miRNA regulation in various biological contexts.

Data integration and visualization are crucial in miRNA research, with tools like Cytoscape and miRBase serving as essential sources. Cytoscape provides an integrative genomic data portal, offering access to genomic information such as miRNA sequence, gene predictions, protein interaction networks, and other biological features. miRBase offers authoritative databases with miRNA sequences annotated with target sequence information, allowing for accurate analysis and interpretation. Cancerous cancer researchers use an integrated approach using bioinformatics tools to understand how miRNAs may play a part in disease. RNA-Seq data from cancerous and normal tissues is processed using miRDeep2, EdgeR, TargetScan, and DIANA-mirPath. These tools detect and quantify miRNAs, identify miRNAs with varying expression levels, and perform pathway analysis to reveal biological implications. Cytoscape visualizes complex miRNA-target interaction networks within cancer pathways, providing therapeutic targets. This innovative methodology identifies key miRNAs used and reveals functional roles within cancer pathways, revealing possible therapeutic targets. These bioinformatics tools are essential for miRNA research specifically related to cancer studies as in detail it is summarized in Table 4.

Table 4: Overview of Bioinformatics Tools and Processes in miRNA Research and Analysis.

Aspect	Tools	Tools	References
Identification and Quantification of miRNAs	miRDeep2, miRExpress	miRDeep2, miRExpress	[111]
miRNA Target Prediction	TargetScan, miRanda	TargetScan, miRanda	[112]
miRNA-mRNA Interaction and Network Analysis	DIANA-TarBase, miRTarBase	DIANA-TarBase, miRTarBase	[113]
Functional Enrichment Analysis	DIANA-mirPath, miRWalk	DIANA-mirPath, miRWalk	[114]
Differential Expression Analysis	edgeR, DESeq	Utilizing statistical models, identify miRNAs with significant expression changes across various conditions.	[115]
Data Integration and Visualization	Cytoscape, miRBase	Integrate and visualize miRNA, mRNA, and protein interaction data to gain greater insight into complex regulatory networks.	[116]
Example of Usage in Cancer Research	miRDeep2, edgeR, TargetScan, DIANA-mirPath, Cytoscape	Gather RNA-Seq data from cancerous/normal tissues; identify and quantify miRNAs; compare expression levels among gene targets; analyze differential expression and target gene targeting patterns, as well as visualize interaction networks.	[117]

Ethical Considerations and Challenges in miRNA Diagnostics

The miRNA diagnostics, which analyze genetic material, raise concerns about patient privacy and security. Safeguards should be in place to protect confidentiality and prevent unauthorized access. Informed consent is crucial, as patients should be fully informed about the purpose, benefits, limitations, and potential risks of testing. Proper genetic counseling should be offered to help patients understand the implications of test results and make informed decisions about medical interventions or family planning. Psychological implications of miRNA diagnostics, particularly if they reveal increased disease risks or genetic predispositions, should be addressed. Access to appropriate psychological support and counseling services is essential. Ethical considerations include ensuring clinical validity and utility of miRNA tests, which require rigorous validation studies. Prioritizing patient autonomy, privacy, data protection, informed consent, and equitable access can ensure the responsible and beneficial use of miRNA diagnostics in clinical practice [118].

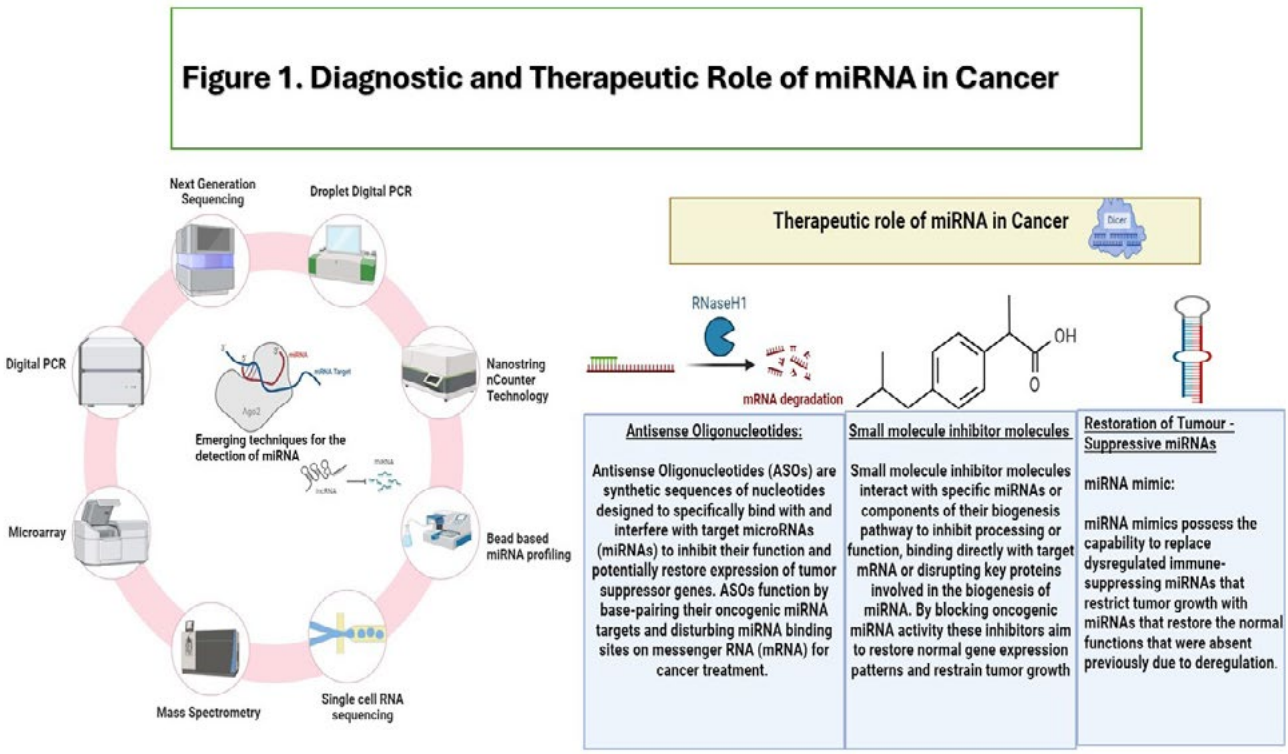
The miRNA mimic therapy is a novel cancer treatment approach that requires patients to be informed about its workings, benefits, risks, and experimental nature. Informed consent is crucial for patients to make informed decisions, which may require additional resources or consultations with healthcare professionals. Patients should be informed about alternative treatments and their right to withdraw at any point without affecting future care. Communication should be in an easily understandable language, avoiding technical jargon and using visual aids or metaphors. Understanding the patient's cultural background and beliefs is essential for their perception of advanced genetic therapies and treatment decisions. Discussions should also address the potential psychological impact of the treatment, including access to psychological support. Effective communication is an ongoing process, with regular updates on treatment progress, new findings, and concerns [119]. The development of miRNA therapeutics, particularly for cancer treatment, is a complex field with regulatory and ethical challenges. The approval process involves extensive preclinical and clinical trials, requiring clear guidelines for standardization and quality control. Ethical considerations include informed consent, patient understanding, data privacy, and potential long-term effects. Intellectual property rights and high costs could hinder research and development. Balancing risks and benefits, maintaining public trust, and fostering innovation are crucial for miRNA therapy advancement. A dynamic approach is needed to ensure patient safety and equity [120].

Conclusion

This review has examined the prominent function of microRNAs (miRNAs) in cancer research and their potential in diverse biological applications. The miRNAs, being non-coding RNA molecules, play a vital role in regulating gene expression by finely adjusting the amounts of gene expression through their interaction with messenger RNA (mRNA) molecules. Altered expression of miRNAs has been detected in various cancer types, indicating their role in the onset and advancement of cancer. Dysregulated miRNAs can serve as tumor suppressors by suppressing the production of oncogenes, or as oncogenes themselves by stimulating tumor development and metastasis. Furthermore, the disruption of miRNAs can affect crucial cellular pathways implicated in the progression of cancer, including as cell growth, programmed cell death, and the formation of new blood vessels.

Significantly, miRNAs have shown promise as significant diagnostic and prognostic indicators for cancer. Distinct miRNA expression profiles have been discovered that facilitate the distinction between healthy and malignant tissues. The differential expression patterns can be identified in diverse clinical samples, including tissue biopsies or liquid biopsies, providing a non-invasive method for early cancer identification [23]. Moreover, some miRNAs have demonstrated potential as prognostic markers, enabling the anticipation of patient outcomes and their reaction to therapies. To establish distinct miRNA signatures for different forms of cancer, it is necessary to conduct additional validation studies, considering the variability of tumors and patient groups. Conducting extensive clinical studies is necessary to validate the clinical usefulness of miRNAs as biomarkers for cancer diagnosis, prognosis, and therapy response as it is shown in Figure 1.

Figure 1: Diagnostic and Therapeutic Role of miRNA in Cancer.



Furthermore, continuous research endeavours are focused on creating novel therapeutic approaches that utilize the abnormal expression of miRNAs in cancer cells, hence creating opportunities for precise and targeted treatments. It is imperative to thoroughly examine the ethical implications and challenges associated with miRNA diagnostics and treatments. Essential factors to address include obtaining informed permission, effective patient communication, ensuring data privacy, and providing fair access to miRNA testing and therapy. The presence of regulatory systems and intellectual property rights will create further difficulties, requiring endeavors to guarantee thorough validation, standardization, and accessibility of miRNA-based therapeutics. Despite the difficulties encountered, the knowledge acquired from miRNA research holds significant ramifications for personalized medicine, illness detection, prediction, and therapy. miRNAs possess the capacity to transform cancer management by acting as crucial regulators and biomarkers in cancer research. Gaining insight into the distinct functions of miRNAs in various cancer types has the potential to enhance diagnostic methods, facilitate the development of tailored treatment strategies, and ultimately improve patient outcomes. Further examination and verification of miRNAs in cancer research will aid in the progress of precision medicine and the creation of innovative treatments.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

References

1. Dwivedi S, Purohit P, Vasudeva A, Kumar M, Agrawal R, Sheikh NA, Misra R, Kishore S, Misra S. Chapter 9 - Gene therapy and gene editing in healthcare. In: Barh D, editor. Biotechnology in Healthcare. Academic Press; 2022.p.147–175. https://www.sciencedirect.com/science/article/pii/B9780323898379000061.
2. Dwivedi S, Purohit P, Sharma P. MicroRNAs and Diseases: Promising Biomarkers for Diagnosis and Therapeutics. Indian J Clin Biochem. 2019;34:243–245. doi: 10.1007/s12291-019-00844-x. PMID: 31391712.
3. Jorge AL, Pereira ER, de Oliveira CS, Ferreira E dos S, Menon ETN, Diniz SN, Pezuk JA. MicroRNAs: understanding their role in gene expression and cancer. Einstein (Sao Paulo). 19:eRB5996. doi: 10.31744/einstein_journal/2021RB5996. PMID: 34287566.
4. Dwivedi S, Singh V, Sen A, Yadav D, Agrawal R, Kishore S, Misra S, Sharma P. Vitamin D in Disease Prevention and Cure-Part I: An Update on Molecular Mechanism and Significance on Human Health. Ind J Clin Biochem. 2024 doi: 10.1007/s12291-024-01251-7.
5. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne). 2018;9:402. doi: 10.3389/fendo.2018.00402. PMID: 30123182.
6. Peng Y, Croce CM. The role of MicroRNAs in human cancer. Sig Transduct Target Ther. 2016;1:1–9. doi: 10.1038/sigtrans.2015.4.

7. Otmani K, Lewalle P. Tumor Suppressor miRNA in Cancer Cells and the Tumor Microenvironment: Mechanism of Deregulation and Clinical Implications. Front Oncol. 2021;11:708765. doi: 10.3389/fonc.2021.708765. PMID: 34722255.
8. Svoronos AA, Engelman DM, Slack FJ. OncomiR or Tumor Suppressor? The Duplicity of MicroRNAs in Cancer. Cancer Res. 2016; 76:3666–3670. doi: 10.1158/0008-5472. CAN-16-0359. PMID: 27325641.
9. Feng Y-H, Tsao C-J. Emerging role of microRNA-21 in cancer (Review). Biomedical Reports. 2016; 5:395–402. doi: 10.3892/br.2016.747.
10. Zhou K, Liu M, Cao Y. New Insight into microRNA Functions in Cancer: Oncogene-microRNA-Tumor Suppressor Gene Network. Front Mol Biosci. 2017;4:46. doi: 10.3389/fmolb.2017.00046. PMID: 28736730.
11. Wang Y, Zhou S, Fan K, Jiang C. MicroRNA-21 and its impact on signaling pathways in cervical cancer. Oncol Lett. 2019; 17:3066–3070. doi:10.3892/ol.2019.10002. PMID: 30867735.
12. Ruiz-Manriquez LM, Carrasco-Morales O, Sanchez Z EA, Osorio-Perez SM, Estrada-Meza C, Pathak S, Banerjee A, Bandyopadhyay A, Duttaroy AK, Paul S. MicroRNA-mediated regulation of key signaling pathways in hepatocellular carcinoma: A mechanistic insight. Front Genet. 2022;13:910733. doi: 10.3389/fgene.2022.910733. PMID: 36118880
13. Syed SN, Brüne B. MicroRNAs as Emerging Regulators of Signaling in the Tumor Microenvironment. Cancers (Basel). 2020;12:911.doi:10.3390/cancers12040911. PMID: 32276464.
14. Mortoglou M, Wallace D, Buha Djordjevic A, Djordjevic V, Arisan ED, Uysal-Onganer P. MicroRNA-Regulated Signaling Pathways: Potential Biomarkers for Pancreatic Ductal Adenocarcinoma. Stresses. 2021;1:30–47. doi: 10.3390/stresses1010004.
15. Dwivedi S, Sharma P. Cancer Stem Cells: Future Possibilities for Cancer Therapy. Ind J Clin Biochem. 2023; 38:149–150. doi: 10.1007/s12291-023-01133-4.
16. Ho PTB, Clark IM, Le LTT. MicroRNA-Based Diagnosis and Therapy. Int J Mol Sci. 2022; 23:7167. doi: 10.3390/ijms23137167. PMID: 35806173.
17. Sharma P, Dwivedi S. Prospects of Molecular Biotechnology in Diagnostics: Step Towards Precision Medicine. Indian J Clin Biochem. 2017;32(2):121-123. doi: 10.1007/s12291-017-0650-9. Epub 2017. PMID: 28428685
18. Neagu M, Constantin C, Cretoiu SM, Zurac S. miRNAs in the Diagnosis and Prognosis of Skin Cancer. Front Cell Dev Biol. 2020;8:71. doi: 10.3389/fcell.2020.00071. PMID: 32185171.
19. de Ronde MWJ, Ruijter JM, Lanfear D, Bayes-Genis A, Kok MGM, Creemers EE, Pinto YM, Pinto-Sietsma S-J. Practical data handling pipeline improves performance of qPCR-based circulating miRNA measurements. RNA. 2017;23:811–821. doi: 10.1261/rna.059063.116. PMID: 28202710.
20. Dwivedi, Shailendra & Khattri, Sanjay & Pant, Kara. (2012). Recent Advances in molecular diagnostic approaches for microbial diseases.
21. Dwivedi S, Purohit P, Misra R, Lingeswaran M, Vishnoi JR, Pareek P, Misra S, Sharma P. Single Cell Omics of Breast Cancer: An Update on Characterization and Diagnosis. Indian J Clin Biochem. 2019;34(1):3-18. doi: 10.1007/s12291-019-0811-0. Epub 2019 Jan 8. PMID: 30728668.
22. Khamina K, Diendorfer AB, Skalicky S, Weigl M, Pultar M, Krammer TL, Fournier CA, Schofield AL, Otto C, Smith AT, et al. A MicroRNA Next-Generation-Sequencing Discovery Assay (miND) for Genome-Scale Analysis and Absolute Quantitation of Circulating MicroRNA Biomarkers. Int J Mol Sci. 2022;23:1226. doi: 10.3390/ijms23031226. PMID: 35163149.
23. Dwivedi S, Goel A, Sadashiv, Verma A, Shukla S, Sharma P, Khattri S, Pant KK. Molecular Diagnosis of Metastasizing Breast Cancer Based Upon Liquid Biopsy. In: Barh D, editor. Omics Approaches in Breast Cancer: Towards Next-Generation Diagnosis, Prognosis and Therapy. New Delhi: Springer India; 2014:425–459. https://doi.org/10.1007/978-81-322-0843-3_22.
24. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 2005;433:769–773. doi: 10.1038/nature03315.
25. Lawless N, Foroushani ABK, McCabe MS, O'Farrelly C, Lynn DJ. Next Generation Sequencing Reveals the Expression of a Unique miRNA Profile in Response to a Gram-Positive Bacterial Infection. PLOS ONE. 2013;8:e57543. doi: 10.1371/journal.pone.0057543.
26. Dwivedi S, Pareek P, Vishnoi JR, Sharma P, Misra S. Downregulation of miRNA-21 and cancer stem cells after chemotherapy results in better outcome in breast cancer patients. World J Stem Cells. 2022;14:310–313. doi: 10.4252/wjsc.v14.i4.310. PMID: 35662862.
27. Bica-Pop C, Cojocneanu-Petric R, Magdo L, Raduly L, Gulei D, Berindan-Neagoe I. Overview upon miR-21 in lung cancer: focus on NSCLC. Cell Mol Life Sci. 2018;75:3539–3551. doi: 10.1007/s00018-018-2877-x. PMID: 30030592
28. Hu J, Huang S, Liu X, Zhang Y, Wei S, Hu X. miR-155: An Important Role in Inflammation Response. J Immunol Res. 2022:7437281. doi: 10.1155/2022/7437281. PMID: 35434143

29. Bazrgar M, Khodabakhsh P, Prudencio M, Mohagheghi F, Ahmadiani A. The role of microRNA-34 family in Alzheimer's disease: A potential molecular link between neurodegeneration and metabolic disorders. Pharmacological Research. 2021;172:105805. doi: 10.1016/j.phrs.2021.105805.
30. Thakral S, Ghoshal K. miR-122 is a Unique Molecule with Great Potential in Diagnosis, Prognosis of Liver Disease, and Therapy Both as miRNA Mimic and Antimir. Curr Gene Ther. 2015;15:142–150. PMID: 25537773.
31. Zhou S, Jin J, Wang J, Zhang Z, Freedman JH, Zheng Y, Cai L. miRNAS in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges. Acta Pharmacol Sin. 2018;39:1073–1084. doi: 10.1038/aps.2018.30.
32. Korpal M, Kang Y. The emerging role of miR-200 family of microRNAs in epithelial-mesenchymal transition and cancer metastasis. RNA Biol. 2008;5:115–119. PMID: 19182522.
33. Chen Z, Guo X, Sun S, Lu C, Wang L. Serum miR-125b levels associated with epithelial ovarian cancer (EOC) development and treatment responses. Bioengineered. 2020;11:311–317. doi: 10.1080/21655979.2020.1736755. PMID: 32107956.
34. MicroRNA in lung cancer—a novel potential way for early diagnosis and therapy | Journal of Applied Genetics doi: https://doi.org/10.1038/s12276-023-01050-9. https://link.springer.com/article/10.1007/s13353-023-00750-2.
35. Gu J, Xu H, Chen Y, Li N, Hou X. MiR-223 as a Regulator and Therapeutic Target in Liver Diseases. Front Immunol. 2022;13:860661. doi: 10.3389/fimmu.2022.860661. PMID: 35371024.
36. Wu X, Cui X, Yue C, Liu X, Mo Z. Expression of miR-92a in colon cancer tissues and its correlation with clinicopathologic features and prognosis. Am J Transl Res. 2021;13(8):9627-9632. PMID: 34540088.
37. Pritchard CC, Kroh E, Wood B, Arroyo JD, Dougherty KJ, Miyaji MM, Tait JF, Tewari M. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila). 2012;5:492–497. doi: 10.1158/1940-6207.CAPR-11-0370. PMID: 22158052.
38. McDonald JS, Milosevic D, Reddi HV, Grebe SK, Algeciras-Schimnich A. Analysis of circulating microRNA: preanalytical and analytical challenges. Clin Chem. 2011;57(6):833-840. doi: 10.1373/clinchem.2010.157198. Epub 2011. PMID: 21487102.
39. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods. 2010;50:298–301. doi:10.1016/j.ymeth.2010.01.032. PMID: 20146939.
40. Smith MD, Leemaqz SY, Jankovic-Karasoulos T, McAninch D, McCullough D, Breen J, Roberts CT, Pillman KA. Haemolysis Detection in MicroRNA-Seq from Clinical Plasma Samples. Genes (Basel). 2022 Jul 21;13(7):1288. doi: 10.3390/genes13071288. PMID: 35886071
41. Kirschner MB, Kao SC, Edelman JJ, Armstrong NJ, Vallely MP, van Zandwijk N, Reid G. Haemolysis during sample preparation alters microRNA content of plasma. PLoS One. 2011;6(9): e24145. doi: 10.1371/journal.pone.0024145. PMID: 21909417
42. Chugh P, Dittmer DP. Potential pitfalls in microRNA profiling. Wiley Interdiscip Rev RNA. 2012;3(5):601-16. doi: 10.1002/wrna.1120. 2012 PMID: 22566380
43. Grasedieck, S., Schöler, N., Bommer, M. et al. Impact of serum storage conditions on microRNA stability. Leukemia 26, 2414–2416 (2012). https://doi.org/10.1038/leu.2012.106
44. Keller A, Leidinger P, Bauer A, ElSharawy A, Haas J, Backes C, Wendschlag A, Giese N, Tjaden C, Ott K, et al. Toward the blood-borne miRNome of human diseases. Nat Methods. 2011;8:841–843. doi: 10.1038/nmeth.1682.
45. Mall C, Rocke DM, Durbin-Johnson B, Weiss RH. Stability of miRNA in Human Urine Supports its Biomarker Potential. Biomarkers Med. 2013;7:623–631. doi: 10.2217/bmm.13.44.
46. Dwivedi S, Purohit P, Misra R, Pareek P, Vishnoi JR, Misra S, Sharma P. Methods for Isolation of High Quality and Quantity of miRNA and Single Cell Suspension for Flow-Cytometry from Breast Cancer Tissue: A Comparative Analysis. Indian J Clin Biochem. 2019;34(1):39-44. doi: 10.1007/s12291-017-0719-5. PMID: 30728671.
47. Faraldi, M., Gomarasca, M., Sansoni, V. et al. Normalization strategies differently affect the circulating miRNA profile associated with the training status. Sci Rep 9, 1584 (2019). https://doi.org/10.1038/s41598-019-38505-x
48. Linsen SE, de Wit E, de Bruijn E, Cuppen E. Small RNA expression and strain specificity in the rat. BMC Genomics. 2010;11:249. doi: 10.1186/1471-2164-11-249.
49. Md G, Rm S, A E, Pm G, Aj B, S S, Pl DH, K T, A C, S L, et al. Comprehensive multi-center assessment of small RNA-seq methods for quantitative miRNA profiling. Nature biotechnology. 2018;36. doi: 10.1038/nbt.4183. PMID: 30010675.
50. Sarantopoulou, D., Tang, S.Y., Ricciotti, E. et al. Comparative evaluation of RNA- Seq library preparation methods for strand-specificity and low input. Sci Rep 9, 13477 (2019). https://doi.org/10.1038/s41598-019-49889-1
51. Sarantopoulou D, Tang SY, Ricciotti E, Lahens NF, Lekkas D, Schug J, Guo XS, Paschos GK, FitzGerald GA, Pack AI, et al. Comparative evaluation of RNA-Seq library preparation methods for strand-specificity and low input. Sci Rep. 2019;9:13477. doi: 10.1038/s41598-019-49889-1.

52. Filipów S, Łaczmański Ł. Blood Circulating miRNAs as Cancer Biomarkers for Diagnosis and Surgical Treatment Response. Front Genet. 2019;10:169. doi: 10.3389/fgene.2019.00169. PMID: 30915102.
53. Dwivedi S, Samdariya S, Chikara G, Goel A, Pandey R, Pareek P, et al. Molecular biotechnology for diagnostics. J Pharm Bioallied Sci. 2016;8(4):275-285. doi:10.1201/b19543-16
54. Kim T, Croce CM. MicroRNA: trends in clinical trials of cancer diagnosis and therapy strategies. Exp Mol Med. 2023;55:1314–1321. doi: 10.1038/s12276-023-01050-9.
55. Muñoz JP, Pérez-Moreno P, Pérez Y, Calaf GM. The Role of MicroRNAs in Breast Cancer and the Challenges of Their Clinical Application. Diagnostics. 2023;13:3072. doi: 10.3390/diagnostics13193072.
56. Paranjape T, Slack FJ, Weidhaas JB. MicroRNAs: tools for cancer diagnostics. Gut. 2009;58:1546–1554. doi: 10.1136/gut.2009.179531. PMID: 19834118.
57. Wang H, Peng R, Wang J, Qin Z, Xue L. Circulating microRNAs as potential cancer biomarkers: the advantage and disadvantage. Clin Epigenetics. 2018;10:59. doi: 10.1186/s13148-018-0492-1. PMID: 29713393.
58. Dwivedi S, Shukla KK, Gupta G, Sharma P. Non-invasive biomarker in prostate carcinoma: a novel approach. Indian J Clin Biochem. 2013;28:107–109. doi: 10.1007/s12291-013-0312-5. PMID: 24426194.
59. Tribolet L, Kerr E, Cowled C, Bean AGD, Stewart CR, Dearnley M, Farr RJ. MicroRNA Biomarkers for Infectious Diseases: From Basic Research to Biosensing. Front Microbiol. 2020;11:1197. doi: 10.3389/fmicb.2020.01197. PMID: 32582115.
60. Nagy Á, Lánczky A, Menyhárt O, Győrffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Sci Rep. 2018;8:9227. doi: 10.1038/s41598-018-27521-y.
61. Cardinali B, Tasso R, Piccioli P, Ciferri MC, Quarto R, Del Mastro L. Circulating miRNAs in Breast Cancer Diagnosis and Prognosis. Cancers (Basel). 2022;14:2317. doi: 10.3390/cancers14092317. PMID: 35565446.
62. Oda S, Yokoi T. Recent progress in the use of microRNAs as biomarkers for drug-induced toxicities in contrast to traditional biomarkers: A comparative review. Drug Metabolism and Pharmacokinetics. 2021;37:100372. doi: 10.1016/j.dmpk.2020.11.007.
63. Dwivedi S, Purohit P, Misra R, Lingeswaran M, Vishnoi JR, Pareek P, et al. Application of single-cell omics in breast cancer. In: Barh D, Azevedo V, editors. Single-cell omics. Academic Press; 2019:69-103. doi: 10.1016/B978-0-12-817532-3.00005-0.
64. Condrat CE, Thompson DC, Barbu MG, Bugnar OL, Boboc A, Cretoiu D, Suciu N, Cretoiu SM, Voinea SC. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. Cells. 2020;9:276. doi: 10.3390/cells9020276. PMID: 31979244.
65. Qi Y, Lai Y-L, Shen P-C, Chen F-H, Lin L-J, Wu H-H, Peng P-H, Hsu K-W, Cheng W-C. Identification and validation of a miRNA-based prognostic signature for cervical cancer through an integrated bioinformatics approach. Sci Rep. 2020;10:22270. doi: 10.1038/s41598-020-79337-4.
66. Miroshnichenko S, Patutina O. Enhanced Inhibition of Tumorigenesis Using Combinations of miRNA-Targeted Therapeutics. Front Pharmacol. 2019;10:488. doi: 10.3389/fphar.2019.00488. PMID: 31156429
67. Lima JF, Cerqueira L, Figueiredo C, Oliveira C, Azevedo NF. Anti-miRNA oligonucleotides: A comprehensive guide for design. RNA Biol. 2018;15:338–352. doi: 10.1080/15476286.2018.1445959. PMID: 29570036.
68. Baumann V, Winkler J. miRNA-based therapies: Strategies and delivery platforms for oligonucleotide and non-oligonucleotide agents. Future Med Chem. 2014;6:1967–1984. doi:10.4155/fmc.14.116. PMID: 25495987.
69. Zhang Y, Lu L, Song F, Zou X, Liu Y, Zheng X, Qian J, Gu C, Huang P, Yang Y. Research progress on non-protein-targeted drugs for cancer therapy. Journal of Experimental & Clinical Cancer Research. 2023;42:62. doi: 10.1186/s13046-023-02635-y.
70. Xiong H, Veedu RN, Diermeier SD. Recent Advances in Oligonucleotide Therapeutics in Oncology. Int J Mol Sci. 2021;22:3295. doi:10.3390/ijms22073295. PMID: 33804856.
71. Hong DS, Kang Y-K, Borad M, Sachdev J, Ejadi S, Lim HY, Brenner AJ, Park K, Lee J-L, Kim T-Y, et al. Phase 1 study of MRX34, a liposomal miR-34a mimic, in patients with advanced solid tumours. Br J Cancer. 2020;122:1630–1637. doi:10.1038/s41416-020-0802-1.
72. Di Martino MT, Arbitrio M, Caracciolo D, Cordua A, Cuomo O, Grillone K, Riillo C, Caridà G, Scionti F, Labanca C, et al. miR-221/222 as biomarkers and targets for therapeutic intervention on cancer and other diseases: A systematic review. Molecular Therapy - Nucleic Acids. 2022;27:1191–1224. doi:10.1016/j.omtn.2022.02.005.
73. Huang C-K, Bär C, Thum T. miR-21, Mediator, and Potential Therapeutic Target in the Cardiorenal Syndrome. Front Pharmacol. 2020;11:726. doi:10.3389/fphar.2020.00726. PMID: 32499708.
74. Fan R, Xiao C, Wan X, Cha W, Miao Y, Zhou Y, Qin C, Cui T, Su F, Shan X. Small molecules with big roles in microRNA chemical biology and microRNA-targeted therapeutics. RNA Biol. 2019;16:707–718. doi:10.1080/15476286.2019.1593094. PMID: 30900502.
75. Nguyen LD, Wei Z, Silva MC, Barberán-Soler S, Zhang J, Rabinovsky R, Muratore CR, Stricker JMS, Hortman C, Young-Pearse TL, et al. Small molecule regulators of microRNAs identified by high-throughput screen coupled with high-throughput sequencing. Nat Commun. 2023;14:7575. doi:10.1038/s41467-023-43293-0.

76. Monroig-Bosque P del C, Chen L, Zhang S, Calin GA. Small Molecule Compounds Targeting miRNAs for Cancer Therapy. Adv Drug Deliv Rev. 2015;81:104–116. doi: 10.1016/j.addr.2014.09.002. PMID:25239236.
77. Liu G-H, Chen T, Zhang X, Ma X-L, Shi H-S. Small molecule inhibitors targeting the cancers. MedComm (2020). 2022;3:e181. doi: 10.1002/mco2.181. PMID: 36254250.
78. Cainap C, Balacescu O, Cainap SS, Pop L-A. Next Generation Sequencing Technology in Lung Cancer Diagnosis. Biology (Basel). 2021;10:864. doi: 10.3390/biology10090864. PMID: 34571741.
79. Saliminejad K, Khorram Khorshid HR, Soleymani Fard S, Ghaffari SH. An overview of microRNAs: Biology, functions, therapeutics, and analysis methods. J Cell Physiol. 2019;234:5451–5465. doi: 10.1002/jcp.27486. PMID: 30471116.
80. Huang S, Guo W, Tang Y, Ren D, Zou X, Peng X. miR-143 and miR-145 inhibit stem cell characteristics of PC-3 prostate cancer cells. Oncol Rep. 2012; 28:1831–1837. doi: 10.3892/or.2012.2015. PMID: 22948942.
81. Menon A, Abd-Aziz N, Khalid K, Poh CL, Naidu R. miRNA: A Promising Therapeutic Target in Cancer. Int J Mol Sci. 2022; 23:11502. doi: 10.3390/ijms231911502. PMID: 36232799.
82. Gaj T, Schaffer DV. AAV-mediated delivery of CRISPR/Cas systems for genome engineering in mammalian cells. Cold Spring Harb Protoc. 2016;2016:10.1101/pdb.prot086868. doi: 10.1101/pdb.prot086868. PMID: 27803249.
83. Chakraborty C, Sharma AR, Sharma G, Lee S-S. Therapeutic advances of miRNAs: A preclinical and clinical update. Journal of Advanced Research. 2021;28:127–138. doi: 10.1016/j.jare.2020.08.012.
84. Chen L, Hong W, Ren W, Xu T, Qian Z, He Z. Recent progress in targeted delivery vectors based on biomimetic nanoparticles. Sig Transduct Target Ther. 2021;6:1–25. doi: 10.1038/s41392-021-00631-2.
85. Yamauchi Y, Greber UF. Principles of Virus Uncoating: Cues and the Snooker Ball. Traffic. 2016;17:569–592. doi: 10.1111/tra.12387. PMID: 26875443.
86. Shang R, Lee S, Senavirathne G, Lai EC. microRNAs in action: biogenesis, function and regulation. Nat Rev Genet. 2023;24:816–833. doi: 10.1038/s41576-023-00611-y.
87. Zhang J-H, Du A-L, Wang L, Wang X-Y, Gao J-H, Wang T-Y. Episomal Lentiviral Vector-Mediated miR-145 Overexpression Inhibits Proliferation and Induces Apoptosis of Human Esophageal Carcinomas Cells. Recent Pat Anticancer Drug Discov. 2016;11:453–460. doi: 10.2174/1574892811666160822161157. PMID: 27549139.
88. Khan MB, Ruggieri R, Jamil E, Tran NL, Gonzalez C, Mugridge N, Gao S, MacDiarmid J, Brahmbhatt H, Sarkaria JN, et al. Nanocell-mediated delivery of miR-34a counteracts temozolomide resistance in glioblastoma. Molecular Medicine. 2021;27:28. doi: 10.1186/s10020-021-00293-4.
89. Dasgupta I, Chatterjee A. Recent Advances in miRNA Delivery Systems. Methods Protoc. 2021;4:10. doi: 10.3390/mps4010010. PMID: 33498244.
90. Shah V, Shah J. Recent trends in targeting miRNAs for cancer therapy. Journal of Pharmacy and Pharmacology. 2020;72:1732–1749. doi: 10.1111/jphp.13351.
91. Rupaimoole R, Han H-D, Lopez-Berestein G, Sood AK. MicroRNA therapeutics: principles, expectations, and challenges. Chin J Cancer. 2011;30:368–370. doi: 10.5732/cjc.011.10186. PMID: 21627858.
92. Davey MG, Davies M, Lowery AJ, Miller N, Kerin MJ. The Role of MicroRNA as Clinical Biomarkers for Breast Cancer Surgery and Treatment. Int J Mol Sci. 2021;22:8290. doi: 10.3390/ijms22158290. PMID: 34361056.
93. Hussen BM, Hidayat HJ, Salihi A, Sabir DK, Taheri M, Ghafouri-Fard S. MicroRNA: A signature for cancer progression. Biomedicine & Pharmacotherapy. 2021;138:111528. doi: 10.1016/j.biopha.2021.111528.
94. Foss FM, Querfeld C, Porcu P, Kim YH, Pacheco T, Halwani AS, DeSimone J, William BM, Seto AG, Ruckman J, et al. Phase 1 trial evaluating MRG-106, a synthetic inhibitor of microRNA-155, in patients with cutaneous t-cell lymphoma (CTCL). JCO. 2017;35:7564–7564. doi: 10.1200/JCO.2017.35.15_suppl.7564.
95. Grundy J, Heinlein C, Kersjes K, Liu K, Owen T, Pandya S, Pagarigan R, Liu S. SP257 treatment with the microRNA-21 inhibitor RG-012 given with and without ramipril delays renal impairment progression and prolongs survival when initiated up to chronic kidney disease (CKD) stage 3 in a mouse model of Alport syndrome. Nephrology Dialysis Transplantation. 2016;31:i173. doi: 10.1093/ndt/gfw164.01.
96. Kunden RD, Khan JQ, Ghezelbash S, Wilson JA. The Role of the Liver-Specific microRNA, miRNA-122 in the HCV Replication Cycle. Int J Mol Sci. 2020;21:5677. doi: 10.3390/ijms21165677. PMID: 32784807.
97. Zarone MR, Misso G, Grimaldi A, Zappavigna S, Russo M, Amler E, Di Martino MT, Amodio N, Tagliaferri P, Tassone P, et al. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Sci Rep. 2017;7:17949. doi: 10.1038/s41598-017-18186-0. PMID: 29263373.
98. Witten L, Slack FJ. miR-155 as a novel clinical target for hematological malignancies. Carcinogenesis. 2020;41:2–7. doi: 10.1093/carcin/bgz183.
99. Xiong R, Sun X-X, Wu H-R, Xu G-W, Wang G-X, Sun X-H, Xu M-Q, Xie M-R. Mechanism research of miR-34a regulates Axl in non-small-cell lung cancer with gefitinib-acquired resistance. Thorac Cancer. 2020;11:156–165. doi: 10.1111/1759-7714.13258. PMID: 31777195.
100. Ling H, Fabbri M, Calin GA. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. Nat Rev Drug Discov. 2013;12:847–865. doi: 10.1038/nrd4140. PMID: 24172333.

101. Mangolini A, Ferracin M, Zanzi MV, Saccenti E, Ebnaof SO, Poma VV, Sanz JM, Passaro A, Pedriali M, Frassoldati A, et al. Diagnostic and prognostic microRNAs in the serum of breast cancer patients measured by droplet digital PCR. Biomarker Research. 2015;3:12. doi: 10.1186/s40364-015-0037-0.
102. Ji J, Anwar M, Petretto E, Emanueli C, Srivastava PK. PPMS: A framework to Profile Primary MicroRNAs from Single-cell RNA-sequencing datasets. Briefings in Bioinformatics. 2022;23:bbac419. doi: 10.1093/bib/bbac419.
103. Armstrong DA, Green BB, Seigne JD, Schned AR, Marsit CJ. MicroRNA molecular profiling from matched tumor and bio-fluids in bladder cancer. Molecular Cancer. 2015;14:194. doi: 10.1186/s12943-015-0466-2.
104. Ren A, Dong Y, Tsoi H, Yu J. Detection of miRNA as Non-Invasive Biomarkers of Colorectal Cancer. International Journal of Molecular Sciences. 2015;16:2810–2823. doi: 10.3390/ijms16022810.
105. Bostjancic E, Zidar N, Glavac D. MicroRNA microarray expression profiling in human myocardial infarction. Dis Markers. 2009;27:255–268. doi: 10.3233/DMA-2009-0671. PMID: 20075508.
106. Li J, Kho AT, Chase RP, Pantano L, Farnam L, Amr SS, Tantisira KG. COMPSRA: a Comprehensive Platform for Small RNA-Seq data Analysis. Sci Rep. 2020;10:4552. doi: 10.1038/s41598-020-61495-0.
107. Want A, Staniak K, Grabowska-Pyrzewicz W, Fesiuk A, Barczak A, Gabryelewicz T, Kulczyńska-Przybik A, Mroczko B, Wojda U. Optimized RT-qPCR and a novel normalization method for validating circulating miRNA biomarkers in ageing-related diseases. Sci Rep. 2023;13:20869. doi: 10.1038/s41598-023-47971-3. PMID: 38012217.
108. Kasai A, Kakihara S, Miura H, Okada R, Hayata-Takano A, Hazama K, Niu M, Shintani N, Nakazawa T, Hashimoto H. Double In situ Hybridization for MicroRNAs and mRNAs in Brain Tissues. Front Mol Neurosci. 2016;9:126. doi: 10.3389/fnmol.2016.00126. PMID: 27920667
109. Borel F, Konstantinova P, Jansen PLM. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. Journal of Hepatology. 2012;56:1371–1383. doi: 10.1016/j.jhep.2011.11.026.
110. Kullolli M, Knouf E, Arampatzidou M, Tewari M, Pitteri SJ. Intact MicroRNA Analysis Using High Resolution Mass Spectrometry. J Am Soc Mass Spectrom. 2014;25:80–87. doi: 10.1007/s13361-013-0759-x. PMID: 24174127.
111. Pritchard CC, Cheng HH, Tewari M. MicroRNA profiling: approaches and considerations. Nat Rev Genet. 2012;13:358–369. doi: 10.1038/nrg3198. PMID: 22510765.
112. Shi J, Dong M, Li L, Liu L, Luz-Madrigal A, Tsonis PA, Del Rio-Tsonis K, Liang C. mirPRo–a novel standalone program for differential expression and variation analysis of miRNAs. Sci Rep. 2015;5:14617. doi: 10.1038/srep14617.
113. Riolo G, Cantara S, Marzocchi C, Ricci C. miRNA Targets: From Prediction Tools to Experimental Validation. Methods Protoc. 2020 Dec 24;4(1):1. doi: 10.3390/mps4010001. PMID: 33374478
114. Vlachos IS, Paraskevopoulou MD, Karagkouni D, Georgakilas G, Vergoulis T, Kanellos I, Anastasopoulos I-L, Maniou S, Karathanou K, Kalfakakou D, et al. DIANA-TarBase v7.0: indexing more than half a million experimentally supported miRNA: mRNA interactions. Nucleic Acids Res. 2015;43: D153-159. doi: 10.1093/nar/gku1215. PMID: 25416803.
115. Vlachos IS, Zagganas K, Paraskevopoulou MD, Georgakilas G, Karagkouni D, Vergoulis T, Dalamagas T, Hatzigeorgiou AG. DIANA-miRPath v3.0: deciphering microRNA function with experimental support. Nucleic Acids Res. 2015;43:W460–W466. doi: 10.1093/nar/gkv403. PMID: 25977294.
116. Dobricic V, Schilling M, Schulz J, Zhu L-S, Zhou C-W, Fuß J, Franzenburg S, Zhu L-Q, Parkkinen L, Lill CM, et al. Differential microRNA expression analyses across two brain regions in Alzheimer's disease. Transl Psychiatry. 2022;12:1–9. doi: 10.1038/s41398-022-02108-4.
117. Jung D, Kim B, Freishtat RJ, Giri M, Hoffman E, Seo J. miRTarVis: an interactive visual analysis tool for microRNA-mRNA expression profile data. BMC Proceedings. 2015;9:S2. doi: 10.1186/1753-6561-9-S6-S2.
118. Garcia-Moreno A, Carmona-Saez P. Computational Methods and Software Tools for Functional Analysis of miRNA Data. Biomolecules. 2020;10:1252. doi: 10.3390/biom10091252. PMID: 32872205.
119. Johnson SB, Slade I, Giubilini A, Graham M. Rethinking the ethical principles of genomic medicine services. Eur J Hum Genet. 2020;28:147–154. doi: 10.1038/s41431-019-0507-1.
120. Iacomino G. miRNAs: The Road from Bench to Bedside. Genes (Basel). 2023;14:314. doi: 10.3390/genes14020314. PMID: 36833241.