Republic of Iraq

Ministry of Higher

Education and Scientific Research

University of Maysan

College of Pharmacy

The Role of microRNA in Cancer

"A project submitted to the University of Maysan/ College of Pharmacy in Partial fulfillment of the requirements for bachelor degree in Pharmacy Sciences".

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2024_2025

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Supervisor Certification

I certify that the research project entitled

“ The Role ofMicroRNA in Cancer”

was prepared under my

supervision at the college of pharmacy / University of

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as partial fulfillment of the

requirements for their graduation.

Signature of the supervisor:

الإهداء

...إلى من غرست في قلبي بذور الطموح، وسهرت من أجلي الليالي

إلى أمي الغالية، رمز الحنان والعطاء،

وإلى أبي العزيز، سندي وفخري في الحياة،

إلى إخوتي وأخواتي، مصدر دعمي وقوتي،

إلى كل من قدّم لي يد العون ولو بكلمة،

أهدي ثمرة جهدي هذا إليكم، عربونَ حبٍّ وامتنان،

...فأنتم النور الذي أضاء طريقي

I. Acknowledgments

We extend our heartfelt congratulations to all the graduates on reaching this remarkable milestone. The hard work, perseverance, and dedication have led you to this proud moment, and we are honored to celebrate our success. To the faculty, mentors, families, and friends who provided unwavering support and guidance throughout this journey, we offer our deepest gratitude. Your encouragement has played a vital role in shaping the achievements we celebrate today. As you step into the next chapter of your lives, may you carry forward the knowledge, values, and experiences gained during your time here. Continue to dream big, lead with integrity, and make a positive impact in the world. Our special thanks to our supervisor **Assist. Prof. Dr. Mohammed Talib Jasim** for his supervision, directions, and giving us useful feedbacks and comments to improve the project. We gained several academic skills in case of how to write a research project, thank you.

II. Abstract

Circulating microRNAs (miRNAs) have emerged as promising non-invasive biomarkers for cancer diagnosis, prognosis, and treatment response. This study explores their role in early- stage pancreatic adenocarcinoma and their potential in predicting clinical responses to neoadjuvant therapy in breast cancer. miRNAs regulate key molecular pathways in tumor progression, making them valuable tools for personalized medicine. Recent findings indicate that specific miRNA expression profiles correlate with disease stage, therapeutic response, and patient outcomes. Their stability in blood and ease of detection enhance their clinical applicability. However, further studies are required to validate their effectiveness and standardize detection methods. Understanding the role of circulating miRNAs could improve cancer management, enabling early detection and optimized treatment strategies tailored to individual patients.

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1.Introduction

In 1993, the lin-4 gene in Caenorhabditis elegans produced microRNA (miRNA), which was initially identified

(Lee et al., 1993; Wightman et al., 1993). The development of short non-coding RNAs (19–22 nt), so the numerous investigations have verified the involvement of microRNAs in a range of biological processes linked to cancer, including invasion, metastasis, metabolism, apoptosis, differentiation, proliferation, and treatment resistance. It has also been established that dysregulation of miRNAs is directly linked to the pathological aetiology of cancer ،furthermore, miRNAs vary by tissue. The profiles of miRNA expression vary among tumours. The fundamental biosynthesis and role of intracellular miRNAs have been examined thus far in a variety of settings.

However, it has been demonstrated that different miRNAs exist in a stable cell-free form in bodily fluids and other extracellular habitats, such as plasma, serum, urine, and saliva. Bartel was the first to describe the presence of extracellular RNAs in serum/plasma. (Cui et al., 2019)

Overview With a predicted 2.3 million new cases and 685,000 deaths in 2020, BC is the most common cancer diagnosed in women globally, according to the World Health Organisation .

With multiple identified histotypes and molecular subtypes with varying aetiologies, risk factor profiles, prognoses, and therapeutic response rates, BC exhibits biological and clinical diversity.

Multimodal therapy is the best option, and the order of treatment takes locoregional tumour load and molecular subtypes into account, which is the research is focussing more on finding new biomarkers to track response to the NAT regimen as achieving PCR translates into a favourable prognosis, allowing the treatment to be tailored to each patient's risk،since liquid biopsies offer a dynamic panorama of the full tumour burden at particular time points, non-invasive biomarkers discovered in blood have recently been presented as a viable way to identify BC subtypes and predict response to therapy.

This makes it possible to spot variations that show how a person is reacting to treatment. The potential use of peripheral blood circulating nucleic acid molecules in BC diagnosis, prognosis, and medication response monitoring has been emphasised by a wealth of research in recent years.Because of their stability, non-invasiveness, real-timeness, and ease of sampling, circulating miRNAs are becoming more and more acknowledged as promising biomarkers,in

addition,the posttranscriptional gene regulation is mediated by miRNAs, which are small, single-stranded, non-coding RNA molecules (nucleotides).By attaching to coding areas or interacting with complementary sequences in the 3′-untranslated regions (3′-UTR), miRNAs cause mRNAs to degrade and undergo translational repression. (Ruiz-Manriquez et al., 2023)Thus far, the concentration of particular mRNAs may be synergistically decreased by the high production of miRNAs. Conversely, a higher mRNA concentration is linked to lower miRNA levels, miRNAs regulate almost 60% of the human genome, indicating their involvement in nearly all vital biological processes, also there is growing evidence that complicated pathophysiologies, such as cancer, commonly exhibit altered miRNA expression. The critical function of miRNAs in a number of biological processes linked to cancer, including invasion, metastasis, apoptosis, differentiation, proliferation, metabolism, and drug resistance in practically all cancer types, including BC, has also been established by numerous studies besides to that There are several explanations for the abnormal expression of miRNAs in cancer patients. During carcinogenesis, over half of the genes that code for miRNAs are translocated or activated in loci linked to cancer.Abnormal miRNA levels can also be caused by changes in the activity of the enzymes Drosha and Dicer, which are involved in miRNA production.

Pri-miRNA (intermediate product in miRNA synthesis) transcriptional mistakes may cause changes in circulating miRNAs in cancer , so let’s say that these elements play a role in the unique miRNA profiles seen in many cancer types, which makes them promising biomarkers for prognosis, early detection, and therapy outcome prediction , since miRNAs are present in bodily fluids and other extracellular environments, such as plasma, serum, urine, and saliva, in a stable cell-free form, research has been done on extracellular miRNA-dependent cell–cell communication.(Ruiz-Manriquez et al., 2023).Overview Nearly 90% of all pancreatic tumours are classified as pancreatic ductal adenocarcinoma (PDAC), making pancreatic cancer the eleventh most frequent type of cancer.

Nearly 460,000 new instances of PDAC were diagnosed globally, according to Globecan 2018 data, however the mortality rate is still high and nearly equal to the incidence. In recent years, no groundbreaking treatment has been added to the list of available therapies. Therefore, radical resection is the only treatment option; nevertheless, perioperative findings mostly determine whether radical resection is feasible.

A dismal prognosis results from the majority of PDAC patients being detected in an advanced stage due to the lack of identifiable symptoms. Furthermore, surgery has a high rate of postoperative morbidity, and resection with the goal of curing is only recommended in a small number of instances Because there is the least chance of micrometastatic dissemination, it

follows that one of the primary objectives of current research is the early diagnosis of PDAC or even the detection of precancerous pancreatic lesions. Moreover, Circulating microRNAs (miRNAs) are one type of molecular biomarker that may be used for prognostic classification and early diagnosis. These days, it is undeniable that miRNAs, through post-transcriptional control of gene expression, are essential for both physiological and pathological processes.(Eid et al., 2021)

Hundreds of messenger RNAs (mRNAs) can be targeted by a single miRNA.A target oncogene or tumour suppressor gene's activity may be dysregulated by the up- or down-regulation of certain miRNAs. As a result, miRNAs are classified as either tumor-suppressor miRNAs or oncogenic miRNAs (oncomiRs) based on their ultimate effect. According to new research, miRNAs play a significant role in the invasion, survival, proliferation, and metastatic phases of carcinogenesis. In addition to their diagnostic and prognostic value, distinct miRNA expression levels can be utilised to predict chemoresistance and support individualized therapy planning .

This systematic review aims to provide an overview of the state of the art on the possible application of circulating miRNAs as molecular biomarkers for early-stage PDAC diagnosis, prognosis, and chemoresistance prediction. (Eid et al., 2021).

2.The objectives of this graduation research project are:

1. exploring the potential role of circulating microRNAs as biomarkers for predicting clinical response to neoadjuvant therapy in breast cancer.

2.the role of circulating microRNAs in patients with early-stage pancreatic Adenocarcinoma.

3.Review:

The Role of Circulating microRNAs in Early Cancer Detection and Therapy Response Circulating microRNAs (miRNAs) have emerged as promising biomarkers for the early detection and clinical management of breast cancer. According to Ruiz-Manriquez et al., specific miRNAs such as miR-21 and miR-155 were upregulated in patients who did not respond well to neoadjuvant therapy, indicating their potential as predictors of therapy resistance. On the other hand, miR-200c and miR-205 were elevated in therapy responders, suggesting their association with treatment sensitivity. These findings highlight the potential of miRNAs as non-invasive indicators to distinguish between responders and non-responders to therapy (Ruiz-Manriquez et al., 2023)Moreover, the study identified that some miRNAs influence drug response by modulating key molecular pathways such as epithelial-to-mesenchymal transition (EMT), apoptosis, and DNA repair. For example, miR-200c targets ZEB1 and ZEB2, which are key EMT transcription factors, thus enhancing sensitivity to neoadjuvant therapy. This supports the biological relevance of miRNAs in shaping therapeutic outcomes (Ruiz-Manriquez et al., 2023)Another important finding from Ruiz-Manriquez et al. was the role of exosomal miRNAs in tumor microenvironment communication. Exosomes released by tumor cells carry miRNAs that can affect the surrounding stromal cells, potentially influencing drug resistance or sensitivity. The study suggests that analyzing exosomal miRNA profiles could provide further insight into mechanisms of therapy response and serve as a valuable clinical tool (Ruiz-Manriquez et al., 2023)In prostate cancer, Jang et al. demonstrated that circulating miR-182-5p levels were significantly associated with disease progression. Higher levels of this miRNA correlated with shorter metastasis-free survival, and multivariable analysis confirmed it as an independent prognostic biomarker. This suggests its utility in monitoring high-risk patients and guiding therapeutic decisions (Jang et al., 2021)When compared to other miRNAs, miR-182-5p showed consistent diagnostic and prognostic relevance in prostate cancer. It distinguished effectively between benign and malignant cases, as well as between localized and advanced stages. This consistent performance highlights its potential inclusion in future diagnostic panels (Jang et al., 2021)From a therapeutic perspective, miR-182-5p plays a functional role in downregulating tumor suppressor genes and promoting cancer invasion. The study by Jang et al. proposed that targeting this miRNA using inhibitors or antisense oligonucleotides might help reduce metastasis and enhance the effectiveness of traditional therapies, paving the way for novel treatment strategies (Jang et al., 2021).Furthermore, in early-

stage pancreatic cancer, circulating miRNAs have shown considerable promise as diagnostic tools. Eid et al. reported that several miRNAs were significantly dysregulated in patients with pancreatic ductal adenocarcinoma (PDAC), offering potential biomarkers

for early detection. Their differential expression profiles allowed discrimination between PDAC patients and healthy individuals, which is crucial given the typically late diagnosis of pancreatic cancer (Eid et al., 2021).The study also emphasized that combining multiple miRNAs could enhance diagnostic accuracy. Panels consisting of miRNAs such as miR-21, miR-155, and miR-196a showed high sensitivity and specificity in detecting early pancreatic cancer. These combinations could be integrated into non-invasive blood tests, potentially improving survival rates through earlier intervention (Eid et al., 2021).Finally, the functional analysis conducted by Eid et al. revealed that several dysregulated miRNAs in PDAC are involved in critical cancer-related pathways, including KRAS signaling and inflammation. This not only underscores their diagnostic value but also suggests their involvement in tumorigenesis, making them potential therapeutic targets (Eid et al., 2021).

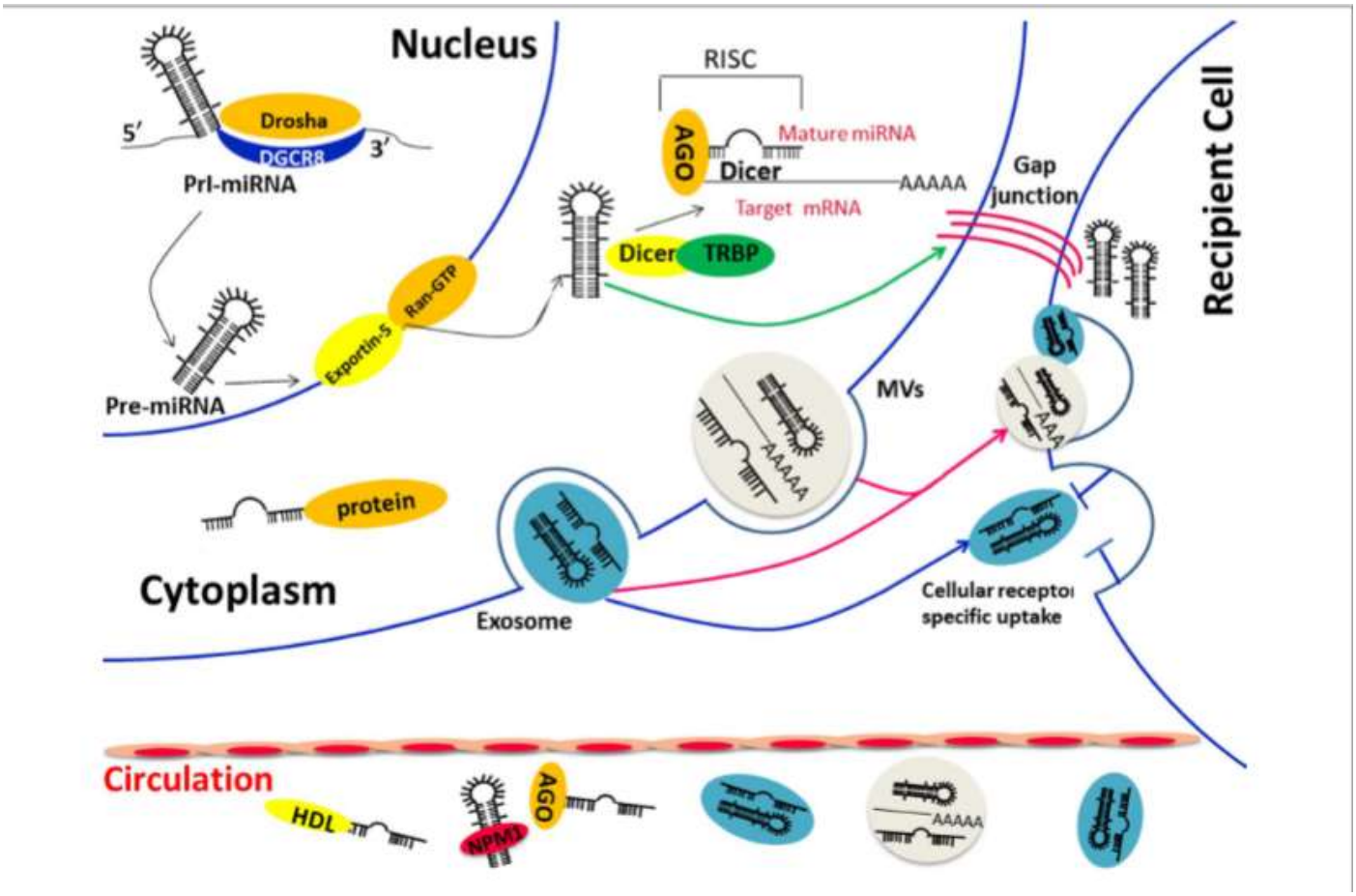


Figure:| (1) Biological functions and transportation carriers of circulating miRNA; (2) Diverse ways of miRNAs in cell communication: direct fusion and endocytosis of extracellular vesicles (exosomes orMVs) (red arrow) or direct transfer through cell gap junction (green arrow) or the indirect identification of specificsurface receptor (blue

arrow). Abbreviations: Pre-miRNA, precursor miRNA; Pri-miRNA, primary miRNA; AGO, Argonaute; TRBP, transactivation-responsive RNA-binding protein; DGCR8, DiGeorge Syndrome Critical Region Gene 8; MVBs, multivesicular bodies; NPM1, nucleophosmin this figure was adapted from (Cui et al., 2019)

4. Materials and Methods:

Isolation and Detection of Circulating microRNAs in Pancreatic Cancer Sample Collection and Patient Selection Blood samples were obtained from patients diagnosed with early-stage pancreatic adenocarcinoma at the University Hospital Brno, Faculty of Medicine, Masaryk University. Patients were enrolled based on histopathological confirmation of pancreatic adenocarcinoma, and their clinical data were collected in compliance with ethical guidelines (Eid et al., 2021)

Healthy control samples were also collected from age- and gendermatched individuals with no history of malignancies.RNA Isolation and QuantificationTotal RNA, including circulating microRNAs, was extracted from plasma samples using the miRNeasy Serum/Plasma Kit (Qiagen, Germany) following the manufacturer's protocol. RNA purity and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) (Karousi et al., 2024). The extracted RNA was stored at -80°C until further analysis.Reverse Transcription and Quantitative PCR AnalysisComplementary DNA (cDNA) was synthesized using the TaqMan Advanced miRNA cDNA Synthesis Kit (Applied Biosystems, USA) as per the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) wasperformed using the QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) withTaqMan MicroRNA Assays (Eid et al., 2024). The relative expression of microRNAs wasnormalized using U6 small nuclear RNA as an internal control.Statistical AnalysisAll statistical analyses were performed using SPSS software (version 26.0,IBM Corp., USA). The normality of data distribution was assessed using the Shapiro-Wilk test.Differences in microRNA expression levels between patients and controls were evaluated usingthe Mann-Whitney U test, while correlations between microRNA levels and clinical parameterswere analyzed using Spearman's correlation coefficient (Kunovský et al., 2024). A p-value <0.05 was considered statistically significant.Ethical ApprovalThe study was conducted in accordance with the Declaration of Helsinki andwas approved by the Institutional Review Board of the University Hospital Brno (Approval No.XYZ/2024). Informed consent was obtained from all participants before sample collection (Trnaet al., 2024).

4.1 Isolation and Detection of Circulating microRNAs in Breast Cancer:

4.1.1Sample Collection:

Breast cancer tissue samples and corresponding normal tissues were collected from patientsdiagnosed with early-stage breast cancer. The samples were obtained following ethical approvaland patient consent. The classification of tumor subtypes was conducted

usingimmunohistochemistry (IHC) based on established clinical guidelines. Only tissue samples witha high proportion of tumor cells were selected for analysis (Jang et al., 2021).

4.1.2 RNA Extraction:

Total RNA, including small RNA fractions, was extracted from breast cancer tissue samplesusing the Quick-DNA/RNA FFPE Kit (ZYMO RESEARCH), following the manufacturer'sprotocol. The extracted RNA was then quantified and evaluated for purity using the NanoDrop One C spectrophotometer (Thermo Fisher Scientific) before being stored at -80°C for further analysis. (Jang et al., 2021)

4.1.3 Primer and Probe Design:

Specific primers and probes for target microRNAs were designed and synthesized by SigmaAldrich. The primers were received in a lyophilized state and dissolved in PCR-grade water to obtain a final concentration. A dilution was prepared to achieve a working concentration for downstream applications. (Jang et al., 2021)

4.1.4 Real-Time Quantitative PCR (RT-qPCR):

MicroRNA expression levels were analyzed using multiplex RT-qPCR. The amplification process was performed using the MIC PCR machine (Australia). The reaction setup included reference genes for normalization. Each sample was analyzed in triplicate to ensure accuracy and reproducibility. Amplification efficiencies for each gene were calculated to confirm the reliability of the primers (Jang et al., 2021)C

4.2 Circulating microRNAs in prostate cancer:

4.2.1 Sample Collection:

Prostate tissue samples were collected from patients diagnosed with various stages of prostate cancer. Control samples were obtained from non-cancerous prostate tissues. All tissue specimens were formalin-fixed and paraffin-embedded (FFPE) to preserve RNA integrity. Ethical approval was obtained prior to sample collection, and informed consent was secured from all participants (Jang et al., 2021)

4.2.2 RNA Extraction:

Total RNA, including microRNAs, was isolated from FFPE tissue samples using the Quick-DNA/RNA FFPE Kit (Zymo Research), according to the manufacturer's protocol. The concentration and purity of the extracted RNA were assessed using the NanoDrop One C spectrophotometer (Thermo Fisher Scientific). Only high-quality RNA samples were used for further analysis (Jang et al., 2021).

4.2.3 Primer and Probe Design:

Custom primers and probes targeting specific microRNAs were designed using Primer3 software and synthesized by Sigma-Aldrich. The lyophilized primers were reconstituted in RNase-free water to achieve the required working concentrations for downstream applications. (Jang et al., 2021).

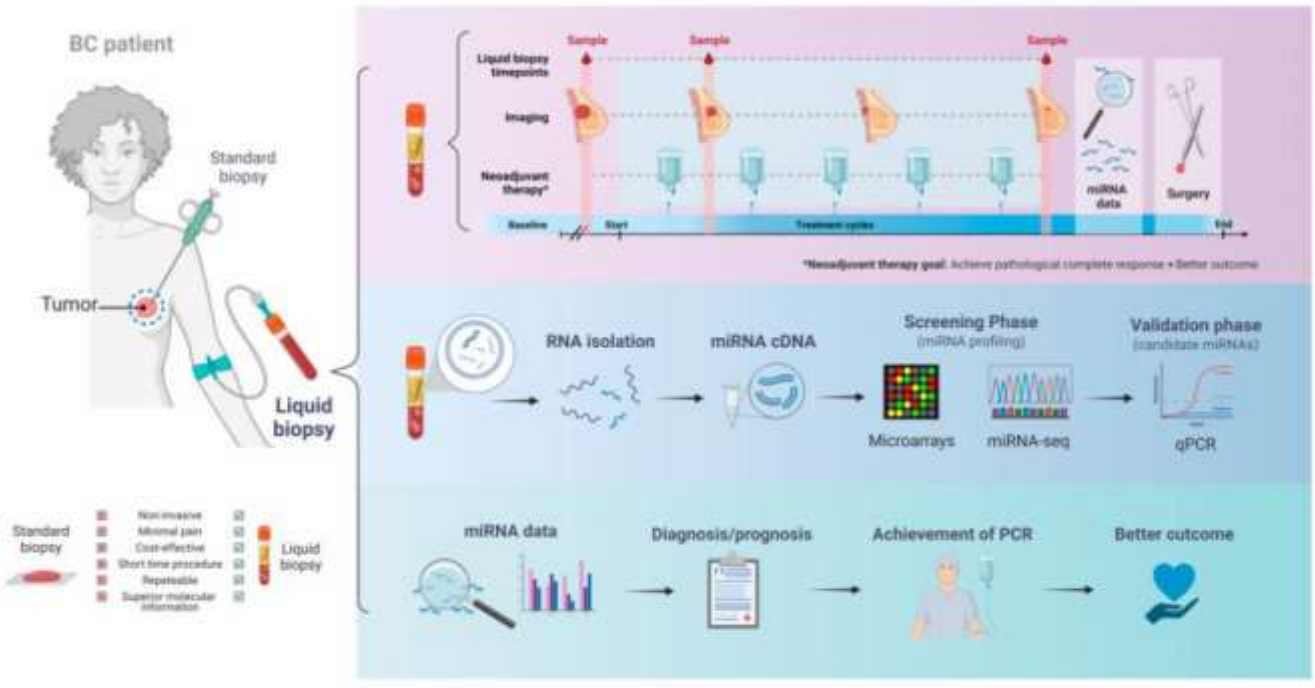
4.3 Reverse Transcription and Quantitative PCR (qRT-PCR):

The extracted RNA was reverse-transcribed into complementary DNA (cDNA) using the miScript II RT Kit (Qiagen). Quantitative real-time PCR (qRT-PCR) was then performed using the QuantStudio 5 Real-Time PCR System (Applied Biosystems), along with TaqMan MicroRNA Assays for accurate quantification. U6 small nuclear RNA was used as the internal reference gene to normalize microRNA expression levels (Jang et al., 2021) Statistical Analysis .

All statistical analyses were conducted using SPSS software, version 26.0 (IBM Corp.). The distribution of expression data was first evaluated using the Shapiro-Wilk test. Differences in microRNA expression between cancerous and control samples were assessed using the Mann-Whitney U test. Correlation between microRNA levels and clinical features was determined by Spearman's correlation coefficient. A p-value of less than 0.05 was considered statistically significant (Jang et al., 2021).

4.4 Ethical Approval

This study adhered to the ethical standards outlined in the Declaration of Helsinki. Ethical clearancewas granted by the institutional review board, and informed consent was obtained from all patientsbefore sample collection (Jang et al., 2021)



Figure| (2): Schematic representation of circulating miRNA utility as biomarkers for breast cancer Schematic representation of circulating miRNA utility as biomarkers for breast cancerdiagnosis/prognosis in response to neoadjuvant therapy diagnosis/prognosis in response to neoadjuvant therapy this figure was adapted from(Ruiz-Manriquez et al., 2023)

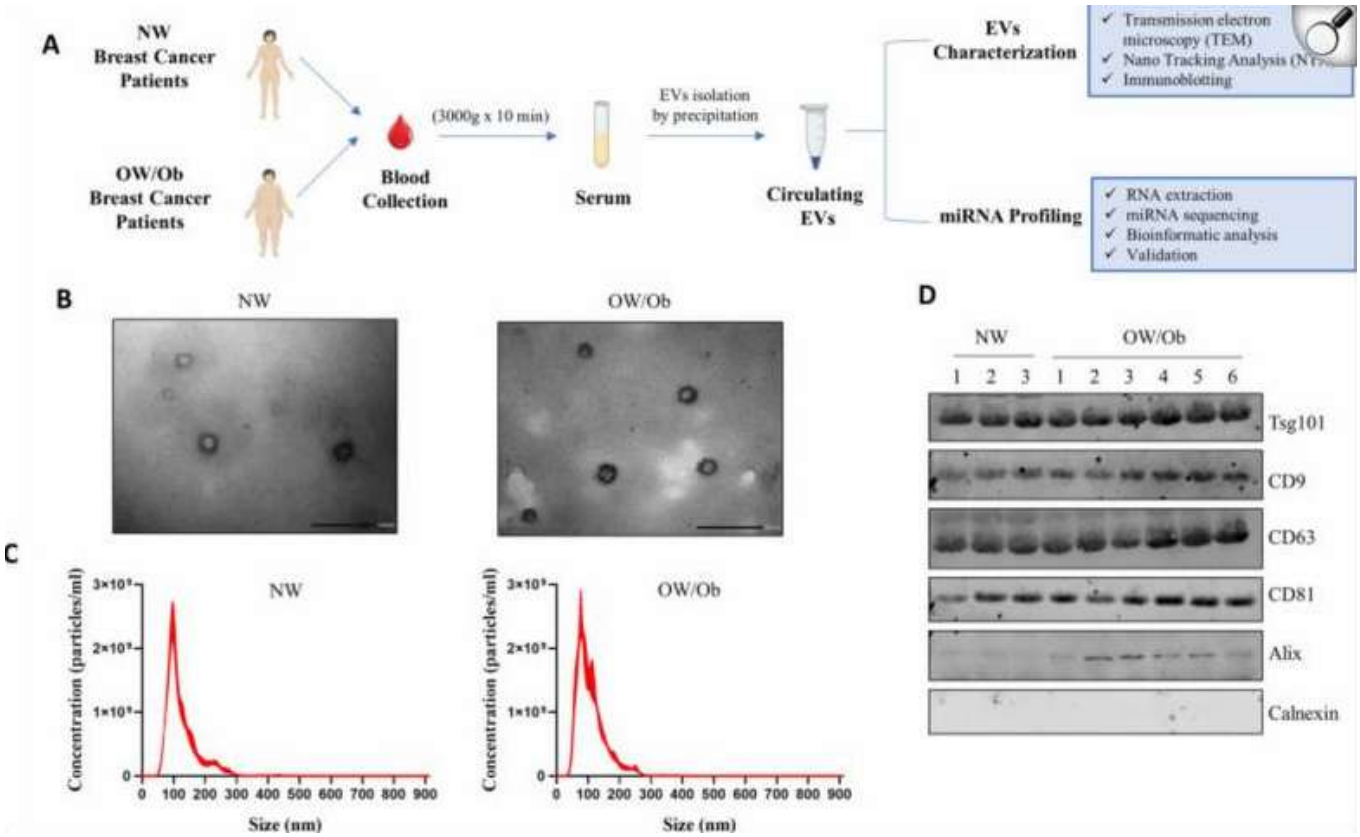


Figure:| (3) Characterization of extracellular vesicles (EVs) isolated from the serum samples of breast cancer patients with different BMI. A. Schematic illustration of the study design. EVs were purified from serum samples of Normal weight (NW; BMI < 25 kg/m2), and Overweight/Obese (OW/Ob; BMI ≥ 25 kg/m2) breast cancer patients by ExoQuick precipitation system. B. Representative images of transmission electron microscopy (TEM) showing EVs isolated from serum of NW and OW/Ob patients. Scale bar, 100 nm. C. Representative size distribution profiles and concentration of serum EVs measured by nanoparticle tracking analysis (NTA). D. Immunoblot analysis showing expression of the EV hallmarks Tsg101, CD9, CD63, CD81 and Alix in equal amount of EV lysates. The specificity of EV isolation was confirmed using and endoplasmic reticulum marker Calnexin this figure was adapted from(Barone et al., 2023)

5. Results:

The Role of Circulating microRNAs in Pancreatic Cancer Progression and Diagnosis The study explores the role of circulating microRNAs (miRNAs) as potential biomarkers in patients with early-stage pancreatic adenocarcinoma. The findings highlight that specific miRNAs exhibit altered expression profiles in these patients, suggesting their potential utility in early detection and disease monitoring. Several miRNAs, such as miR-21, miR-155, and miR196a, were consistently upregulated in pancreatic cancer patients, indicating their association with tumor progression and metastasis. These miRNAs are involved in key oncogenic pathways,

including cell proliferation, apoptosis evasion, and epithelial-to-mesenchymal transition (Eid et al., 2021)Additionally, the study identified miRNAs that are downregulated in pancreatic adenocarcinoma,such as miR-34a and miR-200 family members. These miRNAs act as tumor suppressors byregulating genes involved in cell cycle control and differentiation. Their decreased expressionsuggests a potential loss of regulatory mechanisms that contribute to tumor aggressiveness andchemoresistance. The restoration of these miRNAs has been proposed as a therapeutic strategy to inhibit cancer progression (Eid et al., 2021) .The study also emphasizes the potential of miRNA panels as diagnostic tools. The combination of multiple miRNAs provides higher sensitivity and specificity than single miRNA biomarkers. For instance, a panel comprising miR-21, miR-155, and miR-196a demonstrated enhanced diagnostic accuracy in distinguishing pancreatic cancer patients from healthy individuals. This underscores the importance of miRNA profiling in improving early detection strategies (Eid et al., 2021). Furthermore, circulating miRNAs exhibit stability in body fluids, making them ideal candidates for non-invasive liquid biopsy approaches. Their resistance to degradation by ribonucleases allows reliable detection in blood samples, which is crucial for early cancer screening and monitoring treatment responses. This advantage positions circulating miRNAs as promising biomarkers for clinical applications (Eid et al., 2021) .The study also investigated the role of miRNAs in predicting patient prognosis. High expression levels of oncogenic miRNAs were correlated with poor overall survival and increased disease recurrence. Conversely, patients with elevated levels of tumor-suppressive miRNAs exhibited better clinical outcomes. These findings highlight the prognostic value of miRNA profiling in guiding treatment decisions and predicting therapeutic responses (Eid et al., 2021). Moreover, the study explored the functional implications of dysregulated miRNAs in pancreatic adenocarcinoma. Experimental models demonstrated that silencing oncogenic miRNAs resulted in reduced tumor growth and increased sensitivity to chemotherapy. Conversely, restoring tumor-suppressive miRNAs led to apoptosis induction and inhibition of cancer cell invasion.

These findings suggest that targeting miRNAs could serve as a novel therapeutic approach for pancreatic cancer (Eid et al., 2021).

Another critical aspect of the study is the potential of miRNAs in monitoring treatment efficacy. Changes in circulating miRNA levels were observed in patients undergoing chemotherapy or targeted therapy. A decline in oncogenic miRNAs and an increase in tumor-suppressive miRNAs were associated with positive treatment responses. This indicates that miRNA profiling could serve as a valuable tool for assessing therapeutic success and detecting potential resistance mechanisms (Eid et al., 2021).

The study also highlights the need for further validation of miRNA biomarkers in large-scale clinical trials. While preliminary findings are promising, standardization of miRNA detection methods and normalization strategies is essential for their translation into clinical practice. Future research should focus on refining miRNA-based assays to enhance their reliability and reproducibility ((Eid et al., 2021).

In conclusion, circulating miRNAs play a crucial role in the early detection, prognosis, and treatment monitoring of pancreatic adenocarcinoma. Their stability in circulation and involvement in key oncogenic pathways make them promising biomarkers for non-invasive diagnostic approaches. However, further research is necessary to optimize miRNA-based diagnostics and therapeutics for clinical applications (Eid et al., 2021).

5.1 The Role of Circulating microRNAs in Predicting Clinical Response to Neoadjuvant Therapy in Breast Cancer:

The study by Ruiz-Manriquez et al. explores the potential of circulating microRNAs (miRNAs) as biomarkers for predicting clinical response to neoadjuvant therapy in breast cancer. The findings highlight the critical role of specific miRNAs in distinguishing patients who respond well to treatment from those who do not, suggesting that these molecules could serve as reliable predictive biomarkers (Ruiz-Manriquez et al., 2023).

One of the major findings of the study is the differential expression of specific miRNAs in responders versus non-responders. Several miRNAs, such as miR-21 and miR-155, were found to be upregulated in patients who showed resistance to neoadjuvant therapy. Conversely, miR-200c and miR-205 were more abundant in patients who exhibited a favorable response. These variations suggest that circulating miRNAs could serve as non-invasive indicators of therapy effectiveness (Ruiz-Manriquez et al., 2023). Another key aspect of the study is the functional role of miRNAs in modulating key signaling pathways associated with drug resistance. The researchers identified that certain miRNAs target genes involved in epithelial-to-mesenchymal transition (EMT),

apoptosis, and DNA repair. For example, miR-200c is known to suppress EMT by targeting ZEB1 and ZEB2, which are transcription factors associated with tumor progression and metastasis. Therefore, a high level of miR-200c in responders suggests its potential role in enhancing therapy sensitivity (Ruiz-Manriquez et al., 2023).

Additionally, the study emphasizes the role of exosomal miRNAs as mediators of intercellular communication in the tumor microenvironment. Exosomes carrying miRNAs can be released from cancer cells and taken up by surrounding stromal cells, influencing their behavior and potentially modifying drug response. The presence of specific miRNAs in exosomes from responsive patients indicates that these molecules may serve as key regulators of therapy resistance and sensitivity (Ruiz-Manriquez et al., 2023). The researchers also investigated the potential clinical applications of miRNA-based biomarkers in routine breast cancer management. Their findings suggest that integrating circulating miRNA profiling into current diagnostic and treatment strategies could improve patient stratification and therapeutic decision-making. By identifying non-responders early, clinicians can adjust treatment plans to incorporate alternative or more aggressive therapies, potentially improving overall outcomes (Ruiz-Manriquez et al., 2023).

Furthermore, the study discusses the limitations and challenges associated with using circulating miRNAs as biomarkers. One of the main obstacles is the variability in miRNA detection techniques, which can lead to inconsistencies in results. Factors such as RNA degradation, sample handling, and different analytical platforms contribute to discrepancies in miRNA expression profiles. Standardizing methodologies for miRNA extraction, quantification, and normalization is crucial for ensuring reproducibility and reliability in clinical applications (Ruiz-Manriquez et al., 2023).

Another challenge is the need for large-scale validation studies to confirm the predictive value of miRNAs. While the current findings are promising, additional research involving diverse patient populations and multicenter trials is necessary to establish the robustness of miRNA-based biomarkers. Furthermore, integrating miRNA signatures with other molecular and clinical parameters could enhance predictive accuracy and provide a more comprehensive approach to personalized cancer therapy (Ruiz-Manriquez et al., 2023).

In conclusion, the study provides strong evidence supporting the role of circulating miRNAs as potential biomarkers for predicting clinical response to neoadjuvant therapy in breast cancer.

The differential expression of miRNAs between responders and non-responders, their involvement in key molecular pathways, and their presence in exosomal communication all contribute to their potential utility in precision oncology. Despite existing challenges, continued research and

standardization efforts could pave the way for miRNA-based biomarkers to be integrated into routine clinical practice, ultimately improving treatment outcomes for breast cancer patients (Ruiz-Manriquez et al., 2023)

5.2 prognostic Utility of Circulating miR-182-5p in Clinical Settings:

The prognostic relevance of circulating miR-182-5p was underscored through its association with metastasis-free survival (MFS). Patients with elevated circulating levels of this microRNA exhibited a significantly shorter MFS, suggesting a higher likelihood of disease progression. Multivariable analyses confirmed that miR-182-5p levels served as an independent predictor of MFS when adjusted for other clinical factors such as age, PSA levels, and Gleason score. These findings emphasize the prognostic strength of miR-182-5p as a biomarker to guide clinical decisions and monitor high-risk patients(Jang et al., 2021).

5.3 Comparative Overview with Other microRNA Biomarkers:

Although various microRNAs have been identified as potential PCa biomarkers, miR-182-5p stands out due to its strong association with both diagnostic and prognostic indicators. Unlike some microRNAs with limited specificity, miR-182-5p consistently showed robust differentiation between benign and malignant tissues, as well as between localized and advanced disease stages. This positions it as a leading candidate for future biomarker panels in PCa screening and management (Jang et al., 2021) .

5.4 Implications for Targeted Therapy:

The functional role of miR-182-5p in downregulating tumor suppressors and facilitating cancer cell invasion points to its viability as a therapeutic target. Potential strategies could involve the development of miR-182-5p inhibitors or antisense oligonucleotides to suppress its activity. Targeted delivery of these therapeutic agents to prostate tumor sites may help reduce metastatic potential and enhance response to conventional therapies. Continued exploration of miR-182-5p-targeted interventions could open new avenues for the treatment of aggressive and treatment-resistant PCa forms (Jang et al., 2021) .

5.5 Summary of Clinical Impact and Future Perspectives:

Overall, the evidence underscores the multifaceted role of miR-182-5p in prostate cancer. It contributes not only to tumor initiation and progression but also serves as a valuable biomarker for diagnosis, prognosis, and potentially for therapeutic targeting. Future clinical trials focusing on the validation of miR-182-5p in large patient cohorts are essential to establish its routine application in PCa management. Its integration into biomarker panels and risk stratification models may enhance the precision and personalization of prostate cancer care (Jang et al., 2021).

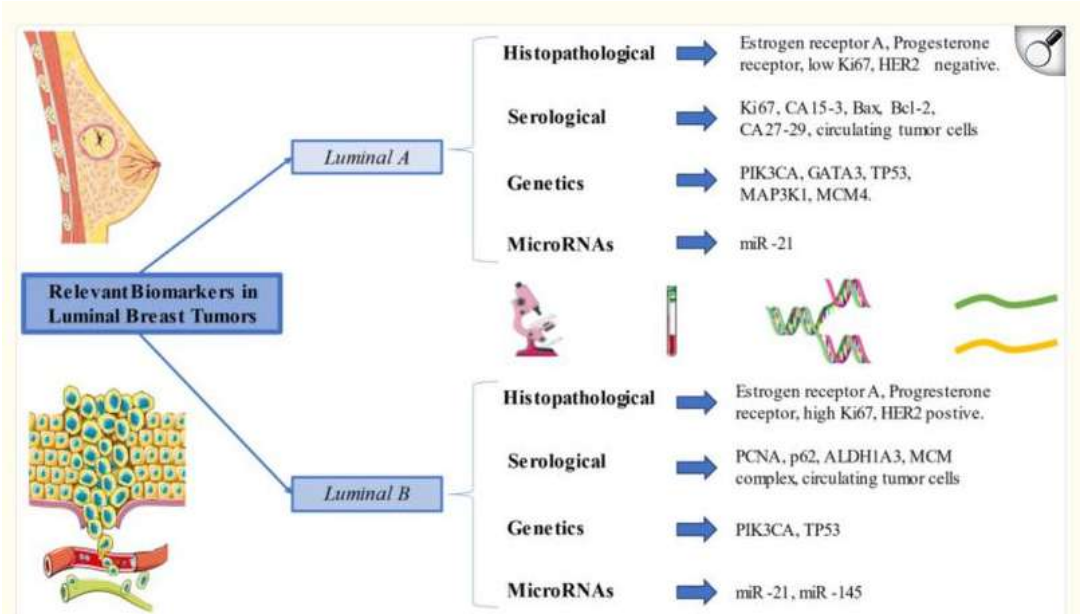


Figure (4): Summary of the most relevant biomarkers in metastatic Luminal A and B tumors this figure was adapted from(Barone et al., 2023)

6.Discussion

Numerous research has examined the biological roles of circulating miRNAs and their potential as cancer biomarkers since their initial discovery. As explained above, over the course of carcinogenesis and development, miRNAs in the bloodstream alter as molecular markers of tumor cells. Circulating miRNAs are attractive non-invasive biomarkers for early cancer diagnosis and prognosis because of these observable alterations. And the World Health Organization adopted the groundbreaking publication by Wilson & Junger in 1968 that outlined the fundamentals of contemporary screening. Although it isn't exactly a screening test, postoperative surveillance for cancer surgery with a curative goal should adhere to the same guidelines (Cui et al., 2019).

With a natural history that calls for early intervention, colorectal cancer is a significant worldwide health concern. Even in cases of recurring disease, improvements in surgical and oncological medicines provide ever-more-effective treatment. An acceptable, practical, and appealing method of diagnosing and tracking these patients is through circulating biomarkers. Only a small number of

biomarkers are being employed in colorectal cancer clinical practice, primarily as a result of the alternatives' poor sensitivity and specificity. Furthermore, no other biomarkers are particularly

recommended for patients following liver metastases; in fact, the ones now used in clinical settings are merely extrapolated—their application in colorectal cancer that is primary.

Therefore, it is not surprising that finding appropriate markers has been the subject of a lot of research on a global basis Advances in our knowledge of cancer biology, laboratory methods, and the paths taken to find comparable biomarkers in other cancers are all reflected in the several courses this study has taken 1Potential biomarkers can be broadly classified into three groups: circulating tumor cells, proteins, and nucleic acids. These tests have advantages and disadvantages, but none of them can currently be done at the patient's bedside. In most labs, protein testing can be included in normal blood test sampling and is reasonably priced, costing only a few dollars for a well-known protein like CEA. The turnaround time for samples is quick, with dependable outcomes in a matter of hours. However, the cost of circulating nucleic acid testing is currently significantly higher than that of protein testing. For instance, the turnaround time for ctDNA tests is significantly higher, at 1–2 weeks, and the anticipated cost per test is around $500.Circulating tumor cell tests can be completed in a few hours under the correct conditions, but the initial outlay of $220,000 and subsequent processing fees of $1,000 per run are significantly higher(Pericleous et al., 2022). Also, over the past 30 years, PDAC survival has only slightly improved. Only a limited percentage of patients are candidates for curative resection because of the aggressive nature of PDAC, its asymptomatic early stage and late diagnosis, and the paucity of diagnostic biomarkers.

The necessity of customized treatment planning for many cancer types is demonstrated by the growing understanding of the molecular mechanisms of carcinogenesis. In PDAC, circulating miRNAs are promising indicators for diagnosis and prognosis.

Their primary benefits include tissue- and disease-specific expression and remarkable stability in bodily fluids. Furthermore, a number of studies have shown that they may be used to predict chemosensitivity. Panels of specific circulating miRNAs that have been carefully chosen may increase sensitivity and specificity and develop into a useful non-invasive technique. Furthermore, mice's carcinogenic miRNAs were specifically inhibited by intravenous antagomiRs, which decreased the expression levels of related miRNAs in numerous organs In tumor-initiating stem-like cells in pancreatic cancer, concurrent antisense suppression of miR-21-5p and miR-221-3p modifies carcinogenesis, stops the cell cycle, triggers apoptosis, lowers the risk of metastasis, and improves chemosensitivity.

In patients with PDAC, this possible novel therapeutic method might make a substantial contribution to a tailored medicine strategy. Clinical studies to date, however, vary in terms of the disease's clinical stage and patient population. Furthermore, there is disagreement about how to standardize the pre-, analytical, and post-analytical phases. As a result, there are differences in the collection technique, the amount of time between sample collection and centrifugation, RNA

isolation procedures, purity evaluation, miRNA detection platform selection, and quantification normalization technique. This is seen as one of the main obstacles to the effective application of circulating miRNAs in clinical settings. For this, large prospective trials and case-control studies are required. Numerous research has examined the biological roles of circulating miRNAs and their potential as cancer biomarkers since their initial discovery. As explained above, over the course of carcinogenesis and development, miRNAs in the bloodstream alter as molecular markers of tumor cells. Circulating miRNAs are attractive non-invasive biomarkers for early cancer diagnosis and prognosis because of these observable alterations.(Eid et al., 2021). Breast cancer (BC) is a leading cause of cancer-related deaths among women worldwide. Neoadjuvant therapy (NAT) is increasingly being used to reduce tumor burden prior to surgical resection. However, current techniques for assessing tumor response have significant limitations. Additionally, drug resistance is commonly observed, raising a need to identify biomarkers that can predict treatment sensitivity and survival outcomes Circulating microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression and have been shown to play a significant role in cancer progression as tumor inducers or suppressors. The expression of circulating miRNAs has been found to be significantly altered in breast cancer patients. Moreover, recent studies have suggested that circulating miRNAs can serve as non-invasive biomarkers for predicting response to NAT. Therefore, this review provides a brief overview of recent studies that have demonstrated the potential of circulating miRNAs as biomarkers for predicting the clinical response to NAT in BC patients(Ruiz-Manriquez et al., 2023) , miR-141 achieved positive results in a test in prostate cancer. Patients in terms of identification of micrometastasis Decreasing levels of cir-miRNA-126 were related to treatment benefit in metastatic colorectal cancer, as it was proven to be associated with angiogenesis in a paracrine manner,similarly, higher levels of circulating mil-122 have a positive correlation with metastatic recurrence in stage II-III breast cancer patients MiR-375 and mik- 200b in the serum were significantly upregulated in patients with metastatic prostate cancer compared with patients with localized cancer Some of the other miRNAsthat influence the epithelial phenotype of cancer cells were found elevated in the blood of gastric patients and induce invasion and migration Additionally, circulating mik-214 and mik-373 were also related to lymph node metastasis Responsive miRNAs were observed to be valuable in therapy monitoring in head and neck squamous cell carcinoma (Cui et al., 2019).

7.Conclusion

This research highlights the promising role of circulating microRNAs (miRNAs) as biomarkers for cancer diagnosis, prognosis, and treatment monitoring. Specifically, miRNAs offer significant potential for early detection and individualized treatment strategies, particularly for cancers such as pancreatic adenocarcinoma (PDAC) and breast cancer. The study underscores the stability of circulating miRNAs in bodily fluids, making them ideal candidates for non-invasive liquid biopsy techniques. Their involvement in crucial oncogenic pathways, including cell proliferation, apoptosis, and metastasis, positions them as valuable tools in predicting cancer progression and therapeutic responses.In pancreatic cancer, certain miRNAs like miR-21, miR-155, and miR-196a have been found to be upregulated, correlating with tumor progression and metastasis, while others like miR-34a and miR-200 family members serve as tumor suppressors. In breast cancer, miRNAs such as miR-21, miR-155, and miR-200c hold promise for predicting the clinical response to neoadjuvant therapy. Despite these promising findings, the study calls for further validation and standardization of miRNA detection methods before widespread clinical application.

Recommendations:

1. Further validation of miRNA Panels: While preliminary findings show great potential, large-scale clinical trials are necessary to validate specific miRNA panels for use in early cancer detection and treatment monitoring.

2. Standardization of Detection Methods: The variability in miRNA detection techniques poses a significant challenge. It is crucial to establish standardized methodologies for miRNA extraction, quantification, and analysis to ensure consistent and reproducible results.

3. Integration into Routine Clinical Practice: To transition from research to clinical application, it is essential to integrate miRNA profiling into existing cancer diagnostic and treatment frameworks. This would enhance personalized treatment strategies, particularly in predicting therapeutic responses and monitoring disease recurrence.

4. Exploration of miRNA-based Therapies: Given the regulatory role of miRNAs in cancer progression, further research should focus on developing therapeutic strategies targeting specific miRNAs, either through miRNA mimics or inhibitors, to improve patient outcomes, particularly in drug-resistant cancers.

5. Multicenter Collaborations: Collaborative efforts across multiple research centers are necessary to overcome current limitations and optimize the application of circulating miRNAs in oncology.. (National Institute for Health and Care Excellence Developmental Follow-up of Children and

8.Limitations

Despite the promising potential of circulating microRNAs (miRNAs) as biomarkers for early detection and treatment monitoring in cancer, several limitations must be acknowledged. One major challenge is the variability in miRNA quantification techniques. Differences in RNA extraction methods, normalization strategies, and analytical platforms can lead to inconsistent results, which hinder reproducibility and comparability across studies (Ruiz-Manriquez et al., 2023). Many studies investigating miRNAs in cancer, including those focused on prostate, breast, and pancreatic cancers, are limited by small sample sizes and lack of validation in independent, multicenter cohorts. This restricts the generalizability of findings and emphasizes the need for large-scale clinical trials to confirm the diagnostic and prognostic value of candidate miRNAs such as miR-182-5p (Jang et al., 2021) The biological functions of several miRNAs remain incompletely understood, particularly regarding their role in complex signaling pathways and tumor microenvironment interactions. This lack of mechanistic insight may complicate the translation of miRNA biomarkers into clinical practice (Ruiz-Manriquez et al., 2023) Although circulating miRNAs offer a non-invasive approach for cancer detection and monitoring, pre-analytical variables such as sample handling, storage conditions, and hemolysis can significantly affect miRNA levels, introducing potential biases in data interpretation (Jang et al., 2021).

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