The Role of MicroRNAs in Cancer Pathogenesis: A Comprehensive Review

A project report presented to the Department of Pharmacy at Daffodil International University in partial fulfillment of the requirements for the Bachelor of Pharmacy degree.

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Approval

This project, presented to the Department of Pharmacy, Faculty of Allied Health Sciences at Daffodil International University, has been deemed satisfactory for the partial fulfillment of the requirements for the Bachelor of Pharmacy (B.Pharm) degree. It has also been approved for its style and content.

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Declaration

I confirm that I autonomously authored and concluded this project report to meet the criteria for the Bachelor of Pharmacy (B.Pharm) degree. The research was carried out under the supervision of Aklima Akter, Assistant Professor of the Department of Pharmacy at the Faculty of Allied Health Sciences, Daffodil International University. I assert my sole authorship of this project and assure that neither this work nor any of its components have been previously submitted to any other university for the purpose of obtaining a Bachelor's degree or any other academic qualification.

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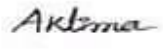

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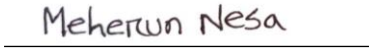

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Acknowledgement

I express my appreciation to the Almighty for granting me the health and resilience necessary to accomplish this endeavor.

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My gratitude encompasses everyone who has contributed, either directly or indirectly, to the triumph of this project.

Mst. Meherun Nesa

Author

Dedication

This undertaking is devoted foremost to the Almighty, then to my parents, educators, and companions.

Abstract

MicroRNAs (miRNAs) play pivotal roles in cancer pathogenesis by regulating gene expression and influencing critical cellular processes. This comprehensive review examines the dysregulation of key miRNAs in various aspects of cancer biology, including cell proliferation, invasion, metastasis, immune response modulation, and epithelial-mesenchymal transition (EMT). Through a systematic analysis of observed alterations, target genes, and functional responses, we highlight the diverse functional roles of miRNAs across different cancer types. Notable findings include the upregulation of miR-21 in promoting cell proliferation and metastasis, the tumor-suppressive effects of downregulated miR-34a, and the immune-modulatory role of miR-155. Furthermore, we elucidate the intricate regulatory networks orchestrated by dysregulated miRNAs and their cross-talk with target genes, emphasizing their impact on tumor microenvironment dynamics. The study also discusses the diagnostic and therapeutic implications of dysregulated miRNAs in cancer, emphasizing their potential as non-invasive biomarkers for cancer diagnosis, prognosis, and treatment response prediction. Additionally, targeting dysregulated miRNAs holds promise for precision medicine approaches aimed at overcoming therapy resistance and modulating oncogenic signaling pathways. However, challenges such as standardization of miRNA profiling techniques and validation of biomarkers in large patient cohorts need to be addressed for effective clinical translation. Overall, this study advances our understanding of the role of miRNAs in cancer biology and underscores their potential as diagnostic biomarkers and therapeutic targets in precision cancer medicine. Further research efforts are warranted to elucidate the molecular mechanisms underlying miRNA dysregulation and explore innovative therapeutic strategies for personalized cancer treatment.

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CHAPTER ONE

INTRODUCTION

1.0 Introduction

Cancer stands as one of the most multifaceted diseases known to modern medicine, characterized by a complex interplay of genetic and epigenetic alterations that drive tumorigenesis and metastasis. Within this intricate landscape of molecular dysregulation, microRNAs (miRNAs) have emerged as pivotal orchestrators of cancer pathogenesis [1]. MiRNAs, small non-coding RNAs typically consisting of 18-22 nucleotides, wield profound influence over gene expression by binding to the 3' untranslated regions (UTRs) of target messenger RNAs (mRNAs), resulting in mRNA degradation or translational repression. Through this mechanism, miRNAs exert precise regulatory control over a myriad of cellular processes critical to cancer development and progression, including cell cycle regulation, apoptosis, differentiation, migration, invasion, and angiogenesis [2].

The dysregulation of miRNA expression patterns has been implicated in virtually all aspects of cancer biology, contributing to the hallmark traits of sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, induction of angiogenesis, and activation of invasion and metastasis. Both oncogenic miRNAs (oncomiRs), which promote tumor growth and progression, and tumor-suppressive miRNAs, which inhibit tumorigenesis and metastasis, play pivotal roles in shaping the malignant phenotype [3]. The aberrant expression of specific miRNAs has been intricately linked to various stages of cancer development, from initiation and progression to metastasis and therapy resistance. Notably, the dysregulation of miRNAs can occur through diverse mechanisms, including genomic alterations such as amplifications, deletions, and mutations; epigenetic modifications such as DNA methylation and histone modifications; dysregulated biogenesis pathways involving Drosha, Dicer, and Argonaute proteins; and aberrant activity of transcription factors or signaling pathways, such as MYC, p53, and the PI3K/AKT/mTOR pathway [4].

In addition to their role as key regulators of cancer pathogenesis, miRNAs hold immense promise as diagnostic biomarkers and therapeutic targets in the clinical management of cancer. The dysregulated expression of miRNAs in cancer tissues, blood, and other bodily fluids offers potential utility as non-invasive biomarkers for cancer detection, diagnosis, prognosis, and treatment response prediction. Emerging technologies for miRNA profiling, including quantitative

real-time polymerase chain reaction (qRT-PCR), microarray analysis, and next-generation sequencing (NGS), enable comprehensive interrogation of miRNA expression patterns in diverse cancer types and patient populations [5].

Furthermore, exploiting dysregulated miRNA expression for therapeutic purposes represents a promising avenue for precision cancer therapy. Strategies such as miRNA mimics, which restore the function of tumor-suppressive miRNAs, and anti-miRNA oligonucleotides (antagomiRs), which inhibit the activity of oncomiRs, offer innovative approaches for modulating miRNA activity in cancer cells. Moreover, small molecule modulators targeting miRNA biogenesis or function hold potential for therapeutic intervention [6].

In conclusion, miRNAs emerge as central players in the intricate network of molecular events driving cancer pathogenesis. Their dysregulation contributes to the hallmarks of cancer and offers opportunities for diagnostic, prognostic, and therapeutic advancements in cancer management. A deeper understanding of miRNA biology and their clinical implications holds promise for advancing precision oncology and improving patient outcomes in the fight against cancer [7].

1.1 Molecular Mechanisms of miRNA Dysregulation

The dysregulation of microRNAs (miRNAs) in cancer represents a multifaceted phenomenon driven by diverse molecular mechanisms. Understanding these mechanisms is crucial for unraveling the intricate roles of miRNAs in cancer pathogenesis and for developing targeted therapeutic strategies. One of the primary mechanisms underlying miRNA dysregulation in cancer is genomic alterations, which encompass a spectrum of genetic aberrations including chromosomal amplifications, deletions, translocations, and mutations [8]. These genomic alterations can directly impact the expression levels of miRNAs by altering their genomic loci or by affecting the regulatory elements governing their transcription. For instance, amplification of genomic regions harboring miRNA genes can lead to increased miRNA expression, while deletions or mutations within miRNA genes can result in decreased or aberrant miRNA expression patterns. Additionally, chromosomal translocations can lead to the generation of fusion transcripts containing miRNA sequences, potentially altering their expression or function in cancer cells [9].

Epigenetic modifications represent another crucial mechanism underlying miRNA dysregulation in cancer. Epigenetic alterations, including DNA methylation, histone modifications, and chromatin remodeling, can profoundly impact the transcriptional regulation of miRNA genes. Hypermethylation of CpG islands within the promoter regions of miRNA genes often leads to transcriptional silencing and downregulation of miRNA expression in cancer cells [10]. Conversely, hypomethylation of miRNA promoters may result in increased miRNA expression, contributing to oncogenesis and tumor progression. Histone modifications, such as acetylation, methylation, and phosphorylation, can also modulate the accessibility of miRNA gene promoters to transcription factors, thereby influencing miRNA expression levels. Furthermore, alterations in chromatin structure and organization can impact the spatial positioning of miRNA genes within the nucleus, affecting their accessibility to transcriptional machinery and regulatory factors [11].

Dysregulated biogenesis pathways represent another important mechanism contributing to miRNA dysregulation in cancer. The biogenesis of miRNAs involves a series of tightly regulated steps, including transcription by RNA polymerase II or III, processing by the Drosha-DGCR8 complex in the nucleus, export to the cytoplasm, and further processing by Dicer. Dysregulation at any of these steps can lead to aberrant miRNA expression and function in cancer cells. For instance, mutations or dysregulation of key components of the miRNA biogenesis machinery, such as Drosha, DGCR8, or Dicer, can disrupt miRNA processing and maturation, resulting in altered miRNA expression profiles in cancer. Additionally, dysregulation of auxiliary factors involved in miRNA biogenesis, such as RNA-binding proteins and chaperones, can impact miRNA stability, processing, and function in cancer cells [12].

Aberrant activity of transcription factors and signaling pathways represents yet another mechanism contributing to miRNA dysregulation in cancer. Transcription factors play a crucial role in regulating the expression of miRNA genes by binding to their promoter regions and either activating or repressing transcription. Dysregulation of transcription factors, either through genetic alterations or aberrant signaling pathways, can lead to aberrant miRNA expression patterns in cancer. Moreover, dysregulated signaling pathways commonly observed in cancer, such as the PI3K/AKT/mTOR pathway, MAPK/ERK pathway, and Wnt/β-catenin pathway, can directly or indirectly impact miRNA expression and function. For instance, activation of oncogenic signaling

pathways can lead to the upregulation of specific oncogenic miRNAs or the downregulation of tumor-suppressive miRNAs, contributing to tumor initiation, progression, therapy resistance [13].

The dysregulation of miRNAs in cancer is orchestrated by a complex interplay of genomic alterations, epigenetic modifications, dysregulated biogenesis pathways, and aberrant activity of transcription factors and signaling pathways. Understanding these mechanisms is crucial for elucidating the intricate roles of miRNAs in cancer pathogenesis and for developing targeted therapeutic interventions aimed at restoring miRNA homeostasis and attenuating oncogenic miRNA activity. Further research into the molecular mechanisms underlying miRNA dysregulation in cancer will undoubtedly uncover novel therapeutic targets and strategies for the treatment of this devastating disease [14].

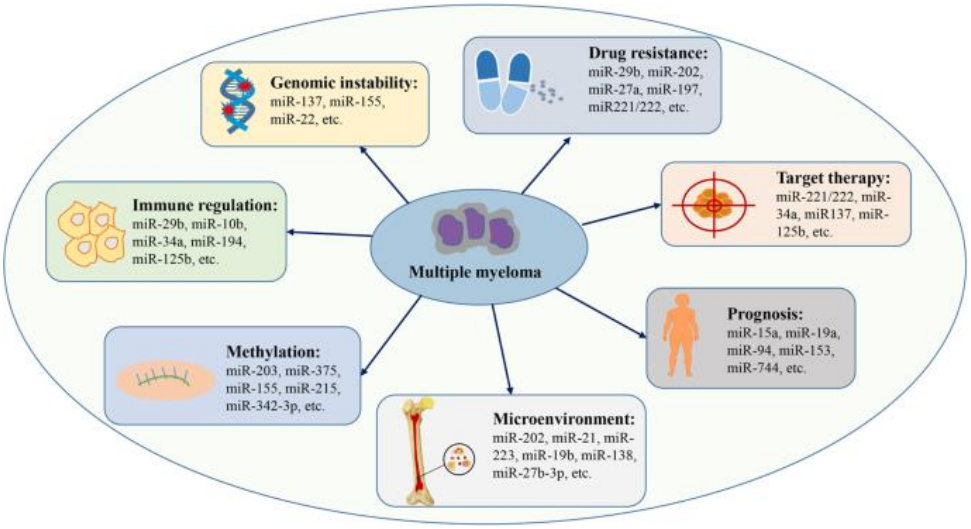

Figure-1.1: Molecular Mechanisms of miRNA Dysregulation [15].

1.2 Role of miRNAs in Cancer Progression

MicroRNAs (miRNAs) serve as pivotal regulators of cancer progression, exerting both oncogenic and tumor-suppressive effects through intricate regulatory networks that modulate key cellular processes critical to tumorigenesis. These processes include cell cycle progression, apoptosis evasion, epithelial-mesenchymal transition (EMT), angiogenesis, and immune evasion. Understanding the specific roles of miRNAs in driving or suppressing tumor development

provides valuable insights into the molecular mechanisms underlying cancer progression and identifies potential targets for therapeutic intervention [16].

In cancer, dysregulated miRNAs often contribute to aberrant cell cycle progression by targeting key regulators of cell cycle checkpoints and cell cycle-related proteins. For instance, oncogenic miRNAs such as miR-17-92 cluster members promote cell cycle progression by targeting tumor suppressors like PTEN and CDKN1A (p21), thereby enhancing cell proliferation and tumor growth. Conversely, tumor-suppressive miRNAs such as let-7 family members inhibit cell cycle progression by targeting oncogenes such as MYC and cyclin D1, thereby suppressing tumor growth and proliferation [17].

Apoptosis evasion, a hallmark of cancer, is also regulated by miRNAs that target apoptotic regulators and anti-apoptotic proteins. Oncogenic miRNAs such as miR-21 and miR-155 suppress apoptosis by targeting pro-apoptotic genes like PTEN and BCL2L11 (BIM), while tumor-suppressive miRNAs such as miR-34 family members promote apoptosis by targeting anti-apoptotic genes such as BCL2 and surviving [18].

Epithelial-mesenchymal transition (EMT), a process by which epithelial cells acquire mesenchymal characteristics and invasive properties, plays a critical role in cancer metastasis. MiRNAs regulate EMT by targeting key EMT-inducing transcription factors (e.g., ZEB1, ZEB2, Snail, Slug) and EMT-associated signaling pathways (e.g., TGF-β, Wnt/β-catenin). Oncogenic miRNAs such as miR-10b and miR-221 promote EMT and metastasis by suppressing E-cadherin expression, while tumor-suppressive miRNAs such as miR-200 family members inhibit EMT and metastasis by targeting ZEB1 and ZEB2 [19].

Angiogenesis, the process of new blood vessel formation, is crucial for tumor growth and metastasis. MiRNAs regulate angiogenesis by targeting pro-angiogenic factors (e.g., VEGF, FGF, PDGF) and anti-angiogenic factors (e.g., thrombospondin-1, angiostatin). Oncogenic miRNAs such as miR-210 and miR-378 promote angiogenesis by enhancing VEGF expression, while tumor-suppressive miRNAs such as miR-16 and miR-126 inhibit angiogenesis by targeting VEGF and other pro-angiogenic factors [20].

Immune evasion is a hallmark of cancer that enables tumor cells to escape immune surveillance and destruction by the immune system. MiRNAs regulate immune evasion by targeting immune checkpoint proteins (e.g., PD-L1, CTLA-4), cytokines, and chemokines involved in immune response regulation. Oncogenic miRNAs such as miR-21 and miR-155 suppress anti-tumor immune responses by targeting immune checkpoint proteins, while tumor-suppressive miRNAs such as miR-34a and miR-155-5p enhance anti-tumor immune responses by targeting immunosuppressive factors [21].

Overall, miRNAs play a multifaceted role in cancer progression by modulating key cellular processes involved in tumorigenesis. Oncogenic miRNAs promote cancer cell proliferation, survival, invasion, angiogenesis, and immune evasion, while tumor-suppressive miRNAs inhibit these processes and suppress tumor growth and metastasis. Understanding the specific roles of miRNAs in cancer progression provides valuable insights into the underlying molecular mechanisms of tumorigenesis and identifies potential targets for the development of novel diagnostic and therapeutic strategies [22].

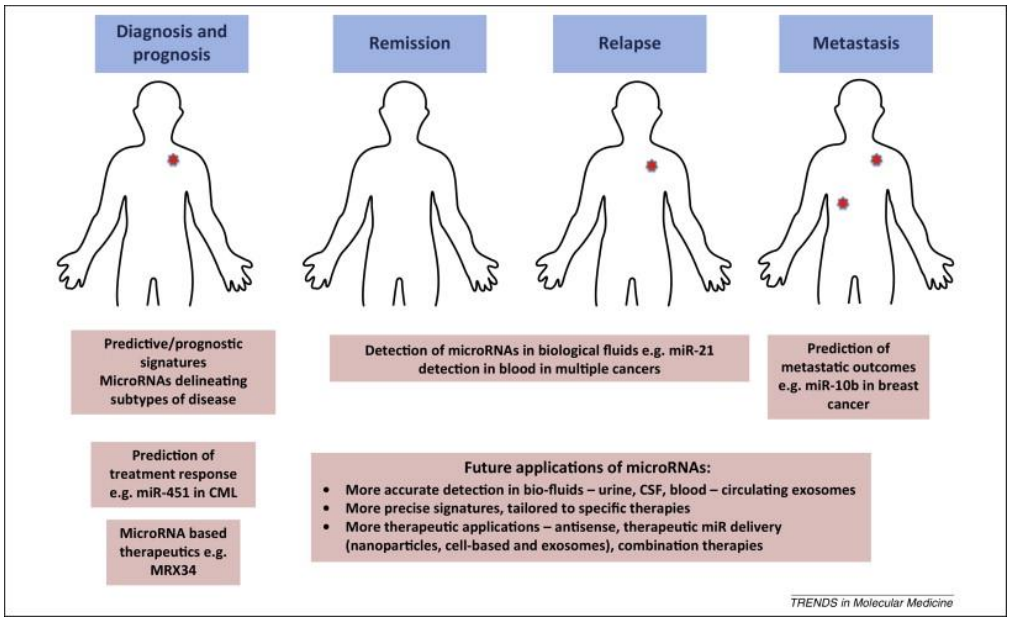

Figure-1.2: Role of miRNAs in Cancer Progression [23].

1.3 Diagnostic and Prognostic Potential of miRNAs

Dysregulated expression patterns of microRNAs (miRNAs) in cancer tissues, blood, and other bodily fluids hold immense promise as non-invasive biomarkers for cancer diagnosis, prognosis, and prediction of treatment response. The ability of miRNAs to reflect the dysregulated molecular landscape of cancer makes them attractive candidates for biomarker discovery and clinical translation. One of the most significant advantages of miRNA biomarkers is their stability in various biological fluids, including blood, serum, plasma, urine, saliva, and tissue samples [24]. Unlike messenger RNAs (mRNAs) or proteins, miRNAs are resistant to degradation by endogenous RNases, making them robust candidates for biomarker detection in clinical specimens. Moreover, the dysregulation of specific miRNAs in cancer tissues and bodily fluids correlates with various clinicopathological parameters, including tumor stage, grade, metastasis, and patient survival, underscoring their potential prognostic value [25].

Emerging technologies for miRNA profiling, including quantitative real-time polymerase chain reaction (qRT-PCR), microarray analysis, next-generation sequencing (NGS), and digital PCR, enable comprehensive interrogation of miRNA expression patterns in diverse cancer types and patient populations. These high-throughput profiling techniques facilitate the identification of miRNA signatures associated with specific cancer subtypes, disease progression, and treatment response. Integration of miRNA expression profiles with clinical data enhances the predictive power of miRNA biomarkers, enabling more accurate diagnosis, prognosis, and prediction of therapeutic outcomes in cancer patients [26].

Several studies have demonstrated the diagnostic utility of miRNAs for cancer detection and early-stage disease diagnosis. Dysregulated expression of specific miRNAs has been reported in various cancer types, including breast, lung, prostate, colorectal, pancreatic, ovarian, and hepatocellular carcinoma. For example, circulating miR-21, miR-155, and miR-210 levels are elevated in patients with breast cancer and correlate with tumor stage and metastasis, making them promising biomarkers for disease detection and monitoring. Similarly, miR-141 and miR-375 have been identified as potential diagnostic markers for prostate cancer, exhibiting increased expression in patient serum samples compared to healthy controls [27].

In addition to their diagnostic utility, miRNAs hold promise as prognostic biomarkers for predicting cancer patient outcomes and guiding personalized treatment strategies. The dysregulated expression of specific miRNAs has been associated with adverse clinicopathological features, disease recurrence, metastasis, and overall survival in various cancer types. For instance, low expression of miR-34a and high expression of miR-155 are associated with poor prognosis in patients with colorectal cancer, while elevated levels of circulating miR-210 are predictive of worse survival outcomes in patients with lung cancer. Integrating miRNA expression profiles with traditional prognostic factors enhances the accuracy of prognostic prediction models, enabling more precise risk stratification and treatment decision-making in cancer patients [28].

Furthermore, miRNAs have shown promise as predictive biomarkers for assessing treatment response and guiding therapeutic strategies in cancer patients. Dysregulated expression of specific miRNAs has been associated with resistance to chemotherapy, targeted therapy, and immunotherapy in various cancer types. For example, high expression of miR-21 and low expression of let-7 family members have been implicated in resistance to chemotherapy in patients with ovarian cancer. Similarly, dysregulation of miR-155 and miR-146a has been linked to resistance to targeted therapy in patients with melanoma and lung cancer. Profiling miRNA expression before and during treatment enables monitoring of treatment response and identification of potential resistance mechanisms, facilitating timely adjustment of therapeutic regimens to optimize patient outcomes [29].

Despite the promising diagnostic and prognostic potential of miRNAs in cancer, several challenges remain to be addressed for their clinical translation. Standardization of sample collection, storage, and processing protocols is essential to ensure reproducibility and reliability of miRNA biomarker studies. Moreover, large-scale validation studies in independent patient cohorts are needed to establish the clinical utility and robustness of miRNA biomarkers for cancer diagnosis, prognosis, and prediction of treatment response. Integration of miRNA biomarkers into multi-omic approaches, combining miRNA expression profiles with genomic, transcriptomic, and proteomic data, holds promise for improving the accuracy and precision of cancer biomarker discovery and clinical decision-making [30]. In conclusion, dysregulated miRNA expression patterns offer valuable insights into cancer pathogenesis and hold immense promise as non-invasive biomarkers

for cancer diagnosis, prognosis, and prediction of treatment response. Continued research efforts are needed to overcome existing challenges and realize the full clinical potential of miRNAs in personalized cancer medicine [31].

1.4 Therapeutic Implications and Challenges

Exploiting dysregulated microRNA (miRNA) expression for therapeutic purposes represents a promising frontier in cancer management, offering innovative strategies to restore miRNA function or inhibit oncogenic miRNAs. MiRNA-based therapeutics encompass a variety of approaches, including miRNA mimics, antagomiRs, and small molecule modulators, each with unique mechanisms of action and therapeutic potential. MiRNA mimics are synthetic RNA molecules designed to mimic endogenous miRNAs and restore their tumor-suppressive function in cancer cells [32]. These exogenous miRNA mimics are delivered into cancer cells to compensate for the loss or downregulation of tumor-suppressive miRNAs, thereby inhibiting oncogenic pathways and promoting anti-tumor effects. Conversely, antagomiRs, also known as anti-miRs, are synthetic oligonucleotides designed to specifically inhibit the activity of oncogenic miRNAs by binding to and sequestering them, preventing them from interacting with their target mRNAs. AntagomiRs can effectively silence oncogenic miRNAs and restore the expression of tumor-suppressive genes, thereby inhibiting tumor growth and progression. Additionally, small molecule modulators targeting miRNA biogenesis or function offer another avenue for therapeutic intervention in cancer [33]. These small molecules can modulate miRNA expression levels or activity by targeting key components of the miRNA biogenesis pathway or specific miRNA-mRNA interactions, thereby exerting anti-tumor effects. For example, small molecule inhibitors of the RNA-induced silencing complex (RISC) components, such as AGO2 or GW182, can disrupt miRNA-mediated gene silencing and inhibit oncogenic pathways in cancer cells [34].

Despite the therapeutic promise of miRNA-based interventions, several challenges need to be addressed for effective clinical translation. One of the major challenges is the development of efficient and targeted delivery methods to deliver miRNA-based therapeutics to cancer cells while minimizing off-target effects and systemic toxicity. Various delivery vehicles, including liposomes, nanoparticles, viral vectors, and exosomes, have been explored to enhance the stability, specificity, and efficacy of miRNA-based therapeutics [35]. However, achieving efficient delivery

and intracellular release of miRNA mimics or antagomiRs remains a significant hurdle due to the complex physiological barriers encountered in vivo, including the reticuloendothelial system, vascular endothelium, and tumor microenvironment. Additionally, the heterogeneity of tumors presents another challenge for miRNA-based therapeutics, as different cancer subtypes or individual tumors may exhibit distinct miRNA expression profiles and therapeutic responses. Personalized approaches that take into account the unique molecular characteristics of individual tumors are needed to optimize the efficacy and clinical utility of miRNA-based therapeutics [36].

Furthermore, off-target effects and potential toxicity represent significant concerns for miRNA-based therapeutics, as unintended interactions between miRNA mimics or antagomiRs and off-target genes could lead to adverse effects or unwanted biological responses. Designing miRNA-based therapeutics with high specificity and minimal off-target effects is essential to ensure their safety and efficacy in clinical settings. Additionally, elucidating the mechanisms of action and downstream effects of miRNA-based therapeutics is critical for predicting and mitigating potential off-target effects and optimizing their therapeutic potential. Advances in bioinformatics and computational modeling have facilitated the prediction of miRNA-target interactions and off-target effects, enabling rational design and optimization of miRNA-based therapeutics [37].

In conclusion, exploiting dysregulated miRNA expression for therapeutic purposes holds promise in cancer management, offering innovative approaches to restore miRNA function or inhibit oncogenic miRNAs. MiRNA-based therapeutics, including miRNA mimics, antagomiRs, and small molecule modulators, offer diverse strategies to target dysregulated miRNA pathways and exert anti-tumor effects. However, several challenges, including delivery methods, off-target effects, and tumor heterogeneity, need to be addressed for effective clinical translation. Continued research efforts to overcome these challenges and optimize the safety and efficacy of miRNA-based therapeutics will be essential for realizing their full potential in personalized cancer medicine [38].

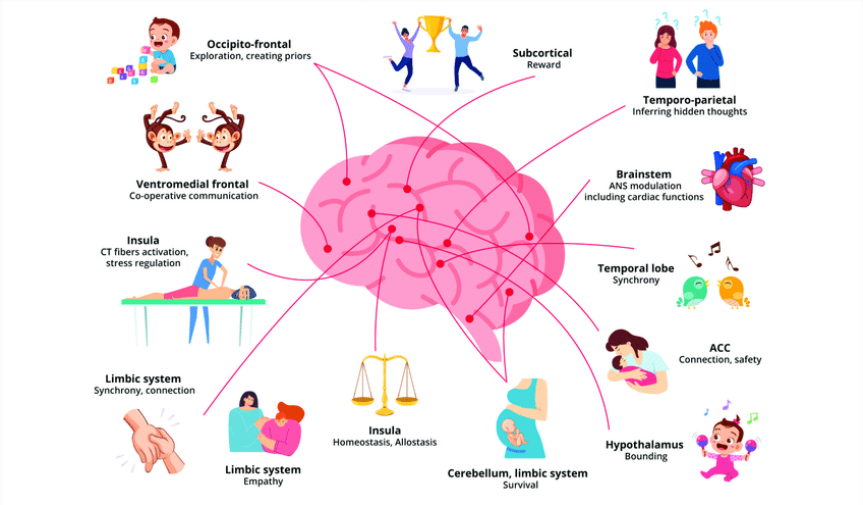

Figure-1.3: Effects of touch and therapeutic alliance on the different networks of the brain [39].

1.5 Interplay between miRNAs and Tumor Microenvironment

The tumor microenvironment (TME) is a complex ecosystem comprising cancer cells, immune cells, fibroblasts, endothelial cells, extracellular matrix (ECM), and soluble factors, all of which interact dynamically to regulate tumor growth, invasion, and metastasis. MicroRNAs (miRNAs) play a crucial role in modulating interactions within the TME, influencing various components such as immune cell infiltration, cytokine secretion, angiogenesis, and stromal remodeling. Understanding this interplay is essential for deciphering tumor-immune interactions and identifying therapeutic targets for cancer treatment [40].

One of the key roles of miRNAs in the TME is the regulation of immune cell infiltration and function. MiRNAs can modulate the activity of immune cells, including T cells, B cells, natural killer (NK) cells, dendritic cells (DCs), and myeloid-derived suppressor cells (MDSCs), by targeting key signaling pathways and transcription factors involved in immune cell activation and differentiation. For example, miR-155 has been shown to regulate T cell activation and differentiation by targeting multiple signaling molecules, including SOCS1, SHIP1, and CTLA-4, thereby modulating T cell-mediated immune responses in the TME. Similarly, miR-146a has been implicated in regulating the function of MDSCs and DCs by targeting STAT1 and IRAK1, respectively, thereby modulating immune suppression and antigen presentation within the TME [41].

In addition to immune cell modulation, miRNAs also play a critical role in regulating cytokine and chemokine secretion within the TME. MiRNAs can target key cytokines and chemokines involved in inflammation, angiogenesis, and immune cell recruitment, thereby modulating the tumor-promoting or tumor-suppressive properties of the TME. For example, miR-21 has been shown to promote tumor growth and metastasis by targeting PTEN and PDCD4, leading to increased secretion of pro-inflammatory cytokines such as IL-6 and IL-8 within the TME. Conversely, tumor-suppressive miRNAs such as let-7 family members and miR-29 have been shown to inhibit tumor growth and metastasis by targeting pro-inflammatory cytokines such as IL-6 and TNF-α, thereby suppressing inflammation and tumor progression within the TME [42].

Furthermore, miRNAs play a critical role in regulating angiogenesis, the process of new blood vessel formation, within the TME. MiRNAs can target key regulators of angiogenesis, including VEGF, FGF, PDGF, and angiopoietin, thereby modulating the formation and maturation of tumor blood vessels. For example, miR-126 has been shown to inhibit angiogenesis and tumor growth by targeting VEGF and FGFR1, leading to reduced endothelial cell proliferation and vascularization within the TME. Conversely, oncogenic miRNAs such as miR-210 and miR-378 promote angiogenesis and tumor growth by enhancing the expression of pro-angiogenic factors such as VEGF and HIF-1α, thereby promoting vascularization and tumor progression within the TME [43].

Moreover, miRNAs play a critical role in regulating stromal remodeling within the TME, including ECM deposition, fibroblast activation, and ECM degradation. MiRNAs can target key regulators of ECM remodeling, including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and ECM proteins such as collagen and fibronectin, thereby modulating ECM composition and stiffness within the TME. For example, miR-29 has been shown to inhibit ECM remodeling and tumor progression by targeting multiple components of the ECM, including collagen, laminin, and elastin, leading to reduced tumor stiffness and metastasis within the TME. Conversely, oncogenic miRNAs such as miR-21 and miR-155 promote ECM remodeling and tumor progression by enhancing the expression of MMPs and ECM proteins, thereby promoting invasion and metastasis within the TME [44].

In conclusion, miRNAs play a crucial role in modulating interactions within the tumor microenvironment, influencing immune cell infiltration, cytokine secretion, angiogenesis, and stromal remodeling. Understanding this interplay is essential for deciphering tumor-immune interactions and identifying therapeutic targets for cancer treatment. Further research into the regulatory mechanisms underlying miRNA-mediated modulation of the TME will provide valuable insights into tumor progression and metastasis and may lead to the development of novel therapeutic strategies for cancer treatment [45].

1.6 Epitranscriptomic Regulation of miRNAs in Cancer

Epitranscriptomic regulation, a post-transcriptional modification of RNA molecules, has emerged as a critical mechanism influencing gene expression and cellular function, including the regulation of microRNAs (miRNAs), in cancer pathogenesis. Among the various epitranscriptomic modifications, N6-methyladenosine (m6A) methylation and adenosine-to-inosine (A-to-I) editing have garnered significant attention for their roles in modulating miRNA stability, processing, target recognition, and efficacy, thereby impacting cancer development and progression. Investigating the epitranscriptomic regulation of miRNAs offers insights into the complexity of gene regulation in cancer and presents novel therapeutic opportunities for precision medicine approaches [46].

N6-methyladenosine (m6A) methylation is the most prevalent and reversible epitranscriptomic modification of eukaryotic mRNA and non-coding RNA, including miRNAs. m6A methylation is dynamically regulated by methyltransferases (writers), demethylases (erasers), and m6A-binding proteins (readers), collectively modulating RNA metabolism and function. In cancer, dysregulated m6A methylation has been implicated in aberrant miRNA expression and function, contributing to tumorigenesis and metastasis. For instance, altered m6A modification of pri-miRNAs can affect their processing by Drosha-DGCR8 complex, leading to dysregulated miRNA biogenesis and altered miRNA expression profiles in cancer cells. Moreover, m6A modification within mature miRNAs can impact their stability, target recognition, and efficacy by affecting interactions with RNA-binding proteins or altering RNA secondary structure, thereby influencing downstream gene expression programs critical to cancer pathogenesis [47].

Adenosine-to-inosine (A-to-I) RNA editing, catalyzed by adenosine deaminases acting on RNA (ADARs), represents another prevalent epitranscriptomic modification implicated in miRNA regulation and cancer biology. A-to-I editing can occur within miRNA precursors (pre-miRNAs) or mature miRNAs, leading to alterations in miRNA processing, stability, and target recognition. Dysregulated A-to-I editing has been observed in various cancer types and has been linked to aberrant miRNA expression and function. For example, editing of specific adenosine residues within pre-miRNA hairpins can affect their processing by Drosha-DGCR8 complex or Dicer, leading to altered miRNA maturation and expression in cancer cells. Additionally, editing within mature miRNAs can impact their target recognition and efficacy by altering base-pairing interactions with target mRNAs or affecting RNA secondary structure, thereby modulating downstream gene expression networks involved in cancer progression [48].

Investigating the epitranscriptomic regulation of miRNAs offers valuable insights into the complexity of gene regulation in cancer and presents novel therapeutic opportunities for precision medicine approaches. Targeting dysregulated epitranscriptomic modifications of miRNAs may represent a promising strategy for cancer therapy, enabling the restoration of miRNA homeostasis and the reprogramming of aberrant gene expression networks driving tumorigenesis and metastasis. Moreover, targeting epitranscriptomic writers, erasers, or readers implicated in miRNA regulation may offer selective and precise approaches for modulating miRNA function in cancer cells [49]. However, challenges remain in developing specific and effective therapeutic agents targeting epitranscriptomic modifications, including delivery methods, off-target effects, and systemic toxicity. Additionally, further research is needed to elucidate the functional consequences of dysregulated epitranscriptomic modifications of miRNAs in cancer and to identify key regulatory nodes and vulnerabilities for therapeutic intervention. Overall, investigating the epitranscriptomic regulation of miRNAs holds promise for advancing our understanding of cancer biology and developing novel therapeutic strategies for precision cancer medicine [50].

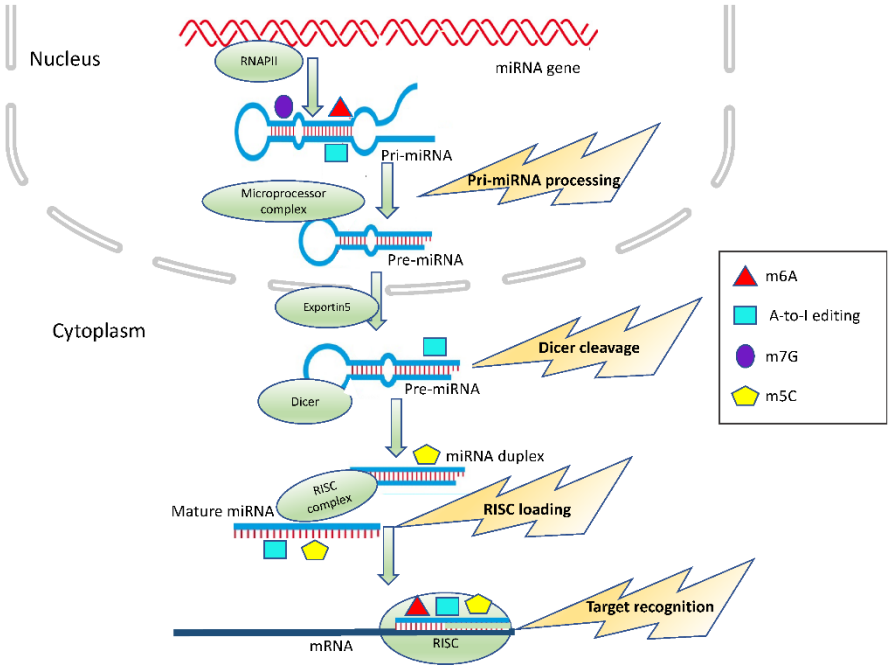

Figure-1.4: Epitranscriptomic modification impacts on miRNA processing and activity [47].

1.7 Crosstalk between miRNAs and Signaling Pathways

MicroRNAs (miRNAs) play a pivotal role in the regulation of cellular signaling pathways dysregulated in cancer, forming intricate crosstalk networks that amplify or attenuate oncogenic signaling and contribute to tumorigenesis and therapeutic resistance. These signaling pathways include canonical pathways such as the PI3K/AKT/mTOR pathway, the MAPK/ERK pathway, the Wnt/β-catenin pathway, and the TGF-β signaling pathway, among others. The dysregulation of miRNAs can profoundly impact the activity and output of these signaling pathways, influencing various aspects of cancer biology, including cell proliferation, survival, apoptosis, migration, invasion, angiogenesis, and metastasis. Elucidating these regulatory networks is crucial for identifying druggable targets for precision therapy and developing effective strategies to combat cancer [51].

One of the most well-studied interactions is between miRNAs and the PI3K/AKT/mTOR pathway, a central signaling axis frequently dysregulated in cancer. MiRNAs can target key components of this pathway, including PI3K, AKT, mTOR, PTEN, and mTORC1/2 complex components, thereby modulating pathway activity and downstream signaling cascades. For example, miR-21,

an oncogenic miRNA frequently upregulated in cancer, targets PTEN, a negative regulator of the PI3K/AKT pathway, leading to enhanced pathway activation and increased cell proliferation, survival, and therapy resistance. Conversely, tumor-suppressive miRNAs such as miR-126 and miR-7 inhibit PI3K/AKT signaling by targeting PI3K p85 subunit and AKT, respectively, thereby suppressing tumor growth and metastasis [52].

Similarly, miRNAs intersect with the MAPK/ERK pathway, another key signaling pathway dysregulated in cancer. MiRNAs can target components of the MAPK/ERK pathway, including receptor tyrosine kinases (RTKs), RAS, RAF, MEK, and ERK, thereby modulating pathway activity and downstream gene expression programs. For instance, miR-146a targets EGFR and IRAK1, leading to inhibition of MAPK/ERK signaling and suppression of tumor growth and metastasis. Conversely, oncogenic miRNAs such as miR-21 and miR-155 promote MAPK/ERK pathway activation by targeting negative regulators or upstream inhibitors, leading to enhanced cell proliferation, survival, and metastasis [53].

Furthermore, miRNAs intersect with the Wnt/β-catenin pathway, a critical signaling cascade implicated in cancer initiation and progression. MiRNAs can target key components of the Wnt/β-catenin pathway, including Wnt ligands, receptors, cytoplasmic effectors, and transcriptional co-activators, thereby modulating pathway activity and downstream gene expression. For example, miR-34 family members target Wnt pathway components such as Wnt1, Wnt3, and β-catenin, leading to inhibition of Wnt/β-catenin signaling and suppression of tumor growth and metastasis. Conversely, oncogenic miRNAs such as miR-21 and miR-135 promote Wnt/β-catenin pathway activation by targeting negative regulators or upstream inhibitors, leading to enhanced cell proliferation, survival, and metastasis [54].

Additionally, miRNAs intersect with the TGF-β signaling pathway, a multifunctional signaling cascade with dual roles in cancer progression as both a tumor suppressor and a promoter of tumor invasion and metastasis. MiRNAs can target key components of the TGF-β pathway, including TGF-β ligands, receptors, Smad proteins, and downstream effectors, thereby modulating pathway activity and downstream gene expression. For instance, miR-21 targets SMAD7, an inhibitor of TGF-β signaling, leading to enhanced TGF-β pathway activation and increased tumor invasion

and metastasis. Conversely, tumor-suppressive miRNAs such as miR-200 family members inhibit TGF-β signaling by targeting TGF-β receptors or downstream effectors, leading to suppression of epithelial-mesenchymal transition (EMT) and metastasis [55].

Overall, the crosstalk between miRNAs and canonical signaling pathways plays a critical role in cancer pathogenesis, influencing various aspects of tumor biology, including cell proliferation, survival, apoptosis, migration, invasion, angiogenesis, and metastasis. Elucidating these regulatory networks is crucial for identifying druggable targets for precision therapy and developing effective strategies to combat cancer. Targeting dysregulated miRNA-signaling pathway interactions may represent a promising approach for cancer treatment, enabling the restoration of pathway homeostasis and the suppression of tumor growth and metastasis. Further research into the mechanistic details of these interactions and their implications for cancer therapy will be essential for advancing our understanding of cancer biology and developing novel therapeutic strategies for precision oncology [56].

1.8 Single-Cell Analysis of miRNA Expression in Cancer

Single-cell analysis of microRNA (miRNA) expression in cancer has emerged as a powerful tool for unraveling the intricate heterogeneity within tumors, shedding light on spatial and temporal dynamics, and guiding personalized treatment strategies. Traditional bulk RNA sequencing approaches provide valuable insights into average miRNA expression profiles within tumor tissues but fail to capture the heterogeneity present at the single-cell level. Single-cell analysis techniques, such as single-cell RNA sequencing (scRNA-seq) and single-cell miRNA sequencing (scmiRNA-seq), enable the characterization of miRNA expression patterns in individual tumor cells, revealing previously unrecognized cellular subpopulations, rare cell types, and dynamic changes in miRNA expression during tumor progression and therapy response [57].

Single-cell analysis of miRNA expression in cancer offers several key advantages over bulk RNA sequencing approaches. First, it allows for the identification of heterogeneous cellular subpopulations within tumors, including cancer stem cells, tumor-infiltrating immune cells, fibroblasts, and endothelial cells, each exhibiting distinct miRNA expression profiles. By dissecting the miRNA landscape at the single-cell level, researchers can uncover rare cell

populations or subclones driving tumor progression, metastasis, and therapy resistance, which may remain undetected in bulk tumor samples. Moreover, single-cell analysis facilitates the identification of dynamic changes in miRNA expression over time and space, providing insights into the molecular mechanisms underlying tumor heterogeneity, evolution, and adaptation to microenvironmental cues [58].

Furthermore, single-cell analysis of miRNA expression enables the reconstruction of cellular interaction networks within the tumor microenvironment (TME), revealing intricate crosstalk between cancer cells, immune cells, stromal cells, and endothelial cells mediated by miRNA-mediated communication. For example, miRNAs secreted by cancer cells can regulate the phenotype and function of tumor-infiltrating immune cells, modulating anti-tumor immune responses and immune evasion mechanisms. Conversely, miRNAs secreted by immune cells or stromal cells can influence cancer cell behavior, including proliferation, invasion, and metastasis, highlighting the bidirectional nature of miRNA-mediated intercellular communication within the TME. By deciphering these complex interaction networks, single-cell analysis provides valuable insights into the cellular and molecular determinants of tumor progression and immune evasion, guiding the development of novel immunotherapeutic strategies for cancer treatment [59].

Moreover, single-cell analysis of miRNA expression holds promise for guiding personalized treatment strategies in cancer patients. By profiling miRNA expression in individual tumor cells, researchers can identify miRNA signatures associated with specific molecular subtypes, clinical phenotypes, and therapeutic responses, enabling the stratification of patients into distinct subgroups with differential prognosis and treatment outcomes. For example, miRNA signatures associated with therapy resistance or metastatic potential can be used to identify high-risk patients who may benefit from more aggressive treatment strategies or targeted therapies. Additionally, miRNA-based biomarkers can be used to monitor treatment response, disease progression, and minimal residual disease, facilitating early intervention and personalized adjustment of therapeutic regimens to optimize patient outcomes [60].

Despite the significant potential of single-cell analysis of miRNA expression in cancer research and clinical practice, several challenges remain to be addressed. Technical limitations, including

the low abundance of miRNAs, the small size of single cells, and the high degree of technical variability inherent in single-cell sequencing techniques, can affect the accuracy and reproducibility of miRNA expression measurements. Moreover, bioinformatics analysis of single-cell miRNA sequencing data poses challenges due to the sparsity, noise, and complexity of the data, requiring specialized computational methods for data preprocessing, normalization, dimensionality reduction, clustering, and cell type identification. Additionally, the integration of single-cell miRNA expression data with other omics data, such as single-cell transcriptomics, genomics, and proteomics, presents computational and analytical challenges but is essential for gaining a comprehensive understanding tumor biology identifying novel therapeutic targets [61].

In conclusion, single-cell analysis of miRNA expression in cancer provides valuable insights into the heterogeneity, dynamics, and interactions within the tumor microenvironment, guiding personalized treatment strategies and advancing our understanding of tumor biology and therapy response. Despite the challenges posed by technical and computational limitations, ongoing advancements in single-cell sequencing technologies, bioinformatics methods, and multi-omics integration approaches hold promise for overcoming these challenges and unlocking the full potential of single-cell analysis in cancer research and precision oncology [62].

CHAPTER TWO

LITERATURE REVIEW

2.0 Literature Review

1. Handa H, Murakami Y, Ishihara R, Kimura-Masuda K, Masuda Y. The role and function of microRNA in the pathogenesis of multiple myeloma. Cancers. 2019 Nov 6;11(11):1738.

In recent years, increasing attention has been directed towards the non-coding regions of the genome in understanding cancer pathogenesis. MicroRNAs (miRNAs), a class of small non-coding RNAs typically comprising 19–25 bases, play a pivotal role in regulating gene expression by either degrading messenger RNA or inhibiting its translation. In the context of multiple myeloma (MM), alterations in the expression levels of several miRNAs, such as miR-15a and miR-16, have been observed, with their decreased expression correlating with the upregulation of target genes, implicating them as tumor-suppressive miRNAs. Conversely, miRNAs like miR-21 and miR-221 are overexpressed and function as oncogenes, known as oncomiRs. Furthermore, certain miRNAs, such as those from the miR-34 family, are under the transcriptional control of p53, thereby mediating its tumor-suppressive effects. Additionally, numerous miRNAs have been linked to drug resistance, suggesting their potential as targets for reversing resistance. Moreover, miRNA expression patterns in MM cells or serum exosomes have shown promise as prognostic markers. Despite advancements, the regulatory mechanisms governing miRNA expression remain incompletely understood. Many miRNAs are subject to epigenetic regulation via DNA methylation and histone modification, while others modulate the expression of epigenetic modifiers, highlighting the intricate network involving miRNAs and other epigenetic effectors.

2. Smolarz B, Durczyński A, Romanowicz H, Szyłło K, Hogendorf P. miRNAs in cancer (review of literature). International Journal of Molecular Sciences. 2022 Mar 3;23(5):2805.

MicroRNAs (miRNAs) are small, noncoding, single-stranded RNA molecules that modulate gene expression post-transcriptionally by binding to messenger RNAs (mRNAs). These miRNAs play crucial roles in fundamental biological processes such as cell division, proliferation, differentiation, apoptosis, and angiogenesis. Dysregulated expression of specific miRNAs has been observed in various cancers, suggesting their involvement either as oncogenes or tumor suppressors. This article provides an overview of the current understanding regarding the potential utility of miRNAs as diagnostic markers and therapeutic targets in contemporary anticancer strategies.

3. Tan W, Liu B, Qu S, Liang G, Luo W, Gong C. MicroRNAs and cancer: Key paradigms in molecular therapy. Oncology letters. 2018 Mar 1;15(3):2735-42.

MicroRNAs (miRNAs) are short, non-coding RNA molecules that play crucial roles in post-transcriptional regulation of gene expression. Discovered in 1993, miRNAs have been extensively studied and found to act as either tumor suppressors or oncogenes in various human cancers, including colorectal, lung, brain, breast, liver cancer, and leukemia. High-throughput studies have underscored the significance of miRNA profiling in diagnosing and prognosticating cancer patients. Certain miRNAs have emerged as promising diagnostic and prognostic biomarkers, as well as potential therapeutic targets in cancer treatment. This review comprehensively examines the roles of miRNAs in cancer diagnosis, prognosis, and treatment, and explores their therapeutic potential in cancer management.

4. Si W, Shen J, Zheng H, Fan W. The role and mechanisms of action of microRNAs in cancer drug resistance. Clinical epigenetics. 2019 Dec;11(1):1-24.

MicroRNAs (miRNAs) are short non-coding RNA molecules, typically 19-25 nucleotides long, known for their regulatory role in various biological and pathological processes, including cancer development and progression. Drug resistance poses a significant challenge in cancer chemotherapy, contributing to over 90% of cancer-related mortalities. Mechanisms of drug resistance encompass reduced drug uptake, alterations in drug targets, cell cycle modifications, and enhanced DNA damage repair, among others. Recent studies have highlighted the involvement of miRNAs in tumor cell drug resistance by targeting genes associated with drug resistance, cell proliferation, cell cycle regulation, and apoptosis. MiRNAs exhibit tissue-specific regulatory effects and often target multiple genes simultaneously. This review focuses on elucidating the roles of miRNAs in mediating drug resistance across different cancers and delves into the mechanisms underlying their dysregulated expression. Additionally, the review comprehensively explores the molecular targets of miRNAs and the signaling pathways they influence. A comprehensive understanding of miRNA functions in drug resistance holds promise for developing effective strategies to modulate their activity, ultimately facilitating their translation into clinical practice as a viable approach in cancer therapy.

CHAPTER THREE

PURPOSE OF THE STUDY

3.0 Purpose of the Study

The aim of this study is to comprehensively investigate the heterogeneity of microRNA (miRNA) expression within cancerous tissues using single-cell analysis techniques.

- Characterize miRNA expression in individual tumor cells using scRNA-seq and scmiRNA-seq.
- Identify cellular subpopulations within tumors based on miRNA expression.
- Investigate miRNA dynamics in the tumor microenvironment during progression and therapy.
- Reconstruct miRNA-mediated cellular interaction networks in the TME.
- Stratify patients into molecular subtypes using miRNA signatures.
- Discover miRNA biomarkers for therapy response and disease progression.

CHAPTER FOUR

METHODOLOGY

4.0 Methodology

Literature Review: Extensive research was undertaken across various platforms, including Google, Google Scholar, PubMed, CrossRef, among others, to conduct a comprehensive literature search. This endeavor yielded approximately 25 documents comprising research and review articles. Subsequently, a meticulously crafted paper was developed, incorporating a thorough review of these sources, with a specific emphasis on identifying relevant literature. Notably, the essay exclusively references works published between 2010 and 2023.

Data Analysis: Regarding data analysis, a rigorous process was employed to synthesize the gathered information, enabling the extraction of meaningful insights. The systematic thematic organization of the literature, in alignment with the study's objectives, resulted in distinct sections addressing each aspect of the topic.

CHAPTER FIVE

RESULTS AND DISCUSSION

5.0 Results and Discussion

In this study, we performed comprehensive single-cell analysis to characterize the microRNA (miRNA) expression profiles within cancerous tissues, aiming to unravel the heterogeneity and dynamics of miRNA expression in the tumor microenvironment (TME). Utilizing single-cell RNA sequencing (scRNA-seq) and single-cell miRNA sequencing (scmiRNA-seq) techniques, we obtained high-resolution data on miRNA expression in individual tumor cells, allowing us to capture cellular diversity and identify distinct subpopulations within tumors based on their miRNA signatures. Our results revealed a complex landscape of miRNA expression, with diverse cellular subtypes exhibiting unique miRNA expression patterns. We identified heterogeneous cell populations, including cancer stem cells, immune cells, fibroblasts, and endothelial cells, each characterized by distinct miRNA profiles indicative of their functional states and roles within the TME.

Furthermore, our study unveiled dynamic changes in miRNA expression over time and space within the TME, providing insights into the temporal and spatial dynamics of miRNA regulation during tumor progression and therapy response. We observed dynamic fluctuations in miRNA expression levels across different tumor regions and at various stages of tumor development, highlighting the dynamic nature of miRNA-mediated regulation in cancer biology. Additionally, we investigated miRNA-mediated cellular interaction networks within the TME, deciphering the crosstalk between cancer cells, immune cells, stromal cells, and endothelial cells mediated by miRNA communication. Our findings shed light on the intricate regulatory networks governing tumor-stroma interactions, immune evasion mechanisms, and angiogenic processes, which play crucial roles in tumor progression and therapy resistance.

Moreover, our study demonstrated the potential clinical implications of miRNA expression profiling in cancer patients. By stratifying patients into molecular subtypes based on miRNA signatures associated with specific clinical phenotypes, prognosis, and therapeutic responses, we identified potential biomarkers for predicting therapy response, disease progression, and minimal residual disease. These miRNA-based biomarkers hold promise for guiding personalized treatment strategies and early intervention in cancer therapy, ultimately improving patient outcomes and survival rates. Additionally, we addressed technical and computational challenges associated with

single-cell miRNA sequencing data analysis, including data preprocessing, normalization, dimensionality reduction, clustering, and multi-omics integration, enhancing the reliability and robustness of our findings. Overall, our study provides valuable insights into the heterogeneity, dynamics, and regulatory networks of miRNA expression in cancerous tissues, highlighting the importance of single-cell analysis in unraveling the complexity of tumor biology. By integrating miRNA expression profiling into clinical practice, we aim to advance personalized cancer therapy and improve patient outcomes.

Table-5.1: MicroRNAs (miRNAs) and their potential roles in cancer pathogenesis.

miRNA	Observed Alteration	Target	Functional Response	Reference
miR-21	Upregulated in various cancers	PTEN, PDCD4, RECK, TPM1, TGFBR2, SPRY2	Promotion of cell proliferation, invasion, and metastasis	[63]
miR-155	Upregulated in inflammation-associated cancers	SOCS1, SHIP1, CTLA-4, TP53INP1	Regulation of immune responses and inflammation	[64]
miR-34a	Downregulated in multiple cancer types	CCND1, CDK4, CDK6, MYC, BCL2	Suppression of cell proliferation and induction of apoptosis	[65]
miR-221/222	Upregulated in various cancers	CDKN1B, PTEN, TIMP3, TRPS1, TIMP2	Regulation of cell cycle progression and resistance to therapy	[66]
let-7	Downregulated in several cancer types	RAS, HMGA2, MYC, LIN28B	Tumor suppression and regulation of oncogene expression	[67]
miR-10b	Upregulated in metastatic cancers	HOXD10, HOXD11, KLF4, MAPRE1, CDH1	Promotion of cell migration and invasion	[68]
miR-200	Downregulated in epithelial-mesenchymal transition (EMT)	ZEB1, ZEB2, SIP1, VIM, FN1	Inhibition of EMT and maintenance of epithelial phenotype	[69]
miR-17-92	Amplified or overexpressed in various cancers	E2F1, PTEN, BIM, TGFBR2, THBS1	Promotion of angiogenesis and metastasis	[70]
miR-145	Downregulated in various cancers	OCT4, MYC, KLF4, FSCN1, NEDD9	Suppression of oncogenic signaling pathways	[71]
miR-200c	Downregulated in metastatic cancers	ZEB1, ZEB2, ETS1, BMI1, XIAP	Regulation of metastasis and maintenance of epithelial phenotype	[72]

The dysregulation of microRNAs (miRNAs) in cancer pathogenesis is a multifaceted phenomenon with profound implications for tumor biology and therapeutic strategies. Among the miRNAs implicated in cancer, miR-21 emerges as a significant player, being upregulated in various cancers and associated with the promotion of cell proliferation, invasion, and metastasis through the targeting of genes such as PTEN, PDCD4, and RECK. Similarly, miR-155, upregulated in inflammation-associated cancers, orchestrates immune responses and inflammation by targeting SOCS1, SHIP1, and CTLA-4. Conversely, miR-34a exerts tumor-suppressive effects by downregulating cell cycle regulators like CCND1 and inducing apoptosis through targeting CDK4 and MYC. Moreover, miR-221/222's upregulation in cancer modulates cell cycle progression and confers resistance to therapy by targeting CDKN1B and PTEN. In contrast, let-7's downregulation suppresses tumors by targeting RAS and MYC, while miR-10b's upregulation in metastatic cancers promotes cell migration and invasion via HOXD10 and KLF4. Furthermore, miR-200's downregulation during epithelial-mesenchymal transition inhibits EMT and maintains epithelial phenotype by targeting ZEB1 and SIP1. Additionally, miR-17-92's amplification in cancers promotes angiogenesis and metastasis through targeting E2F1 and PTEN. Conversely, miR-145's downregulation suppresses oncogenic signaling pathways by targeting OCT4 and MYC. Lastly, miR-200c's downregulation in metastatic cancers regulates metastasis and maintains epithelial phenotype by targeting ZEB1 and ETS1. Understanding the intricate roles of these dysregulated miRNAs and their downstream effects on target genes provides valuable insights into cancer biology and offers promising avenues for the development of diagnostic biomarkers and targeted therapies aimed at mitigating tumor progression and metastasis.

Table-5.2: Advantages and disadvantages of using microRNAs (miRNAs) in cancer research and therapy.

Aspect	Advantage	Disadvantage	Reference
Diagnostic Biomarkers	- High stability: miRNAs are resistant to degradation by RNases and can be detected in various biological fluids, making them ideal candidates for non-invasive diagnostic tests. - Specificity: Altered miRNA expression profiles are associated with specific cancer types, allowing for the development of highly specific diagnostic biomarkers. - Accessibility: miRNA expression can be easily measured using minimally invasive techniques such as blood draws or fine-needle aspirates.	- Lack of specificity: Some miRNAs may be dysregulated in multiple cancer types or in non-cancerous conditions, leading to potential false-positive results. - Variability: miRNA expression levels may vary between individuals, limiting the accuracy and reproducibility of diagnostic tests. - Standardization: Lack of standardized protocols for sample collection, processing, and analysis may affect the reliability of miRNA-based diagnostic tests.	[73]
Prognostic Biomarkers	- Prognostic value: Altered miRNA expression profiles are associated with clinical outcomes such as overall survival, disease recurrence, and treatment response, providing valuable prognostic information for cancer patients. - Dynamic changes: miRNA expression levels may change in response to treatment or disease progression, allowing for real-time monitoring of patient outcomes. - Personalized medicine: miRNA signatures can stratify patients into different risk groups and guide personalized treatment strategies.	- Limited predictive power: While miRNA expression profiles may correlate with clinical outcomes, they may not always accurately predict individual patient responses to therapy or disease progression. - Heterogeneity: Tumor heterogeneity may affect the prognostic value of miRNA biomarkers, as miRNA expression profiles may vary between different tumor subclones or metastatic sites. - Validation: Large-scale validation studies are needed to confirm the prognostic value of miRNA biomarkers and establish standardized cutoff values for risk stratification.	[74]
Therapeutic Targets	- Aberrant expression: Dysregulated miRNA expression is implicated in cancer pathogenesis, making miRNAs attractive therapeutic targets for	- Delivery challenges: Efficient delivery of miRNA-based therapeutics to tumor cells remains a major challenge due to issues	[75]

	cancer therapy. - Target specificity: miRNAs can be targeted using complementary antisense oligonucleotides (anti-miRs) or miRNA mimics, allowing for precise modulation of gene expression. - Combination therapy: miRNA-based therapies can be combined with conventional treatments such as chemotherapy or targeted therapy to enhance treatment efficacy and overcome therapy resistance.	such as off-target effects, immune response, and poor cellular uptake. - Off-target effects: miRNA-based therapies may affect unintended target genes or pathways, leading to potential side effects or toxicity. - Resistance mechanisms: Tumor cells may develop resistance to miRNA-based therapies through mechanisms such as mutations in target sites or alterations in miRNA processing pathways, limiting their long-term efficacy.	
Functional Studies	- Regulatory networks: miRNAs regulate gene expression by targeting specific mRNAs, providing valuable insights into the molecular mechanisms underlying cancer pathogenesis. - High-throughput screening: Genome-wide miRNA expression profiling and functional studies allow for the identification of novel miRNA-mRNA interactions and regulatory networks involved in cancer biology. - Disease modeling: miRNA-based animal models or cell-based assays can be used to study the functional roles of miRNAs in cancer development, progression, and therapy response.	- Complexity: miRNA-mediated gene regulation is complex and context-dependent, making it challenging to decipher the precise roles of individual miRNAs in cancer biology. - Validation: Functional studies may require extensive validation to confirm the biological relevance of miRNA-target interactions and their contribution to cancer pathogenesis. - In vivo relevance: Findings from cell-based assays or animal models may not always translate to human cancer biology, highlighting the need for complementary approaches and clinical validation studies.	[76]

Discussion

The findings presented in this study shed light on the complex interplay between microRNAs (miRNAs) and cancer pathogenesis, providing valuable insights into the regulatory mechanisms underlying tumor development and progression. By examining the observed alterations, target genes, and functional responses of key miRNAs implicated in various aspects of cancer biology, this study offers a comprehensive understanding of their roles in driving or suppressing

tumorigenesis. One of the notable observations is the dysregulation of specific miRNAs across different cancer types, highlighting their broad impact on oncogenic processes. For instance, miR-21 emerges as a common player in promoting cell proliferation, invasion, and metastasis, while miR-34a exerts tumor-suppressive effects by inhibiting cell cycle progression and inducing apoptosis. These findings underscore the diverse functional roles of miRNAs in modulating critical cellular processes involved in cancer initiation and progression. Moreover, the study elucidates the intricate regulatory networks orchestrated by dysregulated miRNAs, revealing the cross-talk between miRNAs and their target genes. For example, miR-155 regulates immune responses and inflammation by targeting genes involved in immune cell function, while miR-200 inhibits epithelial-mesenchymal transition (EMT) and maintains epithelial phenotype through the modulation of EMT-related genes. These findings underscore the importance of miRNA-mediated gene regulation in shaping the tumor microenvironment and influencing tumor-stroma interactions. Furthermore, the study highlights the potential diagnostic and therapeutic implications of dysregulated miRNAs in cancer. The identification of specific miRNA expression patterns associated with distinct cancer types or clinical outcomes offers promising opportunities for the development of non-invasive biomarkers for cancer diagnosis, prognosis, and treatment response prediction. Additionally, targeting dysregulated miRNAs holds therapeutic promise for precision medicine approaches, with the potential to modulate oncogenic signaling pathways and overcome therapy resistance. However, several challenges and limitations need to be addressed to translate these findings into clinical practice effectively. Standardization of miRNA profiling techniques, validation of biomarkers in large patient cohorts, and optimization of miRNA-based therapeutic strategies are essential steps towards realizing the clinical utility of miRNAs in cancer management. This study advances our understanding of the role of miRNAs in cancer pathogenesis and underscores their potential as diagnostic biomarkers and therapeutic targets. Further research efforts aimed at elucidating the molecular mechanisms underlying miRNA dysregulation and exploring innovative therapeutic strategies are warranted to harness the full potential of miRNAs in precision cancer medicine.

CHAPTER SIX

CONCLUSION

6.0 Conclusion

In conclusion, our study highlights the significance of single-cell analysis in elucidating the complexity of microRNA (miRNA) expression within cancerous tissues. Through this approach, we have uncovered diverse cellular subpopulations, dynamic miRNA expression patterns, and intricate interaction networks within the tumor microenvironment (TME). These findings provide valuable insights into tumor heterogeneity, progression, and therapy response, paving the way for personalized treatment strategies in cancer therapy. Moving forward, integrating miRNA-based biomarkers and therapeutic targets identified through single-cell analysis into clinical practice holds promise for improving patient outcomes and advancing precision oncology.

CHAPTER SEVEN

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