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MICRORNAS IN CANCER: ONCOMIRS, TUMOR SUPPRESSORS, BIOMARKERS AND THERAPEUTIC STRATEGIES

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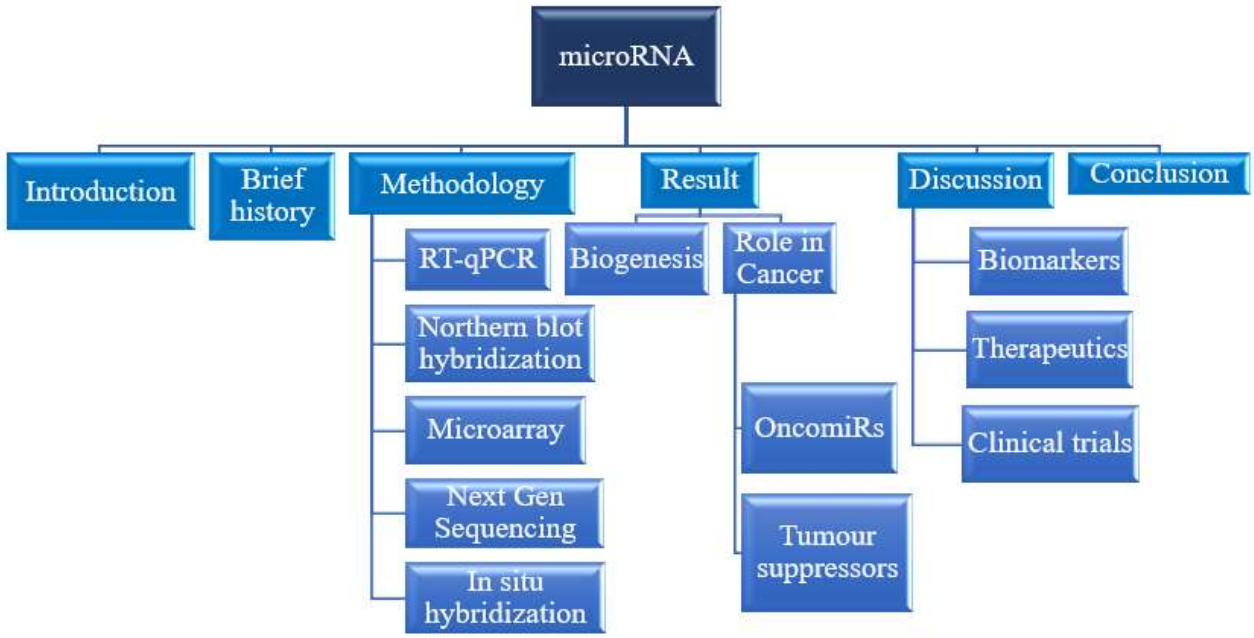
Abstract
MicroRNAs (miRNAs) are small non-coding RNAs that play a significant role in various biological and pathological processes, including cell proliferation and apoptosis. Several miRNAs have been implicated in human cancer through methods such as Northern blot analysis, qRT-PCR, miRNA microarray and Next Generation Sequencing. Research has shown that miRNAs can function as either oncogenes or tumor suppressors making them potential targets for innovative therapeutic strategies. Despite their potential, miRNA-based therapeutics faces significant challenges and setbacks in clinical trials. Significant issues include the need to reduce immunogenic reactions and determine optimal dosing for effective therapeutic outcomes. A thorough risk evaluation of miRNA therapeutics is essential to prevent overdosing and reduce off-target effects. Nevertheless, the therapeutic potential of miRNAs for cancer is clear and further research is necessary to establish their efficacy and safety as clinical treatments, paving the way for miRNA-based therapies to become viable options in cancer treatment. This review discusses the role of miRNAs as oncogenes or tumor suppressors, their potential as biomarkers and their therapeutic strategies.
Keywords: MicroRNAs, cancer, oncogenes, therapeutic strategies, clinical trials.

Introduction

MicroRNAs are small, non-coding RNAs that consist of ~23 nucleotides and are primarily involved in mRNA regulation by promoting its degradation and also in balancing the protein levels (Wang *et al.,* 2016; Etheridge *et al.,* 2011; Chevillet *et al.,* 2014; Kai *et al.,* 2018). They were discovered in 1993 and were described as "mediators of temporal pattern formation" in *Caenorhabditis elegans* (Wightman *et al.,* 1993; Lee *et al.,* 1993; Lee *et al.,* 2001; Lau *et al.,* 2001; Hydbring *et al.,* 2013). Studies have shown that miRNA sequences comprise up to 1% of the human genome (Friedman *et al.,* 2009).The principal function of miRNAs in humans is to regulate gene expression (O'Brien *et al.,* 2018) by mRNA degradation and regulation of transcription and translation through both canonical and non-canonical mechanisms (Kai *et al.,* 2018). The canonical mechanism involves the binding of miRISC, containing the miRNA guide strand, to the 3′-UTR of the target mRNA (Chevillet *et al.,* 2014). This occurs according to the miRNA's seed sequence (the first 2-7 nucleotides from the 5′ end). This binding initiates mRNA deadenylation, translation repression, and eventually, degradation (Bartel, 2009; Huntzinger *et al.,* 2011; Eichhorn *et al.,* 2014). However, approximately 60% of miRNA-mRNA interactions in human cells are non-canonical (Helwak *et al.,* 2013), suggesting that their chains are not always complementary (Jonas *et al.,* 2015). This suggests that a single miRNA can target many mRNAs and a single mRNA can have multiple miRNA binding sites, possibly regulating various biological processes (Chevillet *et al.,* 2014).One of the major global health concerns is cancer. Cancer development involves five important stages which include initiation, promotion, malignant conversion, progression and metastasis. Various oncogenes and tumor suppressor genes have been identified but the molecular mechanisms responsible for cancer development and its prevention remain evasive. Remarkably, more than 50% of miRNA genes are located in cancer-associated genomic regions or fragile sites (Calin *et al.,* 2004), implying a major role of miRNAs in cancer pathogenesis.Currently, thousands of miRNAs have been predicted across animals, plants and viruses by implementing various methods (Zhang *et al.,* 2006b) which include experimental techniques (Lee *et al.,* 2001), computational approaches (Brown *et al.,* 2005), and expressed sequence tag (EST) and genomic survey sequence (GSS) analysis (Zhang *et al.,* 2005; Zhang *et al.,* 2006a). However, only a few have been experimentally proven (Griffiths-Jones *et al.,* 2006). The computational evaluation

shows that miRNAs may add up to more than 1% of the total protein-coding genes (Lai *et al.,* 2003; Lim *et al.,* 2003a; Lim *et al.,* 2003b) and over 30% of protein-coding genes may be miRNA targets (Berezikov *et al.,* 2005; Lewis *et al.,* 2005).Recent developments have elevated our understanding of the origins, functions and therapeutic applications of miRNA. Studies have revealed that levels of some circulating miRNAs involved in angiogenesis and cardiac muscle contractility are determined by the magnitude and duration of exercise, thereby specifying their role in mediating the physiological cardiac adaptation to exercise (Baggish *et al.,* 2011; Clauss *et al.,* 2016; Cui *et al.,* 2017). Furthermore, miRNAs play an important role in creating induced pluripotent stem cells (iPS) where they are repressed by reprogramming factors, converting average fibroblasts into iPS cells (Ranganathan *et al.,* 2014).Nevertheless, the most promising role of miRNAs is their prospect as biomarkers. Evidence indicates their important role as biomarkers in cancer through exosome-mediated intercellular communication (Wong *et al.,* 2019; Zhang *et al.,* 2018; Gao *et al.,* 2018), in neurology for the diagnosis and prognosis of Alzheimer's disease (Wiedrick *et al.,* 2019), spinal cord injury (Tigchelaar *et al.,* 2019), epilepsy (Raoof *et al.,* 2018) or neurodegenerative diseases (Sheinerman *et al.,* 2013). This review provides a concise overview of miRNA biogenesis and highlights their dual roles as oncogenes and tumor suppressors with specific examples. It also explores the potential of miRNAs as biomarkers and their possible therapeutic strategies and their applications, citing some notable clinical trials. Finally, it concludes by addressing the current challenges faced in miRNA-based therapies and outlining their prospects.



Brief History

In 1993, Lee *et al.,* made a groundbreaking discovery that significantly advanced our understanding of gene regulation. They identified microRNAs (miRNAs), a class of non-coding RNAs that play a crucial role in the regulation of gene expression, in the nematode *Caenorhabditis elegans*. They found that the down regulation of the lin-14 protein in these organisms was essential for the transition from the first larval stage (L1) to the second larval stage (L2). This process depended on the transcription of another gene, lin-4. Intriguingly, the lin-4 gene did not encode a protein but instead produced two small RNAs, approximately 21 and 61 nucleotides long. The longer sequence formed a stem-loop structure, serving as a precursor for the shorter RNA. Subsequent studies by Lee *et al.,* 1993 and Wightman *et al.,* 1993 revealed that the smaller RNA had antisense complementarity to multiple sites in the 3′ untranslated region (UTR) of lin-14 mRNA and this binding between complementary sites diminished lin-14 protein levels without changing mRNA levels. These studies proposed a model in which base pairing between numerous lin-4 small RNAs and the complementary sites in the 3′ UTR of lin-14 mRNA causes translational repression of lin-14 thereby facilitating progression from L1 to L2 in *C. elegans* development. Initially, this mechanism of gene regulation was thought to be unique to *C. elegans* but later in 2000, two independent groups discovered that a small RNA called let-7 was important for transitioning from a later larval stage to adult in *C. elegans* (Reinhart *et al.,* 2000; Slack

et al., 2000). Homologs of let-7 were discovered in many organisms, including humans (Pasquinelli *et al.*, 2000), marking a pivotal expansion of miRNA research. Following these discoveries, there was a huge surge in research where numerous laboratories started cloning many small RNAs from humans, flies and worms. These non-coding RNAs, 19 to 24 nucleotides long, were derived from a long precursor with a stem-loop structure (Bartel, 2004). It was found that many of these miRNAs were evolutionary conserved across species and showed cell-type specificity. This recognition and validation of miRNAs led to extensive research aimed at identifying new miRNAs which resulted in the discovery of numerous miRNAs across different species of plants and animals. In 2002, the miRNA registry, miRBase, was established as the primary online repository for miRNA sequences, annotation, nomenclature, and target prediction information (Griffiths-Jones, 2004; Griffiths-Jones *et al.*, 2008). The latest edition, miRbase version 22, includes 38,589 entries representing hairpin precursor miRNAs that express 48,885 mature miRNA products across 271 different species. Despite these advances, the biological significance of many annotated miRNAs remains unknown and requires further validation.

Methodology

Research has explored molecular testing to improve the diagnostic process and create effective treatment plans. The goal is to identify miRNAs involved in common diseases worldwide and discover new ones associated with these conditions. However, this endeavour has proven challenging due to the recent emergence of miRNA research. There are still uncertainties regarding detection limits, the range of concentrations in body fluids, and how these parameters change with factors such as age, gender and health status (Gillespie *et al.,* 2018).

RT-qPCR:

Quantitative reverse transcriptase PCR (RT-qPCR) is widely considered the gold-standard method for quantifying miRNAs (Gillespie *et al.,* 2018; Chen *et al.,* 2018; Yuan *et al.,* 2018). The primary PCR technique used is stem-loop RT-based TaqMan microRNA assay, which is known for its high sensitivity and specificity (Chen *et al.,* 2011). It is a two-step process, in which the first step requires the binding of miRNA molecules with primers at their 3'-end, followed by stem-loop reverse transcription. The second step involves real-time PCR to quantify the targeted miRNAs (Chen *et al.,* 2005).

Other available methods include direct RT-based and poly (A) tailing-based SYBR miRNA assays (Chen *et al.,* 2011), but these techniques may have sensing errors for the samples used and contamination risk during the amplification steps (Gillespie *et al.,* 2018).

Northern blot hybridization:

Another method for quantitative assessments of miRNAs is northern blot hybridization, which involves the separation of total RNA on a polyacrylamide gel, that possesses the property of denaturation, followed by its transfer on a nylon membrane after which the RNA undergoes UV cross-linking and finally, hybridization with a radioactive substance (Pacak *et al.,* 2016; Barciszewska-Pacak *et al.,* 2015; Kruszka *et al.,* 2014; Smoczynska *et al.,* 2019). However, this technique is labor-intensive, requires large amounts of RNA and may omit rare types of miRNAs (Smoczynska *et al.,* 2019). Efforts have been made to improve the method, thereby leading to the usage of lower quantities of RNA and shorter execution times (Pall *et al.,* 2008; Wang *et al.,* 2010; Varallyay *et al.,* 2007).

miRNA microarray:

While northern blotting is widely used for miRNA analysis, it has limitations, which include unequal hybridization efficiency (Thomson *et al.,* 2004), and difficulty in detecting multiple miRNAs simultaneously. It is important to compare miRNA expression patterns between cancerous and normal cells in cancer research. Thereby, suggesting that it is more useful to have methods that detect all miRNA expressions at the same time. Two-colour fluorescence-based microarray technology (DNA microarray) has been adapted for miRNA detection. Modified DNA microarray technology is used by various laboratories to form miRNA microarray technology (Babak *et al.,* 2004; Barad *et al.,* 2004; Liang *et al.,* 2004; Liu *et al.,* 2004; Nelson *et al.,* 2004; Thomson *et al.,* 2004). Thomson *et al.,* 2004 developed a custom dual-channel miRNA microarray platform and used it to study the expression levels of 124 mammalian miRNAs. They found that the expression patterns of miRNAs are quite different between adult mouse tissue and embryonic stem cells (Thomson *et al.,* 2004). Lu *et al.,* 2005

generated a strategy to detect miRNA expression profiles in various human cancers. To solve the issues about probe specificity in miRNA microarray analysis, they performed hybridization in solution after which they quantified the polymer heads which were hybridized to miRNA species using multicolour flow sorting. This method can be used to find out miRNA expression profiles even in poorly differentiated tumors in cancers. This method helps us to enhance our understanding of the relationship between cancerous and normal tissues (Liang *et al.,* 2005; Liu *et al.,* 2004). Additionally, it has been extensively used to examine miRNA roles in cancer (Calin *et al.,* 2005; Iorio *et al.,* 2005; Lu *et al.,* 2005) and has identified several miRNA oncogenes and tumor suppressors.

***In situ* hybridization (ISH) and Next-Generation Sequencing (NGS):**

These are additional methods for identifying miRNAs. *In situ* hybridization uses radioactive, fluorescent or dioxygenin probes to bind the target RNA allowing comparison of miRNA expression in different cells. However, it is labour-intensive, time-consuming and susceptible to errors (Javelle *et al.,* 2012). Next-generation sequencing is a highly accurate technique capable of detecting single miRNAs with the accuracy of one nucleotide, but its high costs limit its broader accessibility (Smoczynska *et al.,* 2019).

Result

Biogenesis of miRNA:

An overview of miRNA biogenesis is illustrated in **Fig 1**. Initially, miRNA transcripts are transcribed by RNA polymerase II as long primary miRNAs (pri-miRNAs), which are several hundred nucleotides long and feature a 5′ guanosine cap and a 3′ polyadenylated tail. These can be non-coding or reside within the introns of coding genes. The pri-miRNA is then processed into ~70- to 120-nucleotide precursor RNA (pre-miRNA) by a multiprotein complex known as Microprocessor (**Fig. 1A**). This complex includes Drosha, a ~160-kDa nuclear RNase III enzyme (Lee et al., 2003) that is highly conserved in animals but not in plants (Wu *et al.,* 2000). Drosha dimerizes with DGCR8 (also known as Pasha) to form the functional Microprocessor complex (**Fig. 1A**) (Denli *et al.,* 2004; Gregory *et al.,* 2004; Han *et al.,* 2004; Landthaler *et al.,* 2004). The transcribed pre-miRNA, characterized by a 5′ phosphate and a ~2-nucleotide 3′ overhang, is then transported to the cytoplasm by exportin 5 (Exp-5), a Ran-dependent nuclear transport receptor (**Fig. 1B**) (Lund *et al.,* 2004; Yi *et al.,* 2003). In the cytoplasm, the pre-miRNAs are further processed into mature ~18- to 23-nucleotide duplexes by Dicer-1 with the help of protein kinase RNA activator and transactivation response RNA binding protein (**Fig. 1C, D**) (Chendrimada *et al.,* 2005; Lee *et al.,* 2006; Lee *et al.,* 2002). The two miRNA strands are separated based on factors like thermodynamic asymmetry and stability of base pairing at the 5′ end. One strand, known as the guide strand, combines with Argonaute (AGO) proteins and other RNA-binding proteins forming an RNA-induced silencing complex (RISC) (**Fig. 1E**) (Schwarz et al., 2003). The miRNA strand with stable base pairing at the 5′ end (known as the passenger or miR* strand) is usually degraded (Czech *et al.,* 2009; Okamura *et al.,* 2009). The guide strand, typically the one with the most unstable base pairing at the 5′ end, directs the complex to the mRNA target through sequence complementarity, resulting in its degradation or translational repression (**Fig. 1F**). Ago2 proteins have been localized to P-bodies where miRNAs with their mRNA targets are stored for degradation or translational repression (Castilla-Llorente *et al.,* 2012). Recent evidence also indicates that miRNA biogenesis can occur independently of the Microprocessor. Examples include pre–miRNA-like hairpins called “Mirtrons” that form from spliced and debranched short hairpin introns, as well as some small nucleolar RNAs (snoRNAs) and endogenous short hairpin RNAs (shRNAs) (Babiarz *et al.,* 2008; Ender *et al.,* 2008; Okamura *et al.,* 2007; Saraiya *et al.,* 2008).

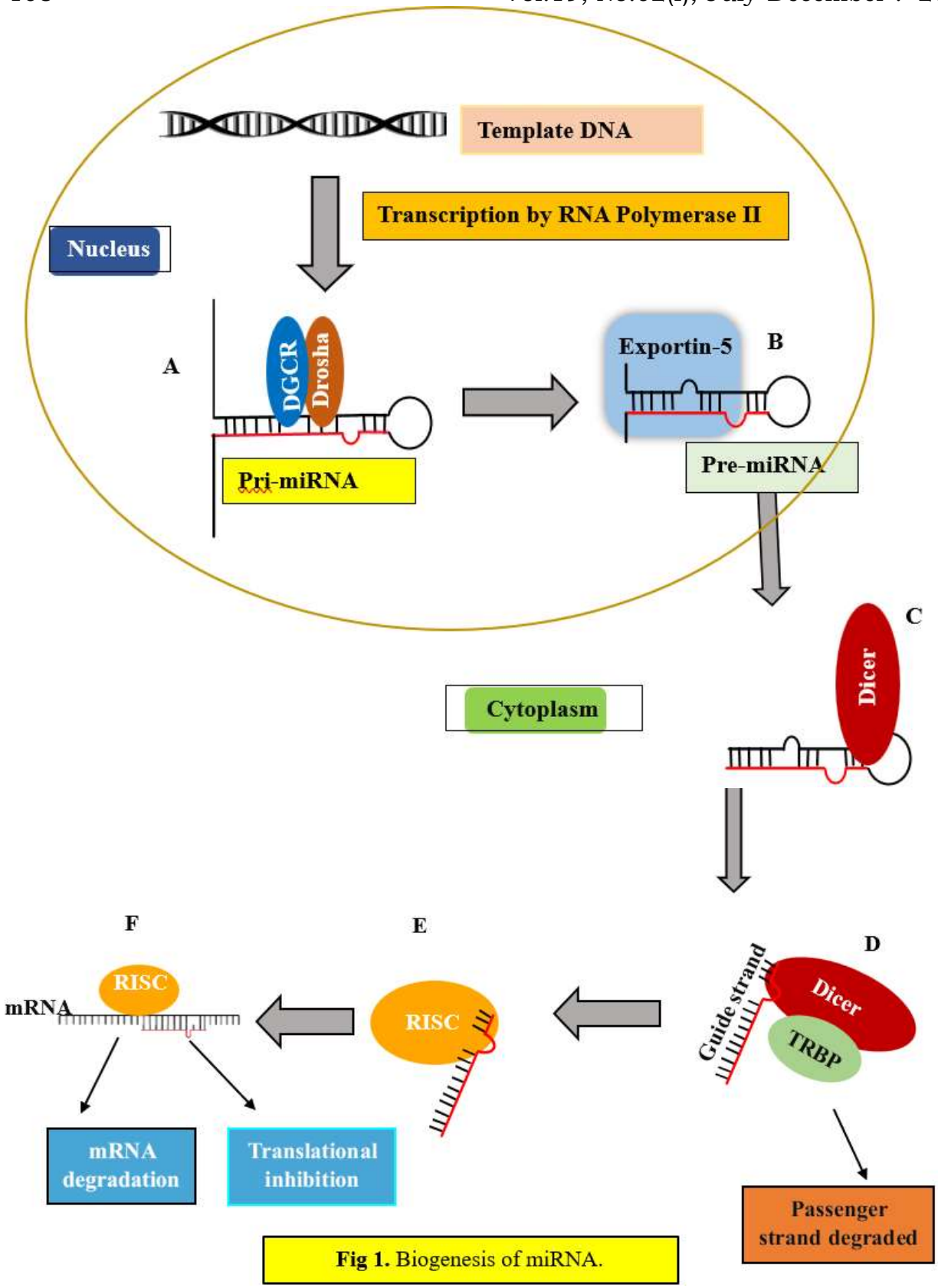


Fig 1. Biogenesis of miRNA.

Role of miRNAs in Cancer

Oncogenic miRNAs (OncomiRs):

OncomiRs are miRNAs that are overexpressed in tumors, leading to the repression of tumor suppressor mRNAs therefore promoting tumor cell proliferation and metastasis (**Figure 2**) (Svoronos *et al.,* 2016). They can down regulate the expression of pro-apoptotic genes, thus inhibiting programmed cell death and promoting cell survival, a key characteristic of cancer. Some oncomiRs also promote angiogenesis by targeting genes involved in angiogenic signaling pathways, thereby facilitating tumor growth and metastasis (Chakrabortty *et al.,* 2023). Numerous oncomiRs with various roles in cancer growth have

been identified. The miR-17-92 cluster (miRs-17, -18a, -19a, -20a, -19b, and -92a) down regulates. PTEN (phosphate and tensin homolog), E2F, the transforming growth factor-β (TGF-β) signalling pathway, B cell lymphoma/leukaemia 2-like protein 11 (BCL2L11) and thrombospondin-1 (TSP-1) are down regulated by the miR-17-92 cluster which includes miR-17, miR-18a, miR-19a, miR-19b, miR-20a, and miR-92a [**103**]. This cluster promotes tumor growth and is overexpressed in small-cell lung cancer, colon cancer, hepatocellular carcinoma, thyroid cancer, colorectal adenoma organoids, and renal cell carcinoma (Zhu *et al.,* 2015; Tsuchida *et al.,* 2011; Takakura *et al.,* 2008; Hayashita *et al.,* 2005; Martens-de *et al.,* 2022; Lu *et al.,* 2022).Additionally, miR-21 is associated with the down regulation of PTEN, Tropomyosin 1 (TPM1), and programmed cell death 4 (PDCD4). Overexpression of miR-21 has been reported in various cancers, including breast, ovarian and colon cancer (Menon *et al.,* 2022; Ozgun *et al.,* 2013; Echevarria-Vargas *et al.,* 2014).

Tumor-suppressor miRNAs (TS-miRNAs):

Tumor-suppressor miRNAs are miRNAs that inhibit cancer progression (**Figure 2**) (Svoronos *et al.,* 2016). Their down regulation promotes cancer development and proliferation. They can directly target and inhibit the expression of oncogenes, which promote tumorigenesis when overexpressed or mutated (Calin *et al.,* 2004). They are often located in cancer-associated genomic regions or fragile sites, making them more susceptible to mutations. Their down regulation can result from dysfunctional proteins involved in their biogenesis or genetic alterations (Macfarlane *et al.,* 2010). Reduced expression of key miRNA biogenesis components such as Drosha, DiGeorge Critical Region 8 (DCGR8) and Dicer significantly decreases miRNA production leading to a more transformed cell phenotype (Yang *et al.,* 2010; Qu *et al.,* 2016; Baradaran *et al.,* 2019; Wen *et al.,* 2011; Zhao *et al.,* 2018; Link *et al.,* 2016).For instance, loss of TS-miRNA, miR-16, is associated with the progression of chronic lymphocytic leukemia, gastric, prostate, and pancreatic tumors (Xia *et al.,* 2008; Shen *et al.,* 2012; Musumeci *et al.,* 2011; Cimmin *et al.,* 2005; Calin *et al.,* 2002). The Let-7 family miRNAs are tumor suppressors that target the Ras and Myc oncogenes (Johnson *et al.,* 2005). Ectopic expression of the Let-7 miRNA family has been shown to induce cell death in lung cancer cells (Kumar *et al.,* 2008). Korourian *et al.,* 2017 revealed that miR-31 functions as a tumor suppressor gene in gastric cancer tumorigenesis. It down regulates RhoA, thus decreasing carcinogenesis. This study suggested that increasing miR-31 levels could serve as a potential therapeutic target for the treatment of gastric cancer in the future.

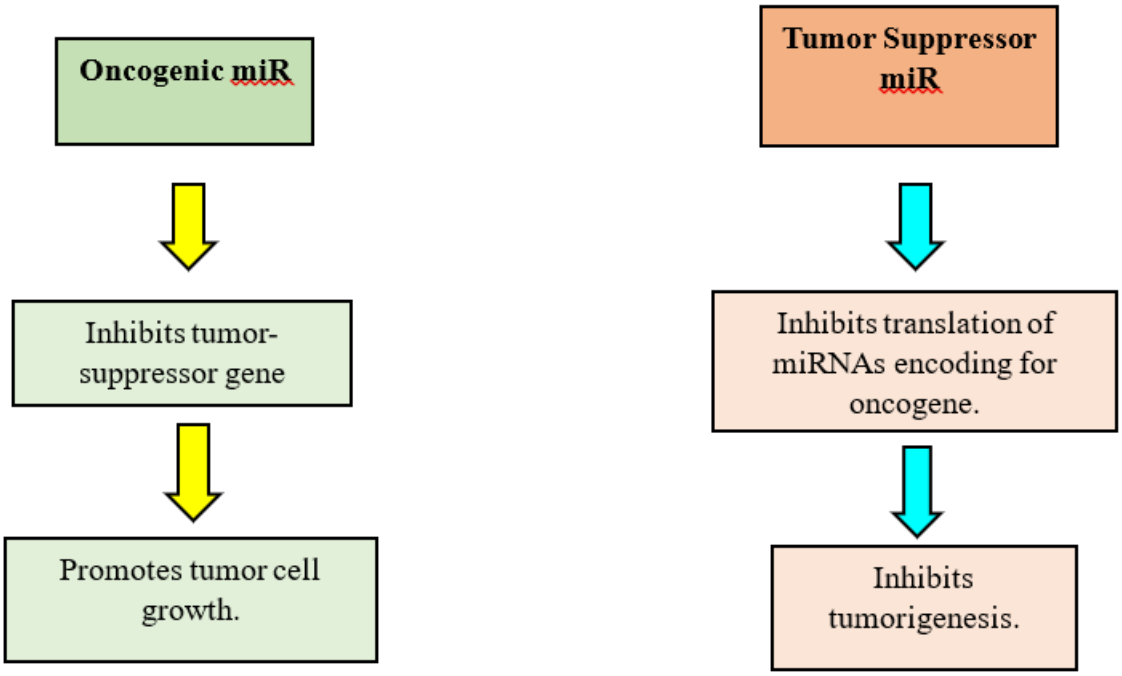


Fig 2. miRNAs as oncomiRs and tumor suppressors.

Discussion

miRNAs as Potential Cancer Biomarkers:

Advancements in techniques like microRNAome sequencing, microRNA-specific quantitative PCR and *in vivo* antisense technologies are believed to greatly influence clinical oncology in the future. Identifying sensitive and specific biomarkers, especially those detectable in body fluids, is essential for the early diagnosis of cancer. Early detection through minimally invasive screening tests can significantly enhance treatment effectiveness and reduce healthcare costs. Current research indicates the important role of miRNAs in cancer development and progression, making them valuable tools as cancer biomarkers. Studies have demonstrated that miRNAs can be stably detected in plasma and serum. Due to their release into circulation and remarkable stability, specific miRNA levels in plasma and other biological fluids can serve as diagnostic and prognostic biomarkers for various diseases, including cancer (**Figure 3**) (Takasaki, 2014). The challenge in using miRNAs as diagnostic tools, however, lies in detecting specific miRNAs of interest accurately. A novel DNA nanomachine was developed, recently, to selectively visualize miR-21 in cancer cells (Agarwal et al., 2021). This nanomachine uses a zeolite imidazole framework-8 (ZIF-8) metal-organic framework (MOF) combined with two hairpin probes (Y1 and Y2) labelled with fluorescent dye for signal amplification. In the acidic extracellular environment, the MOF decomposes, releasing the hairpin molecules. These molecules are then captured by targeted miR-21, triggering catalytic hairpin assembly (CHA) amplification. This allows the detection of the miRNA at a remarkably low sensitivity (27pM) (Kai *et al.,* 2018). Such advancements highlight the potential of targeting miRNAs to develop highly sensitive diagnostics for cancer cells (Yao *et al.,* 2022). Comparative analyses of miRNA expression profiles between healthy tissues and tumor samples have revealed unique patterns that can distinguish tumor from non-tumor cells indicating that miRNA expression profiling could serve as an efficient diagnostic tool (Yanaihara *et al.,* 2006).

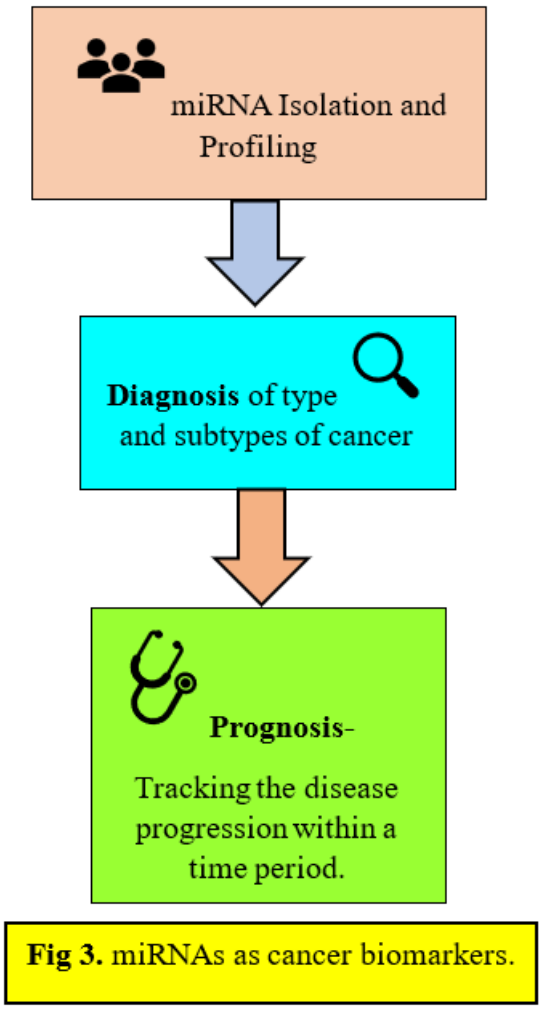


Fig 3. miRNAs as cancer biomarkers.

a. Diagnosis:

Circulating miRNAs have emerged as promising biomarkers for the diagnosis and early detection of various cancers. They are readily available in body fluids such as urine, plasma and saliva, making them suitable for non-invasive diagnosis. For example, urinary miRNA biomarkers have shown

significant potential for early detection of colorectal cancer. A study demonstrated that levels of miR-129-1-3p and miR-566 were significantly increased in urine samples from patients in primary tumor tissues compared to normal tissues (Iwasaki *et al.,* 2022). Similarly, exosomal miRNAs have been investigated for their diagnostic potential. Exosomes are small vesicles secreted by cells that contain various molecular components, including miRNA. A study revealed that elevated levels of exosomal miR-23a and miR-1246 could detect colon cancer in its early stages and differentiate between healthy and malignant tissues (Ogata-Kawata *et al.,* 2014). This highlights the utility of exosomal miRNAs as biomarkers for early cancer diagnosis, offering a minimally invasive diagnostic tool. In melanoma, circulating plasma miRNAs have shown promise as potential biomarkers. Levels of miR149-3p, miR150-5p and miR193a-5p were reported to be significantly higher in plasma samples from melanoma patients compared to healthy individuals (Fogli *et al.,* 2017). Oesophageal cancer is another area where miRNA biomarkers in body fluids have been explored. Overexpression of miR-144, miR-10b, miR-451 and miR-21 was reported in saliva from oesophageal cancer patients compared to healthy patients (Xie *et al.,* 2013). Detecting miRNAs in saliva offers a non-invasive and easily accessible method for early cancer diagnosis, which could significantly improve patient prognosis.

b. Prognosis:

MicroRNA (miRNA) expression profiles have emerged as important clinical tools, providing valuable prognostic information about patient outcomes. These profiles can correlate with survival rates across different tumor types, even in early pathological conditions. For example, low levels of the let-7 miRNA family and high levels of miR-155 are associated with poor prognosis in lung cancer (Yanaihara *et al.,* 2006). Let-7 miRNAs are known to function as tumor suppressors and their reduced expression is linked to increased tumor growth. MiR-155 is often up regulated and is implicated in promoting tumor growth and metastasis. In another study on lung cancer, high levels of miR-137, miR372 and miR-182 correlated with poor prognosis, while high levels of miR-221 and let-7a appear to have protective effects. These miRNA expression profiles not only provide prognostic information but also serve as predictors of tumor recurrence (Yu *et al.,* 2008). In ovarian cancer patients, high levels of circulating cell-free miRNAs (cf-miRNA), such as miR-92a, miR-200c, miR-320b, miR-320c, miR-335, miR-375 and miR-486, are significantly associated with adverse clinical features, suggesting that these cf-miRNAs can serve as independent prognostic markers (Gahlawat *et al.,* 2022). The potential of cf-miRNAs as independent prognostic markers is significant. They offer a minimally invasive method to monitor disease progression, assess treatment efficacy and predict patient outcomes.

c. Tumor Staging:

MicroRNAs (miRNAs) provide crucial clinical information by differentiating disease stages, including localized and metastatic cancer. Their expression profiles correlate with disease progression, helping to choose the right therapeutic interventions and monitor strategies based on the patient's unique miRNA expression profile. In prostate cancer, overexpression of miR-141, miR-200 and miR-375 is associated with increased tumor aggressiveness and metastasis (Farran *et al.,* 2018; Shukla *et al.,* 2017). Similarly, in colon cancer, elevated levels of miRNA-122 and some miRNA-200 family members in the plasma of patients are indicative of metastatic disease (Maierthaler *et al.,* 2017).

d. Treatment Resistance:

miRNAs serve as valuable indicators of sensitivity or resistance to chemotherapeutic agents, providing information that can guide treatment strategies. For example, overexpression of miR-34a, miR-205 and miR-31 is associated with increased sensitivity to taxanes. Contrarily, miR-106b overexpression is linked to radiotherapy resistance, while miR-449a overexpression increases sensitivity to it (Li *et al.,* 2011; Ni *et al.,* 2017; Thieu *et al.,* 2014). Triple-negative breast cancers (TNBC) are particularly aggressive and rapidly develop chemotherapy resistance. Genotoxic treatments like doxorubicin (Dox) significantly increase miR-181a expression in TNBC cells, which in turn promote metastasis. This miRNA is regulated by the transcription factor STAT3. BAX, a direct target of miR-181a, is suppressed, reducing apoptosis and promoting cell invasion. Consequently, elevated miR-181a levels contribute to chemotherapy resistance and increased metastatic potential in TNBC.Targeting miR-181a with specific antagonists could potentially increase TNBC cell sensitivity to chemotherapy and reduce metastasis. By inhibiting miR-181a, the expression of BAX would be restored, promoting apoptosis

and reducing cell invasion. This approach represents a promising therapeutic strategy to overcome chemotherapy resistance in TNBC and improve patient outcomes (Niu *et al.,* 2016).

Various miRNAs Therapeutic Strategies:

miRNA-based therapies:

Endogenous miRNAs are essential for maintaining cellular homeostasis by regulating gene expression. In cancer cells, genomic and transcriptomic alterations can disrupt miRNA expression profiles, leading to widespread transcriptional changes. These disruptions can result in the up regulation of oncogenes and/or down regulation of tumor suppressors, facilitating metastasis (Bartel, 2009).Most miRNA-targeted cancer therapies focus on restoring or inhibiting dysregulated miRNAs (Raue *et al.,* 2021). Restoration involves reintroducing miRNAs that are down regulated in cancer cells to inhibit oncogene expression, while inhibition targets overexpressed miRNAs to prevent the suppression of tumor suppressor genes. Recently, miRNA-based detargeting strategies have been used for cell and tissue-specific targeted therapies (Dhungel *et al.,* 2018). This innovative approach utilizes the binding sites of down regulated miRNAs in cancer cells to prevent the therapy from targeting normal cells, thus minimizing off-target effects. This strategy is particularly beneficial for genetic therapies that involve therapeutic gene transfer.

The above-mentioned approaches are detailed below:

1. miRNA replacement:

Restoring down regulated miRNAs in cancer is one approach to target metastasis. This can be achieved using miRNA mimics (Hosseinahli *et al.,* 2018), which are small synthetic RNA duplexes containing an antisense strand identical to the endogenous miRNA. The sense strand can be chemically modified to increase stability and enhance cellular uptake. The sense strand may also contain many mismatches to minimize off-target effects. These mimics are loaded into the RISC complex and inhibit downstream targets (**Fig. 4a**) (van Rooji *et al.,* 2014). MiRNA mimics have been widely studied for therapeutic purposes in both *in vitro* and *in-vivo* cancer models. For example, a combined delivery of miR-195-5p, miR-101-3p and miR-338-5p mimics effectively reduced tumor growth and the number of metastatic nodules in animal models of lung cancer (Liu *et al.,* 2021).

2. miRNA inhibition:

Another approach is to inhibit up regulated oncomiRs, restoring silenced tumor suppressors (Nguyen *et al.,* 2017).MiRNA inhibitors are single-stranded oligonucleotides complementary to an endogenous miRNA. These inhibitors bind to the miRNA and prevent its incorporation into the RISC complex (**Fig. 4b**) (Rupaimoole *et al.,* 2017). Several types of miRNA inhibitors have shown therapeutic advantages both *in vitro* and *in vivo* including antisense oligonucleotides (ASOs) (Ge *et al.,* 2019), antagomirs (Xie *et al.,* 2020), miRNA sponges (Tay *et al.,* 2015), miRNA masks (Zhang *et al.,* 2016), locked nucleic acid (LNA) anti-miRNAs (Nedaeinia *et al.,* 2016) and small miRNA Zippers (Meng *et al.,* 2017).

a. Synthetic Antisense Oligonucleotides (ASOs):

ASOs are single-stranded, chemically modified DNA molecules, 20-25 nucleotides long, with a full complementarity to a target miRNA. ASOs form an ASO-miRNA duplex that can lead to miRNA cleavage and up regulation of the target mRNA thus, inhibiting the binding of mature miRNA to its target mRNA (Bajan *et al.,* 2020). ASOs have been approved by the FDA for treating Duchenne muscular dystrophy and spinal muscular atrophy through exon-skipping strategies to restore the dystrophin expression (Rinaldi *et al.,* 2018). In cancer therapy and metastasis inhibition, pre-clinical studies have been shown with ASOs. For example, Ge *et al.,* 2019 designed an ASO to target miR-21, which is overexpressed in NSCLC and regulates the activity of PTEN. The ASO-based drug was successful in reducing miR-21 expression and inducing apoptosis in H1650 NSCLC cell lines. ASOs targeting miR-21 and miR-10b in triple-negative breast cancer (TNBC) cell lines and tumor xenografts induced cancer apoptosis and inhibited tumor growth and metastasis in a TNBC mouse model (Devulapally *et al.,* 2015) [**31**].

b. Antagomir antisense oligonucleotides:

Antagomirs are artificially synthesized single-stranded RNA, 23 nucleotides long, complementary to a miRNA. They can be chemically modified with a cholesterol moiety for greater stability. In a

pancreatic cancer mouse model, a cholesterol-modified CXCR4 antagonist delivered with nanoparticles via an intraperitoneal delivery to localize efficacy and limit systemic side, demonstrated reduced metastatic activity and complete inhibition of liver metastasis when antagomirs against miR-210 and siRNA against KRAS were co-delivered (Xie *et al.,* 2020).

c. miRNA sponges antisense oligonucleotide:

miRNA sponges are short, synthetic transcripts mimicking the 3′ UTR of mRNAs targeted by the miRNA, acting as decoys to inhibit miRNA regulation of their target mRNAs (**Fig. 4c**) (Tay *et al.,* 2015). Successful miRNA sponging has been reported for a single miRNA (Ebert *et al.,* 2010). Liang *et al.,* 2016 designed a miRNA sponge plasmid that targets miR-10b in metastatic breast cancer cell lines, thus, inhibiting cancer growth, proliferation, migration and invasion. Furthermore, in another study, a multi-potent miRNA sponge inhibited these four oncogenes, miR-155, miR-21, miR-221 and miR-222 and promoted anti-tumor effects in human breast cancer and pancreatic cancer cells, thus, demonstrating greater efficacy in inhibiting proliferation compared to single miRNA-targeted sponges (Jung *et al.,* 2015).

d. miRNA-masking antisense oligonucleotide:

miRNA-Masking (miR-Mask) oligonucleotides protect mRNAs from miRNA-mediated repression by shielding the miRNA binding sites on the mRNA (**Fig. 4d**). This approach requires full complementarity for better specificity and inhibits miRNA-mediated repression without affecting miRNA expression. Zhang *et al.,* 2016 studied the effects of a miR-mask designed to complement the miR-522 binding site within DENND2D and discovered reduced cell migration and invasion in NSCLC cells.

e. Locked nucleic acid (LNA) antisense oligonucleotide:

Locked nucleic acid anti-miRs (LNA-i-miR) are chemically modified oligonucleotides. They are formed by connecting the 2′ oxygen and 4′ carbon to create an extra methylene bridge, locking the ribose ring, thus providing higher thermal and *in vivo* stability and greater binding affinity to mRNA targets. LNA targeting miR-21 reduced invasiveness and inhibited the proliferation of human colorectal adenocarcinoma cells (Nedaeinia *et al.,* 2016). Furthermore, Lima *et al.,* 2018 developed an approach in which LNA was efficient even when they were delivered at low doses. They targeted miR-9 with LNAs which promoted CDH1 expression, re-establishing E-cadherin in human gastric cancer cells. Another study demonstrated that the delivery of LNA-i-miRs against miR-663a and miR-4787-5p decreased TGFβ1-induced EMT, thus, reducing tumor burden and metastasis in an orthotopic mouse model of pancreatic cancer (Mody *et al.,* 2016).

f. Small RNA zippers:

Small RNA zippers are oligonucleotides complementary to the second and the first halves of a miRNA that are synthesized and delivered into the cells. They form a duplex of multiple miRNA copies to inhibit miRNA function. These have increased affinity, specificity and stability. Using miRNA zippers in breast cancer cell lines resulted in 70–90% inhibition of miR-221 and miR-17 rescuing their target genes and reversing the oncogenic effects of miR-221. The *in vivo* applications of miRNA zippers have not been tested yet (Meng *et al.,* 2017).

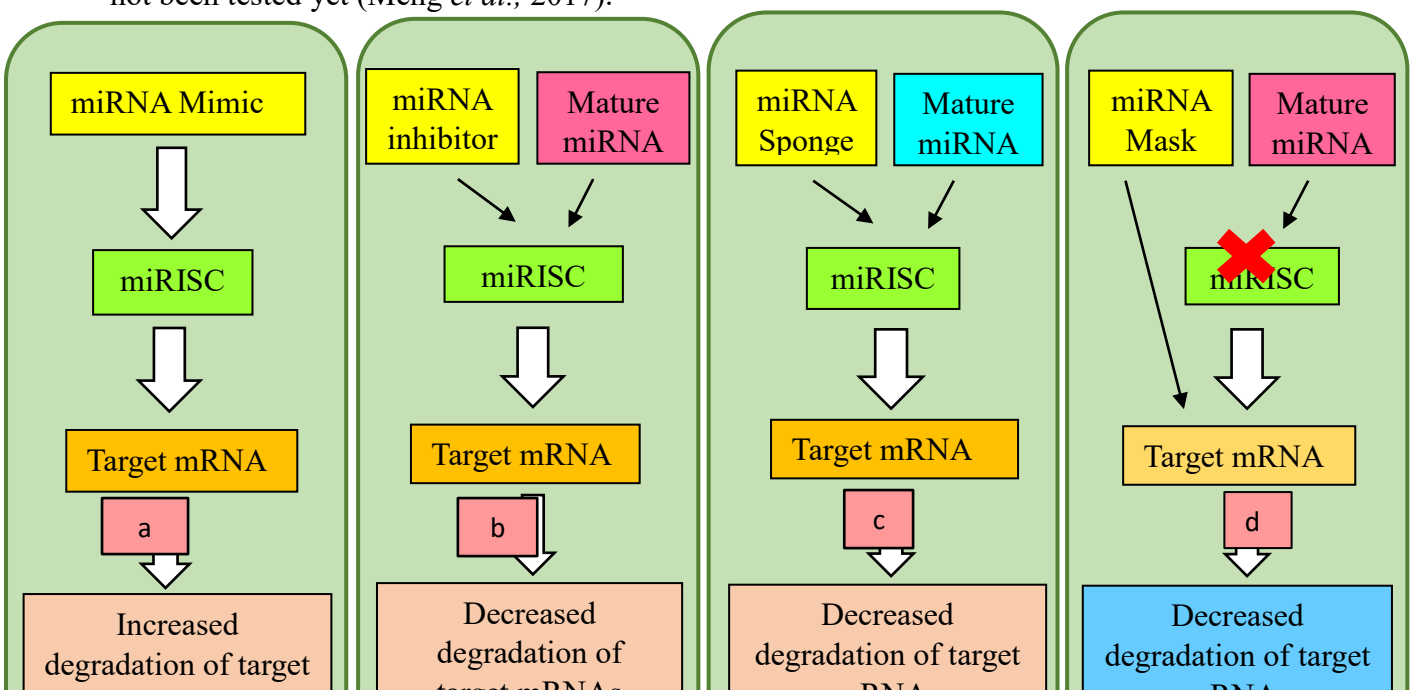


Fig 4. miRNA-based therapies.

Clinical trials

Despite their promising potential, there are no accepted miRNA pharmaceuticals cited on **ClinicalTrials.gov**. This is due to difficulties faced in developing miRNA-based drugs for cancer treatment. A notable example of a miRNA drug terminated in clinical trial phase I and whose planned phase II study was withdrawn, was **MRX34** from Mirna Therapeutics, Inc. This trial is elaborated below:

1. **Official title**: A Multicenter Phase I Study of MRX34, MicroRNA miR-RX34 Liposomal Injection.

i. **Clinical Trial Identifier (ID)**: NCT01829971

ii. **Phase of Clinical Trial**: Phase 1

iii. **Objective**: To assess the MRX34 safety in primary liver cancer patients or patients with other selected solid tumors, replenish depleted miR-34, and restore its function in the p53/WNT cellular pathways.

iv. **Study Design**:

a. Type of Study: Interventional.

b. No. of Participants: 155.

c. Duration of Study:

- Study Start Date: 04-2013
- Study Completion Date: 05-2017

v. **Study Location**:

Countries:

- US (Arizona and Texas).
- Republic of Korea (Seoul).

vi. **Patient Population**:

a. Inclusion Criteria:

- Age: 18 years or older
- Patients with histologically confirmed viral-related hepatocellular carcinoma, small-cell lung cancer, non-cutaneous/ non-uveal melanoma, ovarian cancer, triple-negative breast cancer, sarcoma, bladder cancer and renal cell carcinoma.
- Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1
- Acceptable liver function:

○ Total bilirubin ≤ 1.5 times the upper limit of normal (ULN); for patients with hepatocellular carcinoma only, total bilirubin ≤ 3 mg/dL.

○ Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) ≤ 5 x ULN.

- Acceptable renal function:

○ Serum creatinine ≤ 1.5 times the ULN or calculated creatinine clearance ≥ 60 mL/min/1.73 m2 for patients with creatinine levels above 1.5 times the institutional normal.

- Acceptable hematological status:

○ Absolute Neutrophil Count (ANC) ≥ 1500 cells/mm3.

○ Platelet count ≥ 100,000 plts/mm3 (without transfusion); ≥ 75,000 plts/mm3 for patients with hepatocellular carcinoma.

- Haemoglobin ≥ 9 g/dL
- For hematologic malignancy patients, blood count values cited above do not apply.

- Prothrombin time (PT) or International Normalized Ratio (INR) ≤ 1.25 x ULN; for patients with hepatocellular carcinoma only, INR <1.7 or prothrombin time (PT) or < 4 seconds above ULN (i.e. Child-Pugh Score is no greater than 1 for the coagulation parameter); for patients with hepatocellular carcinoma only, serum albumin > 2.8 g/dL (i.e. Child-Pugh Score for albumin is no greater than 2). For the hematologic malignancy patients, the coagulation and albumin status cited above do not apply.
- For hepatocellular carcinoma patients only- Child-Pugh Class A (score 5-6) disease. The score for hepatic encephalopathy must be 1; the score for ascites must be no greater than 2 and clinically irrelevant; for the determination of the Child-Pugh Class.

b. Exclusion Criteria:

- Myocardial infarction within the past 6 months, unstable and/or symptomatic arrhythmia or ischemia on ECG.
- Active, uncontrolled bacterial, viral or fungal infections requiring systemic therapy.
- Pregnant or nursing women.
- Known HIV infection.
- Serious non-malignant disease (e.g., hydronephrosis, liver failure, heart failure, or other conditions) that could compromise protocol objectives.
- Patients with a recent history of hemorrhage or predisposition to hemorrhage.
- Patients who require therapeutic doses of coumadin-type anticoagulants (maximum daily dose of 1mg allowed for port line patency).
- Patients with cirrhosis classified as Child-Pugh B or C.
- Patients with central nervous system (CNS) metastasis. Intrathecal chemotherapy is allowed for CNS prophylaxis or therapy.
- Patients with contraindications to dexamethasone.

c. Gender: All genders.

d. Age: 18 years and older (Adult, Older Adult)

vii. Intervention:

a. Drug: MRX34

b. Administration Route: Intravenous.

c. Dosage: MRX34 administered daily for 5 consecutive days with 2 weeks off (total of 21 days) for 3 cycles followed by a no-treatment observation period.

viii. Results: Common adverse events among 85 patients included fever [72% of patients at all grades, with 4% experiencing severe (Grade 3) fever], chills (53% of patients, with 14% experiencing chills), fatigue (51% of patients, with 9% experiencing severe fatigue), back/neck pain (36% of patients, with 5% experiencing severe pain), nausea (36% of patients, with 1% experiencing severe nausea) and dyspnoea (25% of patients, with 4% experiencing severe dyspnoea). The recommended phase 2 dose was 70 mg/m^2 for hepatocellular carcinoma and 93 mg/m^2 for non-hepatocellular carcinoma cancers. Pharmacodynamics results showed miR-34a delivery to tumors and dose-dependent modulation of target gene expression in white blood cells. Three patients had partial responses and 16 had stable disease lasting ≥4 cycles (median 19 weeks, range 11-55) (Hong et *al.,* 2020).

ix. Status: Terminated

x. Reason for termination: Five immune-related serious adverse events.

xi. Conclusion: MRX34 treatment with dexamethasone premedication, demonstrated a manageable toxicity profile for most patients and some clinical activity. Despite the early closure of the trial due to serious immune-mediated adverse events resulting in four patient deaths, the dose-dependent modulation of target genes provided proof-of-concept for miRNA-based cancer therapy.

Another clinical trial that highlighted the need for further research to optimize the efficacy of the treatment is elaborated on below:

2. Official title: MesomiR 1: A Phase I Study of Intravenously Administered Epidermal Growth Factor Receptor -Targeted, EnGeneIC Delivery Vehicle (EDV)-Packaged, miR-16 Mimic

(TargomiRs) for Patients with Malignant Pleural Mesothelioma (MPM) and Advanced Non-Small Cell Lung Cancer (NSCLC) Failing on Standard Therapy.

i. Clinical Trial Identifier (ID): NCT02369198

ii. Phase of Clinical Trial: Phase 1

iii. Objective: To evaluate the safety, optimal dosage and efficacy of TargomiRs in patients with recurrent malignant pleural mesothelioma (MPM) and non-small cell lung cancer (NSCLC).

iv. Study Design:

a. Type of Study: Interventional.

b. No. of Participants: 27(Actual).

c. Duration of Study:

- Study Start Date: 09-2014
- Study Completion Date: 04-01-2017

v. Study Location: New South Wales, Australia.

vi. Patient Population:

a. Inclusion Criteria:

- Histological or cytological documentation of MPM or NSCLC and evidence of EGFR expression in tumor tissue.
- Progression during or following the administration of standard 1st, 2nd or 3rd line therapy regimens.
- Patient must have at least one measurable lesion according to the RECIST 1.1 for NSCLC and modified RECIST criteria for MPM.
- Male or female patients at least 18 years of age. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
- Life expectancy of at least 3 months.
- Women of childbearing potential and men must agree to use adequate contraception from the time of signing of the informed consent form until at least 3 months after the last study drug administration.
- Total bilirubin < 1.5 x the upper limit of normal (ULN), ALT and AST < 2.5 x ULN, Amylase < 1.5 x ULN, Serum creatinine < 1.5 x ULN, GFR > 60 ml/min/m2, INR/PTT < 1.5 x ULN (patients who are therapeutically treated with an agent such as warfarin or heparin will be allowed to participate provided that no prior evidence of underlying abnormality in coagulation parameters exists. Close monitoring with at least weekly evaluations will be performed until INR/APTT is stable (before administering the first dose)).
- Platelet count > 100.000 and < 800.000, Haemoglobin (Hb) > 9 g/dl, Absolute Neutrophil count (ANC) > 1500/mm3. Alkaline phosphatase limit < 2.5 x ULN.

b. Exclusion Criteria:

- Previous phase I drug treatment for the current diagnosis. Previous or concurrent cancer that is distinct in primary site or histology from MPM or NSCLC within 10 years from the date of screening EXCEPT for curatively treated cervical cancer *in situ*, non-melanoma skin cancer and superficial bladder tumors (Ta, Tis and T1).
- Presence of Salmonella antibodies.Herbal supplements (such as St John's Wort), nutritional supplements and also (multi)-vitamins taken within the last 30 days before dosing on Day 1 (and continued use, if appropriate), must be reviewed and approved by the local investigator.
- Major surgical procedures in the last four weeks. Pregnancy or breastfeeding. Congestive heart failure > New York Heart Association (NYHA) class 2. Unstable angina (angina at rest) or new-onset angina (< 3 months).Myocardial infarction less than 6 months before eligibility screening.
- Cardiac arrhythmias requiring anti-arrhythmic therapy (beta-blockers or digoxin are permitted).
- Uncontrolled hypertension (Systolic blood pressure > 150 mmHg or diastolic pressure > 90 mm Hg despite optimal medical management).

- Arterial or venous thrombotic or embolic events such as cerebrovascular accident (including Transient Ischemic Attacks), deep vein thrombosis or pulmonary embolism within 6 months before the screening radiographic studies.
- On-going infection > grade 2 NCI-CTCAE version 4.0 HIV infection. Chronic hepatitis B or C. Patients with a seizure disorder requiring medication. Symptomatic brain metastasis (es). The patient must not be undergoing acute steroid therapy or steroid tapering (Chronic steroid therapy is acceptable provided that the dose is stable for one month before and following screening radiographic studies).
- Patients with a history of bleeding diathesis: Any hemorrhage or bleeding event of CTCAE Grade 3 within 4 weeks of the proposed start of study medication.
- Renal failure requiring hemo-or peritoneal dialysis. Substance abuse, medical, psychological or social conditions that may interfere with the patient's participation in the study or evaluation of the study results.
- Known hypersensitivity to bacterial proteins. Any medical condition that is unstable or could jeopardize the safety of the patient and his/her compliance in the study.
- Unresolved toxicity higher than NCI-CTCAE (version 4.0) Grade 2 attributed to any prior therapy/procedure excluding alopecia.

c. Gender: All.

d. Age: 18 Years and older (Adult, Older Adult).

vii. Intervention:

- Drug: TargomiRs
  - TargomiRs consist of three main components:

1. **A miR-16-based microRNA mimic**. The miR-16 family has been implicated as a tumor suppressor in a range of cancer types. This mimic is a double-stranded, 23-base pair, synthetic RNA molecule.
2. **Drug delivery vehicle - EDVs**. EDVs are non-living bacterial minicells (nanoparticles). They function as leak-resistant micro-reservoir carriers that allow efficient drug packaging.
3. **Targeting moiety**. The EDVs are targeted to EGFR-expressing cancer cells with an anti-EGFR bispecific antibody.

- Administration Route: Intravenous
- Dosage: Phase 1 Planned dose levels
  - Dose level 1: 5 billion once a week.
  - Dose level 2: 5 billion twice a week.
  - Dose level 3: 5 billion once a week with cardiac monitoring.
  - Dose level 4: 2.5 billion twice a week with cardiac monitoring.
  - Dose level 5: Same as dose level 3 with dexamethasone challenge.

All patients start with a micro dose of one billion, increasing their dose over 2 weeks and reach their phase 1 dose level on week 3.

viii. Results: In this trial a total of 27 patients were enrolled between September 29, 2014 and November 24, 2016, of which 26 patients received at least one TargomiR dose (one patient died before treatment could begin). Five dose-limiting toxicities were observed: infusion-related inflammatory symptoms and coronary ischemia in two patients who received 5×10^9 TargomiRs twice weekly, anaphylaxis and cardiomyopathy in two patients who received 5×10^9 TargomiRs once weekly with reduced dexamethasone prophylaxis and non-cardiac pain in one patient who received 5×10^9 TargomiRs once weekly. The maximum tolerated dose was established to be 5×10^9 TargomiRs once weekly. TargomiR infusions were accompanied by transient lymphopenia in 25 [96%] of 26 patients, temporal hypophosphatemia in 17 [65%] of 26 patients, elevated aspartate aminotransferase or alanine aminotransferase levels in six [23%] of 26 patients and increased alkaline phosphatase blood concentrations in two [8%] patients. Cardiac events were noted in five patients: three patients had electrocardiographic changes, one patient had ischemia and one patient experienced Takotsubo cardiomyopathy. Out of the 22 patients assessed for response by CT, one had a partial response, 15 had stable disease and six had progressive disease. The proportion of patients who achieved an objective response was one of 22 and the duration of the objective response in that patient was 32

weeks. The median overall survival was 200 days and during the trial, there were 21 deaths, 20 of which were related to tumor progression and one due to bowel perforation (van Zandwijk *et al.*, 2017).

ix. Status: Completed.

Conclusion

The above-mentioned clinical trials illustrate the several challenges faced, such as toxicity and immunogenicity, in developing miRNA-based therapeutics. These pose significant barriers to fully utilizing the therapeutic potential of miRNAs and translating this potential into effective treatments. Furthermore, for the successful implementation of miRNA therapeutics, the development of improved targeted delivery systems is important. These systems must be capable of specifically targeting and delivering miRNA therapeutics to the desired tissues or cells (Chakraborty *et al.,* 2020). The future of RNA-based therapies heavily relies on these targeted delivery mechanisms, which include lipid and polymer nanoparticles, cellular or extracellular vesicle packaging, hybrid systems, and viral vectors. These methods aim to improve the therapeutic efficacy of miRNA therapies while decreasing potential side effects (Seyhan, 2024). Moreover, the sensitivity, specificity, selectivity, toxicity and overall clinical applicability of miRNA therapeutics need to be tackled. Given that each miRNA can regulate multiple genes, a single miRNA might impact many cellular pathways by interacting with multiple targets, a phenomenon known as "too many targets for miRNA effect" (TMTME) (Zhang *et al.,* 2021). Conversely, each mRNA can be regulated by multiple miRNAs. While these properties make miRNAs a powerful therapeutic class, they also present significant challenges in managing adverse effects observed in clinical trials (Diener *et al.,* 2022). Zhang *et al.,* 2021 suggested that adverse events in clinical trials involving miRNA therapeutics might be due to these broad-ranging effects of miRNAs. Additionally, the types of miRNA can differ throughout different stages of cancer, complicating target prediction. However, this variability might be advantageous for associating specific miRNAs with particular cancer stages; thus, suggesting the necessity of novel approaches for predicting and validating miRNA targets (Seyhan, 2024).Another issue that needs to be tackled includes immunogenic reactions. Although viral delivery systems enhance cellular uptake and miRNA expressions, they are linked with various side effects, including immunogenicity (Monahan *et al.,* 2021). A better understanding of the prevalence of these immunogenic reactions from viral transfer systems is important, specifically clarifying the extent to which these reactions occur. Many miRNAs are abnormally expressed in various cancers, functioning either as oncogenes or tumor suppressors, making them potential biomarkers (Seyhan, 2024), therapeutic targets and therapeutics (Li *et al.,* 2017). However, there are significant challenges regarding sensitivity, specificity, selectivity and off-target effects, as each miRNA regulates multiple targets and each target is regulated by multiple miRNAs, leading to undesired toxicity and limiting their therapeutic use. Most miRNA therapeutics are still in the preclinical or early phases of human clinical trials. It remains to be seen how other miRNA therapeutics will perform in human trials concerning toxicity or side effects. Some clinical trials with miRNA therapeutics have reported serious adverse events. For instance, **MRX34**, a miRNA liposomal injection developed by Mirna Therapeutics, Inc. was withdrawn (NCT02862145) or terminated (NCT01829971) from a phase 1 clinical trial for melanoma, due to serious adverse events (Beg et al., 2017; Hong et *al.,* 2020; Desantis *et al.,* 2020). Consequently, multiple challenges must be solved to bring therapeutic miRNAs into clinical practice. These include determining miRNA specificity, sensitivity and selectivity to intended targets, reducing immunogenic reactions and adverse events, determining optimal dosing for desired therapeutic effects while minimizing side effects (Diener *et al.,* 2022) and developing better methods for targeted delivery. Despite these challenges, the potential of miRNA therapeutic approaches for various diseases is evident. Further research will be necessary to determine whether miRNAs can effectively be used as therapeutics or therapeutic targets in clinical implementations.

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secondary data sources related to miRNA. The authors also state that they really hope that their citations of contributions to the techniques of miRNA assessment, biosynthesis, efficacies against cancer and as tumor suppressors, clinical trials as well as prospective therapeutic functions covering the years 1993 to 2023, would not be overlooked.

Authors Contribution

Every author has made a contribution to this research project.

Conflict of Interest

The writers declare that they have no conflicts of interest and reaffirm their dedication to openness.

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