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The linkage between microRNA and cancer and its delivery as cancer therapy: A mini-review

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ABSTRACT

The central dogma of molecular biology was no longer "central" after ground-breaking discoveries conveyed gene expression involves more complex physiological functions in cancer pathogenesis over the last decade. MicroRNAs (miRNAs) are short non-coding RNA that regulate gene expression, affecting key molecular pathways involved in sustaining the proliferative signalling for tumour development, evasion of cellular death, invasion, angiogenesis, as well as metastasis in a plethora of cancer types. MiRNA expression is dysregulated in human cancer through a number of processes, including miRNA gene amplification or deletion, faulty miRNA transcriptional regulation, dysregulated epigenetic alterations, and flaws in the miRNA biogenesis machinery. As a result, the current progress of treatment intervention focuses on modifying the miRNA levels in cancer therapeutics. Nevertheless, the mode of delivery and current management of miRNA therapies remains one of the many questions that need to be addressed. Here, we provided a comprehensive mini-review outlining the role of miRNA in cancer as well as its mode of delivery which includes liposomes, viral vectors, inorganic material-based nanoparticles, and cell-derived membrane vesicles. Likewise, the regulation of miRNA in other diseases and their challenges in translational research was also thoroughly discussed.

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Introduction

microRNA (miRNA) was first found by researchers as 'junk' or non-significant type of RNA that does not contribute to any importance of cellular mechanism but in later years, it was shown to bring a significant impact in various cell activities such as proliferation, migration, differentiation, apoptosis, and key regulators in pathogenesis of diseases, particularly in cancer. The advance of technology in molecular science helps to understand the actual role of miRNA as in the case of the genetic analysis of developmental timing in the nematode, *Caenorhabditis* elegans, postulating the identification of the cloned let-7 and lin-4 miRNA genes as antisense translational repressors of mRNAs encoding for proteins function in the heterochronic developmental timing pathway of the worm (1). The cloning of those genes was made to reinstate their normal function as their mutation causes failure in executing developmental switches which consequently causes abnormal repetition of certain larval stages (1).

Biogenesis of miRNA

In general, miRNAs are made up of 19 to 24 ribonucleotides which categorised them as small single-stranded RNA (2). They control and regulate the expression of genes by targeting mRNA at the post-transcriptional level (3). In the canonical pathway of miRNA biogenesis, precursor miRNA undergoes several biochemical processes starting from the miRNA gene transcription by RNA polymerase II, producing an initial long primary transcript called, pri-miRNA as can be seen in Figure 1 (4). Further processing by nuclear RNase III enzyme comprising of DROSHA-DGCR8 complex results in the removal of its stem-loop structure thus, leads to the formation of pre-miRNA (4). Exportin-5 and Ran provide a shuttle system for exporting the pre-miRNA to the cytoplasm where the Dicer-TRBP complex protein will further process it (4). One strand (i.e. guide strand) of the double-stranded precursor miRNA will serve as a template for RNA-induced silencing complex (RISC) while another strand (i.e. passenger strand) will be cleaved by Argonaute 2 (Ago2) protein (5). Once the mature miRNA is formed, it can bind complementarily at the 3'-UTR regions of its target mRNA causing either inhibition of mRNA translational process (i.e. protein will not be produced as a result) or degradation of mRNA, depending on the miRNA-mRNA complementary binding strength (6,7). The best-matched targets will result in the latter outcome (8). It is noteworthy that there are only 30-40% of genes are regulated by miRNAs despite previous findings postulating that a specific miRNA can regulate hundreds of genes (9). Additionally, the target sites of the

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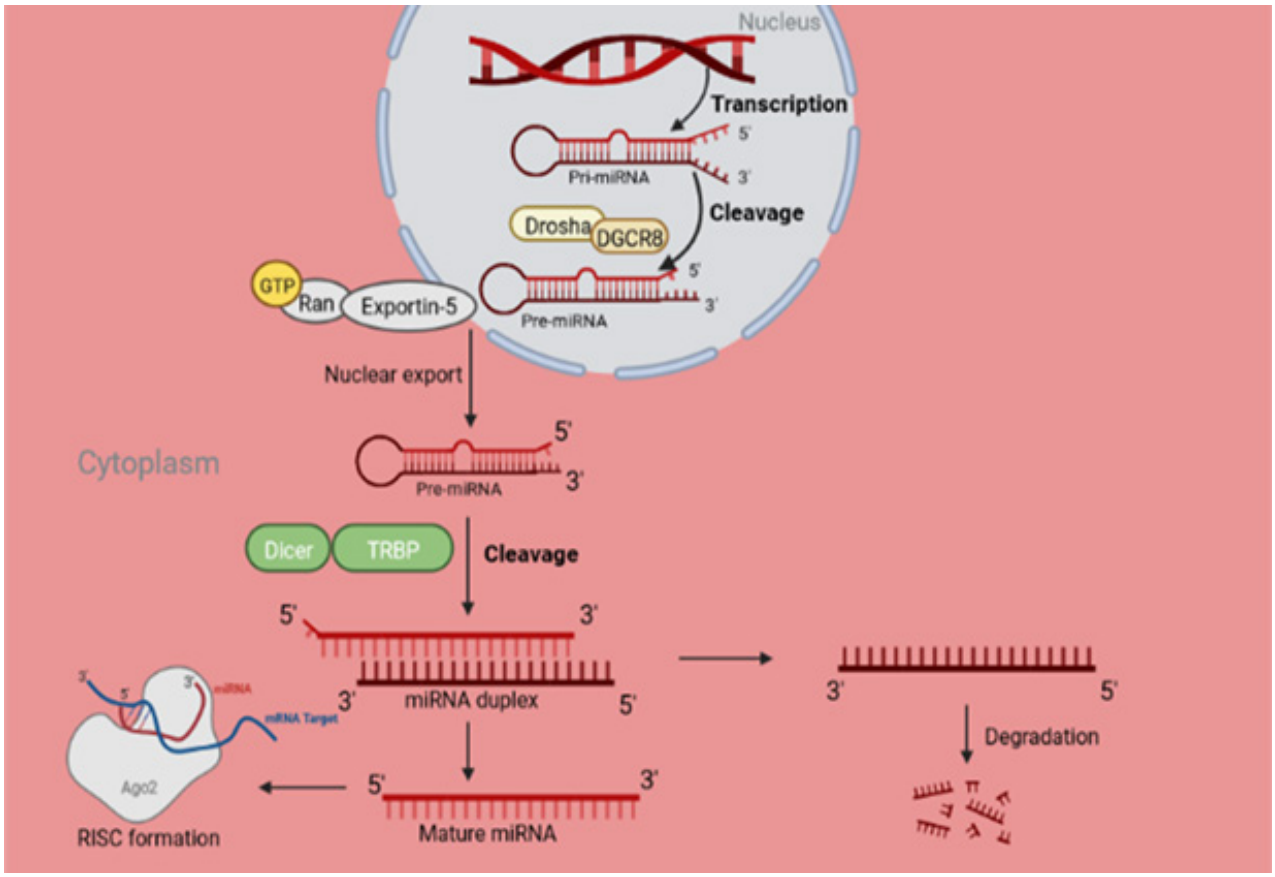

Figure 1. Biogenesis of mature miRNA starts from DNA transcription in the nucleus in the form of primary miRNA before it is processed into pre-miRNA structure and transported to the cytoplasm of the cell by exportin 5-Ran-GTP complex for its further processing.

miRNAs are often selectively conserved within the 3'UTR though in some rare cases, miRNA may bind to the non-3'UTR region (9).

miRNA and cancer hallmarks: A therapeutic linkage

Research on miRNA has been in a progressive state since the discovery of its association in cancer cellular pathways. Additionally, the distinct expression level of miRNA in cancer patients could serve or exploit as a tool for diagnostic and prognostic purposes. The first cancer-associated miRNA was discovered by Calin and his colleague in their study as they identified mir-15a and mir-16-1 within the intron of the deleted lymphocytic leukaemia 2 (DLEU2) gene that was downregulated in chronic lymphocytic leukaemia patients (10). Further studies revealed that these miRNAs act as tumor suppressors in apoptosis and cell cycle regulation pathways by repressing the B-cell lymphoma 2 (Bcl-2) gene and cyclin-related genes (e.g. cyclin D1 and cyclin E1), respectively (10).

The alteration of miRNA expression in myriad types of neoplasia establishes distinct trends of miRNA signature in certain cancers. The hallmarks of cancer consist of six fundamental properties focusing on sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, as well as inducing angiogenesis and cell invasion (11). Given the aberrant miRNA expression in malignancies, miRNA dysregulation may impact the hallmarks of cancer during tumour initiation and development. It is important to note that miRNA can act either as an oncogene or a tumour suppressor depending on their target genes. Here, the significance of the altered miRNA expression in cancer hallmarks was highlighted.

Sustaining proliferative signalling

Constitutive cell proliferation is the primary attribute of cancer cells during tumorigenesis. In particular, cell cycle progression is regulated not only by internal mechanisms but also via extracellular signal molecules in order to strike a balance between stimulating and restraining cell growth (12). Cells become malignant when their proliferation or division becomes uncontrolled (13). Multiple scientific researches have revealed that certain miRNAs physiologically integrate into this critical machinery, leading to the evasion of growth suppressors and sustaining proliferative signals. Several genes and proteins, particularly kinases and kinase receptors, are implicated in these processes and continue to be studied as possible therapeutic targets (14).

Cyclin-dependent kinases (CDK) are one of the renowned kinase proteins that is highly regulated by miRNA and vital for cell cycle machinery. It is commonly deregulated in cancer, causing tumour progression and proliferation. The first evidence by Hatfield et al., (2005) demonstrated that miRNAs were vital for *Drosophila* germline stem cells (*Dicer-1*) to engage in the G1/S checkpoint. The CDK inhibitors i.e. Dacapo (p21/p27 family) were significantly increased in *Dicer*-deficient germline stem cells which contradicts the regulation of miRNA in cell cycle progression (15). In addition, miR-221/222 could positively affect CDK inhibitor of $p27^{Kip1}$ in glioblastoma cells and subsequently confirmed in other immortalised cell lines including primary cells. In nasopharyngeal carcinoma, upregulation of miR-663 (i.e. oncogenic) promotes G1/S

transition as it targets $p21^{C1P1}$ (16). Recent findings demonstrated that miR-27a-3p was higher in nasopharyngeal cancer patients compared to healthy tissues. miR-27a-3p acts upon MAPK10 which consequently results in promoting cell proliferation, migration and invasion of the 5-8 F cells (17). Apart from being CDK inhibitors, the regulation of CDK and cyclin was also orchestrated by miRNAs e.g. miR-545 inhibited cyclin D1 and CDK4, hence halting the cell cycle progression of the lung cancer cells (18). On the other hand, the mir-17-92 cluster involves in cell cycle regulation by modulating the E2F1 expression as well as targeting c-Myc-induced apoptosis through tumor protein p53 pathway. In addition, it also promotes cancer-cell survival by activating the protein kinase B signalling pathway (i.e. through inhibition of phosphatase, tensin homolog, and RB2 genes) (19,20).

Resisting cell death

It has been established that resisting apoptosis of malignant cells is mediated by miRNAs (21,22). Tumour cells develop a number of ways to restrict or avoid apoptosis e.g. the loss of the p53 tumour suppressor is the most prevalent among them. Upregulation of Bcl-2 family proteins i.e. anti-apoptotic regulators, repression of proapoptotic proteins, and the inhibition of the death pathway caused by extrinsic ligands are some of the distinct strategies to avoid apoptosis (23).

Several miRNAs have been discovered to have a role in regulating the p53 functions as in the case of miR-192, miR-194, and miR-215 expression in multiple myeloma (i.e. p53 positive regulators) (24). They are transcriptionally activated by p53 to restrict Mdm2 production as well as prevent the p53 degradation hence, their downregulation is crucial for the development of multiple myeloma (24). Of note, the dysregulation of other p53-regulated miRNAs results in apoptosis resistance to cancer cells. For instance, the downregulation of the miR-17–92 cluster results in hypoxia-induced apoptosis while its excessive expression confers evasion of cell death (25). Besides that, several miRNAs act upon pro-survival regulators such as Bcl-2 and Bcl-xL as well as proapoptotic factors like Bax, Bim, and Puma (26). Denoyelle and his colleagues discovered that miR-491-5p causes apoptosis in ovarian cancer cells by directly suppressing Bcl-xL expression and promoting Bim accumulation (27). In another study, Zhang and his colleagues displayed that miR-221/222 inhibited cell death in human glioma cells by targeting PUMA production, indicating that miR-221/222 is a promising therapeutic target for glioblastoma intervention (28). Other miRNAs such as miR-204, miR-148a, and miR-365 also regulate the Bcl-2 expression which once targeted, results in the induction of apoptosis (29).

Cell invasion and metastasis

Aggressive cancer confers tumour cells the capacity to invade local tissues and distal areas, resulting in metastasis which contributes to cancer morbidity and death. Epithelial-mesenchymal transition (EMT) is the initial process of the metastatic cascade, characterised by the suppression of E-cadherin and the activation of multiple genes involved in motility and invasion (30). The changes in the shape and mobility of epithelial cells caused by EMT result in loss of cell-cell contact, allowing them to migrate into surrounding tissues and distant organs (31).

Metastasis-promoting miRNA such as miR-181b-3p has been shown to induce EMT through the downregulation of mesenchymal marker expression, migration, and invasion in breast cancer cells (32). Moreover, it was also postulated that the miR-181b-3p directly targets YWHAG which is responsible for Snail upregulation (EMT master regulator) and E-cadherin downregulation. Besides that, miR-374a has been shown to induce metastasis in breast cancer via targeting negative regulators of the Wnt/-catenin pathway such as WIF1, PTEN, and WNT5A (33). Additionally, miR-135a is also known for its high expression in metastatic breast cancer, causing factors for enhanced migration and invasion by targeting upon HOXA10 and FOXO1 in HSSCC and hepatocellular carcinoma (HCC) patients, respectively (34). Other than that, metastasis-suppressing miRNA such as the miR-29 family (miR-29a/b/c) displayed the inhibition of migration and invasion of HSSCC via focal adhesion laminin-integrin pathways (LAMC2, ITGA6, and LOXL2) (35). In addition, downregulation of miR-29c in metastatic lung cancer slows down the extracellular matrix breakdown (i.e. less target of integrin β1 and metalloproteinase 2 (MMP2)) thus, inhibit or reduce the metastasis process (36). Other than that, miR-21 targets and causes downregulation of tumour suppressor genes such as programmed cell death 4 (PDCD4), a key component in the RAS-RAF-MAPK pathway which results in an increased expression level of urokinase receptor (u-PAR) and the subsequent plasmin-mediated degradation of ECM components such as fibrin and collagen IV hence, promoting invasion and metastasis in colorectal cancer (6,37). In addition, the involvement of miRNA in metastasis was also postulated in previous studies as mir-141, mir-200b, and mir-200c (i.e. mir-200 family) could suppress zinc-finger-enhancer binding protein 1 (ZEB1) translation, which prevents the event of epithelial-mesenchymal transition (EMT) of the cancer cell (38,39). It is noteworthy that they also target the stem cell genes such as zinc finger E-box binding homeobox 2 (ZEB2), SOX2, Kruppel-like factor 4, polycomb Suz12, and polycomb complex protein BMI-1 (40,41).

Regulation of angiogenesis

Angiogenesis is a physiological process involving the formation of new blood vessels from the existing vasculature in order to meet the demands for food and oxygen in tumour development (42). In response to a hypoxic condition, a heterodimer protein i.e. hypoxia-inducible factor (HIF) will regulate the expression of several genes, including miRNAs (43). Cancer cells in a hypoxia state (i.e. low oxygen level) promotes the growth of new blood vessels through angiogenic factor such as vascular endothelial growth factor alpha (VEGFA), which can be related to the mir-107 activity (44,45). The loss of chromosomes 10q copy number results in mir-107 downregulation which consequently perturbs its normal function in inhibiting hypoxia-inducible factor-1 beta (HIF1B), hence increasing the level of VEGF (46). Therefore, it is anticipated for miRNAs-targeted HIF or VEGF to have a large influence on angiogenesis. Interestingly, diverse research on profiling miRNA-related angiogenesis have been documented for almost all human malignancies (47). For example,

miR-126 ectopic expression inhibited CD97 (G-coupled receptor) expression that promotes cell invasion and angiogenesis via integrin signalling while miR-126 and miR-126* affect breast cancer metastasis through the cell and non-cell autonomous processes as well as targeting the pro-angiogenic insulin-like growth factor binding protein 2 (IGFBP2), phosphatidylinositol transfer protein cytoplasmic 1, and c-Mer tyrosine kinase genes (48–50). In a mouse xenograft model, miR-497 inhibits tumour development and angiogenesis (51). Recent research has found that miR-578 and miR-573 could regulate the HIF1-mediated angiogenesis and have a variable expression in BRCA1/2-associated breast cancer (52). Besides breast cancer, some angiomiRs (angiogenic miRNAs) such as miR-126, miR-21, miR-210, miR-106a, miR-155, miR-182, and miR-424 have been shown to exhibit altered expression levels in non-small cell lung cancer (NSCLC) (53). Regulation of angiogenesis could also be related to the upregulation of the mir-17-92 cluster targeting the anti-angiogenic thrombospondin-1 (THBS1) (54). Despite that, miR-29b targets transcription factor 7-like 2 (TCF7L2), SNAIL, and B-cell CLL lymphoma 9-like protein (BCL9L) with the aim to lower β-catenin translocation to nuclei in SW480 colon cancer cell line (55,56). Ectopic expression of miR-29b lowers the capacity of colon cancer to promote vasculature in vitro, indicating that miR-29b is involved in angiogenic processes (56). Concurrently, preserving miR-29b expression inhibits CRC tumour invasion and metastasis by restoring EMT and target MMP2 and T-cell lymphoma invasion and metastasis 1 (TIAM1) (57).

miRNA role as a tumor suppressor

In spite of its oncogenic status, functional studies revealed that miRNA can function as tumor suppressor as well. For instance, a previous study showed that mir-34 suppresses CDK4/6, cyclin E2, E2F5, and bcl2, halting the cell cycle of the unhealthy cells from progressing and directing them into apoptosis, similar to the role of p53 (58). Its tumor-suppressor role is strengthened with the evidence of decreasing levels of mir-34 in various types of cancer, mainly due to epigenetic silence of mir-34 and loss of chromosome 1p36 (59). Additionally, other miRNAs including mir-192, mir-194-2, and mir-215 have been linked with the apoptosis pathway as they are the downstream target of TP53 (60). The loss or inactivation of the TP53 gene is the main cause of their down-regulation and loss of function (24). Other than that, mir-491 and mir-195 are also related to the apoptosis pathway as their functional activity was revealed to repress the transcription and translation of anti-apoptotic protein BCL2 and BCL-XL (61). Loss of chromosome 9p and 17p were shown to cause a decreased expression of these miRNAs, respectively hence affecting their activity (60). In addition, other miRNA like let-7 was observed to have a distinct low expression level in colon cancer (62). Restoring it to normal expression level permit its normal function as tumor-suppressor where a decrease of RAS protein expression level and reduced tumor growth was observed (62). However, mir-143 showed an inverse correlation between RAS expressions in CRC (i.e. general reduction of mir-143 in colorectal adenomas) as reported in previous studies (63,64).

Current approaches using miRNA therapies

There are two major approaches that have been done to restore the down-regulated miRNA back to its normal function or inhibit the over-expressed oncogenic miRNA in a way to treat and intervenes in the malignant cells. One is through miRNA suppression therapy and the other is miRNA replacement therapy. Examples of miRNA suppression therapy include anti-miRNA oligonucleotides (AMOs), miRNA sponges, and miRNA masks while miRNA mimics are used in miRNA replacement therapy to compensate for the loss or under-expressed miRNAs (65).

anti-miRNA oligonucleotides (AMOs)

They are single-stranded RNA molecules, designed to bind complementarily to miRNA hence, preventing miRNA-mRNA base-pairing (i.e. silencing of miRNA) (66). Chemical modification such as 2′-O-methyl-(2′-OMe), locked nucleic acid (LNA) oligonucleotides, amino-modified oligonucleotides, and peptide nucleic acids (PNAs) on AMOs is critical in order to enhance the stability for in vivo delivery and cellular uptake efficiency of AMOs as to avoid ribonucleases in blood from degrading naked RNA and engulfed by the reticuloendothelial system (RES) (9). Apart from providing nuclease resistance, chemical modifications also make certain the AMOs have high-affinity binding toward the target miRNA (67). Furthermore, modification of cholesteryl functionality at 3’ end of the nucleic acid could improve the AMOs pharmacokinetic properties (e.g. half-life in serum and cellular uptake) (68). A previous study reported that AMOs delivered at the intravenous site of animals managed to silence mir-16, mir-122, mir-192, and mir-194 efficiently (69). Moreover, Ma and his colleagues reported that the use of mir-10b antagomirs in vivo could significantly reduce the expression level of the corresponding miRNA in a mouse mammary tumor model thus, inhibiting metastasis from occurring and providing therapeutic effects to the animal (70).

miRNA ‘sponges’

Similar to the miRNA antisense inhibitor oligonucleotides and gene knockouts, miRNA sponges are considered as popular subject or tool in the miRNA loss-of-function study. Endogenous miRNA ‘sponges’ are the non-coding RNA molecules (e.g. circular RNAs (CircRNAs) and long non-coding RNAs (lncRNAs)) where they complementary bind to with miRNA response elements (MREs) resulting in sequestration of miRNA (i.e. buffering the activity of miRNAs on physiologically relevant targets) (71). As a result, the number of oncogenic miRNAs targeting mRNAs would be reduced. miRNA sponges are transcripts that contain multiple (typically 4–10 separated by a few nucleotides) tandem-binding sites to a miRNA of interest (72). They can form either a perfect match with miRNA or have slight mismatches at their middle sequences. A perfect match of miRNA-sponge combination shows some inhibitory activity where it is bound to be cleaved by Ago2-mediated endonucleolytic while the mis-paired combination of miRNA-sponge at 9-12 nucleotides positions is considered more effective in sequestering miRNAs (71). It is important to note that miRNA sponges occur naturally in plants and animals but their immense impor-

tance and growing demand for regulating miRNA activity for research studies and future clinical use has led to its engineering and custom-designed in the laboratory as newly gene vector-encoded sponges (i.e. synthetic miRNA sponges transcribed from mammalian expression vectors). A few important aspects need to be considered in designing the miRNA sponge as an increase in binding site number would increase their susceptibility to degradation (71). Other than that, the risk of recombination during cloning and introducing unintended binding motifs for other regulatory factors need to be measured as well though those concerns could be overcame by introducing variations in the bulged mismatches and the spacers (71).

There are many miRNA sponges have been studied and characterised for their functions in the past. For instance, early findings by Ebert and his colleagues indicated the de-repression of miRNA targets by miRNA sponges in vitro. Meanwhile, in a bone marrow transplantation study utilising miRNA sponge for mir-223 was shown to have a similar effect to the one using the mir-223 knockout mouse (i.e. produced similar phenotypes and characteristics). It is of importance to note that several miRNAs have seed family members at different loci that may have overlapping functions, which makes it difficult to study the role of such miRNAs using loss-of-function mutants as all of these loci may need to be targeted to accurately study them (73). In that particular case, miRNA sponges could utilize a common seed sequence in their structure and can therefore inhibit multiple miRNAs at once. For example, circRNA7 could target miRNA-7 in the mouse tissues while also serving as a mir-138 sponge that acts upon the sex-determining region gene of chromosome Y (74). miRNA sponge is convenient and offers advantages over the gene knockout technique as there is no necessity for knocking out each miRNA individually or breeding animals to generate the complete knockout strains as well as applicable to various model organisms and cell lines (71).

miRNA mask

miRNA masks are 22-nucleotides single-stranded oligoribonucleotides with 2′-O-methyl-modifications and act as competitive inhibitors of miRNA from forming the miRNA-mRNA complex (75). In that sense, the miRNA mask interacts with its binding sites localised at the 3'-UTR of target gene mRNA through a fully complementary mechanism (i.e. miRNA mask-mRNA combination) (75). This miRNA-mediated therapy approach is considered as supplement for the AMOs and is very useful in impeding the binding of oncogenic miRNAs to their target mRNAs which consequently leads to the reactivation of the previously repressed genes. miRNA masking oligonucleotides were used for the first time in 2012, evaluating the regulation of miRNA-196a2 towards TP63 protein expression and its relation in breast cancer cell proliferation (76). This approach has been used ever since, discovering other miRNA-mRNA functional relationships including miRNA-203 and the LASP- 1 gene, miRNA-29-b-1 and the SPIN1 gene, and miRNA- 27a and the calreticulin (CALR) gene (76). Previous in vitro studies highlight the promising application of miRNA masking oligonucleotides in cancer therapy. For example, miRNA masks managed to suppress cancer cell proliferation and induce apoptosis by preventing the interaction between miRNA-522 and DENND2D mRNA and can also inhibit angiogenesis by blocking the binding of the miRNA-30 family to Delta-like 4 (DLL4) mRNA (77).

miRNA mimics

miRNA mimics is a biological tool used in miRNA replacement therapy that focuses on replenishing the down-regulated miRNA in diseased cells back to its expression level similar to the one in normal cells hence, bringing about the restoration of cellular pathways regulated by these miRNAs back to normal. These chemically modified double-stranded RNA molecules mimic the endogenous mature miRNA, possessing a 'guide strand' and 'passenger strand' (78). The 'guide strand' is identical to the miRNA of interest while the modification is made on the 'passenger strand' by attaching it to cholesterol for cellular uptake purposes (78). Once introduced into cells, this RNA fragment can bind specifically to its target gene and produce posttranscriptional repression, more specifically translational inhibition of the gene. Unlike endogenous miRNAs, miRNA mimics act in a gene-specific fashion (i.e. binding/targeting their specific mRNA) and have been used as an exogenous tool to study gene function by targeting mRNA through miRNA-like actions in mammalian cells. For instance, regression of the proliferation rate of cancer cells was observed when let-7 mimics were re-introduced in cultured lung cells and reduced the tumor size in vivo (79). Other than that, delivery of mir-34a mimics in a lipid-containing formulation increases the level of mir-34a leading to the suppression of its known target genes and thus, inhibits the growth of lung tumor (80). Interestingly, several miRNA mimics such as TargomiRs and MRG-201 are currently undergoing clinical trials (81). The former is a miRNA-16 mimic loaded inside minicells which aimed to treat malignant pleural mesothelioma while the latter is a miRNA-29 mimic that has been utilised as an anti-tumor vehicle possessing a significant tumor suppressive role in myeloid leukaemia, oesophageal squamous cell carcinoma, and gastric cancer (76,82). Of note, the use of miRNA mimics causes less unwanted off-target effects due to its gene-specific function. Moreover, it is easy to be utilised as it can be delivered using lipid- or polymer-based nanoparticles for systemic delivery in vivo but also possesses some disadvantages as it is considered unstable, has a transient effect, and may require repeated supplementation (83).

Delivery of miRNA therapy

miRNA therapy can be administered or delivered through several approaches including liposomes, viral vectors, polymeric nanoparticles, and cell-derived membrane vesicles to increase stability and enhance the pharmacokinetic behaviour of miRNA oligonucleotides. Each approach may possess advantages over another and few limitations in clinical perspectives.

Liposomes

Lipid-based carriers are widely used and by far the most popular approach for delivering nucleic acids in vivo and can be easily chemically modified to conjugate with targeting moieties and fluorescent probes (84). This ap-

proach consists of a mixture of lipids with cationic head groups, which are amphiphilic molecules (i.e. hydrophilic head and hydrophobic tail) and helper lipids, including some with polyethylene glycol chains for masking of the surface charge (84). A previous study showed that 'bubble liposomes', which are miRNA-126-loaded polyethylene glycol (PEG)-modified liposomes combined with entrapping ultrasound could promote angiogenesis and improved blood flow in an experimental hindlimb ischemia model (85). Of note, the addition of a functional group, PEG to the lipid nanocomplexes resulted in increased delivery efficiency as it can escape phagocytosis of the reticuloendothelial system (RES) when administered systemically (86). Other than that, previous studies reported the use of mir-133b and mir-29b with a mixture of DOTMA, cholesterol, and PEG lipid for lung cancer therapy while premir-107 with DAB, cholesterol, and PEG lipid had been used to treat HSSCC (87,88). Despite that, a neutral lipid emulsion could also be utilised to deliver miRNA as in the case of synthetic miRNA-34a and let-7 in treating the non-small cell lung cancer (NSCLC) mouse model whereby their administration resulted in reduced tumor size with a concomitant increase in tissue levels of the miRNA (89). As lipid formulations (structures and compositions) has been optimised, loading capacity and delivery efficiency are not the issues anymore but inherent toxicity and the uptake and re-release efficiency are still a major concern (90).

Viral vector

There are various viral vectors that have been constructed to deliver miRNA including adenovirus, retrovirus, lentivirus, and adeno-associated virus. The use of viral vectors is beneficial as they can transfer genes into different tissues and cause long-term expression. However, it is important to note that certain virus is only suitable for certain purposes than other due to their distinct characteristics (91). For example, adenoviruses are non-enveloped viruses containing double-stranded DNA genomes which show hepatotropic (i.e. affinity towards liver) characteristic hence, is advantageous for liver-targeted gene delivery (92). This is in line with another study utilising anti-HBV pri-miRNA mimics (pri-miRNA-31/5 or pri-miRNA31/5-8-9) which show short-term blockade effect towards hepatitis B virus (93). Of note, deletion of all of adenoviruses protein-coding sequences is necessary before utilising them as a viral vector to ensure safety and reduce immunogenicity in vivo as well as to upregulate transgene efficiency (94). Other than that, a previous study reported that the oncolytic adenovirus vector namely, AdCN205 expressing interleukin-24 and miRNA-34a showed antitumor effects against hepatocellular carcinoma (HCC) models (95). Meanwhile, retroviruses have been used as a vector for delivering genes of interest due to their capability in converting the RNA into double-stranded DNA by using the host reverse transcriptase enzyme before integrating the DNA into the host genome (known as provirus DNA), which leads to the persistent expression of the inserted gene fragment (96). Of note, most of the retroviral vectors are constructed based on the Moloney murine leukaemia virus (MMLV) and the gene of interest like miRNA is inserted within the long terminal repeats (LTRs) region of the retrovirus to enable its expression (97). A previous study reported the administration of MMLV-mir-21 significantly improved the expression levels of miRNA-21 in adult mouse cardiac fibroblasts (93). Similar to retroviruses, lentiviruses can also stably insert themselves into the genome of recipient cells, which leads to sustained gene expression (98). For instance, administration of lentivirus vector encoding miRNA-133b improved functional recovery of spinal cord-injured mice while injection of lentiviral vector-mediated miRNA 101 sponge at the intrahippocampal mitigated the overproduction of soluble β-amyloid precursor protein in hippocampal neurons (99,100). On the other hand, the non-enveloped single-stranded DNA adeno-associated virus (AAV) has also been reported to cause sustained gene expression. For instances, mir-196a delivery using AAV vector could enhance the decay of mRNA androgen receptor by silencing Elav-like family member 2 (CELF2) thus, ameliorated the spinal and bulbar muscular atrophy phenotype in mice (101). Additionally, administration of an artificial miRNA replacing mir-26a with AAV was effective against progression of hepatocellular carcinoma in mouse model (102).

Inorganic material-based nanoparticles

Properties of gold nanoparticles (AuNPs) such as biocompatibility, ease of functionalisation, either with thiol or amino groups (favourable for miRNA entrapment), and tuneable size and shape have made them a useful tool in drug delivery (103). For example, administration of covalent conjugated-thiol antagomir-miRNA-155 to AuNPs via tail vein injection promoted M2 macrophage polarization, reduced inflammatory mediators, and restored cardiac function in an ovariectomised diabetic murine model (93). Furthermore, a non-toxic gold-based formulation has also been developed to obtain excellent miRNA cellular uptake through endocytosis and intracellular delivery (104). Other than that, silica and mesoporous silica nanoparticles are also favourable as miRNA and anti-miRNA carriers due to their thermostability and biocompatibility as well as having large surface area and pore volume (105). Silica nanoparticles are deemed as effective miRNA carriers for cancer research studies as previous studies reported the inhibition of neuroblastoma growth by silica NPs carrying a tumor suppressor mir-34a while mesoporous silica nanoparticles carrying anti-miRNA-155 conjugated with polymerised dopamine and aptamer successfully inhibited tumor growth in vivo (105,106).

Cell-derived membrane vesicles

Exosomes are a type of membrane vesicle of 40-120 nm in diameter size that is produced by many cell types such as epithelial, dendritic, and immune cells (107). They are primarily derived from late endosomes that play role in intercellular communication, antigen presentation, and RNA shuttling (108). miRNAs have been successfully delivered using exosomes to treat diseases such as cardiac diseases, muscular disorders, and cancer (109). Their low cytotoxicity and antigenicity made them ideal vehicles for nucleic acid drugs, particularly for miRNA administration in vivo (i.e. they can circumvent endocytosis and escape from phagocytosis by the RES) (110). For instance, anticancer drugs along with miR-21 inhibitors were co-delivered by exosomes to circumvent drug resistance and

improve the efficacy of colon cancer treatment (111). Interestingly, enrichment of exosomes with miRNA can be achieved by transfecting mesenchymal stem cells with the miRNA of interest as in the case of anti-miRNA-375 that restrict apoptosis during islet transplantation in humanized mice (112).

miRNAs in other diseases

Sepsis is a severe illness caused by aberrant host responses to pathogenic microorganisms that include active inflammatory responses and result in multiple organ failure (113). A significant body of work demonstrated that abnormal miRNA expression is associated to both sepsis and non-infective systemic inflammatory response syndrome (SIRS). Yao and his colleagues discovered that miR-25 levels declined in sepsis patients and that lower levels were correlated to an increase in overall mortality (114). The results were reproduced to validate the findings and displayed that miR-25 was downregulated in sepsis models that included caecal ligation and puncture in rodents, as well as lipopolysaccharide-induced cardiomyocytes (115). Another example is miR-155, a negative regulator of inflammatory response that was induced during *Francisella tularensis* infection, resulting in the translational suppression of MyD88 and SHIP-1 i.e. suppressed the release of endotoxin-stimulated TNF and impaired the innate inflammatory response (116).

Myocardial infarction (myomiR) families such as miR-1, miR-208a/b, miR-133, and miR-499 are examples reported in cardiovascular diseases i.e. acute myocardial infarction (AMI). For example, miR-21, a muscle-specific miRNA is elevated in cardiac and skeletal muscles, indicating necrotic activities of cardiac myocytes (117). Besides that, another study reported that miR-133 is significantly higher in AMI patients, with plasma recovering activities of a 4-fold increase after a week (118). This contradicts earlier studies reporting no significant difference in miR-133 levels between AMI patients and normal individuals (119). The miR-133a plasma levels might be useful for the clinical prognosis of ST-elevation myocardial infarction as the higher the levels, the higher the risk of major myocardial damage, severe reperfusion injury, and decreased myocardial salvage (120).

Challenges of miRNA in translational research

Delivery methods and administration routes, dose problems, and off-target complications are all key hurdles in the development of miRNA-based therapeutics for tumour and other disorders. Considering a large number of preclinical investigations in *in vivo* cancer, there are only a limited number of miRNA candidates have successfully reached translational research. Extensive pharmacokinetics investigations in animals may offer a fundamental understanding for predicting miRNA mimics/antimiRs interactions. Nanotechnologies offer versatile platforms for efficient biomolecule delivery using polymers, lipid compounds, as well as inorganic nanomaterial to improve therapeutic efficacy, reduce the effective dose, and reduce the risk of systemic adverse complications (121). Polymeric biomaterials are designed for particular purposes with surface functionalisation, high active payload, and low toxicity due to their synthetic malleability. MiRNA mimics or inhibitors might therefore be protected from the injection site and delivered to the intended location. This approach is similar to how endogenous miRNAs are naturally protected by extracellular vesicles like exosomes. However, the suitability of nanocarrier formulations for drug delivery is determined by a number of factors, including their average diameter and polydispersity index. Controlling and confirming these characteristics is critical for nanoparticle circulation time, biodistribution, and cellular absorption in order for them to be used effectively in therapeutic applications (121).

In addition, the absence of defined regulatory and safety criteria for quality control has slowed the development of these products toward successful clinical translation. The extensive use of innovative polymeric nanomaterials, advanced polymeric-based nano-formulations, and chemical changes necessitates the development of suitable regulatory guidelines to aid in the evaluation of miRNA drugs. The economic cost is another barrier to the clinical translation of miRNA-based therapeutics as compared to existing anti-cancer medicines, owing to the high cost of both miRNA biological products and developing nanocarriers which are more advance and expensive than traditional medications (121).

Conclusion

MiRNAs govern the genotypic expression in myriad types of cancer pathways involving cancer hallmarks. Acting as tumor suppressors or oncogenes, aberrant miRNA expression in cancer cells manifests the capacity to sustain proliferative signals, elude growth suppressors, resisting cell death, activate invasion and metastasis, as well as promote vascular growth via angiogenesis. Moreover, the pleiotropic function targeting miRNA suggests a promising route for future cancer therapies, in vitro and in vivo. The manipulation of miRNA in cancer therapies are relatively convenient as they compose of small non-coding RNA which offers great opportunity in clinical translation. Besides that, the development of safe and efficient miRNA deliveries is the key concept for a successful output from bench to bedside. MiRNA vehicles via liposomes, viral vectors, nanoparticles, and the newly emerging delivery i.e. secreted exosomes portray an encouraging future for cancer therapeutics. Nonetheless, specific target delivery and off-target complications remain the biggest challenges for successful outcomes using miRNA. It is sure that further researches are still warranted to boost the understanding of miRNA-related cancer prognosis, diagnosis, and therapies.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. List of microRNAs, target genes, and their involvement in pathways that lead to cancer occurrence.

miRNA	Expression profile	Target mRNA/ genes	Pathway involved	Cancer	References
mir-15a	Down-regulated	BCL2, MCL1, CCND1, WNT3A	Apoptosis control, cell cycle regulation	Prostate cancer, pituitary adenomas	(24,122)
mir-16-1	Down-regulated				
mir-221/222	Up-regulated	CDK inhibitor of $p27^{Kip1}$, p57, TRPS1, PUMA	Cell cycle regulation, epithelial-mesenchymal transition, apoptosis	Hepatocellular carcinoma, breast cancer, glioblastoma	(28,123)
mir-663	Up-regulated	$p21^{C1P1}$, TUSC2	G1/S transition of cell cycle regulation, cell proliferation, invasion, and migration	Nasopharyngeal carcinoma, ovarian cancer	(16,124)
mir-27a-3p	Up-regulated	MAPK10	Cell proliferation, migration, and invasion	Nasopharyngeal cancer	(17)
mir-545	Down-regulated	Cyclin D1, CDK4	Cell cycle regulation	Lung cancer	(18)
mir-17-92	Up-regulated	E2F1, p53, tensin homolog, RB2	Cell cycle regulation, apoptosis pathway, protein kinase B signalling pathway	Lung, breast, pancreatic cancer	(19,20,125)
mir-192	Down-regulated	Mdm2, p53	Cell proliferation, apoptosis	Multiple myeloma	(24)
mir-194	Down-regulated				
mir-215	Down-regulated				
mir-491-5p	Down-regulated	Bcl-xl	Apoptosis pathway	Ovarian cancer	(27)
mir-204	Down-regulated	Bcl-2	Apoptosis pathway	Prostate cancer, endometrial carcinoma	(126)
mir-148a	Down-regulated			Colorectal cancer	(127)
mir-365	Down-regulated			Hepatocellular carcinoma	(128)
mir-181b-3p	Up-regulated	YWHAG	Epithelial-mesenchymal transition	Breast cancer	(32)
mir-374a	Up-regulated	WIF1, PTEN, and WNT5A	Wnt/-catenin pathway	Breast cancer	(33)
mir-135a	Up-regulated	HOXA10, FOXO1	Migration, invasion	HSSCC, hepatocellular carcinoma	(34)
mir-29a/ b/c	Down-regulated	LAMC2, ITGA6, LOXL2	Focal adhesion laminin-integrin pathway	HSSCC	(35)
mir-21	Up-regulated	PDCD4	RAS-RAF-MAPK pathway	Colorectal cancer	(6,37)
mir-200 family	Down-regulated	ZEB1	Epithelial-mesenchymal transition	Breast cancer	(38,129,130)
mir-107	Down-regulated	HIF1B	Angiogenesis	Colorectal cancer	(46)
mir-126	Down-regulated	CD97, IGFBP2, phosphatidylinositol transfer protein cytoplasmic 1, and c-Mer tyrosine kinase genes	Angiogenesis	Breast cancer	(48–50)
mir-578	Down-regulated	HIF-1	Angiogenesis	BRCA1/2-associated breast cancer	(52)
mir-573	Down-regulated				
mir-29b	Down-regulated	TCF7L2, SNAIL, BCL9L	β-catenin translocation, angiogenesis	Colorectal cancer	(55,56)
mir-34	Down-regulated	CDK4/6, cyclin E2, E2F5, and Bcl-2	Apoptosis pathway	Hepatocellular carcinoma	(58,131)
mir-195	Down-regulated	Bcl-2, Bcl-xl	Apoptosis pathway	Colorectal cancer	(61,132)

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