Recent trends in targeting miRNAs for cancer therapy

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Abstract

Objectives MicroRNAs (miRNAs) are a type of small noncoding RNA employed by the cells for gene regulation. A single miRNA, typically 22 nucleotides in length, can regulate the expression of numerous genes. Over the past decade, the study of miRNA biology in the context of cancer has led to the development of new diagnostic and therapeutic opportunities.

Key findings MicroRNA dysregulation is commonly associated with cancer, in part because miRNAs are actively involved in the mechanisms like genomic instabilities, aberrant transcriptional control, altered epigenetic regulation and biogenesis machinery defects. MicroRNAs can regulate oncogenes or tumour suppressor genes and thus when altered can lead to tumorigenesis. Expression profiling of miRNAs has boosted the possibilities of application of miRNAs as potential cancer biomarkers and therapeutic targets, although the feasibility of these approaches will require further validation.

Summary In this review, we will focus on how miRNAs regulate tumour development and the potential applications of targeting miRNAs for cancer therapy.

Introduction

The small noncoding RNAs that regulate the gene expression post-transcriptionally are called microRNAs (miRNAs).[1] They are endogenous to the cell[2] and are typically 20–22 nucleotides long.[3] Through various experimental studies, it is now established that miRNA dysregulation has a profound effect on the development and progression of cancer in humans. They control a wide array of biological processes, including carcinogenesis through the transcriptional activation of oncogenes. miRNAs act on numerous target RNAs by identifying the complementary region in the 3′ untranslated region (UTR) and through this mechanism regulate various biological processes such as cell differentiation, proliferation and apoptosis,[4] via feedback mechanisms.[5]

Ambros et al. discovered miRNA in *Caenorhabditis elegans* (*C. elegans*) called *lin-4*, which regulates *lin-14* protein expression for the first time and thus laid the foundation for miRNA research.[6] Thereafter, Reinhart et al.[7] showed that *let-7* negatively regulates *lin-14* protein expression by RNA–RNA sequence-specific interaction on the 3′ UTR of the heterochronic gene *lin-41*. This study has revealed that these small noncoding RNAs bind to a sequence-specific target mRNA and alters its expression. Deregulation of a

single or a small subset of miRNAs has shown to have a profound effect on the expression pattern of a huge pool of mRNAs.[8,9] Consequently, several other studies have showcased that miRNAs are highly conserved across the domains of life, suggesting miRNAs have a general regulatory role post-transcriptionally.[10-12] All this research has established miRNAs as a key regulator of cellular functions by specific interaction with epigenetic modifiers, proteins, transcription factors and RNP complexes.[13–15]

Calin et al.[16] showed for the first time the role of miRNA in human cancer by carrying out the studies on B-cell chronic lymphocytic leukaemia (CLL) cells. Two miRNA genes, miR-15a and miR-16-1, are very frequently found to be deleted in CLL cells. Furthermore, it was found that mir-15 and mir-16-1 genes act as a tumour suppressor by repressing bcl-2 protein, thereby inducing apoptosis.[16] This study established the role of miRNAs in cancer. Since then, several other groups have revealed the importance of miRNA in the development and progression of cancer.[17,18]

The advent of next-generation sequencing and miRNA profiling methods has greatly facilitated our understanding of miRNAs for the purposes of cancer identification, classification, diagnosis and prognosis. In this review, we describe the role of miRNAs in cancer and the emerging role of miRNAs as therapeutic targets. Finally, we discuss

about the challenges in miRNA research and its clinical applications.

miRNA biogenesis/biosynthesis and mechanism of regulation

The biogenesis of miRNAs is highly conserved. miRNAs are encoded into the genome in different ways, either by clusters of multiple precursors or by expression from intergenic transcripts, each encoding only a single strand of pre-miRNA (which then adopts a hairpin-like secondary structure).[19] Transcription of pri-miRNA is carried out typically by RNA polymerase II; however, some are processed by RNA polymerase III.[20,21] pri-miRNA is then translocated within the nucleus where DGCR8 (RNA-binding protein) and DROSHA (a type III RNase) like endonuclease enzymes cut the transcribed sequence and result into an 80–100 nucleotide long pre-miRNA sequence.[22,23] The Ran/GTP/Exportin-5 complex then exports the pre-miRNA from the nucleus to the cytoplasm.[24] In the cytoplasm, the cytoplasmic ribonuclease (RNase III) enzyme called Dicer is present and cleaves the pre-miRNA into a double-stranded mature miRNA strand.[25]

As a result of this processing method, the released mature single strand miRNA binds to Argonaute 2 (AGO 2) resulting in a complex called the RNA-induced silencing complex (RISC). This complex has an intrinsic capacity of binding to typical 3′UTRs that are specific to their cytosolic mRNA targets. Binding to mRNAs is based on the complementarity between the base-pairing at the 5′ end of mature miRNA or open reading frame and the cytosolic mRNA molecule, with the binding site known as seed region, being about 6–8 bp long from the 5′ end of the miRNA. The short length of the binding site enables the miRNA to target a large number of different mRNAs.[26–28] miRNA biogenesis is regulated by methyltransferase like 3, which by methylating pri-miRNAs, marks them, and enables DGCR8-based identification and processing, eventually resulting in a mature miRNA.[29] (Figure 1).

To have a deeper understanding of how miRNA-mRNA base-pairing regulates gene expression, Helwak et al. used an unbiased technique called CLASH. They found additional noncanonical binding cluster which was independent of the seed region and interaction complexity. Once the interaction of mRNA and miRNA is formed, imperfect complementarity leads to translational repression, whereas perfect complementarity leads to mRNA degradation.[30,31]

miRNA in tumours have shown to act as a ligand, upregulating various types of signalling pathways. Toll-like receptor I was found to be affected by miRNA in natural killer cells by modulation of a nuclear factor-kB signalling pathway.[32] For example, the miR-21/miR-29a was secreted by the tumour cells and signalled to immune cells

by binding TLR8, inducing a pro-metastatic inflammatory response, which might contribute towards tumour growth and tumour metastasis.[33] Thus, in many ways, any alteration to miRNA biogenesis significantly influences various cancer-related mechanisms and pathways.

Role of miRNA in cancer

In the last few years of research, miRNAs have been established as a novel cell component differentially expressed in pathological and normal cells.[34] Recently, advances have demonstrated the importance of miRNAs in cancer biology through their regulation of gene expression. miRNA acts as a helper in facilitating tumour invasion, growth, immune invasion and angiogenesis.[35,36] These findings have highlighted possible miRNA-based biomarkers associated with cancer that can be detected in various body fluids and would allow for less invasive detection and monitoring of cancer.[37]

The first example of alteration of miRNA levels in cancer was reported in CLL when a cluster of miR-15 and miR-16 was identified at 13q14.3, which is frequently deleted in CLL.[16] Hanahan and Weinberg[38] have established the role of miRNA as a hallmark in several different types of cancer by studying the 'tumour microenvironment'. Different types of tumours show specific miRNA signatures which help in the discrimination of various cancer types.[39] Through multiple studies, various cancer-associated targets and their respective miRNAs have been well-characterized (see Table 1).

Regulation by miRNAs is mainly carried out by two different functions: (1) the homeostatic maintenance of gene regulation, which is highly cell-type dependent and (2) cell fate specification and the preservation of cell identity through feedback mechanisms.[34] In response to stress, changes in miRNAs assist cells in adapting to the altered conditions in their microenvironment.[19] This has been observed in the case of glioblastoma, wherein low miR-451 levels correlate with low glucose levels. miR-451 regulates the AMP-activated protein kinase pathway activation and suppression, which in turn regulates the cell survival and mammalian target of rapamycin-activated cell proliferation.[40] microRNA-specific genetic alterations are observed in cancer cells leading to a modification in target binding, processing and post-transcriptional changes in 3′UTR of mRNA.[41]

microRNA regulation of mRNA is lost in cancer cells during mRNA splicing due to deletion of 3′UTR, single nucleotide polymorphism and mutations.[42] Mutations causing a reduction in efficiency of miRNA processing machinery lead to a significant reduction in the total amount of mature miRNA in the cell. Often, low levels of mature miRNA are observed in tumours,[39] which
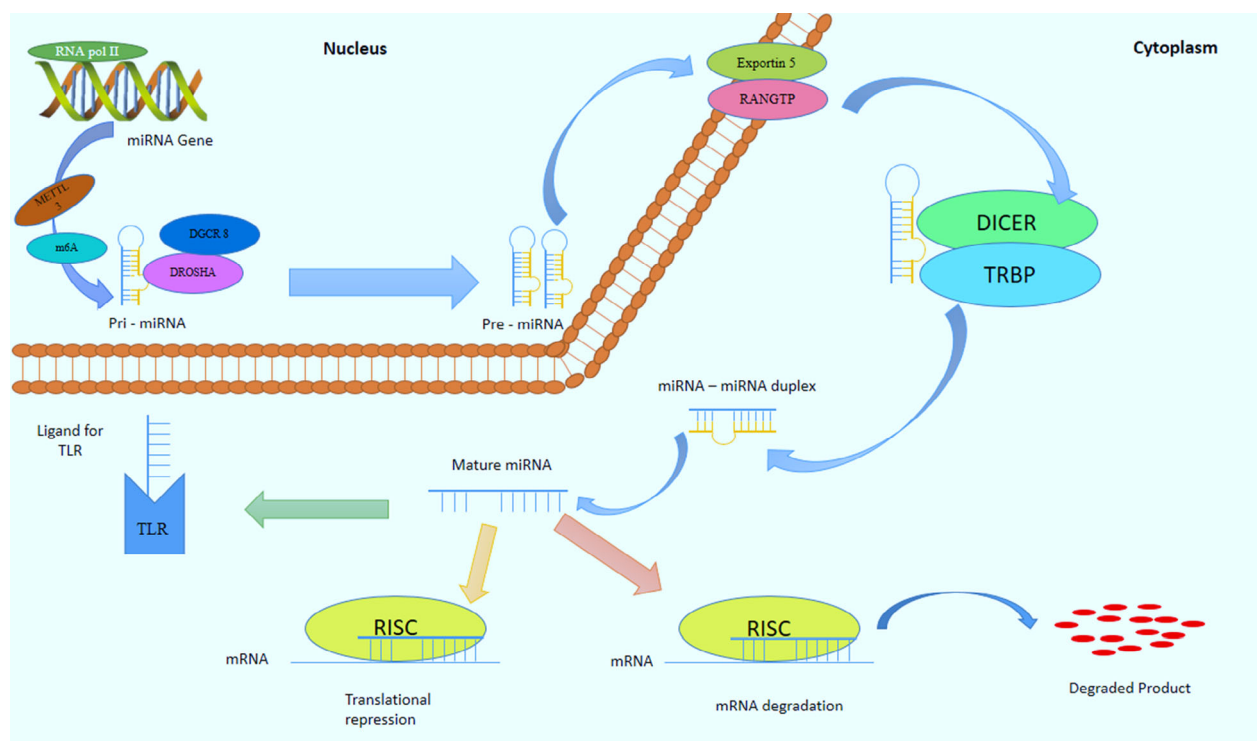
Figure 1 miRNA biosynthesis and regulation. microRNA is transcribed by RNA polymerase II to yield pri-miRNAs which is cleaved by a complex of Drosha and DGCR8 leading to the formation of a hairpin-like structure called pre-miRNA. The exportin-5-Ran-GTP exports this structure from the nucleus to the cytoplasm. Here, the multiprotein complex of TRBP (trans-activation-responsive RNA-binding protein) and the RNase Dicer cleaves it to form a mature microRNA sequence. This mature miRNA strand is incorporated into the RISC (RNA-induced silencing complex), which consisting of AGO2 (Argonaute 2) and GW182. Facilitated by this complex, it partially binds to complementary sequences in the 3' UTR of target mRNAs, thereby controlling mRNA translational repression or degradation.). [Colour figure can be viewed at wileyonlinelibrary.com]

may be caused by genetic loss, epigenetic silencing and changes in the biogenesis pathway or through transcriptional repression.[43,44] The same can also be observed in some microsatellite unstable cancers. Here, mutations in exportin-5 (XPO5) lead to trapping of pre-miRNAs inside the nucleus, preventing further processing of miRNAs.[45]

Reduced levels of DICER expression have been found in various human carcinomas like lung cancer, ovarian cancer and CLL.[46–48] Binding of BCDIN3D (Bicoid-interacting 3, domain-containing) regulates O-methylation of 5'monophosphate leading to an alteration in miRNA processing, as that methyl mark is required for efficient cleavage by DICER, and therefore negatively regulates miRNAs.[49] For example, in ovarian cancer reduced Dicer expression has a direct correlation with drug resistance marker and poor drug therapy outcome.[50] In contrast to these findings, overexpression of DICER has been implicated in prostate cancer progression.[51] Furthermore, the amplification of the Drosha locus is observed in oesophageal cancer.[52] These suggest that it is important to establish, in multiple cancer types, both the frequency of these

mutations and alterations in the miRNA expression signature.

Mechanisms of miRNA dysregulation in cancer

In human malignancies, high irregularity in the miRNA expression level is observed in cancerous cells as compared to the normal cells. The major causes of these alterations of miRNA expression in the cancer are summarized in Figure 2:

Amplification or deletion of genes encoding miRNAs

The alteration of miRNA expression levels in malignant cells is thought to be caused by gene amplification, deletion or translocation. For instance, amplification of gene representing miR-17-92 clusters is observed in lung cancer and B-cell lymphomas.[17,18] Conversely, in B-cell CLL patients, there is a loss of miR-15a/16-1-related genes at 13q14 chromosome.[16] Similarly, the deletion of

Table 1 Regulatory role of miRNA in different cancer types

Type of cancer	MicroRNA	Effect on CSCs property	References
Lung	miR-34a	Inhibitory effect by targeting CD44	[179]
	miRNA-200b	Inhibition of HDAc 1 and Suz-12	[180]
Breast	Let-7	Inhibits self-renewal and dedifferentiation by targeting RAS and HMGA2	[181]
	miR-200 family	Inhibits EMT, self-renewal and mammosphere formation	[182]
			[183]
			[64]
	miR-22	Reduces expression of miR-200 family	[184]
Leukaemia (AML and MDS)	miR-22	Promotes self-renewal	[184]
AML – acute myelogenous leukaemia			
MDS – myelodysplastic syndrome			
Prostate	miR-34a	Inhibits self-renewal and metastasis by targeting CD44	[185]
	miR-320	Inhibits Wnt signalling pathway	[186]
	miR-25	Inhibitory effect by targeting cytoskeleton αv- and α6-integrin	[187]
Liver	miRNA-150	Inhibitory effect by targeting c-Myb	[188]
Pancreas	miR-200c	Inhibitory effect by targeting ZEB1 and E-cadherin	[189]
Brain	miR-17	Promotes cell proliferation of CD133+	[190]
Colon	miR-451	Inhibits tumorigenicity and self-renewal bt targeting COX-2	[191]

miR-143 and miR-145 is observed at 5q33 region in lung cancer patients.[53] However, translocations are also observed, as in the T-cell acute lymphoblastic leukaemia translocation of miRNA-17-92 causes overexpression of this miRNA.[54]

These data have been further confirmed by array-based comparative genomic hybridization technique for 227 specimens representing human breast cancer, ovarian cancer and other melanomas.[55] Furthermore, whole-genome sequencing of these samples showcased that a high amount of miRNA genes is located in cancer-associated genomic regions, that is tumour suppressor genes, oncogene or common breakpoint regions. Thus, specific regions in the genome are responsible for the altered miRNA expression profiles due to deletion, amplification and translocation of certain specific genomic sites.
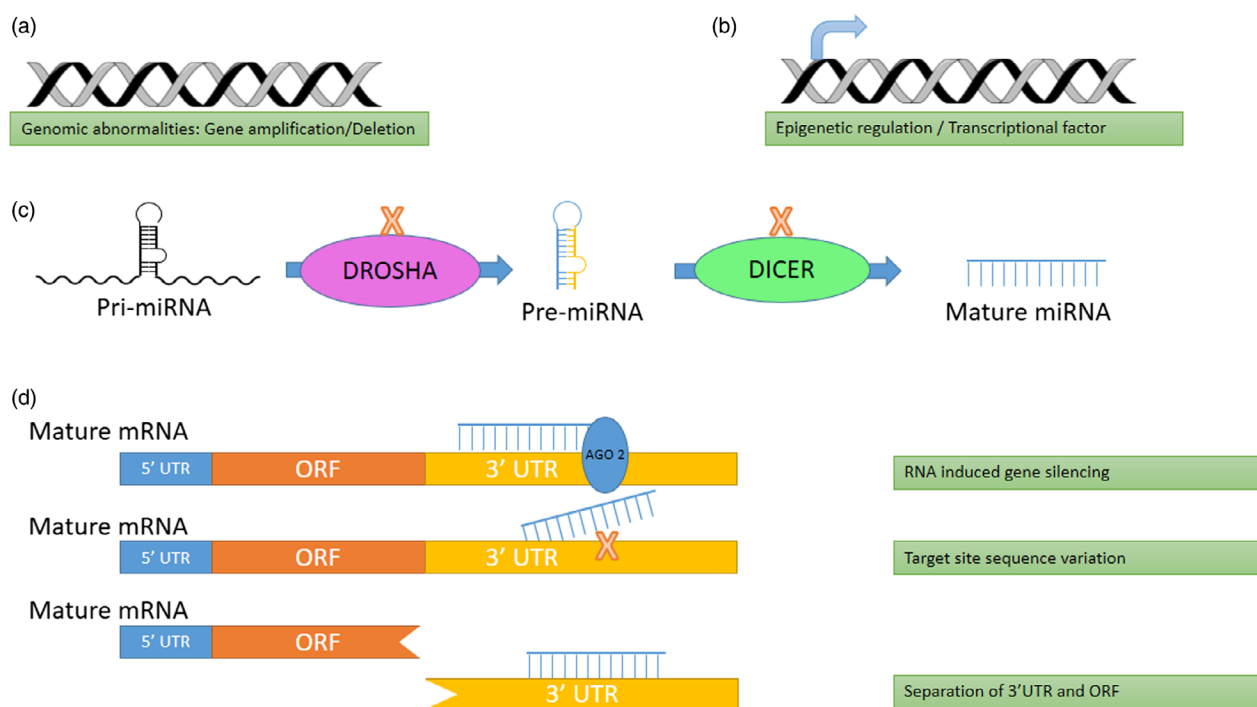
Figure 2 Different mechanisms of miRNA deregulation in cancer. [Colour figure can be viewed at wileyonlinelibrary.com]

Dysregulated epigenetic change

Abnormal epigenetic modifications like tumour suppressor genes hypermethylation, variation in histone modification pattern and global DNA hypomethylation are the characteristic features of cancer cells.[56] A high proportion of miRNA loci is associated with CpG islands, indicating the role of DNA methylation-based epigenetic regulation of miRNA expression.[57] One example is the epigenetic silencing of miR-223 expression by AML1/ETO (AML fusion protein) via CpG methylation.[58] Seventeen miRNAs were upregulated by more than threefold in DNA methylation and histone acetylation inhibitor-treated T24 bladder cancer cells. Of these 17 miRNAs, miR-127 (embedded in CpG Island) was highly upregulated in treated cancer cells as compared to normal cells, simultaneously downregulating the proto-oncogene BCL6. These results suggest that miRNA expression-based tumour suppression can be achieved by the use of DNA methylation and histone acetylation inhibitor treatment.[59] Furthermore, DNA hypomethylation-mediated upregulation of potential oncogenic miRNA has been exhibited through various studies.[60,61]

The miRNA and epigenetic mechanisms have been shown to have a strong relationship with cancer as miR-29 expression inhibit the expression of DNMT3A and DNMT3B,[62] genes required for regulating the DNA methylation. Restoration of miR-29 levels in NSCLC (non-small-cell lung cancer) caused derepression of CpG island methylation-silenced tumour suppressor genes. EZH2 a type of histone methyltransferase is targeted by miR-101 leading to target gene silencing and regulates cancer cell's survival and metastasis.[63] SUZ12 a polycomb repressor complex 2 component is targeted by miR-200 family, having an ability of cancer stem cell (CSC) formation. Loss of miR-200 expression consequently leads to increased expression and binding of SUZ12, H3-K27 tri-methylation and E-cadherin gene repression.[64]

The miR-148a and miR-34b/c were discovered by Lujambio et al.[65] as a hypermethylation-specific silencer of cancer cells with decreased tumour growth and metastasis formation. All these examples demonstrate the role and importance of epigenetic regulation by miRNAs and its ability to alter DNA methylation and histone acetylation levels of the described genes, thus, showcasing its utility as cancer diagnostic or prognostic biomarkers.

Transcriptional control of miRNA

Almost half of the genes representing miRNAs are present in the introns of protein-coding genes or the long noncoding RNA genes and have their associated promoters and enhancers.[66] Transcription of genes that solely encode miRNAs is performed by RNA polymerase II.[21] miRNAs are mainly transcribed as a polycistronic message since the miRNA gene is present in a clustered form. A plethora of RNA polymerase II-associated transcription factor governs several miRNA genes by a single factor, generally via a complex circuit of feedback and feed-forward loops.

It is evident from multiple studies that transcription factors like c-Myc and p53 govern the expression of miRNAs in different cancers. Generally, c-Myc is upregulated because of miR-17-92 cluster activation, regulating apoptosis and cell proliferation of malignant cells.[67] Furthermore, c-Myc downregulates the transcriptional activity of miR-15a, miR-26, miR-29, miR-30 and let-7 families of tumour-suppressive miRNAs.[43] This is thought to be a result of the feedback loop where c-Myc regulates miR-122 by binding to its promoter, whereas Tfdp2 and E2f1 are indirectly inhibited by miR-122, thus inhibiting c-Myc transcription. Thus, showcasing the importance of this feedback loop in the development of carcinoma.[68] In nonsmall-lung cancer, expression of miR-221/miR-222 clusters is controlled by hepatocyte growth factor receptor c-MET, which in turn controls AP1 and ELK-1 transcriptional factors, initiating a negative feedback loop with miR-27a.[69,70] A similar loop is observed where miR-148a-5p/miR-363-3p gene promoter is directly targeted by c-Myc, repressing their expression. These also promote the progression of cell cycle, specifically from G1 to S phase. As a response, c-Myc expression is directly inhibited by miR-148a-5p and destabilized by miR-363-3p via direct targeting of ubiquitin-specific protease.[71]

A synergistic type of loop is observed between p53 and miR-34, imparting a tumour-suppressive activity.[72] It is shown that p53 directly binds with mir-34a gene promoter and triggers the apoptosis process.[73,74] As a feedback response miR-34a directly targets SIRT1 and downregulates it, in turn SIRT1 via deacetylation negatively regulates p53 and prevents transcriptional dependent apoptosis by p53. However, an increase in transcriptionally independent p53-mediated apoptosis is observed.[75] The expression of miR-107,[76] miR-605[77] and miR-1246[78] is also regulated by p53. p63, a p53 family member, is capable of regulating Dicer1 transcription. In tumours with a p63 deficiency, very low Dicer1 expression level is observed, leading to levels of low mature miRNAs, the consequence of which is an increased tendency for metastasis.[79]

Defects in miRNA biogenesis machinery

Dysregulation of enzymes and/or cofactors, like Dicer, Drosha, DGCR8 and exportin 5 that are involved in the biogenesis pathways, significantly affects the overall mature miRNA levels. As evident in both *in-vitro* and *in-vivo* models, when Dicer1 and Drosha were partially deleted, faster tumorigenesis was observed in different types of tumours.[80] Drosha processing has emerged to be a critical

step in the regulation of miRNAs in both cancers and in embryonic development.[81] Similar results can be observed in Dicer dysregulation, as in colorectal cancer cells Dicer1 impairment has led to higher tumour metastasis and initiation capacity.[82] Furthermore, increased median survival has been witnessed in ovarian cancer patients with high mRNA levels of Dicer and Drosha.[83] Conversely, the reduced patient survival rate can be correlated with decreased Dicer expression levels.[47,84]

Argonaute proteins (AGO) play a central role in RNA-silencing, and their dysregulation can have serious implications in cancer. The loss of human EIF2C1/hAgo1 gene has been observed in Wilms' tumour of the kidney.[85] Low AGO2 expression has been evident in melanomas as compared to primary melanocytes.[86] On the other hand, a high AGO2 expression has been observed in primary gastric cancer patients.[87] Lin28, a highly conserved RNA-binding protein that modulates the processing of miRNA let-7, has been implicated in oncogenesis, cell pluripotency and developmental timing.[88] Exportin 5 (XPO5) is a dsRNA-binding protein that is responsible for the export of pre-miRNA from the nucleus to the cytoplasm. A truncated version of the XPO5 gene is unable to export pre-miRNA from the nucleus, and as a result, pre-miRNA is trapped in the nucleus, leading to low mature miRNA processing.[45] Interestingly, XPO5 function restoration normalizes miRNA processing and also provides tumour suppressor activity. It is noteworthy that various other miRNAs are capable of regulating miRNA processing. In aggressive breast cancers, the miR-103/107 family of miRNAs targets DICER and thus reduces the level of the global miRNA. In summary, the key mechanisms linking miRNAs to cancer are chromosomal abnormalities, transcriptional changes, nuclear receptors and defects in miRNA biogenesis.

Altered miRNA expression in tumours

Tumours acquire the ability to resist apoptosis, dodge growth suppressors, maintain proliferative signalling, empower replicative immortality, provoke angiogenesis and initiate invasion and metastasis.[38] miRNA profiling of these tumours has shown abnormal expression as compared to the normal tissues and hence is believed that dysregulated miRNAs function as either tumour suppressor genes or oncogenes depending on the gene target, affecting any of the above-mentioned hallmarks. The balance between extracellular signalling molecules and intracellular processes controls the cell cycle progression. Through different studies, it has been apparent that miRNAs are integrated into multiple cell proliferation pathways, therefore sustaining proliferation and evades growth suppression in cancerous cells.

The E2F proteins, in a cell cycle-dependent fashion, are key cell proliferation regulators, which are in turn regulated by miRNAs. In the G1 to S transition period, E2F1-mediated induction of gene transcription has been observed.[89] Several different types of cancer were observed in E2F1-/- mice, suggesting the role of E2F1 as a tumour suppressor. E2F1 translation is inhibited by miR-17-92 cluster post c-Myc activation.[67] E2F2 and E2F3 translation are also regulated by the miR-17-92 cluster.[90] A feedback mechanism regulates the expression of miR-17-92 cluster and E2F to achieve cell cycle progression in normal cells.[91] In tumorous conditions, disruption in the feedback loop can be observed due to miR-17-92 cluster overexpression, leading to cell proliferation.[92] miRNAs also regulate cyclins, cyclin-dependent kinases (CDKs) and CDKs inhibitors on whom the cell cycle progression is dependent.

Dicer-1 knockout in germline stem cells of Drosophila blocked the transition from G1 to S phase, demonstrating the importance of miRNAs in this transition.[93] Furthermore, in this context increased expression levels of CDK inhibitors (Dacapo) of the p21/27 family were also observed, suggesting that downregulation of the protein by miRNAs would boost cell cycle progression. Cdk inhibitor p27^{kip1} is directly targeted by miR-221/222 in glioblastoma cells.[94] In cancerous cells, high expression of miR-221/222 speeds up cell proliferation, and its low expression causes G1 cell cycle arrest. These data are well correlated with both primary tumour samples and cancer cell line studies.[95–97] Moreover, the upregulation of the miR-221/222 is observed in various human tumours, confirming the findings that Cdk inhibitor p27^{kip1} regulation is a part of an oncogenic programme. The miRNA family of miR-302, miR-663 and miR-24 regulates the p21^{CIP1} and p16^{INK4a} other than p27^{Kip1}.[98,99] miR-663 and p21^{CIP1} form a loop at the molecular level and are responsible for cell proliferation in nasopharyngeal cancer.[100] miRNAs also regulate the expression of cyclins and Cdks, as the expression levels of CDK4 and cyclin D1 are decreased by miRNA-545 in lung cancer cells as a consequence of cell cycle arrest.[101] miRNAs also regulate a variety of signalling pathways thereby affecting cell proliferation. For example, miR-486 affects cell proliferation and migration by targeting p85α, IGF1 and IGF1R of phosphoinositide-3-kinase (PI3K) and insulin growth factor (IGF) signalling pathways.[102] Thus, concurrent and extensive indications of altered miRNAs have been implicated in cancer, representing as a candidate target for treating cancer.

Targeting key cancer-related pathways

Cell cycle and cell proliferation as targets

It has been well-established that miRNAs have a key role in controlling cell proliferation, altering various regulatory pathways, and, hence, have a profound effect on

carcinogenesis. Oncogenic miRNAs are typically overexpressed and act as a facilitator for cancerous cells to enter and progress through the cell cycle. miRNAs that suppress tumour growth are typically lost during cancer and, hence, normally help in inducing cell cycle arrest.[103]

The retinoblastoma (pRb) pathway has a significant effect on the regulation of the cell cycle and is affected in a variety of human cancers.[104,105] It acts by repressing the transcription factor family E2F, which governs the gene expression of genes essential for cell cycle progression.[106] Cyclin-dependent kinases mediated phosphorylation of pRb leads to activation of transcription of genes by E2Fs. Specific kinases and cyclins form complex with active CDKs and aid in the progression of the cell cycle through its sequential phases.[107] These important cell cycle components (i.e. CDKs and cyclins) are targeted by growth-restricting miRNAs, acting on growth diminishing pathways such as p53 or by growth-enhancing mitogenic pathways such as RAS/RAF/ MAPK.[107,108] For example, miR-20a, miR-125b and miR-17-92 clusters possess a tumour-suppressing function by targeting the E2F transcription factor.[67,109,110]

The miRNAs regulate cell cycle inhibitors, which negatively regulates the CDKs, as shown by the CDK inhibitors from the cip/kip family. miR-106b and miR-17-92 families act upon p21, which is a potent CDK inhibitor and a primary mediator of the downstream cell cycle's G1 phase arrest of the p53 gene. Wu et al. experimentally demonstrated that about 28 miRNAs have the potential to target the 3'UTR region of p21 mRNA by a luciferase assay.[111] Similarly, p27 and p57 are controlled post-transcriptionally by miRNAs. In particular, p57 is controlled by the miR221/222 cluster.[96,112] Thus, miRNAs have a significant impact on cancerous cell entry and progression through the cell cycle.

Senescence as target

Senescence is an irreversible exit from the cell cycle. It is mainly of two different types, replicative and premature senescence. The replicative senescence occurs due to the shortening of telomeres, and premature senescence occurs due to higher oxidative stress levels, DNA damage signalling or increased oncogene expression levels.[113] miRNAs negatively regulate cell cycle progression and hence plays a role in the induction of senescence. For example, the senescence inducers p16 and p19 are repressed by HMGA2, which is in turn a primary target of miRNA let-7.[114–116] The miR-24 is downregulated in replicative senescence.[98] miR-34a of the miR-34 family regulates p53, acting as an important regulator of senescence by targeting at multiple sites.[75,117,118] A complex feedback loop is formed between miR-34a and p53 de-acetylating enzyme SIRT1, regulating the transcription and activity of miR-34a.[75,119,120] p53 is not the sole regulator of the miR-34a,

as miR-34a is also regulated by ELK1 of ETS family.[121] Furthermore, four different clusters of miRNA, let-7a-d, let-7i, mir-106b-25 and mir-15b-16-2 are induced by E2F1 and E2F3 during the transition from G1 to S phase. Actually, they inhibit this transition by inhibiting various E2F gene targets and cell cycle promoters.[122] As a highly heterogeneous process, the initiation, maintenance and regulation of senescence involve multiple regulators and factors. miRNAs being able to affect multiple genes and pathways can suitably regulate senescence, which might function as the promoter of senescence.

Cancer stem cells as Target

The CSCs theory proposes that a rare population of cells that possess stem cell-like properties is responsible for cancer.[123] There are a set of protein-coding genes, specifically surface markers, that are involved in asymmetrical cell division and self-renewal of CSCs. miRNAs play a key role in tumorigenicity, drug resistance and asymmetrical cell division of CSCs.[124] An upregulation of the oncogenic miRNAs and downregulation of the tumour suppressor gene have been observed to be responsible for its effects on CSCs.[92] The molecular analysis revealed that oncogene targeting miRNAs are present at fragile sites and are sensitive to loss or reduction of miRNAs, in turn leading to the upregulation of specific oncogenes. Ultimately, these changes affect numerous cancer progression-related processes such as metastasis, anti-apoptotic, tissue invasion and drug resistance.[125]

In recent years, a new strategy of cancer therapy includes specifically targeting CSCs. There are significant obstacles to this approach, as it is of utmost importance that CSCs and other cancerous cells need to be selectively identified by various molecular differences and markers. The cell-based targeted delivery of the miRNA inhibitors or miRNAs mimics is the most effective form of treatment. CSCs differ from normal stem cells with regard to the expression of CSC markers, as well as by glycosylation patterns.[126] This allows a further point of distinction, and so, the development of antibody-conjugated nanoparticles or liposomes against the CSC-specific glycans will enable to delivery of CSC-suppressing miRNAs selectively. Hence, miRNAs act as the functional markers of CSCs and further studies will potentially reveal the role of miRNAs in CSC biology specifically in diagnosis, prognosis and treatment, thereby enhancing the current cancer treatment regime and reducing side effects.

Enabling cancer cell's sensitization to drugs

Only 1–2% of the human genome is protein-coding; however, 70–80% of the human genome is transcribed into

RNA. This is indicative of the importance of noncoding RNAs in the regulation of protein production.[127–129] In principle, targeting miRNAs could alter the protein levels for genes relevant to cancer cell biology. Low protein levels correlate with poor drug efficacy for small molecules. Thus, miRNA targeting by small molecule inhibitors would inhibit oncogenic expression.[2]

A selective pri-miR-515 inhibitor was designed by Costales et al. called Targaprimir-515 inhibiting mature miR-515 biogenesis, leading to higher expression of sphingosine kinase 1 (SK1) and sphingosine-1-phosphate (S1P) protein that are associated with cell migration and proliferation. More significantly, they witnessed high expression levels of human epidermal growth factor receptor 2 (HER2) in HER2-negative MCF-7 cells, showcasing the sensitization of MCF-7 cells towards Herceptin (HER2 targeting drug) post-treatment. The specificity of this small molecule was revealed, as about 99.7% of the genes were unaffected and the healthy breast epithelial cells called MCF-10A were unaffected by pri-mir-515 inhibitor treatment as they lack miR-515.[130]

Similarly, various miRNAs are involved in enhancing the potency of anticancer drugs. For instance, miR-27b sensitizes a broad spectrum of anticancer drugs in liver and kidney cancer. This is achieved by increasing miR-27b levels, as it is generally deleted in both liver and kidney cancer. miR-27b sensitization to anticancer drugs is achieved in patients with high levels of CYP1B1 or p53 wild type, as the miR-27b aids in the activation of p53-induced apoptosis and drug detoxification via CYP1B1.[131] Various miRNAs that sensitize different drugs are summarized in Table 2.

Finally, miRNAs are attractive targets in cancer therapy because of their virtue of nonobvious ways to post-transcriptionally control oncogene expression with such small molecules that increase drug response and efficacy.

miRNA as cancer biomarker

MicroRNAs possess very high stability in the biological fluids, and their differential expression has been closely corelated to cancer patient's prognosis or treatment response. Circulating miRNAs are secreted from tumorous tissue into the surroundings and are protected from endogenous RNase activity.[132] Various circulating miRNAs are being used as a tool for the diagnosis and prognosis of cancers, also aiding in distinguishing tumour subtypes.[133] Micro-RNAs are found in abundance in the exosomes which provides them the stability and plays a crucial role in cancer development and progression.[134–136]

Various cancer-specific miRNAs entrapped in exosomes (30–120 nm membrane-derived vesicles) have been discovered from serum, plasma and body fluids providing an easy and early diagnostics of cancer. Exosomes enable the cell-to-cell communication and miRNA secretion from one cell to another, and cancer cells by this mechanism regulate the physiological and immune response of the surrounding cells.[137] Jin et al showed the role of overexpressed miR-181-5p, miR-361-5p and miR-30a-3p found from plasma exosome in lung adenocarcinoma (LUAD).[138] Tanaka et al using microarray technology showed the role of gastric juice-derived exosomal hsa-miR-933 in functional dyspepsia. Urdinez et al.[139] discovered the role of miR-143/145 in chondrosarcoma as diagnostic biomarker.

However, the specificity of the miRNAs towards a specific disease condition is questionable, as they can regulate the expression profiles of several types of mRNA. The human miRNA disease database was analysed to discover

Table 2 miRNAs that enhance drug efficacy in various cancer

MicroRNA	Sensitized anticancer drug	Cancer type	Target pathway	References
miR-101	Doxorubicin	Hepatocellular carcinoma	Apoptosis via. Mcl-1 targeting	[192]
miR-153	Arsenious acid	Chronic myeloid leukaemia	Bcl2 Downregulation	[193]
miR-126	Vincristine and Adriamycin	Gastric cancer	Directly targeting EZH2	[194]
miR-200c	Vincristine	NSCLC	BCL2	[195]
	Cisplatin		BCL2/ZEB1	
	Cetuximab		ZEB1	
miR-451	Cisplatin	Lung cancer	Mcl-1	[196]
miR-1	Doxorubicin	Lung cancer	CASP3/CASP7	[197]
	Etoposide		MRP1/ABCC1	[198]
miR-134	Doxorubicin	Small-cell lung cancer	MRP1/ABCC1	[198]
miR-128-2	Doxorubicin	NSCLC	E2F5	[199]
miR-133b	Gemcitabine	Lung cancer	MCL1, BCL2L2	[200]
miR-7	Gefitinib	Lung cancer	EGFR, RAF-1	[201]
miR-103	Gefitinib	Lung cancer	PRKCE	[202]
miR-203	Gefitinib	Lung cancer	SRC	[202]
miR-130a	TRAIL	NSCLC	MET targeting	[203]

the specificity of the miRNAs towards the disease. The graph below shows that many miRNAs are not cancer-specific, but are commonly dysregulated in several cancer types especially mir-21. Complex miRNAs signature is disease specific, yet are independently reproduced and rarely validated.[140] Although miRNAs hold a very high diagnostic potential, several challenges are needed to be addressed for translating it from bench to patient care. Challenges like bias in high-throughput approaches, reproducibility and degree of specificity are of key concern. For clinical application, miRNA-based multiplexed tests are warranted for the large-scale application (Figure 3).

miRNA-based cancer therapeutics

Tumour progression leads to a consequent decrease in tumour-suppressive miRNAs expression, progressing the oncogenic signalling pathway. Hence, replenishing the tumour-suppressive miRNAs at the site of the tumour is an attractive option. Alternatively, miRNA antagonists (anti-miRs) are used to target oncomiR-dependent tumours.[141]

miRNAs not only target the tumour-promoting stromal cells but also target endothelial cells and fibroblast cells constraining angiogenesis and fibrosis.[142,143] miRNAs, as natural antisense nucleotides, has minimal toxicity and immune response as compared to the protein-based drug and plasmid DNA-based gene therapy. The double-stranded miRNAs attach with the RISC complex, where the AGO2 protein causes cleavage of passenger strand, while the guide strand binds to the target mRNA.[144] Hence, while designing the miRNA antagonist or miRNA mimics, the properties of the miRNA guide play a crucial role. As miRNAs are having unprotected 3′-hydroxyl and 5′-phosphate ends, they are easily degraded by ribonucleases, making its expression transient and short half-life.[145] Argonaute 2 protein or naturally occurring extracellular vesicles are vastly used to overcome the miRNA stability-related problems. Over the last decade, nanoparticle-based delivery system is being exploited to provide tumour-specific miRNA delivery. Organic lipid-based nanoparticles (LNPs), inorganic compounds like gold, silica and polyamidoamine (PAMAM) dendrimers are some of the
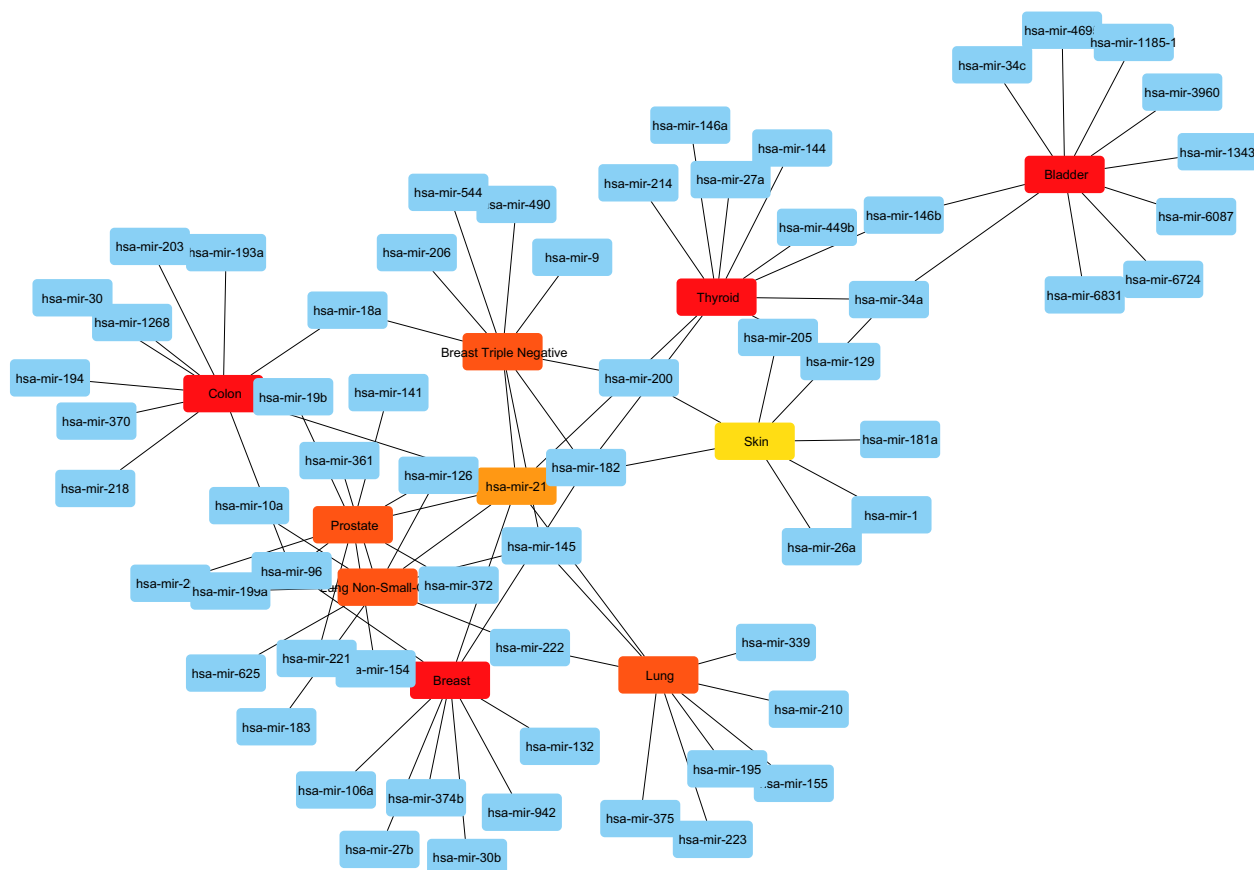

Figure 3 Human miRNA Disease Database (HMDD) disease network. Hub nodes in the combined network for common cancer types and their interaction with miRNA. Hub nodes are indicated with a colour scheme from high interaction (red) to interactive (yellow). [Colour figure can be viewed at wileyonlinelibrary.com]

prominent miRNA delivery vehicles that emerged as a result of efforts of scientists across the globe. Similarly, virus-based miRNA delivery systems involving retrovirus, adenovirus, lentivirus and adeno-associated virus have been developed considerably.[146]

miRNA mimic having its 5′ end complementary to the 3′ UTR of the target gene mimics the endogenous mature miRNA. Thereby, enabling the restoration of the lost or downregulated tumour suppressor miRNA.[147] Cationic LNPs combined with miR-634 mimic (miR-634-LNPs) showed a significant reduction in pancreatic tumour growth as compared to control (miR-LNPs). However, LNP-treated group showed a relative increase in AST levels, indicating LNP-associated toxicity.[148] Many attempts are being made to use the mesenchymal stem cells for cancer therapy, using its ability to secrete abundant chemokines and growth factors.[149] Extracellular vesicles released from the mesenchymal stem cells have been engineered for systemic or oral or intratumoral administration of various miRNA mimics such as miR-379 and miR-146b.[150,151] MSC-EV-miR-185 was tested by applying topically at the carcinoma site in animal models for its ability to curb inflammation and oral squamous cell carcinoma cases.[152] miR-185 regulates the AKT pathway, thereby causing an increase in expression of the cleaved caspase 3 and 9, boosting apoptosis.[153]

Anti-miRs affect cancer-related pathways by blocking the oncomiR. Anti-miR is generally based upon the antisense oligonucleotides or by locked nucleic acids (LNAs) they possess the complementary sequence to that of target miRNA. For example, miR-21 blocks PTEN by activating the PI3K pathway, ultimately leading to inhibition of apoptosis. Anti-miR-21 treatment to the breast cancer cells showed a profound activation of the apoptotic factors, controlling their proliferation.[154] Similarly, Yin et al.[155] focused on the CSCs that are responsible for the cancer's aggressiveness, metastasis and drug resistance. Using a three-way junction (3WJ) motif as a framework for nanoparticles, it carried anti-miR-21 LNAs along with RNA aptamer binding to CD133 receptor. These nanoparticles were able to selectively target triple-negative breast cancer cells, causing a reduction in miR-21 expression and its downstream processes.[155]

Various studies focusing on the miRNA-based sensitization of the tumours that are resistant to the available cancer therapies are underway. Silica nanoparticles with the combination of oxaliplatin and miR-204-5p were tested for colon cancer, resulting in significant decrease in tumour growth via induction of apoptosis.[156] Shah et al.[157] using hydrophilic polyethylene glycol in conjugation with poly-lactic-co-glycolic acid nanoparticles (PLGA-PEG-NPs) comprising of antisense-miR-21 in combination with orlistat (anti-obesity drug) were able to show a drastic decrease

in IC$_{50}$ values as compared to monotherapy for TNBC. Using gold-iron oxide nanoparticles with PEG-T7 peptide (T7-poly-GIONs), the anti-miR-21 and miR-100 were loaded for the glioblastoma multiforme treatment along with systemic temozolomide, increased the overall survival rate in the animals.[158] Thus, these findings corroborate the benefits of the miRNA-based therapy for cancer as monotherapy or as a combinatorial therapy and, thereby, facilitating to lay down a foundation for the development of a viable and potent treatment option for advanced stage cancer.

miRNA-based clinical trials

A number of miRNAs are currently in clinical trials. These are being studied for their use as biomarkers, in disease classification and progression, for synergy with other drugs and as prognostic tools. Studies investigating the miRNAs mimics and anti-micro-RNA construct as potential cancer therapies are being carried out. miRNAs have been evaluated for their potential application of decreasing tumour's drug resistance, for example, in small-cell lung cancer, miR-100 has been shown to have a chemo-resistant property (Table 3).[159]

In chemo-resistant ovarian cancer, miR-199b-5p is epigenetically silenced.[160] Anti-miR is also involved in advanced miRNA-based trials as implicated in hepatitis C therapy, and anti-miR-122 (Miravirsen) is under clinical trial.[161] Miravirsen has a complementary sequence of that to miR-122, and it imparts degradation resistance and high target affinity, due to its LNA structure. Other than targeting the mature miRNAs, it also targets pre- and pri-miRNAs, aiding its therapeutic action.[162] MRX34 was the first miRNA-based therapy for cancer and it mimics miR-34a, which suppresses the tumour, acting downstream on the p53 gene.[163,164]

With these different studies under investigation, resistance to miRNA therapy has emerged as a potential problem, which can be solved with the help of combinatorial therapy or by anti-miRNA-based therapy.[165] Though various clinical trials are underway, miRNA-based therapy is still in its primary stage and side effects are still needed to be evaluated. Systemic side effects might also be a possibility that has to be further investigated. Other miRNA processing alteration might be observed due to the external introduction of replacement miRNAs in the cell, and all such possibilities can only be revealed in the coming future and can only be elucidated by clinical trials.

Future trends and challenges

Owing to the extensive research across the globe, the domain of miRNA-based therapeutics is continuously

Table 3 Cancer-related clinical trials with a significant role of miRNA in it (ClinicalTrials.gov)

Disease	Trial	Reference
Breast cancer	Circulating miRNAs as biomarkers of hormone sensitivity in breast cancer (MIRHO)	NCT01612871
Brain Tumours	Establishment of a signature of circulating microRNA as a tool to diagnosis of primary brain tumours in adults (MIRNA)	NCT03630861
Lung cancer	Plasma microRNA profiling as first-line screening test for lung cancer detection: a prospective study (BIOMILD)	NCT02247453
Lung cancer	Addition of microRNA blood test to lung cancer screening low dose CT	NCT03452514
Skin cancer	Expression levels of microRNA processing enzymes dicer and drosha in epithelial skin cancer	NCT00849914
Cancer	Clinical validation of the role of microRNA-binding site mutations in cancer risk, prevention and treatment	NCT02253251
Brain tumours	Establishment of a signature of circulating microRNA as a tool to aid diagnosis of primary brain tumours in adults (miRNA)	NCT03630861
Bladder cancer	The potential role of microRNA-155 and telomerase reverse transcriptase in diagnosis of nonmuscle invasive bladder cancer and their pathological correlation	NCT03591367
Kidney cancer	Anti-IMP3 autoantibody and microRNA signature blood tests in finding metastasis in patients with localized or metastatic kidney cancer	NCT00806650
Nonsmall-cell lung cancer	Interventional study to identify a signature of response to chemotherapy	NCT00864266
Haematologic cancer	Observational studies of biomarker of expression profiles in initiation, progression and treatment response	NCT01108159
Ovarian cancer	Observational studies of biomarker of response to treatment	NCT01391351

evolving. As a result, deep insight into miRNA function and biogenesis will result in better development of miRNA-based therapies. Further research focusing on leveraging the benefits of target diversity and preventing the off-target effects is the need of the hour. Currently, many companies are exploring the possibility of the use of miRNA-based therapeutics for treating cancer. For the application of exosomal miRNA in cancer diagnostics, issues like exosomal origin must be addressed as the tumour-derived exosomes are required to be separated from the other body fluid derived exosomes. Secondly, the standard protocol for separation and detection of exosomes should be established as the current methods represent several drawbacks such as contamination from other biological molecules and aggregation. Thirdly, the normalization methods must be well established and globally acceptable, so that the comparison of the data from different studies can be easily compared.[166] The literature review reveals disparities among the results of several studies, questioning the reliability and reproducibility of miRNA-based therapeutics in humans.[167] The inconsistencies in the results can also be attributed to the potential difference in the sample size, time of sampling, miRNA quantification and normalization procedure and comorbid conditions.[168] In cancer patients, elevated levels of miRNAs are observed into the circulatory stream as compared to their healthy counterparts. Hence, the use of an equal volume of the sample, rather than using the same amount of total RNA for a reliable detection study, is recommended. Further, for normalization, housekeeping transcripts like SNORD and U6 derived from cells or tissues are generally used. However,

their reliability is questionable as these transcripts are highly sensitive to RNase activity.[169] Normally, unaffected individuals, without any history of the early and late-stage disease, are used as a control in all these studies. But, validation studies are suggestive that dysregulation of the miRNAs is generally in late disease stages.[170] Therefore, confounding factors like age and sex of the patients must be taken into consideration, as they are recognized to modify miRNAs expression.[171] Such complexities can be addressed by performing a meta-analysis; however, the above-mentioned parameters must be kept constant and a standard operating procedure should be followed. Also, data acquisition at multiple time points should be considered, as this can provide vital information about the potential confounders.

The miRNA-based therapeutics offers several problems, like (1) degradation by nucleases,[172] (2) nominal cell membrane transport,[173] (3) endosomal entrapment,[174] (4) poor target tissue delivery[174] (5), innate immune reaction activation,[175] (6) unwanted off-target and toxic effects[175] and (7) poor binding affinity for complementary sequences.[176] One of the major problems related to the *in-vivo* application of miRNA therapeutics is tissue-specific delivery. Further, cellular uptake of synthetic oligonucleotide in a sufficient amount is required for achieving sustained target inhibition.[177] The unmodified 'naked' oligonucleotides are unstable in bodily fluids or tissues and are most vulnerable to cellular and serum nucleases.[177] Size and negative charge of the oligonucleotides prevent transport through the cell membrane, causing poor cellular uptake.[177] Also considering the fact that, miRNAs regulate

many genes, the potential off-target effect of the miRNA therapeutics is of major concern, as they can do more harm than benefits. The miRNAs control various major cancer-related pathways, and it also has a role in various developmental and regulatory pathways. Hence, have the potential to cause serious unwanted side effects.

Various strategies to enhance target delivery like viral, nonviral and chemical modifications are suggested. However, these modifications have significantly improved the target delivery of oligonucleotides, but has also reduced biological activity and increased toxicity.[178] Nanoparticle-based delivery is widely being tested, and the efforts to decrease their toxicity and cellular accumulation are also underway. Furthermore, future studies to provide deep insight concerning pharmacokinetics and pharmacodynamics for miRNA-based cancer therapy are warranted in order to develop therapies that achieve desired therapeutic concentration in the target cell and tissues.

The next goal for miRNA-based cancer therapy is to develop novel delivery methods with improved antisense and miRNA mimic's chemical design. miRNA profiling of the control and drug treated patients are allowing us to develop distinct drug-specific miRNA maps. These maps can be used to develop treatment strategies for reprogramming cancer patient's miRNAome. Also, the use of miRNAs to sensitize the currently available chemotherapeutic agents is an exciting strategy. Novel miRNA delivery systems, nanoparticulate formulations and exosomes can be focused upon to bypass current challenges faced by miRNA-based therapeutics. Targeting the miRNAs to normalize the disturbed miRNA network in cancer patients seems to be a rational and reliable strategy, holding a high level of potential for success. In the future, based on the patient's miRNAome, it will be possible to develop unique miRNA antagonists or mimics and thereby achieve the goal of personalized cancer therapy.

Concluding remarks

Since the discovery that the deletion of miRNAs was linked with CLL,[16] researchers all around the world started investigating the role of miRNAs in different types of cancer and the cause behind the dysregulation of miRNA expression. This revealed the role of different mechanisms such as miRNA gene deletion or amplification,[16,17] epigenetic

factors,[57] abnormal control of gene transcription[67] and altered biogenesis[80] of miRNAs. miRNAs have a huge pool of targets but are thought to have a tumorigenic effect by altering specific targets and acting as an oncogene or as a tumour suppressor. Several miRNA inhibitors and miRNA mimics are under clinical study, holding a promise for their therapeutic use. The role of noncoding RNAs such as circular RNAs and long noncoding RNAs is being studied to understand the underlying mechanisms of cancer disease.

With the advent of technologies such as next-generation sequencing, various roles that can be played by miRNAs as a biomarker for diagnosis,[133] detection[134] and prognosis[136] are being evaluated. Various miRNA signatures specific to the cancer types have emerged, some of which are being assessed in different clinical trials. The majority of our understanding of the functions of miRNAs are based on cell culture models, which have their limitations. So, studies in a large group of patients are essential to be carried out for its better understanding and use as a therapeutic strategy for cancer treatment.

Strategies such as antisense oligonucleotides to inhibit miRNAs, tumour and CSC-targeted nanoparticle therapy[126] and combination therapy with the chemotherapeutic agents[158] are highly promising for their clinical implication and a step forward for cancer personalized medicine.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Author contribution

Vandit Shah collected and analysed the literature and drafted the manuscript. Jigna Shah supervised and revised the manuscript. Both the authors read and approved the final manuscript.

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